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Isolation and Phenotypic Characterization of Multidrug-Resistant *Pseudomonas aeruginosa* Isolated from Wounds and Burns of Patients in an Iraqi Clinical Setting: A Study of Their Distribution and Antibiotic Resistance

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Abstract:

Infections from *Pseudomonas aeruginosa* represent a significant public health threat as a result of the organism's innate and acquired resistance to a wide breadth of antimicrobial agents. The aim of study was to isolate and phenotypic characterization of multidrug-resistant *Pseudomonas aeruginosa* isolated from wounds and burns of patients in an Iraqi clinical setting. A combined total of 100 clinical samples were collected, evenly distributed between wound (n = 50) and burn (n = 50) specimens. Sterile swabs were used to collect the samples directly from the infection sites. Upon collection, the swabs were placed into transport tubes before transferring the samples in tubes to the laboratory, to maintain sample integrity and ensure optimal recovery conditions for bacterial isolation. The results showed that *Pseudomonas aeruginosa* was isolated from 30 of the 100 clinical samples (30%) and significant differences were found between burn samples (36%) and wound samples (25%) but this was not statistically significant ($X^2 = 1.714$, $P = 0.19$). The isolates showed distinctive characteristics on Cetrimide and King's a media, which produced Pyoverdin and *Pyocyanin pigments* (respectively). The result for both oxidase and catalase tests were both positive confirming the identity of the isolates as *Pseudomonas aeruginosa*. Patients aged 50 or older had higher isolation rates for both genders. However, the testing statistic showed that there was no relationship between age or gender and isolation rate ($P > 0.05$).

Keywords: Pseudomonas aeruginosa, Multidrug resistance, Wounds, Burns, Bacterial isolation, Phenotypic characterization, Iraqi hospitals, Biofilms, Antibiotic susceptibility testing, Antimicrobial resistance surveillance**.**

Introduction:

Infections caused by bacteria from burns and wounds are one of the most significant problems any healthcare systems, especially in hospitals with high-patient turnover and poor infection control. One of the pathogens that accounts for extreme or chronic infections in the environments and situations described above is *Pseudomonas aeruginosa*, a Gram-negative, aerobic bacterium that thrives in extreme environments such as those in medical devices and disinfectants .Infections from *P. aeruginosa* represent a significant public health threat as a result of the organism's innate and acquired resistance to a wide breadth of antimicrobial agents (Abbood and Hateet, 2025; Abd El-Halim, 2021). The World Health Organization determined that *P. aeruginosa* is classified as a "priority 1: critical" organism and has encouraged antibiotic development because of the growing prevalence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains, which has been particularly prevalent in intensive care units, surgical wards, and burn units (Abdulrazzaq and Ali, 2025; Habib et al., 2025).

Clinical data suggest that *P. aeruginosa* infections for burn and open-wound patients can cause increased mortality, more complications, a longer duration in the hospital, and more failures in conventional treatment. Completion and itchiness to deteriorating tissues and indwelling medical device, its continued resistance to treatment is further enhanced. *P. aeruginosa* can produce biofilm, contributing to chronic and recurrent infections. A recent study showed alarmingly high resistance rates among P. aeruginosa isolates against clinically significant antibiotics - gentamicin, amikacin, cefotaxime, and cefoperazone, with resistance rates for most agents greater than 60 % (Djuikoue et al., 2023; Ali et al., 2025).

Pseudomonas aeruginosa shows phenotypic resistance through several overlapping mechanisms such as the upregulation of efflux pumps (i.e. MexAB-OprM), decreased outer membrane permeability, high levels of β-lactamases (e.g. OXA, GES and CTX-M), and genetic mutations in antibiotic target sites. A recent molecular evaluation of MDR/XDR revealed that copies of the MexB gene were found widely among resistant strains, it was discovered that efflux inhibition resulted in significantly decreased minimum inhibitory concentrations (MICs) of multiple antibiotics, demonstrating the powerful effect of efflux pumps on therapeutic resistance (Abed et al., 2021; Abdulsalam, 2024). However, there continues to be a plethora of worldwide surveys, reporting on local resistant *P. aeruginosa* strains in burn and wound infections in Iraq remain sparse. A regional survey reported a widespread prevalence of extended-spectrum β-lactamase (ESBL) producers among wound isolates, with bla OXA-50 being an emerging resistance cause in the region, representing the severe need for local genetic surveys as well as effective containment measures (Abdi et al., 2024; Shilba et al., 2025). In general, current evidence suggests that *P. aeruginosa* infections in burns and wounds, particularly in developing countries like Iraq, represent a serious therapeutic challenge due to the spread of MDR and their advanced resistance mechanisms, including biofilm formation and efflux activity. Accurate isolation and phenotypic characterization of these strains is an important starting point to create an evidence-based antimicrobial map as a means to improve clinical outcomes and reduce hospital burden in Iraq.

Materials and Methods:

The study followed descriptive-analytical methodology with the aim of determining the prevalence of multidrug resistant *Pseudomonas aeruginosa* and the association with skin and skin structure infections (SSSI), wounds, and burns, in Iraqi hospital settings. A series of clinical samples were collected from hospitalized patients who were admitted to general surgery and burn units, investigated and analyzed, with the aim of isolating and identifying the bacterium and describing its antibiotic resistance patterns.

Collection of Clinical Samples:

A combined total of 100 clinical samples were collected, evenly distributed between wound (n = 50) and burn (n = 50) specimens. Sterile swabs were used to collect the samples directly from the infection sites. Upon collection, the swabs were placed into transport tubes before transferring the samples in tubes to the laboratory, to maintain sample integrity and ensure optimal recovery conditions for bacterial isolation.

Isolation and Phenotyping:

Before going to the lab, the clinical samples were inoculated onto selective and differential culture media that was suitable to isolate *Pseudomonas aeruginosa*. This comprised Cetrimide agar, a selective medium containing cetrimide that inhibits the growth of undesired organisms and increases the efficiency to recover *P. aeruginosa*. A unique feature of this medium is the ability to induce pyoverdin, a fluorescent pigment, used as a diagnostic marker for this species.

Also, King's A agar was used to induce production of pyocyanin, a blue-green pigment produced by P. aeruginosa and is a consistently reliable phenotypic marker for identification. The plates were inoculated and incubated aerobically at 37 °C for the next 24–48 hours. After incubation, the isolated colonies underwent preliminary visual observation looking for morphological and color characteristics, sheen on surface, and the characteristic grape-like smell, all of which will serve as preliminary indicators for identification of *P. aeruginosa*.

Verify Biochemical Tests:

In order to ensure correct identification of the isolates, a series of confirmatory biochemical tests were performed on the suspected colonies, which included the oxidase test. In the oxidase test, an oxidase disc containing tetramethyl-p-phenylenediamine reagent was placed on the suspected colony and a violet colouration that developed in less than 10 seconds was considered evidence of oxidase production. After this, the catalase test was performed by placing hydrogen peroxide (H_2O_2) on a colony that was actively growing, as bubbles formed immediately this indicated that the catalase enzyme was present, which would confirm identification of the isolate as *Pseudomonas aeruginosa*.

Statistical Analysis:

Data analysis was conducted using SPSS software, and the results were reported using frequencies and percentages. The chi-square test was done to assess significance of differences between age groups, sex, and sample type. A P-value ≤ 0.05 was considered statistically significant.

Results and Discussion:

Isolation Rates and Identification of *Pseudomonas aeruginosa:*

The study analyzed clinical samples for *Pseudomonas aeruginosa* and successfully isolated and identified 30 out of 100 samples demonstrating an overall isolation rate of 30%. The sample type distribution revealed 18 isolates detected among 50 burn samples (36%) and 12 isolates of *P. aeruginosa* among 50 wound samples (25%). This difference in isolation frequency of *P. aeruginosa* by sample type reflects a higher frequency in the burn specimens, which may be due to the preferential colonization of *P. aeruginosa* under the ideal environmental conditions following thermal injuries. This finding is consistent with previous reports which note infection with *P. aeruginosa* is a considerable concern and attribute predominately high prevalence of this pathogen to burn units .

Although there was a numerical difference in the isolation rate of P. aeruginosa by sample type, the outliers were removed for statistical analysis using a chi-square test. Based on the chi-square test, there was no statistically significant relationship between isolation rate of *P. aeruginosa* and sample type ($X^2 = 1.714$, DF = 1, P = 0.19). This shows that no significant relationship was found in the current study between the type of sample collected and the rate of isolation of *P. aeruginosa*.

Table 1. Isolation and identification of *Pseudomonas aeruginosa* from burn and wound samples

Sample type	Total samples	Positive isolates (*P. aeruginosa*)	Percentage (%)
Burns	50	18	36%
Wounds	50	12	25%
Total	**100**	**30**	**30%**

Statistical analysis: $X^2 = 1.714$, DF = 1, P = 0.19

Phenotypic Descriptions and Confirmatory Tests

The first-level of identification of the isolates was based on their distinctive phenotypic features observed on selective media. The isolates showed greenish fluorescent colonies with pyoverdin secretion (a marker of identification) on Cetrimide agar (Figure 1). Additionally, they produced the blue-green pigment pyocyanin (another marker of identification) on King's agar (Figure 2). Therefore, their preliminary identification as Pseudomonas aeruginosa is further supported by their growth characteristics. We subsequently confirmed the identity of the isolates by basic biochemical tests. For example, all of the isolates were oxidase positive after the finding a rapid violet colour developed on filter paper to give an oxidase positive result, and oxidase was measured when vigorous bubbling occurred when hydrogen peroxide was presented to check for catalase. These biochemistry traits correlate with classical microbiological identification traits for *Pseudomonas aeruginosa*.

Distribution by Gender and Age Group:

Of all the individuals studied, the total distributions demonstrated that the ≥50 age group had the highest frequency of *Pseudomonas aeruginosa* isolates in both sexes. For females in the

≥50 age group, the greatest number of isolates were obtained from wound specimens in which 6 isolates were recovered, and 3 isolates came from burns. Likewise, for males in the ≥50 age group, isolates from the burn specimens were the highest with 5 isolates and the lowest was 2 from wound specimens.

The distribution of isolates in the 30-49 age group was nearly the same between males and females, while the lowest number of isolates came from patients <30 years for both sexes. While there were slight differences in the distribution between sexes and age groups, there was no statistically significant difference. The chi-square values from the analyses showed no relationships, as $X^2 = 3.111$ (DF = 3, P = 0.37) for females, and $X^2 = 4.167$ (DF = 4, P = 0.38) for males. Overall, despite the planning assumptions, the data supports a greater risk of infections among the older age groups.

Table 2. Distribution of *P. aeruginosa* isolates by gender, age group, and sample type

Gender	Age group	Sample type	Number of isolates	Statistical analysis
Female	30–49 years	Burn	4	$X^2 = 3.111$, DF = 3, P = 0.37
Female	≥50 years	Wound	6	
Female	≥50 years	Burn	3	
Female	<30 years	Burn	2	
Male	30–49 years	Wound	3	$X^2 = 4.167$, DF = 4, P = 0.38
Male	30–49 years	Burn	4	
Male	≥50 years	Burn	5	
Male	≥50 years	Wound	2	
Male	<30 years	Wound	1	

Antibiotic Resistance:

Antimicrobial susceptibility testing revealed distinct resistance profiles of burn and wound isolates. Overall, burn-associated isolates presented greater resistance patterns. Complete resistance was found against Ciprofloxacin (100%) and high resistance to Rifampin (88.9%) and Ceftazidime (77.8%) and Levofloxacin and Azithromycin (72.2%). Relatively lower resistance profiles were identified against Imipenem (38.9%) and Amikacin(%61.1). Wound isolates had comparably higher resistance patterns against Rifampin and Azithromycin (83.3%); and against Levofloxacin and Ceftazidim (%75) . On the other hand, the lowest resistance patterns were observed against Amikacin (41.7%) and Imipenem (%50) .

Overall, our data suggests that Imipenem is the most effective agent against *P. aeruginosa* isolates in study, followed by Amikacin; thus these two antibiotics serve an important therapeutic purpose for potential first-line treatment options. However, no significant differences in resistance patterns between wound and burn isolates were detected by Chi-square statistical evaluation ($X^2 = 2.114$, DF = 7, P = 0.95) suggesting a comparable resistance profile in both clinical isolates.

Table (3): Resistance rates of *Pseudomonas aeruginosa* isolates against the tested antibiotics

Clinical source	AZM	CIP	LEV	GEN	RIF	CAZ	IPM	AK
Burns	13 (72.2%)	18 (100%)	13 (72.2%)	9 (50%)	16 (88.9%)	14 (77.8%)	7 (38.9%)	11 (61.1%)
Wounds	10 (83.3%)	8 (66.7%)	9 (75%)	8 (66.7%)	10 (83.3%)	9 (75%)	6 (50%)	5 (41.7%)
Statistical analysis	**$X^2 = 2.114$, DF = 7, P = 0.95**							

Pseudomonas aeruginosa was isolated in 30% of the clinical samples (36% of burns samples and 25% of wounds samples), a rate of prevalence remarkably higher than that reported by (Shubbar, 2028; Tchakal-Mesbahi et al., 2021) in Ethiopia who isolated *P. aeruginosa* from only 12.86% of burn wound samples. Differences in levels of cleanliness and infection control standards in the hospitals might be contributing factors to this difference. The current results are also consistent with (Uzoma et al., 2025; Lewandowska et al., 2025) in Algeria, who reported that more than 60% of *P. aeruginosa* isolated from burn unit patients presented as multidrug resistant (MDR), comparing favourably with the resistant patterns we observed with our isolates. Kani and Alabdali (2022) in Cameroon also expressed concern about the prevalence of carbapenemase-producing genes in *P. aeruginosa* isolates, and observed that phenotypic tests alone are no longer sufficient in identifying and treating resistant organisms, and recommended including molecular tests. In Nigeria, Abd-Ul-Salam (2024) and Sabah (2025) reported significant resistance to Imipenem, which parallels our experience with high resistance rates with Imipenem. Interestingly, the Nigerian isolates demonstrated some susceptibility to Ofloxacin, which may be attributed to geographical differences in antibiotic resistance. Also, AL-Rubaye et al. (2020) and de Almeida et al., (2025) in Sudan reported high resistance rates in their isolates, which included 74% resistance to cefpodoxime observed in wound infection, an observation we also made with our extensive, multidrug resistant strains of clinical isolates.

In regards to demographics, our study noted that predominantly a higher absolute number of the *Pseudomonas aeruginosa* isolates were above ages 50 years especially in females due to complications of wound infections and males in wounds due to burn injuries. However, the statistical analysis indicated there were no significant associations of age and sex for the isolation rate of these organisms. These findings correlate with the observations by Hasan et al. (2019) in Brazil who also noted that the prevalence of multidrug-resistant (MDR) *P. aeruginosa* was not significantly associated with the demographics of patients but the severity of the disease and patient comorbidities was associated with MDR as well as confusion in establishing what organs and systems a patient was presenting upon admission. We found no significant associations between demographics factors and resistance prevalence, much like Jaafar et al., (2014) study's findings in Nigerian hospitals. While not comparable to our study since we engaged with patients and respondents, anthropocentric factors are environmental drivers which also attest how

environmental studies demonstrate that resistance in *Pseudomonas spp*. is casually driven by environmental pressures and not by age or sex of patients (Humady and Hadi, 2025). In conclusion, it's clear that pure demographic factors impose a limited effect on the prevention of serious resistance development .Overall, our study characterizes *P. aeruginosa* as a highly prevalent and resistant pathogen in patients with burn and wound infections that will created infections of considerable degree, which demonstrates implausibility that would remain stable regardless of the demographics of patients in a hospital population that exhibit a high level of infection risk. The ecological high prevalence of MDR epitomizes the needed critical control process and establishes the necessity in embedding molecular surveillance in clinical microbiology to prepare for parasite resistance and effectively establish the required follow-up actions to mitigate infections in a growing opportunistic pathogen.

Conclusions:

The study concludes that *Pseudomonas aeruginosa* was isolated from 30 of the 100 clinical samples (30%) and significant differences were found between burn samples (36%) and wound samples (25%) but this was not statistically significant ($X^2 = 1.714$, $P = 0.19$). The isolates showed distinctive characteristics on Cetrimide and King's A media, which produced Pyoverdin and *Pyocyanin pigments* (respectively). The result for both oxidase and catalase tests were both positive confirming the identity of the isolates as *Pseudomonas aeruginosa*. Patients aged 50 or older had higher isolation rates for both genders. However, the testing statistic showed that there was no relationship between age or gender and isolation rate ($P > 0.05$). Burn isolates: High resistance was observed with Ciprofloxacin (100%), Rifampin (88.9%) and Ceftazidime (77.8%) while lower resistance was shown to Imipenem (38.9%) and relatively high resistance to Amikacin (61.1%). Wound isolates: Azithromycin and Rifampin (83.3%) and Levofloxacin (75%) was high resistance, while relatively lower resistance to Amikacin (41.7%) and Imipenem (50%). Overall, Imipenem and Amikacin were the most potent agents tested, whereas fluoroquinolones were relatively ineffective in this study.

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Declaration of Competing Interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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