# Study the Ability of *Pseudomonas Aeruginosa* Isolated from Different Clinical Cases to Biofilm Formation and Detection of Algd Gene

**Hussamsalah al-deen[1], Suaad Khalil Ibrahim[2]**

*[1]Post graduate, [2]Assistant Pro., Department of Biology, College of Education For pure Sciences / Ibn Al – Haitham University of Baghdad, Baghdad – Iraq*

## Abstract

98 samples were collected from various clinical sources included ( Burns, wounds, urines, sputums, blood ) From the city of Baghdad , After performing the biochemical and microscopic examination, 52 isolates were obtained for *Pseudomonas aeruginosa* , 17 (32.7 %) isolates from burn infection , 12 ( 23% ) isolates from Wound infection 11 ( 21.2 % ) isolates from urine infection , 7 ( 13.5 % ) isolates of sputum and 5 ( 9.6 %) isolates from blood . Bacteria susceptibility to form biofilm has been detectedby microtiter plate method, The results showed that 80 % of the bacterial isolates were produced the biofilm with different proportions , **alg D** gene ( alginate production ) has been detected by polymerase chain reaction ( PCR ) Which plays an essential role in the formation of the biofilm , The PCR results showed that the percentage of gene presence was( 95.4 % ) .

***Key words :*** *Pseudomonas aeruginosa , biofilm , alg D*

## Introduction

*P. aeruginosa* is considered an important bacterial species due to its parasitism on humans causing many diseases like Bacteremia , Wound and Burn infection , Urinary tract infection , Endocarditis , Meningitis[(1)]It also affects the digestive system, causing gastroenteritis or diarrhea in children[(2)], *P. aeruginosa* can colonize medical devices which increases its prevalence in health care institutions [(3)]

*P. aeruginosa*has many virulence factors including Exotoxin A , Hemolysin , Protease and Biofilm[(4)] , Bacterial cells tend to form clusters that are resistant to unfavorable conditions despite its presence in most of the time freely [(5)] and these clusters are surrounded by a layer of exopolysaccharides as well as protein and DNA, these clusters are characterized by the presence of channels through which nutrients and cellular secretions are transported[(6)] , the biofilm provides protection from many harsh physical and chemical stresses such as high temperature and drought[(7)] , PH and disinfectants[(8)] and fromthe host's body defenses [(9)] , the biofilm is an important feature for the continuation of infection and when it is formed the bacteria are resistant to antibiotics at a high rate that may reach 1000times higher than that of other non-biofilm types, which makes it difficult to treat[(10)].

**algD** gene responsible for the production of the alginate layer for *P. aeruginosa* and it plays an important role in chronic pneumonia[(11)].

The **algD** gene encodes( GDP-mannose 6-dehydrogenase) an enzyme involved in the alginate biosynthesis pathway [(12)], The alginate layer is considered one of the basic elements for the formation of the biofilm,, its a linear unbranched polymer

composed of ( 1-4 ) linked saccharides D-mannuronic Acid (M) and L-guluronic Acid (G)[13] .

The alginit layer contributes to the structural stability and protection of biofilms[14]As well as providing protection from antibiotics[15] , its overproduction is responsible for the mucosal phenotypic growth that protects bacteria from the harsh environment in the lungs of a patient with cystic fibrosisand causes lung lesions by facilitating the attachment of bacteria to the respiratory tract epithelium[16]Interestingly, this layer is produced mainly in patients with cystic fibrosis .

## Materials and Methods

**Collection of bacterial samples :**

98 samples were collected from different clinical sources ( Burns, wounds, urines, sputums and blood )From the Central Child Hospital and Medical City Hospitals in Baghdad, for the period between 30/9/2020 to 1/1/2021, The samples included: 26 from burns, 25 from wounds, 26 from urine, 12 of sputum and 9 samples of blood.

**Isolation and Identification :**

The bacterium was diagnosed by cultivating it on macConkey agar , cetrimide agar and blood agar, Their phenotypic characteristics were studied in terms of colonies' shape, color, and their odor[17] and Examination microscope( Gram stain ) and biochemical tests ( Oxidase , Catalase , Citrate utilization , Gelatin hydrolysis , Hemolysin and Indole test ) .

**Detection of biofilm formation :**

The ability of *P. aeruginosa* to form biofilm was detected using the microtiter plate method[18] ,the bacterial suspension was prepared using sterile saline and compared with the McFarland standard solution ( 0.5 ) , a 180 µL of Tryptone soy broth ( TSB ) supplemented with additional 1 % glucose and a 20 µL of prepared bacterial suspension was added to each well of microtiter plate(Three replications ) negative control represented only the medium , after 24 hours of incubation at $35^5$C the contents of the wells have been discarded and rinsed gently three timeswith 200µL of sterile phosphate buffered saline ( PH 7.2 )and left the room temperature to dry, cells were fixed with 150 µL of Methanol alcohol for 20 minutes , after discarded of methanol, dried and stained with2% Crystal violet ( 150 µL per well ) for 15 minutes then wells were washed with distilled water.after drying , dye bound to biofilm on well released with 96% ethanol alcohol ( 150 µL per well ) for 20 minutes at room temperature , the optical density was measured using a microtiter plate reader at 570 nm .

**DNA extraction :**

The genomic DNA was extractedfrom preserved bacterial isolates ( 22 isolates ) according to company protocol and using DNA kit ( Promega , USA ) .

**Table (1) : The sequence and source of the gene primers used in the study**

| Target Gene | Primer sequence ( 5' – 3') | Size ( bp ) | Annealing Temperature 5C | Reference |
|---|---|---|---|---|
| alg D | F: 5' - ATGCGAATCAGCATCTTTGGT - 3'<br>R: 5' - CTACCAGCAGATGCCCTCGGC - 3' | 1310 | 60 | ( 19 ) |

**Polymerase chain reaction (PCR ) :**

A PCR mixture for detection ofalgD gene was prepared from 1 µL for each forward and reverse primer , 10 µL of Go taq Green master mix Which was processed by the company ( Promega , USA ) , 5 µL of nuclease free water and 3 µL of DNA template and as shown in the table ( 2 ) .

**Table ( 2 ) : Polymerase chain reaction mixture**

| Master mix components | Volume ( µL ) |
|---|---|
| Master mix | 10 |
| Forward primer | 1 |
| Reverse primer | 1 |
| Nuclease free water | 5 |
| DNA | 3 |
| Total | 20 |

**Agarose gel electrophoresis :**

The PCR product reaction was loaded into the wells then pass an electric current with a difference voltage of 100 volts for 60 minutes , the DNA will be transmitted from cathode to plus anodes poles , the Ethidium bromide – stained bands in gel were visualized using gel imaging system .

## Result and Discussion

**Isolation and Identification :**

After performing the biochemical and microscopic examination , 52 isolates were obtained for *P. aeruginosa* , all the isolates were Cram negative, rods-shaped, the colonies were pale in color when grown onmacConkey agar because it does not have the ability to ferment lactose[20]. On sterimide agar, the colonies were bluish and greenish in color due to their water-soluble biocyanin and bioverdin pigments [21] while a transparent aura formed around the colony when it grew on the blood agar due to the hemolysin[22].

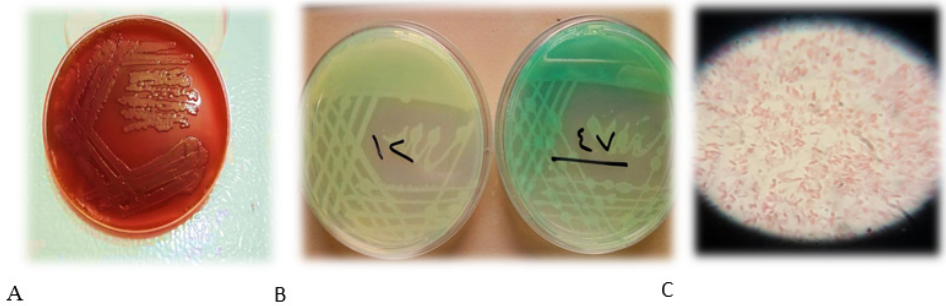


Figure (1): Shape of *P. aeruginosa*bacteria A. blood agar , B. cerimide agar , C. Microscope (oily lens)

**Figure (1): Shape of *P. aeruginosa*bacteria A. blood agar , B. cerimide agar , C. Microscope (oily lens)**

About biochemical tests, all bacterial isolates were positive for the tests : oxidase , catalase , gelatinase , citrate utilization , while were negative for indole , the result of this study were in agreement with[23] .

**The ability to form a biofilm :**

The results of the phenotypic detection showed that 20% of the bacterial isolates were not forming the biofilm while 80% were produced the biofilm in different degrees after comparing it with the negative control.

28.9 % isolates were weakly for Biofilm formation , 24.4 % isolates were moderately and 26.7 % isolates were strong , as shown in Figure (2)

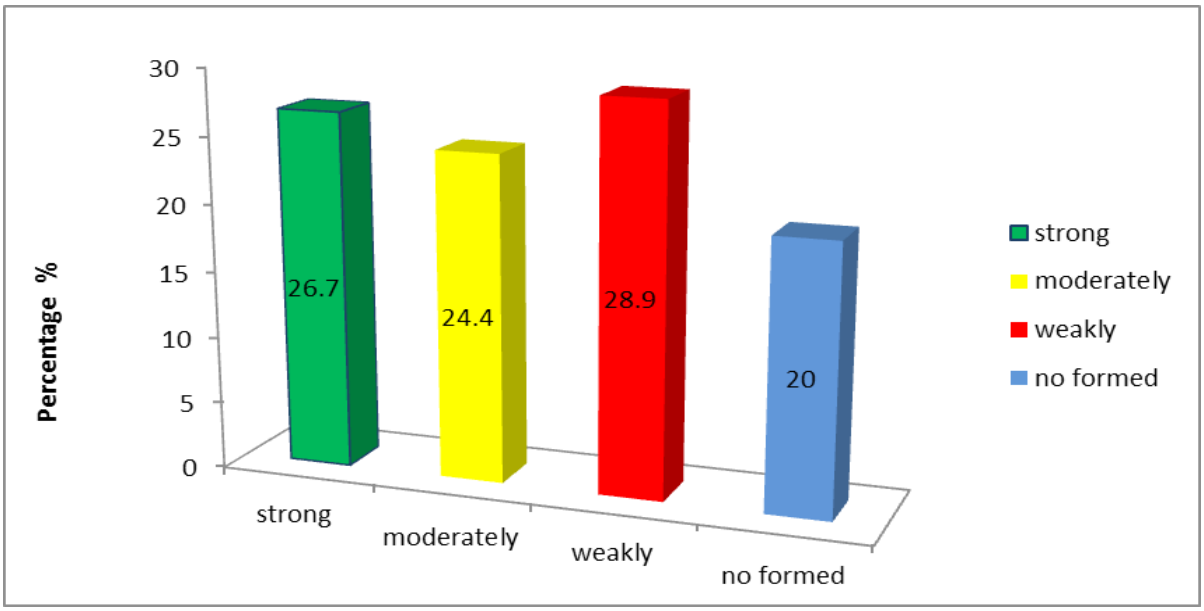


**Figure (2): Percentage of *P. aeruginosa*isolates on biofilm formation.**

The results of this study were in agreement with [24]who found that 14 % not forming the biofilm and 28 % weremoderatelyfor biofilm formation While not in agreement with[25]Who found that 24% of the isolates of *P.aeruginosa* were produced the biofilm .

This difference in the thickness of the biofilm is due to different reasons, perhaps due to the initial number of cells that succeeded in attaching to the wells of the microtiter plate or to the difference in the amount of auto inducers (quorum sensing signal particles ) which is produced from each isolate and play an essential and important role in the formation of biofilms[26] .

**Detection of algD gene :**

The results of the molecular detection of the algD gene that contribute to the production of the alginate layer in *P. aeruginosa*were: all isolates possessed algD gene about ratio 21(95.4 %) and one isolate did not possessed it , shown in figure(3), The results of this study were in agreement with[27] and [28]While not in agreement with[29].

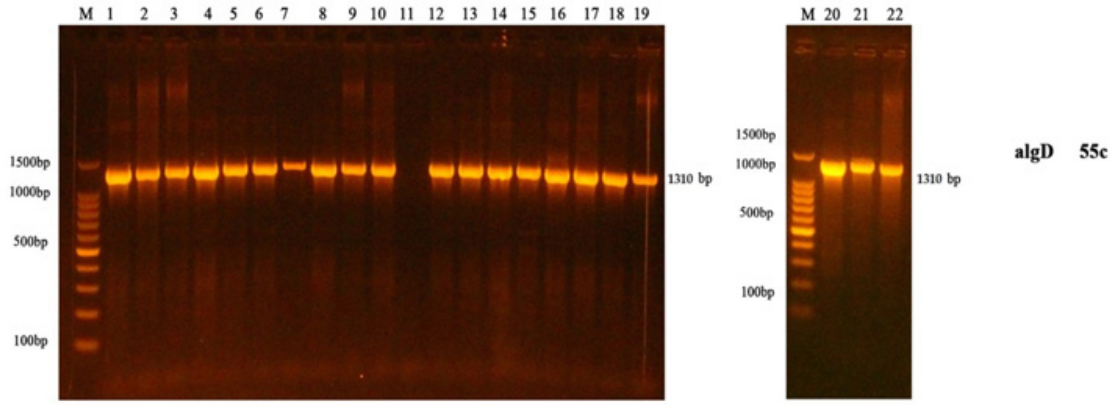


**Figure ( 3 ) : Resuts of algD gene of *P. aeruginosa* on 1,5 % agarose gel electrophoresis , ( M ) DNA ladder 1500 bp**

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of bothMOH and MOHSER in Iraq

**Conflict of Interest:** None

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