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Molecular detection of efflux pumps in *Pseudomonas aeruginosa* obtained from different clinical cases

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Abstract---150 samples were collected from different clinical sources from Al-Diwaniyah city hospitals from September 2021 to January 2022. The samples were grown on MacConkey agar and blood agar medium and incubated at 37°C for 24 hours. Bacterial isolates were diagnosed through culture characteristics, microscopic examination and biochemical tests, as 25 isolates belonging to *P. aeruginosa*. The phenotypic detection of these isolates was conducted and it was found that 16 isolates contain efflux pumps, after which the genetic detection was done by PCR technique. The isolates were subjected to the mexAB-OprM efflux pump detection using PCR technique, and after the replication product was migrated in agarose gel, it was observed that 93.75 % of the isolates possess the *mexA* gene encoding the above efflux pump, and (43.75%) the *oprM* gene encoding the above efflux pump also.

Keywords---efflux pumps, *Pseudomonas aeruginosa*, clinical cases.

Introduction

Pseudomonas aeruginosa is one of the most important Gram-negative bacteria that cause the spread of hospital-acquired infections (Gawish et al., 2013). These bacteria are characterized by the production of pigments such as pyoverdine (fluorescein) and pyocyanin, which show a bluish-green color on the surface of the agar, and this is the most important diagnostic characteristic of *P. aeruginosa* (Ferguson 2007).

These bacteria are widespread and are found in soil, water, and plants, as well as found in small numbers in animals and humans, and are present in the humid environments of hospitals and on the skin of many normal people. The function of this skin is a natural protection for body tissues, when any burn or wound occurs that leads to damage to these tissues and thus Provides an environment for the growth and reproduction of microorganisms, including *P. aeruginosa*. It represents a danger for many patients, including those who suffer from inflammation of burns and wounds, as these bacteria can invade the bloodstream, causing Bacteremia and then Septicemia, and those who suffer from immunodeficiency, as well as infecting patients with leukemia (Jalil et al., 2017).

This bacteria is secondary opportunistic pathogens, as it is characterized by its ability to cause several types of infections in many sites of the body, such as cystic fibrosis, eye infection, bacteremia, ear infection, and urinary tract infection. tract infection (UTI, central nervous system infection, skin infection, endocarditis, bone and joint infection) (Willenbrock et al., 2006). *P. aeruginosa* has a large number of virulence factors, including biofilm formation, the adhesion of *P. aeruginosa* bacteria and multi-layered biofilm formation on the surfaces and tissues of the host is one of the most important virulence factors that increase the danger of bacteria (Mohammed and Al-Marjani, 2015).

The *P. aeruginosa* bacterium represents a threat to many patients, including those who suffer from inflammation of burns and wounds, as this bacteria can invade the bloodstream, causing Bacteremia and then Septicemia, and those who suffer from immunodeficiency, as well as in patients with leukemia (Jalil et al., 2017). Efflux pumps in bacteria increase virulence factors and resistance to most antibiotics, *P.aeruginosa* has six types of efflux pumps (MexAB-OprM, MexCD-OprJ, MexEF-OprN, MexXY-OprM, MexJK-OprM, MexVW-Opr) (Al-Grawi, Al-Absali et al,2021). The mex AB-OprM is the main efflux pump in these bacteria (Lopez, et al, 2017).

Aim of study

Given the importance of *Pseudomonas aeruginosa* bacteria and its relationship to different clinical cases and its resistance to antibiotics, this study aims to reveal the efflux pumps possessed by these bacteria isolated from different clinical cases in Al-Diwaniyah city and its relationship to antibiotic resistance.

Materials and Methods

Sample collection

150 samples were collected from different clinical sources in Al-Diwaniyah city hospitals (Al-Diwaniyah Teaching Hospital, Maternity and Children Hospital, Burns Hospital) from September 2021 to January 2022.

Bacteria isolation

Samples were cultured on Macconkey agar and blood agar medium and incubated at 37°C for 24 hours

Diagnosis of bacterial isolates

1. Cultural traits and microscopic examination

The initial diagnosis of the isolates was carried out based on the phenotypic characteristics that included the shape, color and texture of the colonies growing on the selective media used in the study (Macconkey agar), as well as the type of lysis on the blood agar medium, as well as their ability to analyze lactose sugar or not. The isolates were subjected to microscopic examination, as swabs from these colonies were prepared after re-purification on Macconkey agar and blood agar medium, stained with Gram stain, and examined under an oil lens at 100X magnification.

2. Biochemical tests

Biochemical tests were performed according to the following references: McFaddin (2000), Daboor and his cohorts (2010).

Indol Production Test, Voges Proskauer and Methyl red test, Citrate Utilization test, Motility test, Urease test, Catalase test, Oxidase test test, Triple Sugar Iron Test (TSI).

3. Diagnosis using the Api-20E system and the use of Phytex

The final confirmation of the diagnosis of bacterial isolates was made using the Api20 assay, as well as the use of Vietek because it is considered more accurate in determining the type of bacteria.

Detection of the *mexA* and *oprM* efflux pump gene using PCR technology

DNA was extracted from the bacteria *P.aeruginosa* using a Genomic DNA extraction kit (Geneaid, USA) according to the company's instructions.

Table 1: Primers are used for the diagnosis of *P. aeruginosa*

Gene	Primers
<i>mexA</i> gene	F : CGACCAGGCCGTGAGCAAGCAGC
	R: GGAGACCTTCGCCGCGTTGTCCG
<i>OprM</i> gene	F: GATCCCCGACTACCAGCGCCCCG
	R: GATCCCCGACTACCAGCGCCCCG

The polymerase chain reaction mixture was prepared in PCR tubes and the tubes were transferred to the PCR thermocycler first cycle at 96°C for 5 minutes, followed by 30 cycles at 96°C for 30 seconds, 55°C for 1 minute and 70°C for 2 minutes. The products were multiplied by migrating samples onto agarose gel prepared at a concentration of (1.5)%. The samples were migrated using the ready-made loading buffer in addition to the DNA Ladder migration. Then the products and the DNA Ladder were migrated electrically for one hour, the gel was examined after the migration was completed by exposing it to a UV source and then estimating the molecular sizes of the replicated pieces compared to the position of the bundles in the used DNA Ladder and the replication products.

DNA sequencing of the genes encoded by the efflux pump proteins was performed. 150 samples were taken from different sites of wounds, burns, ear, nose and respiratory tract from patients admitted to the Maternity and Children Hospital, General Teaching Hospital and Burn Hospital in Al-Diwaniyah city. For a period

of 24 hours, 112 (74.6%) bacterial growth appeared. The isolates were diagnosed based on culture and microscopic examinations, as a preliminary diagnosis as shown in the table below. The diagnosis was confirmed by the Phytex device, as 25 isolates belonging to *P.aeruginosa* were diagnosed.

Results and Discussion

Table 2: Biochemical tests for the diagnosis of *P. aeruginosa*

Biochemical test	Result
Oxidase	+
Catalase	+
Indole	—
MR	—
VP	—
Citrate	+

(+) Negative

(-) Negative

MR) Methyl red

VP) Voges Proskauer

16 bacterial isolates were tested to detect the presence of *mexA* gene, and after the replication product was migrated in 1.5% agarose gel, it was noticed that one etching bundle appeared at the same level after exposing the gel to ultraviolet rays. On comparing the multiple bundles with the volumetric guide with bundles of known sizes as in Figure (1), where these bundles came with a size of (316) base pairs.

The results of this study showed that 15 isolates (93.75 %) of the tested study isolates possess the *mex A* gene, and thus our results are in agreement with the findings of Ugwuanyi and his group (2020) as the *mexA* gene was 90% present, while the presence of the *mexA* gene was close to As reached by Al-Grawi and his group (2012), Tomas and his group (2010), Abbas and his group (2018), where the percentages were 100%, 88.2%, 100%, respectively. While the percentage of the gene's presence contradicted what was reached (Al-Obaidi, 2015) in his study, where the percentage reached by it was (68.8%), as well as what was reached by Abdallah and his group (2021), where the percentage of the gene was 54%.

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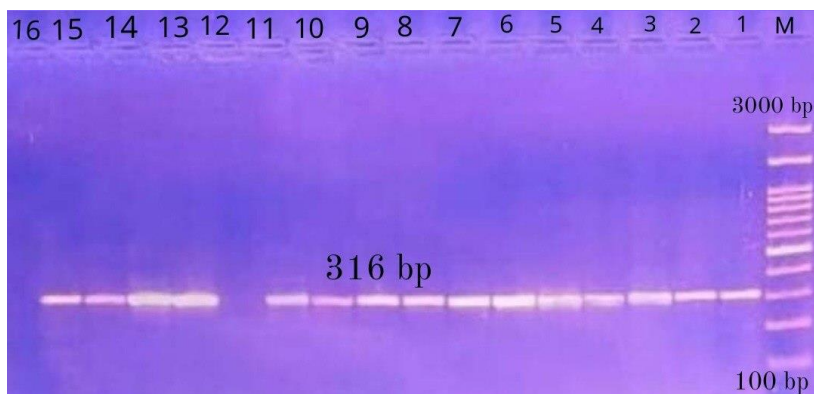


Figure (1) The chromosomal *mexA* gene amplification products of the bacteria under study, electrophoresis was carried out on 1.5% agarose gel at voltage 90 for one hour, column M represents DNA Ladder

While the presence of the *oprM* gene was revealed, and after the electrophoresis, it was observed that one bundle appeared in the pits at the same required level after exposing the gel to ultraviolet rays, evidence of the link of the initiator with its complementary sequence on the binding of the template DNA, when comparing the duplicated bundles with the volumetric guide with The known beam sizes are as shown in Figure (2). These beams are similar to the expected size, which is approximately 247 base pairs, when compared with the results reached (Dumas et.,al 2006).

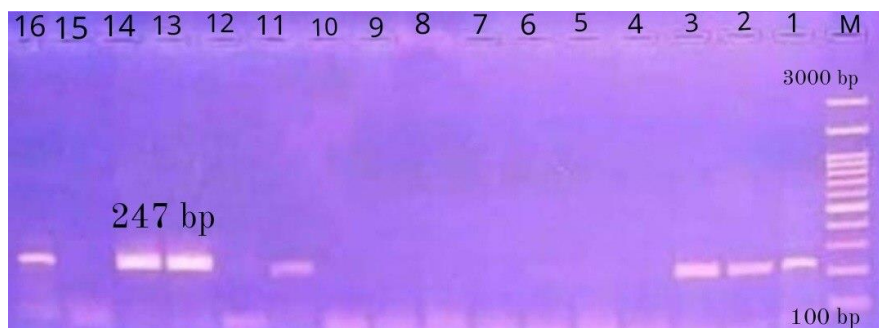


Figure (2) The chromosomal *oprM* gene amplification products for the bacteria under study, electrophoresis was carried out on 1.5% agarose gel at voltage 90 for one hour, column M represents DNA Ladder

The current results of this study showed that 7 isolates (34.75%) of the tested isolates of the study have the *oprM* gene, and the rest of the isolates do not have this gene, and this is similar to what was reached by Abbas and his group (2018)

where the percentage of presence of the gene was 40.5%, and similar to what was reached Murugan and his group (2018) where the gene presence was 35%, while our results contradicted what Abdallah and his group (2021) found, where the gene presence was 8.6%. As the *oprM* protein has a very important role in the flow of antibiotics outside the bacterial cell (Zgurskaya and Nikaido, 2000; Masuda et al., 2000; Chuanchuen et al., 2001).

It is located in the outer membrane of the bacterial cell, and it has an important part in the efflux pumps so that three-segment efflux pumps are effective for antibiotic efflux, and if it is missing, some of the pumps are ineffective (Dikievit et al., 2001; Meletis and Bagkeri., 2013). The efflux pump Mex AB-OprM contributes to the intrinsic resistance to many antibiotics such as beta-lactam antagonists, chloramphenicol, fluoroquinolones, tetracyclines, sulfonamide (Poole, 2001).

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