The Sequencing of hpmB 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 comprised 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 hpmB 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. 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. 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. Alpha (a) 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. 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. 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 carried 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.

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. 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 combiner. 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. mirabilis 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. mirabilis. 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.

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. mirabilis isolates is %100; that is isolated from patients suffering from urinary tract infections. 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. 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. 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. This standard indicates that hpmA is a factor in the pathogenesis of P. mirabilis samples isolated from human urine. Among the other results are those reported by Swihart and Welch, 1990 referring 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 hemolysin on blood agar plates, but Mishara et al. (2001) find that (85.14\%) of *Proteus* isolates produce \(\alpha\)-hemolysis while other isolates produce \(\beta\)-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. mirabilis* 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 *α*-hemolysin at *P. mirabilis*, that it consists of two genes *hpmA* and *hpmB*. *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 *hpmB* gene and PCR condition

| Genes | Sequence (S’ to 3’) | PCR condition | Size (bp) | References |
|-------|---------------------|---------------|-----------|------------|
| hpmB  | F:CAGTGGATTAAGCGAAATG | 95°C 5min 1x<br>95°C 30sec | 422 | (Cestari et al., 2013) |
|       | R:CCTTCAATAGTCTACAAACC | 62°C 30sec 30x<br>72°C 20 sec<br>72°C 5min 1x | | |
### 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 change | Effect |
|--------|----------------------|-----------------|------------------|------------------|--------|
| 1      | Transversion         | 2389107         | ACT>ACA          | Threonine        | Silent |
| 2      | Transition           | 2389062         | TTC>TTT          | Phenylalanine    | Silent |

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

| Sequence ID: CP017085.1 | Length: 4131426 | Range 1: 3196590 to 3196919 |
|-------------------------|-----------------|-----------------------------|
| Score                   | Expect          | Identities                  |
| 610 bits(330)           | 8e-171          | 330/330(100%)               |
|                         | Gaps            | 0/330(0%)                   |
|                         | Strand          | Plus/Plus                   |
| Query 1                 | GAAATTAATCTAATTAATAAGAACAAACTCGTATCAGCAACTGCAAGAAGAAAGCGGTAAAT 60 |
| Sbjct 3196590           | GAAATTAATCTAATTAATAAGAACAAACTCGTATCAGCAACTGCAAGAAGAAAGCGGTAAAT 3196649 |
| Query 61                | ATTTCAACTCCCCAATTTATATTACTGAGTCAAGAAGACTGTTGGCTCTATAAAGAACGGT 120 |
| Sbjct 3196650           | ATTTCAACTCCCCAATTTATATTACTGAGTCAAGAAGACTGTTGGCTCTATAAAGAACGGT 3196709 |
| Query 121               | TATATCACCACCTATTTACTCTGAGGATCTCATCCATTTACTCTCTCTCCTCCTACTTACCT 180 |
| Sbjct 3196710           | TATATCACCACCTATTTACTCTGAGGATCTCATCCATTTACTCTCTCTCCTCCTACTTACCT 3196769 |
| Query 181               | GATCAATGTATTAAGAGTGCTGTATAATTACGCCCTGTGAAAGAAGACTCCTAGCTAGTTAT 240 |
| Sbjct 3196770           | GATCAATGTATTAAGAGTGCTGTATAATTACGCCCTGTGAAAGAAGACTCCTAGCTAGTTAT 3196829 |
| Query 241               | CTCAATAGTTGATATAACACGGGGATATCAATTCAGCTCAATTCCTAGAGGGAGAGGTGTTG 300 |
| Sbjct 3196830           | CTCAATAGTTGATATAACACGGGGATATCAATTCAGCTCAATTCCTAGAGGGAGAGGTGTTG 3196889 |
| Query 301               | TTAGGTCTGATGTATGCTAGAGGGGTGTTGTT 330 |
| Sbjct 3196890           | TTAGGTCTGATGTATGCTAGAGGGGTGTTGTT 3196919 |

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

| Sequence ID: CP020052.1 | Length: 4191021 | Range 1: 2389008 to 2389362 |
|-------------------------|-----------------|-----------------------------|
| Score                   | Expect          | Identities                  |
| 645 bits(349)           | 0.0             | 353/355(99%)                |
|                         | Gaps            | 0/355(0%)                   |
|                         | Strand          | Plus/Minus                  |
| Query 1                 | GAAATTAATCTAATTAATAAGAACAAACTCGTATCAGCAACTGCAAGAAGAAAGCGGTAAAT 60 |
| Sbjct 3196590           | GAAATTAATCTAATTAATAAGAACAAACTCGTATCAGCAACTGCAAGAAGAAAGCGGTAAAT 3196649 |
| Query 61                | ATTTCAACTCCCCAATTTATATTACTGAGTCAAGAAGACTGTTGGCTCTATAAAGAACGGT 120 |
| Sbjct 3196650           | ATTTCAACTCCCCAATTTATATTACTGAGTCAAGAAGACTGTTGGCTCTATAAAGAACGGT 3196709 |
| Query 121               | TATATCACCACCTATTTACTCTGAGGATCTCATCCATTTACTCTCTCTCCTCCTACTTACCT 180 |
| Sbjct 3196710           | TATATCACCACCTATTTACTCTGAGGATCTCATCCATTTACTCTCTCTCCTCCTACTTACCT 3196769 |
| Query 181               | GATCAATGTATTAAGAGTGCTGTATAATTACGCCCTGTGAAAGAAGACTCCTAGCTAGTTAT 240 |
| Sbjct 3196770           | GATCAATGTATTAAGAGTGCTGTATAATTACGCCCTGTGAAAGAAGACTCCTAGCTAGTTAT 3196829 |
| Query 241               | CTCAATAGTTGATATAACACGGGGATATCAATTCAGCTCAATTCCTAGAGGGAGAGGTGTTG 300 |
| Sbjct 3196830           | CTCAATAGTTGATATAACACGGGGATATCAATTCAGCTCAATTCCTAGAGGGAGAGGTGTTG 3196889 |
| Query 301               | TTAGGTCTGATGTATGCTAGAGGGGTGTTGTT 330 |
| Sbjct 3196890           | TTAGGTCTGATGTATGCTAGAGGGGTGTTGTT 3196919 |
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 phylogenetic 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 organism's evolutionary relationships. The identified genetic profile of any bacteria by a specific genotyping method can be as unique as a fingerprint. However, phylogeny estimated from a single gene should be treated with caution. The phylogenetic tree derived from hpmB gene sequences of clinical strains of 2 samples *Proteus*

![Agarose gel electrophoresis](image)

**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 2-10 isolates were positive results.
mirabilis with other sequences is available at NCBI showed in (Fig. 2). As to be seen in 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\(^\text{22}\). 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\(^\text{22}\). Therefore, the use of other genes rather than 16SrRNA gene and the distinction between bacteria at the species level is regarded as a very important issue\(^\text{23}\). 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\(^\text{24}\).

By joining molecular phylogeny with traditional approach such as morphological, physiological and biochemical characteristics, bacteria identification could be achieved in a more accurate way\(^\text{25,26}\).

![Fig. 2. Phylogenetic tree of Proteus species based on hpmB gene sequence analysis.](image-url)
ACKNOWLEDGMENT

None

CONFLICT OF INTERESTS

The authors declare that there is no conflicts of interest.

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