

Molecular Detection of Some Virulence Genes in *Proteus Mirabilis* Isolated from Hillaprovince

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Abstract: This study is concentrated for isolation and identification of 17 *Proteus mirabilis* isolates out of 135 clinical samples from patients attending to Hilla teaching Hospital during a period from October 2014 to February 2015. The isolates obtained from patients with urinary tract infections have been 11(64.7%), whereas that from patients with ear infection 3(17.6%). Besides, the isolates obtained from patients with diarrhea, wound and vaginal infection that each case has percentage 1(5.9%). The isolates obtained have been cultured on selective media and identified by biochemical reaction.

Some important virulence factor to *P. mirabilis* was detected by using molecular techniques include PCR and it was found that only 3(%18) of isolates gave positive result for ure C at 533bp, 6(%35) of them gave positive result for *mrpA* at 565bp, 7(%41) of them gave positive result for *pm1* at 563bp, 8(%47) of them gave positive result for *luxS* at 464bp, 9(%53) of them gave positive result for *rsbA* at 467bp.

Keywords: *P. mirabilis*, PCR, virulence factors gene (*ureC*, *mrpA*, *pm1*, *luxS* and *rsbA*)

1. INTRODUCTION

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 [1].

Besides urinary tract infections, *P. 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 [2,3].

The medical importance of this organism may attribute in its ability to produce a variety of extracellular enzymes such urease, which is responsible for the formation of bladder and kidney stones, the formation of stones around the bacteria prevent antibiotic cure effect. Additionally, the hemolysin is cytotoxic for urinary tract epithelial cells [4].

P. mirabilis expresses several virulence factor involved in infection like adhesins, flagella, toxins, quorum-sensing, enzymes and immune invasion [5].

Urease is very important in *P. mirabilis* pathogenesis. This enzyme catalyzes the formation of kidney and bladder stones or to encrust or obstruct indwelling urinary. The urea-inducible urease gene cluster *ure RDABCEFG* that encodes a multimeric nickel-metalloenzyme, which hydrolysing urea to ammonia and carbon dioxide, thereby increasing the pH and facilitating the precipitation of polyvalent ions in urine (stone formation). This pH alteration is important during *P. mirabilis* catheter colonization, facilitating the bacterial adherence and formation of biofilm incrustation [6].

The *rsbA* gene was regulator of swarming behavior that encodes a sensory, which RsbA may function as a protein sensor of environmental conditions [7]. RsbA was stimulated bio film formation and Extracellular polysaccharide formation [8].

The MR/P fimbriae are the best understood and most important fimbriae of *P. mirabilis*. The MR/P gene cluster is constituted by two transcripts *mrpABCDEFGHIJ* (*mrp* operon) and *mrpI*. The MrpA protein is the main structural subunit of them. The MrpA from two clinical isolates was 100% identical, so the genes encoding MR/P fimbriae may be highly conserved. The genetic organization of the PMF fimbriae operon revealed five functional genes *pmfACDEF*. The mutant adhered significantly less than its parent strain to uroepithelial cells, while kidney colonization was not affected by mutating *pmfA* [6].

The *luxS* genes have been shown to be responsible for the production of autoinducer 2 (AI-2) which plays an important role in other types of cell–cell signaling in bacteria. When *luxR* bound to autoinducer, it enhanced transcription of *luxS* structural operon *luxCDABF*. After *luxS* produced AI-2 signal that used to sense intra and inter species interaction and it's own cell density in a polymicrobial population that play important roles in the regulation of virulence factors [9].

2. MATERIALS AND METHODS

2.1. Isolation and Identification

One hundred thirty five samples were collected from patients with different clinical cases (urine, otitis media, diarrhea, vaginal and wound infections) from patients were admitted to Hill teaching hospital in the period from October 2014 to February 2015. Each specimen was inoculated on selective media and identified by biochemical reaction according to the diagnostic procedures recommended in Forbes *et al.*, [10].

2.2. Molecular Detection of Some Virulence Factors

We using PCR technique for detection of some virulence gene include (*ureC1*, *pm1*, *luxS*, *rsbA*, *mrpA*).

2.3. Extraction of Bacterial DNA

This method was applied according to the genomic DNA purification kit that supplemented by the manufacturing company (Geneaid, UK). The suspension containing DNA was stored at -20 °C until used as template for PCR.

2.4. PCR Amplifications

Detection of virulence genes was performed by amplifying the genes via PCR. Descriptions and sequences of the PCR primers used in this study are displayed in Table-1.

Table 1. The primer sequence that used in present study

Genes name	Primer sequence (5' - 3')	Size bp	Condition	Reference
<i>ureC1</i>	F: CCG GAA CAG AAG TTG TCG CTG GA R: GGG CTC TCC TAC CGA CTT GAT C	533	94°C 3min 94 °C 1min 63°C 30sec 40x 72°C 1min 72°C 7min	[11]
<i>pm1</i>	F: GGA TCA TCT ATA ATG AAA CTG R: CTG ATA ATC AAC TTG GAA GTT	563	94°C 3min 94°C 30sec 40°C 30sec 35x 72°C 30sec 72°C 7min	[12]
<i>luxS</i>	F: GTA TGT CTG CAC CTG CGG TA R: TTT GAG TTT GTC TTC TGG TAG TGC	464	94°C 5min 94°C 60sec 58°C 45sec 40x 72°C 1min 72°C 7min	[13]
<i>rsbA</i>	F: TTG AAG GAC GCG ATC AGA CC R: ACT CTG CTG TCC TGT GGG TA	467	94°C 5min 94°C 60sec 58°C 45sec 35x 72°C 1min 72°C 7min	[13]
<i>mrpA</i>	F: TTC TTA CTG ATA AGA CAT TG R: ATT TCA GGA AAC AAA AGA TG	565	94°C 3min 94°C 30sec 40°C 30sec 40x 72°C 30sec 72°C 7min	[12]

3. RESULTS AND DISCUSSION

3.1. Isolation of *P. Mirabilis*

The most isolates of *P. mirabilis* were obtained from urine 11(64.7%), also the present study show 3(17.6%) of *Proteus mriabilis* were isolated from otitis media. While in stool, wound and vaginal have percentage 1(5.9%) for each one.the reason for urinary tract and vagina infections is due to the proximity of the anal opening to the vagina and urethra.

3.2. Molecular Detection of Virulence Genes

The results of the present study are show that 9(53%) of *Proteusmirabilis* isolates give positive result at 467bp, this result was shown in Figure (1).Which nearly agreed with the results obtained byBadiet *al.*,[13] who found that (70%) *Proteusmirabilis* isolates display *rsbA* genes band so the foundation is common in *P. mirabilis*.

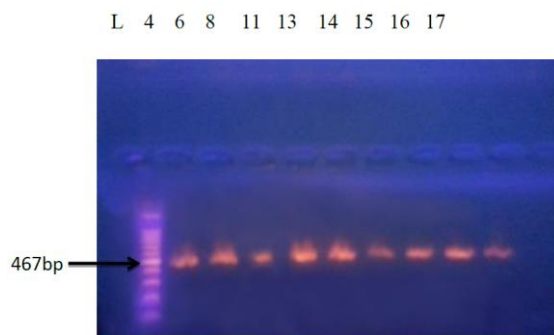


Figure1. illustrations gel electrophoresis of *rsbA* gene that the positive result represents 4, 6, 8, 11, 13, 14, 15, 16, 17 isolates from left to right. L: Ladder with 1500bp.

The results of PCR amplification to specific *ureC* primers indicated that only 3(18%) of isolates gave positive result at 533bp when compared with allelic ladder, this result was shown in Figure (2). The result of Ali and Yousif[14] disagrees with the present study. They reported that PCR product was visible of *ureC* and *ureA* genes were (96.66%), (100%) respectively. So the *ureA* gene is responsible for producing urease enzyme in a (100%) rate.

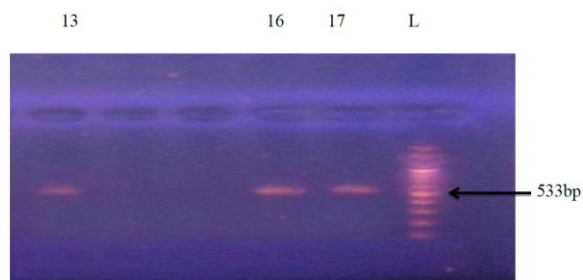


Figure2. Illustrations gel electrophoresis of *ureC* result that the positive band represents the isolates 13, 16 and 17. L: Ladder with 1500bp.

Primers of *luxS* were used for detecting the presence of *luxS* gene in *p. mirabilis* isolates. It has been found that 8(47%) of these isolates contain the genes with the length of 464 bpas shown in Figure (3).The results of the present study are disagreed with the results obtained byBadiet *al.*,[13] who found that (70%) *Proteusmirabilis* isolates display *luxS* genes band.

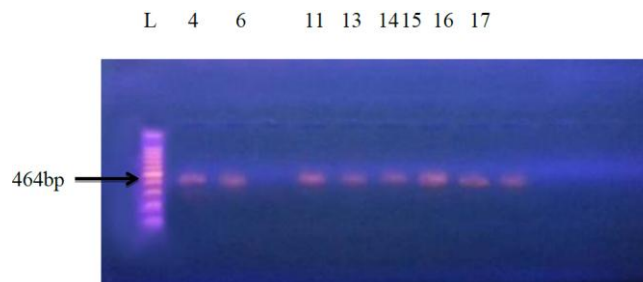


Figure3. illustrations gel electrophoresis of *luxS* gene that the positive result represents 4, 6, 11, 13, 14, 15, 16, 17 isolates from left to right. L: Ladder with 1500bp.

Primers of *mrpA* genes were used for detection the presence of *mrpA* gene in *proteus mirabilis* isolates. It has been found that 6 (35%) of these isolates contain that gene with molecular length 565 base pairs. The amplicon was detected in gel electrophoresis and compared with allelic ladder. This result was shown in figure (4). Liet *al.*, [15] reported that *in vivo* expression of MR/P fimbriae was definite by the PCR-based assay that >90% of bacteria expressed of *mrp* genes. Pearson *et al.*, [16] stated that the genes encoding (MR/P) fimbriae are highly upregulated and expressed *in vivo* compared to *in vitro*.

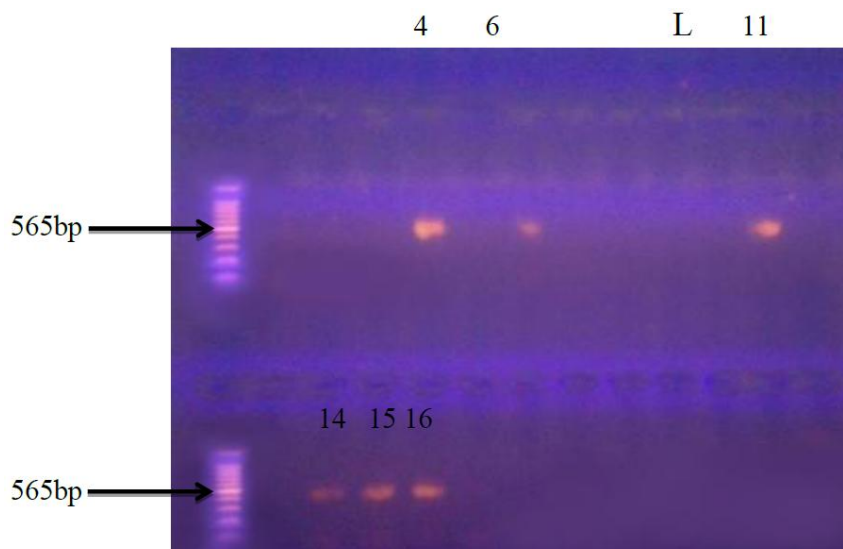


Figure3-4. illustrations gel electrophoresis of *mrpA* gene that the positive result represents 4, 6, 11, 14, 15, 16 isolates from left to right. L: Ladder with 1500bp.

Primers for *pmI* gene were used for detecting the presence of *pmI* gene in *proteus mirabilis* isolates. It has been found that 7 (41%) of these isolates contain the genes with the length of 563 base pairs. The amplicon was detected in gel electrophoresis and compared with allelic ladder. This result was shown in figure (5). The present study was disagree with the result of Sosa *et al.*, [12] that displayed all isolate of *P. mirabilis* (100%) could produce *pmfA* and *mrpA* genes.

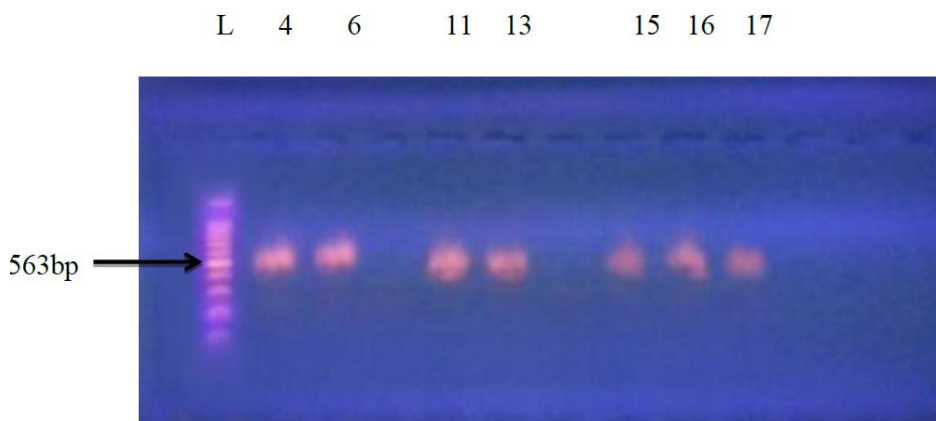


Figure3-5. Illustrations gel electrophoresis of *pmI* gene that the positive result represents 4, 6, 11, 13, 15, 16, 17 isolates from left to right. L: Ladder with 1500bp.

In the present study the *pmI* gene was noticed in seven isolates from different source include one isolate from ear and one other from vaginal and others from urinary tract, however the majority of these gene were detected from urine this result is expected since MR/P and PMF have specific receptor on urinary tract. Also the *mrpA* gene was noticed in six isolates from different source include one isolate from ear and one other from vaginal and others from urinary tract. The present study was matched with Kahdhum [17] who reported that this bacterium isolates from otitis media has (MR/P), (PMF) and (UCA) fimbriae and there is no any differences among isolates in the ability to adhere on uroepithelial cells even they isolated from different sources and its ability to cause different infections. In addition one isolate from vaginal that comprise *mrpA* and *pmI* this indicate that the bacteria may be preceded from urinary tract.

4. CONCLUSIONS

1. *Proteus mirabilis* can be isolated from different clinical positions (urinary tract, otitis media, wounds, vagina, and stool).
2. The total isolates of *Proteus mirabilis* in this study have the ability to hold many virulence factors include: hemolysin, urease, proticin, adherence factors and swarming activity.
3. All isolates of *Proteus mirabilis* were resistant to Imipenem and most isolates are sensitive to Ertapenem and Cefepime/Clavulanic acid.
4. Not all isolates of *Proteus mirabilis* contain the same genes when detected by Molecular technique (PCR), that are responsible for the important virulence factors

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