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Sequencing of *HpmA* Gene in *Proteus mirabilis* of UTIs among rheumatoid arthritis patients

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Abstract

Study has done during February to May 2016 at Baghdad hospitals. There were fifty urine samples collected from rheumatoid arthritis (RA)patients .

Bacteriological investigation of urine samples from RA patients, to isolate and diagnose of *Proteus mirabilis* bacterium that also these patients suffering from UTIs has done. In addition, the study had detected phenotypically *Proteus mirabilis* α-hemolysin activity.

Molecular identification has done by using a specific primer to *hpm A* gene that encode for α-Hemolysin as a virulence factor of *Proteus mirabilis* by using PCR and it was found that 7(%100) of isolates gave positive result, for *hpmA* at709 bp. Three *P. mirabilis* isolates were sequenced for the *hpmA* genes.The *hpmA* genes that were sequenced presented 100%, 99%,100% respectively identity with CP015347.1, CP021550.1 ,CP021550.1 stain at NCBI global databases.

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

INTRODUCTION

Rheumatoid arthritis RA is an autoimmune disease with no certain etiology that could likely be triggered by environmental factors. In genetically susceptible individuals' RA has associated with genetic predisposition. Different immunological and microbiological data results support that there could be a link between urinary tract infections (UTI) and RA, this has mainly caused by Proteus bacterium [1]

Proteus mirabilis is a Gram-negative bacterium [2]. Different set of virulence factors can be detected by *P. mirabilis*, that able to access and colonize the host urinary tract including toxins like hemolysin and its function of pore formation, biofilm formation and regulation of pathogenesis [2]. Since *Proteus mirabilis* had many virulence factors that were important for inflicting UTIs, these factors had an importance role to make an infection in different areas of the urinary tract [3].

Alpha (α) hemolysin *hpmA* has produced by *P. mirabilis* that used to damage the kidney tissues. This α-hemolysin has related to the cell, calcium-independent, former of pores which encodes by two genes, *hpmA* and *hpmB*, that regulate the *HpmA* (166 kDa) proteins [3]. *HpmA* α-hemolysin is responsible for damaging tissue and activating when its N-terminal peptide has cleaved, so the results can be activating *HpmA* (140 kDa) [4].

The objectives of current study were isolating and identifying the *P. mirabilis* from urine samples of RA patients. Detecting the genes encodes of α- Hemolysin by PCR technique, sequencing of gene encoded Proteus haemolysin, genotyping of *P. mirabilis* strains by using genetic markers such α -hemolysin genes and detecting a phylogenic tree of *P. mirabilis* based in the ,*HpmA* gene.

MATERIALS AND METHODS

Midstream patients urine samples have collected and controlled in sterilized wide-container from one hundred RA patients. These RA patients had obtained from Rheumatology consultation clinic / Baghdad Teaching Hospital and they diagnosed by clinic's rheumatologists from February to May 2016 at Baghdad hospitals. Extraction of the *Proteus mirabilis* DNA of the study from bacterial cells using Genomic DNA Mini kit which supplemented by the manufacturing company (Promega, US). DNA electrophoresis in agarose gel, it has performed according to Sambrook, and Russell, 2006 [5].

Thermal cycles program to amplify the DNA:

Specific primers had used for detecting the *Proteus mirabilis* virulence gene encodes for α-hemolysin sequence according to Cestari *et al.*, 2013 [6]. These primers had provided by Promega Company (USA) and prepared according to the information of the supplying company, which listed at table (1).

DNA Sequencing o*f hpmA* gene

Three samples had sequenced through PCR-sequences by Macrogen company/ Korea sending. The nucleotide substitution had determined by comber the data that obtained from gene bank publish which available at NCBI *https://www.ncbi.nlm.nih.gov.*

RESULTS AND DISCUSSION

Single polymerase chain reaction technique was used in investigation of the genes responsible for the virulence factor in *P. mirablis* through the use of pieces of the DNA with limited number of nucleotides (oligonucleotide), which act a primers specialized for virulence genes in *P. mirablis* and it include *hpmA*. *hpmA* gene which responsible for producing hemolycin. *P. mirabilis* α-hemolysin is different from other *Proteus spp*., its organized by two genes, (*hpmA* and *hpmB*), that encode for the *HpmA* and *HpmB* proteins respectively [7][8].

The results of the current study have shown that *hpmA* gene was present in 7 isolates out 7 isolates at rate (100%) from urine samples of RA patient as shown in fig (1). These results matches with the result recorded by Ali and Yousif, (2015) for they mentioned that the rate of this gene in *P. mirablis* isolates has %100, that isolates from Patients that were suffering from urinary tract infection[9]. While Cestari *et al*., 2013 found the ratio of this gene in bacterial isolates 96.24 % presented amplification for the *hpmA* and *hpmB* genes by PCR. [6]. The α-hemolycin acts as a destroyer to the leukocyte membrane through creating small holes in the leukocyte membrane and epithelial cell. So its presence is vital factor in supplying the bacteria with iron and because of having the cytotoxic effects, this could lead to destroy the host kidney tissue [10].

The presence of the *hpmA* gene in the isolates was in line with the descriptions by Uphoff and Welch (1990) who showed the need to cleave the N-terminal peptide of the *HpmA* by *HpmB* to activate and transport the hemolytic *HpmA* protein to outside the cell[8]. This suggested that *HpmA* was a factor in the pathogenesis of *P. mirabilis* samples isolated from human urine [10]. Other result reported by Swihart and Welch,1990 referred to all *P. mirabilis* strains had *HpmA* but *HlyA* has not detected in *P. mirabilis* isolates and has found in only 2 of the 24 *P. vulgaris* strains examined. Since *P. mirabilis* composes most (97%) of the Proteus urinary tract isolates, this suggests that HpmA was the

predominant Proteus hemolysin and might play a role in extra intestinal infections caused by *Proteus spp.* [11].

These positive isolates with *hpmA* gene were also checked to confirm their ability to produce hemolysin on blood agar and it was found that all of the isolates (100%) had the ability to produce hemolysin. These results agree with the results of Sosa *et al*. (2006) and AL-Jumaa *et al*. (2011), who demonstrated that all isolates (100%) of *Proteus* bacterium isolated from different clinical sources exhibit hemolysis on blood agar plates, but Mishara *et al*. (2001) found that (85.14%) of *Proteus* isolates produce β-hemolysis, while other isolates produce α –hemolysis on blood agar plate [12][13][14].

Study results demonstrate that the detection of *hpmA* gene by PCR was sensitive enough to be used for the discovering of these virulence factors produced by *P. mirabilis* and The PCR technique had shown to be precise, fast, cheap and more accurate, therefore this suggests that *HpmA* could use as diagnostic feature of the *P. mirablis* bacterium.

DNA SEQUENCING ANALYSIS

Analysis DNA sequence of *HpmA* gene

Three samples had sequenced through PCR-sequences by Macrogen company/ Korea sending .The nucleotide substitution had determined by comber the data that obtained from gene bank publish which available at NCBI *(https://www.ncbi.nlm.nih.gov).*

The results of gene sequence analysis *HpmA* has shown that there was one polymorphism in 3 isolates of the gene *Hpm A* as shown in fig (2,3,4) and table (1). In the isolation of *P. mirabilis* (MF993443) has found Adenine nucleotide substitutions to Guanine at locus 1004761. However the results shown silent variation, this type of variation do not lead to a change in the sequence of amino acid in the protein and do not alter function of protein as shown in table (1) [15].

Samples (MF993443) presented 99 % identity for *hpmA* compared to the same genes of the CP015347.1. While samples, (MF993444, MF993445) respectively presented 100 % identity for *HpmA* compared to the same genes of the CP021550.1, CP021550.1 respectively strain.

Table (1): Primer sequence of *hpm A gene* and PCR condition.

Genes	Sequence (5' to 3')	PCR condition	Size (bp)	References
hpmA	**F-** GTTGAGGGGCGTTATCAAGAGTC	95 ^{0} C 5min 1x 95 ^{0} C 30sec 62^0 C 30sec 30x 72 ^{0} C 20 sec 72 ^{0} C 5min 1x	709	(Cestari *et al*., 2013)
	R-GATAACTGTTTTGCCCTTTTGTGC			

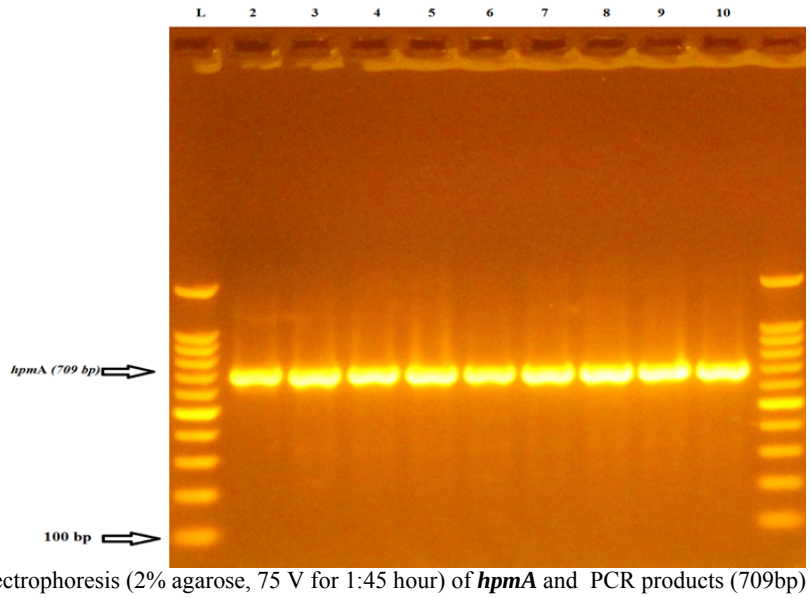

Figure (1): Agarose gel electrophoresis (2% agarose, 75 V for 1:45 hour) of ***hpmA*** and PCR products (709bp) codify for α-hemolysin of *P. mirabilis* isolates. Lane 1DNA ladder) , 100-1100bp molecular marker, lanes 2, 3, 4, 5, 6, 7, 8, 9 and 10 isolates were positive results.

Table (1): Type polymorphism of in the *HpmA* gene sequence in *Proteus mirabilis*

No. Of sample	Type of substitution	Location	Nucleotide	Nucleotide change	Amino acid change	Predicted effect	Range of nucleotide	Sequence ID	Score	Expect	Identities	SOURCE
1	Transition	1004761	**A>G**	**GAA>GAG**	**Glutamic acid> Glutamic acid**	Silent	1004493 to 1005095	ID: CP015347.1	1109	0.0	99%	*Proteus mirabilis HPMA*
2	----------						3580709 to 3581335	ID: CP021550.1	1158	0.0	100%	*Proteus mirabilis HPMA*
3	----------						3580708 to 3581337	ID: CP021550.1	1164	0.0	100%	*Proteus mirabilis HPMA*

Figure (2): Isolate number (1)

Proteus mirabilis strain AOUC-001, complete genome
Sequence ID: CP015347.1Length: 4272433Number of Matches: 1
Related Information
Range 1: 1004493 to 1005095GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
1109 bits(600)	0.0	602/603(99%)	0/603(0%)	Plus/Minus

```
Query  1        ATGTTGGTATCGATGTTGGCGTTAATCTTGATTATAGCGGTGTAACGAAGCCAGTTAAGA  60
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1005095  ATGTTGGTATCGATGTTGGCGTTAATCTTGATTATAGCGGTGTAACGAAGCCAGTTAAGA  1005036

Query  61       AAGCAATCGAAGATGGTGTTAACACAACCAAACCGGGTAACAATACCGATTTAACTAAAA  120
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1005035  AAGCAATCGAAGATGGTGTTAACACAACCAAACCGGGTAACAATACCGATTTAACTAAAA  1004976

Query  121      AAGTTACAGCAAGAGATGCAATTGCTAATTTAGCTAACCTTAGCAATTTAGAGACCCCCA  180
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1004975  AAGTTACAGCAAGAGATGCAATTGCTAATTTAGCTAACCTTAGCAATTTAGAGACCCCCA  1004916

Query  181      ATGTCGGTGTTGAAGTTGGTATTAAAGGTGGTGGTAGTCAGAAATCACAAACCGATAGCC  240
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1004915  ATGTCGGTGTTGAAGTTGGTATTAAAGGTGGTGGTAGTCAGAAATCACAAACCGATAGCC  1004856

Query  241      AAGCCGTTTCAACCTCTATCAATGCAGGAAAAATCAACATTGATAGTAATAATAAGTTAC  300
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1004855  AAGCCGTTTCAACCTCTATCAATGCAGGAAAAATCAACATTGATAGTAATAATAAGTTAC  1004796

Query  301      ATGATCAAGGTACTCACTATCAATCAACCCAAGAGGGTATTTCTCTCACAGCAAACACCC  360
                |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1004795  ATGATCAAGGTACTCACTATCAATCAACCCAAGAAGGTATTTCTCTCACAGCAAACACCC  1004736

Query  361      ACACAAGTGAGGTCGCGCAAGATAAACATCAAACGACATTCCATGAAACAAAAGGCGGTG  420
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1004735  ACACAAGTGAGGTCGCGCAAGATAAACATCAAACGACATTCCATGAAACAAAAGGCGGTG  1004676

Query  421      GACAAGTTGGTGTCAGTACTAAAACGGGCAGTGATATTACCGTTGCTATTAAAGGTGAAG  480
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1004675  GACAAGTTGGTGTCAGTACTAAAACGGGCAGTGATATTACCGTTGCTATTAAAGGTGAAG  1004616

Query  481      GCCAAACAACTGATAACGCCTTAATGGAAACAAAGGCTAAAGGTAGCCAATTTACCTCAA  540
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1004615  GCCAAACAACTGATAACGCCTTAATGGAAACAAAGGCTAAAGGTAGCCAATTTACCTCAA  1004556

Query  541      ATGGCGATATTTCGATTAATGTAGGTGAAGATGCCCATTATGAAGGTGCTCAATTTGATG  600
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1004555  ATGGCGATATTTCGATTAATGTAGGTGAAGATGCCCATTATGAAGGTGCTCAATTTGATG  1004496

Query  601      CAC  603
                |||
Sbjct  1004495  CAC  1004493
```

hemolysin [Proteus mirabilis]
Sequence ID: WP_080047776.1Length: 1577Number of Matches: 1
See 1 more title(s)
Related Information
Identical Proteins-Identical proteins to WP_080047776.1
Range 1: 863 to 1062GenPeptGraphicsNext MatchPrevious Match

Alignment statistics for match #1

Score	Expect	Method	Identities	Positives	Gaps	Frame
398 bits(1023)	3e-126	Compositional matrix adjust.	200/200(100%)	200/200(100%)	0/200(0%)	+3

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Query  3    VGIDVGVNLDYSGVTKPVKKAIEDGVNTTKPGNNTDLTKKVTARDAIANLANLSNLETPN  182
            VGIDVGVNLDYSGVTKPVKKAIEDGVNTTKPGNNTDLTKKVTARDAIANLANLSNLETPN
Sbjct  863  VGIDVGVNLDYSGVTKPVKKAIEDGVNTTKPGNNTDLTKKVTARDAIANLANLSNLETPN  922

Query  183  VGVEVGIKGGGSQKSQTDSQAVSTSINAGKINIDSNNKLHDQGTHYQSTQEGISLTANTH  362
            VGVEVGIKGGGSQKSQTDSQAVSTSINAGKINIDSNNKLHDQGTHYQSTQEGISLTANTH
Sbjct  923  VGVEVGIKGGGSQKSQTDSQAVSTSINAGKINIDSNNKLHDQGTHYQSTQEGISLTANTH  982

Query  363  TSEVAQDKHQTTFHETKGGGQVGVSTKTGSDITVAIKGEGQTTDNALMETKAKGSQFTSN  542
            TSEVAQDKHQTTFHETKGGGQVGVSTKTGSDITVAIKGEGQTTDNALMETKAKGSQFTSN
Sbjct  983  TSEVAQDKHQTTFHETKGGGQVGVSTKTGSDITVAIKGEGQTTDNALMETKAKGSQFTSN  1042

Query  543  GDISINVGEDAHYEGAQFDA  602
            GDISINVGEDAHYEGAQFDA
Sbjct  1043  GDISINVGEDAHYEGAQFDA  1062
```

Figure (3): Isolate number (2)

Proteus mirabilis strain AR_0159, complete genome
Sequence ID: CP021550.1Length: 4055152Number of Matches: 1
Related Information
Range 1: 3580709 to 3581335GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
1158 bits(627)	0.0	627/627(100%)	0/627(0%)	Plus/Minus

```
Query  1        CATCTAAAACTGACAGCTTAAATGTTGGTATCAATGTTGGCGTTAATCTTGATTATAGCG  60
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3581335  CATCTAAAACTGACAGCTTAAATGTTGGTATCAATGTTGGCGTTAATCTTGATTATAGCG  3581276

Query  61       GTGTAACGAAGCCAGTTAAGAAGGCAATCGAAGATGGTGTTAACACAACCAAACCGGGTA  120
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3581275  GTGTAACGAAGCCAGTTAAGAAGGCAATCGAAGATGGTGTTAACACAACCAAACCGGGTA  3581216

Query  121      ACAATACCGATTTAACTAAAAAAGTTACAGCAAGAGATGCAATTGCTAATTTAGCTAACC  180
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3581215  ACAATACCGATTTAACTAAAAAAGTTACAGCAAGAGATGCAATTGCTAATTTAGCTAACC  3581156

Query  181      TTAGCAATTTAGAGACCCCCAATGTCGGTGTTGAAGTTGGTATTAAAGGTGGTGGTAGTC  240
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3581155  TTAGCAATTTAGAGACCCCCAATGTCGGTGTTGAAGTTGGTATTAAAGGTGGTGGTAGTC  3581096

Query  241      AGAAATCAAAAACCGATAGCCAAGCCGTTTCAACCTCTATCAATGCAGGAAAAATCAACA  300
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3581095  AGAAATCAAAAACCGATAGCCAAGCCGTTTCAACCTCTATCAATGCAGGAAAAATCAACA  3581036

Query  301      TTGATAGTAATAATAAGTTACATGATCAAGGTACTCACTATCAATCAACCCAAGAGGGTA  360
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3581035  TTGATAGTAATAATAAGTTACATGATCAAGGTACTCACTATCAATCAACCCAAGAGGGTA  3580976

Query  361      TTTCTCTCACAGCAAACACCCACACAAGTGAGGCCGCGCAAGATAAACATCAAACAACAT  420
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3580975  TTTCTCTCACAGCAAACACCCACACAAGTGAGGCCGCGCAAGATAAACATCAAACAACAT  3580916

Query  421      TCCATGAAACAAAAGGCGGTGGACAAGTTGATGTCAGTACCAAAACGGGCAGTGATATTA  480
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3580915  TCCATGAAACAAAAGGCGGTGGACAAGTTGATGTCAGTACCAAAACGGGCAGTGATATTA  3580856

Query  481      CCGTTGCTATTAAAGGTGAAGGCCAAACAACTGATAACGCCTTAATGGAAACAAAGGCTA  540
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3580855  CCGTTGCTATTAAAGGTGAAGGCCAAACAACTGATAACGCCTTAATGGAAACAAAGGCTA  3580796

Query  541      AAGGTAGCCAATTTACCTCAAATGGCGATATTTCGATTAATGTAGGTGAAGATGCCCATT  600
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3580795  AAGGTAGCCAATTTACCTCAAATGGCGATATTTCGATTAATGTAGGTGAAGATGCCCATT  3580736

Query  601      ATGAAGGTGCTCAATTTGATGCACAAA  627
               |||||||||||||||||||||||||||
Sbjct  3580735  ATGAAGGTGCTCAATTTGATGCACAAA  3580709
```

hemolysin [Proteus mirabilis]
Sequence ID: WP_087741119.1Length: 1577Number of Matches: 1
See 1 more title(s)
Related Information
Identical Proteins-Identical proteins to WP_087741119.1
Range 1: 856 to 1063GenPeptGraphicsNext MatchPrevious Match

Alignment statistics for match #1

Score	Expect	Method	Identities	Positives	Gaps	Frame
413 bits(1062)	2e-131	Compositional matrix adjust.	208/208(100%)	208/208(100%)	0/208(0%)	+3

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Query  3     SKTDSLNVGINVGVNLDYSGVTKPVKKAIEDGVNTTKPGNNTDLTKKVTARDAIANLANL  182
             SKTDSLNVGINVGVNLDYSGVTKPVKKAIEDGVNTTKPGNNTDLTKKVTARDAIANLANL
Sbjct  856   SKTDSLNVGINVGVNLDYSGVTKPVKKAIEDGVNTTKPGNNTDLTKKVTARDAIANLANL  915

Query  183   SNLETPNVGVEVGIKGGGSQKSKTDSQAVSTSINAGKINIDSNNKLHDQGTHYQSTQEGI  362
             SNLETPNVGVEVGIKGGGSQKSKTDSQAVSTSINAGKINIDSNNKLHDQGTHYQSTQEGI
Sbjct  916   SNLETPNVGVEVGIKGGGSQKSKTDSQAVSTSINAGKINIDSNNKLHDQGTHYQSTQEGI  975

Query  363   SLTANTHTSEAAQDKHQTTFHETKGGGQVDVSTKTGSDITVAIKGEGQTTDNALMETKAK  542
             SLTANTHTSEAAQDKHQTTFHETKGGGQVDVSTKTGSDITVAIKGEGQTTDNALMETKAK
Sbjct  976   SLTANTHTSEAAQDKHQTTFHETKGGGQVDVSTKTGSDITVAIKGEGQTTDNALMETKAK  1035

Query  543   GSQFTSNGDISINVGEDAHYEGAQFDAQ  626
             GSQFTSNGDISINVGEDAHYEGAQFDAQ
Sbjct  1036  GSQFTSNGDISINVGEDAHYEGAQFDAQ  1063
```

Figure (4): Isolate number (3)

Proteus mirabilis strain AR_0159, complete genome
Sequence ID: CP021550.1Length: 4055152Number of Matches: 1
Related Information
Range 1: 3580708 to 3581337GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
1164 bits(630)	0.0	630/630(100%)	0/630(0%)	Plus/Minus

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Query  1        AACATCTAAAACTGACAGCTTAAATGTTGGTATCAATGTTGGCGTTAATCTTGATTATAG  60
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3581337  AACATCTAAAACTGACAGCTTAAATGTTGGTATCAATGTTGGCGTTAATCTTGATTATAG  3581278

Query  61       CGGTGTAACGAAGCCAGTTAAGAAGGCAATCGAAGATGGTGTTAACACAACCAAACCGGG  120
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3581277  CGGTGTAACGAAGCCAGTTAAGAAGGCAATCGAAGATGGTGTTAACACAACCAAACCGGG  3581218

Query  121      TAACAATACCGATTTAACTAAAAAAGTTACAGCAAGAGATGCAATTGCTAATTTAGCTAA  180
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3581217  TAACAATACCGATTTAACTAAAAAAGTTACAGCAAGAGATGCAATTGCTAATTTAGCTAA  3581158

Query  181      CCTTAGCAATTTAGAGACCCCCAATGTCGGTGTTGAAGTTGGTATTAAAGGTGGTGGTAG  240
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3581157  CCTTAGCAATTTAGAGACCCCCAATGTCGGTGTTGAAGTTGGTATTAAAGGTGGTGGTAG  3581098

Query  241      TCAGAAATCAAAAACCGATAGCCAAGCCGTTTCAACCTCTATCAATGCAGGAAAAATCAA  300
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3581097  TCAGAAATCAAAAACCGATAGCCAAGCCGTTTCAACCTCTATCAATGCAGGAAAAATCAA  3581038

Query  301      CATTGATAGTAATAATAAGTTACATGATCAAGGTACTCACTATCAATCAACCCAAGAGGG  360
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3581037  CATTGATAGTAATAATAAGTTACATGATCAAGGTACTCACTATCAATCAACCCAAGAGGG  3580978

Query  361      TATTTCTCTCACAGCAAACACCCACACAAGTGAGGCCGCGCAAGATAAACATCAAACAAC  420
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3580977  TATTTCTCTCACAGCAAACACCCACACAAGTGAGGCCGCGCAAGATAAACATCAAACAAC  3580918

Query  421      ATTCCATGAAACAAAAGGCGGTGGACAAGTTGATGTCAGTACCAAAACGGGCAGTGATAT  480
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3580917  ATTCCATGAAACAAAAGGCGGTGGACAAGTTGATGTCAGTACCAAAACGGGCAGTGATAT  3580858

Query  481      TACCGTTGCTATTAAAGGTGAAGGCCAAACAACTGATAACGCCTTAATGGAAACAAAGGC  540
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3580857  TACCGTTGCTATTAAAGGTGAAGGCCAAACAACTGATAACGCCTTAATGGAAACAAAGGC  3580798

Query  541      TAAAGGTAGCCAATTTACCTCAAATGGCGATATTTCGATTAATGTAGGTGAAGATGCCCA  600
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3580797  TAAAGGTAGCCAATTTACCTCAAATGGCGATATTTCGATTAATGTAGGTGAAGATGCCCA  3580738

Query  601      TTATGAAGGTGCTCAATTTGATGCACAAAA  630
               ||||||||||||||||||||||||||||||
Sbjct  3580737  TTATGAAGGTGCTCAATTTGATGCACAAAA  3580708
```

hemolysin [Proteus mirabilis]
Sequence ID: WP_087741119.1Length: 1577Number of Matches: 1
See 1 more title(s)
Related Information
Identical Proteins-Identical proteins to WP_087741119.1
Range 1: 855 to 1063GenPeptGraphicsNext MatchPrevious Match

Alignment statistics for match #1

Score	Expect	Method	Identities	Positives	Gaps	Frame
415 bits(1066)	4e-132	Compositional matrix adjust.	209/209(100%)	209/209(100%)	0/209(0%)	+2

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Query  2    TSKTDSLNVGINVGVNLDYSGVTKPVKKAIEDGVNTTKPGNNTDLTKKVTARDAIANLAN  181
            TSKTDSLNVGINVGVNLDYSGVTKPVKKAIEDGVNTTKPGNNTDLTKKVTARDAIANLAN
Sbjct  855  TSKTDSLNVGINVGVNLDYSGVTKPVKKAIEDGVNTTKPGNNTDLTKKVTARDAIANLAN  914

Query  182  LSNLETPNVGVEVGIKGGGSQKSKTDSQAVSTSINAGKINIDSNNKLHDQGTHYQSTQEG  361
            LSNLETPNVGVEVGIKGGGSQKSKTDSQAVSTSINAGKINIDSNNKLHDQGTHYQSTQEG
Sbjct  915  LSNLETPNVGVEVGIKGGGSQKSKTDSQAVSTSINAGKINIDSNNKLHDQGTHYQSTQEG  974

Query  362  ISLTANTHTSEAAQDKHQTTFHETKGGGQVDVSTKTGSDITVAIKGEGQTTDNALMETKA  541
            ISLTANTHTSEAAQDKHQTTFHETKGGGQVDVSTKTGSDITVAIKGEGQTTDNALMETKA
Sbjct  975  ISLTANTHTSEAAQDKHQTTFHETKGGGQVDVSTKTGSDITVAIKGEGQTTDNALMETKA  1034
Query  542  KGSQFTSNGDISINVGEDAHYEGAQFDAQ  628
            KGSQFTSNGDISINVGEDAHYEGAQFDAQ
Sbjct  1035  KGSQFTSNGDISINVGEDAHYEGAQFDAQ  1063
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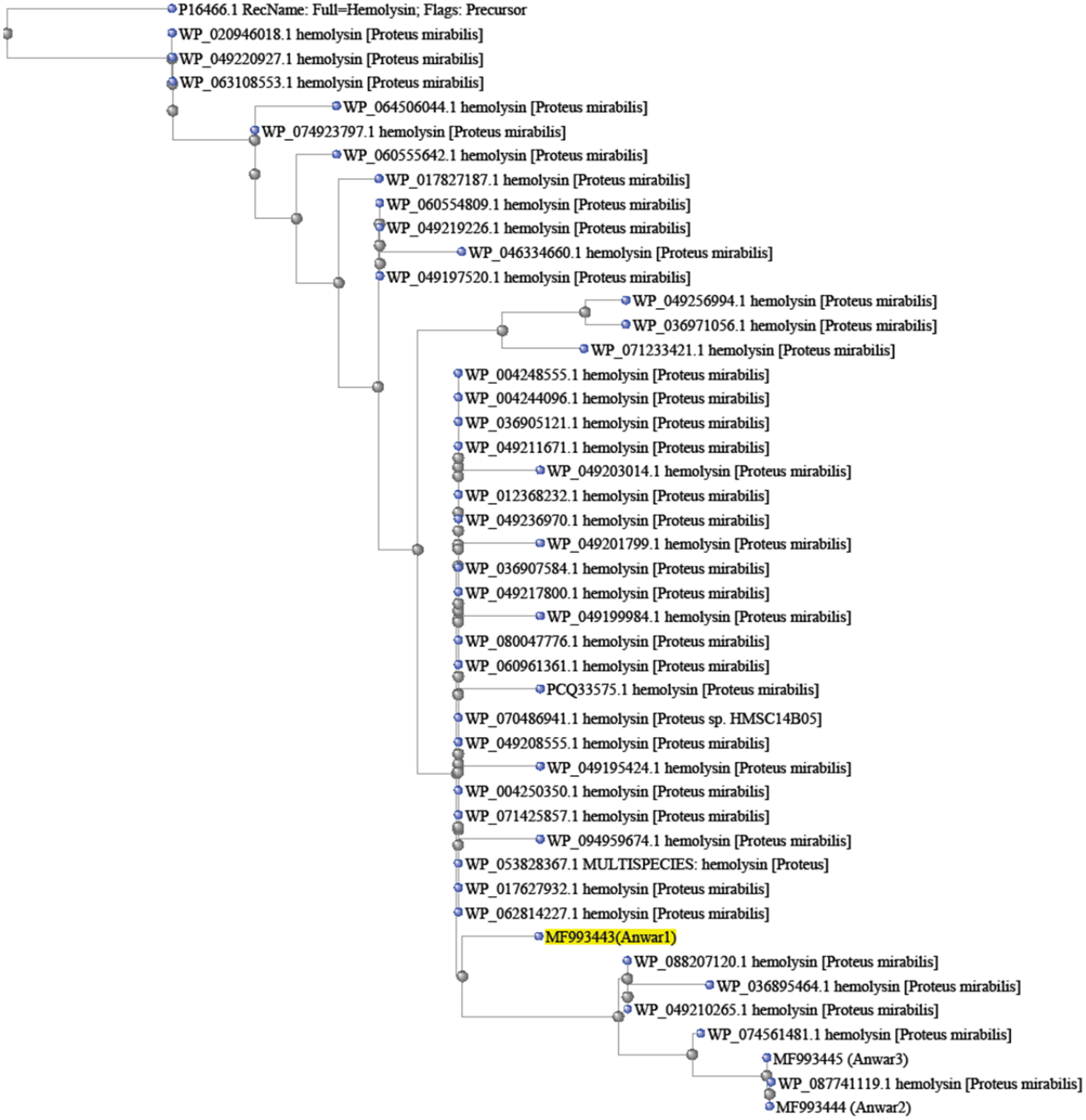
Fig.5: Phylogenetic tree of *Proteus species* based on *HpmA* gene sequence analysis.



Through the sequencing of the α-hemolysin at *P. mirabilis*, it was observed that this hemolysin consists of two genes, *hpmA* and *hpmB* [7]. Detecting and characterizing *P. mirabilis* hemolysin *HpmA* has required to elucidate its importance as a virulence factor, in addition to its probable relationship with other factors produced by *P. mirabilis* that together contribute to cytotoxicity in the UTIs of humans [7].

Mordi and Momoh found a change in the amino acid or replacement with other amino acid might lead to change in the nature of protein or output and thus lead to the emergence of strains resistant or sensitive to antibiotics [16].

In previous studies for *P. mirabilis* strains had used isolates from patients with UTIs in Brazil, from 2000 to 2009.

The *hpmA* and *hpmB* genes had sequenced in the local isolation samples presented 98 % identity for *hpmA* and *hpmB* compared to the same genes of the HI4320 (wild strain) (NCBI GenBank Number NC_010554.1) and when the two samples of this study were compared there was 100 % identity among the genes [17].

Genotyping which works to establish the relationship between bacteria strain on the basis of their genetic content uses many genotyping methods that had became important in the field of genealogy between breeds in the field of epidemiological studies furthermore in field of classification of bacteria, identification of sources, method of infection and the differentiation of strain of high virulent bacteria to prevent their spread and elimination [18].

Other studies whom found molecular mimicry or similarity between *Proteus* and self antigens, patients infected with *Proteus* microbes would produced not only antibodies against this microbe but also against the self tissue molecules [19]. Molecular mimicry sequence ESRRAL in *P. mirabilis* haemolysin, which has the

same shape and charge distribution as the RA susceptibility sequence EQRRAA [20].

Therefore, the study found great importance in the genetic sequence of *P. mirabilis* virulence factors and explored its variation, In conclusion the isolates had polymorphism in *HpmA* genes .

A phylogenic tree of based in the *HpmA* gene

Molecular phylogenetic is the branch of phylogeny that analyses hereditary molecular differences, mainly in DNA sequences, to gain information on an organism's evolutionary relationships [21]. The identified genetic profile of any bacteria by a specific genotyping method can be as unique as fingerprint [21].

However, phylogeny estimated from a single gene should be treated with caution [22][23]. The phylogenetic tree derived from genes *HpmA* gene sequences of clinical strains of 3 *sample Proteus mirabilis* with other sequences available at NCBI showed in (Fig.5). 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 has considered and widely used in bacterial taxonomy because it contains high conservation region which had variable region in different species. Furthermore, the most importance was that 16SrRNA gene could sequenced easily [23].

On the other hand, the sensitivity of this approach has questioned particularly among human bacterial closely related *Enterobacteriaceae*, which includes many common pathogens because of the high degree of conservation in species [23].

Therefore, the using of other genes rather than16S rRNA gene the distinction between bacteria at the species level has very important issue [24].

Results indicated that the *HpmA* gene was a suitable and efficient molecular marker for the distinction of *P. mirabilis* and could used as an alternative molecular tool for examining phylogenetic relationships of the *P. mirabilis* and a powerful tool for the study of different microorganisms.

The genome sequence gives a significant clue to understand the regulatory and metabolic network that link chromosomal genes [15]. In current study the sequence of the *HpmA* genes has used as a molecular clock to estimate relationships among bacteria (phylogeny), but more recently it has also become important as a means to identify an unknown bacterium up to the genus or species level.

By combining of molecular phylogeny with traditional approach, such as morphological, physiological and biochemical characteristics, bacteria identification could achieved more accurately [25] [26].

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