Phenotypic and Molecular Detection of Some Virulence Genes Among Clinical Isolates of Pseudomonas aeroginosa and P. fluorescence

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Abstract

The study was conducted for the period from September 2021 to March 2022, in Ghazi Hariri Hospital, Yarmouk, and the Medical City. The study included collecting 115 samples from inpatients and returning patients, males, and females, of different ages. The samples included (burns, wounds, and urine) to investigate the presence of Pseudomonas aeruginosa and Pseudomonas fluorescens and study its virulence factors phenotypically and at the molecular level, , as Pseudomonas aeruginosa (25) bacterial isolates were isolated from the total (115) isolates, with a percentage of (21.7%), which included (5) isolates from urine, (7) isolates from wounds, and (13) isolates from burns, which represented (20%), (28%), and (52%), respectively, from a total of 25 isolates. The results showed that 25 isolates in each of P. aeruginosa and P.flurosence gave positive isolates for the mexB gene, as the isolates of P. aeruginosa and P.flurosence bacteria isolated from the urine contained 25% and 20% of the MexB gene, respectively. , and by 25% and 60% of wounds, while from burns it was 50% and 20%, respectively. As for the PIcN gene, the study showed that the isolates of P. aeruginosa and P.flurosence bacteria isolated from the urine contained the PIcN gene by 18% and 16%. %, respectively, by 27% and 50% of wounds, while burns were 55% and 34%, respectively.

Also, the isolates of P. aeruginosa and P.flurosence bacteria isolated from the urine contained the LasB gene by 22% and 25%, respectively, and by 26% and 50%, respectively, from wounds, while the percentage of burn isolates reached 52% and 25%. As for the isolates of P. aeruginosa, P.flurosence isolated from the urine contained 20% and 19% of the AprA gene, respectively, and by 25% and 50%, respectively, from wounds, while the percentage of burn isolates reached 55% and 34%. Sequence of total bacterial isolates P. aeruginosa, P.flurosence.

Keyword: *Pseudomona saeruginosa, Pseudomonas fluorescens, mexB, PIcN, Las B. AprA gene.*

INTRODUCTION

The genus Pseudomonas includes many species of bacteria everywhere, and to date, it includes 272 species isolated from many different environments, such as soil, water, air, and sediment, as well as from many species of hosts, such as animals, plants, fungi, and algae (1). Pseudomonas aeruginosa is one of the types of Pseudomonas.spp. It is an opportunistic pathogen that possesses a group

of virulence factors that make it a subject of interest and study for researchers because of the damage it causes to human health and is responsible for some of the injuries that affect it, as it causes many acute and chronic infections such as burns, wounds, and ear infections. External, cornea, and urinary tract, and are also considered pathogens that cause acquired infections, especially in people who suffer from immunodeficiency. Pseudomonas aeruginosa is one of the most widespread types of bacteria and its ability to cause infections among patients with wounds and burns is assisted in this by the multiple virulence factors that it possesses (1), including the formation of biofilms, the production of toxins that cause extensive damage to tissues and then enter the bloodstream and spread to the tissues of the body, as well Because it produced virulence factors, it has high resistance to many antibiotics because it has many means of self-resistance, and it can also develop new resistance when exposed to antibacterial materials, which leads to the emergence of strains characterized by many characteristics, including multi-resistance to antibiotics. The increase in the emergence of multiple antibiotic-resistant bacteria has led many researchers to try to develop new effective antibacterial materials that have a low cost and do not cause resistance. As for the bacteria Pseudomonas fluorescens, it is a non-pathogenic bacterium found in soil and plants (2), and it is a chemo-organotroph for nutrition and respiration, and this bacterium is considered one of the most important factors of biological resistance (3), these bacteria are characterized by being adapted to survive in the soil or settle inside plants, and have a positive effect on both the pathosystem and growth components such as increasing plant height, wet and dry weight, and production, and are characterized by being biofilm producers (4) Enzymes produced by bacteria such as Alkaline Protease and Elastase, where Alkaline Protease unites with Elastase to break down Collagen and Elastin. The enzyme elastase is encoded by the Las B gene, as the Las B enzyme elastase is highly efficient in analyzing proteins and penetrating the cell wall of the host (5). They have a role in analyzing many host proteins, which helps in invasion and invasion. The expression of these genes is associated with species pathogenicity, especially in skin burn patients (6). The LasB protease is encoded by the last gene. P. aeruginosa also has a system of efflux pumps, which are complex protein pumps that form a channel that allows materials to be pumped directly from the cytoplasm of the bacterial cell to the extracellular environment, as these pumps increase the resistance of the germ to antibiotics significantly because the effectiveness of the flow can easily exceed the flow of antibiotics One of the largest multidrug resistance effluxes pumps with a high level of expression is the MexB-OprM efflux pump, which is controlled by the regulatory genes mix and mix (7). The non-glycolytic enzyme (PLcN) Phospholipase C is encoded by the PLcN gene. P. aeruginosa produces two types of hemolysin: the first is called phospholipase C (PLC), which is thermally unstable and has anti-emulsifying activity, and the other is called Rhamnolipid hemolysin, which is thermally stable. Both enzymes may work together to break down lipids and lecithin, and they may also be responsible for tissue invasion via their cytotoxic effect (8).

Aim of study

1. Isolation and identification of P.aeruginosa and P.fluorescen from various clinical sources such as wounds, burns, and urinary tract infections, and studying their sensitivity to antibiotics.

2. Detection of the lasB, MexB, AprA, and PIcN genes in P. aeruginosa and P.fluorescen using polymerase chain reaction (PCR).

Materials and method

1. Collection of bacteria: 115 samples were collected from patients attending and inpatients in Ghazi Hariri, Yarmouk, and Medical City hospitals from the first of September 2021 until the end of March of the year 2020, and the collected samples included (burns, wounds, and urination) and by a sample of both sexes and of different ages, where sterile cotton swabs were used Swabs were used to take burn and wound samples. As for sputum and urine, sterile plastic containers were used. (25) isolated each of Pseudomonas aeruginosa, (10) isolates from P. fluorescens.

2. Identification of bacteria:

Identification of bacteria: identification of the isolates by culturing on media culture including MacConkey agar, Blood agar, Cetrimide agar, Pseudomonas agar, and CHROMagar Orientation then identified by using biochemical tests including (oxidase and catalase test) and further identification by using an API20E system. The diagnosis was confirmed using the Vitek 2 compact system.

Table (1) Preliminary diagnostic test results for Pseudomonas aeruginosa P. fluorescents

P. fluorescents \ 10	*P. aeruginosa* 25	Properties
10	25	Fluorescin
10	25	Oxidase
0	25	Nitrate reduction
10	25	Gelatin liquefaction
10	25	Growth on cetrimide
10	0	Growth on 4C.
0	25	Growth on 42C

3. DNA isolation: A genomic DNA extraction and purification kit prepared by Promega (USA) was used to isolate genomic DNA from diagnosed bacterial isolates.

4. Detection of molecular gene: The method was used to measure the concentration and quantify the purity of DNA using a Nanodrop device, as follows: The sample to be determined for genomic DNA was diluted 100 times using EDTA(TE) Tris dissolving solution. The optical density was measured at a wavelength of 260 nm using a UV-visible spectrophotometer and using a buffer (TE) as a control solution on the basis that a reading at a wavelength of 260 nm, which is equal to 1, is equivalent to a concentration of double-stranded DNA of 50 ng/mL. The best concentration of DNA is between (150-200) ng/ml, according to the following equation: 0.D 260 nm x Dilution factor x 50 ug/ml 100. Measurement of DNA purity:

The purity of the DNA was measured by dividing the optical density measured at the wavelength of 260 nm by the optical density measured at the wavelength of 280 nm, as the best purity of the DNA is between (1.8-2).

Results and discussion

1- Investigation of some virulence factors of Pseudomonas spp.

As in most types of pathogenic bacteria, Pseudomonas bacteria can produce many virulence factors that play an important role in the pathogenicity of these bacteria (23). The production of some virulence factors was detected in the strains of P. fluorescens and P.aeruginosa, under study, namely Biofilm Production, Haemolysin, and Lecithenase, as well as Protease production was investigated, Shows the spread of virulence factors according to the sources of isolation of these bacteria. Biofilm Production is the production of Pseudomonas bacteria of Biofilim mucous living membranes, which is one of the most important virulence factors possessed by

bacterial strains that cause serious infections due to its lack of control due to its adhesion to many surgical tools. These results are consistent with many studies. Manandhar, (2018) mentioned that most of the 41 isolates studied by them were biofilm producers, and there was no difference in biofilm production between the pathogenic isolates that the percentage of enzyme production was 60.5% and 69%, respectively (24).

It is clear from the current results that most of the isolates produced live mucous membranes, because the enzyme is an important virulence factor for bacteria that helps them invade, spread, damage, and resist antibiotics This current study agrees with the study by (25). Where Congo red agar and smooth surface adhesion method were used as two simple methods to examine the biofilms of P. aeruginosa isolates.

Molecular Detection

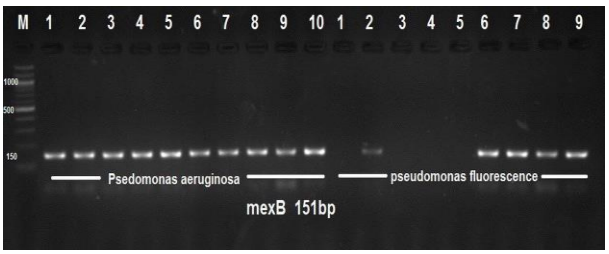
1- Detection of the Mex B gene

The results showed that 25 isolates in each of (P. aeruginosa, and P.flurosence) gave positive isolates see Figure (1). The results of electrophoresis showed the presence of genetic packages with a molecular size of 151 bp compared to the size index of 100 bp in 20 isolates of P. aeruginosa, and 5 isolates of P. fluorescence, as shown in the Figure (1). The results are recorded in Table (2), including the distribution of the Mex B gene in the isolates under study according to the isolate sources. The results showed that the isolates of P. aeruginosa, P. fluorescence isolated from the urine contained the Mex B gene by 25% and 20%, respectively, and P. aeruginosa and P.flurosence isolated from wounds contained the Mex B gene by 25% and 60%, respectively, while the percentage of P. aeruginosa and P.flurosence isolated from wounds was Burns accounted for 50% and 20%, respectively, of the total bacterial isolates, P. aeruginosa and P.flurosence.

Table (2) Distribution of Mex B gene in the isolates under study according to to isolate sources

Total		Gene distribution ***Mex B***				source of isolates
		عدد العزلات الكلي **(10)** *P.flurosence*		عدد العزلات الكلي *P. aeruginosa* **(25)**		
%	**No.**	**%**	**No.**	**%**	**No.**	
24	6	20	1	25	5	**urine**
32	8	60	3	25	5	**Wounds**
44	11	20	1	50	10	**burns**
100	25	100	5	100	20	**total**

Figure (1): Molecular detection of Mex B gene in P. aeruginosa, P.flurosenc using PCR technology



In 2006, researchers Hearn and his group indicated that the MexB gene in P. fluorescence infection (MexB efflux pumps) showed importance in periplasmic domains in determining the substrate specificity of the specific substrate. The MexB flow pump has been demonstrated to discriminate and transfer substrate.

Middlemiss and Poole (2004) found several mutations within the MexB periplasmic and

transmembrane domains that altered substrate selectivity, likely by affecting the tertiary structure or protein-protein interactions within the efflux complex. There is no route through which the substrate enters the central cavity, nor is there a mechanism of substrate selectivity (9). Middlemiss and Poole also identified a mutation in the outer membrane pump-membrane MexB docking protein in P.flurosence that decreased the efflux activity of all antibiotics tested. This mutation in MexB was reported by Middlemiss and Poole (10).

1- Detection of the PIcN gene

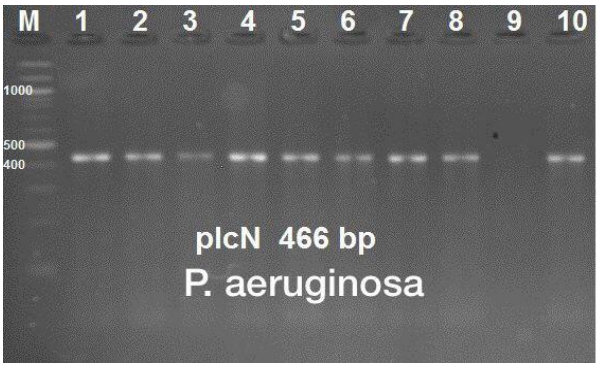
The results showed that 28 isolates in each of (P. aeruginosa, P.flurosence) gave positive isolates Figure (2). The results of electrophoresis showed the presence of genetic packages with a molecular size of 466 bp compared to the size index of 100 bp in 22 isolates of P. aeruginosa, and 6 isolates of P. fluorescence, as shown in the figure (2). The results are recorded in Table (3), which includes the distribution of the PIcN gene in the isolates under study according to the isolate sources. The results showed that the isolates of P. aeruginosa, P. fluorescence isolated from the urine contained the PIcN gene by 18% and 16%, respectively, and P. aeruginosa and P.flurosence isolated from wounds contained the PIcN gene by 27% and 50%, respectively, while the percentage of burn isolates was 55%. And 34%, respectively, of the total bacterial isolates, P. aeruginosa and P.flurosence.

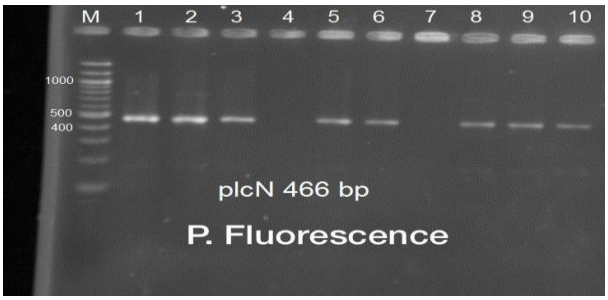
Table (3) Distribution of PIcN gene in the isolates under study according to to isolate sources

Total		Gene distribution ***PIcN***				source of isolates
		عدد العزلات الكلي **(10)** *P.flurosence*		عدد العزلات الكلي *P. aeruginosa* **(25)**		
%	**No.**	**%**	**No.**	**%**	**No.**	
18	5	16	1	18	4	**urine**
32	9	50	3	27	6	**Wounds**
50	14	34	2	55	12	**burns**
100	28	100	6	100	22	**total**

The results of the study almost agreed with the results of Endimiani and others in 2007, who found that (64%) of their isolates isolated from wounds and urine possessed this gene and it was prevalent in the isolates of urine and burns (11), and somewhat with the results of Tanya and others in 2009, who found that (72%) of Their isolates possessed this gene and it was predominant in urine (12), and the results of the current study agreed with the study of Heirmann and others in 2012, , who recorded percentages (88%,) of their isolates isolated from different infection sites of the body (13), there are genes encoding Pseudomonas aeruginosa PLcN, all of which have a role in the degradation of phosphatidylcholine, and the increase in the proportions of these three genes leads to bacteremia, especially if it is present with the protease (14).

Figure (2): Molecular detection of PIcN gene in P. aeruginosa, P.flurosenc using PCR technology





2- Detection of the LasB gene

The results showed that 31 isolates in each of P. aeruginosa), and P.flurosence) gave positive isolates see Figure (3). The results of electrophoresis showed the presence of genetic packages with a molecular size of 300 bp compared to the size index of 100 bp in 23 isolates of P. aeruginosa from a total of 25, 8 isolates of P.flurosence from a total of 10. The results are recorded in Table (4), which includes the distribution of the last gene in the isolates under study according to the isolate sources. The results of the study showed that the isolates of P. aeruginosa, and P.flurosence isolated from urine, it contained the LasB gene by 22% and 25%, respectively, and P. aeruginosa and P.flurosence isolated from wounds contained the LasB gene by 26% and 50%, respectively, while the percentage of burn isolates was 52% and 25%. %, respectively, of the total isolates of P. aeruginosa and P.flurosence.

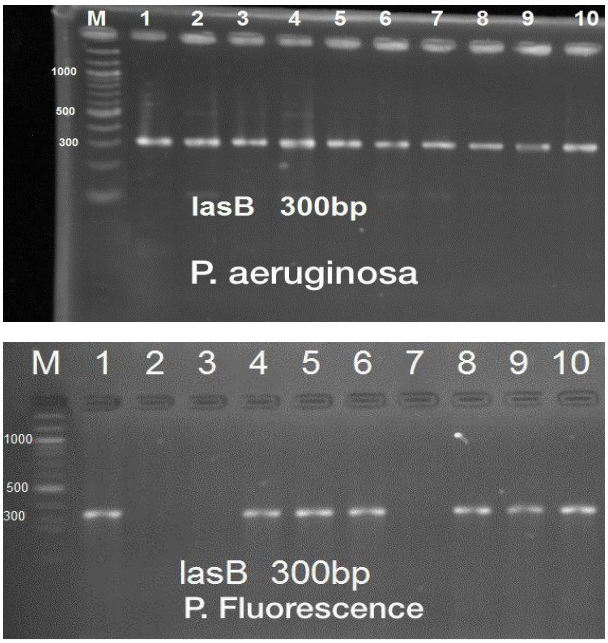
Table (4) Distribution of the LasB gene in the isolates under study according to to isolate sources

Total		gene distribution ***LasB***				source of isolates
		عدد العزلات الكلي **(10)** *P.flurosence*		عدد العزلات الكلي **(25)** *P. aeruginosa*		
%	**No.**	**%**	**No.**	**%**	**No.**	
23	7	25	2	22	5	**urine**
32	10	50	4	26	6	**Wounds**
45	14	25	2	25	12	**burns**
100	31	100	8	100	23	**total**

The current study agreed with that of Bradbury et al. (2010) who reported the presence of this gene in 86% of their isolates and indicated that the protease is important in burn and wound infections (15). As for the study Smith et al. (2006) that they conducted in Australia (30%) of burns and wounds have this gene (16). As for the results of Wolska & Szweda in 2008, they recorded the presence of this gene at a rate of (100%) in their studied isolates (17), while Ghassan et al. in (2005) found the percentage of this gene in (41%) in burn (18). While the isolates of Nikbin et al. (2010), Lama et al. (2012) and Mitov et al. (2010) gave 100% of the last gene. In the current study, we note the predominance of the gene in isolates of wounds and burns (19,20,21).

The results of Al-Shwaikh and Al-Arnawtee showed in 2019 at the University of Baghdad, where the researchers recorded infection with P. aeruginosa bacteria isolated from the urine sample, where the percentage of the LasB gene was 62%, while in wounds and burns it reached 82% and 100%, respectively (5).

Figure (3): Molecular detection of LasB gene in P. aeruginosa, P.flurosenc using PCR technology



The results showed that 26 isolates in each of (P. aeruginosa, and P.flurosence) gave positive isolates in Figure (4). The results of electrophoresis showed the presence of genetic packages with a molecular size of 1017 bp compared with the size index of 100 bp in 20 isolates of P. aeruginosa, 6 isolates of P.flurosence, as shown in the figure (4). The results are recorded in Table (5), which includes the distribution of the AprA gene in the isolates under study according to the isolate sources. The results of the study showed that the isolates of P. aeruginosa, and P.flurosence isolated from The urine contained 20% and 19% AprA gene, respectively, and P. aeruginosa and P.flurosence isolated from wounds contained 25% and 50% AprA gene, respectively, while the percentage of burn isolates reached 55% and 34%. Sequence of total bacterial isolates P. aeruginosa, P.flurosence.

3- Detection of the AprA gene

Table (5) Distribution of the AprA gene in the isolates under study according to to isolate sources

Total		Gene distribution *AprA*				source of isolates
		عدد العزلات الكلي (10) *P.flurosence*		عدد العزلات الكلي *P. aeruginosa* (25)		
%	No.	%	No.	%	No.	
19	5	16	1	20	4	**urine**
32	8	50	3	25	5	**Wounds**
50	13	34	2	55	11	**burns**
100	26	100	6	100	20	**total**

Sabharwal and authors in 2014 reported on their study, in which clinical strains were examined for the prevalence of different virulence factor genes in P. fluorescens and P. aeruginosa. The AprA gene had the lowest incidence with 16.6% of different clinical samples (22).

The large genome and genetic complexity allow P. aeruginosa and P. fluorescens to thrive in diverse environmental conditions. Multiple bacterial virulence factors influence the pathogenesis of P. aeruginosa infection. The combination of virulence factors expressed by each P. aeruginosa strain tends to determine the outcome of an infectious process. However, in clinical situations, it is often difficult to distinguish between simple colonization and infection, and there is no diagnostic tool available to assess the virulence potential of a given isolate (22).

Conclusions:

The DNA chain replication reactions were carried out using specific primers targeting the specific sequence of (Mex B, PIcN, AprA, and LasB) genes. To investigate the possession of

P. aeruginosa bacteria, P.flurosence), of the important virulence genes that they possess.

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