

Research article

Antibiotic Susceptibility Patterns of *Pseudomonas aeruginosa* Isolated from Burn Wound Infections

Marwa Mohammed Talib¹, Lubna Ali Abd Al-Mutalib^{1*}

ABSTRACT

Multi-drug-resistant *Pseudomonas aeruginosa* is one of the biggest challenges facing public health. The ability of these bacteria to develop resistance mechanisms makes it essential to routinely monitor their resistance to antibiotics. The current study aimed to investigate the incidence of burn infections caused by *P. aeruginosa* and to evaluate the susceptibility of these bacterial isolates to several antibiotics commonly used in the treatment of infected wounds. One hundred and fifty swabs were collected from infected burn samples. These swabs were cultured on various differential and selective media. The biochemical tests were used to identify the isolates. The VITIK 2 system was used to confirm the species of isolated bacteria. The Kirby-Bauer method was also used to determine the susceptibility of *P. aeruginosa* isolates to several antibiotics, including amoxicillin (AX), tobramycin (TOB 10), levofloxacin (LEV5), amikacin (AK10), gentamycin (CN10), and cefepime (FBP10), by measuring the diameters of inhibitory zones. The study revealed that the incidence of infected burn wounds caused by *P. aeruginosa* was 20%, and all isolates were resistant to amoxicillin. The highest susceptibility rate was to tobramycin, followed by levofloxacin. The number of bacteria sensitive to gentamicin, amikacin, and cefepime was 13, 11, and 11, respectively. It can be concluded from the current study that the incidence of burn infection with *P. aeruginosa* was 20%, and the highest sensitivity of *P. aeruginosa* was to tobramycin. Thus, we suggest that tobramycin may be a suitable choice for treating infected burn wounds caused by *P. aeruginosa*.

Keywords: Antibiotics, Burn Wound Infections, *Pseudomonas aeruginosa*, Susceptibility.

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1. INTRODUCTION

Burn injuries can be incredibly painful and often require long hospital stays, especially in Low-income countries. When someone suffers a burn, their skin loses its protective layer, which makes it easier for infections to set in. This can significantly complicate things and slow down the recovery process. One of the bacteria often found in burn wounds is *Pseudomonas aeruginosa* [1]. This bacterial species is known for causing infections in hospitals because it can resist treatments effectively and adapt to various environments. Its ability to form biofilms makes it especially tough to deal with during recovery. It's important to understand these risks when treating burn patients [2,3].

P. aeruginosa is a Gram-negative, aerobic, non-fermenting bacillus that can be isolated from moist environments, including hospital surfaces, medical equipment, and topical wound sites. It possesses several virulence factors, including elastases, exotoxins, and pyocyanin, which contribute to tissue damage and immune evasion [4]. Furthermore, this bacterial isolate's resistance to a wide spectrum of antibiotics is attributed to both intrinsic and acquired mechanisms, including low outer membrane permeability, efflux pumps, antibiotic-inactivating enzymes (e.g., β -lactamases), and the ability to acquire resistance genes [5,6].

The outbreak of multidrug-resistant (MDR) *P. aeruginosa* strains

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has become a critical concern in burn units globally, where practical antibiotic treatment is commonly administered before susceptibility profiles are available [7]. Resistance to commonly used antibiotics such as β -lactams, aminoglycosides, and fluoroquinolones complicates therapeutic options, necessitating regular monitoring of resistance trends to guide effective treatment strategies [8]. Bio-surveillance of local antibiotic susceptibility patterns is particularly important in the context of burn wound infections, where delays or failures in therapy can result in sepsis, prolonged healing, graft failure, or death [9].

Numerous studies have shown that *P. aeruginosa* can develop resistance in various hospital environments; however, information about its resistance in burn wound infections is often inconsistent and underreported, especially in developing countries where infection control may be weaker [10]. Conducting susceptibility studies on *P. aeruginosa* isolated from burn infections annually is highly important because the bacteria's resistance to different antibiotics can change over time. It is crucial to understand how *P. aeruginosa* behaves locally in these cases to enable more effective treatment and better patient outcomes [11].

The present study aims to evaluate the antibiotic susceptibility of *P. aeruginosa* isolates obtained from infected burn wounds to different types of antibiotics. The findings of this research may support the development of evidence-based experimental treatment protocols and infection control strategies tailored to local clinical settings.

2. MATERIALS and METHODS

2.1. Sample collection

In the present study, 150 swab samples were collected from inpatients with burn wound infections. The samples were collected from the Medical City Hospital in Baghdad, Iraq. Not all patients received antibiotic treatment 72 h prior to the sample collection date and consented to participate in the study. All cohorts had given consent to participate in the study. The study was conducted after receiving ethical approval from the Ethics Committee of the Department of Biology, College of Science, University of Baghdad. The samples collected from patients were transferred immediately to the clinical lab.

2.2. Isolation and identification

The collected swab samples were inoculated onto MacConkey agar. Lactose non-fermenting colonies were further sub-cultured to examine colony characteristics and pyocyanin pigment production. Cetrimide agar, a selective medium for *P. aeruginosa*, was used to screen for suspected *P. aeruginosa* colonies. Biochemical tests, including catalase and oxidase assays, were performed. Gram staining was used to observe the morphological characteristics of the bacterial cells. The VITEK 2 fluorescence-based identification system (ID-GNB card) was used to confirm the species of the isolated bacteria. For short-term preservation, bacterial isolates were maintained by streaking onto nutrient agar slants and plates, incubated at 37 °C, and then stored at 4 °C for up to one week. For long-term preservation, the isolates were suspended in nutrient broth containing 20% glycerol (Fluka) and stored at -20 °C.

2.3. Standard inoculum of *P. aeruginosa*

The identified bacterial isolates of *P. aeruginosa* were inoculated in Muller-Hinton broth (MHB, Himedia) and incubated for 18 h at 37 °C. The bacterial cell was washed three times with phosphate

buffer saline (PBS; pH 7; 0.1 M). The turbidity is equivalent to a 0.5 McFarland standard tube.

2.4. Kirby–Bauer method

This method was implemented for antimicrobial susceptibility testing. Briefly, standard inocula of bacterial isolates of *P. aeruginosa* (10^8 CFU/ml) (Pa1-Pa30) were spread onto Mueller-Hinton agar (MHA) plates. The plates were used for the sensitivity test. Standard commercial antibiotic discs (six discs were put on each plate). The standard antibiotic discs amoxicillin (AX, 10 μ g), imipenem (IMP 10 μ g), tobramycin (TOB 10 μ g), tetracycline (TE 10 μ g), vancomycin (VA 30 μ g), Levofloxacin (LEV, 5 μ g), Amikacin (AK 10 μ g), gentamycin (GM, 10 μ g), Ceftriaxone (CRO 10 μ g), cefepime (FEP 30 μ g) were checked against all 20 isolates of *P. aeruginosa*. The plate was then incubated for 18 hours at 37°C. The scale was used to measure the inhibition zones. The diameters were compared with the measured diameters of the Clinical and Laboratory Standards Institute (CLSI) breakpoint charts to determine the Sensitive (S), Intermediate (I), and Resistant (R) bacteria to the antibiotics [12,13].

2.5. Statistical analyses

The statistical analysis was conducted, and the graphs were generated utilizing Origin v. 8.6 software (OriginLab, Northampton, USA). The data were presented as means \pm standard error (M \pm SE).

3. RESULTS

3.1. Incidence of infection

The present study showed that a moderate incidence of wound infection with *P. aeruginosa* was found in 20 %. The bacterial isolates obtained from infected burn wounds were identified using biochemical tests and confirmed by the VITIK 2 system.

3.2. Antibiotic susceptibility pattern

Table 1 presents the diameter of the inhibitory zones surrounding the six antibiotic disks. The breakpoints of CLSI were used to interpret the results. Figure 1 shows the number of *P. aeruginosa* that responded to the six antibiotics. The results showed that all isolates were resistant to amoxicillin. Eighty isolates of *P. aeruginosa* were resistant to cefepime, the lowest number of resistance isolates of *P. aeruginosa* was to tobramycin. The highest number of sensitive bacteria was to tobramycin, followed by levofloxacin.

4. DISCUSSION

The previous reports showed that burn wound infections are a significant cause of morbidity and mortality in hospitalized patients who are infected with *P. aeruginosa* [14]. This pathogen is one of the common and challenging pathogens associated with burn wound infections. Its intrinsic resistance mechanisms and ability to acquire further resistance contribute to limited therapeutic options. Evaluating antibiotic susceptibility patterns is crucial for guiding effective treatment strategies and preventing the spread of multidrug-resistant strains [15]. This study investigates the susceptibility of *P. aeruginosa* isolates from burn wounds to commonly used antibiotics, including amoxicillin, tobramycin, levofloxacin, amikacin, gentamicin, and cefepime. Previous studies have shown that aminoglycosides, such as tobramycin and

amikacin, remain partially effective, and resistance to β -lactams, like amoxicillin, is widespread [16]. The present study demonstrated that conducting an annual antibiotics pattern scan on *P. aeruginosa* isolates from infected wounds is highly important, as this bacterium possesses remarkable mechanisms for acquiring antibiotic resistance. This is why bio-surveillance of *P. aeruginosa* resistance isolates is highly important. Previous studies showed that the incidence of burn infection with *P. aeruginosa* in Iraq was 20%, in Pakistan was 53 % [17,18].

Table 1. The diameter of the inhibitory zone of amoxicillin (AX), tobramycin (TOB 10), levofloxacin (LEV5), amikacin (AK10), gentamycin (CN10), and cefepime (FBP10) against thirty isolates of *P. aeruginosa* isolated from infected burn wounds. The CLSI breakpoint charts were followed to determine the sensitivity (S), intermediate (I), and resistance (R) of bacteria to the antibiotics.

	(AX)	TOB10	LEV5	AK10	CN10	FBP10
Pa1	0 (R)	20 (S)	25 (S)	15 (I)	15(S)	18 (S)
Pa2	0 (R)	22 (S)	28 (S)	16 (I)	18(S)	27 (S)
Pa3	0 (R)	20 (S)	29 (S)	17 (S)	18 (S)	24 (S)
Pa4	0 (R)	20 (S)	30 (S)	18 (S)	12(R)	22 (S)
Pa5	0 (R)	24 (S)	24 (S)	17 (S)	17(S)	22(S)
Pa6	0 (R)	20 (S)	16 (S)	15 (I)	0(R)	13 (R)
Pa7	0 (R)	24 (S)	27 (S)	16 (I)	21 (S)	25 (S)
Pa8	0 (R)	22 (S)	32 (S)	18.5 (S)	19 (S)	21.5 (S)
Pa9	0 (R)	20 (S)	25 (S)	13 (R)	19 (S)	13 (R)
Pa10	0 (R)	13 (I)	18 (S)	14 (R)	12 (R)	15 (I)
Pa11	0 (R)	18 (S)	0 (R)	15 (I)	0(R)	0(R)
Pa12	0 (R)	0 (R)	0 (R)	0 (R)	0(R)	0(R)
Pa13	0 (R)	22 (S)	12 (R)	17 (S)	0(R)	12 (R)
Pa14	0 (R)	0 (R)	0 (R)	0(R)	0(R)	0(R)
Pa15	0 (R)	20 (S)	12 (R)	21 (S)	0(R)	0(R)
Pa16	0 (R)	11 (R)	0 (R)	16 (I)	20 (S)	0(R)
Pa17	0 (R)	0 (R)	0(R)	0(R)	0(R)	0(R)
Pa18	0 (R)	13 (I)	30 (S)	15 (I)	12 (R)	13 (R)
Pa19	0 (R)	19 (S)	11 (R)	0 (R)	0(R)	10 (R)
Pa20	0 (R)	22 (S)	0 (R)	15 (I)	0(R)	0(R)
Pa21	0 (R)	22 (S)	26 (S)	17 (S)	15 (S)	22 (S)
Pa22	0 (R)	19 (S)	16 (I)	12 (R)	15 (S)	11 (R)
Pa23	0 (R)	22 (S)	15 (I)	15 (I)	15 (S)	10.5
Pa24	0 (R)	28 (S)	27 (S)	17 (S)	16 (S)	28 (S)
Pa25	0 (R)	19 (S)	25 (S)	17 (S)	17 (S)	23 (S)
Pa26	0 (R)	0 (R)	0 (R)	0(R)	0(R)	9 (R)
Pa27	0 (R)	26 (S)	30 (S)	19 (S)	26 (S)	18 (S)
Pa28	0 (R)	14 (I)	18 (S)	10(R)	11 (R)	0(R)
Pa29	0 (R)	14 (I)	20 (S)	13 (R)	10 (R)	12 (R)
Pa30	0 (R)	13 (I)	19 (S)	10 (R)	12 (R)	11 (R)

P. aeruginosa isolates employ different mechanisms to resist several antibiotics. Their mechanisms are dependent on the type of antibiotic. The resistance to β -lactam antibiotics, such as amoxicillin and cefepime, is dependent on the expression of β -lactamase. *P. aeruginosa* produces AmpC β -lactamase, which hydrolyzes different β -lactam antibiotics. It may also have

extended-spectrum β -lactamases (ESBLs) [19]. The characteristics of the outer membrane of *P. aeruginosa* may play a central role in reducing the permeability of various molecules, including antibiotics, thereby increasing resistance to amoxicillin and cefepime. Overexpression of MexAB-OprM and related systems can actively pump out β -lactams, such as cefepime [20].

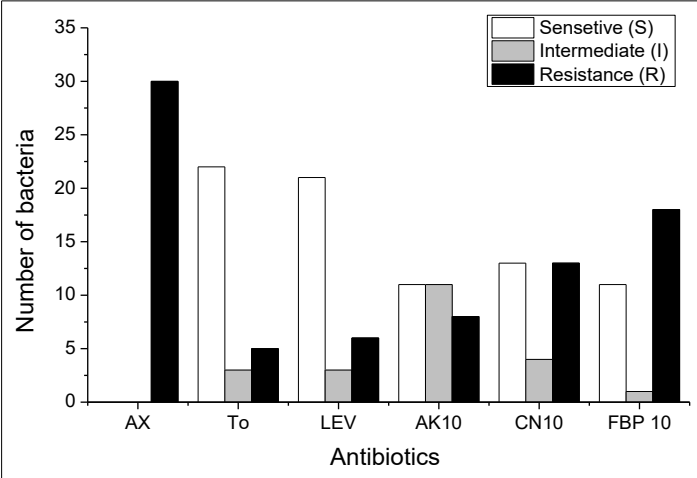


Fig 1. The number of isolates of *P. aeruginosa* that responded to the six antibiotics, amoxicillin (AX), tobramycin (TOB 10), levofloxacin (LEV5), amikacin (AK10), gentamycin (CN10), and cefepime (FBP10)

The resistance of *P. aeruginosa* to aminoglycosides, such as tobramycin, amikacin, and gentamicin, is dependent on various factors, one of which is aminoglycoside-modifying enzymes (AMEs). The methylation of the 16S rRNA in the 30S ribosomal subunit reduces the binding of drugs like tobramycin, gentamicin, and amikacin [21]. Furthermore, efflux pump systems, such as MexXY-OprM, are particularly associated with aminoglycoside resistance [22]. The resistance to fluoroquinolones, such as levofloxacin, is dependent on mutations that may occur in the target enzymes, topoisomerase IV, which reduces the binding of levofloxacin [23]. Similarly to the above, the overexpression of efflux pumps has a role in resistance to fluoroquinolones. Other mechanisms that may contribute to the resistance of *P. aeruginosa* to the aforementioned antibiotics include biofilm formation, which provides a physical barrier to antibiotic penetration and creates a dormant cell population that is tolerant to antibiotics [24]. Thus, the antibiotic resistance of *P. aeruginosa* poses a significant challenge for physicians treating infected burn wounds caused by this bacterium. The ability of this bacterium to resist the antibiotic I has been dramatically modified from time to time; thus, it is highly required to do the antibiotic susceptibility test on the *P. aeruginosa* to evaluate the situation of the resistance of this pathogen to the antibiotic and to determine the level of risk that this bacterium has reached [25, 26, 27].

5. Conclusion

The current study demonstrated that the incidence of wound infection by *P. aeruginosa* is considered moderate to high. The study revealed that *P. aeruginosa* isolates were completely resistant to amoxicillin, indicating that this treatment is no longer effective for infections caused by this pathogen. However, interestingly, the rate of resistance to tobramycin was relatively low. The study also showed that resistance to amikacin and levofloxacin was moderate, indicating that using a combination of more than one antibiotic can play a positive role in treating burn infections caused by *P. aeruginosa*.

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethical Approval

The study was carried out after receiving ethical approval from the ethics committees of the Department of Biology, College of Science, University of Baghdad, with serial number CSEC/1014/0078, 8th November, 2023

Author contributions

Marwa M Talib: Investigation; Project administration; Resources; Supervision; Validation; Roles/Writing - original draft; and Writing - review & editing.

Lubna AA Al-Mutalib: Resources; Supervision; Validation; Roles/Writing Writing - review & editing.

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