Lazm *et al. J Pure Appl Microbiol,* **13**(**1**), 447-453 | March 2019
Article 5353 | https://dx.doi.org/10.22207/JPAM.13.1.49

Print ISSN: 0973-7510; Online ISSN: 2581-690X

RESEARCH ARTICLE

The Sequencing of *hpm*B Gene in *Proteus mirabilis* Among UTIs Patients

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Abstract

The present study was carried out during February to May 2018 in Baghdad hospitals. A sample of urine has been collected from fifty patients with an infection in their urinary tract (UTIs) of both sexes and different ages. Bacteriological investigation of urine samples from UTIs patients is made to isolate and diagnose *Proteus mirabilis* bacterium. In addition, the study detects the phenotypic and genetic characteristic of *Proteus mirabilis* α-hemolysin activity. Moreover, for the study to prove its hypothesis, a molecular detection has been carried out utilizing specific primer to *hpmB* gene which encodes α-Hemolysin as a factor of virulence of *Proteus mirabilis* through the use of PCR. The results show that 7(%100) of isolates are positive for *hpmB* at 422 bp. Two isolates of *P. mirabilis* are sequenced as *hpmB* genes. The ratio of identity of the *hpmB* genes with the CP017085.1 and CP020052.1 stains at NCBI global databases copmrised 100%, 99% respectively.

Keywords: *P. mirabilis,* virulence factors gene *hpmB*, Sequencing.

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(Received: 29 October 2018; accepted: 15 December 2018)

Citation: Anwar M. Lazm, Mohammed S. Jebur, Hussein O. M. Al-Dahmoshi and Noor S. Al-khafaji, The Sequencing of *hpm*B Gene in *Proteus mirabilis* Among UTIs Patients, *J Pure Appl Microbiol.,* 2019; Vol. **13**(**1**):447-453 doi: 10.22207/JPAM.13.1.49

INTRODUCTION

Proteus mirabilis refers to a Gram-negative bacterium[1]. Its harmful virulence factors can be recognized by *P. mirabilis* that is able to access and settle the host urinary tract. These include toxins like hemolysin and its function of pore formation, biofilm formation and regulation of pathogenesis[2]. *Proteus mirabilis* has numerous virulence factors that may inflict UTIs. These factors have an important role in causing an infection in varying spots of the urinary tract[3]. Alpha (α) hemolysin hpmA is created by *P. mirabilis* that leads to an injury in kidney tissues. This α-hemolysin belongs to the cell, independent of calcium, pores-former which is encoded by two genes, hpmA and hpmB, that control the hpmB (63kDa) proteins[3]. hpmA α-hemolysin accountable for destructive and triggering the tissue when its N-terminal peptide is slashed. So, the result can activate hpmA and hpmB that is responsible for hpmA activation and transport[4]. The present study aims to characterize the *hpmB* among *P. mirabilis* isolated from UTIs patients.

MATERIALS AND METHODS

Patients with urinary tract infection are from Baghdad Teaching Hospital during the period from September to October 2018. Midstream urine samples are collected and controlled in sterilized wide-container from one hundred urinary tract infection patients. The extraction of the *Proteus mirabilis* DNA from bacterial cells is carries out by using Genomic DNA Mini kit which supplemented by the manufacturing company (Promega, US). DNA electrophoresis in agarose gel is performed according to Ouda, 2014[5].

Thermic cycles program for amplifying the DNA

Specific primers are used for detecting the *Proteus mirabilis* virulence gene encodes of β-hemolysin sequence according to Cestari et al., 2013[6]. These primers are provided by Promega Company (USA) and prepared according to the information of the supplying company, which is listed below:

DNA Sequencing of *hpmB* gene

The process of sequencing through PCR-sequences included two. This is performed according to Macrogen company/Korea sending. The nucleotide replacement is decided by comber. The data that obtained are from gene bank which is available at NCBI https://www.ncbi. nlm.nih.gov.

RESULTS AND DISCUSSION

The technique used to investigate the genes responsibility for the virulence factor in *P. mirablis* is Single Polymerase Chain Reaction Technique. This has been conducted through using segments of the DNA with restricted number of nucleotides (oligonucleotide). Their action is to be primers specialized for virulence genes in *P. mirablis*., hpmB is also included and it is responsible for producing hemolycin *P. mirabilis* β-hemolysin that is different from other *Proteus* spp. It is organized by two genes, (hpmA and hpmB) that encodes the hpmA and hpmB proteins respectively[7,8].

The present study shows that hpmB gene is present in all 7 isolates at rate (100%) from urine samples of RA patients as shown in fig (1). The results contest the result which is verified by Al-Jumaily and Zgaer. In their study, it is declared that the frequency of this gene in *P. mirablis* isolates is %100; that is isolated from patients suffering from urinary tract infections[9]. While Cestari et al., 2013 find the ratio of this gene in bacterial isolates comprising 96.24 % existing amplification for the hpmA and hpmB genes by PCR.[6]. The α-hemolycin toxin acts as a destroyer to the leukocyte membrane through creating small holes in the leukocyte membrane and epithelial cell. So, its presence is a vital factor in supplying the bacteria with iron and because of having the cytotoxic effects, this could lead to the destruction of the host kidney tissue[10]. Isolates with hpmA gene in them is in compatibility with the characterization presented by Uphoff and Welch (1990) who state the necessity of cleaving the N-terminal peptide of the hpmA by hpmB for the purpose of activating and transporting the hemolytic hpmA protein out of the cell[8]. This standard indicates that hpmA is a factor in the pathogenesis of *P. mirabilis* samples isolated from human urine[10]. Among the other results are those reported by Swihart and Welch,1990 refering that all *P. mirabilis* strains as having hpmA but HlyA is not detected in *P. mirabilis* isolates and is found only in 2 of the 24 *P. vulgaris* strains examined. Since *P. mirabilis* composes most (97%) of the *Proteus* urinary tract isolates, this suggests that hpmA is the predominant *Proteus* hemolysin and

might play a role in extra intestinal infections caused by *Proteus* spp[11]. These positive isolates with hpmB gene are also checked to confirm their ability to produce hemolysin on blood agar and it has been found that all the isolates (100%) have the ability to produce hemolysin. These results agree with the results of Sosa et al. (2006) and AL-Jumaa et al. (2011), who demonstrate that all isolates (100%) of *Proteus* bacterium which are isolated from different clinical sources exhibit hemolysis on blood agar plates, but Mishara et al. (2001) find that (85.14%) of *Proteus* isolates produce α-hemolysis while other isolates produce β –hemolysis on blood agar plate[12,13,14]. The results of the study demonstrate that the detection of hpmB gene by PCR is sensitive enough to be used for discovering these virulence factors produced by *P. mirabilis*. The PCR technique is shown to be precise, fast, cheap and more accurate therefore this suggests that hpmB could be used as a diagnostic tool for *P. mirablis* bacterium.

DNA sequencing analysis

Analyzing DNA sequence of *hpmB* gene

Three isolates were sequenced by Macrogen/Korea. The nucleotide switch is firmed by comber. The data are obtained from gene bank available at NCBI (https://www.ncbi.nlm.nih.gov). The results of gene sequence analysis hpmB show that there are two polymorphism in 2 isolates of the gene hpmB as shown in fig (2) and table (1). In the isolation of *P. mirabilis* (MF993448) Thymine nucleotide substitutions to Adenine is found at locus 2389107 and Cytosine has substitutions to Thymine at locus 2389062 . Finally, the results show nonsense polymorphism as predict . Also, the results show silent variation and this type of variation doesn't change the sequence of amino acid in the protein and doesn't alter protein function as shown in table (1)[15]. Samples (MF993446) show 100 % identity for hpmB in comparison to the same genes of the CP017085.1. While samples, (MF993448) show 99 % identity for hpmB in comparison to the same genes of the : CP020052.1 strain.

It is noticed, through the process of sequencing of the a-hemolysin at *P. mirabilis*, that it consists of two genes hpmA and hpmB[7]. *P. mirabilis* hemolysin hpmA investigation and description is necessary to clarify its significance as a virulence factor. Furthermore, it might have a possible association with other elements that are produced by *P. mirabilis*. These altogether could contribute to cytotoxicity in the UTIs of humans[7]. Mordi and Momoh.(2009) find that a change in the amino acid or replacement with other amino acid may lead to a change in the nature of protein or output and thus lead to the emergence of strains resistant or sensitive to antibiotics[16]. The hpmA and hpmB genes are sequenced from local isolated samples presented 98 % identity for hpmA and hpmB compared to the same genes of the HI4320 (wild strain) (NCBI GenBank Number NC_010554.1). When the samples of the study are compared with each other 100 % identity is found among these genes[17]. The results of the study display that the amino acid remained the same and that indicates variation from the silent type. In contrast, there is a study conducted on hpmB gene like Strauss et al., (1997) find that mutations enhance the function of hpmB (increase in hemolytic activity). This increasing in hemolytic activity could be a result of hpmB activating and secreting more hpmA[18]. Genotyping works to establish the relationship between bacteria strain on the basis of their genetic content uses. Many genotyping methods become important in the field of genealogy, classification of bacteria, identification of sources, method of infection and the differentiation of strain of high virulent bacteria to prevent their spread and elimination[19].

Table 1. Primer sequence of *hpm*B *gene* and PCR condition

Genes	Sequence (5' to 3')	PCR condition	Size (bp)	References
hpmB	F:CAGTGGATTAAGCGCAAATG	95°C 5min 1x 95°C 30sec 62°C 30sec 30x	422	
	R:CCTTCAATACGTTCAACAAACC	72°C 20 sec 72°C 5min 1x		(Cestari *et al*., 2013)

Table 2. Identity of *hpmB* gene sequence in

Range of nucleotide	Sequence ID	Score	Expect	Identities	Source
3196590 to 3196919	CP017085.1	610	0	100%	*Proteus mirabilis* HPMB
2389008 to 2389362	CP020052.1	645	0	99%	*Proteus mirabilis* HPMB

Table 3. Polymorphism of *hpmB* gene sequence

Sample	Type of substitution	Location change	Nucleotide change	Amino acid	Effect
1				None	
2	Transversion	2389107	ACT>ACA	Threonine> Threonine	Silent
	Transition	2389062	TTC>TTT	Phenylalanine> Phenylalanine	Silent

Table 4. Alignment of *hpmB* gene sequence *Proteus mirabilis* strain T18

Sequence ID: CP017085.1Length: 4131426 Range 1: 3196590 to 3196919				
Score	Expect	Identities	Gaps	Strand
610 bits(330)	8e-171	330/330(100%)	0/330(0%)	Plus/Plus

```
Query  1         GAAATTAATCAATTAATAGAACAAAATCGCTATCAGCAACTGCAAGAAAAAGCGGTAAAT  60
Sbjct  3196590   GAAATTAATCAATTAATAGAACAAAATCGCTATCAGCAACTGCAAGAAAAAGCGGTAAAT  3196649
Query  61        ATTTCACCTACCCCAACTTTAATTACTGAGTCAGAACACTGTTTGCCTATAAAAGGCGTT  120
Sbjct  3196650   ATTTCACCTACCCCAACTTTAATTACTGAGTCAGAACACTGTTTGCCTATAAAAGGCGTT  3196709
Query  121       TATATTCAAGGTATTACTTTACTTACTGAGAAGGATCTCAATTCATTATCTCCGTTACCT  180
Sbjct  3196710   TATATTCAAGGTATTACTTTACTTACTGAGAAGGATCTCAATTCATTATCTCCGTTACCT  3196769
Query  181       GATCAATGTATTAAGAGTGCTGATATTAATCGCCTCGTGAAAGAACTCACTCAGCGTTAT  240
Sbjct  3196770   GATCAATGTATTAAGAGTGCTGATATTAATCGCCTCGTGAAAGAACTCACTCAGCGTTAT  3196829
Query  241       CTTCAACATGGTTATATTACCGCGCGTATCCAATTTTTACGTCCTAACCAACATGGCGAA  300
Sbjct  3196830   CTTCAACATGGTTATATTACCGCGCGTATCCAATTTTTACGTCCTAACCAACATGGCGAA  3196889
Query  301       TTAGGTCTGTATGCTATTGAAGGGTTTGTT  330
Sbjct  3196890   TTAGGTCTGTATGCTATTGAAGGGTTTGTT  3196919
```

Table 5. Alignment of *hpmB* gene sequence *Proteus mirabilis* strain AR_0059

Sequence ID: CP020052.1Length: 4191021 Range 1: 2389008 to 2389362				
Score	Expect	Identities	Gaps	Strand
645 bits(349)	0.0	353/355(99%)	0/355(0%)	Plus/Minus

```
Query  1         GAAATTAATCAATTAATAGAACAAAATCGCTATCAGCAACTGCAAGAAAAAGCGGTAAAT  60
Sbjct  3196590   GAAATTAATCAATTAATAGAACAAAATCGCTATCAGCAACTGCAAGAAAAAGCGGTAAAT  3196649
Query  61        ATTTCACCTACCCCAACTTTAATTACTGAGTCAGAACACTGTTTGCCTATAAAAGGCGTT  120
Sbjct  3196650   ATTTCACCTACCCCAACTTTAATTACTGAGTCAGAACACTGTTTGCCTATAAAAGGCGTT  3196709
Query  121       TATATTCAAGGTATTACTTTACTTACTGAGAAGGATCTCAATTCATTATCTCCGTTACCT  180
Sbjct  3196710   TATATTCAAGGTATTACTTTACTTACTGAGAAGGATCTCAATTCATTATCTCCGTTACCT  3196769
Query  181       GATCAATGTATTAAGAGTGCTGATATTAATCGCCTCGTGAAAGAACTCACTCAGCGTTAT  240
Sbjct  3196770   GATCAATGTATTAAGAGTGCTGATATTAATCGCCTCGTGAAAGAACTCACTCAGCGTTAT  3196829
Query  241       CTTCAACATGGTTATATTACCGCGCGTATCCAATTTTTACGTCCTAACCAACATGGCGAA  300
Sbjct  3196830   CTTCAACATGGTTATATTACCGCGCGTATCCAATTTTTACGTCCTAACCAACATGGCGAA  3196889
Query  301       TTAGGTCTGTATGCTATTGAAGGGTTTGTT  330
Sbjct  3196890   TTAGGTCTGTATGCTATTGAAGGGTTTGTT  3196919
```

Table 6. Alignment of hemolysin activation protein [*Proteus mirabilis*]

Sequence ID: WP_074561482.1 Length: 561 Identical proteins to WP_074561482.1

Score	Expect	Method	Identities	Positives	Gaps	Frame
228 bits(582)	2e-70	Compositional matrix adjust.	110/110 (100%)	110/110 (100%)	0/110 (0%)	+1

Query 1	EINQLIEQNRYQQLQEKAVNISPTPTLITESEHCLPIKGVYIQGITLLTEKDLNSLSPLP
180Sbjct 43	EINQLIEQNRYQQLQEKAVNISPTPTLITESEHCLPIKGVYIQGITLLTEKDLNSLSPLP
102Query 181	DQCIKSADINRLVKELTQRYLQHGYITARIQFLRPNQHGELGLYAIEGFV
330Sbjct 103	DQCIKSADINRLVKELTQRYLQHGYITARIQFLRPNQHGELGLYAIEGFV 152

Table 7. Alignment of hemolysin activation protein [*Proteus mirabilis*]

Sequence ID: WP_046334659.1 Length: 561 Identical proteins to WP_046334659.1

Score	Expect	Method	Identities	Positives	Gaps	Frame
244 bits(623)	2e-76	Compositional matrix adjust.	118/118 (100%)	118/118 (100%)	0/118 (0%)	+1

Query 2	RALQDSQREINQLIEQNRYQQLQEKAVNISPTPTLITESEHCLPIKGVYIQGITLLTEKD 181
Sbjct 35	RALQDSQREINQLIEQNRYQQLQEKAVNISPTPTLITESEHCLPIKGVYIQGITLLTEKD 94
Query 182	LNSLSPLPDQCIKSADINRLVKELTQRYLQHGYITARIQFLRPNQHGELGLYAIEGFV 355
Sbjct 95	LNSLSPLPDQCIKSADINRLVKELTQRYLQHGYITARIQFLRPNQHGELGLYAIEGFV 152

Therefore, the study finds a great importance in the genetic sequence of *P. mirabilis* virulence factors. The study finds that variant isolates possess polymorphism in hpmB genes.

A phylogenic tree based on the *hpmB* gene

Molecular phylogenetic is a branch of phylogeny that analyzes hereditary molecular differences mainly in DNA sequences to gain information on an organisms' evolutionary relationships[20]. The identified genetic profile of any bacteria by a specific genotyping method can be as unique as fingerprint[20].

However, phylogeny estimated from a single gene should be treated with caution[21,22]. The phylogenetic tree derived from hpmB gene sequences of clinical strains of 2 samples *Proteus*

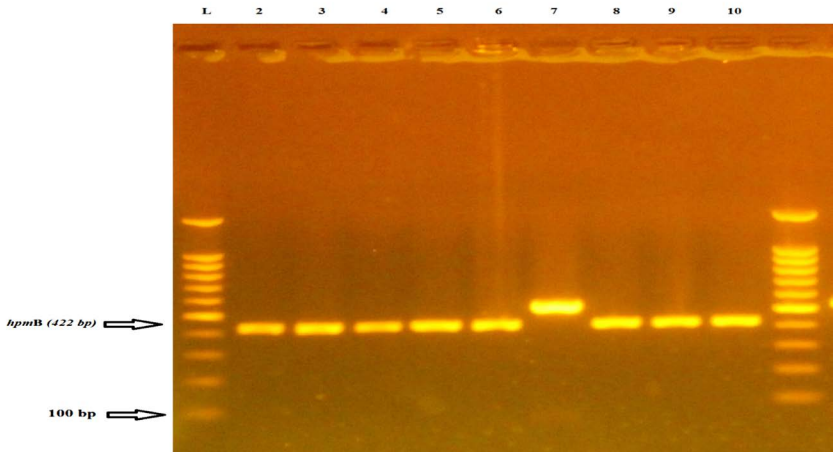

Fig. 1. Agarose gel electrophoresis (2% agarose, 75 V for 1:45 hour) of hpmB and PCR products (422bp) codify for α-hemolysin of P. mirabilis isolates. Lane 1DNA ladder), 100-1100bp molecular marker, lanes 2 ,3,4,5,6,7,8,9,10 isolates were positive results.

mirabilis with other sequences is available at NCBI showed in (Fig.2). As to be seen in the this figure, *P. mirabilis* (MF993443) lies in the same branch of the phylogenetic tree with P.mirabilis (WP_088207120.1).

Sequences of 16SrRNA with the size of 1.5 Kb is considered and widely used in bacterial taxonomy because it contains high conservation region which has variable region in different species. Furthermore, the most important was that 16SrRNA gene which could be sequenced easily[22]. On the other hand, the sensitivity of this approach is questioned particularly among human bacterial closely related to Enterobacteriaceae, which includes many common pathogens because of the high degree of conservation in species[22]. Therefore, the use of other genes rather than16SrRNA gene and the distinction between bacteria at the species level is regarded as a very important issue[23]. Results indicate that since there is an increase in clinical significance of *P. mirabilis*, the choice of effectual molecular methods is of great epidemiological reputation. Bacterial genotyping opened new chances on epidemiological studies by the documentation of clinical and ecological isolates, the assessment of this association, the watching of clone propagation and the classification of bacterial populations within more or less constrained environments[24]. By joining molecular phylogeny with traditional approach such as morphological, physiological and biochemical characteristics, bacteria identification could be achieved in a more accurate way[25,26].

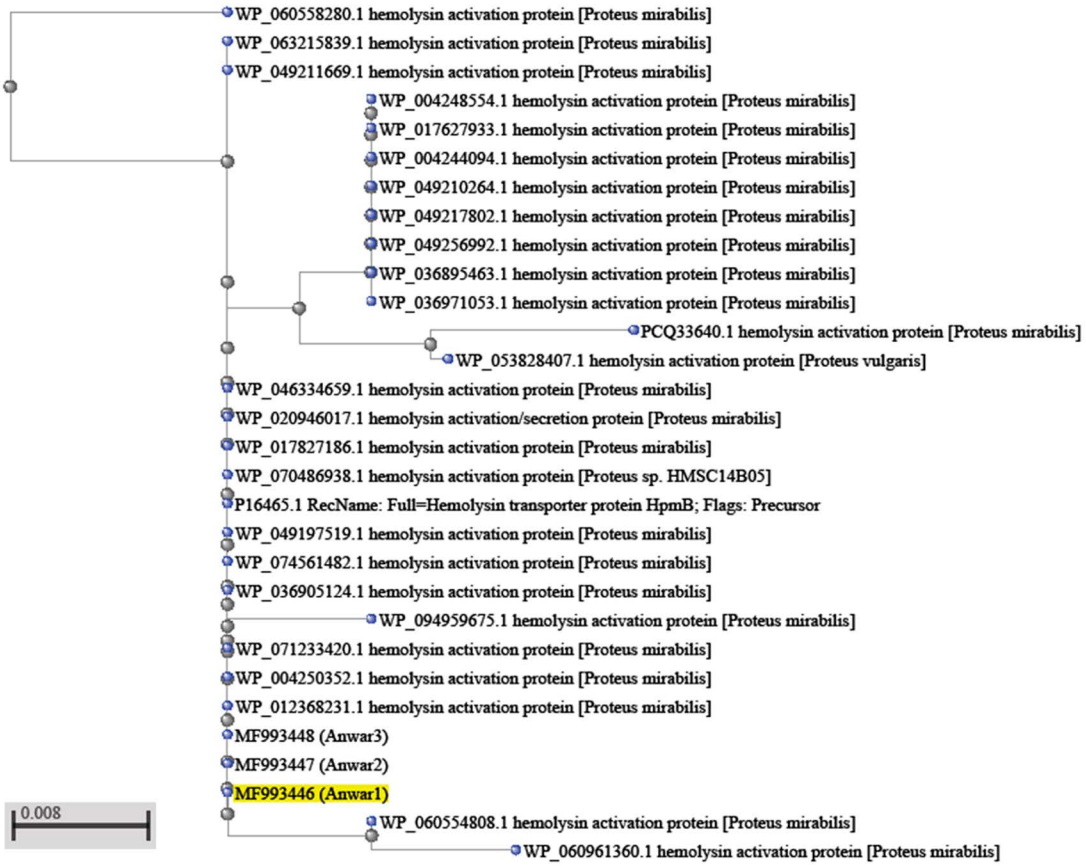

Fig. 2. Phylogenetic tree of *Proteus species* based on *hpm*B gene sequence analysis.

ACKNOWLEDGMENT

None

CONFLICT OF INTERESTS

The authors declare that there is no conflicts of interest.

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