# Determination Of Multi Drug Resistance (MDR) Pseudomonas Aeruginosa Isolated From Clinical Sources

**Zainab Jawad Salman [(1)], Jehan Abdul Sattar Salman [(2)], Raghad Akram Aziz [(3)]**

[(1)(2)] Department of Biology, College of Science, Mustansiriyah University
[(3)] Department of Science, College of Basic Education, Mustansiriyah University

## Abstract

The current study included the isolation and identification of *Pseudomonas aeruginosa* from different clinical sources, as (51) isolates were obtained from (143) samples collected from several hospitals in the city of Baghdad during September 2023 to December 2023. The antibiotic susceptibility test was done for all isolates using eleven antibiotics. After conducting the sensitivity test, the isolates showed resistance to all the antibiotics used in varying proportions, the highest ratio was to Doxycycline which is 90%, followed by Cefepime, Tetracycline, and Ceftazidime which are ranged between (83%, 75%, and 65%), respectively. While ciprofloxacin, gentamacin, and aztreonam showed the lowest rates of resistance, ranging between (18%, 20%, and 22%), respectively. This study was designed to identify multi-antibiotic resistant *P. aeruginosa* isolates and test their ability to produce biofilm. It was found that out of 51 clinical isolates of *P. aeruginosa* bacteria, 26 isolates were multi-resistant to antibiotics, among them, 23 were biofilm producers, while 3 isolates were non-biofilm producers.

**Keywords:** *Pseudomonas aeruginosa*, Antibiotic sensitivity, Biofilm formation, Multi drug resistance

## Introduction:

*Pseudomonas aeruginosa* is a Gram-negative opportunistic pathogen that is aerobic, heterotrophic and motile. It is also a model bacterium for investigating virulence and bacterial social features. It can be found in practically any habitat that is affected by humans or animals, even though it may be isolated in small numbers from a wide range of settings, including as soil and water. It is commonly called a 'opportunistic' disease since it rarely infects healthy people [1]. *P. aeruginosa* displays high flexibility and variability as a microorganism, enabling it to adjust to diverse environmental conditions [2]. The multitude of virulence factors that *P. aeruginosa* possesses allow it to be flexible and adaptable, enabling it to tailor its

response to many environmental stressors [3]. *P. aeruginosa*'s pathogenesis includes a number of virulence factors that exist both inside and outside the cell. These include Exotoxin A (ExoA), elastase, flagella, lipopolysaccharide, pyocyanin, pili, and phospholipase [4].

Treatment failure has emerged as a significant worldwide issue as a result of this bacterium's broad involvement in the development of numerous diseases and the rise in antibiotic resistance [5]. *P. aeruginosa* infection is a common health issue among hospital patients. Increased bacterial infections in Iraq during recent years, combined with great resistance to a wide range of antibiotics, as well as environmental pollution and a lack of health knowledge, have all contributed to increased infection rates of various bacterial isolates [6]. It is the main cause of infections from burn injuries, and swimmer's ear. In addition to ventilator-associated pneumonia, meningoencephalitis, and sepsis. It is a cause of respiratory and urinary tract infections, bacteremia, dermatitis, soft tissue infections, bone and joint infections, gastrointestinal and systemic infections, particularly in patients with severe burns, bed ulcers, and immunocompromised cancer or AIDS patients. It is also the most common colonizer of medical devices, including catheters, nebulizers, and humidifiers [7,8,4]. *P. aeruginosa* is the cause of 10% of hospital-acquired infections globally.

As *P. aeruginosa* is frequently resistant to a variety of antibiotics classes [9], Antimicrobial resistance around the world make antibiotics used to treat P. aeruginosa infections ineffective [10]. Contributes in the increasing development of antibiotic resistance against *P. aeruginosa*, the widespread and uncontrolled use of antibiotics [11]. Where *P. aeruginosa* is highly susceptible to developing resistance against a variety of antibiotics, such as aminoglycosides, β-lactams, fluoroquinolones, and several disinfectants. Its innate and acquired mechanisms account for this. *P. aeruginosa*'s intrinsic resistance involves decreasing membrane permeability, overexpressing efflux pumps that eject antibiotics from the cell, and synthesizing enzymes that break down antibiotics, such as AmpC β-lactamases and aminoglycoside-modifying enzymes.

Furthermore, the formation of *P. aeruginosa* biofilms is thought to be one of the main resistance mechanisms in the bacterium, and these biofilms are essential for shielding the bacteria from host immune reactions and are up to 1000 times more resistant to antibiotics than planktonic cells [12]. So multi-Drug Resistant (MDR) *P. aeruginosa* is a major bacterial pathogen that

causes illness and has a high death rate due to resistance to many medications [5]. Communities of bacteria adhered to surfaces, known as biofilms, are present in industrial, natural, and medicinal environments [13]. *P. aeruginosa* can attach itself to a variety of surfaces and create biofilms that can cause long-term infections by strengthening the bacteria's resistance to immune system function, irradiation treatments, antibiotics, and disinfectants.

Antibiotics have long been a viable option for treating bacterial illnesses since they are affordable and extremely effective against a wide range of bacterial agents. Nonetheless, for a variety of reasons, including overuse and misuse of broad-spectrum antibiotics, bacterial resistance to existing medicines has grown, and MDR strains have rapidly spread globally. Thus, biofilms and *P. aeruginosa's* intrinsic and acquired antibiotic resistance mechanism have increased the predominance of MDR strains in recent years, and there are virtually no fully effective medicines available to combat this bacteria [5].

The current study aimed to determine the MDR *P. aeruginosa* isolated from different clinical sources.

## Materials and Method

### Collection of samples:

*Pseudomonas aeruginosa* were collected from 143 different clinical sources during September 2023 to December 2023 from various hospitals in Baghdad which are the Medical City, Al-Shaheed Al-Sadder Hospital, Specialised Sensitivity Center, Ibn-Albaladi Hospital, Al-Kindy Teaching Hospital, and Al-Kadhimiya Teaching Hospital. Clinical sources of collection were swabs of wounds, sputum, urine, ear, blood, burns, cerebrospinal fluid, bronchial, and respiratory tract infections.

### Identification of *Pseudomonas aeruginosa*:

### Morphological Characteristics

The phenotypic characteristics of the bacterial isolates were studied by growing them on culture media such as Blood agar, MacConkey agar and Cetrimide agar, and incubating them at a temperature of 37 °C for 18 to 24 h, and then observing the shape of the colony, its texture, color, and edges, and the type of decomposition and its production of pigment [14].

### Microscopical Examination

Microscopical examination of the cells of bacterial isolates was carried out by stained using a gram stain and examined under a light microscope to observe the shape of the bacterial cells, their color and arrangement [15].

**Biochemical Tests**

An oxidase and catalase test was performed to test the ability of the isolates to produce the enzymes oxidase and catalase [16].

**Identification using Vitek 2 System:**

Commercial tests (GN VITEK2 Gram negative colorimetric identification kit) for *P. aeruginosa* bacteria (Bio Merieux, France) were also used to confirm the diagnosis.

**Antibiotic Susceptibility Test:**

Susceptibility test was performed for isolates using Kirby-bauer disk diffusion method [17]. The tested antibiotics were aztreonam (ATM; 30 µg), amikacin (AK; 30 µg), imipenem (IPI; 10 µg), doxycycline (DO; 30 µg), tetracycline (TE; 30 µg), tobramycin (TOB; 10 µg), ceftazidime (CAZ; 30 µg), ciprofloxacin (CIP; 10 µg), gentamicin (CEN; 10 µg), cefepime (FEB; 30 µg) and ceftriaxone (CTX; 30 µg). The clear zone's diameter was measured, and the Clinical and Laboratory Standards Institute (CLSI) 2023 was used to evaluate the observations [18].

**Detection of Biofilm Formation:**

To estimate the ability of the isolates to form biofilm, the concave microtiter plate method was used. This method was adopted to quantitatively investigate the ability of bacterial isolates to form biofilms, as described by Mathur et al. (2006) and Kord et al. (2018) [19,20], as follows:

The 96 well microtiter plate with 180 µl of brain heart infusion broth contains 2% sucrose inoculated with 20 µl of the overnight bacterial suspension equivalent to 0.5 McFarland standard in triplicates for each isolate. Wells contain 200 µl of BHI broth with 2% sucrose considered as control wells. The wrapped microtiter plate incubated at 37 C for 24 h. After that the non-adherent bacterial cells were emptied and rinsed the wells three times with distilled water and leave it to dry at room temperature for 15 min. Then 200 µl of crystal violet (0.1%) was added to the wells for 25 min. After removing the crystal violet, wells were rinsed three times with distilled water to remove unbound pigment and and left to dry. Then 200 µl of 96% absolute ethanol were added. The absorbance of each well was measured at 630 nm using ELISA reader. Efficiency of the bacterial isolates in biofilm formation was determined as follows:

ODc means optical density of control,

OD ≤ ODc means (Negative),

ODc < OD ≤ 2 ODc means (Weak),
2ODc < OD ≤ 4 ODc means (Moderate),
4ODc < OD means (High).

**Results and Discussion**

**Isolation and Identification of *P. aeruginosa*:**

All *Pseudomonas aeruginosa* isolates obtained from various clinical sources were identified using cultural characteristics, biochemical tests, and the Vitek 2 system.

After cultivation on blood agar, colonies appeared as black colonies with beta-hemolytic. While its cultivation at 37 C and 18-24 hours on MacConkey agar, the colonies looks like large pale colonies as they do not ferment lactose [16,21]. On cetrimide agar which is special medium for *P. aeruginosa*, the isolate appear as fluorescent green or blue as a result of pyocyanin and pyoverdin pigments production which fluoresces when exposed to ultraviolet rays [22,23] (Figure 1). This bacterium has the ability to produce pigments such as Pyocyanin which are fluorescent blue, pyoverdine which are green, and reddish-brown (Pyorubin) [24]. The isolates demonstrated their ability to develop on solid Cetrimide agar media under aerobic circumstances; due to their resistance to the substance, the bacterial colonies appeared green-yellow and in a mucous form [25].

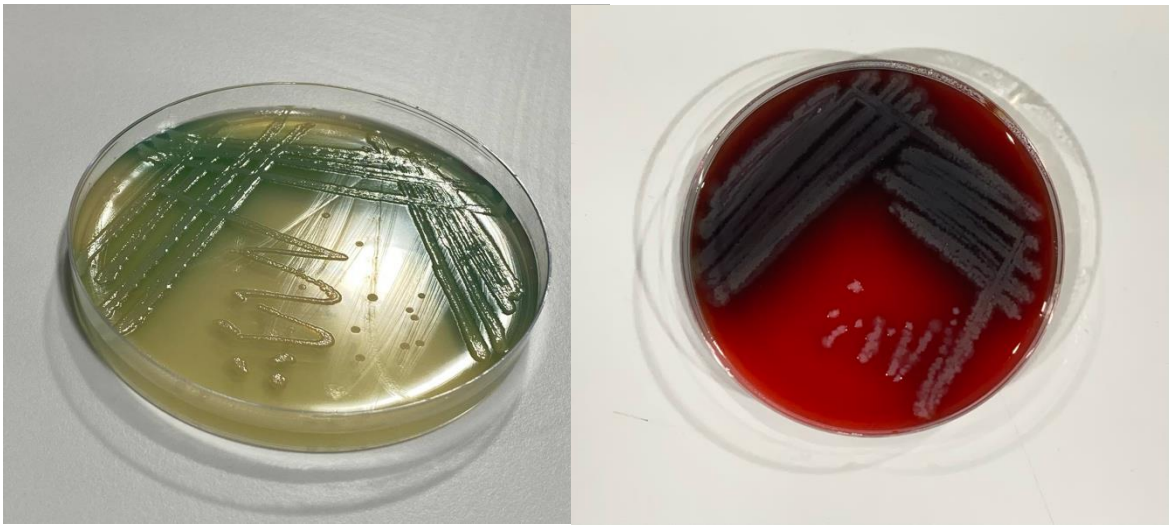

**Figure1: *Pseudomonas aeruginosa* on cetrimide agar and blood agar.**

Microscopic examination showed that *P. aeruginosa* appear as gram-negative small rods in a single cells or in pairs [26].

Examine the ability of isolates to produce oxidase and catalase enzymes was positive for all isolates. The oxidase test is effective for detecting bacteria

that contain cytochrome oxidase, an enzyme that is essential in the electron transport chain. It distinguishes oxidase-negative *enterobacteriaceae* from oxidase-positive *Pseudomadaceae* [27]. The catalase test is also used to identify the catalase enzyme in bacteria, which is responsible for converting hazardous hydrogen peroxide to innocuous oxygen and water, and it is thought to be the most effective at distinguishing across genera [28].

After Vitek's diagnosis, 51 isolates belonging to *P. aeruginosa* were identified from a total of 143 samples.

Most of the isolates obtained came from the wounds, as 19 isolates were obtained from the wounds, followed by 9 isolates that were from the sputum and 7 of the urine, while 6 isolates were from the ear. 3 blood isolates, 3 burns isolates, and 2 cerebrospinal fluids isolates were identified. As for Broncheal and RTI, it was 1 isolate for each (Table 1).

*P. aeruginosa* is one of the important human pathogen that can produce very dangerous illnesses. More importantly, it is a common multidrug-resistant (MDR) gram-negative bacterium that is highly prevalent globally in nosocomial infections and causes pneumonia in hospitalized patients [29]. This bacterium uses its important binding factors, flagella, pili, and biofilms, to thrive on water, various surfaces, and medical devices. As a result, *P. aeruginosa* is widespread in both natural and artificial environments such as lakes, hospitals, and home sink drains [30].

**Table 1: Number of *Pseudomonas aeruginosa* isolated from different clinical sources**

| Samples | No. of samples | No. of *P. aeruginosa* isolates |
|---|---|---|
| Urine | 19 | 7 |
| Blood | 15 | 3 |
| Sputum | 22 | 9 |
| Wounds | 34 | 19 |
| Burns | 12 | 3 |
| Ear | 17 | 6 |
| CSF | 11 | 2 |
| RTI | 5 | 1 |
| Bronchial | 8 | 1 |
| Total | 143 | 51 |

**Antibiotic Susceptibility test:**

Susceptibility test was performed for all isolates using the disk diffusion method toward eleven antibiotics. Antibiotic sensitivity is categorized into sensitive, intermediate, and resistant. The isolates showed resistance to all the antibiotics used: aztreonam, amikacin, imipenem, doxycycline, tetracycline, tobramycin, ceftazidime, ciprofloxacin, gentamicin, cefepime and ceftriaxone and in varying proportions showed variation in the resistance of the isolates to antibiotics, was highest at 90% to Doxycycline and at a lower rate to Cefepime, Tetracycline, and Ceftazidime, reaching 83%, 75%, and 65%, respectively. While the isolates showed close rates of resistance against both Ceftraxone and Amikacin, at 41% and 37%. The lowest resistance rate was against Tobramycin, Imipenem, Aztreonam, Gentamicin, and finally Ciprofloxacin as 25%, 24%, 22%, 20%, and 18% respectively. The results were interpreted according to the CLSI (2023) (Figure 2).

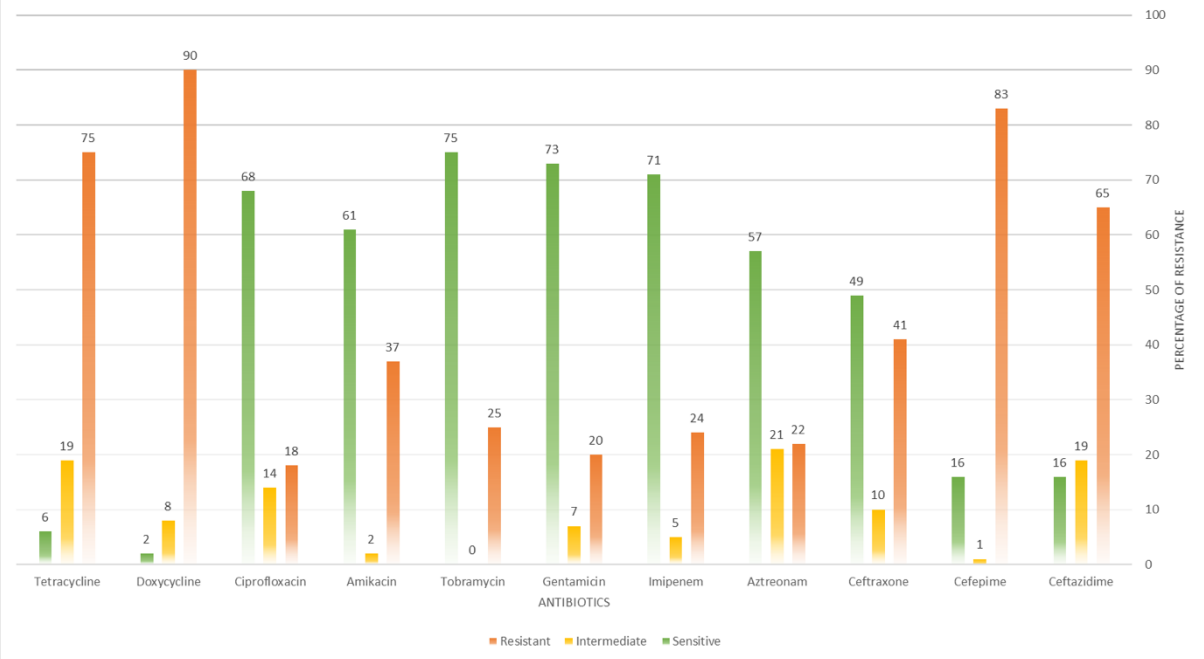


**Figure 2: The percentage of antibiotic resistance of *Pseudomonas aeruginosa* isolates.**

Therefore, the findings of this study indicate that the *P. aeruginosa* isolates under study were more resistant to the antibiotics doxycycline, cefepime, and tetracycline, at a rate of 90%, 83%, and 75%, respectively, while they were more sensitive, at very nearly rates, to tobramycin, gentamycin, imipenem and ciprofloxacin, the percentages were 75%, 73%, 71%, and 68%, respectively.

Cefepime was the second most resistant antibiotic by *P. aeruginosa* bacteria, with a rate of (83%), and this is consistent with a study conducted by [31] which found that P. aeruginosa isolates showed resistance (80%) to tetracycline. Also anther study done by [32] show a similar ratio as (80%).

The third most common antibiotic in the current study to which *P. aeruginosa* isolates showed resistance was tetracycline, where the resistance rate was (75%). Where [33] referred that bacterial isolates were resist to tetracycline (71%), and sensitive (29%) so that these results slightly agree with current study. A study conducted by [29] found that *P. aeruginosa* isolates showed a resistance rate of (78.95%) to tetracycline, and this is consistent with the findings of the current study.

The first highly effective in the current study is Tobramycin, an aminoglycoside derived from *Streptomyces tenebrarius* and is used to treat a variety of Gram-negative bacterial infections. *Pseudomonas* species are very sensitive to its effects. Tobramycin inhibits the development of the 70S complex by binding to a specific site on the bacterial 30S and 50S ribosomes. Because of this, mRNA cannot be translated into protein, resulting in cell death. In the present study, P. aeruginosa isolates were responsive to the antibiotic tobramycin (75%) and resistant (25%). These findings approach near [34] referred to *P. aeruginosa* was relatively susceptible to tobramycin (80%) and resistant (20%).While agree with [35], it indicated that *P. aeruginosa* isolates were sensitive to tobramycin at a rate of (75%).

Gentamicin which belong to aminoglycoside group used to treat various bacterial infections, especially those brought on by Gram-negative organisms [36] was the second highly effective in the current study with (73% efficacy) and (20% resistance). This is consistent with what was stated in [34], where it was found that *P. aeruginosa* isolates were 95% sensitive to gentamicin and 5% resistant, and these results are close to the current study.

The results showed that the antibiotic Imipenem is the most effective antibiotic against the *P. aeruginosa* bacteria, as the resistance rate was 24%, and this agrees with [32], where the resistance rate was 20%, and this percentage is close to what the current study found.

Ciprofloxacin belongs to the quinolone drug class of synthetic chemotherapeutic antibiotics. It's a second-generation fluoroquinolone antibacterial. Ciprofloxacin, which is commonly used to treat *P. aeruginosa* infections, inhibits bacterial infections by interfering with the enzymes that cause DNA to unwind after replication, halting DNA and protein synthesis

[37]. The results of the current study showed that ciprofloxacin ranked fourth in terms of its effectiveness against *P. aeruginosa* bacteria, with a sensitivity rate of 68%, and the bacteria were resistant to it at a rate of 18%. These findings are consistent with those of [38], who discovered that ciprofloxacin was highly sensitive in 57% of instances.

*P. aeruginosa* is a human pathogen that can cause severe illnesses. Furthermore, its internal resistance to several medicines, as well as its potential to develop high-level resistance, making these bacteria difficult to destroy. The resistance mechanism is primarily caused by the coordinated expression of drug efflux pump systems and a low-permeability outer membrane [39]. Because most commercial antibiotics have been used improperly in clinical therapy, so microorganisms have developed resistance to them [12]. Examining alterations in metabolic pathways when exposed to a particular antibiotic could aid in identifying the metabolic routes accountable for microbial resistance [40] Most *P. aeruginosa* isolates were resistant to several antibiotics due to decreased membrane permeability, antibiotic inactivation enzyme production, and expression of the efflux pump system [39].

**Multi-drug resistance:**

Multi drug resistant (MDR) bacteria have become a major issue for human health, as they can endanger the lives of the elderly, children, and immunocompromised persons [39]. Bacterial infection of the wound can cause serious consequences, particularly with multidrug resistant isolates of *P. aeruginosa*, hence detecting the virulence factor structure should be explored for drug design and therapy [6].

Just over half of *P. aeruginosa* isolates, 50.98% (26/51) in the current study, were determined to be multidrug-resistant (MDR) based on the results of antibiotic susceptibility testing, displaying resistance against cephalosporines, aminoglycoside, fluoroquinolones and tetracyclines (at least three classes of antimicrobial medications). A study conducted by [41] showed that the percentage of *P. aeruginosa* isolates identified as MDR was 54% (27/50), and this result is consistent with our current study. Another study [42] showed that (14/21) 66.7% of *P. aeruginosa* isolates had the MDR trait.The outcome supported the discovery of MDR strains of *P. aeruginosa* and is consistent with the findings of Berendonk et al. [43], who discovered that *P. aeruginosa* exhibited multi-resistant to most antibiotics.

*Pseudomonas aeruginosa* is a leading cause of hospital-acquired illnesses. It frequently causes serious and potentially deadly infections that are difficult to treat because this organism has inherited multidrug resistance (MDR) and can develop resistance to the majority of effective antimicrobial drugs [44].

**Biofilm Formation:**

The ability of MDR *P. aeruginosa* isolates to form biofilm was investigated using micro titer plates. The results showed that 23/26 isolates of *P. aeruginosa* were biofilm-forming, 18/26 isolates were weak biofilm-forming and 5/26 were moderately biofilm-forming isolates, while 3 isolates showed their inability to form biofilm (Table 2). That is, the percentage of the isolates' ability to form a biofilm was 88.46%, and this is consistent with [45] as its results indicated that 95.56% of the bacterial isolates have the ability to form a biofilm. It was mentioned [46] that isolates of *P. aeruginosa* bacteria isolated from various clinical sources have the ability to form biofilms at a rate of 92%. Likewise, a study found that *P. aeruginosa* bacteria isolated from the conjunctiva and cornea have the ability to form biofilms at a rate of 85.7% [47].

**Table 2: Biofilm formation of MDR *Pseudomonas aeruginosa* isolates from different clinical sources**

| Sample source | No. of isolates | No biofilm formation | Weak biofilm formation | Moderate biofilm formation | Strong biofilm formation |
|---|---|---|---|---|---|
| Ear | 3 | - | 2 | 1 | - |
| Sputum | 5 | 1 | 3 | 1 | - |
| Urine | 5 | - | 3 | 2 | - |
| Burn | 1 | - | 1 | - | - |
| Wound | 10 | 2 | 7 | 1 | - |
| Blood | 2 | - | 2 | - | - |
| Total | 26 | 3 | 18 | 5 | - |

One essential component of the pathogen's pathogenicity that allows it to colonize both biotic and abiotic environments is the production of biofilms. The formation of biofilms increases antibiotic resistance, making it difficult to completely eradicate infections. Quorum sensing regulates the creation of biofilms and coordinating the synthesis of additional virulence agents, including proteases, exotoxins, and siderophores, which amplify and prolong the overall pathogenicity [48].

The bacteria build a powerful gelatinous biofilm that can adhere to almost any surface. Biofilms in burn and cystic fibrosis patients cause life-threatening skin and lung infections that can lead to death. Additionally, biofilms can lead to ear infections, sinusitis, gum disease, and tooth decay. Biofilms have an exopolysaccharides matrix (EPS) made up of nucleotides, polysaccharides, and proteins. The most efficient technique to eradicate biofilm may be to dissolve its EPS using enzymes like EPS depolymerises. Furthermore, biofilms are considered therapeutic failures because they cause antibiotic resistance [49]. In fact, living in a biofilm most likely reflects the predominant way that bacteria thrive in most environments.

A few particular characteristics define mature biofilms. An extracellular matrix that gives the community shape and protection usually covers biofilm microorganisms. The distinctive architecture of microorganisms growing in biofilms is also characterized by the presence of fluid-filled channels encircling macro colonies, which are composed of hundreds of cells. The ability of biofilm-grown bacteria to withstand a variety of antimicrobial treatments, including clinically important antibiotics, is another well-known trait. The micro titer plate test is a vital tool for the investigation of biofilms because of its simplicity, low prices, and adaptability [13].

*P. aeruginosa* is well known for producing strong biofilms that are extremely resistant to host defenses, disinfectants, and antibiotics. This makes it difficult for the bacteria to be cleared from the body and can result in the development of extremely resistant chronic infections, which can be quite problematic for medicine [3]. Mature *P. aeruginosa* biofilms are distinguished by "capped" mushroom-shaped structures and a sophisticated network of channels that transport nutrients and oxygen and removing waste products [3,50].

Gram-negative *P. aeruginosa* is an opportunistic pathogen that colonizes wounds and lives in a range of settings, including soil and water. Burn wound sepsis, which increases morbidity and mortality, is caused by complex microenvironments contaminated with bacterial pathogens such as *P. aeruginosa*. *P. aeruginosa*'s capacity to create a diverse set of virulence factors, including biofilm formation and resistance to antibiotics, environmental stressors, disinfectants, and heavy metals, improves its pathogenicity [51].

**Conclusion**

Clinical isolates of *Pseudomonas aeruginosa* show MDR and biofilm formation capabilities and the bacterial isolates showed varying resistance to antibiotics, with the highest resistance to doxycycline and the lowest resistance to ciprofloxacin.

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## تحديد بكتريا Pseudomonas aeruginosa متعددة المقاومة للمضادات الحيوية المعزولة من مصادر سريرية

**زينب جواد سلمان [1]، جيهان عبد الستار سلمان [2]، رغد اكرم عزيز [3]**
[2][1] الجامعة المستنصرية، كلية العلوم، قسم علوم الحياة
[3] الجامعة المستنصرية، كلية التربية الاساسية، قسم العلوم

**مستخلص البحث:**
تضمنت الدراسة الحالية عزل وتشخيص بكتيريا *Pseudomonas aeruginosa* من مصادر سريرية مختلفة، حيث تم الحصول على (51) عزلة من (143) عينة جمعت من عدة مستشفيات في مدينة بغداد خلال الفترة من أيلول 2023 إلى كانون الأول 2023. وتم إجراء اختبار الحساسية للمضادات الحيوية لجميع العزلات باستخدام أحد عشر مضادا حيويا. بعد إجراء اختبار الحساسية أظهرت العزلات مقاومة لجميع المضادات الحيوية المستخدمة بنسب متفاوتة وكانت أعلى نسبة للدوكسيسايكلين وهي 90%، يليها السيفيبيم والتتراسايكلين والسيفتازيديم والتي تراوحت بين (83%، 75%، 65%)، على التوالي. فيما اظهرت كل من السبروفلوكساسين و الجنتاماسين و ازتريونام اقل نسب مقاومة تراوحت بين (18%، 20%، و 22%) على التوالي. صممت هذه الدراسة للتعرف على عزلات *Pseudomonas aeruginosa* المقاومة للمضادات الحيوية المتعددة واختبار قدرتها على إنتاج الأغشية الحيوية. وجد أن من أصل 51 عزلة سريرية لبكتيريا *Pseudomonas aeruginosa*، هناك 26 عزلة كانت متعددة المقاومة للمضادات الحيوية، من بينها 23 عزلة منتجة للأغشية الحيوية، في حين كانت 3 عزلات غير منتجة للأغشية الحيوية.

**الكلمات المفتاحية:** الزائفة الزنجارية، الحساسية للمضادات الحيوية، تكوين الأغشية الحيوية، المقاومة المتعددة للمضادات الحيوية