MOLECULAR DETECTION OF SOME VIRULENCE GENE IN *Proteus Mirabilis* ISOLATED FROM URINARY TRACT INFECTION IN IRAQ

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ABSTRACT

This study was concentrated for isolation and identification of 60 (35.2%) *Proteus mirabilis* isolates out of 170 urine samples from patients suffering from urinary tract infection from different hospitals in Baghdad city during a period from September 2020 to January 2021. The isolates were cultivated on selective media and biochemical reactions were used to identify them confirmatory APi 20 E tests. The sixty selected isolates were tested for resistance against four antibiotics. The results shown that there were differences in the antibiotic resistance of isolates. High resistance to nalidixic acid and ampicillin were found among isolates as (75%) and (51%) respectively while the resistance of *Proteus mirabilis* isolates to amikacin and impenem, were(8.3%). Some important virulence factor to *Proteus mirabilis* was detected by using molecular techniques include PCR and it was found that only 18 (60%) of isolates gave positive result for *rsbA* at 467 bp. 27 (90%) of them gave positive result for *luxS* at 464 bp.

Keywords: Api20E, PCR, the gene s*(rsbA and luxS)*, sensitivity test.

مجلة العلوم الزراعية العراقية -2023 :54(3):709-715 الخالدي و ابو ريشة

التحري الجزيئي لبعض جينات الفوعة في *Proteus mirabilis* المعزولة من اصابات المسالك البولية في العراق

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المستخلص

هدفت هذه الدراسة الى عزل وتشخيص 60 (35.2%)عزلة من المتقلبات الرائعة *Proteus mirabilis* من أصل 170 عينة ادرار من المرضى الذين يعانون من التهاب المسالك البولية من مستشفيات مختلفة في مدينة بغداد خلال الفترة من سبتمبر2020 إلى يناير 2021. زرعت العزلات على وسط انتقائي واستخدمت التفاعلات الكيميائية الحيوية للتعرف عليها فضلاعن الاختبارات التأكيدية باستخدام نظام Api 20 E test. تم إجراء اختبار الحساسية الدوائية ل(60) عزله اتجاه(4) مضادات، وقد أظهرت النتائج بان أعلى نسبة مقاومة كانت تجاه كل من مضاد حامض النالدكسك (75%) تبعه مضاد الامبيسلين (51%). أما بالنسبة لمضاد اميكاسين والامبينيم كانت النسبة (8.3%) .تم الكشف عن بعض عوامل الضراوة الهامة لـ *Proteus mirabilis* باستخدام تقنيات جزيئية تشمل تفاعل سلسة البلمرة ووجد أن 18(60%) فقط من العزلات أعطت نتيجة إيجابية لـ *rsbA* عند 467 bp ، (90%) 27 أعطت نتيجة إيجابية لـ *luxR* عند 464 bp.

الكلمات المفتاحية: تفاعل سلسة البلمرة، اختبار الحساسية.

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INTRODUCTION

Bacteria *Proteus mirabilis* causes of many types of infections, more commonly associated with complicated urinary tract infections and bacteremia. It affecting patients with anatomical abnormalities, immunodeficiency and continuing urinary catheterization .(3).Besides urinary tract infections, *Proteus mirabilis* associated with opportunistic infections for pulmonary system, wound, burns, skin, eyes, ears, nose and gastroenteritis. As well causing an autoimmune disease in human who is genetically susceptibility to develop rheumatoid arthritis. (8 , 28). The ability of this organism to create a variety of extracellular enzymes, such as urease, which is responsible for the creation of bladder and kidney stones, and the formation of stones around the bacterium inhibit antibiotic cure effect, may account for its medical value. In addition, urinary tract epithelial cells are cytotoxic to haemolysin.(14). For the importance of *Proteus* spp. as a nosocomial pathogen, the present study was planned to perform the isolation of *Proteus spp*. from different sources and determination of antibiotic sensitivity of the selected (60 isolates). *Proteus mirabilis* expresses adhesins, flagella, toxins, quorum-sensing, enzymes, and immune invasion, among other virulence factors involved in infection.(5). *Proteus mirabilis* encodes many virulence genes involved in infection (1 , 21). Quorum sensing regulates the expression of genes involved in a variety of physiological functions, including swarming motility, type III secretion, exopolysaccharide (EPS) production, and biofilm formation (1, 11). A biofilm can be defined as "a community of microorganisms attached to a suitable surface. (27, 30) .The *rsbA* gene was regulator of swarming behaviour that encodes a sensory, while *rsbA* may function as a protein sensor of environmental conditions.(19). *rsbA* gene was stimulated biofilm formation and Extracellular polysaccharide formation.(15uce an auto inducer2(AI-2), which is thought to be involved in inter-species communication . (6,22 , 24). The *luxS* genes have been shown to be responsible for the production of auto inducer 2 which plays an important role in other types of cell–cell signalling in bacteria. The transcription of the *luxS* structural operon *luxCDABF* was increased when *luxR* coupled to auto inducer. After *luxS* gene produced auto inducer 2 signal, which is used to sense intra- and inter-species interactions as well as its own cell density in a polymicrobial community and plays a crucial role in virulence factor control (10,23,29).

MATERIALS AND METHODS

Bacterial isolation and identification

One hundred seventy samples were collected from patients with urinary tract infection cases from different hospitals (Al-Yarmouk, Central hospital of paediatric hospital) in the period from September 2020 to January 2021.Each specimen was inoculated on selective media and identified by biochemical reaction according to the diagnostic procedures recommended in(9). and according to API 20E confirmatory test**.**

Antibiotic sensitivity test (qualitative disk method): Four antibiotic disks (nalidixic acid-30 µg,ampicillin-10 µg, imipenem-10 µg and amikacin-30 µg) were used to detect the sensitivity of 60 isolates of *P. mirabilis* by using Kirby-bauer method according to (18).

Molecular detection of some virulence factors: We using polymerase chain reaction (PCR) technique for detection of some virulence gene include (*rsbA* and *luxS*). And the genomic DNA extracted by purification kit that supplemented by the manufacturing company (intron biotechnology, Korea).The suspension containing DNA was stored at-20ºC until used as template for PCR.

PCR amplifications

The detection of virulence genes was accomplished using PCR technique.Tabl-2. Descriptions and sequences of the PCR primers used in this study are displayed in Table-1.

Table 1. The primer sequence and that used in present study

Genes name	Primer sequence (5´- 3´)	Size bp	Reference
rsbA	F: TTG AAG GAC GCG ATC AGA CC	467	(4)
	R:ACT CTG CTG TCC TGT GGG TA		
luxS	F: GTA TGT CTG CAC CTG CGG TA	464	(4)
	R: TTT GAG TTT GTC TTC TGG TAGTGC		

***F: Forward Primer, R: Reverse Primer**

Table 2. PCR programs of *rsbA* and *luxS* genes

Genes name	PCR Amplicfiation	T°C	Time	Cycle
rsbA	**Initial denaturation**	**94°C**	**5min**	**1**
	Denaturation	**94°C**	**60sec**	**1**
	Annealing	**58°C**	**45sec**	**35**
	Extension	**72°C**	**1min**	**1**
	Final extension	**72°C**	**7min**	**1**
luxS	**Initial denaturation**	**94°C**	**5min**	**1**
	Denaturation	**94°C**	**60sec**	**1**
	Annealing	**58°C**	**45sec**	**40**
	Extension	**72°C**	**1min**	**1**
	Final extension	**72°C**	**7min**	**1**

RESULTS AND DISCUSSION

Bacterial isolation and identification

The most isolates obtained from urine were *Proteus mirabilis* 60(35.2%).the reason for urinary tract infections is due to the proximity of the anal opening to the vagina and urethra. *Proteus* isolates were firstly identified as related to the genus *Proteus* by swarming phenomenon on blood agar and non-lactose fermenter on macconkey agar and appeared pale (7). Microscopic examination of the bacteria appeared as straight rods and gram negative when it stained with gram stain (9).Several biochemical tests were done to characterize *Proteus* isolates. All the 60 isolates of *Proteus mirabilis* showed positive results to the biochemical tests, catalase, urease and KIA, but all were oxidase of citrate utilization test, negative. These isolates were motile, and all the 60 isolates were indole negative. Also *Proteus* isolates were unable to ferment lactose and maltose. The results of biochemical tests, Table (3), were compared with the characteristics of *Proteus spp.* documented by (9,12, 18) who showed two species of *Proteus* were identified, *P. mirabilis* and *P. vulgaris*

Table 3. Bacteriological and biochemical properties of *Proteus mirabilis*

Identification	Bacteriological Tests andBiochemical Tests	*P. mirabilis*
1	**Swarming on Blood agar**	**+**
2	**lactose fermentation on MacConkey agar**	**Non lactose fermenter**
3	**Catalase production**	**+**
4	**Oxidase production**	**–**
5	**Urease production**	**+**
6	**Indole production**	**–**
7	**Methyl red test**	**+**
8	**VogeusProskauer tests**	**–**
9	**Citrate utilization**	**+**
10	**Kligler iron agar**	**Red slant/yellow butt +H2S ,- gas**

(+):positive result; (-): negative result

For confirmation of the biochemical results, the API 20E strips were used for Enterobacteriaceae identification containing 12 tests . The results revealed that the tested isolate were *P.mirabilis*.

Antibiotic resistance test of *Proteus* isolates: Sixty selected isolates were tested for resistance toward four antibiotics. It was found that isolates differed in their antibiotic resistance. High resistance to Nalidixic acid and ampicillin were found among isolates as 75% and 51% respectively . While resistance of *P. mirabilis* isolates was observed to imipenem and amikacin 8.3 %, as illustrated in figure-1.

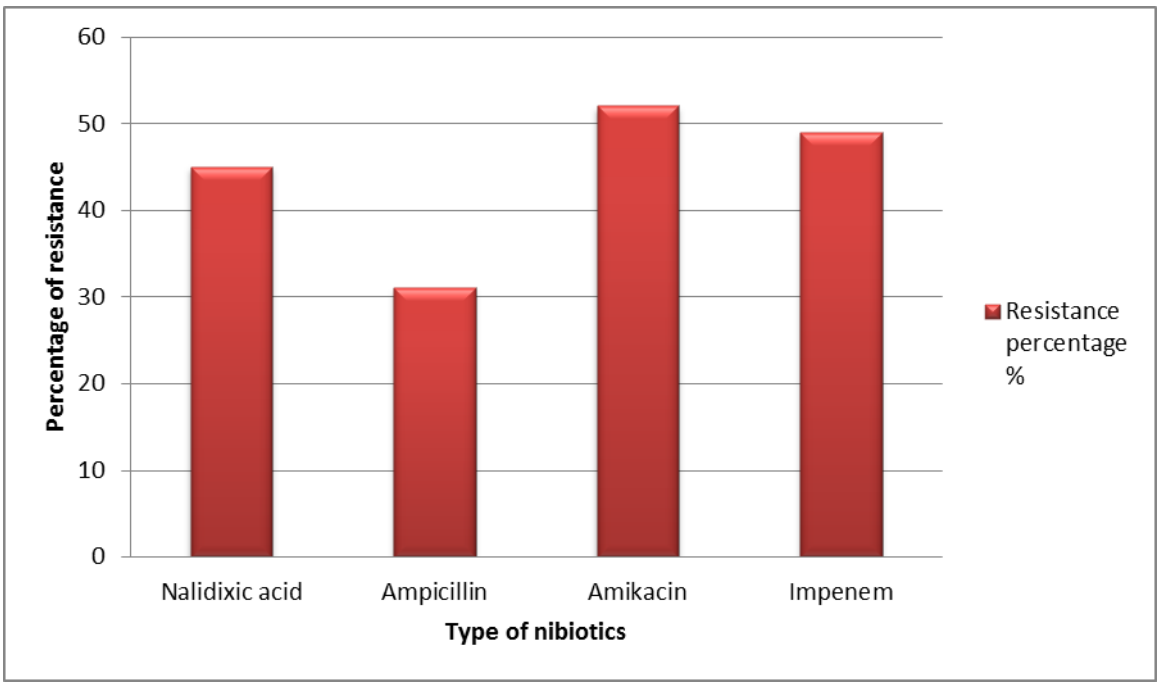

Figure 1. Susceptibility of *P.mirabilis* to Antibiotics

Results showed that (51%) of the isolates were resistant to ampicillin.These observations are in agreement with studies of (16) who found that (62%) of *Proteus* isolates were resistant to ampicillin , and (25) reported that ampicillin has no more effect on any of the isolates of UTI. The resistance to the amikacin and imipenem with percentage (8.3%), Imipenem is uncommon to be used in our country therefore the antibiotic resistance is low and this is result nearly close to 1.6% of (17) .Also this study indicated that (75%) of the isolates were resistant to nalidixic acid. This disagrees with (31) who observed the resistance of the isolates to nalidixic acid was (18%). Multi-drug resistance to *Proteus* isolates could be a result of the extra outer cytoplasmic membrane which contains a lipid bilayer,lipoproteinsand lipopolysaccharide(20). Resistance of *Proteus* to antibiotics was due to selection for drug resistance has been associated with an increased and inappropriate use of antibiotics .There is an irregular use of antimicrobial agents in Iraq.

Molecular detection of virulence genes

The results of the present study are showed that 18 (60%) of *Proteus mirabilis* isolates give positive result at 467bp for *rsbA* gene. this result was shown in Figure (1).Which nearly agreed with the results obtained by (4).who found that (70%) of *Proteus mirabilis* isolates display *rsbA* genes band so it is common in *P.mirabilis.*

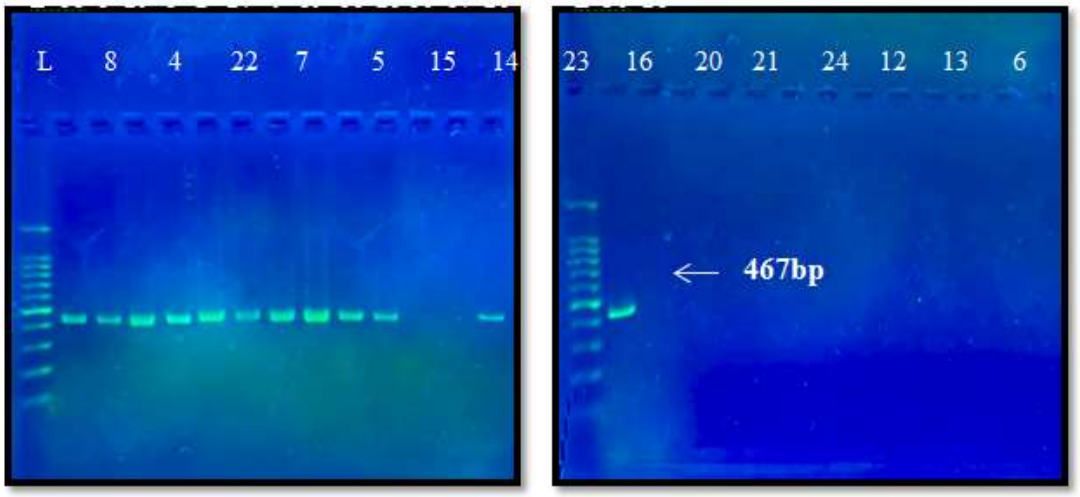

Figure 1- Gel electrophoresis for amplified PCR product of *rsbA* gene of *Proteus mirabilis* with band size 467bp. The product was electrophoresis on 1.5% agarose at 5 volt/cm^2. 1X TBE buffer for 1:30 hours. L: DNA ladder (100).

The presence of the *luxS* gene among *Proteus mirabilis* isolates was detected using *luxS* primers. It has been found that 27 (90%) of these isolates contain the genes with the length of 464 bp as shown in Figure (2).The results of the present study are agreed with the results obtained by (4).who found that (70%) *Proteus mirabilis* isolates display *luxS* genes band.

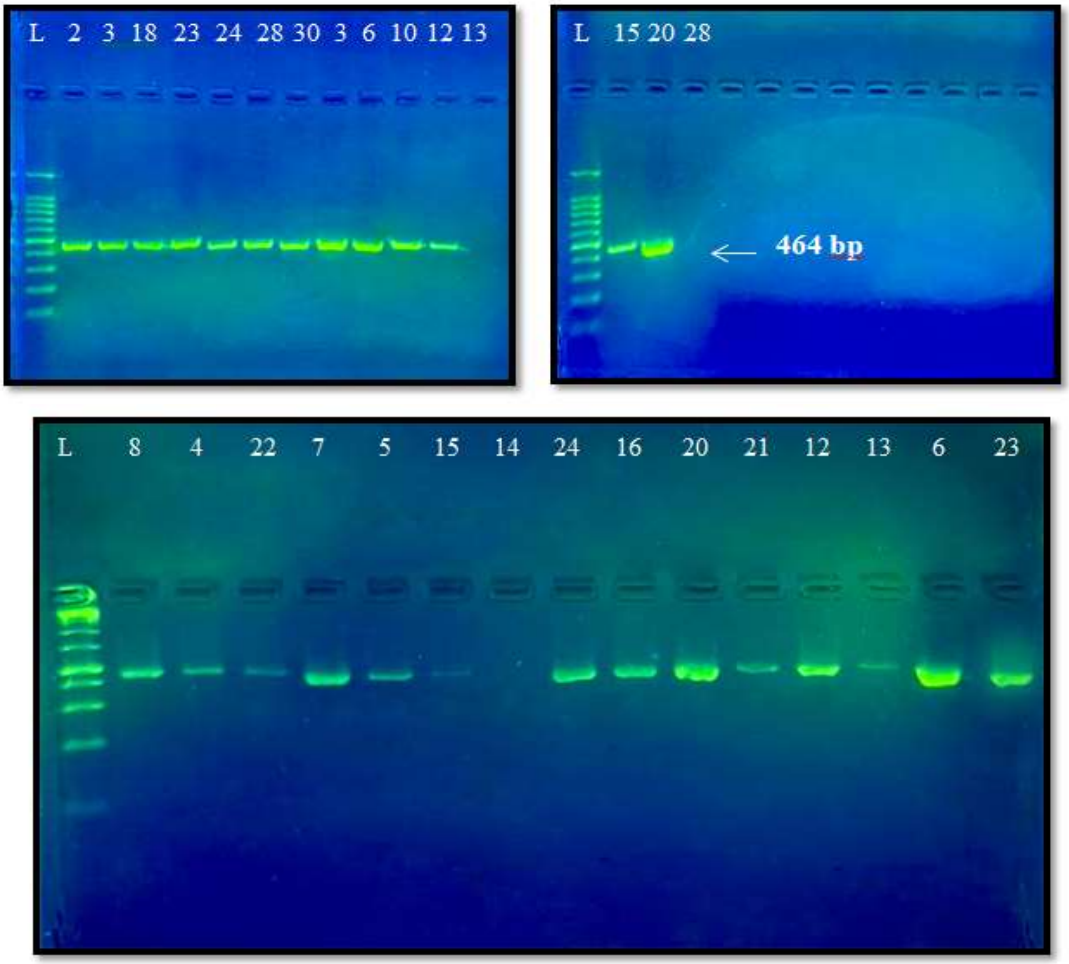

Figure 2. Gel electrophoresis for amplified PCR product of *luxS* gene with band size 464 bp. The product was electrophoresis on 1.5% agarose at 5 volt/cm2. 1X TBE buffer for 1:30 hours. L: DNA ladder (100).

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