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Detecting some virulence genes in *Pseudomonas aeruginosa* isolated from different clinical sources

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ABSTRACT

P. aeruginosa is an opportunistic pathogen capable of infecting almost all tissues of the body due to its possession of a wide variety of virulence factors that contribute significantly to pathogenicity in the host. In the current study, (162) samples are collected from different clinical sources, to detect the spread of *P. aeruginosa* bacteria in these sources and to study some virulence genes possessed by this bacteria. The developing isolates are diagnosed after cultivation on Blood agar and MacConkey agar according to phenotypic, microscopic and culture properties, and the diagnosis is confirmed using API 20E kit. The results of the diagnosis have shown that 33 isolates belong to the type *P.aeruginosa* from the total clinical samples, distributed by (11 isolates for burn injuries, 12 isolates for wounds and 10 isolate samples for urinary tract infections). The sensitivity of the bacterial isolates to 15 antibiotics is tested, and the results have shown that all isolates are resistant to Ceftriaxone, Colistin, and Amoxicillin by 100%, to Ceftazidime and Nalidixic by 96% and 90% respectively, to Gentamicin by 60%, to Tobramycin and Cefepime by 40%, to Pipracillin, Aztreonam, and Meropenem by 33.3%, to Ciprofloxacin, Levofloxacin and Imipenem by 23.3%, and to Norfloxacin by 16.6%. The prevalence of genes encoding the production of the basal protease enzyme aprA and elastase enzyme lasB, is detected using the (PCR) technique. The results reveal the presence of the aprA gene and the lasB gene with a rate of (100), within the genotype of the *P. aeruginosa* isolates under study. Due to their high presence in most of the sources of the studied isolates, these genes can be considered a successful and rapid alternative for diagnosing *P. aeruginosa* by molecular methods based on the polymerase chain reaction (PCR) technique.

Keywords: *P.aeruginosa*, virulence, aprA gene, lasB gene

Introduction

Pseudomonas aeruginosa is an opportunistic pathogen as it is a gram-negative bacteria that do not ferment the sugar lactose. It has a great ability to adapt in different and complex environments, where it is commonly found as parasites on the mucous membranes of human and animal tissues and in large environmental areas such as water, soil and plants. It is at the forefront of the main pollution factors for medical devices and tools, causing what is known as acquired infections [1]. *P. aeruginosa* is also characterized by its versatility, as it can use different types of raw materials for nutrients, because it has multiple and diverse enzymes that enable it to carry out different metabolic processes in different organs of the body. The clear diversity in metabolism is an important factor in giving it wide environmental success [2]. Virulence factors express the degree of pathogenicity caused by bacteria to the cells of the host body, while virulence factors represent the ability of the pathogen to diversify in causing different infections to the host cells, through molecules derived from bacteria that allow entry into the host cells and lead to tissue damage and necrosis.

The special combination of these factors also enables *P. aeruginosa* to display high levels of virulence and high resistance to infection control factors in chronic and acute cases [3]. Its great ability to grow in human cells, invade tissues and produce toxins is due to its effective ability to express many diverse virulence factors [4]. These bacteria possess a large number of genes, maintain a variety of strategies to help it survive in different environments, and play a role in transport, metabolism, environmental sensitivity, and the flow of organic compounds. These genes are highly flexible in nature, allowing it to grow and reproduce in a variety of environments, having four chemo-attractive systems. Moreover, control of the antibiotic resistance gene at molecular levels, such as the use of treatment factors of appropriate concentration, can reduce resistance or release of pathogenic bacteria [5]. There are many motives that call for focusing research in the world on *P. auroginosa*, the most important of which is the seriousness of the disease and the lack of effective treatment, which necessitates the need to improve the efficiency of diagnosis and reduce time and effort.

Although modern technologies are still limited in third world countries, they have provided the best diagnostic tools, including the polymerase chain reaction technique. This technique is characterized by specificity and high speed during the investigation of bacteria in clinical samples and food samples, and obtaining molecular indicators resulting from the molecular investigation of the genes encoding virulence factors and their adoption in diagnostic studies. This is what traditional laboratory diagnostic methods cannot do, especially when dealing with large numbers of these Models [6]. The current study aims at:

- 1- Isolating and identifying *P. aeruginosa* bacteria from various infections (wounds, burns and urine).
- 2- Detecting the spread of genes encoding virulence factors *aprA*, *lasB* in selected isolates from different infection cases by using specialized primers using the DNA polymerase chain reaction (PCR) technique and the relationship of these genes to the site of infection.

Material and Methods of Work

Isolation and identification of *P.aeruginosa*

A number of (162) samples are collected from patients suffering from different pathological conditions, which include wound infections, burns, and urinary tract infection. The samples are obtained from Tikrit Teaching Hospital, Salah Al-Deen General Hospital, during the specified time period from 13/10/2021 to 22/03/2022. These samples are cultured on a blood agar and a MacConkey agar and the dishes are incubated at 37°C for 24 hours. The diagnosis is conducted based on the culturological and microscopic properties and biochemical tests of the bacterial isolates. For the purpose of studying the cultural properties of bacterial isolates, a test of their ability to cultur is started on a number of culture agars, which include MacConkey agar, blood agar, Pseudomonas agar which is the selective medium for *P.aeruginosa*, and Cetrimide agar. They are also cultured on a Nutrient agar, where the culturing properties are diagnosed based on the color and shape of the colonies. A microscopic examination of the developing bacterial cells is also conducted, and the purified colonies are subjected to biochemical tests for the diagnosis of bacterial isolates through the Catalase Test and the Oxidase Test [7]. Diagnosis is confirmed using the API-20E system for final confirmation of *P.aeruginosa* isolates and according to the manufacturer's instructions .

Table 1: Distribution of *P. aeruginosa* according to the source of the isolate

No	source of isolation	number of models	positive samples	Percentage %
1	burns	52	11	21.15
2	wounds	61	12	19.67
3	urine	55	10	18.18
total		168	33	

Genetic Content of Bacterial Isolates

Genomic DNA Extraction

Genomic DNA is extracted by a genomic DNA extraction and purification kit according to the

instructions of the manufacturer (Geneaid) for the bacterial isolates under study and according to the leaflet attached with the extraction kit. A number of (17) isolates are selected depending on their ability to express the phenotypic characteristics studied in the laboratory. These are from the different cases of infections, by 5 isolates from burn infections, 6 isolates from wound injuries, and 6 isolates from urinary tract infections, for the purpose of extracting genomic DNA by means of a genomic DNA extraction and purification kit according to what is stated in the manufacturer's instructions.

Characterization of DNA: The process of characterizing DNA includes the following:

Estimation of DNA Concentration and Purity

The DNA concentration and purity are measured, by adding 2 microliters of the extracted DNA to the Nano spectrophotometer lab instrument. The purity of the DNA is estimated by optical density (OD) (ng/microliter) at wavelengths of 280/ 260 nm. The concentration of the extracted samples under study varies between (278-70) ng/microliter, while the purity of the extracted samples varies between (1.5 - 2).

Electrophoresis of Genomic DNA

The method of preparing the gel is followed and as mentioned in [8] where 2.4 g of agarose is dissolved in 120 ml of buffer (SB) in a water bath and at a temperature of 100 ° C, left to cool to a temperature of 55 ° C, and then 4 µl of Red safe dye is added to the gel and then mixed well. An agarose backing plate (gel former) is prepared and its attachments are firmly fixed. After that, the amount of agarose is poured and the specialized comb is dipped to make wells at the end of the plate. It is left to solidify in a horizontal position, then the drill formation comb is lifted from the hardened agarose. The ends of the plastic plate and its attachments are also lifted , which are temporarily fixed until the agarose is hardened. The plate is then fixed on the supports prepared for it in the relay unit, the gel is covered with a level of 1-2 mm of SB buffer, and then 7 µl of DNA is mixed with 3 microliter of bromophenol blue dye solution (loading buffer) in the case of extracted DNA and without the loading dye. The red safe dye is used with the gel in order to show the bands of results. After that, the process of loading the mixture of DNA and dye is carried out in the gel pits, where it traveled electrically on a horizontal surface under a voltage difference of (80 volts) for a period of (1–2) hour .

Molecular detection of virulence factors genes (*aprA*, *LasB*) by the Polymerase Chain Reaction (PCR) Technique

A number of genes responsible for virulence factors within the hereditary genetic system of *P. aeruginosa* are detected by PCR technique for the bacterial isolates under study, in the presence of basic reaction materials (DNA and dNTPs primer template, buffer and custom primers) and under optimal conditions for the reaction. The time period for implementing the

interaction program has taken approximately (2) hours, after which the reaction products are placed under observation after being tested on agarose gel at a concentration of (2%) to detect the genes under study. Then, DNA replication reactions are performed using specific primers designated to target the

specific sequence of (*aprA*, *LasB*) genes (Table 2), in order to detect the important virulence genes possessed by *P. aeruginosa* isolates. Specific primers manufactured by (Macrogen) are used. The program is approved as described by [9].

Table 2: Nitrogen base sequences for primers used for qualitative endpoint

No.	Target gene	Sequence(5~ 3~)	Product Size(bp)	Primers
1	LasB	-GGAATGAACGAAGCGTTCTC- -GGTCCAGTAGTAGCGGTTGG-	300	lasB F lasB R
2	aprA	-TGTCCAGCAATTCTCTTGC- -CGTTTTCCACGGTGACC-	1017	aprA F aprA R

Results and Discussion

Detecting the *aprA* gene

The detection is carried out for this gene that is responsible for encoding the base protease enzyme. The results of the molecular amplification have shown the results of the polymerase chain reaction (PCR) for the *aprA* gene using primers designated for this gene on agarose gel packaged within the expected size of the gene 1017 (pb for 17/17 isolates with a percentage of (%) 100), as shown in Figure (1).



Fig. 1: Electrophoresis of the product of the polymerase chain reaction (PCR) for the DNA of *P. aeruginosa* isolates isolated from different sources of infection using a specialized initiator of the *AprA* gene on an agarose gel with a concentration of (2%) and a potential difference of 60 volts, for a period of (2 hours).

M path Size guide (100pb DNA ladder)

The pathways (1,2,3,4,5) are the amplification product of the *aprA* gene in the DNA of burns isolates (PsaB1, PsaB2, PsaB3, PsaB4 and PsaB5), respectively.

The pathways (6,7,8,9,10,11) amplify the *aprA* gene in the DNA of wounds isolates (PsaW6, PsaW7, PsaW8, PsaW9, PsaW10, PsaW11), respectively.

The pathways (12, 13, 14, 15, 16, 17) are the result of amplification of the *aprA* gene in the DNA of Urine isolates (PsaU12, PsaU13, PsaU14, PsaU15, PsaU16, and PsaU17), respectively.

Molecular studies indicate that the *aprA* gene is responsible for encoding the production of the special protease enzyme for *P. aeruginosa* bacteria. It has also found that the bacteria producing the protease enzyme are more virulent than the strains that do not produce this enzyme [10]. The molecular detection results in the current study show the possession of all *P. aeruginosa* isolates 17/17 (100%) of the *aprA* gene. The isolates are divided by 5/5 (100%) for burn infections, 6/6 (100%) for wound infections, and 6/6 (100%) for urinary tract infections. The results show

the equal prevalence of *aprA* gene in burns, wounds and urinary tract infections.

The results of the current study are in agreement with the findings of the study [11], which shows that the rate of its bacterial isolates of different sources is 96%.m. These results are in agreement with the studies [12] and [13] which show that all *P. aeruginosa* isolates are producers of the *aprA* gene. It differs from what is stated in the study [14] which has found that there is no *aprA* gene in the of *P. aeruginosa* bacteria isolates. Moreover, it states that the variation in gene expression is due to several reasons, including the participation of other enzymes of the basal protease enzyme in the process of proteolysis, as the abundance or lack of nutrients in the medium in which bacteria grow can be an important factor in the gene expression variation of these isolates [15].

Detecting the *las B* gene

This gene responsible for the production of the *elastase* enzyme is detected within the genotype of *P.aeruginosa* isolates obtained from different clinical locations. The results of the molecular amplification of the polymerase chain reaction (PCR) products of the *las B* gene, which are obtained after completing the agarose gel electrophoresis, are shown within the expected region of this gene (300bp). All isolates (100%) 17/17 possess this gene despite the different sources of isolates obtained as shown in Figure (2).



Fig. 2: Electrophoresis of the DNA polymerase chain reaction (PCR) product of *P. aeruginosa* isolates that are isolated from different sources of infection using a specialized initiator of the *LasB* gene on agarose gel at a concentration of (2%) and a potential difference of 60 volts, for a period of (2 hours).

M path Size guide (100pb DNA ladder)

The pathways (1,2,3,4,5) amplification product of *LasB* gene in the DNA of burns isolates (PsaB1, PsaB2, PsaB3, PsaB4 and PsaB5), respectively.

The pathways (6,7,8,9,10,11) amplification product of LasB gene in the DNA of wounds isolates (PsaW1, PsaW2, PsaW3, and PsaW4), respectively.

Pathways (12, 13, 14, 15, 16, 17) are the result of amplification of LasB gene in the DNA of Urine isolates (PsaU12, PsaW13, PsaW14, PsaW15, PsaW16, PsaW17), respectively.

It is distributed according to the cases of infection to represent 5/5 (100%) of burn infections, 6/6 (100%) of isolates in wound infections, and 6/6 (100%) of urinary tract infections. This result shows the dominance of the LasB gene in all bacterial isolates selected from burns, wounds and urinary tracts. The results of the current study agree with the findings of the study by [16], which has found that all isolates obtained from clinical sources possess the Las B gene with a rate of (100%). The results of the current study also coincide with the findings of the study by [17], which has shown that the rate of isolates owning it is 100% in burns, wounds and urine infections. The results of our current study also agree with what is stated in the study of [18] which has recorded positive results for the presence of lasB with a rate of (95.6%) in burn injuries and (87%) in wound injuries.

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الكشف عن بعض جينات الضراوة في بكتريا *Pseudomonas aeruginosa* المعزولة من مصادر

سريرية مختلفة

هاله عبدالخالق عوض ، اسماء حاضر فاضل

الملخص

الزائفة الزنجارية هي أحد مسببات الأمراض الانتهازية القادرة على إصابة جميع أنسجة الجسم تقريباً نظراً لامتلاكها مجموعة متنوعة من عوامل الفوعة التي تساهم بشكل كبير في الأمراض في المضيف. في الدراسة الحالية ، تم جمع (162) عينة من مصادر طبية مختلفة ، للكشف عن انتشار بكتيريا *P. aeruginosa* في هذه المصادر ودراسة بعض جينات الضراوة التي تمتلكها هذه البكتيريا. تم تشخيص العزلات النامية بعد زراعتها على اوساط زرعية مختلفة حسب الصفات المجهرية والزرعية ، ويتم تأكيد التشخيص باستخدام مجموعة API 20E. أظهرت نتائج التشخيص أن 33 عزلة تنتمي لنوع *P.aeruginosa* من مجموع العينات السريرية موزعة على (11 عزلة من إصابات الحروق ، 12 عزلة من الجروح و 10 عينات من التهابات المسالك البولية). تم اختبار حساسية العزلات البكتيرية لـ 15 مضاد حيوي ، وأظهرت النتائج أن جميع العزلات مقاومة لـ Ceftriaxone ، Colistin ، Amoxicillin بنسبة 100% ، و Ceftazidime و Nalidixic بنسبة 96% و 90% على التوالي ، Gentamicin بنسبة 60% . Tobramycin و Cefepime بنسبة 40% ، و Piperacilli و Aztreonam و Meropenem بنسبة 33.3% و Ciprofloxacin و Levofloxacin و Imipenem بنسبة 23.3% و Norfloxacin بنسبة 16.6%. تم الكشف عن انتشار الجينات المشفرة لإنتاج إنزيم البروتياز القاعدي aprA وإنزيم الإيلاستاز lasB ، باستخدام تقنية (PCR). أظهرت النتائج وجود جين aprA وجين lasB بنسبة (100%) ضمن التركيب الوراثي لعزلات *P. aeruginosa* قيد الدراسة. نظراً لوجودها العالي في معظم مصادر العزلات المدروسة ، يمكن اعتبار هذه الجينات بديلاً ناجحاً وسريعاً لتشخيص *P. aeruginosa* بالطرق الجزيئية المعتمدة على تقنية تفاعل البلمرة المتسلسل (PCR).