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Phenotypic and molecular detection of biofilm production by P. aeruginosa isolated from different clinical cases

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***Abstract*---**This research was performed in the aim of detection the ability of *Pseudomonas aeruginosa* to form a biofilm. So 100 sample were collected from hospitalized and reviewed patients with a different clinical cases including (urinary tract infections, respiratory tract infections, burns, andotitis), both sexes and different ages,at Al-Sadiq hospital in BabilGovernorate, as well as Al-Diwaniyah Teaching hospital, Burns hospital, Women & Childrenhospital in Al-Diwaniyah Governorate.Samples were collected from October 2021 until January 2022. 20 isolate of *Pseudomonas aeruginosa* were obtained. The detection of biofilm formation was performed by using two conventional methods, and all isolates was able to form a biofilms. Thena genetic detection was performed throughthe search for the presenceof*algD*, *pelF*, and *pslD* genes thatencode for a biofilm production. The results showed the presence of *algD*gene in a percentage of 65%, while the presence of *pelF* gene was equal to *psld* gene, which reached a percentage of 70%, while some isolates contained both genes, others contained *pelF* gene and lake of*pslD* gene or vice versa, the percentage of isolates that contained all three genes was 35%, and the percentage of isolates that contained only one gene was 30%.

***Keywords*---**phenotypic, molecular detection, biofilm production, P. aeruginosa.

Introduction

Pseudomonas aeruginosa is a widely distributed bacteria, it could be isolated from different environments as soil, water, plants, human body, and hospitals (1). *Pseudomonas aeruginosa* depending ona multiple metabolic pathways(2),that isallowingthemto usea huge and variety organic compounds (3), and surviving in harsh circumstanceswith aminimal nutritional requirements(4).These bacteria are a common causesof different nosocomial infections (5), that may develop to acute and chronic diseases,like septicemia, pneumonia, bacteremia,keratitis (6), and wound infections in achronic diabetes patients(7).*Pseudomonas aeruginosa* is an opportunistic pathogen that is usually affect immunocompromised people, like those suffering from cancers, burns, or AIDS. So, this bacterium create an increasing threat to human health (8,9). *Pseudomonas aeruginosa*is a highly virulent bacteria with a several different virulence factors, including pigments (pyocyanin & pyoverdin), enzymes (elastase & phospholipase), exotoxins, cell surface structures like capsule and lipopolysaccharideLPS (10,11,12). As well as their ability to form a biofilm, which is represent the most important virulence factor (13).

A recent global estimates says that, about 40- 80% of prokaryotic organisms lives within biofilms as a natural growth pattern, and this is dominate in all habitats of earth's surface except oceans (14). Biofilms formation is a complex collaborative process, that is occur in gradual sequential steps, and characterized by the presence of a chemical communication between bacterial cells within(15).After maturation, the biofilm appears as a three-dimensional structures that contain a micro colonies generated from dividing bacterial cells embedded in an Extracellular Polymeric Substance (EPS), these substance secreted by bacterial cells themselves, it is necessary for packaging, and surface attachment, since biofilms usually formed on surfaces. It isalso provide protection against various external effects such as (mechanical disruptions, host immunity, and oxidation), enhancing antibiotics resistant, facilitating distribution of different materials within the biofilm like (oxygen, nutrients, signaling molecules, inorganic ions, and insoluble compounds) (16). The diffusion occurs easily within the biofilm’s matrix through pores and channels, because of their high water content, that may be reached 97% (17).

Regardless of their location,the presence of bacteria within the biofilms gave them many advantages, that the most important advantage is resistance and high tolerance (18). The lowest metabolic activity, limiting diffusion of antibiotics through EPS, and persister cells creation (a metabolically inert cells that could tolerate all conventional antibiotics), these features give a high resistance capacity, this is rather than intrinsic and acquired resistant mechanisms (19). Biofilm production also considered as the main strategy of bacteria to survive against environmental changes like inappropriate temperature and starvation (20). Finally, the biofilm provide resistance against host immunity (18).

Materials and Methods

Samples collection

100 clinical samples were collected from patients of different cases, from these samples about 20 *Pseudomonas aeruginosa* bacteria were isolated and selected for this study. These isolates had been diagnosed depending on culturing characteristics, biochemical tests, and microscopic examinations according to the reference (21).

Phenotypic detection of biofilm production

Phenotypic detection of biofilm was carried out firstly, by tube method according to (22), the result considered positive when a violet rings are formed. Then, Congo red agar method was carried out according to the reference (23), the result was positive in this method when the color became dark.

Molecular detection of biofilm production

Bacterial DNA were initially extracted by using Geneaid/USA kit, then polymerase chain reaction technique (PCR) was carried out by the primers of the following genes: *algD*, *pslD*, and *pelF* (as these genes encoding for biofilm production), electrophoresis of PCR products then done on agarose gel (1.5% concentration), then UV was used to detect the DNA bands.

Results and Discussion

The results of a phenotypic detection showed that all isolates under study of*Pseudomonas aeruginosa*were producing a biofilms.In the case of a tube method, biofilms appear as a violet ring around the edges of the tubes, figure (1).

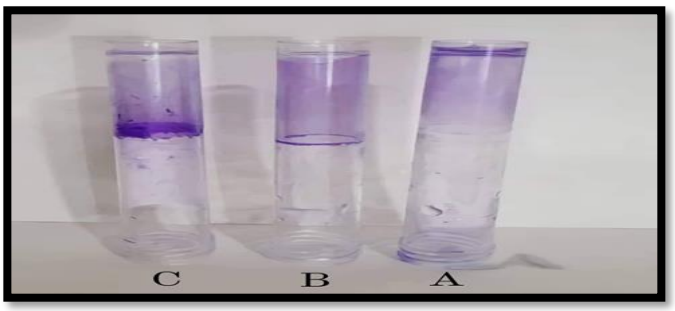

Figure1: biofilm production by tube method, whereas A represents a negative control, while B and C is a positive results

The rings formed as a result ofthe conversion of cells from a suspended state to an adherent state.All suspended cells through washing step were removed, while adhesive cells were stained bycrystal violetwhich was added after washing(24) There aretwo factors responsible for biofilms formation, the first and the most important one is type B hydrophilic LPS, this is produced by *Pseudomonas aeruginosa*depending on their endemic environment, and the second is type IV

pilli(25).In some cases, the ringsappeared more intense,this is due to the ability of these bacteria to form a mucous layer known as pellicle, which is enhances the adhesiveness capacity(26). Biofilm detection by Congo red agar method also showed a positive results, as the colonies of all isolates had been changed from red to dark color, fig (2).

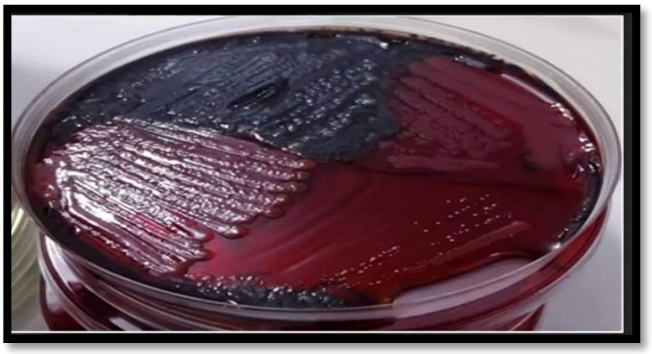

Figure 1 : positive result of biofilm by Pseudomonas aeruginosa colonies on Congo red agar

The Congo red dye represent the indicator of this test, it is reduced atthelate incubationby asecondary metaboliteresulting fromthe metabolism of exopolysaccharides (27),the addition of sucrose in 5% concentration is a key factor todetermine the production exopolysaccharides (28). The molecular detection of biofilm confirmed theresults of phenotypic detection. The percentage of isolates that contained all examined genes (*algD*, *pslD*, and *pelF*) was 35%, and this is equal to the percentage of isolates that contained two genes, while the percentage of isolates that contained just one gene was 30%. The *algD* gene encodes an alginate, the *pelF* gene encodes a pellicle, and the *pslD* gene encodes a polysaccharide synthesis locus

The results showed that the presence of *algD* gene was in a rate of 65% of the total isolates, fig (3).This is compatible with Garallah, *et al* (29) who got a percentage of 65.38%, but not compatible with Yang, *et al* (30), as the presence of this gene was 98.49% in the mentioned study.

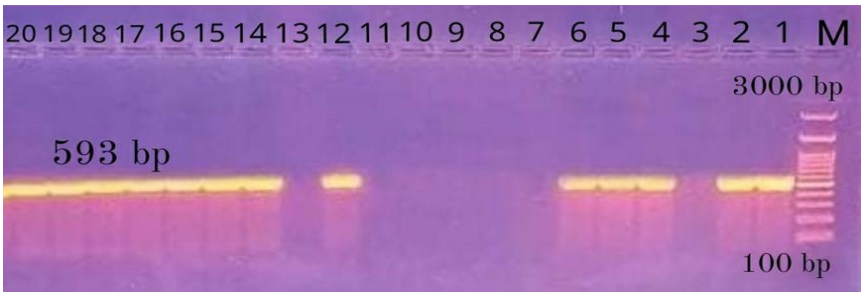

Figure 2: PCR results for the chromosomal algD gene, the electrophoresis done on agarose gel, 1.5% concentration, 70 volts, for an hour. The column M represent the ladder, while columns 1-20 represent the isolates

The results also showed the presence of *pslD* gene in a percentage of 70% , fig (4), this is agreed with Ghadaksaz, *et al* (31), as the percentage of this gene of that study was 83.7%, but didn't agree with Abdelwahab and Yakout (32) whose detected this gene in just 49.7% of their total isolates.

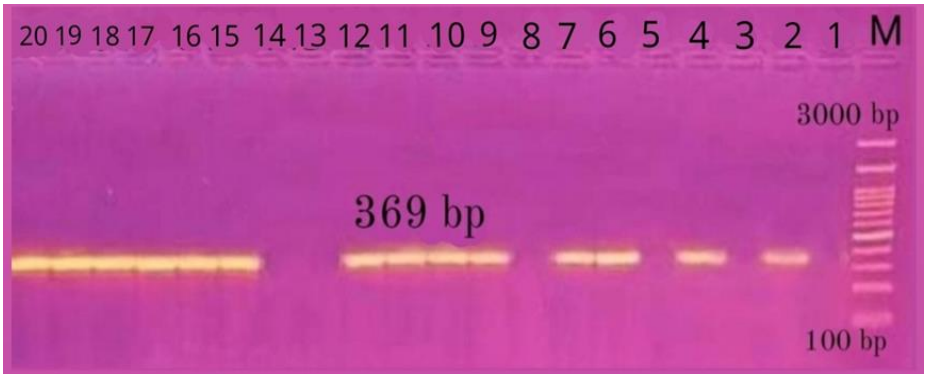

Figure 3 : PCR results for the chromosomal pslDgene, the electrophoresis done on agarose gel, 1.5% concentration, 70 volts, for an hour. The column M represent the ladder, while columns 1-20 represent the isolates

Finally, the results showed the presence of pelF gene in 70% of the total isolates, fig (5), this is close related to the percentage of Pournajaf, et al (33), whose detected this gene in about 57.3% , but far from the results of Yanget al (30), as the gene is found in most of the bacterial isolates of that study 96.48%.

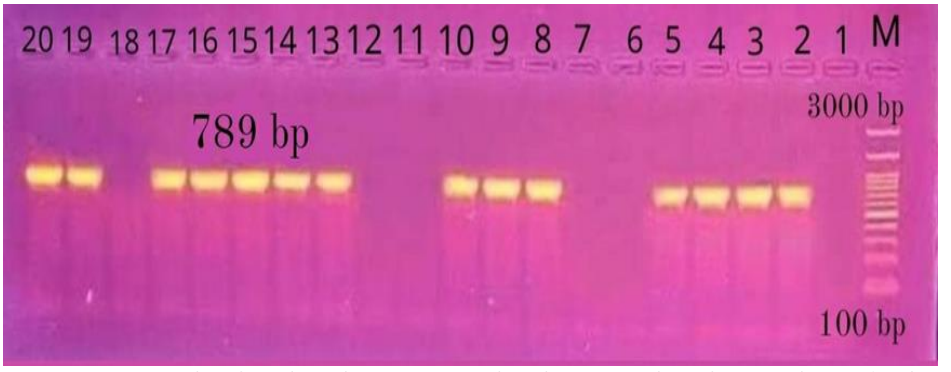

Figure 4 : PCR results for the chromosomal pelFgene, the electrophoresis done on agarose gel, 1.5% concentration, 70 volts, for an hour. The column M represent the ladder, while columns 1-20 represent the isolates

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