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MDR *Pseudomonas aeruginosa* in Burn Units of Iraq: Resistance Trends, Biofilm Quantification Using Microtiter OD, Predictor of Clinical Outcomes

Fatima Hakim Obaid*
Department of medical microbiology. Hammurabi college of medicine, University of Babylon, Hilla 51002, Iraq
***Email:** ham306.samah.sajad@uobabylon.edu.iq

المكورات الزائفة المصلية متعددة مقاومة الأدوية في وحدات الحروق بالعراق: اتجاهات المقاومة، قياس الغشاء الحيوي باستخدام قياس الامتصاص الضوئي في الميكروتيتر، مؤشر على النتائج السريرية

فاطمة حاكم عبيد*
قسم الاحياء المجهرية الطبية، كلية حمورابي للطب، جامعة بابل، الحلة 251002، العراق

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Abstract
This study aimed to evaluate resistance patterns, longitudinal trends, biofilm production, and clinical determinants of mortality for Pseudomonas aeruginosa isolates from burn units. A multicenter longitudinal observational study included burn patients in whom Pseudomonas aeruginosa was first isolated in a non-recurring setting. The study included 360 burn patients. The majority of the cohort was male (63.1%) with a mean age of 31 years (interquartile range 20–44 years) and a mean burned body surface area of 27% (interquartile range 18–41 years). The highest resistance rates were recorded for the following antibiotic classes: ciprofloxacin (61.9%), levofloxacin (59.4%), and aztreonam (57.5%). Resistance rates for carbapenems were 40.3%, for imipenem 43.3%, and for meropenem 43.3%. Bacteria producing biofilms in high density constituted 22.5% of the total bacteria, with a high proportion of multidrug-resistant bacteria (77.8%) and a high proportion of non-drug-resistant bacteria (21.0%) ($p < 0.001$ and $p = 0.002$, respectively). In-hospital mortality was 13.6%, with a higher incidence in DTR (28.6 vs 11.8; $p = 0.002$), a lower incidence in DTR (strong biofilm 19.8 vs 11.7; $p = 0.041$), and a lower incidence in experimentally ineffective biofilm (25.9 vs 8.9; $p < 0.001$). Bacteremia was also present (50.0 vs 9.6; $p < 0.001$). The model demonstrated good discrimination (area under the curve = 0.83). Strong biofilm formation was strongly associated with multidrug resistance/direct immune response and was an independent predictor of mortality. Improving experimental treatment in accordance with more recent unit-specific protocols, antibiotic data, strengthening infection prevention policies, and integrating them with supervisory recommendations are important to reduce harmful procurement.

Keywords: *Pseudomonas aeruginosa*; burns, multidrug resistance, difficult to treat non-responsive, biofilms, carbapenem resistance, mortality, longitudinal trends.

الملخص
هدفت هذه الدراسة إلى تقييم أنماط المقاومة، والاتجاهات الطولية، وإنتاج الأغشية الحيوية، والمحددات السريرية للوفيات لعزلات بكتيريا الزائفة الزنجارية من وحدات الحروق. شملت دراسة رصدية طولية متعددة المراكز مرضى حروق تم عزل بكتيريا الزائفة الزنجارية لديهم لأول مرة في حالة غير متكررة. ضمت الدراسة 360 مريض حروق. كانت غالبية المجموعة من الذكور (63.1%) بمتوسط عمر 31 عامًا (المدى الربيعي 20-44 عامًا) ومتوسط مساحة سطح الجسم المحروق 27% (المدى الربيعي 18-41 عامًا). سُجلت أعلى معدلات المقاومة لفئات المضادات الحيوية التالية: سيبروفلوكساسين (61.9%)، وليفوفلوكساسين (59.4%)، وأزتريونام (57.5%). بلغت معدلات مقاومة الكاربابينيمات 40.3%، والإيميبينيم 43.3%، والميروبينيم 43.3%. شكلت البكتيريا المنتجة للأغشية الحيوية بكثافة عالية 22.5% من إجمالي البكتيريا، مع نسبة عالية من البكتيريا المقاومة للأدوية المتعددة (77.8%) ونسبة عالية من البكتيريا غير المقاومة

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للأدوية (21.0%) (قيمة $p < 0.001$ و $p = 0.002$ على التوالي). بلغت نسبة الوفيات داخل المستشفى 13.6%، مع ارتفاع في معدل الإصابة في حالات مقاومة المضادات الحيوية (28.6 مقابل 11.8؛ قيمة $p = 0.002$)، وانخفاض في معدل الإصابة في حالات مقاومة المضادات الحيوية (الأغشية الحيوية القوية 19.8 مقابل 11.7؛ قيمة $p = 0.041$)، وانخفاض في معدل الإصابة في حالات الأغشية الحيوية غير الفعالة تجريبياً (25.9 مقابل 8.9؛ قيمة $p < 0.001$). كما لوحظ وجود تجرثم الدم (50.0 مقابل 9.6؛ $p < 0.001$). أظهر النموذج تمييزًا جيدًا (المساحة تحت المنحنى = 0.83). ارتبط تكوين الأغشية الحيوية الكثيف ارتباطًا وثيقًا بمقاومة المضادات الحيوية المتعددة/الاستجابة المناعية المباشرة، وكان مؤشرًا مستقلًا للوفيات. يُعد تحسين العلاج التجريبي وفقًا للبروتوكولات الخاصة بالوحدة، وبيانات المضادات الحيوية، وتعزيز سياسات مكافحة العدوى، ودمجها مع توصيات الإشراف، أمورًا بالغة الأهمية للحد من عمليات الشراء الضارة.

الكلمات المفتاحية: الزائفة الزنجارية، الحروق، مقاومة الأدوية المتعددة، صعوبة العلاج وعدم الاستجابة، الأغشية الحيوية، مقاومة الكاربابينيم، الوفيات، الاتجاهات الطولية.

Introduction

Burn injuries are a major public health issue and represent an inequity levelled mainly against the poor and middle-income countries. Globally, burns are linked to significant mortality and prolonged disability and health systems in resource constrained settings are frequently limited in their capacity to address specialized ones as well as infection control and antimicrobial stewardship measures. [1] Beyond the initial tissue damage, loss of the skin barrier, devitalized tissue and the immune dysregulation caused by burns provide a perfect milieu for microbial colonization and toward invasive microbe infection. As a result, burn wound infection and burn associated sepsis remain leading causes of morbidity, extended length of hospitalization and numerous surgical procedures and death in burn centers. Contemporary burn critical-care data have consistently identified quantitative variables such as larger total body surface area (TBSA), inhalation injury and advanced age as important predictors for development of sepsis and sepsis-related mortality, with a strong interconnection of the risk for infection and burn severity and physiologic reserve [2,3]. Within burn units, healthcare-associated infections are further exacerbated by frequent use of devices, multiple wound manipulations, extensive range of antimicrobial effects and higher patient density. Gram-negative pathogens are the predominant pathogens in many burn centers and *Pseudomonas aeruginosa* in particular is very consequential as it is environmentally resilient, easily contaminates moist hospital reservoirs, and can cause outbreaks due to patient to environment to patient transmission. Clinically, *P. aeruginosa* is related to wound infections, bloodstream infections, ventilator-associated pneumonia and invasive septicemia in critically ill hosts. Its inherent resistance (low outer-membrane permeability, inducible AmpC and more than one efflux system), plus its ability to easily acquire more resistance determinants, make the treatment decisions time sensitive and complex. However, recent reviews have highlighted that MDR *P. aeruginosa* is now a constant danger in all healthcare settings with ever-increasing difficulties for treatment and an increasing reliance on the few new combinations of drugs of the beta-lactam group with beta-lactamase inhibitors or alternative drugs in serious infections [4,5,6,7]. Antimicrobial resistance (AMR) in *P. aeruginosa* is also known to be a R&D and public health priority worldwide. The World Health Organization's Bacterial Priority Pathogens List (BPPL) 2024 fully acknowledges the importance of carbapenem-resistant P. *aeruginosa* as a priority pathogen as a result of its burden of disease in healthcare settings and need for continued surveillance and continued innovation of therapeutics and prevention strategies [8,9]. In peace, the Infectious Diseases Society of America (IDSA) have pointed out fresh pointers of 2024 focusing on tough resistance (DTR) in *P. aeruginosa*, noting that clinical management has positioned itself increasingly on correct susceptibility testing, cognition of local epidemiology, and prompt use of effective therapy in the setting of reduce odds of reaction of traditional therapy [10]. Standardized definitions for MDR/XDR/PDR phenotypes further allow for meaningful comparisons across both centers and also from before and after [11], these remain essential for longitudinal trends and benchmarking in burn units where resistance may change quite rapidly, under conditions of

selective antimicrobial pressure. A defining characteristic among *P. aeruginosa* that ties to the concept of its persistence as well as poor outcomes in burn wounds is its ability to form biofilms. Biofilms are structured communities of microorganisms encased in an extracellular matrix and they adhere to biotic and abiotic surfaces. In burn wounds, formation of biofilm can hinder host clearance and decrease myeloid penetration of drugs and also promotes phenotypic tolerance (including persister populations), promoting chronicity, graft failure, recurrent infection and requiring repeat debridement. Evidence from recent clinical studies and systematic analyses of literature supports an association between increased biofilm formation and increased levels of antimicrobial resistance in *P. aeruginosa* suggesting that biofilm phenotype is not only a virulence trait and that it may correlate with clinically relevant resistance patterns and risk of treatment failure [12,13]. For laboratory quantification, the crystal violet microtiter plate procedure is still widely used because it is cheap, scalable and amenable for routine characterization of large collections of isolates, allowing objective optical density (OD) based measurement of adherent biomass, under a standardized growth condition [14]. However, the inter-laboratory variability as well as the methodological heterogeneity (e.g. plate type, staining protocol, solvent, wavelength selection, cut off definitions) could impact the comparability, which is why reproducibility studies and standardization works are celebrated more and more if biofilm metrics are going to be used to inform epidemiology or outcome modeling [15,16,17]. In Iraq, as in many other countries, burn units are under the burden of balancing a high rate of infection and rising rates of AMR with a paucity of robust longitudinal data units which incorporate phenotypic resistance trajectories as well as biofilm quantity and clinical outcomes. Recent studies conducted in Iraq validate this by confirming that MDR *P. aeruginosa* is very commonly seen in patients who develop burns, as well as the need for surveillance studies to provide direct support for empiric therapy policies and stewardship interventions based on local resistance ecology [18,19]. However, most available reports are cross-sectional in form and often try to see the prevalence of resistance, with little or no systematic link between microbiologic phenotypes and patient-level outcomes and predictors of resistance. This gap is clinically important since that burn-unit decisions demand more than the isolated susceptibility proportions: they require time-trended resistance signals (for early detection of emerging threat), objective biofilm profiling (of the potential for persistence), and multivariable outcome models (including the burn severity and care factors. TBSA, inletation injury, timing of infection, exposure to invasive devices, ICU admission and antimicrobial history). Accordingly, the proposed study titled "MDR *Pseudomonas aeruginosa* in Burn Units in Iraq: Longitudinal Resistance Trends, Biofilm Quantification (Microtiter OD), and Clinical Outcome Predictors," is aimed at developing an integrated and practice-relevant evidence base. By combining (i) the longitudinal resistance trend analysis, (ii) the standardization of microtiter OD biofilm quantification and (iii) the prediction of clinical outcome modelling, the aim of the study is to identify the actionable predictors of adverse outcome and to provide the data that can be used directly to guide infection prevention priority setting, update empiric therapy, and antimicrobial stewardship strategies for the Iraqi burn centers.

Material and methods

Study Design and Setting

This study was designed as a multicenter longitudinal observational study which was conducted in burn units of tertiary hospitals of Iraq over a period of 18 months. The research consisted of a combination of a microbiological surveillance study of *P. aeruginosa* isolates and a prospective collection of clinical and outcome data. Reporting of the study followed recommendations based on the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) as a means of enhancing the methodological transparency and reproducibility of the study [20].

Study Population

Burn patients admitted to participating burn units were eligible to be included in the study if they had at least one culture confirmed isolation of *P. aeruginosa* from a clinical specimen (wound swab, tissue biopsy, or blood culture). Only the first isolate associated with a patient and an infectious episode (defined as a 14-day interval) was included so as not to include duplications. Patients excluded from the study were those with isolates that were classified as contaminants, or those that were taken for environmental surveillance without clinical correlation.

Clinical Data Collection

Demographic and clinical variables were obtained from patient records based on a standardized case report form. Collected data were age, sex, total body surface area (TBSA), burn depth, inhalation injury, admission to ICU, use of invasive device, surgery, antimicrobial exposure before culture, and hospital stay. Outcomes which were measured included in-hospital mortality (primary outcome); bacteremia; prolonged hospitalization; and graft failure where applicable. Definitions of healthcare-associated infection have been modified from proven surveillance frameworks in order to ensure consistency among different centers [21].

Collection and Identification of Specimen

Wound samples were collected on sterile swabs following wound cleansing or by tissue biopsy during the surgical debridement procedure. No. Blood cultures were collected in cases envisaged systemic infection clinically. All specimens were handled in the standard microbiologic manner. Isolation was carried out on selective and non-selective medium and presumptive identification was made preceding biochemical confirmation or automated identification systems where available based on the colony morphology, oxidase reaction and pigment production.

Cognitive assessment for Susceptibility to Antimicrobials

Antimicrobial susceptibility testing (AST) was carried out by disk diffusion or automated technique with respect to Clinical and Laboratory Standards Institute (CLSI) M100 performance standards [22] or European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints [23] and any in-house consistency of tests was maintained. The tested panel of antibiotics availed at is piperacillin-tazobactam, ceftazidime, cefepime, aztreonam, imipenem, meropenem, ciprofloxacin, levofloxacin, amikacin, gentamicin, colistin if clinical indicators are present. Quality control was ensured using *P. aeruginosa*, first type of period, 01832,27853,1. Resistance phenotypes (MDR, XDR and DTR) were allocated following international consensus criteria and clinical interpretive frameworks for the difficult to treat resistance [24].

Biofilm Quantification

Biofilm formation was determined by the crystal violet microtiter plate assay. Overnight cultures of *P. aeruginosa* were normalized to McFarland 0.5 degree and diluted to 1:100 dilution in tryptic soy broth. Aliquots were seeded into 96-well polystyrene microtiter plates and incubated at 37 degrees C for 24 hours. Wells were gently washed with phosphate buffered saline to remove planktonic cells, fixed and stained using crystal violet 0.1%. After washing, dissolved dye was measured by an ethanol solubilization and measured for optical density (OD) at 570 nm using a microplate reader. Biofilm production was classified as non-producer, weak, moderate or strong following cut-off values calculated on negative controls (ODc = mean OD of control + 3x standard deviation). Technical triplicates were used for every isolate and 10% of isolates were re-tested to determine reproducibility [25].

The Longitudinal Trend Analysis of Resistance

Proportions of resistance for antimicrobial agents and resistance phenotypes were calculated monthly and quarterly. Temporal trends were estimated using tests of Cohan, Armitage trend and a segmented regression models where suitable. Inter-hospital variability was controlled for with hospital as a random effect in mixed effects logistic regression models [26].

Predicting Outcome Modeling

Multivariable logistic regression was used to predict independent predictors of in-hospital mortality and other adverse outcomes. Candidate variables included TBSA, inhalation injury, ICU stay, MDR/DTR phenotype, biofilm category and appropriateness of first antimicrobial therapy. The performance of the model was evaluated with two techniques: area under curve (AUC-ROC) and calibration plots. Internal validation was done using bootstrap resampling.

Data Management

All data were anonymized and entered into one secure data base. Double data entry was recorded for main variables to reduce the number of errors in transcription. Missing data withdrawn or implemented to multiple imputation, if appropriate.

Ethical Considerations

Ethical approval was received from institutional review boards of hospitals where study participants were recruited. Because bacteria isolates were obtained from routine diagnostic samples, informed consent was waived by the study in line with the national research regulations. Patient confidentiality was maintained throughout the course of the study.

Results and discussion

The study included 360 burn patient episodes with first non-duplicate *Pseudomonas aeruginosa* isolates. The cohort was predominantly male (227/360, 63.1%), with a median age of 31 years (IQR 20–44), indicating that most cases clustered in young and middle-aged adults. Burn severity was moderate-to-high, with a median TBSA of 27% (IQR 18–41). A substantial proportion of patients had full-thickness burns (150/360, 41.7%), reflecting extensive tissue injury that typically requires repeated debridement and grafting and is commonly associated with higher infection risk. Inhalation injury was documented in 82/360 (22.8%), a clinically important factor often linked to ventilatory support and worse prognosis. Nearly half of the cohort required ICU admission (151/360, 41.9%), consistent with high acuity. Prior antibiotic exposure within the defined window was frequent (196/360, 54.4%), suggesting considerable selective pressure that may contribute to MDR emergence. The median time from admission to the first *P. aeruginosa* isolation was 6 days (IQR 3–9), supporting that many episodes likely represented hospital-acquired infection or late colonization rather than early community acquisition.

Table 1: Baseline characteristics (N = 360)

Variable	Value
Age, median (IQR)	31 (20–44)
Male sex, n (%)	227 (63.1)
TBSA %, median (IQR)	27 (18–41)
Full-thickness burns, n (%)	150 (41.7)
Inhalation injury, n (%)	82 (22.8)
ICU admission, n (%)	151 (41.9)
Prior antibiotic use, n (%)	196 (54.4)
Time to first isolate (days), median (IQR)	6 (3–9)

Most isolates were isolated from wound swabs (252/360, 70.0%), affirming the preferred location of clinical reservoir for *P. aeruginosa* identification in this study cohort was burn wound surfaces. Tissue/biopsy specimens accounted for 72/360 (20.0%) and it usually represents deeper sampling in more severe or surgically managed wounds and therefore could be more representative of invasive wound infection compared with superficial swabbing. Blood cultures were used in 36/360 (10.0%), indicating a frequency of isolating samples from blood representing one in ten episodes of undetermined etiology, which is a marker of invasive disease representing an exposure with a strong association with bad outcome in burn populations.

Table 2: Specimen type (N = 360)

Specimen	n (%)
Wound swab	252 (70.0)
Tissue/biopsy	72 (20.0)
Blood culture	36 (10.0)

Non-susceptibility was potentially highest with fluoroquinolones and aztreonam and may vary in usefulness of these agents for empiric coverage of severe burn-unit *P. aeruginosa* infections without known susceptibility. Ciprofloxacin non-susceptibility approached 223/360 (61.9%) and levofloxacin 214/360 (59.4%) suggesting the abundance of fluoroquinolone hinderance. Aztreonam nonsusceptibility was also high at 207/360, or 57.5%. Cephalosporin activity was also reduced significantly with non-susceptibility with ceftazidime 185/360 (51.4%) and cefepime 172/360 (47.8%). Carbapenem non-susceptibility was also clinically significant (imipenem 145/360, 40.3% non-susceptible; meropenem 156/360, 43.3% non-susceptible) which helps build favor for the increasing resistance to carbapenems. In contrast, aminoglycosides maintained comparatively better activity, especially amikacin (113/360, 31.4%) while gentamicin (140/360, 38.9%) and tobramycin (124/360, 34.4%) activity was also lower than beta-lactam and Fluoroquinolones non-susceptibility, this susceptibility pattern is consistent with an environment where several first line anti-pseudomonal classes are often compromised.

Table 3: Non-susceptibility rates (I+R)

Antibiotic	n/N (%)
Piperacillin–tazobactam	158/360 (43.9)
Ceftazidime	185/360 (51.4)
Cefepime	172/360 (47.8)
Aztreonam	207/360 (57.5)
Imipenem	145/360 (40.3)
Meropenem	156/360 (43.3)
Ciprofloxacin	223/360 (61.9)
Levofloxacin	214/360 (59.4)
Amikacin	113/360 (31.4)

More than half of isolates were eligible of MDR, and in our study 192/360 isolates (53.3%) were MDR. Extensive drug resistance (XDR) was also common (64/360, 17.8%), which means that a significant minority of isolates were still susceptible to only one or two classes of antimicrobials. Pan drug resistance (PDR) was uncommon (3/360, 0.8%), indicating that pan drug resistance was uncommon but occurred in non-susceptibility. Importantly, 42/360 (11.7%) belonged to the difficult to treat resistant (DTR) category, which represents a level of resistance patterns known to compromise the reliability of standard options of the first-line therapy, in turn clinically associated with limited choices of effective empiric treatment, and delayed time-to-active therapy. This distribution of phenotypes suggests the impression of a high overall burden of resistance as well as a clinically meaningful subset of the highest treatment challenge.

Table 4 :Resistance categories

Phenotype	n (%)
MDR	192 (53.3)
XDR	64 (17.8)
PDR	3 (0.8)
DTR	42 (11.7)

A definite upward trend in resistance was found for the six quarters. Meropenem non-susceptibility rose from 35.5% in Q1 to 51.2% in Q6 and the statistical test for a trend was statistically significant ($p = 0.004$), which encourages confidence that there is a true increase in non-susceptibility over time and is not a result of random fluctuation. MDR frequency increased from 47.5 to 58.5% between these same time periods ($p = 0.021$), predicting progressive example of gathering multiple classroom resistance organ in the burn sector. DTR was also higher in Q6 (16.4%) than in Q1 (8.3%, $p = 0.038$), suggesting that an increasing proportion of isolates are for which standard empiric anti-pseudomonal regimens are less likely to be active. Together, these longitudinal findings suggest worsened of susceptibility over time (compatible with sustainable antimicrobial selective strain working felony and/or persevering transmission of resistant strains within the burn unit environment).

Table 5 :Quarterly trend analysis

Quarter	Meropenem NS %	MDR %	DTR %
Q1	35.5	47.5	8.3
Q2	39.2	50.0	9.8
Q3	41.9	52.4	11.3
Q4	45.0	54.9	12.7
Q5	48.3	56.7	14.8
Q6	51.2	58.5	16.4
Trend p-value	0.004	0.021	0.038

Biofilm production was frequent in which 81/360 (22.5%) were identified as strong biofilm producers, 115/360 (31.9%) of moderate, 108/360 (30.0%) of weak and 56/360 (15.6%) were non-producers of biofilm. There was a significant gradient between biofilm strength and antimicrobial resistance. MDR prevalence level gradually rose from 30.4% among non-producers to 41.7% among weak producers, 58.3% among moderate producers and 77.8% among strong producers; this association was found to be highly significant ($p < 0.001$) supporting a strong association between higher biofilm biomass and multi-class resistance. A similar trend was obtained for DTR (from 7.1% in non-producers and 7.4% in weak producers up to 11.3% in moderate producers and 21.0% in strong producers; $p = 0.002$). These findings suggest that isolates with strong biofilm formation represent a sociotype of high-risk microbiological isolates that are enriched for resistance phenotypes which are clinically associated with reduced treatment options and potentially worse outcome.

Table 6: Categories and resistance to biofilm

Biofilm category	n (%)	MDR n (%)	DTR n (%)
None	56 (15.6)	17 (30.4)	4 (7.1)
Weak	108 (30.0)	45 (41.7)	8 (7.4)
Moderate	115 (31.9)	67 (58.3)	13 (11.3)
Strong	81 (22.5)	63 (77.8)	17 (21.0)
p-value	-	<0.001	0.002

In-hospital mortality was 13.6% (49/360). Mortality was almost threefold higher in episodes involving DTR isolates (28.6%) than in non-DTR isolates (11.8%) and the difference was significant (p = 0.00 amount of risk.). and mucormycosis used to detect can be clinically relevant. and DTR phenotype is, start to appear, associated with There is clinical evidence. Similarly, a strong biofilm formation was associated with an increased mortality (19.8 vs 11.7% - p value=0.041), which support the clinical relevance of the biofilm phenotype identified beyond a laboratory characterization. The strong crude association with death was seen for inactive empiric therapy (25.9 vs 8.9%; p < 0.001), and was consistent with the notion that delayed active therapy aggravates outcomes in the severely infected. Highly independent of mortality was also bloodstream infection (bacteremia [50.0% vs 9.6%; p < 0.001], invasive disease was an important determinant of fatal outcome in burn patients).

Table 7: Mortality by major factors

Factor	Mortality %	p-value
DTR vs non-DTR	28.6 vs 11.8	0.002
Strong biofilm vs others	19.8 vs 11.7	0.041
Inactive empiric therapy vs active	25.9 vs 8.9	<0.001
Bacteremia vs none	50.0 vs 9.6	<0.001

After adjustment for main clinical and microbiological covariates, several factors were found independently associated with the in-hospital mortality. India each 10% increase in TBSA increased the odds of death 49% (aOR 1.49; 95% CI 1.25-1.78; p shorter 0.001): Confirms that burn size is a major marker of severity. Inhalation injury increased the odds of death by 100% from AOD in general (aOR 2.01; 95% CI 1.09-3.70; p = 0.025), consistent with the disturbance of the state of the respiratory system and the load on the general inflammatory reaction. Bacteremia was the strongest predictor (aOR 4.15; 95% CI 2.05--8.40; p <. 001), suggesting that food-borne progression to bloodstream poses a dramatic increase in terms of fatality. Inactive empiric therapy was again a significant modifiable factor (aOR 2.28; 95% CI 1.22-4.25; p = 0.010), and demonstrates the importance of adopting local empiric regimens that are based on the burn unit antibiogram. Importantly, the DTR phenotype (aOR 2.02 [95% CI 1.01 - 4.05, p = 0.047] and a strong biofilm formation (aOR 1.85 [95% CI 1.01 - 3.38, p = 0.048]) were found to remain independently significant, thus suggesting that resistant phenotypes and biofilm ability are contributors to risk of death beyond burn severity only. The model demonstrated good discrimination (AUC = 0.83), hence indicating that the included predictors provide useful discrimination between survivors and non-survivors.

Table 8: Multivariable predictors (logistic regression model)

Predictor	Adjusted OR	95% CI	p-value
TBSA (per +10%)	1.49	1.25–1.78	<0.001
Inhalation injury	2.01	1.09–3.70	0.025
Bacteremia	4.15	2.05–8.40	<0.001
Inactive empiric therapy	2.28	1.22–4.25	0.010
DTR phenotype	2.02	1.01–4.05	0.047
Strong biofilm	1.85	1.01–3.38	0.048

Model performance: AUC = 0.83

Discussion

This multicenter longitudinal study provides an integrated analysis of antimicrobial resistance (AMR), biofilm-forming capacity, and clinical outcome predictors among *Pseudomonas aeruginosa* isolates from burn units in Iraq. The findings demonstrate a high burden of MDR and DTR phenotypes, a progressive increase in carbapenem non-susceptibility over time, and a strong association between biofilm production, resistance patterns, and adverse clinical outcomes. The MDR prevalence exceeding 50% is consistent with recent global reports from burn and critical-care settings, where *P. aeruginosa* remains one of the dominant pathogens with complex resistance profiles. Similar MDR rates have been documented in burn cohorts from South Asia and North Africa, underscoring the convergence of burn severity, prolonged hospitalization, and selective antimicrobial pressure as drivers of resistance expansion [27,28]. The observed upward trend in meropenem non-susceptibility and DTR frequency across sequential quarters suggests ongoing selective pressure and possible clonal persistence or horizontal dissemination within burn units. Longitudinal increases in carbapenem resistance have been linked to sustained carbapenem consumption and suboptimal infection control adherence in high-risk wards, including burn centers [29,30]. Fluoroquinolone and aztreonam resistance were the most prominent phenotypes in this study, aligning with global surveillance data indicating erosion of activity of these agents against *P. aeruginosa*. In several recent regional surveillance programs, fluoroquinolone non-susceptibility exceeded 60%, mirroring the pattern observed here and reinforcing concerns regarding empirical use of these drugs in severe burn infections without susceptibility confirmation [31]. In contrast, aminoglycosides retained comparatively better activity, a pattern reported in multicenter ICU studies where amikacin often remains one of the last reliable conventional options for *P. aeruginosa* infections [32]. A contribution of this study is the demonstration of a graded relationship between biofilm biomass and resistance phenotypes. Strong biofilm producers were significantly enriched among MDR and DTR isolates, supporting the hypothesis that biofilm formation and antimicrobial resistance are biologically and epidemiologically linked. Experimental and clinical studies have shown that biofilm growth induces phenotypic tolerance through reduced metabolic activity, altered gene expression, and impaired antibiotic penetration, thereby facilitating the survival of resistant subpopulations under antimicrobial pressure [33,34]. Recent burn-specific investigations further indicate that biofilm-dominant infections are associated with delayed wound healing and increased likelihood of graft failure, which may partially explain the higher mortality observed among patients infected with strong biofilm–forming isolates in this cohort [35]. The clinical outcome analysis highlights several independent predictors of mortality. Burn severity, represented by TBSA and inhalation injury, remained the strongest host-related determinants, consistent with contemporary burn outcome models [36]. Importantly, microbiological factors retained significance after adjustment for burn severity. DTR phenotype and strong biofilm formation were independently associated with increased odds of death, underscoring the clinical relevance of phenotypic resistance classification beyond simple susceptibility proportions. These findings are concordant with recent multinational studies showing that infections caused by DTR *P. aeruginosa* are associated with delayed effective therapy and excess mortality compared with non-DTR strains [37]. Inappropriate empiric therapy emerged as a major modifiable risk factor for death. Patients who initially received inactive antimicrobial regimens had more than double the adjusted odds of mortality. This finding reinforces evidence from critical-care cohorts demonstrating that early discordant therapy in Gram-negative sepsis significantly worsens outcomes, particularly when resistant organisms are involved [38]. The combination of rising resistance trends and high rates of inactive empiric therapy suggests an urgent need for local antibiogram updates, burn-unit–specific empirical guidelines, and antimicrobial stewardship interventions tailored to resistance dynamics observed in this population. Bloodstream infection represented the most lethal clinical

presentation in this study, with mortality approaching 50%. This aligns with recent meta-analyses indicating that *P. aeruginosa* bacteremia in burn patients carries substantially higher mortality than wound-limited infection, reflecting both pathogen virulence and host physiological compromise [39]. The coexistence of bacteremia with DTR phenotype or strong biofilm production likely compounds this risk, although this interaction warrants further targeted investigation. From a public health and infection control perspective, the documented longitudinal increase in resistance and the clustering of high-risk phenotypes among strong biofilm producers suggest possible intra-unit persistence and transmission. Environmental reservoirs and contaminated moist surfaces have been repeatedly implicated in sustaining *P. aeruginosa* in burn units, highlighting the need for intensified surveillance, water system monitoring, and surface decontamination strategies [40,41]. Moreover, routine phenotypic biofilm assessment, although not standard practice, may provide additional stratification for infection risk and could be integrated into research-oriented surveillance frameworks [42,43].
This study has limitations. First, molecular characterization of resistance and clonal relatedness was not performed, precluding direct inference regarding transmission pathways. Second, although longitudinal, the observational design limits causal interpretation of associations between biofilm production and clinical outcomes[44,45]. Third, colistin susceptibility testing was available only for a subset of isolates, restricting conclusions regarding last-line agents. Nevertheless, the strength of this study lies in its integration of temporal resistance trends, standardized biofilm quantification, and multivariable outcome modeling in a high-risk clinical population.

Conclusion

This multicenter longitudinal analysis shows the high prevalence of MDR *P. aeruginosa* infections in burn units of Iraq and also confirms rising non-susceptibility to carbapenem and frequency of DTR, indicating a deterioration of the resistance situation. Biofilm formation is prevalent and the strong biofilm producers are overrepresented among MDR/DTR leading to a clinically important relationship between biofilm phenotype and the risk of treatment failure. Clinically, death is influenced by the severity of the burn (TBSA, inhalation injury) and also by infection-related factors, and bacteremia, inactive empiric therapy, DTR phenotype, and strong biofilm formation have been shown to independently predict death.
Strengths
This study combines three high-impact components within the field of burn-unit epidemiology; longitudinal resistance trend analysis, standardized phenotypic quantification of biofilm (microtiter OD) and multivariable analysis of clinical outcomes. The use of first non-duplicate isolates per episode reduces selection bias from repeated sampling phenomena as well as more interpretable resistance proportion. The combination of microbiological and clinical framework gives support for translation to decision-making in the areas of stewardship and infection control.

Limitations

The design of a study with observations limits causal inference. Molecular characterization (clonal relatedness, resistance genes) was not included and therefore could not complete attribution of increasing resistance to categories (transmission pathways) of emergence versus independent emergence. Biofilm quantification was carried out under standardized measured in vitro conditions and thus does not necessarily replicate wound microenvironments. Additionally, some agents that fall into the last resort category were not available for global testing, which may have limited interpretation of susceptibility to newer/reserve agents.

Ethics approval and consent form

The Institutional Review Boards of participating hospitals and/or universities in Iraq gave ethical approval. As isolates were obtained from routine diagnostic specimens and the

analysis of data performed in a de-identified fashion, informed consent was waived/obtained according to the local regulations (specify clearly which applies). Confidentiality was ensured in data collection and data analysis.

Consent for publication

Not applicable/ or to be obtained Where required (de-identified data)

Data availability and materials availability

De-identified datasets and analysis codes will be made available by an Author Method: Corresponding Author Request End User Note on Potential Biases Associated With Data Use and Analysis There could be a risk that data have been de-identified or less tremendous data have been entered or removed for data sharing purposes against Case Report Form Control Questions De-identified Laurent et al Case Report have approvals Data sharing Laurent et al will be contained from data sharing purposes given institutional approvals Policies and terms of data sharing The data sharing will be made here after reasonable request subject to institutional approvals and data sharing policies.

Competing interests

The authors state that they have no competing interests.

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