Bacteriological Study and Investigation of Some Virulence Factors of *Proteus mirabilis* Bacteria Isolated from Urinary Tract Infection Patients in Ramadi City

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Abstract

Twenty isolates of *Proteus mirabilis* were diagnosed with 8.33% of the total of 240 samples that were isolated from urinary tract infections of patients returning to Ramadi General Teaching Hospital and Women's and Children's Hospital, after conducting biochemical tests and confirming them with VITEC 2, the results of the qualitative investigation of the UreR, ZapA, and MrpA genes showed that all isolates (20) of P. mirabilis had these three genes with a percentage of 100%. The results of the nitrogenous bases sequences confirmed the agreement of the diagnostic results of *P. mirabilis* isolates with the isolates recorded in NCBI using the(MEGA7) program with 100% match for each of the MrpA gene isolation number(17) and ZapA gene isolation number 12, as for the ZapA gene, isolation number (2), seven mutations of the type of equivalent transition were shown, and one mutation, a type of non-equivalent substitution, transversion, with a match ratio of 98%, while for the UreR gene for isolates No.(5)and No.(7), it showed four mutations equivalent to the same sites and for the same Sequence ID. With a similar ratio of 99%, it can be inferred in principle that the ZapA, UreA, and MrpA genes were found in the same genus but in other species and strains with the same sequence of nitrogenous bases.

Keywords: *UreR, Proteus mirabilis, PCR, gene*

Introduction

The urinary tract infection UTI is one of the most widespread infections and affects millions annually, the rate of infection among females is higher than that of males, and that urinary tract infection ranks second in terms of the number of infections after respiratory tract infection[(1)]. *Proteus mirabilis* is the most common pathogen for urinary tract infection (UTI), especially in individuals with urinary tract abnormalities or those using urinary catheters[(2)] The pathogenesis of these bacteria is associated with possessing many virulence factors which include the pili(Fimbria), Protease, Flagella,Urease Heamolysin,and multi-sugars adipose (lipopolysacchrie) and called Endotoxin[(3)]. The (UreR) gene can be used very accurately in diagnosing of P. mirabilis bacteria, which is responsible for the production of the Urease enzyme[(4)]. 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 magasium and calcium phosphate in the biofilm formed which in its turn leads to the formation of Crystallinbiofilm 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[(5)]. The gene that encodes the protease enzyme is coded for (Zap A) and possession of P. Mirabilis bacteria for this enzyme helps the bacteria break down the (IgA) antibodies[(6)]. The protease enzyme produced by *P. mirabilis* may be called IgA enzyme due to its activity on serum(IgA1) and (IgA2)[(7)].The(MR/P fimbriae) play a role in the virulence observed during UTIs caused by uropathogenic *P. Mirabilis* strains. Expressions of MR/P fimbriae are increased under

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oxygen limitation, and were suggest that MR/P fimbriae dictate the localization of bacteria in the bladder[8]

The aim of the study was to isolate and diagnose *P. mirabilis* from (UTI) patients and to investigate some genetically determined virulence factors using PCR, and then perform genetic sequences using the Gene Sequencing technique

Materials and Methods

Samples collection

240 samples of medial diuresis were collected from patients suffering from urinary tract infections for both sexes and for all age groups, and samples were taken from the visitors of the Ramadi General Teaching Hospital and the Women's and Children's Hospital in Ramadi for the period from 1/9/2019 to 15/2/2020.

Identification

P.mirablis was primarily identified through its cultivation, culture, and planning method on the medium of MacConkey agar, and observation of the phenomenon of swarming on blood agar and incubated aerobically at 37 ° C for 24 hours, then biochemical tests were performed and confirmed by VITEC2 device.

Genomic DNA Extraction

DNA was extracted from 20 samples of P. mirabilis isolates using a Promega Genomic Extraction Kit.

DNA Primers

The specific prefixes for the UreR, ZapA, and MrpA genes were provided by AlphaDNACanada company as shown in Table (1).

Table 1: Primers and size products.

Target Gene	Nitrogene base sequences 5' -3'	Molecular size bp	Refrence
ZapA-F	-ACCGCAGGAAAACATATAGCCC-3, - 5'	540	12))
ZapA-R	5' - GCGACTATCTTCCGCATAATCA-3,		
UreR-F	5' -GGTGAGATTTGTATTAATGG-3,	225	(22)
UreR-R	ATAATCTGGAAGATGACGAG-3, -,'5		
MrpA-F	5' -TTC TTA CTG ATA AGA CAT TG-3,	565	23))
MrpA-R	5'-ATT TCA GGA AAC AAA AGA TG-3'		

Table (2): Programs of PCR Thermocycling Conditions of gene.

Gene name	Temperature (°C) / Time					Cycles Number
	Initial Denaturation	Cycling Conditions			Final Extension	
		Denaturation	Annealing	Extension		
UreR	940C\4min	940C\40std	58 0C\30sec	720C\30sec	0C/5min 72	40
ZapA	95 0C\2min	30std \950C	59 0C/30sec	C/30sec 720	72 0C/5min	35
MrpA	940C\3min	940 C\30 std	400 C\30sec	720C\30min	720C/5min	40

DNA sequencing

PCR was used to amplify the samples. The results were sent to the Korean company Macrogen and compared with the information in the National Center for Biotechnology, the NCBI. The results were read using the Blas Basic Local Alignment search tool.

Results and Discussion

240 urine samples were collected from patients suffering from urinary tract infections and 200 samples showed a positive growth rate of 83.33% and 40 samples with a rate of (16.67%) negative growth. There were high significant differences between males and females, so there were (135) females (67.6%) and (65) Males (32.40%) and showed the return of 20 isolates of *P. mirabilis* bacteria (8.33%). A study[9] agreed, when they isolated P. mirabilis bacteria from different sources and the percentage of its isolation from urine was(10.7%).

The initial diagnosis of *P. mirabilis* was made by observing the growth of single, pale colonies on MacConkey agar and non-fermented lactose blades with soft and medium-sized edges. On the center of the blood agar, the swarming or anthropogenic odor was observed and it had a distinctive smell similar to the smell of rotten fish.

Microscopic examination of P. mirabilis indicated that it is a short Gram-negative bacillus that does not form spores.

The polymerase chain reaction was performed to detect the UreR gene using a specialized primer for this gene to detect its presence in (20) isolates of *P. mirabilis* which has a volume (225bp), the isolates were relayed (20), and the results of the UerR gene detection showed the presence of this gene in all isolates of *P.mirabilis* bacteria, as shown in **Figure (1)**, this is normal because *P. mirabilis* has a urea-induced enzyme that breaks down urea in urine into ammonia(NH_3) and carbon dioxide (Co_2) which raises the environmental pH, and mediates precipitation of normally soluble polyvalent ions from the urine, specifically precipitation of ammonium, phosphate , calcium, and magnesium ions results in formation of the struvite and carbonate hydroxyapatite crystals that comprise urinary stones[24]. The results of the current study indicated that the percentage of UreR presence is 100%, and it was in agreement with [10] and also similar to what was stated in[11].

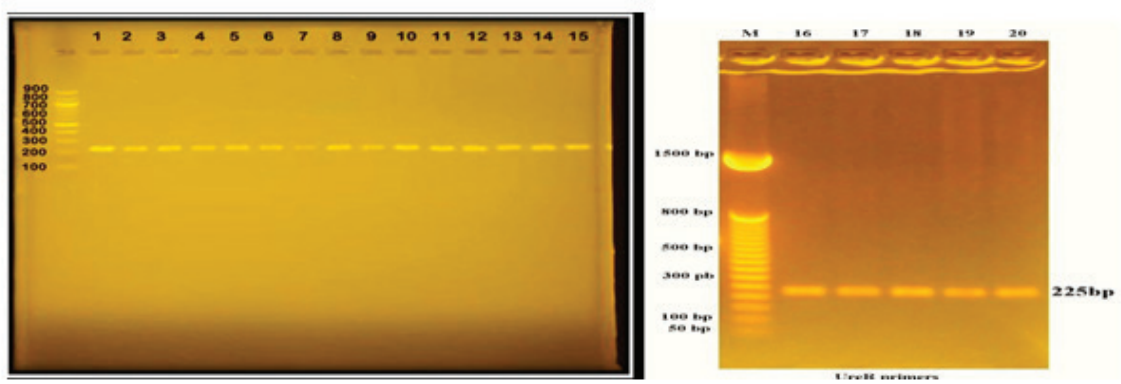

Figure 1:Results of the amplification of UreR gene of proteus mirabilis samples were fractionated on 2% agarose gel electrophoresis stained with Eth.Br. M: 50bp DNA ladder marker. Lanes 1-20 resemble 225bp PCR products.

The ZapA gene was detected by a PCR test using PCR reaction mix primers to detect the ZapA gene which has a volume of 540 bp, then the 20 isolates of *P. mirabilis* were relayed, and the bundles in all isolates showed evidence of the mentioned bacteria's possession of the ZapA gene, and the results of the detection of the ZapA gene were 100%, as shown in **Figure (2),** it agreed with each of the studies [12], [7] and [17], and converged greatly with the results of [14], as the percentage reached 98.2% and disagreed with [15] and [19], as the percentages reached 44.5% and 45.8. %, respectively.

From this it is evident that *P. mirabilis* is specifically able to produce (ZapA) and it has been detected in the urine of patients with UTI, while the non-pathogenic strain of other types of Proteus is less efficient in producing this type of enzyme [7].

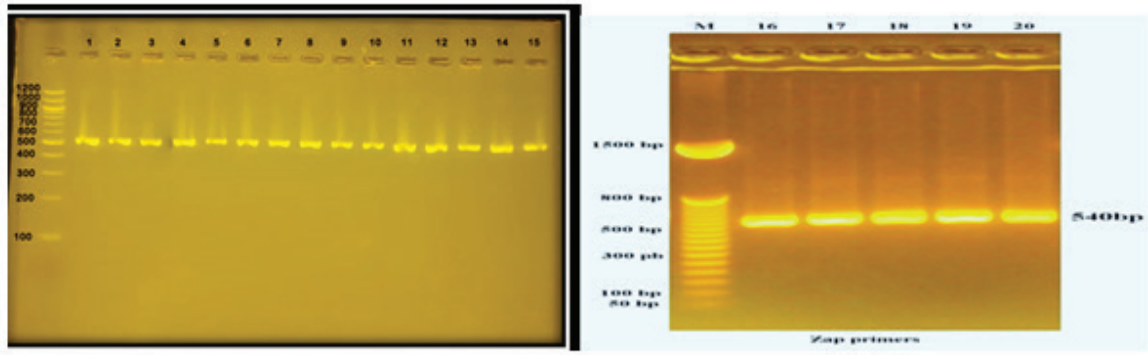

Figure2: Results of the amplification of ZAPA gene of proteus mirabilis samples were fractionated on 2% agarose gel electrophoresis stained with Eth.Br. M: 50bp DNA ladder marker. Lanes 1-20 resemble 540bp PCR products.

A PCR test was conducted to check for the presence of the MrpA gene using specialized primers for this gene for 20 isolates belonging to P. mirabilis bacteria with a size of (565bp), and its presence was 100% as shown in **Figure (3)**, it was agreed with the results of [18] and converged with [13], as the percentage reached 80% ,while [16] confirmed that the percentage of this gene in its isolates reached 90% while it disagreed with [19]. It was found that 45.8% of its isolates possess the MrpA gene and the ZapA gene in P. mirabilis bacteria and not possessed by P. vulgairs, and [20] was found that 6 of the 17 isolates have the MrpA gene, or 35%.

The importance of this type of fimbria is attributed to its role in adherence to uroepithelium cells and thus contributes significantly to urinary tract infection [18] and this is confirmed by a study [21] which revealed the ability of this type of pili(Fimbria)to adhesion, specifically adhesion to uroepithelium cells, tubular and renal epithelial cells. To say that the virulence factors of P. mirabilis have evolved to adapt to the host's environment and increase our understanding of the ability of natural flora to colonize and infect the urinary tract.

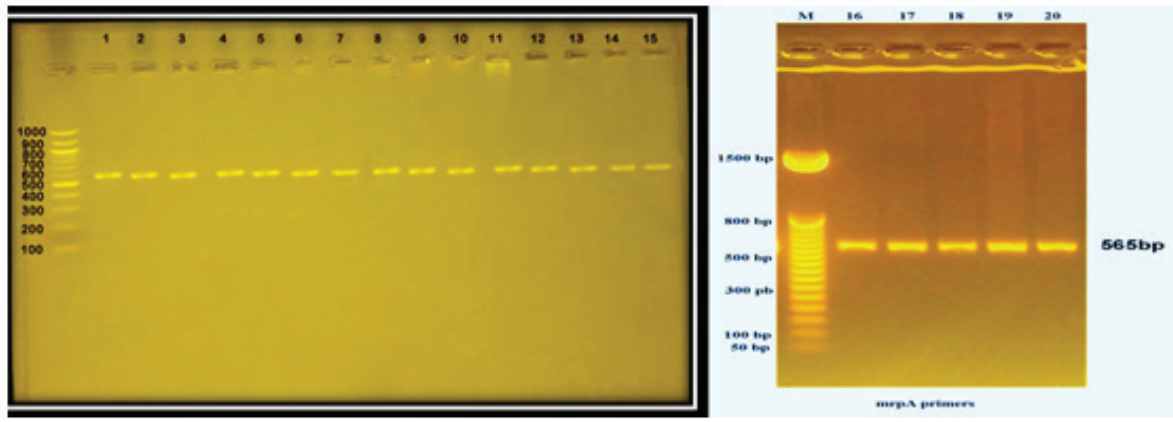

Figure 3:Results of the amplification of mrpA gene of proteus mirabilis samples were fractionated on 2% agarose gel electrophoresis stained with Eth.Br. M: 50bp DNA ladder marker. Lanes 1-20 resemble 565bp PCR products.

The results of the DNA sequences of isolates (12) and (2) of *P.mirabalils* were shown for the purpose of determining the sequences for the nitrogenous bases of the ZapA gene, which were compared with three sequences recorded in the National Center for Informatics (NCBI) using (BLASTN2.6.4), As shown in **Table(3)** ,the results of the comparison with the first sequence that carries the symbol MG748837.1, which is related to *P. merabilis*, showed that there are variations in the sequence of nitrogenous bases and there are eight mutations, seven mutations of the equivalent transition type in which the(C- T) mutation, (T- C) mutation,(G-A) mutation in situ,(A- G) mutation,(G- A) mutation,

(T- C) mutation, and(T- C) mutation at sites(525, 537, 549 , 558, 564. 567,570) sequences and one mutation of an unequal substitution type, so (A- T) was at site (535) and the match was 98%, while a comparison with sequences bearing the code JQ040548.1 for the UreR gene in isolation No. 7 showed that there were variations in the sequence of nitrogenous bases and found two equivalent transition mutation, the first in which(T- C) is transformed at site 108 and the other is an (A- G) mutation at site 220 with a 99% match as found. In isolation No. 15 of the same gene with the same code JQ040548.1, there are also two mutations, a type of equivalent transition(T- C) and (A- G), and the same sites 108, 220, with a match ratio of 99%.

While isolates No. 17 of the MrpA gene and No. 12 of the ZapA gene did not show any genetic mutations , with a match ratio of 100%.

The emergence of some types of mutations in the same strain is attributed to its exposure to mutagenic agents or because of the place of isolation, while strains belonging to other species were found that contain the same zap gene and with the same repetition of the sequence of nitrogenous bases due to the possibility of repeating the gene with the same order of nitrogenous bases between the mentioned models in light of the results of the current study. It is inferred that the genes that showed the mutations can replicate the different types of *P. mirabilis* bacteria with genetic variants as a result of the mutations and these results give an impression after comparisons between genes there are similarities for the repetition of genes between P. mirabilis types and with the same sequence of nitrogenous bases for the mentioned genotypes.

Table 3 : **Type of substitution**

No. Of sample	Type of substitution	Location	Nucleotide	Range of nucleotide	equence ID
ZapA(2)	Transition	525	C-T	290 to 671	MG748837.1
	Transversion	535	A-T		
	Transition	537	T-C		
	Transition	549	G-A		
	Transition	558	A-G		
	Transition	564	G-A		
	Transition	567	T-C		
	Transition	570	T-C		
UreR(7)	Transition	108	T-C	53 to 225	JQ040548.1
	Transition	220	A-G		
UreR(15)	Transition	108	T-C	54to 225	JQ040548.1
	Transition	220	A-G		
ZapA(12)	---------	-------	-------	389 to 753	MG748837.1
MrpA(17)	---------	-------	-------	6 to 381	MT294143.1

Conclusions

P.Mirabilis bacteria is one of the most important causes of urinary tract infection because it has many virulence factors, including urease, protease and fimbriae, which increase its pathogenicity, and this is what was shown by the investigation of the genes of the aforementioned bacterium virulence factors that it possesses these genes in high proportions as well as the comparison of the UreR, MrpA, ZapA gene sequence. is a suitable method for inferring the genetic relationships of P. mirabilis isolates on a molecular basis and in the future. By studying the genetic map, it is possible to learn more about *P. mirabilis* .

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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