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المحترمين

تحية طيبة.....

يسرنا اعلامكم ان هيئة التحرير في اجتماعها المنعقد قد درست نتائج التقويم العلمي لبحثكم المعنون

(Molecular characterization for Some virulant factors of *Proteus Mirabilis* isolated from patients in Al-Qadisiyiah Province).

وفي ضوء ذلك قررت قبول نشره في العدد (2) المجلد (16) من المجلة الذي سيصدر في النصف الثاني للعام 2017

متمنين لكم التوفيق مع فائق الاحترام

أ.د. ضياء حسين جاسم
رئيس هيئة التحرير
2017/10/8

Antimicrobial Susceptibility and Molecular Characterization For Some Virulence Factors of *Proteus Mirabilis* Isolated From Patients In AL-Diwaniya Province / Iraq .

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Abstract : Total of 64 Samples clinical isolates were collected from various sources included urine , ear external swab ,wounds swab , burns swab , high cervical and endometrium cervical swab ,which was taken from (in-patients) and (out-patients) in Maternal , and General Teaching Hospital in AL-Diwaniya city during the period from 1/3/2015 till 30/9/2015 . The resistance of bacterial isolates to 12 different antibiotics was tested and isolates showed different resistance to anti-βata Lactam including penicillin and Amoxicillin / Clavulanic acid by (100%) , cefotaxime by (86%) , and Cephalexin by (90.62%) , Imipenem by (18.75%) , and Meropenem by (15.62%) . Some of the virulence factors have been studied genetically (20 isolate) ,in terms of the genetic aspect , five genes were obtained for virulence factors and at varying rates , as the urease enzyme *ureC* , fimbriae formation *mrpA* , flagella *flaA* , hemolysin enzyme *hpmA* and biofilm formation *luxS* (60 , 40 ,100 , 45 , 55) % , respectively .

Keywords:Antimicrobial susceptibility test , virulence factors genes, PCR, *proteus mirabilis*.

Introduction

Proteus mirabilis is a common causative agent of the severe invasive diseases.This microorganism expresses several virulence factors. *P.mirabilis* can expresses adhesins , flagella, toxins. *Proteus* is a gram-negative bacteria associating to the Enterobacteriaceae family. Which is distinct from other species by swarming in the surface of agar (23). *Proteus* is a broad distribution in the environment that puts up a portion of the normal flora in a gastrointestinal tract of the human. It is ranked third in a cause of hospital-acquired infections (42).The opportunistic human pathogens represented by three species: *P.vulgaris*, *P.mirabilis* , and *P.penneri* (21). It is an important source of hospital-acquired infections (17). *P.mirabilis* the main cause of urinary tract infections (31, 33, 37, 12) , *P.mirabilis* is a common cause of urinary tract infection , most existing in patients with indwelling catheters and urinary tract structural abnormalities (3),respiratory tract and wounds infections (38), burns and digestive tract infections (44), Ear infection and Otitis media (7). *P.mirabilis* has a wide arrange of cell-associated factors , it can secreted a lot of factors, some of these factors have been closely rlated with disease producing potential called virulence factors (16, 39), such as swarming , fimbriae , urease , hemolysin , iron acquisition systems (22), protease and Lipopolysaccharides (LPS) (41). This pathogen have developed several pathogenic factors including which listed above which enable them to colonize , survive and grow in the host organism (16, 40).

Materials and methods

Specimens' collection

different Samples such as urine , wound swab , burn swab , high cervical , endometrium cervical and ear external swab, which was taken from (in-patients) and (out-patients) after taken the approval of the patient's in Maternal and General Teaching Hospital in AL- Diwaniya city during the period from 1/3/2015 till 30/9/2015.The collection process has been conducted according to (26).

Identification of Bacterial Isolates

The isolates were identified according to (18) by using traditional micro-scopic examination (Gram's stain), colony morphological features on MacConkey agar and blood agar, and standard biochemical tests.

DNA extraction

The total genomic DNA of the *P.mirabilis* was isolated using the DNA extraction and purification kit (Geneaid ,USA) according to the manufacturer instructions . DNA preparations were then analyzed by electrophoresis in 1.5% agarose gel .

Polymerase chain reaction

Polymerase chain reaction was used to amplify the entire sequences of the genes studied in this research. The specific primers (Bioneer, Korea) used for the amplification of these genes (45) were shown in (table 1) .

Agarose gel electrophoresis

Theproducts were separated in 1.5% agarose gel in TBE buffer (pH 8) , stained with ethidium bromide, and photographed in ultraviolet light (14) . The electrophoresis result noticed by using gel documentation system .

Detection of the virulence factors of *P. mirabilis*

1-Hemolysin production **4-**Detecting flagella

2-Urease production **5-**Detecting fimbriae

3- Biofilm formation

Table (1) : The primer of some virulence factors genes in this study

Primer	Sequence	Amplicon
Urease *ureC*	**CAAGCCCAAGAAGGTCTCGT**	**517bp**
	CAAGATGCTCGTCCACGGTA	
Fimbriae *mrpA*	**CGGGTTCTGCTTTAGCTGCA**	**359bp**
	GTTTTGAGCAGCACTTGGGG	
Flagella *flaA*	**ATCAATGCAGCTGCGACACT**	**445bp**
	TGAAGTACCCGCTTGTTGCA	
Hemolysin *hpmA*	**AGGTGCTAAACTGCATGCGA**	**270bp**
	ACAAAAGCACCTTGGTTGCC	
Biofilm formation *LuxS*	**ACGTATGTCTGCACCTGCG**	**290bp**
	CCATAGCTGCCTTCCATGCA	

Antimicrobial susceptibility test (AST)

Determination of antimicrobial agents susceptibility by disk diffusion method (35) ,Which was gotten from BDH London, UK ,which was: penicillin (P) , augmentin (AUG) , gentamycin (GM) , cefotaxime (CTX) , cephalexin (CL), amikacin (AK), imipenem (IMP), Meropenem (MEM), Tobramycin (TOB), Kanamycin (K), Netilmicin (NET) and Streptomycin (S).

The inoculum were prepared by growing of *Proteus mirabilis* on dispersed agar plates, then the colonies grow in the plate transferred by loop into a test tube filled with 3 ml of normal saline. The suspensions density was adapted to 0.5 McFarland standards. The plate surface of Muller-Hinton agar (Himedia India) was inoculated with bacteria by a sterile swab. The swab was soaked into the suspension and pressed into the side of the test tube to discard exuberance fluid, then inoculate the Muller-Hinton agar by streaking method. Antibiotic discs were applied to the inoculated agar and incubated at 37ºC overnight. The diameter of zone of growth - inhibition observed was measured and compared to the chart of National Committee for Clinical Laboratory Standards (NCCLS).

Results

Sixty four isolates were identified as *P.mirabilis* consisted of 32 isolates (12.8%) obtained from urine , 17 isolates (9.18%) obtained from external ear, 3 isolates (7.5%) obtained from wounds , 4 isolates (4.7%) obtained from burns and 8 isolates (8.88%) obtained from high cervical and endometrium cervical (table 2).

Table (2) : Distribution of *P.mirabilis* among various clinical sources .

Type of samples	No. of samples (%)
Urine	**32 (12.8)**
External ear	**17 (9.18)**
Wounds	**3 (7.5)**
Burns	**4 (4.7)**
High cervical and endometrium cervical	**8 (8.88)**
Total	**64 (9.84)**

Susceptibility of *P.mirabilis* isolates to different antimicrobial agents

(Table3) shows a higher percentage of resistance to both Penicillin and amoxicillin with clavulanic acid (100%) , while, the lowest resistance with impenem and meropenem (18.75 , 15.62) % , respectively .

Virulence factors of *P.mirabilis*

The results of the PCR for 20 *P.mirabilis* isolates showed that higher percentage of gene *flaA* (100%) , while , the lowest percentage of gene *mrpA* (40%) (table 4) .

Figure (1,2,3,4,5) shows that PCR amplified production for 20 *p.mirabilis* .

Table (3) : Percentage (%) of antimicrobial resistance and sensitivity of *P.mirabilis*

Antibiotics	Resistance isolates		Sensitive isolates	
	No.	(%)	No.	(%)
Penicillin	64	100	0	0
Augmentin	64	100	0	0
Cefotaxime	55	86	9	14.06
Cephalexin	58	90.62	6	9.37
Impenem	12	18.75	52	81.25
Meropenem	10	15.62	54	84.37
Gentamycin	35	54.68	29	45.31
Amikacin	20	31.25	44	68.75
Tobramycin	49	76.56	15	23.43
Kanamycin	52	81.25	12	18.75
Streptomycin	55	85.93	9	14.06
Netlimicin	52	81.25	12	18.75

Table (4) :The numbers and percentages of the genes of Virulence factors for 20 *P.mirabilis* isolates .

Virulence factors genes	No. *P.mirabilis* isolates	(%)
ureC	12	60
mrpA	8	40
flaA	20	100
hpmA	9	45
luxS	11	55

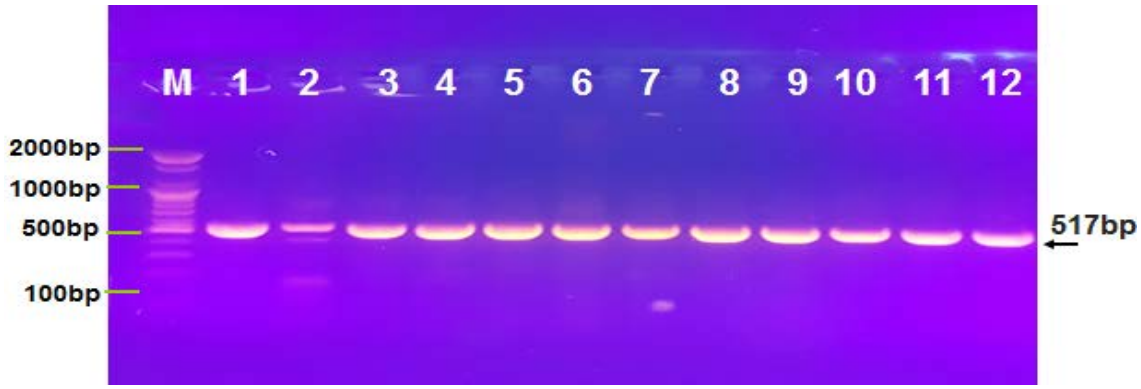

Figure (1): PCR amplified production of *P. mirabilis* isolates using *ureC* gene primers. M:marker ladder (100-2000 bp). Lanes 1-12 the isolates showed positive results with *ureC* gene , (80mA and 100V) for one hour .

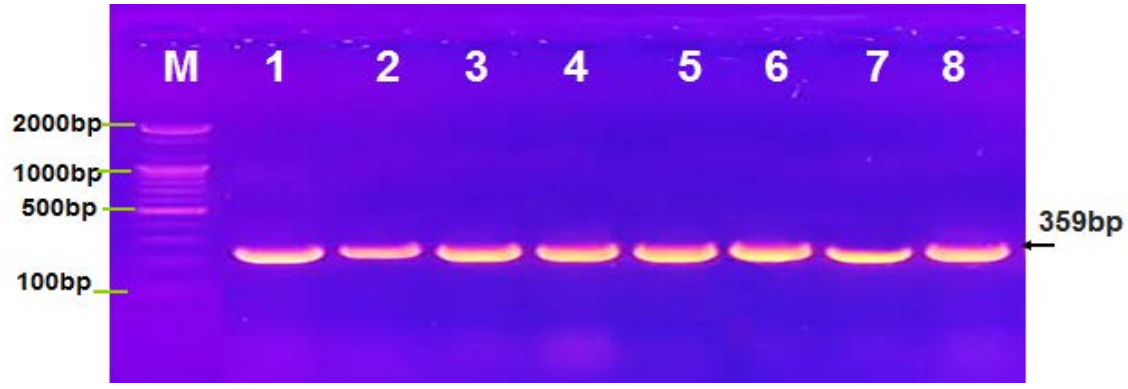

Figure (2): PCR amplified production of P. mirabilis isolates using *mrpA* gene primers. M:marker ladder (100-2000 bp). Lanes 1-8 the isolates showed positive results with *mrpA* gene , (80mA and 100V) for one hour .

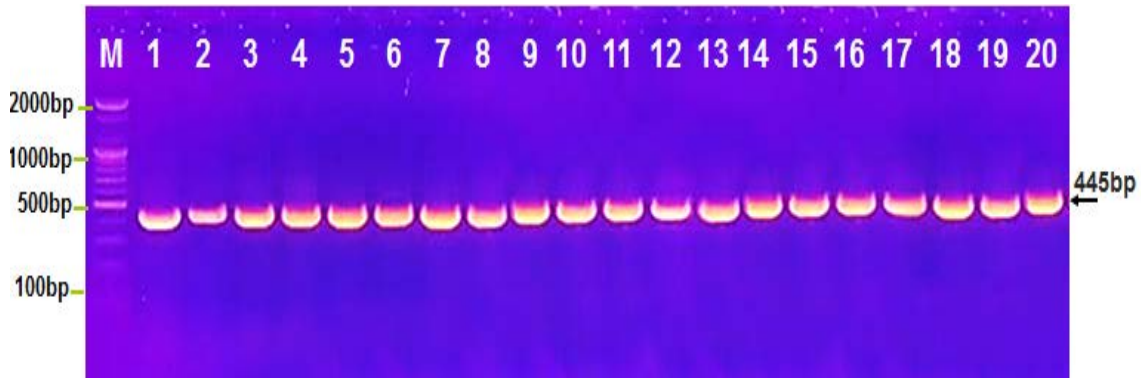

Figure (3): PCR amplified production of *P. mirabilis* isolates using *flaA* gene primers. M:marker ladder (100-2000 bp). Lanes 1-20 the isolates showed positive results with *flaA* gene , (80mA and 100V) for one hour .

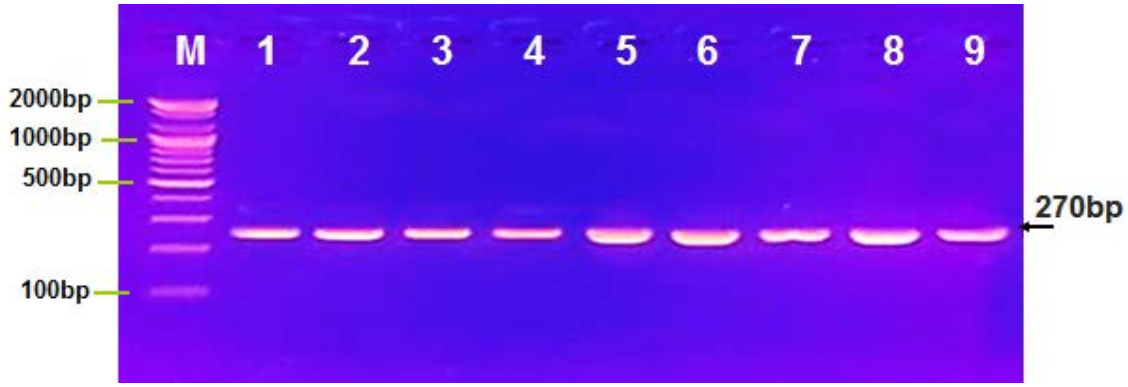

Figure (4): PCR amplified production of *P.mirabilis* isolates using *hpmA* gene primers. M:marker ladder (100-2000 bp). Lanes 1-9 the isolates showed positive results with *hpmA* gene,(80mA and 100V) for one hour .

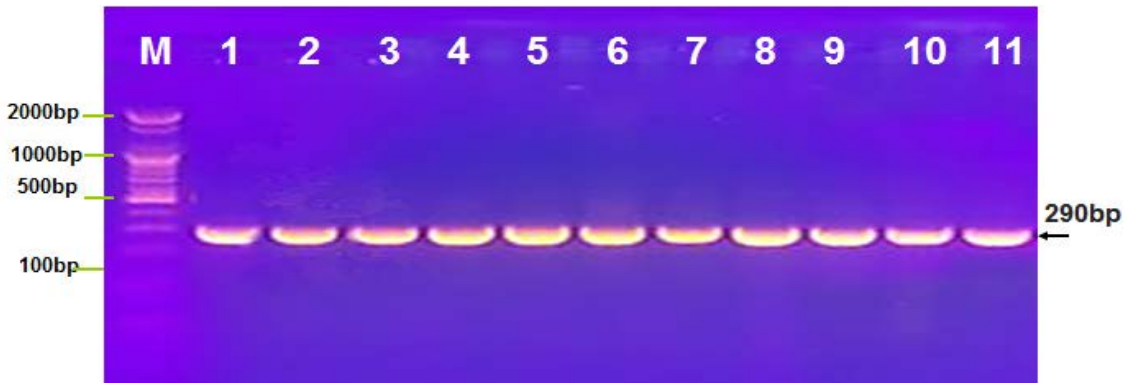

Figure (5): PCR amplified production from extracted DNA of *P.mirabilis* isolates using *luxS* gene primers. M: marker ladder (100-2000 bp). Lanes 1-11 the isolates showed positive results with *luxS* gene ,(80mA and 100V) for one hour**.**

Discussion

Isolation and identification

Sixty four isolates were identified as *P.mirabilis* consisted of 32 isolates (12.8%) obtained from urine , 17 isolates (9.18%) obtained from external ear, 3 isolates (7.5%) obtained from wounds , 4 isolates (4.7%) obtained from burns and 8 isolates (8.88%) obtained from high cervical and endometrium cervical .

We analyzed resistance to 12 antibiotics belonging to two groups: beta-lactams and aminoglycosides all isolates showed microbiological resistance Penicillin , amoxicillin with clavulanic acid (100%) , this result agrees with similar studies both of (10,8) , Cefotaxime (86%), this result agrees with (2) about (83%) ,but this current result not agrees with (4) about (100%), also Cephalexin (90.62%) this result agrees with (10) about (80%), but this current result agrees with (8) about (42.85%). As for antibiotic aminoglycosides Gentmycin about (54.68%) this result agrees with (6) about (50%) but not agrees with (20) about (33%), also Amikacin (31.25%) this

result agrees with (11) about (38.4%) but this current result not agrees with (25,6) about (1.6,5)% , respectively. Tobramycin (76.56%)this result agrees with (5) about (81%) but this current result not agrees with (47) about (33.3%) . also antibiotics Kanamycin , Streptomycin and Netl- imicin about (81.25 , 85.93 , 81.25)% , respectively .

Virulence factors of *P.mirabilis*

The genotypic characters were tested for 20 *P. mirabilis* isolates in this study in order for detection of the virulence factors. The results of the PCR for the isolates of *P. mirabilis* showed that (60%) isolates were positive for the presence of *ureC* gene,this result not agrees with (43) value *ureC* gene about (100%). Urease is one of the most important factors in *P.mirabilis pathogenesis*. In vitro (on basic urea agar), urease hydrolyzing urea to alkaline ammonia and carbon dioxide, there by increasing the pH and will be changing the color of phenol red indicator to pink (19). But *In vivo* (human body) this enzyme catalyzes the formation of kidney and bladder stones or to encrust or obstruct indwelling urinary (16).

In the current study found *mrpA* gene about (40%) , this result agrees with (1) were value *mrpA* gene (35%), but current study not agrees with (28) were vaule gene (8%) . Basic studies showed that serum from mice infected was reacted firmly to MR/P fimbrial preparations, which revealed that these fimbriae are expressed in vivo (32). MR/P fimbriae expression shown to be extremely induced through infection (24).

The results of this study revealed that all *P.mirabilis* isolates (100%) possessed *flaA* gene, this finding is in agreement with other researches (29) and (9) that conducted in Iraq. They reported the presence of *flaA* gene in

Proteus isolates (100%, 86.66%) respectively. While (13) found that (100%) of *P.mirabilis* isolates were carrying *FliL* gene which encodes for the flagellar basal body protein.

In this study the presence of the *hpmA* gene was responsible for hemolycin about (45%),this result agrees with (46) about (46.7%) , while not agrees with (8) about (100%) .

Finally in current study the presence of the *luxS* gene was responsible for biofilm formation about (55%) , this result agrees with (1) who found value *luxS* gene about (47%) .

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التوصيف الجزيئي لبعض عوامل ضراوة *Proteus mirabilis* المعزولة من المرضى في محافظة القادسية

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الخلاصة

تعد بكتريا *Proteus mirabilis* أحد الأسباب الشائعة لعديد من الإمراض ، تستطيع هذه البكتريا ان تعبر عن عوامل الضراوة منها القابلية على الالتصاق والأسواط والسموم . جمعت ٦٤ عزلة من مصادر سريرية متعددة تضمنت الإدرار والأذن الخارجية والحروق والجروح وأعلى عنق الرحم وبطانة الرحم من المرضى الراقدين والمراجعين لمستشفى الولادة والأطفال التعليمي ومستشفى الديوانية التعليمي للفترة من ٢٠١٥/٣/١ إلى ٢٠١٥/٩/٣٠ . أظهرت هذه البكتريا مقاومة متعددة أتجاه ١٢ نوع من المضادات الحيوية ، إذْ أظهرت مقاومة لمضادات البيتالاكتام المتمثلة Penicillin و Amoxicillin / Clavulanic acid بنسبة (100%) و Cefotaxime بنسبة (86%) و Cephalexin بنسبة (90.62%) و Imipenem بنسبة (18.75) و Meropenem بنسبة (15.62%). كذلك قاومت البكتريا المعزولة مضادات مجموعة الامينوكلايكوسيدات وبنسب مختلفة وهي : Gentamycin و Amikacin و Tobramycin و Kanamycin و Streptomycin و Netlimicin (54.68 و 31.25 و 76.56 و 81.25 و 85.93 و 81.25) % وعلى التوالي . درست بعض عوامل الضراوة وراثياً لـ 20 عزلة بكتيرية والمتمثلة بجين انزيم اليوريز *ureC* وجين لتكوين الأهداب *mrpA* وجين لتكوين الأسواط *flaA* وجين انزيم الهيمولايسين *hpmA* وجين لتكوين الغشاء الحيوي *luxS* وأظهرت النتائج امتلاك هذه البكتريا لعوامل الضراوة بنسب (60 و 40 و 100 و 45 و 55) % على التوالي .

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