



*Microbial Science Archives* (ISSN: 2583-1666)

An International Research Journal

Journal homepage: [www.microbial.sciencearchives.org](http://www.microbial.sciencearchives.org)

<https://doi.org/10.47587/MSA.2024.4304>

Research Article



## Evaluation siderophores production and biofilms formation among *Pseudomonas aeruginosa* isolated from clinical infections

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Received: Aug 24, 2024/ Revised: Sep 14, 2024/Accepted: Sep 28, 2024

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### Abstract

*Pseudomonas aeruginosa* is an important opportunistic bacterium, producing biofilm and two groups of siderophores, the important factors allowing host colonization. The present research aimed to study the siderophores production and biofilm formation among *Pseudomonas aeruginosa* isolates in patients with different infections in Tikrit City. 30 *Pseudomonas aeruginosa* isolates were isolated from 80 clinical specimens (for both gender and different age groups) with wound infections, burn infections, otitis infections, and urinary tract infections. Isolates identification based on morphological properties, gram staining, and biochemical tests. Biofilm formation potential of isolates was studied by Congo red agar plate at 37°C for 24 h. Also, isolates tested for Siderophores production by using CAS assay. In this study, the results showed that 30 (37.5%) *Pseudomonas aeruginosa* were isolated from 80 different clinical infections; (6) 20% from burn infections, (10) 33.3% from wound infections, (7) 23.3% from UTI, and (7) 23.3% from otitis infections. The current study showed that 10(33.3%) of isolates were biofilm producers while 20 (66.7%) were non-biofilm producers and the maximum of Biofilm production was in wound samples also the study presented that all tested isolates of *Pseudomonas aeruginosa* were positive for siderophore production. The study showed that *Pseudomonas aeruginosa* associated with variance patients infections. Biofilm is assessed to be answerable for all human infections and hospital-acquired infections. In this study, the biofilm formation differs between *Pseudomonas aeruginosa* strains and has been higher in wound infections. Further, all tested isolates were positive for siderophore production, the differences between producers and non-producer bacteria should increase with increasing iron -limitation because production of siderophores is regulated in response to iron limitation.

**Keywords:** Siderophores Production, Biofilms Formation, *Pseudomonas aeruginosa*, Clinical Infections.

### Introduction

According to the World Health Organization (WHO), *Pseudomonas aeruginosa* has been flagged as the greatest public health concern. Resistance of *Pseudomonas aeruginosa* to antibiotics continues to increase over the years in several regions of the world (Al-Bayati et al., 2021). *Pseudomonas aeruginosa* generally establish in humans, animals, soil and plants, is a common nosocomial bacterium, it is extremely pathogenic for person with comprised immunity and higher

prevalent in the patients with cystic fibrosis (CF) also it has been demonstrated in other organs, such as brain, ear, eye, blood, bone, wounds, burn, gastrointestinal tract, urinary tract and periprosthetic joints. Multiple virulence factors identified in *Pseudomonas aeruginosa* as the essential causation of infection including biofilm formation, exotoxin, elastase, adhesion factors, pyocyanin and siderophore production. *Pseudomonas aeruginosa* composed two specific siderophores, pyochelin (Pch) and pyoverdine (Pvd), for high-affection iron intake from environment and host cell (Takase

et al., 2000; Jeong et al., 2024). Iron molecules that exhibit outside the cell bind these siderophores and transport them inside, coming after admission by receptors of cell membrane (Meyer, 1992). Siderophores serve as virulence factor by affect the host immune system and perform many functions (Beare, 2003). Pch and Pvd each presented for eliminate iron from lactoferrin and transferrin as well as to improve *Pseudomonas aeruginosa* evolution in media involving human serum or these iron-binding proteins (Takase et al., 2000).

Biofilm exists in more than 90% of bacteria, is one of the elected growth states for bacteria, and is described as an aggregation of microorganisms enclosed inside of a self-secreted polymeric extracellular texture matrix connected to a surface invariable. The content of the biofilm matrix differs among variance species. However, it generally comprises nucleic acids, polysaccharides, and proteins. The constancy of *Pseudomonas aeruginosa* biofilm construction is decisive by numerous polysaccharides, which involve pel, alginate, and psI (Vetrivel et al., 2021). In view above, this study is designed to assess the siderophores production and biofilm formation in the variance infections that caused by *Pseudomonas aeruginosa*.

### Sample collection and identification

Thirty *Pseudomonas aeruginosa* isolates in this study were isolated from eighty clinical samples included both genders and different ages with different infections; urinary tract infections, burn infections, wound infections, and otitis infections (from Tikrit Military Hospital, Tikrit, Iraq) during the period from July 2023 to August 2023. Isolation of *Pseudomonas aeruginosa* was done by streaking all samples on the surface of selective medium Cetrimide agar and incubating at 37°C for 24 hrs. Isolates were identified using morphological characterization (shape of colonies on cetrimide agar, MacConkey, and nutrient agar plates as well as pigment production), gram staining, and biochemical reactions including motility test, citrate utilization test, oxidase test, urease test, ornithine decarboxylation test, Vogus Proskauer test, Indole test, Methyl red test and growth at 42°C. These identification methods were done according to (Brown and Smith, 2015).

### Biofilm formation assay

For the detection Biofilm formation among isolates of *Pseudomonas aeruginosa* Congo red agar procedure was performed as described in (Freeman et al., 1989). In this method as a PH indicator, Congo red dye was used. In pH ranges from (3.0 to 5.2) demonstrating a black color. implanted Plates were incubated in an aerobic environment with the Congo red agar for (24 to 48) hours at (37°C). After incubation, colonies appearance with crystalline consistency or blackish, and dark red were expressed biofilm producers while red colonies with a smooth appearance in the midpoint were expressed biofilm non-procedures.

### Siderophores production

Among *Pseudomonas aeruginosa* isolates siderophores production was tested by chrome azurol S (CAS) assay (Ghosh et al., 2015). Over cetrimide agar the isolates were spread and incubated for 48 h at 30°C. Afterwards, by a thin layer of CAS reagent in 0.7% agar the bacterial growth was spread and plates repeatedly incubated for 24h at 30°C forming a yellow orange area all over the colonies reveals siderophore production.

### Results and Discussion

A total of 30 (37.5%) *Pseudomonas aeruginosa* isolates, (6)20% from burn infections, (10) 33.3% from wound infections, (7) 23.3% from UTIs, and (7) 23.3% from otitis infections were isolated from 80 patients. The result of *Pseudomonas aeruginosa* distribution showed that *Pseudomonas aeruginosa* is associated with different patient infections as in Table 1. In the current study, the isolation of these bacteria from wound infections is higher from other infections it may be due to the contamination of these infections from the environment, beds, tools, and contaminated worker's hands. The results of (Midhat and Abed, 2023) reported 25 isolates from *Pseudomonas aeruginosa* which involve 5 (20%) isolates from urine, 7 (28%) isolates from wounds and 13(52%) isolates from burns, and a higher percentage from burns patients. Without a doubt the percentage of *Pseudomonas aeruginosa* in the current study is different from the other studies' results, this may be due to the number of samples, sample type, and difference in the sampling method.

**Table 1. Distribution of *Pseudomonas aeruginosa* isolates according to the different types of infection**

Type of specimen	No. of specimen	No. of <i>P. aeruginosa</i> Isolate %
Wound swab	10	33.3%
Burn swab	6	20%
Urine	7	23.3%
Otitis media swab	7	23.3%
Total	30	100%

The diagnosis of all the isolates is based on culture characteristics (colony morphology, pigment production, and growth at 42°C) Fig.1, microscopic examination (exhibit, single, gram-negative rod shape bacteria, non-spore-forming), and different biochemical tests as in Table 2.



Fig.1 *Pseudomonas aeruginosa* on cetrimide agar

Table 2. Biochemical test for identification of *Pseudomonas aeruginosa* isolates

Biochemical test	Results
Catalase	Positive
Oxidase test	Positive
Urease test	Positive
Simmon Citrate test	Positive
Vogus proskauer test	Negative
Indole test	Negative
Methyl red test	Positive
Motility test	Positive

Table 3. Distribution biofilm formation among *Pseudomonas aeruginosa* isolates in clinical specimen

Clinical specimen	Numbers of isolates	Numbers of isolates forming biofilm
Wound swab	10	6
Burn swab	6	1
Urine	7	1
Otitis media swab	7	2
Total	30	10 (33.3%)

Concerning the biofilm producing capacity prevalence in isolates of *Pseudomonas aeruginosa*, it showed that 10(33.3%) were biofilm producers while 20 (66.7%) were non-biofilm producers, regarding to their doling out these isolates were mostly achieved from wound samples 6 isolates out of a total of 10 samples followed by otitis media samples 2 out of 7 isolates, from burn one isolates out of 6 isolates and finally UTI samples one out of 7 isolates. The maximum of Biofilm production in this study was found in isolates from wound infections Table 3.

The capability of *Pseudomonas aeruginosa* to form biofilm in wounds has been reported by the (Harrison–Balestra et al., 2003), who reported that biofilm production can occasionally be fast by bacteria and the residence of some microorganisms on wounds could induce the progress of biofilms in a duration of (24-48) hours after colonization. The differences in biofilm formation among strains of *Pseudomonas aeruginosa* depend on numerous factors including temperature, the presence of other bacteria, the attachment surface, amino acid and iron, and availability of nutrients (Ali and Wakte, 2016).

Benda et al. (2020) noted lesser results than those established in this research where he noted only 14 out of 86 (16.3%) of isolates were biofilm formers in great shape. Biofilm is the major arresting virulence factor of *Pseudomonas aeruginosa* and has a beneficial effect on chronic disease. In healthcare, biofilm formation deals with about 65-80% of pathogenic infections. Biofilm formation has been highlighted by several studies because biofilm acts as a barricade that limits

antimicrobial entry to cells and improves adaptive resistance (Khan et al., 2024). Biofilm is the predominant cause of invasive infections in burn patients. Although gram-positive bacteria are the initial microorganisms to colonize the location of infection, then other bacteria including *Pseudomonas aeruginosa* colonize these wounds (Alhazmi, 2015). A modern study recorded from Nepal demonstrated that the pervasiveness of *Pseudomonas aeruginosa* infection in burn wounds was 11%, in India, it was about 32.1%, of which 73.8% of isolates have biofilm producers (Kunwar et al., 2021).

Siderophores production by *Pseudomonas aeruginosa* isolates were identified by growing them on CAS plate. The results of culture reveal that all isolates were positive and high for Siderophores production after 24-48 hours of incubation. Similar findings have been reported by (Ali and Wakte, 2016) and also found by (Ali and Vidhale, 2011). Siderophores can be considered as force against host cells (Wilson et al., 2001) establish that *pseudomonas* benefit the siderophores pyoverdine to segregate iron from host cell proteins, such as lactoferrin, leading to death of the host cell. A preceding study noted that the siderophores unit production by *Pseudomonas aeruginosa* (80%) is better than other bacteria such as *Bacillus* spp. (Ghosh et al., 2015). The results of other studies reverse to the present study because the growth medium was variance. Screening for the most active siderophore-producing bacteria is not only for producing a higher unit of siderophore but also the ability to produce a higher siderophore unit at a short incubation period. However, siderophore production

concentration decreases when the iron concentration increases in the medium by *Pseudomonas aeruginosa*, which referring that siderophore production by bacteria for scavenging iron take place under a finite iron situation (Ustiatik et al., 2021). For most living microorganisms, iron is a potential element for its growth because it is service as a catalyst in synthesis of both DNA and RNA, enzymatic processes, oxygen metabolism, electron transfer. Siderophores are transferred towards the outer membrane to bind to specific receptors, the FpvA and FptA outer membrane proteins for pyoverdine and pyochelin transferred (Kadry et al., 2018). The level of ferrous iron required by microorganisms is a nearly  $10^{-7}$  to  $10^{-5}$ M. In order microorganisms siderophore that is a metal-chelating agents primary operative to catch the insoluble ferric iron from variance surroundings. Actually studies demonstrated that gram positive and gram negative bacteria manufactured siderophore underneath iron-destitute situation. It has been recorded that facultative aerobic bacteria *Pseudomonas* can yield siderophores in anaerobic and aerobic conditions. Siderophores first linked with iron ( $Fe^{+3}$ ) securely and through the cell membrane, the complex moves into the cell using the specific siderophores receptors, an outer membrane receptor in gram negative bacteria, a cytoplasmic membrane protein and a periplasmic binding protein relationships to ATP-binding cassette transporter are contributes in the transferred of siderophore iron complex. Despite of the mainly utilization of siderophores is to fund soluble iron to microbe, they as well as carry out many purposes in distinct field such as ecology, biosensor, bioremediation, agriculture, and medicine (Saha et al., 2015).

## Conclusion

The study demonstrated that *Pseudomonas aeruginosa* is closely associated with various patient infections, particularly nosocomial and chronic human infections, due to its ability to form biofilms. The research highlighted that biofilm formation significantly varies among different *P. aeruginosa* strains, with a higher incidence observed in wound infections. Additionally, two types of siderophores—pyoverdine (Pvd) and pyochelin (Pch)—play distinct roles in iron scavenging from the surrounding environment and host cells. All tested isolates were found to produce siderophores, and the distinction between siderophore producers and non-producers becomes more pronounced as iron limitation increases, as siderophore production is tightly regulated by iron availability.

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**How to cite this article**

Khalid, S. A., Khalid, N. A. and Al-bayati, R. A. K. (2024). Evaluation siderophores production and biofilms formation among *Pseudomonas aeruginosa* isolated from clinical infections. *Microbial Science Archives*, Vol. 4(3), 30-34. <https://doi.org/10.47587/MSA.2024.4304>.

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