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RESEARCH ARTICLE

DETECTION OF SOME VIRULENCE FACTORS GENES OF *Proteus mirablis* THAT ISOLATED FROM URINARY TRACT INFECTION

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Abstract

In the present study ,170 urine sample were collect from Patients suffering from urinary tract infection from both sexes and from different ages , who frequents Al-Diwanyia Teaching Hospital, and Al-Diwanyia women and children Hospital in Al-Diwanyia city , the period was from January 2013 to April 2014 to examine *Proteus mirablis* bacteria. And the results of the facial biochemical tests showed that (30%) (%17.64) sample belong to *Proters mirablis* **.**

Some of the genes of virulence factors were investigated using Single and Multiplex Polymerase Chain Reaction Technique and they are *ureA* and *ureC* responsible for producing urease enzyme , *hpmA* ,Which is responsible for producing of hemolycin ,*zapA* Which is responsible for producing protease enzyme , *flaA* ,Which is responsible for flagella. For the rates of the appearance of these genes were (%96.66) , (%100) , (%100) , (%100) and (%86.66).respectively.

INTRODUCTION

Bacteria *Proteus mirablis* is one of the most important species belonging to the genus *Proteus* (1) . It is a negative bacilli to dye Gram negative and belonging to the family of intestinal Enterobacteracea which is one of the most important bacteria spreading in hospitals(2) . *P.mirablis* bacteria cauesee many disease which is in the forefront of urinary tract infection especially in the upper part of it and sometimes infection lead to pylonephritis(3).The bacterial pathogen *P.mirablis* bacteria is considered as second cause after *Escherichia coli* bacteria in making inflammation in the urinary tract infection and frequented in patients admitted to the hospitals and users of urinary catheter for a long time as well as people who suffer from synthetic abnormalities in the urinary tract(4).These bacteria colonize the surfaces of urinary catheter (5) . Urinary tract infection are the most common diseases and are frequent among the human race for it affacts all categories (6) .The pathogenesis of these bacteria is associated with possessing many virulence factors which include the pili (Fimbria) ,Flagella ,Urease , Protease , Heamolysin ,and multi-sugars adipose (lipopolysacchrie) and called Endotoxin(4).The protease and urease enzymes are considered as virulence factors which are produced by all strains of bacteria *Proteus*.spp.And it is considered as diagnostic and differential feature which is characterizes the members of this genus from the rest of intestinal family member (7) .The bacteria *P.mirablis* produces protease enzyme to protect themselves from the body's immune defenses (8).And this enzyme works on breaking down peptides that have effective anti-microbial activity which is one of the body's defenses produced by distal tubule ,Henle's loop and collection channels in the kidney at the beginning of the urinary tract infection (9).It also possesses the ability to form swarming phenom (10).The swarming movement is the important of the virulence factors for *Proteus* bacteria to cause urinary tract infection and can invade different parts of urinary tract by this movement and mediated by flagella which increase the *Proteus* bacterial pathogenesis

making it capable of invasion and colonization of the kidneys(11).In addition to the injury of the urinary tract there are respiratory tract infection and wounds , burns and gut infection when eating food contaminated by it(12).

Materials and Methods

A-Patients and specimens:170 urine samples were collected from patients suffering urinary tract infections: these samples taken from AL- Diwaniya Teaching Hospital and women and childern Hospital for the period from November 2013 to April 2014.

B-Bacterial diagnosis: Isolation of *P.mirablis* bacteria was performed by a surface streak procedure on both blood and MacConkey agar using calibrated loops and incubated aerobically at 37°C for 24 hours .Bacterial identification was made using biochemical test ,namely indole, citrate, oxidase, catalase, urea hydrolysis,H2S production ,lactose fermentation (13).

C- Detection of heamolysin :it was performed according to (14).

D- Detection of extracellular protease production; it was performed according to (15).

E-Genomic DNA Extraction: DNA was extracted of bacteria *P.mirablis* by using (Genomic DNA mini kit) processed from American company and according tosupplying company's instructions.

F-DNA electrophoresis in agarose gel :it was performed according to (16)

g- DNA Primers

Table 1: DNA Primer which purchased from Bioneer (Korea) company

Primer type	Nitrogene base sequences 3-5		Product	Refrence
zapA	F *	ACCGCAGGAAAACATATAGCCC	Pb 540	17
	R **	GCGACTATCTTCCGCATAATCA		
ureC	F	GTTATTCGTGATGGTATGGG	Pb 317	
	R	GTAAAGGTGGTTACGCCAGA		
Urea	F	GATCTGGGCGACATAATCGT	Pb 362	Design in this study
	R	TCACCGGGGATCATGTTATT		
hpmA	F	TGGTATCGATGTTGGCGTTA	Pb 717	Design in this study
	R	GTGGTGCCCACTTTCAGATT		
flaA	F	AGGATAAATGGCCACATTG	Pb 417	Design in this study
	R	CGGCATTGTTAATCGCTTTT		

F*:Forword

R**:Reverse

H- Thermer cycles program to amplify the DNA

Enzyme polymerization reaction was carried out by thermo cycler PCR. This device was programmed for genes under study by the interaction as shown in the **Table 2**.

Table 2: Thermer cycles program for the interaction of single PCR

Cycle NO.	Temperature / Time					Gene
	Final Extention	Cycling Condiation			Initial denaturation	
		extention	annealing	denaturation		

30	72/5min	72/60Sec	59/30Sec	Sec 95/30	Min 95/2	*ZapA*

Table 3 :Thermer cycles program for the interaction of Multiplex PCR

Temperature/ Time					Gene
Final Extension	Cycling condition			Initial denaturation	
	Extension	Annealing	denaturatin		
72/5 min	72/20 sec	56.2/30 sec	95/30 sec	95/2 min	*hpmA ureC*
72/5 min	72/30 sec	54.2/30 sec	95/30 sec	95/3 min	*FlaA UraA*

Results and Discussion

Isolation and diagnosis *P.mirablis*

The present study demonstrated 30 isolate belong to genus *P.mirablis* out of 170 sample collected from patients with urinary tract infection , through the study of some cultural and Microscopical characteristic and biochemical tests as follows.

Cultural characteristic

Developing colones are single pale colones on MacConky agar ,are medium in size and the edges smooth and non-ferment sugar lactose as well as the smell of bacterial growth which is similar to smell of fish rotting and appeared ripple movement or swarming on the blood agar , which is the recipe initial diagnostic for this bacteria. as in **Figure 1**.and then diagnosed on chrom agar and appeared colones brown color as **Figure 2**

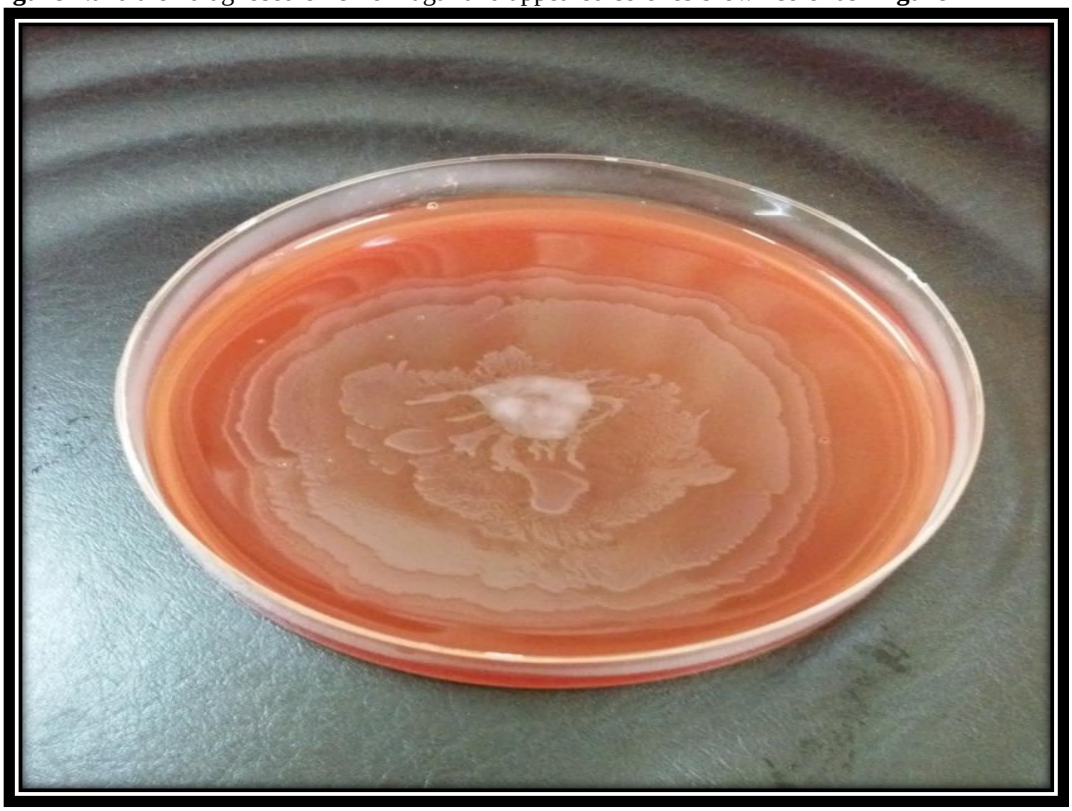

Figure 1 swarming of *P.mirablis* on blood agar

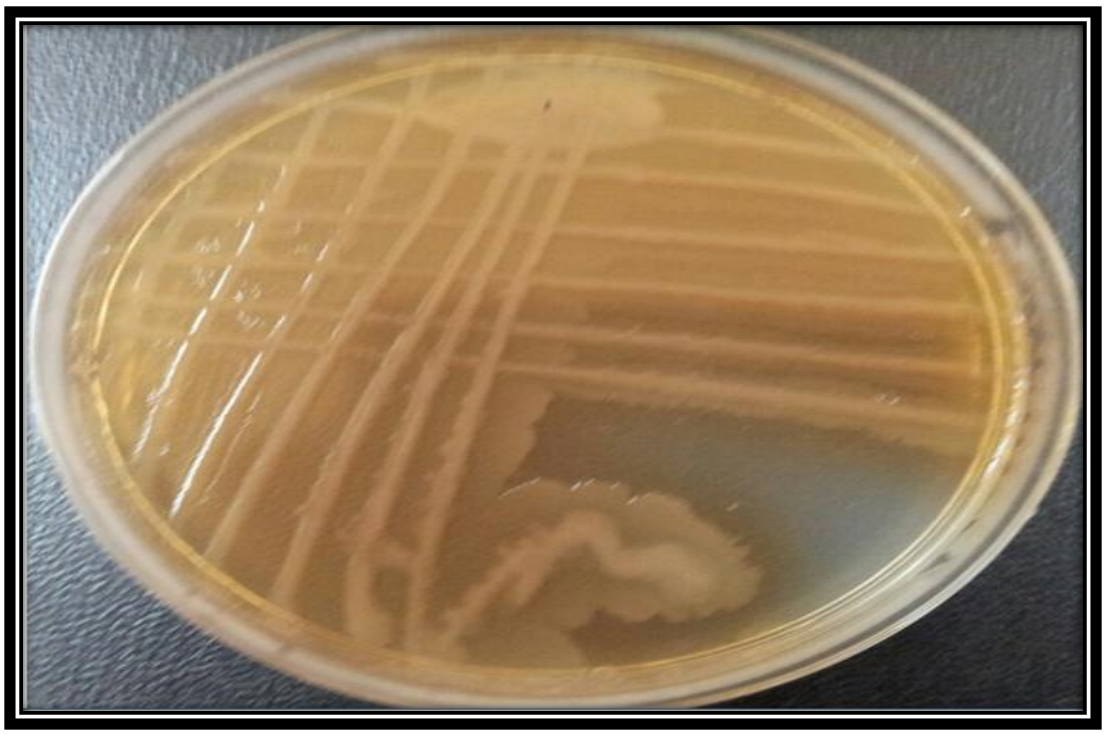

Figure 2: *P.mirablis* on chrom agar

Microscopic characterstic

Microscopic examination of the results showed that the bacterial cells isolate are short bacilli negative to Gram stain ,non forming to spores

Biochemical tests

Proteus isolates represented differences in some biochemical characteristics as shown in table 1-4 . All *Proteus* isolates were oxidase negative,and indol test , catalase positive, and urease positive. But variable results were noticed citrate utilization .

Table 4 : results of biochemical tests of *P.mirablis*

Biochemical test	Results
Catalase	+
Oxidase	-
Vogous-Proskaur	-
Indole	-
Methyl Red	+
Citrate Utilization	+
Urease	+
H2S Production	+
Groth on TSI medium	Acid/Alkaline

+:Positive

-:Negative

Single and Multiplex Polymerase Chain Reaction

Been investigating some of virulence factors for all isolates *P.mirablis* by using Single and Multiplex PCR as figures 3,4,5. As shown 30 isolation rate 100% ownership of each of genes *zapA* , *ureA* ,*hpmA* while show 29 isolate of gene *ureC* and 26 isolate of gene *flaA* .

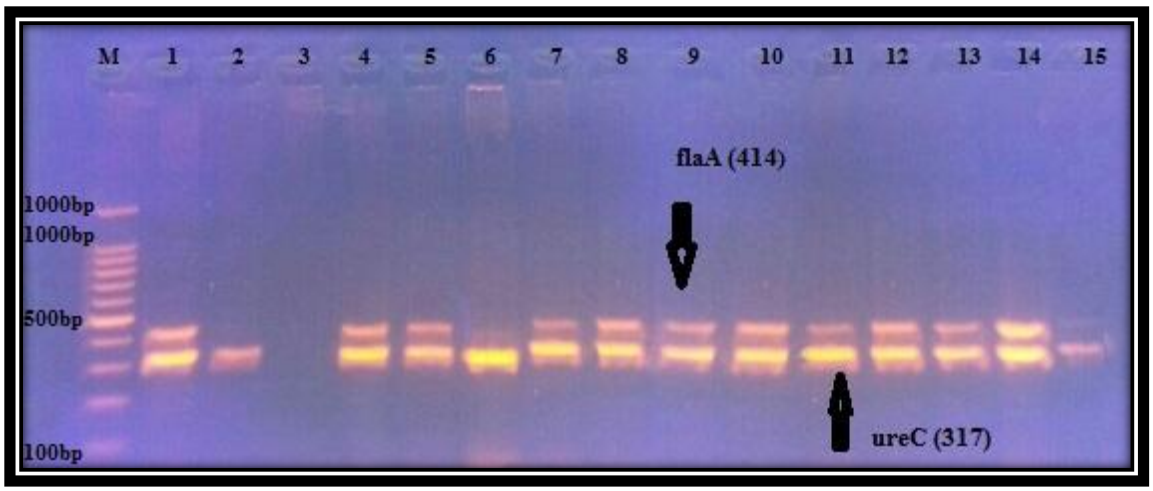

Figure 3:Results of amplified genes *flaA* and *uraC* for *P.mirablis* using Multiplex PCR.M (DNA Ladder 100-1500**) .**

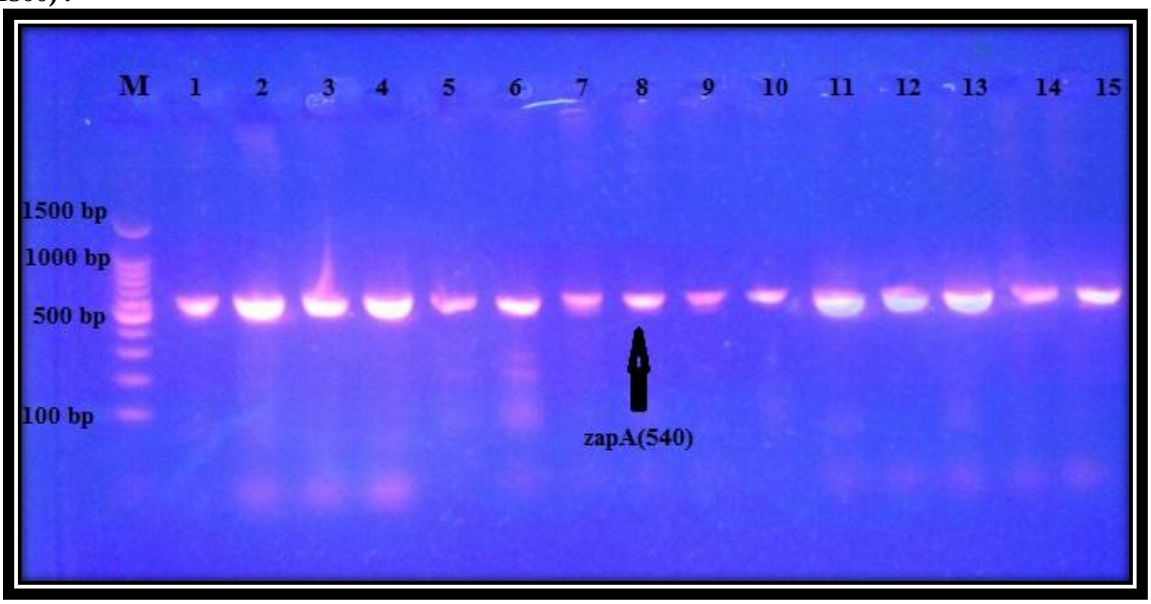

Figure 4 : Results of amplified gene *zapA* for *P.mirablis* using Single PCR .M(DNA Ladder 100-1500**)**

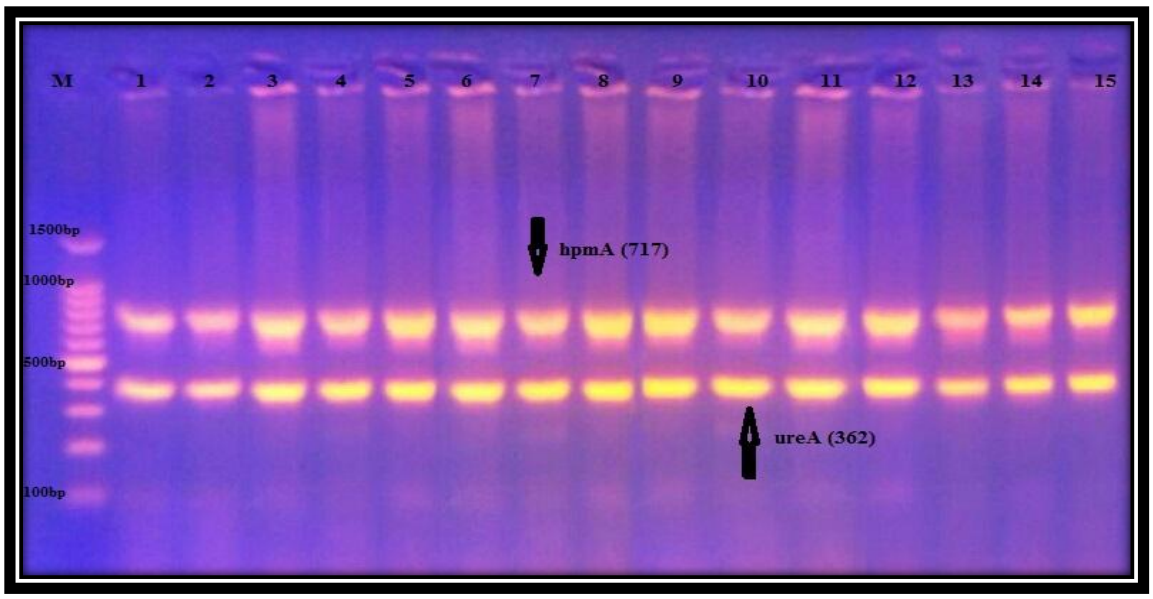

Figure 5:Results amplified genes *hpmA* and *ureA* for *P.mirablis* using Multiplex PCR.M(DNA Ladder 100-1500**).**

Isolation and diagnostsis of *P.mirablis*

Thirty isolate of *P.mirablis* has been diagnosed of the total samples in a rate of (17.64).These sample have been collected from patients with urinary tract infection .The results of the present study were in agree with aprevious study(18) that mentioned that *P.mirablis* was isolate in a rate (%20.6) . also it was accordance with (19) who mentioned that these bacteria were isolate in a rate of 26.3%.

Concerning diagnostic biochemical test that shown in **(table 1)** , it had shown that all isolate respond to catalase and cimon citrate test being the sole source to carbon and this result is similar to(20). These diagnostic isolate give a positive test to methyl-red and a negative one to vogous-proskaure because of not composing Acetyl-Methyl Carbinol out of the moleculer decomposition of sugur and this is identical with (20).

Concerning the indol test ,the result was negative for all isolates under study ;and this test is also used to distinguish between *P.mirablis* and the rest of its genus while the positive result is by composing red ring due to the decomposition of the amino acid (tryptophane) and conversed to indol.Also the isolates gave a negative result to oxidase test because of being unable to produce oxidase enzyme (21) .

Single and Multiplex Polymerase Chain Reaction technique

The technique Single and Multiplex Polymerase Chain Reaction was used in investigation some of the genes responsible for the virulence factore 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 *ureA* , *ureC* , *hpmA*, *flaA* and *zapA*.

ureC gene which is responsible for the production urease enzyme which is regarded as a diagnostic feature of the bacteria *of P.mirablis* . Yet it is considered in the present study as virulence factor which had been diagnosed using Polymerase Chain Reaction in addation to *ureA* , *hpmA*, *flaA* and *zapA*.

The results of the current study show that 29 isolates out of 30 isolates in a rate 96.66% contain *ureC* gene for it has been investigated using the technique of Multiplex PCR As shown in **Figure 3**. The study also shown that all these isolates were productive for *ureA* gene, also it is responsible for producing urease enzyme in a 100% rate as shown in **Figure 5**.Urease enzyme produced from *P.mirablis* is characterized by being more active than urease enzyme produced from other types of bacteria , It works on changing PH urine to basic leading to deposition the calcium and magasium phosphate in the biofilm formed which in its turn leads to the formation of Crystallin biofilm which is the more complex type biofilms for it works to close the catheter urinary and protect the bacteria from antibiotics causing failure to the treatment with antibiotics (10). The results of the present study are compalible with (17) as well as for all its isolates were producing urease enzyme.

hpmA gene which responsible for producing hemolycin is considered as important virulence factor for *P.mirablis* . In this study , *hpmA* has been investigated by using Multiplex PCR technique ,and the results shown that 30 isolate in a rate of 100% had *hpmA* gene as shown in **Figure 5**. And this result is similar to (22) for they mentioned that the rate of this gene in *P.mirablis* isolates was %97.15.

The hemolycin enzyme acts on destroying the leukocyte membrane by making small holes in the membrane of the leukocyte and epithelial cell and its presence is a very important factor in providing the bacteria with iron ; and because of its cytotoxic ,it leads to the destraction of the kidney tissue of the host(23).The results of the present study also showed that all the bacteria isolates possess *zapA* gene which is responsible for producing proteins in a rate of 100% that had been investigated about by using single PCR as shown in **Figure 4** and this result was agree with (17) and (24).

The enzyme protease is regarded as one of the important enzymes which has the ability to break down antibodies of the type IgA and IgG , and thus reducing immune response and for this reason they enzymes accquired great importance being important virulence factor for the bacteria producing them , The *P.mirablis* had the ability to produe case protein enyme of the type (Metaloprotease) which had been discovered in the urine of patients infected in urinary tract infection and other infections . while non pathogenic strain of the genus *Proteus* are less efficient in the production of this type of enzymes (25) .

Concerning the gene responsible for *flaA* which is considered as one of the important virulence factors that help this bacteria to move rapidly , for it has been investigated in this study by using Multiplex PCR technique and the results showed that 26 isolated in a rate of (86.66%) out of 30 isolate of *P.mirablis* were having *flaA* gene as shown in **Figure 3** . Out of what had been presented we can say that pathogen of bacteria developed through virulence factors to be adapted with the host envirument , and the virulence of *P.mirablis* bacteria is increased through our understanding of the ability of normal flora to invade and infect urinal tract.

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