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# Molecular Screening for *luxs* and *pm1* Virulence Genes of *Proteus mirabilis* Isolated from Iraqi Urinary Tract Infection Patients

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**Abstract:** The urinary tract infection (UTI) is defined as infection or colonization of the urinary tract (urethra, bladder, ureter and kidney) by microorganisms and the occurrence is found in both males and females. A total of 103 urine samples were collected from urinary tract infection patients from different hospitals in Baghdad (Baghdad Teaching Hospital, Al-kindy Teching Hospital and private laboratories) in Baghdad at a period of study from beginning of December 2021 to the end of April 2022. Antimicrobial susceptibility testing of the twenty two *P.mirabilis* isolates was screened by disk diffusion method on muller-hinton agar. Isolates were interpreted as susceptible or resistant according to the inhibition zones of the particular antimicrobial Antibiotic sensitivity test were conducted for ten *P. mirabilis* isolates using a types of antibiotics which differ in their action including Ceftazidime (68%), nitrofuranton (100%), Gentamicin (60%), Ciprofloxacine (60%), imipenem (25%),Sulphomethoxazole/ Trimethoprim (14%), Amikacin (10%) , with no resistant to Piperacillin/tazobactum , further diagnosis using *urec* gene for identification *P. mirabilis* ,the results indicated that from 103 urine specimen 22 isolates *P. mirabilis* . Detection results of *luxs, pm1* genes was done: the percentage of present of *luxs* ,*pm1* were 100% .

**Keywords:** *proteus mirabilis*, UTI, Antimicrobial susceptibility.

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## Introduction

A urinary tract infection (UTI) is a collective term for infections that involve any part of the urinary tract. Most of the UTIs are caused by bacteria, but some due to fungi and rarely by viruses' infection (1), *Escherichia coli* is the most common uropathogen one of the most bacterial infections with a variety of infections ranging from a mild self-limiting illness to severe sepsis, with a mortality rate of 20- 40%. The occurrence of sepsis and its associated mortality increases disproportionately with age (2).

The UTIs are most common infections that occure in human. An UTI can happen anywhere in your urinary tract. Our urinary tract is made up of kidneys, ureters, urinary bladder, and urethra. Most UTIs only involve the urethra and bladder, in the lower tract. However, UTIs can involve the ureters and kidneys, in the upper tract also.

Although upper tract UTIs are rarer than lower tract UTIs, but usually more severe (3). The urinary infections are fairly common, like cystitis. Often there is burning or stinging of the urine and urinary frequency is the most common symptom of UTIs (4).

Although the fact, that both genders are susceptible to infection, women are frequently vulnerable due to the anatomy and physiology of urinogenital structures (5).

The bladder urothelium plays an important role in the host innate immune response to UTI, and the fact that women are more prone to getting UTIs, the role of estrogen's effect on the urothelial defense mechanisms, and the shorter distance from the urethral opening to the bladder, increases the chance that a potential uropathogen can rise to the bladder, multiply in the urine, and invade bladder wall or rise further to the kidneys causing UTI (6).

In addition to the above-mentioned bacterial species, *Klebsiella, Proteus, Pseudomonas* and *Enterobacter* are associated with UTI. The bacteria enter the bladder through urethra and the infection can also occur through blood and lymph. The microbial etiology of UTIs is deemed to be well established and frequent. Pathogens like *E. coli* and *S. saprophyticus* are associated with population acquired acute uncomplicated infection whereas *Klebsiella, Enterococcus, Proteus Species, Enterobacter* are known to confer uncomplicated cystitis and phylonephritis(7).

## Materials and methods

In this study, 103 urine specimens were collected from patients suffering from urinary tract infections with different age groups of both genders from different hospitals in Baghdad (Baghdad Teaching Hospital, Al-kindy Teching Hospital and private laboratories) from the beginning of December 2021 to the end of April 2022. Isolates were identified depending on their physical characteristics on the culture media (blood agar ,nutrient agar and MacConkey agar) .That the colonies on the blood agar are identified as *P. mirabilis* according to their swarming phenomenon, also with their distinctive fishy odor, Proteus grow on the blood agar plate in successive waves to form a thin filmy layer of concentric circles (swarming), and on macckongy agar colony appear not to form a swarm, but form smooth, pale, or colorless colonies(8). The identification card for Gram negative bacteria was used to validate the identification of all 22 clinical isolates of *P. mirabilis* using the vitek2 system (IDGNB). The bacterial isolates were inoculated on to Macconcky agar plates and then inocubated over night at 37 C˚. A single colony was then taken and suspended in to solution. The turbidity of the bacterial suspension was adjusted with VITEK 2 densichek (Bio Merienx ) to match the Mcfar land 0.5 standerd in 0.45% sodium chloride . Then the VITEK 2 ID.GN (gram negative) card and the bacterial suspension tubes were manually loaded into the VITEK 2 system. Following steps on the software were done according to the manufacturer's instructions (Bio Merieux, France) (9) .This system used to give good results for identification and confirmation with conventional methods.

## Molecular methods

### DNA extraction

The extraction of the DNA was carried out; *P. mirabilis* isolates were

grown in brain heart broth for 24 hrs. at 37 C°. Genomic DNA was extracted from bacterial growth according to the protocol of Favor Prep Blood /Cultured Cell Genomic DNA extraction mini Kit. Quantus Fluorometer was used to detect the concentration of extracted DNA in order to detect the goodness of samples for downstream applications. Specific primers were used for detecting the *P. mirabilis* bacteria and some virulence genes according to (17, 18, 19). They were prepared according to information of supplying company (Table 1).

**Table (1): The primers sequences and size used for detection of genes**

| Genes | primer Sequences (5→3) | Size (bp) | Ref. |
|---|---|---|---|
| ***UreC*** | F: CCG GAA CAG AAG TTG TCG CTG GA<br>R: GGG CTC TCC TAC CGA CTT GAT C | 533 | 17 |
| ***LuxS*** | F: GTA TGT CTG CAC CTG CGG TA<br>R: TTT GAG TTT GTC TTC TGG TAG TGC | 464 | 18 |
| ***pm1*** | F: GGA TCA TCT ATA ATG AAA CTG<br>R: CTG ATA ATC AAC TTG GAA GTT | 563 | 19 |

**Table 2: PCR program for gene amplification**

| Steps | Cycles | Genes amplification conditions | | |
|---|---|---|---|---|
| | | ***UreC*** | ***luxs*** | ***Pm1*** |
| **Initial denaturation** | 1 cycle | 94 Cº<br>(3 min.) | 95 Cº<br>(5 min.) | 94 Cº<br>(3 min.) |
| **Denaturation** | 40 cycles<br>(*UreC*)<br>35 cycles<br>(*luxs*)<br>30 cycles<br>(*pm1*) | 94 Cº<br>(1 min.) | 95 Cº<br>(30 sec.) | 94 Cº<br>(30 sec.) |
| **Annealing** | | 63 Cº<br>(30 sec.) | 62 Cº<br>(30 sec.) | 40 Cº<br>(30 sec.) |
| **Extension** | | 72 Cº<br>(1 min.) | 72 Cº<br>(20 sec.) | 72 Cº<br>(30 sec.) |
| **Final extension** | 1 cycle | 72 Cº<br>(7 min.) | 72 Cº<br>(5 min.) | 72 Cº<br>(7 min.) |

## Results and discussion

Isolation and identification of isolates were done following their morphology in cultural characteristics and biochemical properties. A total of 103 samples were cultured on medium, blood agar, on MacConkey agar and nutrient agar. The isolates that were obtained from these media were identified according to their shape characters (swarming), which were observed. The DNA was extracted from 22 bacterial isolates that diagnosed by vitek2 system. Total DNA was extracted by using Favor Prep Blood /Cultured Cell Genomic DNA extraction mini kit. The concentration and purity of extracted DNA was measured by Quantifluor (Promega, USA).

## Antimicrobial susceptibility testing

The result of this study agree with what was reached by (10) as the percentage of resistance was (25%) to the antibiotic imipenem is the first antibiotic of this group with high ability and effectiveness against Gram-negative bacteria.

Also Gentamicin shows resistant as (60%), this result agrees with the study of (11). Resistance against Nitrofuranton was (100%) of the isolates, a study (12) in demonstrated that the rate of resistant to nitrofuranton was (100%).

(13) who agree with this study that found that the resistance pattern of the *p.mirabilis* was Ceftazidime (68%), Sulphomethoxazole/Trimethoprim (14%),while no resistance was observed to Piperacillin+tazobactum.

Also this result agrees with the researcher (14) resistant reach 10 % to Amikacin while this result disagrees with (15) were the isolates show high resistance (80%).

The charts described in figure (1) showed that (60 %) of the isolated bacteria were resistant to Ciprofloxacin, which agree with the results recorded by (16) who test the susceptibility against antimicrobial agents for the *P. mirabilis* pathogenic isolates.

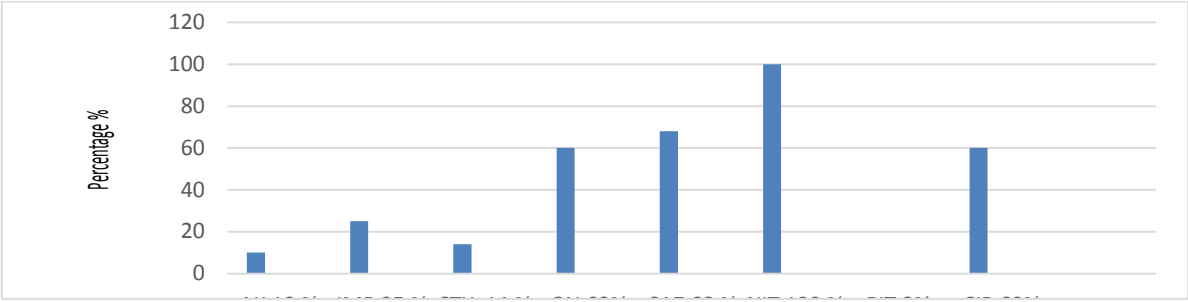


**Figure (1): Percentage of resistance for *P.mirabilis* bacteria isolates to different antibiotic.**

**CAZ =Ceftazidime, PIT = Piperacillin/tazobactum, NIT =Nitrofuranton, GN = Gentamicin ,IMP = Imipenem , STX = Sulphomethoxazole/ Trimethoprim , AK = Amikacin , CIP = Ciprofloxacin .**

For identification of all 22 isolates of *P. mirabilis* depending on use of specific primers for *Urec* gene give the same results when compared with traditional methods and vitek2 system. Product of conventional PCR for 22 isolates detected by using gel electrophoresis as showing in Figure (2).

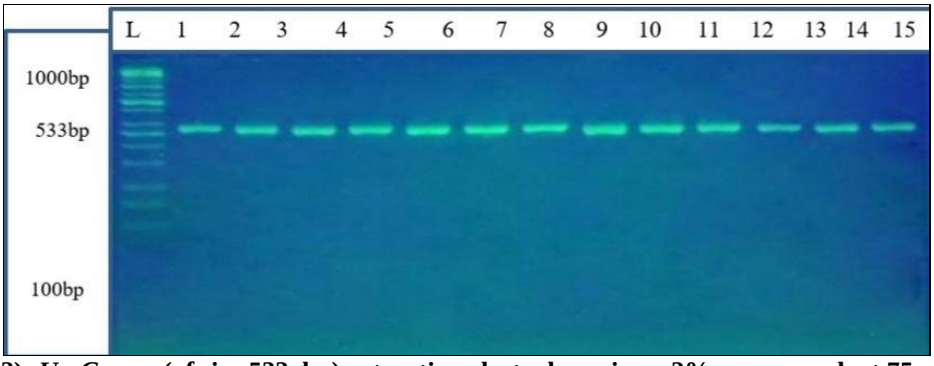


**Figure (2): *UreC* gene (of size 533 bp) extraction electrphorasis on 2% agarose gel at 75 volt for 1 hr.**

Clear correlation was appeared between PCR techniques and culturing methods whereas all positive samples with culturing method gives a positive amplification results with conventional PCR. *luxS* gene is one from group of virulence genes is the quorum sensing (*luxS*, and *rsbA*). The *luxS* gene

produces signal that is used to sense the interaction of species and its cell density in a polymicrobial community that plays critical roles in the virulence genes regulation.. It has been found that percentage reach (100%) of the isolates contain the genes with the length of 464 bp. These studies agree with a previous study by (20). Figure (3).

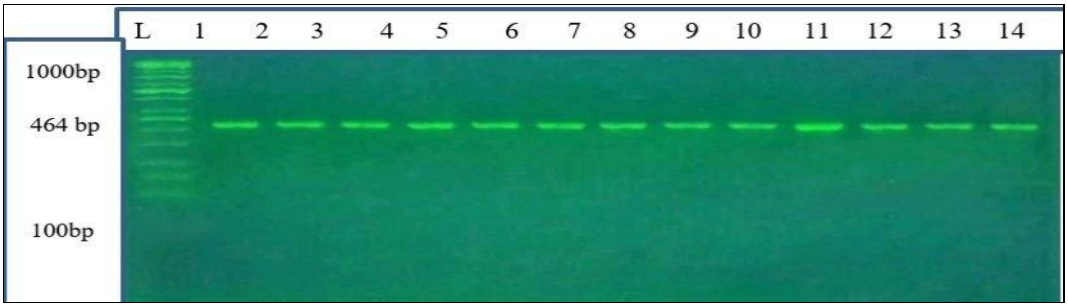


**Figure (3): Agarose gel electrophoresis (2% agarose, 75 V for 1:45 hour) of *luxs* gene PCR products (length of 464 bp) .**

*Proteus mirabilis* strain has the ability to produce many virulence factors that play an important role in human infection. Detection of *pm1* gene encoded with molecular size of 563 bp in percentage 100 % fig (4) , this agree with previous study (20,21).

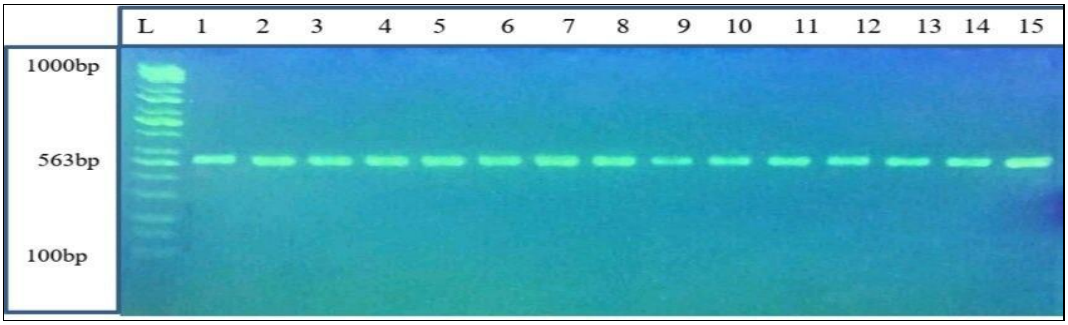


**Figure (4): Agarose gel electrophoresis (2% agarose, 75 V for 1:45 hour) of of *pm1* gene gene PCR products (length 563 bp) .**

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