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Genotypic and Phenotypic Diagnosis of *Pseudomonas aeruginosa* Associated with Urinary Tract Infection in Diabetic Patients and Their Resistance to Certain Antibiotics

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Abstart:

In this study, 125 cases of urinary tract infection were collected from patients of both sexes at Al Hussein Educational Hospital and Diabetes and Endocrinology Specialist Center in Dhi Qar Governorate for the period from October 2017 to March 2018. After isolation and diagnosis, 105 isolates were obtained and the number of *P. aeruginosa* was 75 (71.42%). The isolates were tested to 5 types of antibiotics and 13 isolates for 16S rRNA detection tests. The isolates showed high resistance to antibiotics, Gentamycin, Tetracycline and Vanomycin, where the resistance was 100% while the resistance was low for Tobramycin 33.3% and was very sensitive to the antibiotic Imipenem, where the sensitivity was (86.6 %). The results of 16S rRNA polymerase chain reaction products, was show diagnostic gene within the expected size of the 1500 bp, some isolates were recorded as new strains in the NCBI database.

Key words: Urinary tract infection, Diabetes, sensitivity test, 16S rRNA.

1. Introduction

Diabetes is a common disease worldwide, diabetes has increased significantly over the last 50 years in parallel with obesity as of 2010 there are approximately 285 million people suffering from this disease compared to about 30 million in 1985 (Pasquel and Umpierrez, 2014). Diabetes is a chronic disease, a hyperglycemia that results from an imbalance in insulin production (Zia, *et al*., 2012; Shaikh, *et al*., 2012). This affects the metabolism of proteins, carbohydrates and fats. To functional changes in the cells of the body causing the emergence of complications of diabetes affecting the kidneys, eyes and nervous system and may lead to these deaths sometimes (Frier, *et al*., 1999).

Diabetes is classified into two types: Diabete Insulin-Dependent and Diabete non Insulin-Dependent Diabetes. Insulin-dependent diabetes is also called Type1 diabetes, which is called juvenile diabetes, which occurs as a result of severe deficiency or a total absence of insulin secretion from the pancreas gland is more common in children and young people under the age of 20 (Paltalk, 2002). The second type is known as insulin-free diabetes. It is called adult diabetes. It occurs when the pancreas secretes an insufficient amount of insulin or when the cells begin to resist insulin, which is more prevalent and affects people over

the age of 20 (Nabipour, 2003). Patients with diabetes are more likely to develop UTIs (Shah and Hux, 2003) due to various immune system disorders (Delamaire, *et al*., 1997; Valerius, *et al*., 1982)

Urinary tract infection (UTI) is a condition that results from the injury of one or more organs of the urinary system when one of the microorganisms can pass the strong natural defense line. Despite the strength of this line of defense, urinary tract infection is common and can occur at any stage. Approximately 95% of cases are caused by bacteria entering the urethra and moving to the bladder. In a few cases, bacteria can enter the kidney through blood (Cynthia, 2001). Natural urine is sterile and contains salts, liquids and wastes products. However, the infection usually occurs when bacteria enter the open area of the urethra. The infection is called inflammation of the urethra, after which the bacteria move to the bladder causing inflammation of the bladder and if not treated these injuries, the germs may reach the ureters in preparation for the events of the kidney disease, which is then called kidney inflammation and its pelvis (Pylonephritis) (Jureen, *et al*., 2003).

The incidence of infection in the urinary tract is one of the health problems affecting many members of the human community estimated at millions annually. Studies indicate that the rate of infection of females in these infections is higher than the rate of male injury due to the length of the urethra in males, which reduces the chances of colonization of the bladder by the bacteria (Boyko, *et al*, ., 2005).

There are a number of reasons for the obstruction of the urinary tract and cause the causes of obstruction to the formation of stones in the kidneys, or because of prostate enlargement or the presence of tumors in the urinary system and sometimes because of loss of nerve control of the bladder, where this impedes the natural flow of urine or the presence of congenital defects in Urinary tract is the congenital defect between the bladder and ureter or the so-called Vesicoureteral reflux due to the return of urine to the ureter and then to the kidneys in advanced cases, leading to frequent urinary infections, and thus renal failure and sexual contact through a person infected with bacteria causing urinary tract infection. The decline in immunity due to age (elderly and children) and the incidence of diabetes, which makes patients susceptible to urinary tract infections also the pressure of the uterus on the ureter during pregnancy, may lead to stagnation of urine, especially bacterial kidney inflammation, a so-called Pyelonephirtis and pathogens from the most common urinary tract infection because they possess special characteristics that can cause the infection, including its susceptibility to conjugation and its ability to adhesion and colonization of host tissues and the exchange of genes expressing multiple resistance of antibacterials (Jackson, 2015; Mims *et al*. , 2004; Orenstein and Wong , 1999). *Pseudomonas aeuroginosa* is widespread in nature due to its human, animal and plant diseases and has the ability to live in diverse, free-living environments living in soils, marshes, river waters, coastal and marine areas (Todor, 2004), an important cause of urinary tract infection (Akanji, *et al*., 2011) is not fermented for most sugars, is an opportunistic Pathogens, which is characterized by its ability to cause numerous injuries in various sites in the body, including urinary tract infections, eye infections, ear infections, skin infections, and central nervous system infections. (Nelson and Reginald, 2007). *P. aeruginosa* has many virulence factors that enable it to settle in the host's body causing disease and resistance to defensive means Cellular, of these factors biofilms, capillaries (Brook, *et al*., 2010).

Aim of the study

Molecular diagnosis of *P. aeruginosa* isolated from urinary tract infection in diabetics and tested for certain antibiotics.

2. Materials and Methods:

a) Samples Collection

A total of 125 Urine samples were collected from patints of both sexes for the period from October 2017 to March 2018. Samples were taken from patients who visited the Al Hussein Educational Hospital and the Diabetes and Endocrinology Specialist Center. Patints history was recorded in a special form (gender, age). The samples were collected from midstream urine, In special sterile tubes with a tight lid and a size of 10 ml and transferred to the Microbiology Laboratory at the College of Education for Pure Sciences to be planted in development media for subsequent experiments.

b) Samples Culture

Initial Culture was carried out on nutrient agar, blood agar and MacConkey agar where the samples were planted in loop and incubated at 37 ° C for 24 hours (Forbes *et al*., 2002).

c) Identification of Bacterial Isolates

Bacterial isolates were identified based on their microscopic characteristics and biochemical tests. The bacterial isolates developed in the blood agar MacConkey agar, Nutrient agar media were initially identified based on their phenotypic characteristics in terms of colony size, color and strength as well as their ability to analyze red blood cells on blood agar. Bacterial isolates were subjected to microscopic examination. Several swabs were prepared to each bacterial Culture and stainingGram stain and examined under the oily lens to observe the cell's response to the dye as well as its shape and arrangement (Forbes et al., 2002).

d) Antibiotics Sensitivity Test

The method of the disks was used on 15 isolates of *P. aeruginosa*, a method used in hospitals, using a combination of antibiotics, Tetracycline, Imipenem, Tobramycin, Gentamycin and Vanomycin,. The comparison was made if the bacteria were sensitive or resistant according to international standards in (CLSI, 2012).

e) DNA Extraction

The total DNA of the bacterial isolates was extracted for use in molecular diagnosis using a DNA extraction kit produced by Promega (USA). The electrophoresis was performed on gel agarose with 1% concentration of DNA extracted by 100v and 60 minutes after dyeing with 5 mg/ml of ethidium bromide and gel examination by UV-Transilluminator and direct images.

f) Polymerase chain reaction

PCR technique was used to amplify the 16R rRNA encoding of study isolates using the primers shown in Table (1).

Table (1) Sequences and concentrations of the primers and the expected size of the gene (Lane, 1991)

Primer	Nucleotide sequence	Pb	Product size Pmol/µl	Size Gene
F1492	5- GGT TAC CTT GTT ACG ACT T- 3	19	100	1500
R27	5-AGA GTT TGA TCC TGG CTC AG- 3	20	100	1500

Table (2) Components of polymerization reaction mixture and sizes. Follow the program shown in Table (2) to amplify PCR DNA samples.

Table (2) PCR work program

Stage	Steps	Temperature (°C)	Time (Minute)	Number of courses
First	Initial denaturation	94	3	1
the second	Denaturation 2	94	30	32
	Annealing	56	30	
	Extention 1	72	1.5	
Third	Extention 2	72	10	1

Electrophoresis replication products of the samples were detected on the agarose gel and the DNA ladder was placed in the first well of the gel for the purpose of locating the 16S rRNA gene in the migrated DNA. After the migration process, the gel was examined by exposing it to ultraviolet light to see the molecular sizes of the multiplying pieces and then photographed directly.

3. Results

A total of 125 samples were collected from people with diabetes, of which 92 samples showed a growth of 73.6%. After morphological and biochemical tests and diagnosis using vitek2, 105 isolates were obtained and the number of isolates was *P. aeroginosa* 75 isolation by (71.42%).

3-1 Molecular diagnostics using PCR technique

❖ **Detection of 16S rRNA gene**

The results of the 16 S rRNA gene amplification showed that the *P. aeruginosa* gave a positive result of the gene and that the size of the gene was 1500bp and Figure 1 showed the 60-minute electrophoresis (100V) For PCR products.

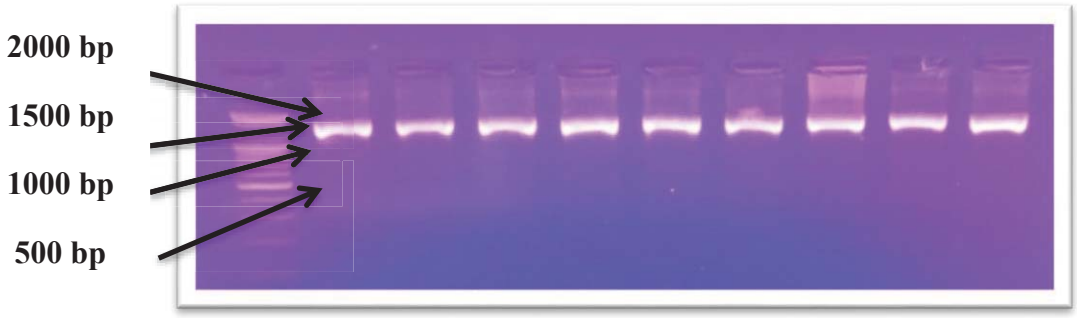

Figure (1) Electrophoresis for 16S rRNA PCR products on 1% agarose gel for *P. aeruginosa*

❖ **DNA Sequencing**

DNA sequencing results for bacterial isolates showed that 16S rRNA sequence has the similarity between the isolates studied with other isolates recorded in the gene bank. They were compared with

the data available in the gene bank based on the highest E-value ratio. With the other breeds 96% and 99% of isolates selected in this study. The present study showed that 13 isolates gave a positive result for sequencing DNA of 16S rRNA gene. Five isolates were registered in the gene bank as new strains as shown in Table (3).

Table (3): Proportion of identical bacterial species isolated with bacterial species in the gene bank

Strains	Accession numbers
***P.aeruginosa* strain Ha11**	**MH470328**
***P.aeruginosa* strain Ha12**	**MH470329**
***P.aeruginosa* strain Ha13**	**MH470333**
***P. aeruginosa* strain HA15**	**MH489000**
***P.aeruginosa* strainHa16**	**MH489002**

0/136(0%)

3-2 Antibiotics Sensitivity Test

The current study showed a significant increase in the resistance ratios of *P.aeruginosa* isolates to antibiotics Gentamycin, Tetracycline and Vanomycin, reached (100%). The current study showed that the resistance of *P.aeruginosa* isolates to the antibiotic Tobramycin was 33.3%. It was very sensitive to the antibiotic Imipenem, with a sensitivity rate of 86.6%, as shown in Table (4).

Table (4) The percentage of certain isolates of *P.aeruginosa* and antimicrobial resistance.

Antibiotics	Resistance isolates		Sensitive isolates	
	No.	%	No.	%
Tetracycline	15	100	-	-
Gentamycin	15	100	-	-
Vanomycin	15	100	-	-
Tobramycin	5	33.3	10	66.6
Imipenem	2	13.3	13	86.6

4. Discussion

The results of the current study showed that positive growth rate of samples of people with diabetes was (73.6%), which is a study of the study conducted by Atiya and colleagues (2016) where the growth rate of bacterial study of the types of diabetes the first and the second 78.5% and 75, respectively. Studies showed that people with diabetes had more urinary tract infections than those without diabetes (Zamanzad and Moezzi, 2006). This is because people with diabetes are more likely to have urinary tract infections because of weak immunity. There are fewer white cells and T cells that fight bacteria and viruses in the body, as well as impaired metabolic control of diabetes and incomplete bladder secretion due to autonomic neuropathy (Truzzi, *et al.*, 2008) which leads to increased bacterial infections, and this is also consistent with the results of Chang *et al* (2003) and Goswam *et al.* (2001). On the other hand the results of the

Maharjan *et al*. (2015) study were the lowest of the current study, with positive growth of 10.37% in diabetic patients. This difference may be explained by the distribution of pathogens on the basis of different areas of study or the reason for the number or size of samples.

They were ratios of *P. aeroginosa* was high perhaps because they have a role in the infection of the urinary tract Bonadio *et al*., (2006). *P.aeroginosa* is a common pathogen in the infection of urinary tracts (Sleigh and Timbury, 1998). The dominance of these bacteria indicates their role in urinary tract infection to the adhesion factors of these bacteria and their resistance to many antibacterial agents (Brook, *et al*., 2007: Goering, *et al*., 2008).

P. aeruginosa bacteria are the most frequent among gram-negative bacteria in patients with UTIs in the United States (Gaynes and Edwards, 2005). And bacteria *P. aeruginosa* is the most common type in urinary tract infections (Ehinmidu, 2003). Also, the presence of virulence factors helps in the incidence of urinary tract infection when conditions are available, especially in the case of weakened immunity (Typas, 2007). The results of the current study differ in terms of the dominance of *P. aeruginosa* bacteria in it with the results of Raouf and Tawfiq (2015) in Tikrit, where the proportion in their study 30%. Bacterial isolates were identified by phenotypic, biochemical and Vitek 2 tests. The results of tests on bacterial isolates as reported in the working methods were consistent with those of the approved diagnostic systems (Koneman, *et al*., 1997; Colle *et al*., 1996) . The molecular study included DNA extraction of the bacterial species selected by electrophorses using the KIT method. DNA extracted from isolates was used to induce 16SrRNA gene. The isolates of *P. aeruginosa* were selected as a positive result of 16S rRNA. This result was agreed with researchers who indicated that 500bp DNA sequencing for the 16SrRNA gene is sufficient to diagnose both bacterial species separately Claesson *et al*., (2009) Nazia *et al*., 2012 and Vimal, *et al*. (2016). All new strains were recorded in the NCBI database.

Bacteria resistance varied for many antibiotics. In the current study, *P.aeruginosa* isolates showed a marked increase in antibiotic resistance. The resistance of *P.aeruginosa* was high for a number of antibiotics. The resistance was 100% Gentamycin. This study is consistent with Naqvi, *et al* (2005) with a high resistance to Gentamycin (95%). The results of the present study also agree with what happened Lyamuya, *et al* (2011) where the percentage of resistance to *P.aeruginosa* 100% antibiotic followed by the resistance of *P.aeruginosa* to the antibiotic Tobramycin (33.3%). The approach was similar to that of the Adeli and Ramahi study (48%). This result is not consistent with Varaiya *et al*. (2008) for the authors Tobramycin, Gentamycin. The study recorded a resistance rate of 10% each. It was 100% resistant to Tetracycline. The result was similar to that obtained by the Al Faham (2017). In the study, it was 87.5% and agreed with the study of ABD (2001). Where the percentage of isolates resistant to antibiotic Tetracycline was 100%. It was also sensitive to Imipenem (86.6%) which is similar to the results of Bonadio, *et al* (2006). The resistance of *P.aeruginosa* to Vanomycin was 100%.

Several studies have shown that bacteria that cause urinary tract infection are usually resistant to many antibiotics (Richard, *et al*., 1997). Perhaps the reason is that more bacterial species isolated from these infections possess special genes based on chromosome DNA where they encode the character of resistance to antibiotics, and has the ability to transfer resistance to other bacterial species through specific mechanisms such as bacterial conjugation (Dijkshoorn and Ursing, 2000 ; Stuart, 1997).

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