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Incidence and Multidrug Resistance of *Pseudomonas Aeruginosa* Isolated from Clinical Samples in Diyala

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Abstract. Background: *Pseudomonas aeruginosa* is a gram-negative bacterium that plays a key role in the pathogenesis of immunocompromised individuals. *Pseudomonas aeruginosa* is a common cause of hospital-acquired infections, particularly in intensive care units and burn wards. This bacterium is characterized by its extreme resistance to antibiotics, which results primarily from the expression of inducible chromosomal beta-lactamase enzymes and the production of efflux pumps, which may be constitutive or inducible. It also has the unique ability to evolve resistance to virtually all available antimicrobials through mutation, in addition to its natural resistance. Objective: The study aimed to identify the most appropriate antibiotics for treating *Pseudomonas aeruginosa* isolated from hospitals. Materials and Methods: 160 clinical samples of burn and wound infections, urinary tract infections, otitis media infections, sputum, blood, and vaginitis and throat infections were collected from patients admitted to Baqubah Teaching Hospital, Al-Batool Teaching Hospital, outpatient clinics, women's clinics, and the consulting clinic in the governorate. Diyala, during the period from 3\11/2022 to 15\4/2023. Results: Screening using conventional methods and biochemical tests revealed that (26) isolates (16.25%) were *P. aeruginosa*. All isolates recorded positive results for both the catalase and oxidase tests, while the results of the IMViC tests in all isolates showed negative results for the methyl red, indole, and Voges-Proskauer tests, and positive results for the citrate consumption test. Susceptibility testing was performed using eight types of antibiotics, and the resistance and sensitivity of the isolates were verified using the standard disk diffusion method (Kirby Power). The resistance rate was 80.76% for ticarcillin, 53.84% for cefepime, 38.46% for ciprofloxacin, 46.15% for piperacillin, 25.92% for amikacin, 30.76% for tobramycin, and 30.23% for imipenem. Conclusion: The resistance of *Pseudomonas aeruginosa* to antibiotics has increased over time, leading to the emergence of new strains that are classified according to their degree of resistance into: multidrug-resistant strains and extensively resistant strains.

Highlights:

1. Pseudomonas aeruginosa is a major cause of hospital-acquired infections with high resistance to antibiotics.

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2. The study revealed a high incidence of multidrug-resistant (MDR) strains, especially in wound and ear infections.
3. Ticarcillin and Cefepime showed the highest resistance, while Amikacin was the most effective antibiotic.

Keywords: Pseudomonas aeruginosa, Antibiotic Resistance, Multidrug Resistance, Clinical Samples, Diyala

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Introduction

There are multiple causes of hospital-acquired infections, and *Pseudomonas aeruginosa* is the leading cause of this type of infection. It is also the second most common Gram-negative pathogen isolated from patients [1]. *Pseudomonas aeruginosa* bacteria are arranged singly or in short chains, containing one or more polar flagella, and are positive for the enzymes catalase and oxidase. *Pseudomonas aeruginosa* is similar to members of the Enterobacteriaceae family, but differs from them in that it is an obligate aerobe, obtaining energy by the oxidation of carbohydrates rather than fermentation. It is called non-fermentable because of its inability to ferment glucose [2].These bacteria can cause a wide range of acute and chronic infectious diseases, and their main sites of infection are the gastrointestinal tract, respiratory tract, urinary tract, ear infections, burns, and wounds. They can reach the bloodstream through these sites, leading to a systemic infection called bacteremia. Blood [3]. *P.aeruginosa* is the second most common cause of nosocomial pneumonia, the third most common cause of nosocomial urinary tract infections, and the seventh most common cause of nosocomial bacteremia [4]. *Pseudomonas aeruginosa* is a major cause of life-threatening nosocomial infections as it is a malignant agent that tends to develop Resistant to most antibiotics [5]. Symptoms of this infection are general inflammation and sepsis, especially in burn patients, where the host's skin is destroyed, and patients with a weakened immune system, including those infected with HIV or immunosuppressed cancer patients [6].

Antibiotics are natural organic substances that have the ability to inhibit the growth of other organisms and are produced by microorganisms in certain concentrations [7]. Bacterial resistance to antibiotics is a major public health problem as the indiscriminate use of antibiotics to treat infections caused by *P.aeruginosa* has led to the emergence of strains with multiple resistance to different antibiotics. It has become one of the major problems, especially among hospitalized patients, causing economic loss in healthcare centers because it leads to larger amounts of treatment and longer duration [8]. Resistance of *P.aeruginosa* to antibiotics and antiseptics

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is a major problem worldwide due to indiscriminate and unrestricted use of antibiotics [9]. It is also possible that this resistance occurs for several reasons, including the production of beta-lactamase enzymes and the impermeability of its cell wall, as well as the possibility of it containing and acquiring resistance genes from other bacterial species through plasmids and elements transferable by bacterial conjugation, and the occurrence of genetic mutations in genes that lead to the development of Resistance to these antibiotics and antiseptics and causing treatment failure of infections caused by *P.aeruginosa* bacteria [10], [11]. *P.aeruginosa* bacteria also possess many virulence factors that have an impact on their pathogenesis, including the flagellum, the fourth pili [12], the hemolysin enzyme, and the hemolysin enzyme. Hemolytic enzyme. phospholipases, proteolytic enzymes and elastases [13], as well as toxins represented by exotoxin A, which are responsible for the fever and shock associated with sepsis, with the production of pigments such as pyoverdine and pyocyanin [14]. **The aim of study:** Determine the appropriate treatment to eradicate *P. aeruginosa* isolated from clinical sources.

Methods and Materials

A. Collection of samples

Samples were collected under specialized medical supervision, and included 160 samples from various medical conditions, different ages, and both sexes, for the period from March 9, 2022 to April 15, 2023. These included: 35 vaginal infections, 25 middle ear infections, 25 wound infections, 21 urinary tract infections, 19 burns, 14 blood samples, 13 sputum samples, and 8 throat samples from Baqubah Hospital, Al-Batool Teaching Hospital, and private outpatient clinics. They were cultured on MacConkey agar, blood agar, and Pseudomonas agar after ensuring their safety. It was ensured that antibiotics had not been taken for at least three days to isolate and initially diagnose the bacteria.

B. Diagnosis of isolates

2.1 Appearance examinations

Bacterial colonies have phenotypic characteristics when they grow on blood agar, MacConkey agar, and Pseudomonas agar, and these

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characteristics help to diagnose the characteristics of the colonies in terms of the colonies' shape, size, color, and odor emanating from them. Isolates grown in MacConkey medium are pale in color because they are unable to ferment lactose. They also appear green on Pseudomonas selective medium, which only allows the growth of Pseudomonas aeruginosa and inhibits the growth of other bacteria [2].

2.2 Microscopic examinations

Microscopic examination of bacterial isolates using a smear, stained with Gram stain, and then examined under a light microscope using an oil lens to observe the reaction of the isolates to the stain and determine the shape and arrangement of the cells [15].

2.3 Biochemical examinations

Biochemical tests for diagnosing *Pseudomonas aeruginosa* included the catalase test, the oxidase test, and the IMViC tests, which are four tests (citrate utilization test, indole test, Voges-Proskauer test, and methyl red test). Based on the method [16].

2.4 Antibiotic susceptibility testing

Susceptibility testing was performed for the bacterial isolates under study using the Kirby Bauer method, by preparing a suspension of bacterial isolates by taking (3-5) cultures growing on nutrient agar medium and aged (18-24) hours in a test tube containing (3) ml of solution. Physiological saline and the density was compared with a standard turbidity solution. Then they were left for 5-10 minutes to dry, then the antibiotic discs were placed on the inoculated agar-Muller-Hinton medium using sterile forceps, gently pressed and incubated at 37°C for 24 hours. After incubation, the results were recorded by measuring the diameter of the inhibition zone in millimeters around each disc and then comparing them to the standard tables provided in [17]. The antibiotics used against *P. aeruginosa* were as follows: amikacin (30µg), cefepime (30µg), ticarcillin (75µg), ciprofloxacin (5µg), imipenem (10µg), piperacillin (100µg), and tobramycin (10µg).

2.5 Statistical Analysis

Percentages were used to find bacterial prevalence values among clinical sources and to find the percentage of *Pseudomonas aeruginosa* resistance to antibiotics.

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Results

A. P.aeruginosa Bacterial isolation

A total of (26) isolates of *P.aeurginosa* bacteria were obtained from (160) samples, with an infection rate of 16.25%. They were collected from different isolation sources, at different ages, and from both sexes. It was collected from patients suffering from infections of wounds, burns, otitis media, urinary tract, blood, vaginal and throat infections, and sputum for the period between 3\11/2022 and 15\4/2023 from Baqubah Teaching Hospital, Al-Batool Teaching Hospital, and the consulting clinic in the governorate. Diyala. The samples were cultured on MacConkey agar medium and blood agar medium, then they were cultured on Pseudomonas agar medium for the purpose of purification, and their diagnosis was confirmed by conducting biochemical tests.

Pseudomonas aeruginosa bacteria were diagnosed based on the morphological characteristics of the bacterial isolates on each of the culture media used for diagnosis. The bacterial colonies on the blood agar medium appeared gray in color, completely hemolytic, of the beta-blood type, evidence of the bacteria's ability to produce the enzyme hemolysin [18], while colonies on MacConkey agar appeared large and convex with irregular edges, some were sticky, had a distinct odor, and were pale in color due to their inability to ferment the sugar lactose [19], and all isolates appeared in a light green color when grown on Pseudomonas agar medium, as this medium is considered selective for Pseudomonas bacteria, and these results are similar to previous studies in Iraq [20], and the results of the researcher [21]. As for the results of microscopic examination of Gram-stained bacterial isolates, it was found that there were Gram-negative bacillary cells, and this is consistent with what was mentioned [21]. Some biochemical tests were conducted to identify and confirm the isolates, and the results showed that all *P.aeruginosa* isolates gave A positive result for the oxidase test and the catalase test, due to the ability of the bacteria to produce the enzymes oxidase and catalase, and a negative result for the methyl red test, the indole test, and the Fuchs-Proskauer test, and a positive test for the citrate test [19].

B. Distribution of *Pseudomonas aeruginosa* in clinical samples

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The (26) isolates were classified according to location after they were diagnosed according to what we mentioned. It is noted from Table (1) that the largest percentage of isolates were among wound infection samples (7) isolates (28%), followed by otitis media infections, which amounted to (6) isolates (24%) of the total otitis media isolates, while The percentage of burn infections was (4) isolates (21%), in urinary tract infections the percentage was (3) isolates (14.2%), while the prevalence of bacteria in sputum and vaginal infections was (2) isolates (5.7%), and finally, the prevalence in throat and blood samples of one isolate reached [(1)12.5% and (1) 7.1%), respectively, of the total.

Table (1): Number of isolates and percentages of *P.aeruginosa* according to the source of isolation

Sample type	Total number of samples	number of *P.aeruginosa*	Percentage %*
Otitis media	25	6	%24
Burns	19	4	%21
Wounds	25	7	%28
Urine	21	3	%14.2
Sputum	13	2	%7.6
Vagina	35	2	%5.7
Blood	14	1	7.1
Throat	8	1	%12.5
Total summation	160	20	%16.25

C. Antibiotic susceptibility test results for *P. aeruginosa* bacteria

Antimicrobial susceptibility tests were conducted on 26 isolates of *P.aeruginosa* isolated from different clinical sources using the Kirby-Bauer disk method against seven different antibiotics, and the results were compared according to what was stated in [17]. *P. aeruginosa* isolates

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showed different levels of resistance to each antibiotic, and the percentage of resistance to Piperacillin was 46.15%, Ticarcillin was 80.76%, Cefepime was 53.84%, Tobramycin was 30.76%, Amikacin was 25.92%, Ciprofloxacin was 38.46%, and Imipenem was 38.46%. 30.23%, as shown in Figure (1).

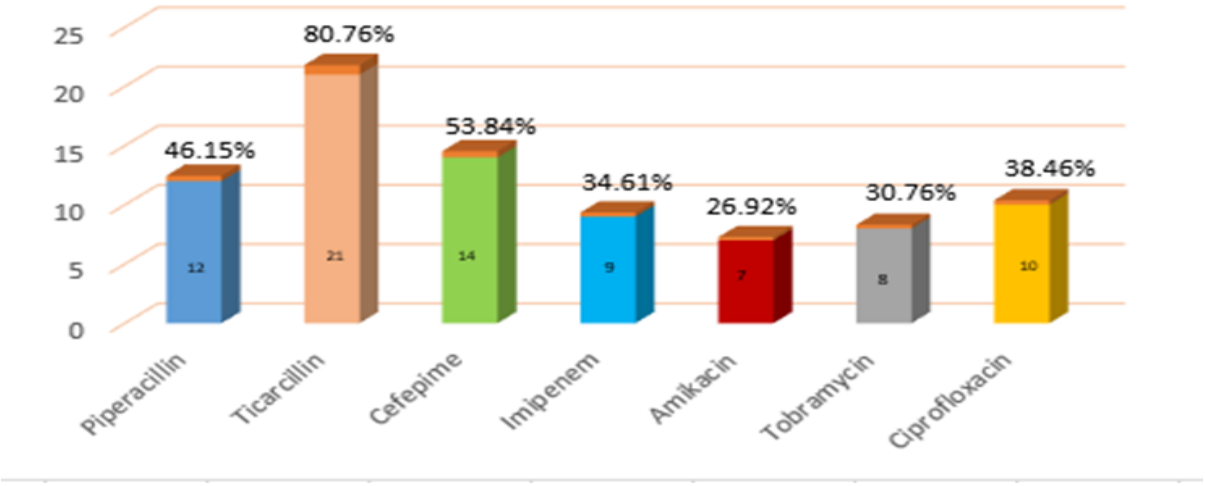

Figure (1): Antibiotic susceptibility testing of *Pseudomonas aeruginosa* isolates

Discussion

The percentage of *Pseudomonas aeruginosa* isolated from wounds reached 24.48% according to the study of researcher Al-Saadi (2020) in Baqubah, and thus it was close to the results of this study. [22]. Wounds are an important port of entry for the germ into the body, especially since wound contamination is common in our environment due to the ability of this germ to Living in water and soil, as well as injuries that originate in the hospital as a result of surgical wounds [23]. The results of the study by Muhammad *et al*. (2020) were similar to our results, which amounted to 26% of middle ear infections, as well as to what was obtained by [24], who isolated *Pseudomonas aeruginosa* from middle ear infections at a rate of 25%. The reason for the difference in isolation rates is due to individual differences in the socioeconomic awareness of patients, the health culture of patients, and the geographical area. The burn isolations in this study are similar to what Muhammad *et al.* (2020) [21] reported, which amounted to 25%. The reason

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is that damaged skin cells provide a suitable environment for *Pseudomonas aeruginosa*, which then colonizes tissues and begins to form biofilms on infected surfaces. This increases the duration of infection, making treatment difficult [25], [26].

The isolates collected from urinary tract infections in this study agreed with the results of [21] and the results of researcher [27], which amounted to (14.28% and 12.24%), respectively. This bacteria is one of the common pathogens of urinary tract infections, and the presence of these bacteria in the urinary tract is through limited factors such as urinary catheters. The most important characteristic of these bacteria is their ability to colonize the surfaces of urinary catheters and form biofilms. Therefore, when catheterization is performed, these bacteria are transmitted to the patient's urinary system, causing urinary tract infections and resistance to many antibiotics through their presence within the dense biofilm, and that most of these patients are elderly or suffer from immunosuppression [28]. The isolation rate of bacteria from vaginitis was 5.7%, and this result was consistent with the study of [29], in which the isolation rate was 5%. As for the percentage of *P.aeruginosa* isolates in the larynx, it amounted to 12.5%, and it was close to the result of [21], who found the percentage of bacteria isolated in the larynx to be 14.49%. The reason may be due to the fact that these bacteria are considered secondary opportunistic pathogens, as they take advantage of the opportunity for an imbalance to occur. General or local in one of the body's mechanical or immune defenses, or both [30]. The percentage of isolates in the blood was 7.1% and agreed with the result of [29], which was 8.5%. There are several reasons for the difference in isolation rates and types of *P.aeruginosa* in local and international studies, including sample size, different sample collection seasons, isolation times and isolation source, and differences in the geographical location of the sample and the number of samples. And other influencing factors, as well as the most important factor is antibiotics and their misuse, attention to cleanliness and the type of sterilizers and disinfectants used in hospitals.

The results showed that the resistance rate shown by *P.aeruginosa* isolates towards the group of penicillins represented by the antibiotic Piperacillin (12) amounted to 46.15%, as the results of this study are

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consistent with the findings of [31] and [32], as they recorded a resistance rate. 44% for the antibiotic Piperacillin, and researchers Raouf and Tawfiq (2015) [33] reached a resistance rate of 41.53%, while the results reached by [34] and [35] contradicted them, as they recorded a resistance rate of 74.07% and 0%, respectively.

P.aeruginosa isolates also showed high resistance to the generation of resistance to fourth-generation cephalosporin antibiotics, including Cefepime, to which the resistance rate (14) was 53.84%. This result was similar to the results of local and international studies, as [36] in Erbil obtained a resistance rate to the antibiotic. Cefepime reached 61.5%, while [37] in Egypt, and [38] in Spain found the rate of resistance to this antibiotic to be 42% and 43.4%, respectively.

In this study, we note that the high rate of resistance of *P.aeruginosa* isolates towards penicillins and cephalosporin antibiotics, such as Ceftazidime, Piperacillin, and Cefepime, is due to the ability of *P.aeruginosa* bacteria to produce beta-lactamase enzymes, such as broad-spectrum beta-lactamase enzymes (ESβLs), which work to degrade penicillins and cephalosporins in particular. whose genes are carried either on chromosomes or on plasmids in many types of bacteria, which leads to multiple resistance to different antibiotics [39]. The widespread and indiscriminate use of these antibiotics by patients, in inappropriate doses, increases the resistance of bacterial species to these antibiotics, which has led to the emergence of many strains characterized by multi-drug resistance [40]. The high rates of resistance of bacteria in general to antibiotics is a man-made problem that is widespread worldwide, but is clearly evident in developing countries of the world compared to developed countries [41].

The results of the study showed that the antibiotics Tobramycin and Amikacin, which are part of the group of aminoglycosides, have varying effectiveness against the bacteria *P.aeruginosa*, as the rate of resistance to both the antibiotics Tobramycin and Amikacin was (8) 30.76% and (7) 26.92%, respectively, in [29] found that the resistance rate of *P.aeruginosa* bacteria to the antibiotic Tobramycin was 36.2%. Also, the researcher [42] found in their study conducted in Iran, who found that the resistance rate to this antibiotic was 32.94%, and this result is consistent with the result of the

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current study. The reason for resistance to aminoglycosides is due to the production of Aminoglycoside Modifying Enzymes (AMEs) by *P.aeruginosa* bacteria, such as N-acetyl transferase and Phosphotransferase, and the genes of these enzymes are carried on the chromosome or plasmid [43], in addition to the occurrence of resistance. Also due to chromosomal mutations or a change in membrane permeability [13].

The current study recorded the rates of resistance shown by the *P.aeruginosa* bacteria to fluorinated anti-quinolones, which are known for their high effectiveness in resisting the growth of these bacteria. The rate of resistance to the antibiotic Ciprofloxacin (10) was 38.46%. This result was similar to [44], as the rate of resistance was For this antibiotic, it was 37.9%, while in a study conducted in Iran, [42], found that the resistance rate was 23.5%. The resistance of *P.aeruginosa* bacteria to fluorinated quinolones is due to a mutation in the DNA gyrase enzyme or works to inhibit DNA synthesis by stopping the action of the DNA gyrase enzyme [45]. *P.aeruginosa* isolates in the current study recorded a resistance rate of (9) 34.61% and 30.23% to the antibiotic Imipenem, which belongs to the group of carbapenems. The results of the current study agree with what was recorded by [46], who indicated that the resistance rate to this antibiotic was 31.6%. They also agreed with the results of researcher [47] in his study conducted in Baghdad Governorate, where the resistance rate reached 30%. Carbapenems are characterized as being among the most effective antibiotics for treating infections caused by *P.aeruginosa* bacteria due to their permeability through the outer membrane of the bacteria, as well as their stability against hydrolysis by beta-lactamase enzymes [48], as resistance to Carbapenems increases. By the bacterium *P.aeruginosa*, it has become a serious problem in countries around the world, as this resistance is linked to the presence of several factors, including oxacilinase enzymes, mobile genetic elements, and metallo-beta-lactamase enzymes (MBL), and many previous studies in Iraq have indicated this [49], [50], [51]. Finally, the *Pseudomonas aeruginosa* bacteria showed a very high resistance to the antibiotic Ticarcillin-clavulanate, which belongs to the beta-lactam combination, as the resistance rate was (21) 80.76%, with [52] [29] who obtained a resistance rate. 79% and 89.6%, respectively.

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Conclusions

The highest percentage of *P. aeruginosa* bacteria was isolated from wound infections. The bacterial isolates that were studied showed varying resistance to antibiotics. The highest resistance was to Ticarcillin and Cefepime, and the lowest percentage of resistance was to Amikacin. Most of the P. aeruginosa isolates under study showed Multi-resistance to MDR and XDR, which poses a major therapeutic challenge.

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