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Molecular study of *Proteus mirabilis* bacteria isolated from urine and wounds in hospitals Al-Najaf province

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Abstract

Background: This study aims to find out the relationship between biochemical test and molecular in diagnosis of *Proteus mirabilis* and correlation between them calibrated and the effects on the patients of the difference age groups. **Material and method:** The collected of 60 from patients attending (Al-Najaf hospital/Central healthy Laboratories. Samples were wounds swabs and urine. The specimens were directly streaked onto Macconkey and blood agar sand and Incubated at 37ºC for 24 hours. Also the isolates were identified depending on morphological and biochemical tests as compared with vitak 2. Polymerase chain reaction (PCR) was used to detect the ureC and zap genes in the genomes of the bacterial strains. **Results**: A total of 60 cases samples were collected from patient attended AL-Najaf Hospital / Central healthy laboratories. Forty five (75%) out of 60 patient by Vitak 2, and isolated of *Proteus mirabilis* from 42 cases of urine while 18 cases of wound patients. By Vitak test, the highest frequency of *Proteus* in urine [17/19 (89.5%)] was detected among the patients of the 1st age group (10-30 yr.), followed by the patients of the 2nd age group (31-51yr.) [15/19(78.9%)], while the lowest frequency [1/4(25%)] was seen in the patients of the 3rd age group. While showed the highest frequency of positive in wound were the 2nd age group (31-51yr.) [6/7(85.7%)] and then the patients of the 1st age group (10-30yr.) [4/4(100%)], while the lowest frequency was seen in the patients of the 3rd age group [2/7(28.6%)]. The percent of urine and wound patients that positively by PCR were 54.5% in urine and 83.3% in wound .This appear the compared of between positive and negative tests with *Proteus mirabilis* by PCR detected that isolated from urine and wound patients . The detection of *Proteus mirabilis* genes by PCR technique for positive patients were recorded in *ureC* gene (33.3 %),*zapA* (44.5%) and *aac(6')-Ib* (22.2%) in urine while in wound were recorded *ureC* gene (50%), *zapA* (30%) and *aac(6')-Ib* (20%). **Conclusion:** The correlation between of the Vitak test and biochemical tests with molecular test. **Recommendations**: To detect genotypes that lead to aminoglocides resistance.

Keywords: *Proteus mirabilis*, PCR , *ureC, zapA and aac(6')-Ib.*

Introduction

Proteus mirabilis, a gram-negative enteric bacterium, occurs as vegetative swimmer cells and hyper flagellated swarmer cells (1). Individuals suffering from urinary tract infections (UTI) caused by *P. mirabilis* often develop bacteria urea, kidney and bladder stones, catheter obstruction due to stone encrustation, acute pyelonephritis, and fever (2).

P. mirabilis is one of the most common causes of UTI in individuals with long-term indwelling catheters, complicated UTI, and bacteremia among the elderly (3). As the aging population expands, more individuals will be at risk for *P. mirabilis* UTI (4).

These infections of the urinary tract occur in an ascending manner (3). Uropathogenic microorganisms

contaminate the per-urethral area, enter the bladder through the urethra, and establish an initial colony. These bacteria have specific adhesion and motility phenotypes that allow them to ascend to the bladder against the flow of urine, which normally prevents bacterial invasion at low infectious doses. After initial colonization, *P. mirabilis* ascends the urethras and initiates an interaction with epithelial cells of the renal pelvis, which allows colonization of the kidney (5). In some cases, bacteria breach the one-cell-thick renal tubular epithelial barrier and enter the bloodstream (6). *Proteus* spp are the causative agent of a variety of opportunistic nosocomial infections including those of the respiratory tract, ear, nose, skin, burns, and wounds, it may also cause gastroenteritis (7).

Proteus species (*P. mirabilis*, *P. vulgaris*, and *P. penneri*) are important pathogens of The urinary tract and primary infectious agent in patients with indwelling urinary Catheters (8).

Individuals suffering from urinary tract Infections caused by *Proteus mirabilis* often develop bacteriuria, cystitis, and kidney And bladder stones, and catheter obstruction due to stone encrustation, and acute pyelonephritis (1) .

For the importance of *Proteus spp*. as a nosocomial pathogen. UTIs are among the most frequently occurring human bacterial infections, accounting for about 20·% of all infections acquired outside the hospital. Almost 90% of UTIs are ascending, with bacteria gaining access to the urinary tract via the urethra to the bladder and then to the upper part of the urinary tract (9).

Materials and Methods

Sample Collection

Total of 60 samples were collected from patients atten ding (Al-Najaf hospital/Central healthy Laboratories. Samples were of wounds swabs and urine. The specimens were directly streaked onto Macconkey and blood agar sand and incubated at 37ºC for 24 hours.

Identification of the Isolates

Isolates were identified depending on morphological and biochemical tests as compared with vitak 2 Test.

Biochemical test:

The diagnosis of *Proteus mirabilis* were used of all biochemical tests reactions by Vitak 2.

Measurement of hemolytic activity

The hemolytic activity of the Proteus strains was tested by two methods. The first was the blood plate method, where the zone of hemolysis produced by the colony of tested bacteria was observed.

DNA extraction kit

The diagnosis of *Proteus mirabilis* were used of promaga kits.

Detection of genes

Polymerase chain reaction (PCR) was used to detect the ureC, zapA and aac(6')-Ib genes in the genomes of the bacterial strains. Matrix DNA was prepared by warming bacterial cells at 100°C for 5 min and removing solid debris by centrifugation. The ureC primers used were forward: 5'-GTTATTCGTGATGGTATGGG-3 and reverse: 5'-ATAAAGGTGGTTACGCCAGA-3. The PCR cycles were: denaturation at 95°C for 1 min, annealing at 52°C for 1 min, and extension at 72°C for 1 min, repeated 35 times. For zapA the primers were forward: 5'-ACCGCAGGAAAACATATAGCCC-3 and reverse: 5'-GCGACTATCTTCCGCATAATCA-3. The PCR cycles were: denaturation at 95°C for 1 min, annealing at 53°C for 1 min, and extension at 72°C for 1 min, repeated 35 times and The aac(6')-Ib primers used were forward: 5'-TATG AGTGGCTAAATCGA-3 and reverse: 5'-CCCGCTTTCTCGTAGCG-3. The PCR cycles were: denaturation at 94°C for 2 min, annealing at 55°C for 40sec, and extension at 72°C for 1 min, repeated 45 times (must be used of PCR gradient). The products were separated in 1.5% agarose gel in Tris-acetate. ethylenediamine tetra-acetic acid (TE), stained with ethidium bromide, and photographed in ultraviolet light by electrophoresis at70v for 90min(Gel document) .

Statistical analysis

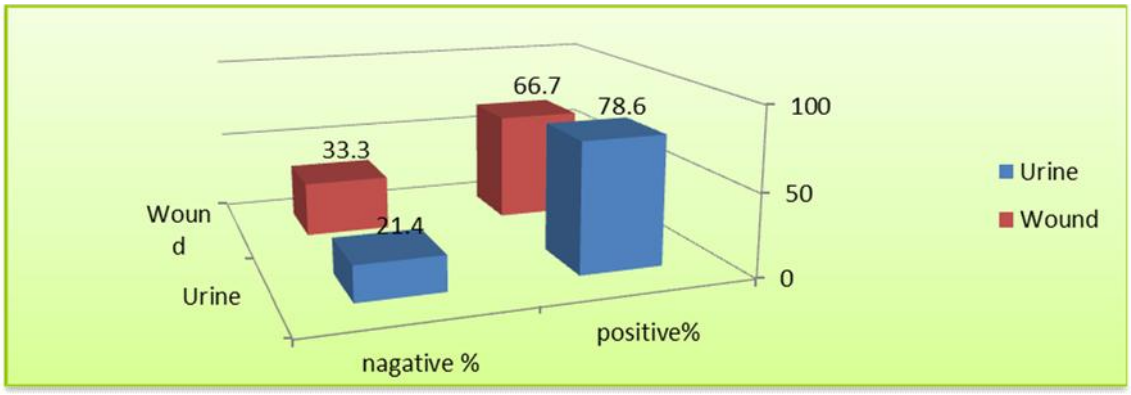
Statistical tests were performed using by (Version 9; SPSS Inc.,) software. Data were p Values <0.05 were considered statistically significant.

Results

A total of 60 cases samples were collected from patient attended AL-Najaf Hospital / Central healthy laboratories . Forty five (75%) out of 60 patient by Vitak 2, and isolated of *Proteus mirabilis* from 42 cases of urine while 18 cases of wound patients (Table-1 and Figure-1).

(Table-1) Prevalence of *Proteus mirabilis*

Bacteria / Infections	*Proteus mirabilis*					
	+ve No.	%	-ve No.	%	Total No.	%
Urine	33	78.6	9	21.4	42	100%
Wound	12	66.7	6	33.3	18	
Total	45	75	15	25	60	
P<0.001						



(Figure-1) prevalence of *Proteus mirabilis*.

Distribution of *Proteus mirabilis* with different age groups

By Vitak test, the highest frequency of *Proteus* in urine [17/19 (89.5%)] was detected among the patients of the 1st age group (10-30 yr.), followed by the patients of the 2nd age group (31-51yr.) [15/19(78.9%)], while the lowest frequency [1/4(25%)] was seen in the patients of the 3rd age group.

By Vitak test, patients of the 2nd age group (31-51yr.), showed the highest frequency of positive in wound [6/7(85.7%)] in compare to the patients of the 1st age group (10-30yr.) [4/4(100%)], while the lowest frequency was seen in the patients of the 3rd age group [2/7(28.6%)].

Table 2 **Distribution of *Proteus mirabilis* with different age groups**

Bacteria / Age groups	*Proteus mirabilis*											
	Urine		Wound		Urine		Wound		Urine		Wound	
	+ve No.	%	+ve No.	%	-ve No.	%	-ve No.	%	Total No.	%	Total No.	%
10-30	17	89.5	4	100	2	10.5	0	0	19	100%	4	100%
31-51	15	78.9	6	85.7	4	21.1	1	14.3	19		7	
52-62	1	25	2	28.6	3	75	5	71.4	4		7	
Total	33	78.6	12	66.7	9	21.4	6	33.3	42		18	
P<0.001												

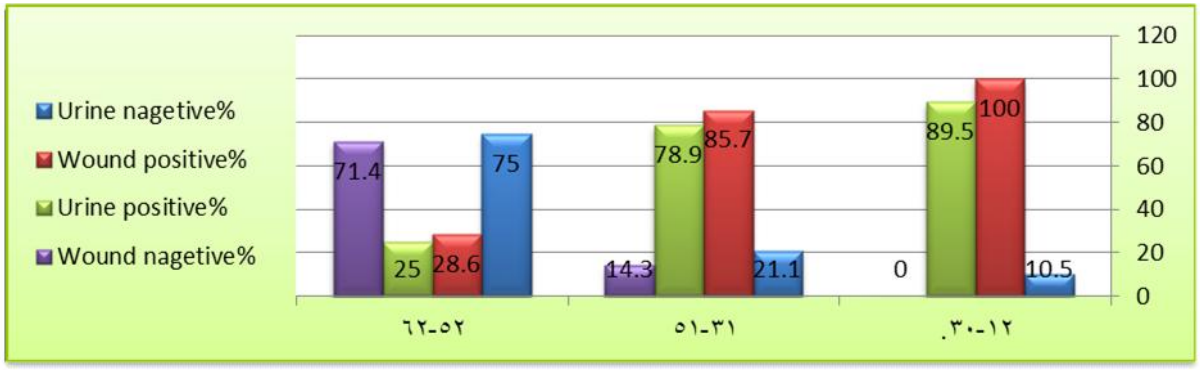


Figure 2 Distribution of *Proteus mirabilis* with different age groups.

Estimation of positive cases by PCR technique according to the type of specimen

The percent of urine and wound patients that positively by PCR were 54.5% in urine and 83.3% in wound .This table appear the compared of between positive and negative tests with Proteus mirabilis by PCR detected that isolated from urine and wound patients.

(Table-3) Estimation of positive cases by PCR technique according to the type of specimen.

Type of sample	Positive		Negative	
	No.	%	No.	%
Urinary (33 cases)	18	54.5%	15	45.5%
Wound (12 cases)	10	83.3%	2	16.7%
P<0.05				

The detection of *Proteus mirabilis* genes by PCR technique for positive patients.

Statistically, there significant differences were between Urinary and wound samples in giving positive results by PCR as revealed in (table-4). Table appear of compared between wound and urinary samples with genotyping that higher percent were recorded in ure1 gene (33.3 %), zapA (44.5%) and aac(6')-Ib (22.2 %) in urine while in wound were recorded ure1 gene (50%),zapA (30%) and aac(6')-Ib (20 %).

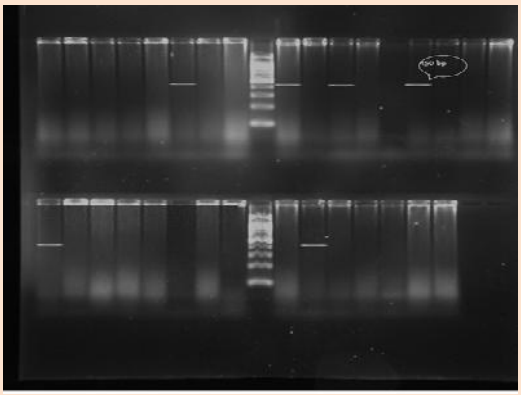
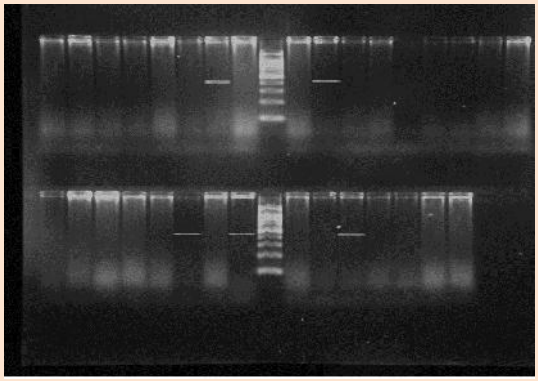
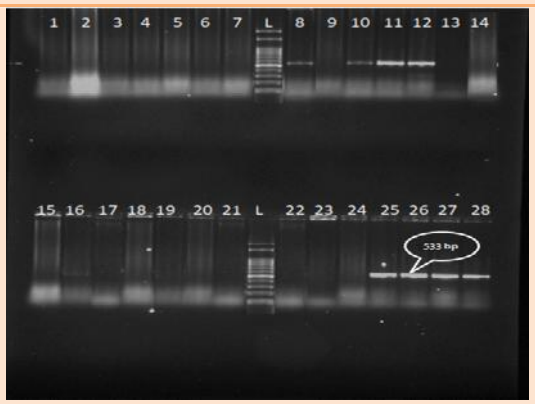
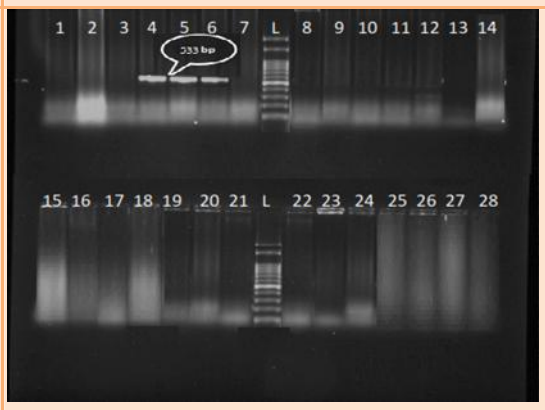
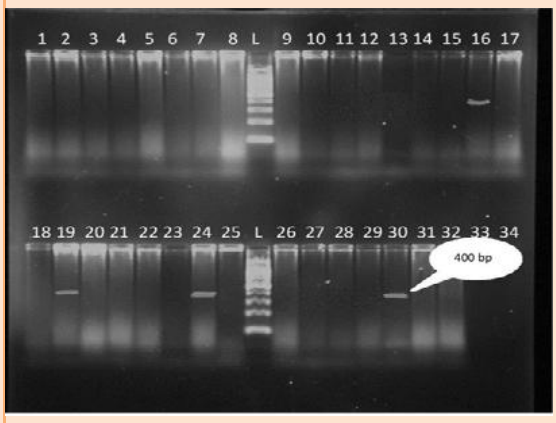
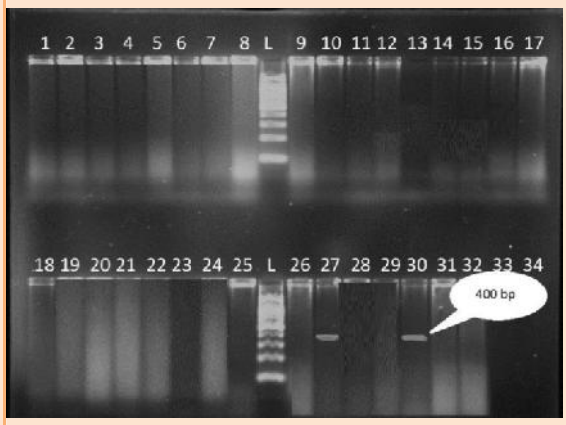
(Table-4) The detection of *Proteus mirabilis* genes by PCR technique for positive patients.

Proteus mirabilis genes		PCR			
		Urine		Wound	
		NO.(18 cases)	%	NO.(10cases)	%
Urc1	**+ve**	6	33.3%	5	50%
zapA	**+ve**	8	44.5%	3	30%
aac(6')-Ib	**+ve**	4	22.2%	2	20%
		P<0.001			

Deletion of PCR products:

Gel electrophoresis of PCR amplified products from extracted DNA isolates from (urine and wound) and amplified with *ureC* primers (bp). And amplified with *zapA* primers (533 bp) and amplified with *aac(6')-Ib* primes (400 bp) are shown in **Appendix-1** .

Appendix-1

Figure	Urine	Wound
Urc1		
zapA		
aac(6')-Ib		

Discussion

Prevalence of *Proteus mirabilis*

The percentage of *Proteus mirabilis* was 45(75%) and demonstrated in the table 1 and figure 1. The percentage of *Proteus mirabilis* which obtained from the urine specimens 33 (78.6)% which is a higher percentage compare d with wounds samples. In the study of *Proteus mirabilis* were a percentage of 56.9% that isolated from urine and wounded (8).

The reason of the difference in isolate percentages may be due to the differences in size and number of hospitals surveyed as well as The season of collecting samples and medication taken before sampling. The bacteria has numerous virulence factors that are important for causing UTI and several of these factors. Appear to be more important for establish infection in different areas of the urinary Tract. These virulence factors include adherence capability, unease production and flagella (9).

Molecular test

The technique Polymerase Chain Reaction was used in investigation some of the genes responsible for the virulence factored in *P.mirabilis* through the use of pieces of the DNA with limited number of nucleotides (oligonucleotide) which act a primers specialized for virulence genes in *P.mirabilis*, and it include *ureC*, *aac(6)-lb* and *zapA*. *ureC* gene which is responsible for the production urease enzyme which is regarded as a diagnostic feature of the bacteria *of P.mirabilis* as shown table (4). Yet it is considered in the present study as virulence factor which had been diagnosed using Polymerase Chain Reaction in addition to *aac(6)-lb* and *zapA* The results of the current study show that 28 isolates out of 45 isolates in a rate 62.2% As shown in Figure 3. The study also shown that all these isolates were productive for *urec* gene, also it is responsible for producing urease enzyme. Urease enzyme produced from *P.mirabilis* 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 magnesium 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 compatible with (11) 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.mirabilis* . In this study, *zapA* has been investigated by using PCR technique, and the results shown that 8 isolate in a rate of 44.5% had *zapA* gene as shown in table (4). And this result is similar to (12) for they mentioned that the rate of this gene in *P.mirabilis* isolates.

Conclusion

The correlation between of the Vitak test and biochemical tests with molecular test.

Recommendations: To detect genotypes that lead to aminoglocides resistance.

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