

Original Paper

Assessment of the Antibiotic Resistance Patterns and Biofilm Formation in Clinical Isolates of *Pseudomonas aeruginosa* from Iraqi Hospitalized Patients

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ABSTRACT

Pseudomonas aeruginosa is a leading cause of hospital-acquired infections; its multidrug resistance and strong biofilm production complicate treatment. This study aimed to identify *P. aeruginosa* in clinical samples from hospitals in Baghdad, Iraq, and to relate antibiotic susceptibility to quantitative biofilm production. In a cross-sectional study, 150 clinical samples were collected from Baghdad hospitals. Isolates were identified by culture, biochemical tests and the VITEK 2 system. Susceptibility to 10 antimicrobial agents was tested by the Kirby–Bauer disc diffusion method according to CLSI guidelines, and biofilm formation was quantified by the crystal violet microtiter plate method at 570 nm. Of the 150 samples, 100 isolates were confirmed as *P. aeruginosa*. Males accounted for 54% of cases and females for 46% ($p = 0.236$). Resistance was highest to trimethoprim (95%), followed by gentamicin (58%), ceftazidime (56%), piperacillin and amikacin (52% each) and levofloxacin (45%), whereas colistin was the most effective agent (85% susceptible) ($p < 0.0001$). Overall, 62% of isolates showed a resistant phenotype, comprising extensively drug-resistant (XDR, 42%), multidrug-resistant (MDR, 15%) and pandrug-resistant (PDR, 5%) isolates. All isolates (100%) formed biofilm, predominantly as moderate producers (62%), followed by strong (29%) and weak (9%) producers ($p = 0.0001$ for the association with gender). Biofilm production was significantly associated with several clinical sources, with levofloxacin resistance (Fisher's exact test, $p = 0.0046$) and with the overall XDR resistance profile ($p = 0.0001$). Clinical *P. aeruginosa* isolates from Baghdad hospitals therefore show widespread antibiotic resistance and universal biofilm formation, highlighting the urgent need for effective antimicrobial stewardship.

KEYWORDS: *Pseudomonas aeruginosa*, Biofilm, Antibiotic resistance, Colistin, Microbial identification, VITEK 2 Compact system.

1-INTRODUCTION

Pseudomonas aeruginosa is a highly adaptable, opportunistic Gram-negative pathogen. It is a major problem in clinical practice because of its complex antibiotic resistance mechanisms and its ability to form biofilms, and it causes serious hospital-acquired infections, particularly in intensive care units, burn centres, neonatal wards and oncology units [1,2]. The organism produces a wide range of infections, including pneumonia, bloodstream infection, urinary tract infection, wound infection and burn sepsis [1,3]. Its minimal nutritional requirements and high metabolic flexibility allow it to colonise many hospital niches, such as medical devices, ventilators, urinary catheters and hospital water systems [4].

The number of isolates of this species that are resistant to antimicrobial agents is rising, and multidrug-resistant (MDR) and extensively drug-resistant (XDR) variants are now common worldwide. Infections caused by these strains are a serious clinical problem: they prolong hospital stay, increase costs and raise patient morbidity and mortality [5].

A recent global analysis has recognised antimicrobial resistance as one of the greatest threats to public health in this century, driven mainly by Gram-negative bacteria [6,7]. The World Health Organization has responded by placing carbapenem-resistant *P. aeruginosa* (CRPA) and related pathogens on its critical priority list of pathogens in urgent need of new antibiotics [8], a point reiterated in subsequent reviews that underline the severity of the situation [9].

P. aeruginosa possesses several cooperating resistance mechanisms: intrinsic, acquired and adaptive [10]. Its outer membrane is highly impermeable, and drug entry is regulated by specific porins such as outer membrane porin D (OprD); when OprD is absent or under-expressed, carbapenems are poorly taken up by the cell [11].

The bacterium also possesses active efflux pumps, such as MexAB-OprM and MexXY-OprM, which extrude antibiotics from the cell [12] and can remove numerous classes of drugs [13]. In addition, the organism can acquire new resistance genes, including β -lactamase genes, by horizontal gene transfer [14].

Adaptive resistance provides a further layer of protection: the bacterium can alter its gene expression in response to stress, allowing it to withstand antibiotics, low oxygen and nutrient limitation [10]. These changes are usually transient but reduce the likelihood of successful treatment. The combination of intrinsic, acquired and adaptive systems makes this pathogen very difficult to control [5], which explains why many infections fail to resolve with the initial agents used.

Biofilm formation is another important defence mechanism of this pathogen. The bacterium can switch between motile and attached states [15]. Cells within a biofilm are embedded in a self-produced matrix of extracellular polymeric substance (EPS) [16], which acts as both a physical and a chemical barrier: it slows the penetration of antibiotics into the community and allows some cells to enter a dormant "persister" state [17]. Biofilm cells can survive extremely high drug concentrations, up to 100–1000 times the normal inhibitory dose [18], which is why biofilm-associated infections are so difficult to eradicate [19].

Medical devices are especially difficult to manage because the bacterium readily attaches to implants, catheters and tubing [20] and forms biofilms that are extremely difficult to remove, often requiring device removal and prolonged antibiotic therapy. Device-associated biofilm infections are frequent in intensive care units (ICUs) [21], increasing both patient harm and treatment cost.

Biofilm formation is a regulated process linked to the signalling mechanism known as quorum sensing [22], through which cells sense their own population density and switch on genes for matrix production and virulence. Because resistance is a major clinical problem and there is a strong relationship between biofilm formation and resistance [23], the aim of this study was to relate the antibiotic resistance profiles of clinical *P. aeruginosa* isolates to their quantitative biofilm production.

2-MATERIALS AND METHODS

2.1. Clinical sample collection

This was a cross-sectional study. A total of 150 clinical samples were collected from Iraqi patients between November 2025 and March 2026 at four hospitals in Baghdad: Ibn Al-Baladi Hospital, Imam Ali Hospital, Ghazi Al-Hariri Hospital and Baghdad Teaching Hospital.

Of the 150 clinical samples processed, 100 isolates were identified as *P. aeruginosa* on the basis of colony morphology, biochemical testing and VITEK 2 confirmation. The remaining 50 samples yielded other bacterial species or no growth and were excluded from further analysis. Clinical sources included burn swabs, urine, respiratory secretions, wound and tissue swabs, ear swabs, blood, skin swabs and stool. Patients' age, sex and clinical condition were documented. Samples were collected aseptically under strict hygiene procedures.

2.2. Identification of *P. aeruginosa*

Colony morphology after aerobic growth was used for primary screening. Samples were cultured on MacConkey, blood and cetrimide agars at 37 °C for 24 h, and pyocyanin production and the characteristic odour were noted.

Oxidase and catalase tests were used for biochemical confirmation: for the catalase test, a single colony was placed on a slide with a few drops of 3% hydrogen peroxide, and for the oxidase test a portion of the colony was transferred to filter paper moistened with oxidase reagent. Final confirmation at species level was performed with the automated VITEK 2 Compact system (bioMérieux, France) using the microbial ID/AST system. Bacterial suspensions were adjusted to 0.5 McFarland and loaded into Gram-negative (GN) identification cards.

2.3. Antibiotic susceptibility testing

Antibiotic susceptibility testing (AST) was performed by the Kirby–Bauer disc diffusion method on Mueller–Hinton agar according to CLSI guidelines [24,25]. A 0.5 McFarland bacterial suspension was spread evenly over the agar surface. Ten antibiotics were evaluated: norfloxacin (NX), piperacillin (PRL), ceftazidime (CAZ), trimethoprim (TMP), levofloxacin (LEV), meropenem (MRP), imipenem (IMP), gentamicin (CN), amikacin (AK) and colistin (CT; modified protocol, see Section 2.3.1). Plates were incubated for 24 h at 37 °C, after which the zones of inhibition were measured and interpreted.

Isolates were categorised as multidrug-resistant (MDR), extensively drug-resistant (XDR) or pandrug-resistant (PDR) according to the international consensus criteria of the European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC) [26].

Trimethoprim was retained in the panel despite the well-documented intrinsic resistance of *P. aeruginosa* to this agent [27,28], because it forms part of the standard empirical antibiogram panel used across the participating Iraqi hospitals; its inclusion allows the resistance rate reported here to serve as a local surveillance reference point rather than as a treatment recommendation (see Discussion).

2.3.1. Modified susceptibility testing for colistin

Although standard Kirby–Bauer disc diffusion on Mueller–Hinton agar is effective for most antibiotics, it has major limitations for colistin because of the large molecular size and poor agar diffusion of this drug [29]. To determine colistin resistance more accurately, a modified disc diffusion test on modified Mueller–Hinton agar was employed; several methods and agar concentrations were trialled before the adopted method was selected. The adapted protocol used different agar concentrations (0.5 g, 0.75 g and 1.25 g per 100 mL of Mueller–Hinton broth) to optimise diffusion of the colistin molecule through the medium, and the bacterial suspension was spread evenly with an L-shaped spreader to ensure a strictly uniform inoculum.

2.4. Detection of biofilm (microtiter plate method)

Biofilm production was quantified in 96-well flat-bottomed microtiter plates as described by Stepanović et al. [30]. Each well received 180 µL of brain heart infusion broth supplemented with 0.5% glucose, followed by 20 µL of the standardised bacterial suspension. Plates were incubated for 48 h at 37 °C. After incubation, non-adherent cells were removed by washing the wells three times with phosphate-buffered saline (PBS). The attached biofilm was stained with 0.1% crystal violet for 15 min; excess dye was removed and the wells were air-dried. The bound dye was solubilised with 33% glacial acetic acid, and the optical density (OD) was measured at 570 nm in an ELISA microplate reader.

2.5. Statistical analysis

Data were analysed using SPSS version 26. Categorical data are presented as frequencies and percentages. The chi-square test was used to compare percentages between groups, and Fisher's exact test was applied where expected cell counts were below 5. The relationship between resistance and patient age was evaluated by Spearman's rho correlation. A p-value < 0.05 was considered significant and a p-value < 0.01 highly significant; values > 0.05 were considered non-significant (NS).

3-RESULTS

3.1. Isolate confirmation and demographic data

Of the 150 clinical samples, 100 were identified as *P. aeruginosa*. Infections occurred in both sexes: males accounted for 54% of cases and females for 46%. The difference between age groups and sexes was non-significant ($p = 0.236$), indicating that both sexes were affected at a similar rate.

3.2. Distribution of isolates by clinical source

The isolates were recovered from a wide range of clinical sources (Table 1, Figure 1). Burn swabs were the most common source (29%), followed by urine (17%). Sputum and ear swabs each contributed 12%, and wound samples accounted for 10%. The difference between sources was highly significant ($p = 0.0001$), confirming that burns are a key reservoir of this pathogen.

Table (1) Distribution of *P. aeruginosa* isolates according to the source of specimens (n = 100)

Source	No. of isolates	Percentage (%)
Burn	29	29.0
Urine	17	17.0
Sputum	12	12.0
Ear swab	12	12.0
Wound	10	10.0
Blood	8	8.0
Skin swab	5	5.0
Endotracheal tube (ETT)	2	2.0
Palatal tissue	1	1.0
Tissue	1	1.0
Stool	1	1.0
Pus	1	1.0
Cystic fibrosis	1	1.0
Total	100	100

Chi-square test, $p = 0.0001$ (highly significant, $P \leq 0.01$).

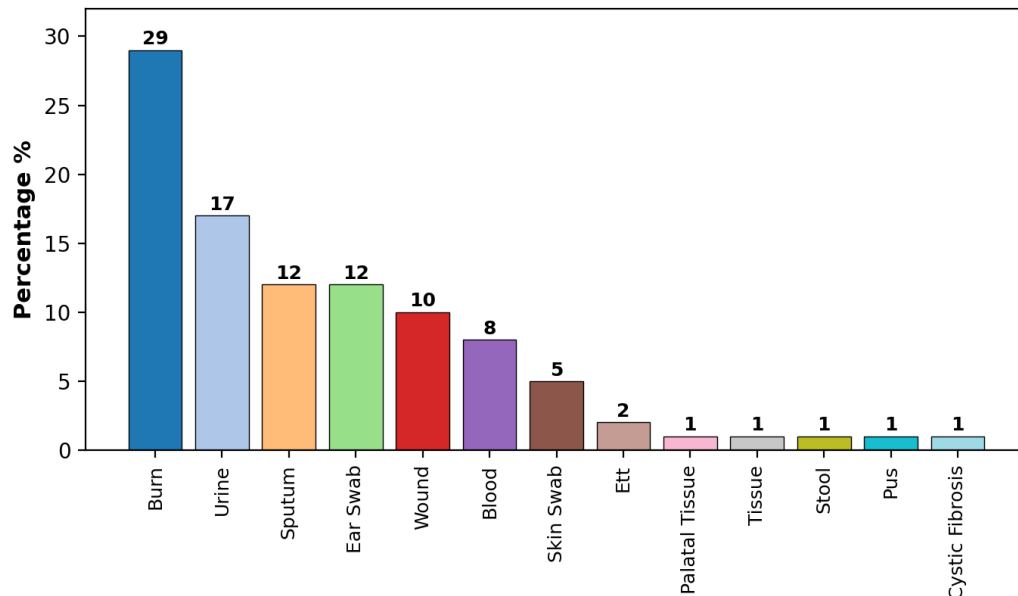


Figure (1): Prevalence of *P. aeruginosa* isolates according to the source of specimens.

3.3. Antibiotic susceptibility pattern

P. aeruginosa is widely recognised for its formidable intrinsic and acquired resistance to multiple antibiotic classes, which makes its clinical eradication highly challenging [5]. Antibiotic susceptibility testing revealed substantial multi-class resistance among the 100 clinical isolates (Table 2, Figure 2). Several agents showed higher resistance than susceptibility rates: isolates were predominantly resistant to trimethoprim (95%), followed by gentamicin (58%), ceftazidime (56%), piperacillin (52%), amikacin (52%) and levofloxacin (45%). The mean resistance rate across all ten agents was approximately 50.6%. Conversely, colistin emerged as the most effective antimicrobial agent, with 85% of isolates susceptible. Favourable susceptibility was also retained for norfloxacin (58%), meropenem (52%) and imipenem (48%). Differences between susceptibility categories were highly significant for all agents ($p < 0.01$), indicating a severe resistance burden in the studied strains.

Table (2) Antibiotic resistance pattern of the *P. aeruginosa* isolates (n = 100)

Antibiotic	Resistant, n (%)	Intermediate, n (%)	Susceptible, n (%)	P-value
Norfloxacin (NX)	41 (41.0)	1 (1.0)	58 (58.0)	0.0001 **
Piperacillin (PRL)	52 (52.0)	15 (15.0)	33 (33.0)	0.0001 **
Ceftazidime (CAZ)	56 (56.0)	10 (10.0)	34 (34.0)	0.0001 **
Trimethoprim (TMP)	95 (95.0)	0 (0.0)	5 (5.0)	0.0001 **
Levofloxacin (LEV)	45 (45.0)	18 (18.0)	37 (37.0)	0.0008 **
Meropenem (MRP)	46 (46.0)	2 (2.0)	52 (52.0)	0.0001 **
Imipenem (IMP)	46 (46.0)	6 (6.0)	48 (48.0)	0.0001 **
Gentamicin (CN)	58 (58.0)	21 (21.0)	21 (21.0)	0.0001 **
Amikacin (AK)	52 (52.0)	21 (21.0)	27 (27.0)	0.0002 **
Colistin (CT)	15 (15.0)	0 (0.0)	85 (85.0)	0.0001 **

Chi-square test; ** = highly significant ($P \leq 0.01$).

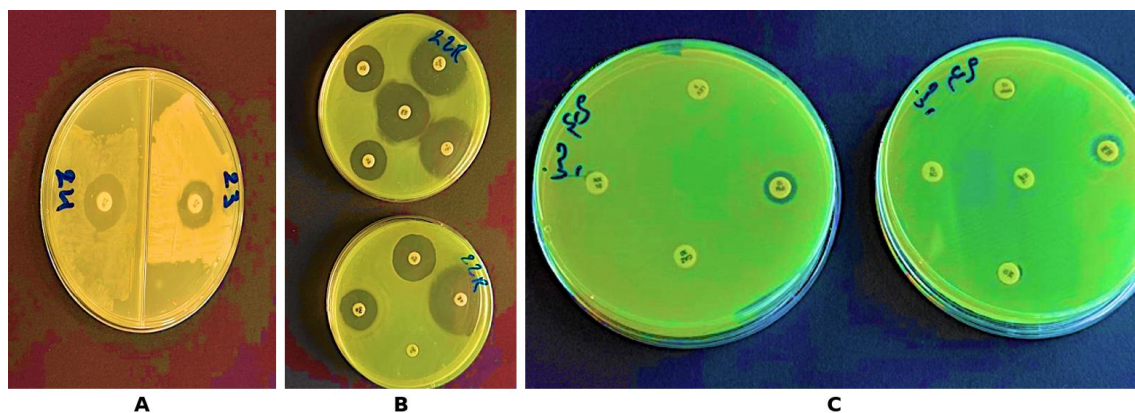


Figure (2): Disc diffusion susceptibility testing of *P. aeruginosa* isolates. (A) Colistin susceptibility testing on modified Mueller–Hinton broth supplemented with 1.25% agar, showing a clear zone of inhibition around the disc. (B) An isolate with a susceptible pattern to a panel of tested antibiotics on standard Mueller–Hinton agar, with distinct, wide zones of inhibition. (C) An isolate with a resistant pattern, characterised by the absence of inhibition zones and bacterial growth extending to the edges of the discs.

3.4. Multidrug resistance classification

Isolates were grouped by resistance profile. Sensitive isolates accounted for 38%, MDR isolates for 15%, XDR isolates, the largest resistant group, for 42%, and PDR isolates for 5%. In total, 62% of isolates showed a resistant phenotype (Table 7). This high proportion supports the aim of documenting the local resistance burden.

3.5. Biofilm formation

Every isolate produced biofilm (100%). Isolates were grouped by biofilm score into strong, moderate and weak producers (Figure 3). Moderate producers formed the largest group (62%), strong producers accounted for 29% and weak producers for the smallest share (9%). This universal biofilm formation is a central finding of the study.

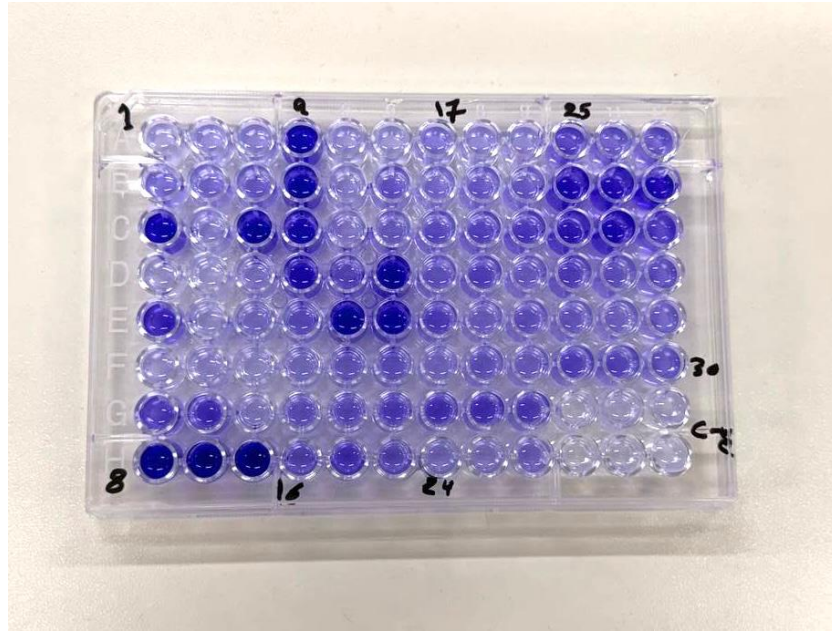


Figure (3): Qualitative overview of the microtiter plate biofilm assay. The variation in crystal violet staining distinguishes different biofilm-forming capacities, from minimal (weak) to robust (strong) staining, alongside negative controls.

Biofilm strength was also examined by gender (Table 3); moderate producers were the most common category in both males and females. The association between gender and biofilm formation was highly significant ($p = 0.0001$).

Table (3) Distribution of isolates by biofilm-forming ability and gender

Gender	Weak (0.20)	Moderate (0.4)	Strong (0.5)	Total	P-value
Male	5	33	16	54	0.0001 **
Female	4	29	13	46	
Total	9	62	29	100	

Chi-square test; ** = highly significant ($P \leq 0.01$).

3.6. Biofilm production in relation to patient age and clinical source

Biofilm strength was compared across age groups (Table 4). Moderate producers were common in every age group, and the association between age group and biofilm strength was not significant ($p = 0.917$). A separate Spearman's rho analysis, however, revealed a positive correlation between patient age and antibiotic resistance ($\rho = 0.074$, $p = 0.006$): older patients tended to carry more resistant isolates, even though biofilm strength did not change with age.

Table (4) Distribution of isolates by biofilm-forming ability and age group

Age group (years)	Weak	Moderate	Strong	Total	P-value
≤ 20	1 (5.9%)	12 (70.6%)	4 (23.5%)	17 (100%)	0.917 NS
21–40	2 (6.1%)	20 (60.6%)	11 (33.3%)	33 (100%)	
41–60	4 (10.8%)	23 (62.2%)	10 (27.0%)	37 (100%)	
> 60	2 (15.4%)	7 (53.8%)	4 (30.8%)	13 (100%)	
Total	9 (9.0%)	62 (62.0%)	29 (29.0%)	100 (100%)	

Chi-square test; NS = non-significant ($P > 0.05$).

The relationship between biofilm production and clinical source is shown in Table 5. Isolates from burns, urine, sputum and ear swabs showed highly significant associations with biofilm formation ($p = 0.0001, 0.0079, 0.0094$ and 0.0098 , respectively). Wound and skin swab isolates showed significant associations ($p = 0.0317$ and 0.0483), whereas isolates from blood, endotracheal tubes, palatal tissue, tissue, stool, pus and cystic fibrosis specimens showed non-significant associations.

Table (5) Association of specimen source with biofilm production

Source	Weak	Moderate	Strong	Total	P-value
Burn	2	16	11	29	0.0001 **
Urine	3	11	3	17	0.0079 **
Sputum	1	8	3	12	0.0094 **
Ear swab	1	8	3	12	0.0098 **
Wound	0	6	4	10	0.0317 *
Blood	1	5	2	8	0.2263 NS
Skin swab	1	4	0	5	0.0483 *
ETT	0	2	0	2	0.2452 NS
Palatal tissue	0	1	0	1	0.6522 NS
Tissue	0	1	0	1	0.8502 NS
Stool	0	0	1	1	0.6522 NS
Pus	0	0	1	1	0.6522 NS
Cystic fibrosis	0	0	1	1	0.6522 NS
Total	9	62	29	100	

* = significant ($P \leq 0.05$); ** = highly significant ($P \leq 0.01$); NS = non-significant; ETT: endotracheal tube.

3.7. Relationship between antibiotic resistance and biofilm production

The resistance pattern for each antibiotic was compared across the biofilm groups (Table 6). Using Fisher's exact test on the per-isolate data (necessary because expected cell counts were < 5 in several categories), only levofloxacin showed a significant association between the resistant/intermediate profile and biofilm strength ($p = 0.0046$); the results for the remaining nine antibiotics, including piperacillin, colistin, norfloxacin and meropenem, were non-significant.

Table (6) Antibiotic resistance pattern in relation to biofilm production in *P. aeruginosa* isolates

Antibiotic	Profile	Weak (n = 9)	Moderate (n = 62)	Strong (n = 29)	Total (n = 100)	P-value
NX	S	5 (55.6%)	31 (50.0%)	22 (75.9%)	58 (58.0%)	0.1402 NS
	I	0 (0.0%)	1 (1.6%)	0 (0.0%)	1 (1.0%)	
	R	4 (44.4%)	30 (48.4%)	7 (24.1%)	41 (41.0%)	
PRL	S	3 (33.3%)	16 (25.8%)	14 (48.3%)	33 (33.0%)	0.2658 NS
	I	2 (22.2%)	10 (16.1%)	3 (10.3%)	15 (15.0%)	
	R	4 (44.4%)	36 (58.1%)	12 (41.4%)	52 (52.0%)	
CAZ	S	3 (33.3%)	20 (32.3%)	11 (37.9%)	34 (34.0%)	0.6464 NS
	I	2 (22.2%)	5 (8.1%)	3 (10.3%)	10 (10.0%)	
	R	4 (44.4%)	37 (59.7%)	15 (51.7%)	56 (56.0%)	
TMP	S	0 (0.0%)	4 (6.5%)	1 (3.4%)	5 (5.0%)	1.0000 NS
	I	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
	R	9 (100.0%)	58 (93.5%)	28 (96.6%)	95 (95.0%)	
LEV	S	5 (55.6%)	24 (38.7%)	8 (27.6%)	37 (37.0%)	0.0046 **
	I	0 (0.0%)	6 (9.7%)	12 (41.4%)	18 (18.0%)	
	R	4 (44.4%)	32 (51.6%)	9 (31.0%)	45 (45.0%)	
MRP	S	3 (33.3%)	29 (46.8%)	20 (69.0%)	52 (52.0%)	0.1690 NS
	I	0 (0.0%)	2 (3.2%)	0 (0.0%)	2 (2.0%)	
	R	6 (66.7%)	31 (50.0%)	9 (31.0%)	46 (46.0%)	
IMP	S	6 (66.7%)	29 (46.8%)	13 (44.8%)	48 (48.0%)	0.2956 NS

CN	I	0 (0.0%)	2 (3.2%)	4 (13.8%)	6 (6.0%)	0.9684 NS
	R	3 (33.3%)	31 (50.0%)	12 (41.4%)	46 (46.0%)	
	S	1 (11.1%)	13 (21.0%)	7 (24.1%)	21 (21.0%)	
AK	I	2 (22.2%)	13 (21.0%)	6 (20.7%)	21 (21.0%)	0.8600 NS
	R	6 (66.7%)	36 (58.1%)	16 (55.2%)	58 (58.0%)	
	S	2 (22.2%)	18 (29.0%)	7 (24.1%)	27 (27.0%)	
CT	I	2 (22.2%)	11 (17.7%)	8 (27.6%)	21 (21.0%)	0.4849 NS
	R	5 (55.6%)	33 (53.2%)	14 (48.3%)	52 (52.0%)	
	S	8 (88.9%)	50 (83.9%)	27 (93.1%)	87 (87.0%)	
	I	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
	R	1 (11.1%)	12 (16.1%)	2 (6.9%)	13 (13.0%)	

S: susceptible; I: intermediate; R: resistant; NX: norfloxacin; PRL: piperacillin; CAZ: ceftazidime; TMP: trimethoprim; LEV: levofloxacin; MRP: meropenem; IMP: imipenem; CN: gentamicin; AK: amikacin; CT: colistin. Percentages are of the column total. P-values were calculated by Fisher's exact test (two-sided, 3 × 3 table per antibiotic) because of expected cell counts < 5 across the biofilm categories; ** = highly significant (P ≤ 0.01); NS = non-significant.

The overall resistance profile was also related to the level of biofilm production (Table 7). This association was highly significant (p = 0.0001). The sensitive (p = 0.0001), XDR (p = 0.0001) and PDR (p = 0.0497) groups showed significant relationships, whereas the MDR group did not (p = 0.2174). In particular, most XDR isolates were moderate (28; 66.7%) or strong (11; 26.2%) producers, with only 3 (7.1%) weak producers, confirming that high-level (XDR) resistance was predominantly associated with moderate-to-strong biofilm capacity.

Table (7) Relationship between resistance profile and biofilm production level

Resistance profile	Weak	Moderate	Strong	Total	P-value
Sensitive	2 (5.3%)	24 (63.2%)	12 (31.6%)	38	0.0001 **
MDR	3 (20.0%)	6 (40.0%)	6 (40.0%)	15	0.2174 NS
XDR	3 (7.1%)	28 (66.7%)	11 (26.2%)	42	0.0001 **
PDR	1 (20.0%)	4 (80.0%)	0 (0.0%)	5	0.0497 *
Total	9 (9%)	62 (62%)	29 (29%)	100	0.0001 **

* P ≤ 0.05; ** P ≤ 0.01; NS = non-significant; MDR: multidrug-resistant; XDR: extensively drug-resistant; PDR: pandrug-resistant.

4-DISCUSSION

The management of hospital-acquired infections is increasingly challenging, and *P. aeruginosa*, a member of the ESKAPE group of pathogens, is a particularly versatile threat. It differs from other nosocomial bacteria in its remarkable ecological success, which stems from its genetic plasticity and metabolic adaptability. This versatility enables the bacterium to shift between acute, invasive infection and chronic, recalcitrant forms within the clinical milieu. The pathogen dynamically and selectively modifies its physiological state, for example in response to empirical antibiotic therapy and host immunity, which complicates patient management and increases the disease burden in tertiary care [31].

The present study identified 100 clinical isolates of *P. aeruginosa*. Demographic analysis showed more infections in males (54%) than in females (46%). This male predominance may be explained by greater occupational risk, outdoor activity and trauma exposure leading to hospitalisation and nosocomial infection [32]. By age, the 41–60-year group had the highest infection frequency, followed by the 21–40-year group; the increased susceptibility of the 41–60-year group is likely explained by gradual decline of the immune system, a higher burden of chronic comorbidities (diabetes, cardiovascular disease and others) and greater exposure to surgery or prolonged hospitalisation [32]. This demographic pattern is consistent with findings elsewhere, such as a study from Nasiriyah by Saud et al., which likewise found more *P. aeruginosa* infections in males than in females.

The isolates showed a descending pattern of resistance across the antibiotics tested. The highest resistance was to trimethoprim (95%), followed by gentamicin (58%), ceftazidime (56%), piperacillin and amikacin (52% each), meropenem and imipenem (46% each), levofloxacin (45%) and norfloxacin (41%). This descending order, with

trimethoprim and gentamicin at the top, closely matches recent findings of other Iraqi researchers investigating local isolates and points to the empirical failure of these agents in Iraq generally and in Baghdad specifically [33]. The very high resistance to trimethoprim observed here (95%) is best interpreted in light of the well-documented intrinsic (chromosomally encoded) resistance of *P. aeruginosa* to this agent [27,28]; the organism is not considered a clinically relevant target for trimethoprim in most international susceptibility panels. Trimethoprim was nonetheless retained in the antibiogram because it is included in the standard empirical panel used by the participating Iraqi hospitals for local antimicrobial resistance surveillance, and because reporting it allows this dataset to be compared directly with other Iraqi hospital antibiograms that also test it. Accordingly, the 95% resistance figure should be read as confirmation of the organism's expected intrinsic resistance and as a local surveillance reference point, rather than as evidence that trimethoprim was ever a viable treatment option for these infections.

The high level of resistance to these commonly used therapeutic agents is concerning and reflects strong selection pressure from empirical antibiotic use in Iraqi hospitals. Notably, colistin was the most effective antimicrobial agent, with only 15% of isolates resistant and 85% fully susceptible, confirming its importance as a last-resort therapeutic option. The modified diffusion protocol used for colistin in this study (Section 2.3.1), which accounts for the large size of the molecule, was adopted specifically because the standard Kirby–Bauer method under-detects colistin susceptibility [29].

The multidrug resistance analysis showed that the largest proportion of isolates (42%) were XDR, followed by sensitive isolates (38%), MDR (15%) and PDR (5%) isolates. PDR represents the most advanced and formidable resistance phenotype, more severe than XDR. The resistance level in Baghdad tertiary hospitals is notably higher than that reported from other provinces, probably reflecting a high degree of localised evolutionary adaptation [34]. Several local selective pressures may explain the high rates of resistant phenotypes observed in Baghdad, including aggressive empirical use of broad-spectrum antibiotics, which allows bacteria to develop more complex resistance mechanisms such as over-expression of multidrug efflux pumps (e.g. MexAB-OprM), and the capacity to build thick, highly protective biofilms in the hospital environment.

One of the major findings of this study was that 100% of isolates were able to form biofilm. On the basis of quantitative OD values, most isolates were moderate producers (OD 2–4; 62 isolates, 62%), 29 isolates (29%) were strong producers (OD > 4) and 9 isolates (9%) were weak producers (OD 1–2); no isolate fell into the non-producer category (OD < 1). The EPS matrix of the biofilm forms an almost impenetrable barrier that renders the cells highly tolerant of antibiotics. These findings corroborate the work of Ali and Abdul-Lateef [35], who showed that resistance and strong biofilm formation were highly correlated with the XDR and PDR phenotypes.

This study has some limitations. The reference strain *P. aeruginosa* ATCC 27853 was not available in our laboratory during the experimental phase, so a dedicated quality-control zone diameter could not be recorded for the modified colistin disc diffusion test; although the modified agar concentration parameters established by Uwizeyimana et al. [29] were followed meticulously, the colistin results should therefore be regarded as presumptive and require confirmation by broth microdilution. In addition, resistance and biofilm formation were characterised phenotypically only, and molecular analysis of resistance determinants and biofilm-associated genes was not performed.

CONCLUSION

The present study has shown that clinical isolates of *P. aeruginosa* from hospitalised patients in Baghdad, Iraq, are highly resistant to the tested antimicrobials and possess a high capacity to form biofilm. High resistance was observed to trimethoprim, gentamicin, ceftazidime, piperacillin and amikacin, whereas colistin was the most effective antimicrobial agent against the majority of isolates. Notably, the XDR phenotype emerged as the

predominant resistance pattern, clearly distinct from the MDR phenotype (15%); this high prevalence confirms that the pathogen is becoming a growing therapeutic challenge in the Iraqi healthcare system.

All isolates were able to form biofilm, and quantitative analysis showed that moderate biofilm producers represented the largest share. Biofilm production correlated significantly with certain clinical specimen types, with resistance to some antimicrobial agents and with the overall antimicrobial resistance phenotype. The biofilm-forming ability of XDR isolates is consistent with the hypothesis that biofilm formation is a significant factor in bacterial persistence, antimicrobial tolerance and the establishment of chronic hospital-acquired infections.

These findings highlight the need for continuous local surveillance of antimicrobial resistance, regular monitoring of bacterial biofilm-forming capacity, and the establishment of comprehensive antimicrobial stewardship and infection-prevention programmes in Iraqi hospitals. Future studies should include molecular analysis of resistance determinants and biofilm-associated genes, such as carbapenemase genes and quorum-sensing/biofilm regulatory genes, to better understand the mechanisms of multidrug resistance and persistent infection. Such data will be essential for developing effective treatment models and containing highly resistant strains of *P. aeruginosa*.

DECLARATIONS

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