Al-Mustansiriyah Journal of Science
ISSN: 1814-635X (print) 2521-3520 (electronic)

ORIGINAL ARTICLE OPEN ACCESS

Phenotypic and Molecular Characterization of Antimicrobial Resistance and Virulence Determinants in *Pseudomonas aeruginosa* Isolated from Burn Patients

Maad Abdulla Mansur [a], and Mohammed Hakeem Khalaf [b]

[a] Department of Community Health, Kirkuk Technical Medical Institute, Northern Technical University, Kirkuk, Iraq
[b] Department of Pharmacy Techniques, Kirkuk Technical Medical Institute, Northern Technical University, Kirkuk, Iraq

CORRESPONDENCE
Maad Abdulla Mansur
maadabdullahm@ntu.edu.iq

ARTICLE INFO
Received: Mar. 18, 2026
Revised: Jun. 07, 2026
Accepted: Jun. 13, 2026
Published: Jun. 30, 2026

ABSTRACT: ***Background:*** *The pathogen "Pseudomonas aeruginosa (P. aeruginosa)" is extremely hazardous for people with weak immune systems because it has several virulence factors and can resist antibiotics.* ***Objective:*** *To isolate and molecularly identify P. aeruginosa from burn and wound infections and to evaluate its antimicrobial resistance patterns, ESBL production, and virulence characteristics.* ***Methods:*** *A total of 150 burn and wound samples were collected from patients at Erbil West Emergency Hospital and processed immediately. P. aeruginosa was isolated using selective media and confirmed molecularly by PCR targeting rpoB and oprL genes. Virulence genes (nan1, plcH, exoT, toxA, aprA) were detected by PCR. Antimicrobial susceptibility testing was performed against 14 antibiotics using the disk diffusion method. ESBL production was detected using the Double Disc Synergy Test (DDST). Hemolytic and proteolytic activities were evaluated using blood agar and skim milk agar, respectively, while pigment production was assessed on cetrimide agar.* ***Results:*** *Out of 150 samples, 40 P. aeruginosa isolates were identified. Imipenem showed the highest efficacy, while complete resistance was observed against AMP, CTX, P, AMC, and C. Resistance rates were ATM (32.5%), MEM (42.5%), TOB, AK, and CN (45%), CIP (62.5%), CAZ (67.5%), and TE (80%). ESBL production was detected in 26 isolates (65%). β-hemolysis was observed in 25 isolates (62.5%), and strong proteolytic activity was detected in 35 isolates (87.5%). Typical pigment production was observed on cetrimide agar.* ***Conclusions:*** *The study reveals a high prevalence of MDR and ESBL-producing P. aeruginosa with considerable virulence potential in burns and wounds. Imipenem still holds its position as the most promising therapeutic agent; this emphasizes the need for continuous surveillance and proper antibiotic use.*

KEYWORDS: *Pseudomonas aeruginosa; Burns; Antibiotics; Virulence genes; ESBL*

INTRODUCTION

Pseudomonas aeruginosa (P. aeruginosa) has the ability to adapt quickly, which allows it to invade nearly any tissue in the human body that it comes across. This microorganism usually provides the base for infections that are obtained in a clinical environment, especially in people with a weakened immune system or in a hospital environment [1]. This pathogen has been known to cause severe infections in a hospital setting, resulting in a worsening of patient outcomes and increased risk of mortality [2]. Patients suffering from burns, those on mechanical ventilation, and cystic fibrosis (CF) patients are more susceptible to *P. aeruginosa* infection. For those suffering from CF, this bacterium is known to be a major cause of mortality [3]. The management of *P. aeruginosa* infection is becoming increasingly difficult because this organism is able to develop intrinsic resistance and also acquire extra resistance to various antibiotics [4]. In recent times, strains that display multidrug resistance, such as extended spectrum beta-lactamases (ESBLs) and metallo-beta-lactamases (MBLs), have been spreading rapidly and have become a public health problem [5]. Infections with such pathogens are often

severe and can be fatal [6]. Continuous selection of resistant strains of bacteria by prolonged exposure to β-lactam antibiotics has accelerated the development and diversification of the spectrum of activity of the β-lactamases, enhancing their ability to inactivate third- and fourth-generation cephalosporins such as ceftazidime, cefotaxime, and cefepime, and the monobactam aztreonam. These enzymes are now referred to as extended-spectrum β-lactam [7]. The detection of ESBL-producing bacteria may be performed by two major categories of methods: phenotypic methods, in which the activity of the enzyme is determined by the hydrolysis of particular types of β-lactam antibiotics, and genotypic methods, in which molecular-based tests are used for the detection of the ESBL-encoding gene [8]. Moreover, in many hospital lab settings, the Double Disk Diffusion Test, Phenotypic Confirmatory Double Disk Test, and ESBL E-Test are commonly used, mainly because of their simplicity, rapidity, and cost-effectiveness compared to the genetic testing methods. One notable characteristic of *P. aeruginosa* is the fact that it displays a distinctive blue-green color in over 90% of the strains [8]. This pigmentation is a result of the release of pyocyanin (PCN), a phenazine that has commonly been found in the ear effusions or secretions of infected patients. PCN has antimicrobial activity and allows for the competitive advantage of *P. aeruginosa* against other microbes in the environment and in the clinic. The synthesis of PCN is primarily controlled by the Las and Rhl quorum sensing pathways, with the GacA-GacS and Vfr pathways making minor contributions [9]. Therefore, owing to the changing nature of antibiotic efficacy over time and place, existing information on the antimicrobial response of *P. aeruginosa* in a given geographic location can help make more informed treatment decisions [10]. This microorganism is known to develop resistance to various forms of treatment, which limits treatment options for clinicians. Resistance is also increasing due to the spread of resistant strains among people [11].

MATERIAL AND METHODS

Collection of Samples

The total number of clinical specimens obtained was 150. These specimens were obtained from burn patients of different ages: children, young adults, and elderly people. They were obtained from West Erbil Emergency Hospital. For each specimen obtained, basic demographic information was obtained right away. These pieces of information include the patient's age and sex. Additional clinical data recorded for each patient included: (i) burn degree (first, second, or third degree) assessed clinically by the attending physician according to the depth of tissue injury; (ii) total body surface area (TBSA) affected, estimated using the rule of nines; (iii) duration of hospitalization prior to wound sampling; and (iv) history of prior antibiotic use within the four weeks preceding admission, including the specific agent(s), duration, and route of administration. Patients who had received antibiotic therapy prior to sampling were noted, as prior antibiotic exposure is a recognized risk factor for colonization with multidrug-resistant and ESBL-producing organisms. After that, the obtained swab was inserted into transport media and then subjected to analysis and processing within 30 minutes of sampling. Subsequently, these obtained specimens were cultured using Cetrimide agar and Pseudomonas agar plates. In this case, a disposable swab was uniformly streaked across these two media in sterile petri dishes.

Biochemical Testing for *P. aeruginosa*

Several biochemical assays were performed to identify the bacterial isolates obtained from burn specimens. These included tests for catalase activity, citrate utilization, oxidase production, urea hydrolysis, DNase activity, gelatinase production, motility, and protease activity.

Pigment Production

The bacterial isolates were streaked onto Pseudomonas isolation agar (PIA F) to assess the production of pyoverdin (fluorescein). The resulting colony colors were observed and documented.

Disk Diffusion Test

The disk diffusion antibiotic susceptibility test was done according to the CLSI standards (CLSI, M100-S34, 34th edition, 2024) [12]. 0.5 mL of phosphate-buffered saline (PBS) was used to suspend three to four colonies from an overnight culture on cetrimide agar. A turbidity equal to the 0.5 McFarland criterion was attained by adjusting the suspension. A sterile cotton swab was put into the

inoculum and rotated against the tube wall to remove extra liquid within 15 minutes after adjustment. In order to ensure uniform distribution of bacteria, Mueller Hinton agar plates were streaked in three different directions. This was done by rotating the plates approximately 60 degrees between streakings. After streaking, plates were allowed to settle for three to fifteen minutes. This step is followed by the application of antibiotic discs. Plates were incubated at 37 °C for 18 to 24 hours [12].

1 Extended Beta Lactamase (ESBL) Activity Detection

The double disk synergy test (DDST) was used to screen the *P. aeruginosa* isolates for the development of extended spectrum beta-lactamases (ESBLs). Distribution of bacterial suspensions, standardized to a turbidity of 0.5 McFarland, onto Mueller-Hinton agar plates was accomplished by the use of sterile cotton swabs. Cefotaxime (CTX), ceftazidime (CAZ), and aztreonam (ATM) disks were positioned 16–20 mm (center-to-center) from an amoxicillin-clavulanic acid (AMC) disk in the middle of the plate. After that, the plates were incubated at 37 °C for an entire night in order to look for any increase of inhibitory zones that would indicate the formation of ESBL [13].

Molecular Identification & Detection of Certain Virulence Genes

DNA was extracted from overnight cultures grown on BHI broth according to (Addbio genomic DNA extraction kit), PCR amplification was carried out using a thermal cycler (BioRad). ID genes (*rpoB* and *oprL*) were used to confirm its *P. aeruginosa*. Certain virulence genes in *P. aeruginosa* were characterized (*nan1*, *toxA*, *exoT*, *plcH*, and *aprA*), as shown in Table 1.

Table 1. Primers used in the current study

Amplified genes	Coding Genes	Primers	Reference
rpoB	beta (β) subunit of bacterial RNA polymerase	F- CAGTTCATGGACCAGAACAACCCG R- ACGCTGGTTGATGCAGGTGTTC	[14]
nan1	neuraminidase (sialidase)	F- AGGATGAATACTTATTTTGAT R- TCACTAAATCCATCTCTGACCCGATA	[15]
toxA	Exotoxin A	F- CTGCGCGGGTCTATGTGCC R- GATGCTGGACGGGTCGAG	[16]
exoT	Exoenzyme T	F- AATCGCCGTCCAACTGCATGCG R- TGTTCGCCAGAGGTACTGCTC	[17]
oprL	outer membrane lipoprotein L	F- ATGGAAATGCTGAAATTCGGC R- CTTCTTCAGCTCGACGCGACG	[18]
plcH	Phospholipase C (hemolytic)	F- GAAGCCATGGGCTACTTCAA R- AGAGTGACGAGGAGCGGTAG	[19]
aprA	Alkaline Protease A	F- GTCGACCAGGCGGCGGAGCAGATA R- GCCGAGGCCGCCGTAGAGGATGTC	[20]

PCR was carried out in 25 μL volume reaction mixtures containing 1 μL of each primer, 3 μL of crude template DNA and of 12 μL Qiagen master mix and 8 μL of free nuclease water. The cycling conditions used for each gene are detailed in Table 2. Then PCR products were separated by gel electrophoresis on 2% agarose gel containing 10 μg/mL safe dye.

RESULTS AND DISCUSSION

Collection of *P. aeruginosa* Isolates

A total of 150 clinical samples were collected from patients admitted to West Erbil Emergency Hospital. Among these, forty isolates were initially identified as *P. aeruginosa*, representing 26.6% of the total samples. The remaining 110 samples (73.4%) yielded other bacterial species, including Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Escherichia coli, and Proteus mirabilis, or showed no significant bacterial growth on selective media. A series of confirmatory tests was subsequently conducted to verify that all recovered isolates were *P. aeruginosa*.

Table 2. Cycling conditions for conventional PCR

Genes	Amplicon size	Denaturation	2nd Denaturation	Annealing	Extension	Final extension
rpoB	760 bp	94 ℃ 3 min	94 ℃ 1 min	58 ℃ 1 min	72 ℃ 2 min	72 ℃ 2 min
nan1	1316 bp	94 ℃ 3 min	94 ℃ 1 min	58 ℃ 1min	72 ℃ 1 min	72 ℃ 10 min
toxA	270 bp	94 ℃ 3 min	94 ℃ 1 min	63 ℃ 1.5 min	72 ℃ 1 min	72 ℃ 5 min
exoT	150 bp	95 ℃ 5 min	95 ℃ 30 sec	55 ℃ 30 sec	72 ℃ 30 sec	72 ℃ 5 min
oprL	500 bp	94 ℃ 3 min	94 ℃ 30 sec	57 ℃ 1 min	72 ℃ 1 min	72 ℃ 7 min
plcH	307 bp	94 ℃ 3 min	94 ℃ 40 sec	60 ℃ 1 min	72 ℃ 1 min	72 ℃ 5 min
aprA	993 bp	94 ℃ 5 min	94 ℃ 35 sec	58 ℃ 1 min	72 ℃ 1 min	72 ℃ 5 min

Identification of *P. aeruginosa*

1 Biochemical Tests for *P. aeruginosa*

P. aeruginosa can be distinguished from other bacterial species by its ability to grow at 42 °C. Most colonies produce a characteristic grape-like odor due to the compound amino acetophenone. The positive oxidase and catalase tests also help in differentiating *P. aeruginosa* from members of the Enterobacteriaceae family. In this study, all isolates turned violet or purple within 10 seconds, which confirmed that they were oxidase-positive. The isolates were also able to hydrolyze urea slowly, used Simmons' citrate, and were motile. On TSI agar, none of the bacteria produced gas or hydrogen sulfide (H_2S), and both the slant and butt were red. All isolates were positive for DNase activity. Gelatin liquefaction distinguished *P. aeruginosa* from other species by revealing the organism's ability to hydrolyze gelatin. Among the isolates, 35 (87.5%) exhibited proteolytic activity, 15 (37.5%) showed α-hemolysis, and 25 (62.5%) displayed β-hemolysis , as shown in Table 3.

Table 3. Biochemical tests for *P. aeruginosa*

Biochemical tests	Result	%
Catalase	+	100
Citrate	+	100
Oxidase	+	100
Urease	+	100
Gelatin hydrolysis	+	100
Proteolytic activity	+	87.5
Hemolysis activity	+	37.5 for (α), 62.5 for (β)
DNase	+	100
TSI	−	100
Motility	+	100

Distribution of Burn Wounds According to Age and Gender

Table 4 summarizes the distribution of burn patients by age and gender. Among the study population, 22 patients (55%) were female and 18 (45%) were male. The difference in burn incidence between genders was not statistically significant ($\chi^2 = 0.40$, $p = 0.527$), though a trend toward higher prevalence in females was observed. The highest percentage of female patients was recorded among the age group of 11–20 years, which accounted for 9 patients (22.5%), while the lowest percentage of female patients was recorded among the age group of 31–40 years, with only 1 patient (2.5%). Among male patients, the largest number, 7 (17.5%), was seen in the 21–30 years age group, whereas the smallest, 2 patients (5%), were observed in the 41–50 years age group. While some studies have reported a higher incidence of burns among males compared to females [21], our findings are more consistent with research from Libya, where females were more frequently affected than males [22]. Other studies have shown contradictory results. In one study, it was observed that 70% of the patients with burns were male and 30% were female. This contradicts the results obtained in the present

study [23]. Some studies [24], [25] have confirmed the results obtained in the present study. In such studies, it has been observed that more female patients are affected with the condition than male patients [26], [27]. In other studies, it has been observed that male patients are more likely to be affected with the condition than female patients. A study by [28] also reported that 75% of burn cases occurred in females compared to 25% in males, which is comparable to our results. In our study, the elevated incidence of burns among women may be ascribed to their prolonged engagement in domestic activities, such as cooking and food preparation, thereby increasing their exposure to open flames. Additionally, in some cases, social or personal crises may lead women to self-inflicted burns as a last resort when no other solutions are available. From a microbiological perspective, hormonal differences may also play a role: estrogen has been shown to modulate innate immune responses and alter the composition of skin microbiota, potentially influencing susceptibility to *P. aeruginosa* colonization in burn wounds. Furthermore, the predominance of females in younger age groups (1–20 years) in this study may reflect accidental flame burns during domestic activities, which is consistent with patterns reported across Middle Eastern populations.

Table 4. Incidence of burn according to age and gender

Age	Gender		Total No.
	Male No.	Female No.	
1-10	6 (15%)	8 (20%)	14 (35%)
11-20	3 (7.5%)	9 (22.5%)	12 (30%)
21-30	7 (17.5%)	4 (10%)	11 (27.5%)
31-40	0 (0%)	1 (2.5%)	1 (2.5%)
41-50	2 (5%)	0 (0%)	2 (5%)
Total	18 (45%)	22 (55%)	40

Color Production by *P. aeruginosa* Isolates

The isolates were cultured on cetrimide agar to assess pigment production. When grown on this selective medium at 37 °C, the resulting colony colors were recorded. Cetrimide agar is known to stimulate pyocyanin production, a pigment commonly associated with *P. aeruginosa*. In this study, the isolates produced yellow-green pigmentation indicative of fluorescein and blue-green pigmentation corresponding to pyocyanin, as summarized in Table 5. Pigment production is an important phenotypic characteristic of *P. aeruginosa* [29]. Representative colony morphology and pigment production observed on cetrimide agar are illustrated in Figure 1. The type of growth medium strongly influences pigment synthesis, with different media favoring the production of specific pigments in pseudomonads. These pigments also contribute to virulence: they act as pro-inflammatory agents, disrupt the normal function of human nasal cilia, impair the epidermal barrier, and interfere with lymphocyte activity, potentially through inhibition of electron transport [30].

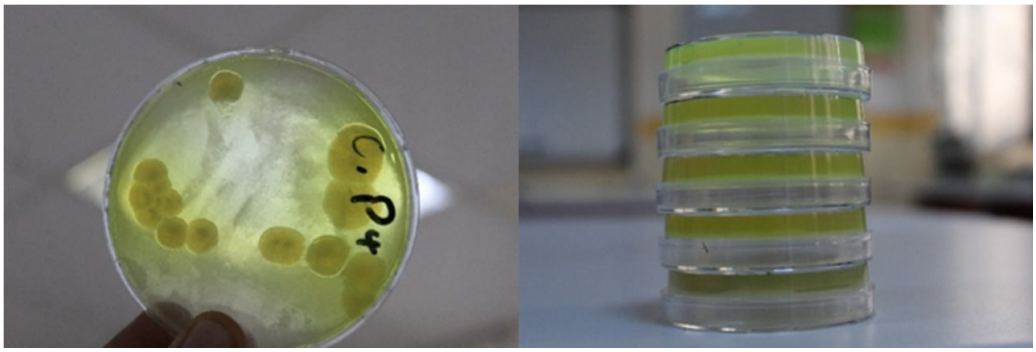

Figure 1. *P. aeruginosa* showing different pigment production on cetrimide agar

Table 5. The pigments' production by *P. aeruginosa* isolates

Pseudomonasisolation No	**Phenotypic characteristic**		**Smooth or Rough colonies**
	PLA	**Mucoid-non mucoid**	
P 1	Blue-Green Colonies	Non-mucoid	R
P 2	Blue-Green Colonies	Non-mucoid	R
P 3	Blue-Green Colonies	Non-mucoid	R
P 4	Blue-Green Colonies	Non-mucoid	R
P 5	Blue-Green Colonies	Mucoid	S
P 6	Blue-Green Colonies	Non-mucoid	R
P 7	Blue-Green Colonies	Non-mucoid	R
P 8	Blue-Green Colonies	Non-mucoid	R
P 9	Blue-Green Colonies	Non-mucoid	R
P 10	Blue-Green Colonies	Non-mucoid	R
P 11	Blue-Green Colonies	Non-mucoid	R
P 12	Blue-Green Colonies	Mucoid	S
P 13	Blue-Green Colonies	Non-mucoid	R
P 14	Blue-Green Colonies	Non-mucoid	R
P 15	Yellow- Green Colonies	Mucoid	S
P 16	Yellow- Green Colonies	Mucoid	S
P 17	Blue-Green Colonies	Mucoid	S
P 18	Blue-Green Colonies	Mucoid	S
P 19	Blue-Green Colonies	Mucoid	S
P 20	Blue-Green Colonies	Non-mucoid	R
P 21	Blue-Green Colonies	Non-mucoid	R
P 22	Blue-Green Colonies	Non-mucoid	R
P 23	Blue-Green Colonies	mucoid	S
P 24	Blue-Green Colonies	Non-mucoid	R
P 25	Blue-Green Colonies	Non-mucoid	R
P 26	Blue-Green Colonies	mucoid	S
P 27	Yellow- Green Colonies	mucoid	S
P 28	Blue-Green Colonies	Non-mucoid	R
P 29	Blue-Green Colonies	mucoid	S
P 30	Blue-Green Colonies	mucoid	S
P 31	Blue-Green Colonies	mucoid	S
P 32	Yellow- Green Colonies	mucoid	S
P 33	Yellow- Green Colonies	mucoid	S
P 34	Yellow- Green Colonies	mucoid	S
P 35	Yellow- Green Colonies	mucoid	S
P 36	Yellow- Green Colonies	Non-mucoid	R
P 37	Yellow- Green Colonies	Non-mucoid	R
P 38	Yellow- Green Colonies	Non-mucoid	R
P 39	Yellow- Green Colonies	mucoid	S
P 40	Blue-Green Colonies	Non-mucoid	R

Antimicrobial Sensitivity Screening Test for *P. aeruginosa*

A total of forty *P. aeruginosa* isolates were tested for resistance against 14 commonly used antibiotics, including amikacin, amoxicillin-clavulanic acid, ampicillin, cefotaxime, penicillin, ciprofloxacin, chloramphenicol, gentamicin, imipenem, meropenem, tetracycline, ceftazidime, aztreonam, and tobramycin. It is worth noting that ampicillin, penicillin, and amoxicillin-clavulanic acid were included for epidemiological documentation purposes only; *P. aeruginosa* exhibits intrinsic resistance to these agents due to the low permeability of its outer membrane and constitutive expression of efflux pumps (MexAB-OprM), and these antibiotics are therefore not recommended for clinical use against this pathogen. Their inclusion was intended to confirm the identity of the organism and serve as a phenotypic internal control for the susceptibility panel. The clinically relevant resistance data are therefore focused on the anti-pseudomonal agents (carbapenems, antipseudomonal penicillins, cephalosporins, fluoroquinolones, aminoglycosides, and aztreonam). The antibiotic resistance patterns of these isolates are summarized in Table 6. Representative zones of inhibition observed during antibiotic susceptibility testing are shown in Figure 2. The results demonstrated considerable variation in resistance among the isolates. Resistance rates ranged from 92.8% for isolates P1, P2, P18, P19, P22, P25, P26, P31, P32, P38, and P39 to 35.7% for P15 and P17. The remaining isolates showed intermediate resistance levels, with percentages varying from 85.7% for P3, P5, P20, P21, P24, P30, P34, and P40, to 42.8% for P6, P12, P14, P16, P28, P33, P35, P36, and P37.

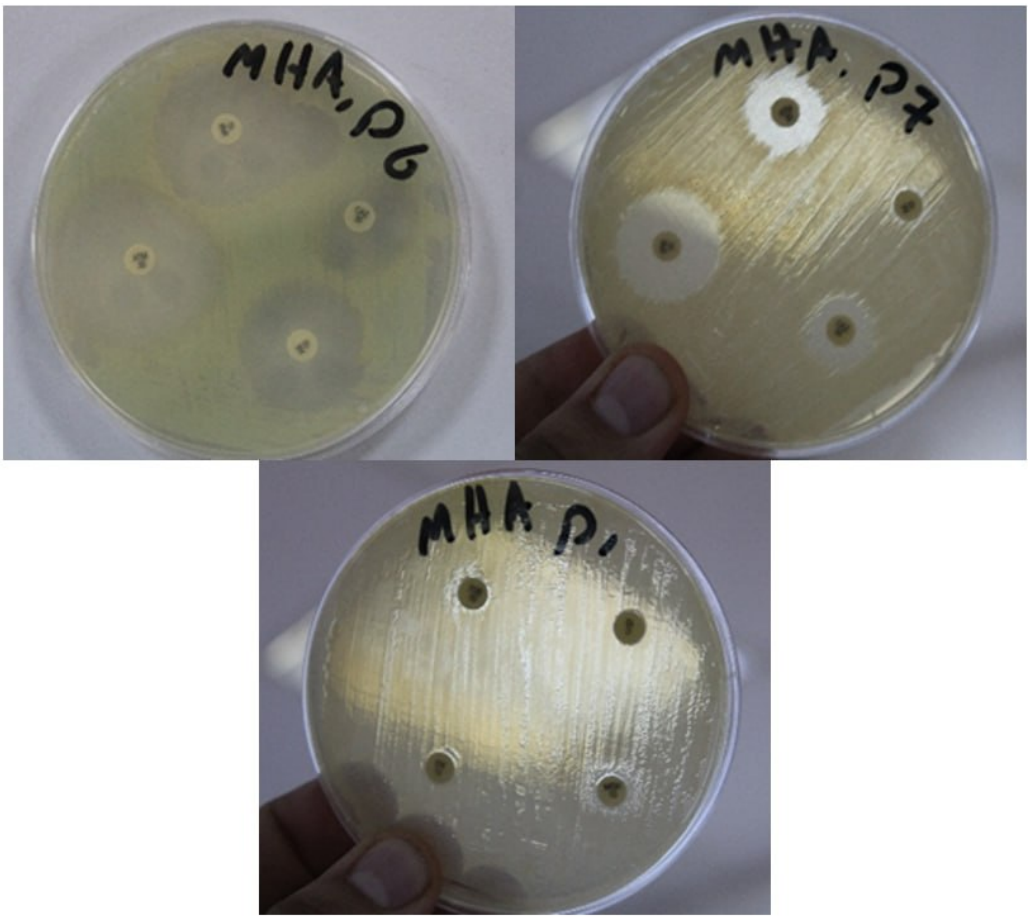

Figure 2. The antibiotic sensitivity test against *P. aeruginosa* showed various zones of inhibition

Table 6. Antibiotic resistance pattern for *P. aeruginosa* under study

No of isolates	% of resistance	Antibiotics at final concentration													
		P	**CTX**	**TE**	**CN**	**C**	**IMP**	**MEM**	**ATM**	**CIP**	**AK**	**CAZ**	**AM**	**AMC**	**TOB**
P1	92.8	R	R	R	R	R	S	R	R	R	R	R	R	R	R
P2	92.8	R	R	R	R	R	S	R	R	R	R	R	R	R	R
P3	85.7	R	R	R	R	R	S	R	S	R	R	R	R	R	R
P4	78.5	R	R	R	R	R	S	R	S	R	R	S	R	R	R
P5	85.7	R	R	R	R	R	S	R	S	R	R	R	R	R	R
P6	42.8	R	R	S	S	R	S	S	S	R	S	S	R	R	S
P7	78.5	R	R	R	R	R	S	R	S	R	R	S	R	R	R
P8	78.5	R	R	R	R	R	S	R	S	R	R	S	R	R	R
P9	78.5	R	R	R	R	R	S	R	S	R	R	S	R	R	R
P10	78.5	R	R	R	R	R	S	R	S	R	R	S	R	R	R
P11	71.4	R	R	R	R	R	S	S	S	R	R	S	R	R	R
P12	42.8	R	R	S	S	R	S	S	S	R	S	S	R	R	S
P13	78.5	R	R	R	R	R	S	R	S	R	R	S	R	R	R
P14	42.8	R	R	R	S	R	S	S	S	S	S	S	R	R	S
P15	35.7	R	R	S	S	R	S	S	S	S	S	S	R	R	S
P16	42.8	R	R	S	S	R	S	S	S	S	R	S	R	R	S
P17	35.7	R	R	S	S	R	S	S	S	S	S	S	R	R	S
P18	92.8	R	R	R	R	R	S	R	R	R	R	R	R	R	R
P19	92.8	R	R	R	R	R	S	R	R	R	R	R	R	R	R
P20	85.7	R	R	R	R	R	S	R	S	R	R	R	R	R	R
P21	85.7	R	R	R	R	R	S	R	S	R	R	R	R	R	R
P22	92.8	R	R	R	R	R	S	R	R	R	R	R	R	R	R
P23	71.4	R	R	R	R	R	S	S	R	S	S	R	R	R	R
P24	85.7	R	R	R	R	R	S	S	R	R	R	R	R	R	R
P25	92.8	R	R	R	R	R	S	R	R	R	R	R	R	R	R
P26	92.8	R	R	R	R	R	S	R	R	R	R	R	R	R	R
P27	71.4	R	R	R	R	R	S	S	S	R	R	S	R	R	R
P28	42.8	R	R	S	S	R	S	S	S	R	S	S	R	R	S
P29	71.4	R	R	R	R	R	S	S	S	R	R	S	R	R	R
P30	85.7	R	R	R	R	R	S	S	R	R	R	R	R	R	R
P31	92.8	R	R	R	R	R	S	R	R	R	R	R	R	R	R
P32	92.8	R	R	R	R	R	S	R	R	R	R	R	R	R	R
P33	42.8	R	R	S	S	R	S	S	S	R	S	S	R	R	S
P34	85.7	R	R	R	R	R	S	S	R	R	R	R	R	R	R
P35	42.8	R	R	S	S	R	S	S	S	R	S	S	R	R	S
P36	42.8	R	R	S	S	R	S	S	S	R	S	S	R	R	S
P37	42.8	R	R	S	S	R	S	S	S	R	S	S	R	R	S
P38	92.8	R	R	R	R	R	S	R	R	R	R	R	R	R	R
P39	92.8	R	R	R	R	R	S	R	R	R	R	R	R	R	R
P40	85.7	R	R	R	R	R	S	S	R	R	R	R	R	R	R

ESBL Production in *P. aeruginosa*

All *P. aeruginosa* isolates were screened for extended-spectrum β-lactamase (ESBL) production. Among the 40 isolates tested, 26 (65%) were positive for ESBL production, while 14 (35%) were negative. The main findings are summarized in Table 7.

Table 7. Number and percentage of ESBL producing in *P. aeruginosa* isolates

Production of ESBL	*P. aeruginosa* Isolates	
	Number	Percentage
Positive	26	65 %
Negative	14	35 %
Total	40	100 %

Past studies have shown that the ESBL resistance in *P. aeruginosa* varies geographically, ranging from 25% [31], in one instance, to 17.7% in patient samples [32]. In this current research, 65% of the *P. aeruginosa* strains produced ESBL enzymes, which contribute to the progression of infectious diseases. The strains have shown poor patient outcomes, mortality risks, and the absence of therapeutic options in the face of multi-drug resistance, which is a worrying trend in patients with pre-existing medical conditions. Patients who have long hospital stay durations, severe illness, compromised immunity, and the use of catheters in the urinary tract have shown a high likelihood of carrying the ESBL-producing strains [33]. This is in line with previous studies. Mushtaq *et al.* (2021) found the prevalence of ESBL-producing bacteria in the range of 65% [34], while Gales *et al.* (2023) [35], and Rezai (2018) observed the prevalence of 45% and 43.3%, respectively [36]. In recent times, the spread of ESBL-producing bacteria has accelerated globally [37]. In a different study [1], it was observed that "20.5% of *P. aeruginosa* isolates were found to harbor extended-spectrum β-lactamases, with approximately 95% producing TEM-type β-lactamases (Temoniera). Resistance to these β-lactam antibiotics often co-exists with reduced susceptibility to aminoglycosides or fluoroquinolones, making the therapeutic management of infections caused by this pathogen, which contributes significantly to nosocomial morbidity, more complicated." ESBL-mediated resistance can result from a variety of mechanisms, which include "the action of metallo-β-lactamases, reduced antibiotic accumulation due to porin mutations, loss of specific outer membrane proteins, and efflux." [38]. Plasmids are considered essential in the transmission of ESBL genes, which has resulted in the worldwide spread of antimicrobial resistance. The extensive use of β-lactam antibiotics has put pressure on the development of defense mechanisms in bacteria, including the production of ESBL enzymes, emphasizing the need to review the guidelines for testing and reporting in the laboratory [39]. The notably high ESBL prevalence of 65% in the current study warrants specific attention to local infection control policies at Erbil West Emergency Hospital. This finding suggests that prior broad-spectrum β-lactam antibiotic use, Cefotaxime (100% resistance) and Ceftazidime (67.5% resistance), may be a key driver of ESBL selection in this burn unit. Institutional measures should include mandatory ESBL screening for all burn wound isolates, restriction of empirical third-generation cephalosporin use, implementation of antibiotic stewardship programs, and contact precautions for ESBL-positive patients to prevent nosocomial dissemination.

Proteolytic Activity of *P. aeruginosa* Isolates

The proteolytic activity of 40 *P. aeruginosa* isolates was investigated on a skim milk agar plate, and 35 of these isolates (87.5%) showed proteolytic activity. However, Elnegery *et al.* (2025) [40] reported that 70% of *P. aeruginosa* isolates, out of a total of 50, showed protease activity. Similarly, Zhang *et al.* (2020) [41] reported that 30% of *P. aeruginosa* isolates possessed protease activity, whereas Grbavčić *et al.* (2011) [42] reported that 40% of *P. aeruginosa* isolates possessed protease activity. Many *P. aeruginosa* strains are known for their production of enzymes that can hydrolyze gelatin and casein. However, it has been noted [43]. Importantly, in the current study the high rate of proteolytic activity (87.5%) directly correlates with the high prevalence of the *aprA* gene (90%), which encodes alkaline protease, a metalloprotease that degrades host proteins, including elastin, fibronectin, and immunoglobulins, directly mediating the caseinolytic and gelatinolytic activities observed on skim milk agar. This strong genotype-phenotype concordance confirms that *aprA* gene expression is functionally active in burn wound isolates and serves as a major virulence determinant in burn wound pathogenesis at this institution.

Hemolytic Activity of *P. aeruginosa* Isolates

In this study, 15 out of 40 *P. aeruginosa* isolates (37.5%) exhibited α-hemolytic activity, while 25 isolates (62.5%) demonstrated β-hemolysis, as summarized in Table 6. Hemolysin production contributes to the pathogenicity of *P. aeruginosa* in burn infections by exerting toxic effects on white blood cells and fibroblasts [44]. Earlier research spotted comparable results. El-Mahdy with El-Kannishy (2019) showed that twelve *P. aeruginosa* samples from urine infections broke down red blood cells [44]. On top of that, Mohamed *et al.* (2022), as well as Sperandio's team (2010), noticed hemolysin production in 60% and 65% of *P. aeruginosa* strains taken from different medical cases [45], [46]. The alpha type of this toxin is seen as a harmful trait, possibly involved in infections outside the gut [46]. The high rate of β-hemolysis (62.5%) observed in the current study correlates strongly with the high prevalence of the *plcH* gene (87.5%), which encodes a hemolytic phospholipase C that cleaves phosphatidylcholine and sphingomyelin in the host cell membrane, thereby releasing erythrocytes and facilitating iron acquisition from hemoglobin, an essential nutrient for bacterial proliferation in the iron-poor burn wound environment. This genotype-phenotype concordance confirms that *plcH* is a principal genetic determinant of the hemolytic phenotype in these burn wound isolates, and its high prevalence underscores the capacity of these strains to cause extensive tissue destruction and evade host immune defenses.

Molecular Identification & Detection of Certain Virulence Genes

The traditional identification relied on phenotypic methods; it's still accurate, especially when dealing with this bacterium. For more confirmation, usual biochemical tests are used to confirm the bacterium, but molecular assays have shown superiority to the traditional ones. In the current study, *P. aeruginosa* was identified using the molecular marker *rpoB* throughout traditional PCR. All 40 (100%) isolates showed clear bands after they were amplified and run on gel electrophoresis, as illustrated in Figure 3. A study done by Jawher and Hassan (2022) [47] and Ali and Abdulrahman (2020) [48] agrees with our findings.

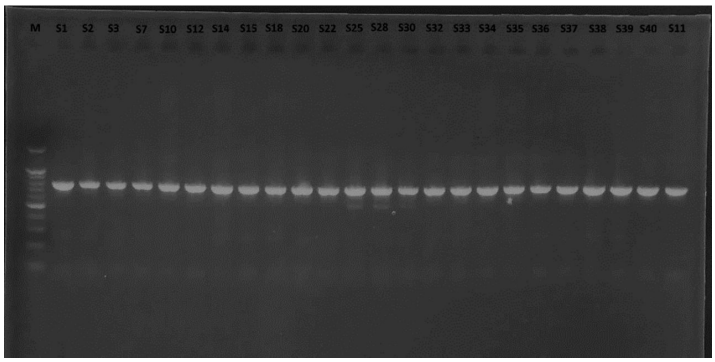

Figure 3. Gel electrophoresis of PCR product. M: ladder 100 bp DNA ladder, lanes (S1-S40) positive result at 759 bp of *rpoB* as an ID gene for *P. aeruginosa*

Another gene used to confirm the bacteria, *oprL* is one of the genes which encodes for outer membrane lipoprotein. Another function of this gene is making the bacteria more resistant to antibiotics, which makes it difficult to be treated. All samples were tested for the presence of this gene, and all 40 (100%) isolates showed positive bands, as shown in Figure 4. Studies conducted by Lavenir *et al.* (2007) and Khattab *et al.* (2015) showed an agreement with our result with 100% of all samples [14], [49].

P. aeruginosa exhibits various virulence genes like *toxA*, responsible for the production of exotoxin A. These toxins are translation inhibitors, meaning they stop protein synthesis during the elongation step, which modifies the EF2. If a cell stops producing protein, it leads to cell death (necrosis), which allows the bacteria to destroy host tissues and enter deeper in the body. In the current study, *toxA* was detected in 28 (70%) (as in Figure 5) of *P. aeruginosa*, which is similarly shown by Nikbin *et al.* (2012) (90%) and El Husseini (80%) isolated from burned patients, respectively [50], [51].

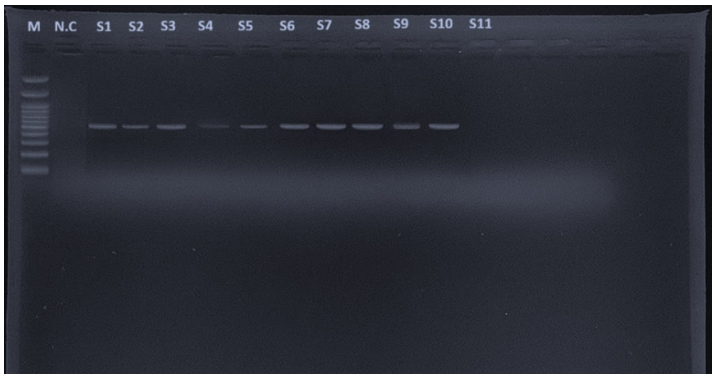

Figure 4. Gel electrophoresis of PCR product. M: ladder, 100 bp DNA ladder, N.C. (negative control), lanes (S1-S11) positive result at 500 bp of *oprL* as a virulence gene for *P. aeruginosa*

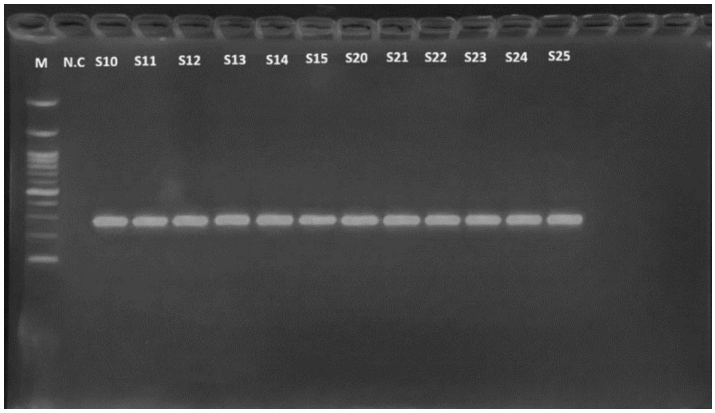

Figure 5. Gel electrophoresis of PCR product. M: ladder, 100 bp DNA ladder, N.C. (negative control), lanes (S10-S25) positive result at 270 bp of *toxA* as a virulence gene for *P. aeruginosa*

Phospholipid is one of the human cell membrane components and lung surfactants; during the infection with *P. aeruginosa*, which expresses the *plcH* gene, it causes a break in the skin of the host cells, like lung cells, which causes them to burst. In the current study, *P. aeruginosa* showed a very high percentage of the *plcH* gene was detected in 35 (87.5%), as shown in Figure 6; the prevalence of this gene in clinical samples implies the essentiality of a factor for the survival of *P. aeruginosa*. The same results were observed by Jasim and Hussein (2023) [52], and other research by Mackinder *et al.* (2024) was also were nearly to the current study [53].

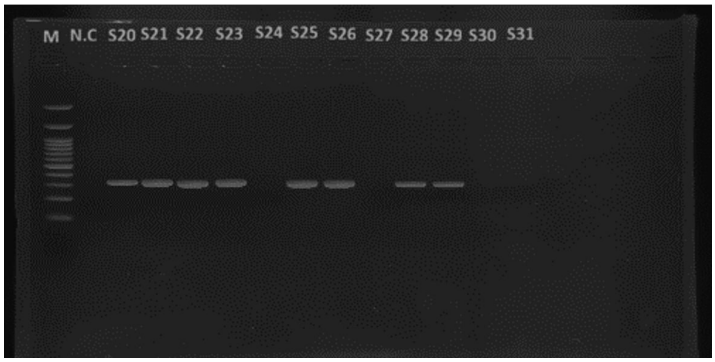

Figure 6. Gel electrophoresis of PCR product. M: ladder 100 bp DNA ladder, N.C. (negative control), lanes (S20-S31) positive result (except S24, S27, S30, S31) at 307 bp of *plcH* as a virulence gene for *P. aeruginosa*

Regarding the *nan1* gene in this study, it has been found in 27 (67.5%) (as illustrated in Figure 7) of *P. aeruginosa*; this result correlates with studies done by Nikbin *et al.* (2012) and Edward *et al.*

(2023) [50], [54]. One of the functions of the *nan1* gene is assisting the bacteria to form a biofilm, which makes the bacteria multidrug resistant and makes it difficult to eradicate.

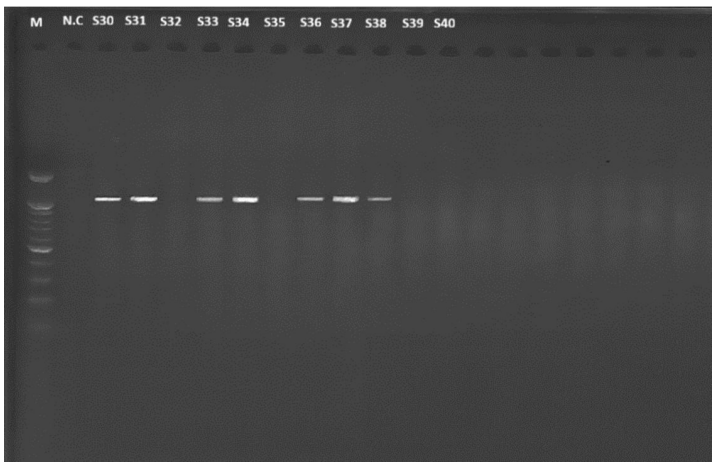

Figure 7. Gel electrophoresis of PCR product. M: ladder 100 bp DNA ladder, N.C. (negative control), lanes (S30-S40) positive result (except S32, S35, S39, S40) at 1316 bp of *nan1* as a virulence gene for *P. aeruginosa*

One of the most problematic issues during infection with *P. aeruginosa* is the *exoT* gene present in this bacterium, acting as antiphagocytosis, which prevents the macrophages from engulfing the bacteria. Another issue with this gene is inhibiting the wound healing process, which is why *P. aeruginosa* infections are chronic and slow to heal. In our study, the gene was present in 35 *P. aeruginosa*s (87.5%), as shown in Figure 8; the same results were obtained by Jurado *et al.* (2021) and El Husseini *et al.* (2024) [51], [55].

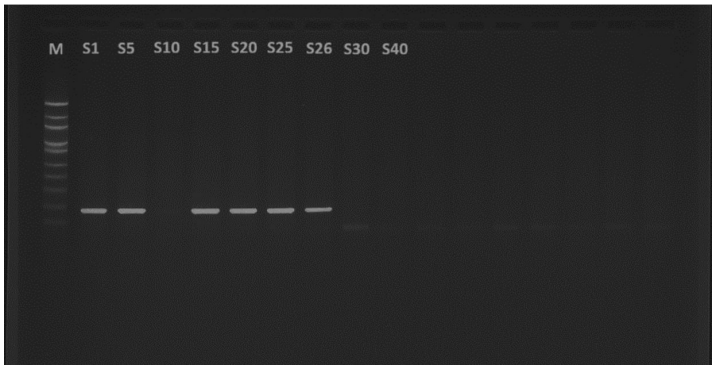

Figure 8. Gel electrophoresis of PCR product. M: ladder 100 bp DNA ladder, lanes (S1, S5, S15, S20, S25, S26) positive result (except S10, S30, S40) at 150 bp of *exoT* as a virulence gene for *P. aeruginosa*

The current study showed a high presence of the *aprA* gene detected in burned patients, with 90%, as illustrated in Figure 9; therefore, expression of the *aprA* gene is likely related to the burn wound environment, where the abundant availability of denatured proteins and necrotic tissue provides a rich substrate for alkaline protease activity. The high expression of *aprA* in burn patients correlates with the extensive tissue damage characteristic of burn injuries, facilitating bacterial invasion of deeper tissues and degradation of host immune components such as immunoglobulins and complement factors and contributing to the chronicity and severity of burn wound infections. The metalloprotease encoded by *aprA* also inactivates flagellin and type IV pili, impairing neutrophil-mediated killing, a mechanism particularly detrimental in the immunocompromised state of burn patients. These findings phenotypically correlate with the high rate of proteolytic activity (87.5%) observed in the current study, confirming that *aprA* gene expression is a major driver of the observed protease phenotype in burn isolates. Carey *et al.* (2024) agreed with our findings [56].

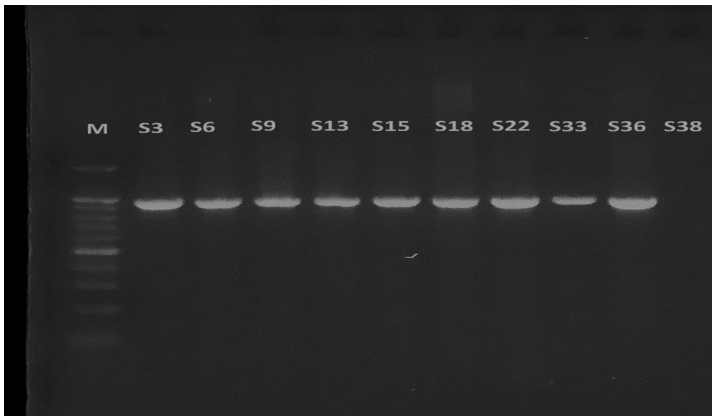

Figure 9. Gel electrophoresis of PCR product. M: ladder 100 bp DNA ladder, lanes (S3, S6, S9, S13, S15, S18, S22, S33, S36) positive result (except S38) at 993 bp of *aprA* as a virulence gene for *P. aeruginosa*

CONCLUSION

This study demonstrates a high prevalence of multidrug-resistant and ESBL-producing *P. aeruginosa* in burn patients at Erbil West Emergency Hospital, with females representing the majority of cases (55%), likely reflecting their greater exposure to domestic fire hazards. *P. aeruginosa* isolates exhibited high resistance to ceftazidime (67.5%), ciprofloxacin (62.5%), and meropenem (42.5%), while complete resistance was recorded for ampicillin, penicillin, amoxicillin-clavulanic acid, cefotaxime, and chloramphenicol antibiotics, to which *P. aeruginosa* is intrinsically resistant. Imipenem retained the highest efficacy among tested carbapenems, consistent with its established activity against ESBL-producing *P. aeruginosa* strains, as documented by Mushtaq *et al.* Clinicians at Erbil West Emergency Hospital should consider imipenem as a preferred empirical option for severe burn wound infections pending susceptibility results, while de-escalating or avoiding ceftazidime given the high local resistance rate (67.5%). A large number of ESBL-producing *P. aeruginosa* could be tied to misuse or heavy antibiotic use over time. Most *P. aeruginosa* isolates harbored multiple virulence genes (*aprA* 90%; *plcH* and *exoT* 87.5%), which directly correspond with the high rates of proteolytic (87.5%) and hemolytic (62.5% β-hemolysis) activities observed phenotypically, confirming the pathogenic potential of these burn isolates. These findings underline the critical need for continuous local antimicrobial surveillance, strict antibiotic stewardship, and infection control measures at Erbil West Emergency Hospital to curb the spread of MDR *P. aeruginosa* and improve clinical outcomes in burn patients.

SUPPLEMENTARY MATERIAL

None.

AUTHOR CONTRIBUTIONS

Maad Abdulla Mansur: Conceptualization, Validation, Methodology, Writing–original draft. Mohammed Hakeem Khalaf: Investigation, Formal Analysis, Writing–review and editing.

FUNDING

This research received no external funding.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

ACKNOWLEDGMENTS

The authors would like to thank Salahaddin University-Erbil for its support in the present work.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DECLARATION OF GENERATIVE AI USE

During the preparation of this manuscript, the authors used Grammarly for grammar checking and language polishing. After using this tool, the authors thoroughly reviewed and revised the generated content, taking full responsibility for the accuracy and integrity of the final manuscript.

REFERENCES

[1] N. Ahmed, Z. Ali, M. Riaz, B. Zeshan, J. I. Wattoo, and M. N. Aslam, "Evaluation of antibiotic resistance and virulence genes among clinical isolates of *Pseudomonas aeruginosa* from cancer patients," *Asian Pacific Journal of Cancer Prevention*, vol. 21, no. 5, pp. 1333–1338, 2020, doi: 10.31557/apjcp.2020.21.5.1333.

[2] R. Gharieb, M. Saad, M. Khedr, A. El Gohary, and H. Ibrahim, "Occurrence, virulence, carbapenem resistance, susceptibility to disinfectants and public health hazard of *Pseudomonas aeruginosa* isolated from animals, humans and environment in intensive farms," *Journal of Applied Microbiology*, vol. 132, no. 1, pp. 256–267, 2021, doi: 10.1111/jam.15191.

[3] N. Sathe, P. Beech, L. Croft, C. Suphioglu, A. Kapat, and E. Athan, "*Pseudomonas aeruginosa*: Infections and novel approaches to treatment "knowing the enemy" the threat of *Pseudomonas aeruginosa* and exploring novel approaches to treatment," *Infectious Medicine*, vol. 2, no. 3, pp. 178–194, 2023, doi: 10.1016/j.imj.2023.05.003.

[4] M. D. Younus, O. F. Bahjat, and S. A. Rashid, "Antibiotic susceptibility pattern, molecular characterization of virulence genes among *Pseudomonas aeruginosa* isolated from burn patients," *Cihan University-Erbil Scientific Journal*, vol. 5, no. 1, pp. 36–41, 2021, doi: 10.24086/cuesj.v5n1y2021.pp36-41.

[5] M. D. Younus, Z. F. A. Abdulrahman, O. F. Bahjat, N. L. Ali, S. A. Rashid, and A. S. Alkhouri, "Plasmid profiling, genetic site determination of antibiotic resistance gene of *Pseudomonas aeruginosa* isolated from burned patients," in *4th International Conference on Biological & Health Sciences (CIC-BIOHS'2022)*, Cihan University-Erbil, Jul. 2022, pp. 68–75, doi: 10.24086/biohs2022/paper.538.

[6] R. F. Polse, H. M. Khalid, and W. M. S. Mero, "Distribution of bla_{oxa-10}, bla_{per-1}, and bla_{SHV} genes in ESBL-producing *Pseudomonas aeruginosa* strains isolated from burn patients," *Scientific Reports*, vol. 13, no. 1, Art no. 18402, 2023, doi: 10.1038/s41598-023-45417-4.

[7] F. Piri, K. Amini, and M. Mohammadi, "Inhibitory effect of iron oxide nanoparticles on CTX-M gene expression in extended-spectrum βeta-lactamase-producing *Pseudomonas aeruginosa* isolated from burn patients," *Journal of Advances in Medical and Biomedical Research*, vol. 30, no. 140, pp. 289–294, 2022, doi: 10.30699/jambs.30.140.289.

[8] M. K. Pattanayak, S. Tomar, A. Gaur, and S. Goel, "Phenotypic detection of multidrug-resistance *Pseudomonas aeruginosa* isolated from various clinical samples," *International Journal of Health Sciences*, vol. 6, no. S6, pp. 883–893, 2022, doi: 10.53730/ijhs.v6ns6.9709.

[9] T. Wang, X. Du, L. Ji, Y. Han, J. Dang, J. Wen, Y. Wang, Q. Pu, M. Wu, and H. Liang, "*Pseudomonas aeruginosa* T6SS-mediated molybdate transport contributes to bacterial competition during anaerobiosis," *Cell Reports*, vol. 35, no. 2, Art no. 108957, 2021, doi: 10.1016/j.celrep.2021.108957.

[10] M. T. Jasim, A. F. A. Alzubaidi, and S. M. D. Al-Rubaye, "Isolation and identification of *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria from burns and wounds patients in Diyala governorate," *International Journal of Health Sciences*, vol. 6, no. S9, pp. 273–282, 2022, doi: 10.53730/ijhs.v6ns9.12222.

[11] B. Magalhães, B. Valot, M. M. H. Abdelbary, G. Prod'hom, G. Greub, L. Senn, and D. S. Blanc, "Combining standard molecular typing and whole genome sequencing to investigate *Pseudomonas aeruginosa* epidemiology in intensive care units," *Frontiers in Public Health*, vol. 8, Art no. 3, Jan. 2020, doi: 10.3389/fpubh.2020.00003.

[12] V. B. Nehmatulla, "Isolation and identification of candida species and some superficial mycosis associated with diaper dermatitis in Erbil city," *Polytechnic Journal*, vol. 11, no. 1, pp. 98–103, 2021, doi: 10.25156/ptj.v11n1y2021.pp98-103.

[13] M. Kaur and M.-P. Mingeot -Leclercq, "Maintenance of bacterial outer membrane lipid asymmetry: Insight into MlaA," *BMC Microbiology*, vol. 24, no. 1, Art no. 186, 2024, doi: 10.1186/s12866-023-03138-8.

[14] M. A. Khattab, M. S. Nour, and N. M. ElSheshtawy, "Genetic identification of *Pseudomonas aeruginosa* virulence genes among different isolates," *Journal of Microbial & Biochemical Technology*, vol. 7, no. 5, pp. 274–277, 2015, doi: 10.4172/1948-5948.1000224.

[15] I. Mitov, T. Strateva, and B. Markova, "Prevalence of virulence genes among Bulgarian nosocomial and cystic fibrosis isolates of *Pseudomonas aeruginosa*," *Brazilian Journal of Microbiology*, vol. 41, no. 3, pp. 588–595, 2010, doi: 10.1590/s1517-83822010000300008.

[16] F. Faraji, M. Mahzounieh, A. Ebrahimi, F. Fallah, O. Teymournejad, and B. Lajevardi, "Molecular detection of virulence genes in *Pseudomonas aeruginosa* isolated from children with cystic fibrosis and burn wounds in Iran," *Microbial Pathogenesis*, vol. 99, pp. 1–4, Oct. 2016, doi: 10.1016/j.micpath.2016.07.013.

[17] B. Noomi, "Detection of virulence factors of *Pseudomonas aeruginosa* in different animals by using bacteriological and molecular methods," *Iraqi Journal of Veterinary Sciences*, vol. 32, no. 2, pp. 205–210, 2019, doi: 10.33899/ijvs.2019.153851.

[18] I. S. Aljebory, "Genetic detection and identification of some virulence factors genes among *Pseudomonas aeruginosa* samples in Kirkuk province-Iraq," *International Journal of Pharmaceutical Quality Assurance*, vol. 10, no. 3, pp. 147–150, 2019, doi: 10.25258/ijpqa.10.3.8.

[19] A. I. Cotar, M. C. Chifiriuc, O. Banu, and V. Lazar, "Molecular characterization of virulence patterns in *Pseudomonas aeruginosa* strains isolated from respiratory and wound samples," *Biointerface Research in Applied Chemistry*, vol. 3, no. 2, pp. 551–558, 2013, [Online]. Available: https://www.researchgate.net/publication/236330033_Molecular_characterization_of_virulence_patterns_in_Pseudomonas_aeruginosa_strains_isolated_from_respiratory_and_wound_samples.

[20] G. İnat, B. Sırıken, C. Başkan, İ. Erol, T. Yıldırım, and A. Çiftci, "Quorum sensing systems and related virulence factors in *Pseudomonas aeruginosa* isolated from chicken meat and ground beef," *Scientific Reports*, vol. 11, no. 1, Art no. 15639, 2021, doi: 10.1038/s41598-021-94906-x.

[21] S. Phan, C. H. Feng, R. Huang, Z. X. Lee, Y. Moua, O. J. Phung, and J. R. Lenhard, "Relative abundance and detection of *Pseudomonas aeruginosa* from chronic wound infections globally," *Microorganisms*, vol. 11, no. 5, Art no. 1210, 2023, doi: 10.3390/microorganisms11051210.

[22] M. Lichtenberg, T. H. Jakobsen, M. Kühl, M. Kolpen, P. Ø. Jensen, and T. Bjarnsholt, "The structure–function relationship of *Pseudomonas aeruginosa* in infections and its influence on the microenvironment," *FEMS Microbiology Reviews*, vol. 46, no. 5, Art no. fuac018, 2022, doi: 10.1093/femsre/fuac018.

[23] N. Mathlouthi, C. Al-Bayssari, A. El Salabi, S. Bakour, S. Ben Gwierif, A. A. Zorgani, Y. Jridi, K. Ben Slama, J.-M. Rolain, and C. Chouchani, "Carbapenemases and extended-spectrum β-lactamases producing *Enterobacteriaceae* isolated from Tunisian and Libyan hospitals," *The Journal of Infection in Developing Countries*, vol. 10, no. 7, pp. 718–727, 2016, doi: 10.3855/jidc.7426.

[24] S. N. Mahaseth, L. Chaurasia, B. Jha, and R. K. Sanjana, "Prevalence and antimicrobial susceptibility pattern of *Pseudomonas aeruginosa* isolated from various clinical samples in a tertiary care hospital," *Janaki Medical College Journal of Medical Science*, vol. 8, no. 2, pp. 11–17, 2020, doi: 10.3126/jmcjms.v8i2.33972.

[25] B. L. Aghaee, M. Khan Mirzaei, M. Y. Alikhani, A. Mojtahedi, and C. F. Maurice, "Improving the inhibitory effect of phages against *Pseudomonas aeruginosa* isolated from a burn patient using a combination of phages and antibiotics," *Viruses*, vol. 13, no. 2, Art no. 334, 2021, doi: 10.3390/v13020334.

[26] B. Hosien, H. Belhaj, and A. Atia, "Characteristics of antibiotic-resistant bacteria in Libya based on different source of infections," *Libyan International Medical University Journal*, vol. 7, no. 2, pp. 039–044, 2022, doi: 10.1055/s-0042-1759621.

[27] A. Radhika, J. Lakshmi, K. Ariyanachi, and V. Sakthivadivel, "Detection of metallo beta-lactamase (MBL) producing *Pseudomonas aeruginosa* in a Tertiary Care Hospital, Ghanpur, Medchal, India," *Maedica - A Journal of Clinical Medicine*, vol. 17, no. 1, pp. 134–142, 2022, doi: 10.26574/maedica.2022.17.1.134.

[28] N. S. Budayanti, D. N. Aisyah, N. N. D. Fatmawati, N. M. A. Tarini, Z. Kozlakidis, and W. Adisasmito, "Identification and distribution of pathogens in a major tertiary hospital of Indonesia," *Frontiers in Public Health*, vol. 7, Art no. 395, Jan. 2020, doi: 10.3389/fpubh.2019.00395.

[29] M. B. Sannathimmappa, V. Nambiar, R. Aravindakshan, and N. M. Al-Kasaby, "Profile and antibiotic-resistance pattern of bacteria isolated from endotracheal secretions of mechanically ventilated patients at a tertiary care hospital," *Journal of Education and Health Promotion*, vol. 10, no. 1, Art no. 195, 2021, doi: 10.4103/jehp.jehp_1517_20.

[30] J. Alamu, L. Kakithakara Vajravelu, B. Venkatesan, and J. Thulukanam, "Correlation of phenotypic and genotypic virulence markers, antimicrobial susceptibility pattern, and outcome of *Pseudomonas aeruginosa* sepsis infection," *Microbial Pathogenesis*, vol. 170, Art no. 105716, Sep. 2022, doi: 10.1016/j.micpath.2022.105716.

[31] Z. Asmare, M. A. Reta, Y. Gashaw, E. Getachew, A. Sisay, M. Gashaw, E. Tamrat, A. A. Kidie, W. Abebe, T. Misganaw, et al., "Antimicrobial resistance profile of *Pseudomonas aeruginosa* clinical isolates from healthcare-associated infections in Ethiopia: A systematic review and meta-analysis," *PLOS ONE*, vol. 19, no. 8, Art no. e0308946, 2024, doi: 10.1371/journal.pone.0308946.

[32] B. Schwartz, K. Klamer, J. Zimmerman, P. B. Kale-Pradhan, and A. Bhargava, "Multidrug resistant *Pseudomonas aeruginosa* in clinical settings: A review of resistance mechanisms and treatment strategies," *Pathogens*, vol. 13, no. 11, Art no. 975, 2024, doi: 10.3390/pathogens13110975.

[33] A. E. Laudy, P. Róg, K. Smolińska-Król, M. Ćmiel, A. Słoczyńska, J. Patzer, D. Dzierżanowska, R. Wolinowska, B. Starościak, and S. Tyski, "Prevalence of ESBL-producing *Pseudomonas aeruginosa* isolates in Warsaw, Poland, detected by various phenotypic and genotypic methods," *PLOS ONE*, vol. 12, no. 6, Art no. e0180121, 2017, doi: 10.1371/journal.pone.0180121.

[34] S. Mushtaq, D. Meunier, A. Vickers, N. Woodford, and D. M. Livermore, "Activity of imipenem/relebactam against *Pseudomonas aeruginosa* producing ESBLs and carbapenemases," *Journal of Antimicrobial Chemotherapy*, vol. 76, no. 2, pp. 434–442, 2020, doi: 10.1093/jac/dkaa456.

[35] A. C. Gales, G. Stone, D. F. Sahm, M. G. Wise, and E. Utt, "Incidence of ESBLs and carbapenemases among Enterobacterales and carbapenemases in *Pseudomonas aeruginosa* isolates collected globally: Results from ATLAS 2017–2019," *Journal of Antimicrobial Chemotherapy*, vol. 78, no. 7, pp. 1606–1615, 2023, doi: 10.1093/jac/dkad127.

[36] M. S. Rezai, F. Ahangarkani, A. Rafiei, A. Hajalibeig, and M. Bagheri-Nesami, "Extended-Spectrum beta-lactamases producing *Pseudomonas aeruginosa* isolated from patients with ventilator associated nosocomial infection," *Archives of Clinical Infectious Diseases*, vol. 13, no. 4, Art no. e13974, 2018, doi: 10.5812/archcid.13974.

[37] M. F. Kengne, A. T. Mbaveng, O. Karimo, B. S. T. Dadjo, O. D. Tsobeng, W. J. T. Marbou, and V. Kuete, "Frequency of fecal carriage of ESBL resistance genes in multidrug-resistant *Pseudomonas aeruginosa* isolates from cancer patients at Laquintinie Hospital, Douala, Littoral Region, Cameroon," *International Journal of Microbiology*, vol. 2024, no. 1, Art no. 7685878, 2024, doi: 10.1155/2024/7685878.

[38] N. Saleh, H. U. Abdu, and I. Yusuf, "Disinfectant activity of *Eucalyptus globulus* leaves extracts against MRSA and ESBL producing *Pseudomonas aeruginosa* isolated from hospital environment," *Bima Journal of Science and Technology Gombe*, vol. 8, no. 2A, pp. 65–73, 2024, doi: 10.56892/bima.v8i2.660.

[39] W. O. Ordu, C. Mbata, and M. T. Pius, "Molecular profiling of antibiotic-resistant *Pseudomonas aeruginosa* isolated from patients with urinary tract infections in Rivers State University teaching hospital," *International Journal of Pathogen Research*, vol. 14, no. 1, pp. 27–41, 2025, doi: 10.9734/ijpr/2025/v14i1338.

[40] A. A. Elnegery, W. K. Mowafy, T. A. Zahra, and N. T. Abou El-Khier, "Study of quorum-sensing *LasR* and *RhlR* genes and their dependent virulence factors in *Pseudomonas aeruginosa* isolates from infected burn wounds," *Access Microbiology*, vol. 3, no. 3, Art no. 211, 2021, doi: 10.1099/acmi.0.000211.

[41] D. Zhang, J. Palmer, K. H. Teh, M. M. A. Calinisan, and S. Flint, "Milk fat influences proteolytic enzyme activity of dairy pseudomonas species," *International Journal of Food Microbiology*, vol. 320, Art no. 108543, May 2020, doi: 10.1016/j.ijfoodmicro.2020.108543.

[42] S. Grbavčić, D. Bezbradica, L. Izrael-Živković, N. Avramović, N. Milosavić, I. Karadžić, and Z. Knežević-Jugović, "Production of lipase and protease from an indigenous *Pseudomonas aeruginosa* strain and their evaluation as detergent additives: compatibility study with detergent ingredients and washing performance," *Bioresource Technology*, vol. 102, no. 24, pp. 11 226–11 233, 2011, doi: 10.1016/j.biortech.2011.09.076.

[43] M. R. Marwa, A.-T. Marwa, and E. A. Amal, "Phenotypic and genotypic characterization of *Pseudomonas aeruginosa* isolates from Egyptian hospitals," *African Journal of Microbiology Research*, vol. 10, no. 39, pp. 1645–1653, 2016, doi: 10.5897/ajmr2016.8254.

[44] R. EL-Mahdy and G. El-Kannishy, "Virulence factors of carbapenem-resistant *Pseudomonas aeruginosa* in hospital-acquired infections in Mansoura, Egypt," *Infection and Drug Resistance*, vol. 12, pp. 3455–3461, Nov. 2019, doi: 10.2147/idr.s222329.

[45] W. F. Mohamed, A. A. Askora, M. M. H. Mahdy, E. A. EL-Hussieny, and H. M. Abu-Shady, "Isolation and characterization of bacteriophages active against *Pseudomonas aeruginosa* strains isolated from diabetic foot infections," *Archives of Razi Institute*, vol. 77, no. 6, pp. 2187–2200, 2022, doi: 10.22092/ari.2022.359032.2357.

[46] D. Sperandio, G. Rossignol, J. Guerillon, N. Connil, N. Orange, M. G. Feuilloley, and A. Merieau, "Cell-associated hemolysis activity in the clinical strain of *Pseudomonas fluorescens MFN1032*," *BMC Microbiology*, vol. 10, no. 1, Art no. 124, 2010, doi: 10.1186/1471-2180-10-124.

[47] I. M. Jawher and M. G. Hasan, "Molecular identification of *Pseudomonas aeruginosa* in meat at Mosul city retails using PCR technique," *Iraqi Journal of Veterinary Sciences*, vol. 36, no. 4, pp. 1083–1087, 2022, doi: 10.33899/ijvs.2022.133086.2173.

[48] M. D. Y. Ali and Z. Abdulrahman, "Molecular identification, susceptibility pattern, and detection of some virulence genes in *Pseudomonas aeruginosa* isolated from burn patients," *Plant Archives*, vol. 20, no. 1, pp. 2573–2580, 2020, [Online]. Available: https://www.researchgate.net/profile/Mustafa-Younus/publication/352121253.

[49] R. Lavenir, D. Jocktane, F. Laurent, S. Nazaret, and B. Cournoyer, "Improved reliability of *Pseudomonas aeruginosa* PCR detection by the use of the species-specific *ecfX* gene target," *Journal of Microbiological Methods*, vol. 70, no. 1, pp. 20–29, 2007, doi: 10.1016/j.mimet.2007.03.008.

[50] V. Nikbin, M. M. Aslani, Z. Sharafi, M. Hashemipour, F. Shahcheraghi, and G. Ebrahimipour, "Molecular identification and detection of virulence genes among *Pseudomonas aeruginosa* isolated from different infectious origins," *Iranian Journal of Microbiology*, vol. 4, no. 3, pp. 118–123, 2012, [Online]. Available: https://pmc.ncbi.nlm.nih.gov/articles/PMC3465536/pdf/IJM-4-118.pdf.

[51] N. El Husseini, J. A. Carter, and V. T. Lee, "Urinary tract infections and catheter-associated urinary tract infections caused by *Pseudomonas aeruginosa*," *Microbiology and Molecular Biology Reviews*, vol. 88, no. 4, Art no. e00066-22, 2024, doi: 10.1128/mmbr.00066-22.

[52] A. K. Jasim and A. A. Hussein, "Detection genotyping virulence factor among *Pseudomonas aeruginosa* isolated from burns patients," *International Journal of Advanced Multidisciplinary Research and Studies*, vol. 3, no. 4, pp. 1209–1214, 2023, [Online]. Available: https://www.multiresearchjournal.com/admin/uploads/archives/archive-1693288980.pdf.

[53] J. R. Mackinder, L. A. Hinkel, K. Schutz, K. Eckstrom, K. Fisher, and M. J. Wargo, "Sphingosine induction of the *Pseudomonas aeruginosa* hemolytic phospholipase C/sphingomyelinase (PlcH)," *Journal of Bacteriology*, vol. 206, no. 3, Art no. e00382-23, 2024, doi: 10.1128/jb.00382-23.

[54] E. A. Edward, M. R. El Shehawy, A. Abouelfetouh, and E. Aboulmagd, "Prevalence of different virulence factors and their association with antimicrobial resistance among *Pseudomonas aeruginosa* clinical isolates from Egypt," *BMC Microbiology*, vol. 23, no. 1, Art no. 161, 2023, doi: 10.1186/s12866-023-02897-8.

[55] I. Jurado-Martín, M. Sainz-Mejías, and S. McClean, "*Pseudomonas aeruginosa*: An audacious pathogen with an adaptable arsenal of virulence factors," *International Journal of Molecular Sciences*, vol. 22, no. 6, Art no. 3128, 2021, doi: 10.3390/ijms22063128.

[56] J. N. Carey, S. Lamont, D. J. Wozniak, A. A. Dandekar, and M. R. Parsek, "Quorum sensing regulation of Psl polysaccharide production by *Pseudomonas aeruginosa*," *Journal of Bacteriology*, vol. 206, no. 12, Art no. e00312-24, 2024, doi: 10.1128/jb.00312-24.