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Biofilm generation and antibiotic resistant profile of extensive and multidrug resistant *Pseudomonas aeruginosa* from burn patients in Ahvaz: A cross-sectional study

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Abstract

Background and Aims: Multidrug and extensive drug-resistant *Pseudomonas aeruginosa* was extracted from burn patients referring to burn centers in southwest Iran so that biofilm generation and antibiotic resistance could be investigated.

Methods: A specific primer was used to confirm all our considered 110 *P. aeruginosa* culture-positive reports on 345 burn patients. The resistance of *P. aeruginosa* to seven antibiotics and Colistin with minimum inhibitory concentration (MIC) was assessed. Biofilm formation was assessed by the phenotypic study of specimens under Congo red agar and microtiter plate assays.

Results: One hundred and 10 clinical *P. aeruginosa* isolates taken from burn wound infections were validated. Among *P. aeruginosa* isolates, Piperacillin, Ceftazidime, Maeropenem, Gentamycin, and Gatifloacin had the highest resistance to antibiotics, while Ticarcillin-Clavulanic acid and Ceftolozane-Tazobactam showed the least resistance. MICs were then evaluated via the E test. Seven isolates were resistant to colistin. Colistin reference MICs for multidrug-resistant *P. aeruginosa* prevalence was 38%, while it was 22% for extensively drug-resistant (XDR) *P. aeruginosa*. One *P. aeruginosa* was pandrug-resistant (PDR). Under Congo red agar test, 66 isolates (67%) formed biofilms and black colonies, whereas 44 isolates (50%) had red colonies. In MTP, 76% formed biofilm. 40%, 32%, 21% of the isolates were strong, moderate, and weak biofilm formers, respectively, while 43% did not form biofilms.

Conclusion: The *P. aeruginosa* resistance to antimicrobial agents has largely challenged the control of the infection. Accordingly, a higher resistance occurred when the isolates were transferred to the patients. Less than 50% *P. aeruginosa* samples generated strong biofilms. Consequently, hygienic measurements are essential to inhibit *P. aeruginosa* transmission to hospitalized patients.

KEYWORDS

antibiotics, antibiotic resistance, burn wound, drug resistance, *P. aeruginosa*

1 | INTRODUCTION

Burn wounds might be life threatening and severe burn patients are usually at the risk of failure of different organs, and in those who survive the acute phase, infections are still the most common cause of mortality. More than 75% of deaths due to burn in severely burned patients can be attributed to sepsis, infection complications, and inhalation injury.[1] About 180,000 burn-related deaths happens each year, most of which in disadvantaged developing countries. Infection complications are responsible for around 73% of casualties in the first 5 days after burning.[2] In Iran, reports on burn-related death rates range from 1.4/100,000 to 9.7/100,000 with case fatalities from 2% to 14% irrespective of study population.[3] Some risk factors associated with infection initiation are skin tissue disruption, burnt area expansion, immunocompromised impact, and lengthy hospitalization. By the growth of microorganisms in the initially-sterile burn wounds, infection would then occur depending on the wound's nature and extension and microorganism's species and number.[4] *Pseudomonas aeruginosa* as a leading factor in the induction of serious infections over the wound.[5] *P. aeruginosa* is indeed among the top 10 virulent nosocomial pathogens worldwide. Treatment of the virulent *P. aeruginosa* infection has also been restricted to antimicrobial-robust strains dissemination.[6] *P. aeruginosa* survival in nosocomial ambiences is increased by strengthening innate resistance mechanisms like efflux pumps overexpression and acquiring foreign genetic agents like plasmids.[7] Due to their fast adaptation capability under environmental stresses, *P. aeruginosa* strains can also resist all β-lactam agents.[8] Accordingly, the control of the gram-negative multidrug-resistant (MDR) A. *P. aeruginosa* that is highly resistant to antibiotics has been a great challenge.[9] By the entrance of the opportunistic *P. aeruginosa* pathogen into the host tissue, it produces biofilm and causes infection concomitantly.[10] During biofilm production process, due to the irreversible attachment and growth of microorganisms on the surface, extracellular polymers are formed, which increases matrix formation and attachment leading to the change of phenotype of the organisms for rate of growth and transcription of gene.[11] This study considered the infestation of drug-resistant strains of *P. aeruginosa* over burnt wound patients in Ahvaz, Iran.

2 | MATERIALS AND METHODS

2.1 | Ethics approval and consent to participate

The research obtained the approval of Research Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (No: IR. AJUMS.RE .1399.669). In line with the Declaration of Helsinki, 1975, this observational study was performed without any interventions. All subjects, freely given, informed written consented to participate in the study.

2.2 | Specimen collection and microbiological procedures

The study was done in Taleghani Hospital's Burn Center as the main clinic in Ahvaz, Iran, in May 2019–November 2021. To this end, after removing the duplicates, 110 *P. aeruginosa* culture-positive reports on wound 345 patients were considered. Patients with burn infections who had a hospitalization period of equal to or over 48 h were examined. According to the American Burn Association, wound colonization is defined as the presence of a low concentration of bacteria on the surface without invasion or systemic manifestations. When there is more than 10^5 of tissue in the wound, a wound infection exists. When more than 10^5 of tissue in the burn wound causes the formation of pus and separation of the eschar, loss of graft with the involvement of tissue, or the presence of systemic sepsis, then it is called invasive infection.[12] Thus, any patties with any wound colonization were excluded from the study. For clinical examinations, the isolates were soon moved to Microbiology Department in Jundishapur University of Medical Sciences. For isolates confirmation, we employed conventional and biochemical tests like culturing on Eosin-Methylene Blue (EMB) agar (Biolife), Cetrimide agar, blood agar, TSI, oxidation fermentation (OF) test, and pigment production in Mueller Hinton Agar (Biolife) and growth at 42°C.[13]

2.3 | Polymerase chain reaction (PCR) test to confirm *P. aeruginosa*

The boiling method was used to extract genomic DNA from isolates. A few bacterial colonies grown overnight on nutrient agar (Merck) were suspended in microtubes containing 500 µL of Tris-EDTA buffer. The microtubes were placed in cub lock microtube incubators (Denville Scientific) for 5 min at 95°C, and then centrifuged at 14,000 rpm for 10 min at 4°C. The supernatant was used as the DNA template in the PCR assays. The quality and average DNA yield were assessed using Nano Drop Spectrophotometer PROMO (Thermo Scientific).[14]

Amplification reactions were set up as detailed by Mohammed et al.[15] The following *16S rRNA* specific primer set was used (Sigma-aldrich): 16S forward primer: 5'-AGAGTRTGATCMTYGCTWAC-3'; 16S reverse primer: 5'-CGYTAMCTTWTTACGRCT-3'. Following optimization, reaction mixes (100 µL) were set up as follows: 10 mM Tris/HCl, pH 8·3; 50 mM KCl; 2·5 mM MgCl2; 200 µM (each) dATP, dCTP, dGTP and dTTP; 1·25U Taq DNA polymerase (Genei Bangalore, India); 0.1 µM (each) primer; and 4 µL DNA template. Reaction mixtures, following a "hot start," were subjected to the following empirically optimized thermal cycling parameters: 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 2 min, followed by a final extension at 72°C for 5 min. Positive (*P. aeruginosa* ATCC 27853 DNA) and multiple negative (water) amplification controls were included in every set of PCRs. PCR products were run on 1.5% agarose gel and were afterward visualized under UV lamp.

2.4 | Drug susceptibility testing (DST)

Using disk diffusion based on Clinical and Laboratory Standards Institute (CLSI) procedures (2020), *P. aeruginosa* isolates resistance to seven antibiotics classes was evaluated. In antibiotic discs, seven antibiotic classes were observed: PENICILLINS (100 µg Piperacillin), B-LACTAM COMBINATION AGENTS (100/10 µg Piperacillin-tazobactam), (30/20 µg Ceftazidime-avibactam), (30/10 µg Ceftolozane-tazobactam), (75/10 µg Ticarcillin-clavulanate), CEPHEMS (30 µg Ceftazidime), (30 µg Cefepime), MONOBACTAMS (30 µg Aztreonam), CARBAPENEMS (10 µg Doripenem), (10 µg Imipenem), (30 µg Meropenem), AMINOGLYCOSIDES (10 µg Gentamicin,), (10 µg Tobramycin), (30 µg Amikacin), (30 µg Netilmicin), FLUOROQUINOLONES (5 µg Ciprofloxacin), (5 µg Levofloxacin), (10 µg, Lomefloxacin), (10 µg Norfloxacin), (5 µg Ofloxacin), and (5 µg Gatifloxacin). The *P. aeruginosa* phenotypes were pandrug-resistant (PDR), MDR, and extensively drug-resistant (XDR) based on International Expert proposal for Interim Standards.[16]

2.5 | Minimum inhibitory concentration (MIC)

Colistin MICs were measured by E-test strips (Liofilchem) and interpreted based on CLSI (2021) guidelines. Once the MICs were measured with the E-test method, the *P. aeruginosa* isolates with a MIC equal to or lower than 2 µg/mL were assumed to be moderate, while MICs equal to or higher than 4 µg/mL were considered resistant. *Escherichia coli* ATCC 25,922 was used as quality control for antimicrobial susceptibility testing.

2.6 | Biofilm production assessment under phenotype approach

2.6.1 | Biofilm production in 96-well microplate

Biofilm production extent was assessed by considering the capability of attachment to polystyrene microplate.[21] Using the computation system introduced by Stepanovic et al., we defined cut-off optical density (ODc) as three standard deviations (SD) over the average OD of inoculated medium (negative control): $ODc = average\ OD\ of\ negative\ control + (3 \times SD\ of\ negative\ control)$.[22] For each microplate, the ODc was separately measured. For a better result description, we categorized the strains as $OD \leq ODc = non\_biofilm\ producers; ODc$.[17]

2.6.2 | Congo red agar (CRA) test

To estimate *P. aeruginosa* ability to form biofilms, we conducted isolates cultivation on brain-heart infusion agar with Congo red (0.08% w/v) (Sigma-Aldrich) with 3.6% (w/v) sucrose supplementation. The inoculated plates were then exposed to aerobic conditions at 35°C. According to Freeman et al.'s methodology, strong-biofilm and moderate-biofilm forming organisms create black colonies with and without a dry crystalline consistency, respectively. Besides, smooth red colonies with occasional darkening in the middle parts are associated with nonbiofilm strains. *P. aeruginosa* (PA01) were utilized as biofilm-positive.[18,19]

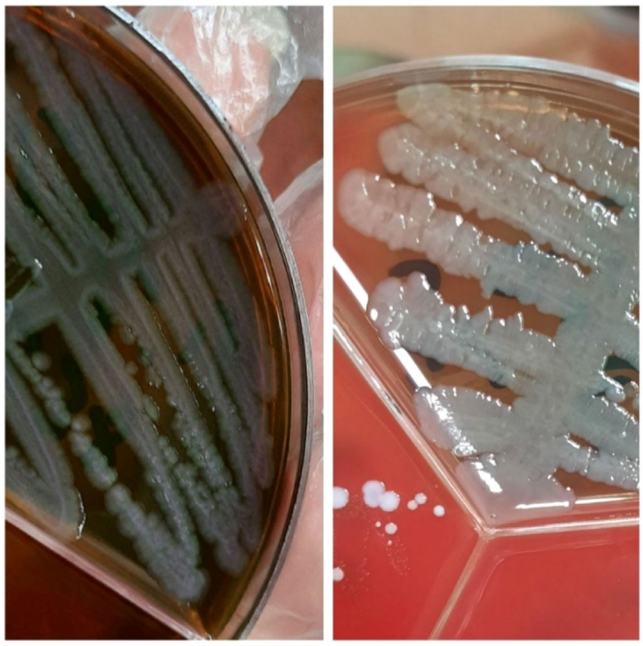

FIGURE 1 *Pseudomonas aeruginosa* colonies on blood agar medium after 24 h in aerobic conditions at 37°.

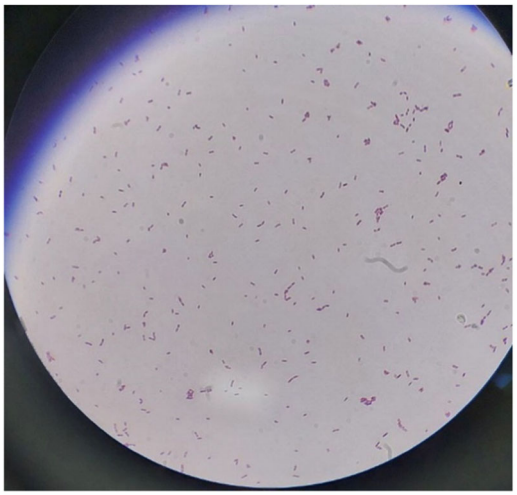

FIGURE 2 *Pseudomonas aeruginosa* in the form of rods and single, double and short chains under the microscope.

2.6.3 | Data analysis

Via SPSS software, v.22 (SPSS Inc.), we studied the connections between categorical variables, such as biofilm properties and

TABLE 1 Patients and results of biofilm formation, antibiotic resistance and *Pseudomonas aeruginosa* isolates.

ID	G	Duration of hospitalization	MTP	Congo red	ARPs	Colistin minimum inhibitory concentration (MIC)	Mu ltidrug-resistant (MDR)	Exte nsively drug-resistant (XDR)	Pan drug-resistant (PDR)
1	29-F	First 48 h	Moderate	+	NET, TOB, MEM, DOR, ATM, CAZ, FEP, TIM,C/T, CZA, TZP, PIP, GN, AMK, LVX, NOR, OFX, GAT	3	-	+	-
2	33-M	Third week	Strong	+	NET, TOB, MEM, DOR, ATM, CAZ, FEP, TIM,C/T, CZA, TZP, PIP, GN, AMK, LVX, NOR, OFX, GAT	2.5	-	+	-
3	39-M	Last of first week	Strong	+	NET, TOB, MEM, DOR, ATM, CAZ, FEP, TIM,,C/T, CZA, TZP, PIP,E, GN,T, AMK, LVX, NOR, OFX, GAT	8(R)	-	-	+
4	27-F	Fourth week	Strong	+	NET, TOB, MEM, DOR, ATM, CAZ, FEP, TIM,C/T, CZA, TZP, PIP, CIP, GN, AMK, LVX, NOR, OFX, GAT	1	-	+	-
5	27-M	First 48 h	Moderate	+	NET, TOB, MEM, DOR, ATM, CAZ, FEP, TIM,C/T, TZP, PIP, CIP, CZA, GN, AMK, LVX, NOR, OFX, GAT	1	-	+	-
6	28-F	Fourth week	Strong	+	NET, TOB, MEM, DOR, ATM, CAZ, FEP, TIM,C/T, CZA, TZP, PIP, GN, AMK, LVX, NOR, OFX, GAT	3	-	+	-
7	45-M	Second week	Moderate	+	NET,T OB, MEM, DOR, ATM, CAZ, FEP, TIM, CZA, TZP, PIP, C D, E, T, C/T, GN, AMK, L VX, NOR, O FX, GATCD	2.5	-	+	-
8	35-F	First 48 h	Strong	+	NET, TOB, MEM, DOR, ATM, CAZ, FEP, TIM, TZP, PIP, CZA,C/T, GN, AMK, LVX, NOR, OFX, GAT	2.5	-	+	-
9	33-M	Fourth week	Weak	+	NET, TOB, MEM, DOR, ATM, CAZ, FEP, TIM,C/T, CZA, TZP, PIP, GN, AMK, LVX, NOR, OFX, GAT	1	-	+	-
10	42-F	Second week	Strong	+	NET, TOB, MEM, DOR, ATM, CAZ, FEP, TIM, CZA, TZP, PIP, CIP,C/T, GN, AMK, LVX, NOR, OFX, GAT	0.75	-	+	-
11	47-M	Last of first week	Moderate	-	NET, TOB, MEM, DOR, ATM, CAZ, FEP, TIM, CZA, TZP, PIP,T,C/T, GN, AMK, LVX, NOR, OFX, GAT	1	-	+	-

TABLE 1 (Continued)

ID	G	Duration of hospitalization	MTP	Congo red	ARPs	Colistin minimum inhibitory concentration (MIC)	Mu ltidrug-resistant (MDR)	Exte nsively drug-resistant (XDR)	Pan drug-resistant (PDR)
12	43-F	Fourth week	Strong	+	NET, TOB, MEM, DOR, ATM, CAZ, FEP, TIM, CZA, TZP, PIP, GN,T,C/T, AMK, LVX, NOR, OFX, GAT	2.5	-	+	-
13	22-M	First 48 h	Strong	+	NET, TOB, MEM, DOR, ATM, CAZ, FEP, TIM, PIP, TZP, CIP, CZA,C/T, GN, AMK, CIP, LVX, NOR, OFX, GAT	0.75	-	+	-
14	28-F	Third week	Moderate	-	NET, TOB, MEM, DOR, ATM, CAZ, FEP, TIM, TZP, PIP, CIP, CZA,C/T, GN, AMK, LVX, NOR, OFX, GAT	3	-	+	-
15	32-M	Last of first week	-	-	NET, TOB, MEM, DOR, ATM, CAZ, FEP, TIM, TZP, PIP, CZA, GN, AMK, CIP, LVX, NOR, OFX, GAT	0.75	-	+	-
16	36-F	Third week	Strong	+	NET, TOB, MEM, IPM, DOR, ATM, CAZ, FEP, TIM, TZP, PIP, CIP, CZA, GN, AMK, LVX, NOR, OFX, GAT	0.75	-	+	-
17	21-M	Fourth week	-	-	NET, TOB, MEM, IPM, DOR, ATM, CAZ, FEP, TIM, CZA, TZP, PIP, CD, GN, AMK, CIP, LVX, NOR, OFX, GATCD	0.75	-	+	-
18	51-M	First 48 h	Moderate	-	NET, TOB, MEM, IPM, DOR, ATM, CAZ, FEP, TIM, CZA, TZP, PIP, CIP, CD, GN, FOX, AMK, LVX, NOR, OFX, GATCD	3	-	+	-
19	53-M	Fourth week	Strong	+	NET, TOB, MEM, IPM, DOR, ATM, CAZ, FEP, TIM, CZA, PIP, SXT, C, CD, GN, TZP, AMK, CIP, LVX, NOR, OFX, GATCD	2.5	-	+	-
20	36-M	Last of first week	Weak	-	NET, TOB, MEM, IPM, DOR, ATM, CAZ, FEP, TIM, CZA, TZP, PIP, CIP, GN, AMK, LVX, NOR, OFX, GAT	0.75	-	+	-
21	37-F	Third week	Weak	-	NET, TOB, MEM, IPM, DOR, ATM, CAZ, FEP, TIM, CZA, TZP, PIP,C, RP, GN, AMK, CIP, LVX, NOR, OFX, GAT	0.75	-	+	-
22	40-M	Second week	Moderate	+	NET, TOB, MEM, IPM, DOR, ATM, CAZ, FEP, TIM, CZA, TZP, PIP,E, GN, AMK, CIP, LVX, NOR, OFX, GAT	0.75	-	+	-

(Continues)

TABLE 1 (Continued)

ID	G	Duration of hospitalization	MTP	Congo red	ARPs	Colistin minimum inhibitory concentration (MIC)	Mu ltidrug-resistant (MDR)	Exte nsively drug-resistant (XDR)	Pan drug-resistant (PDR)
23	34-M	Fourth week	-	-	NET, TOB, MEM, IPM, DOR, ATM, CAZ, FEP, TIM, CZA, TZP, PIP,E, GN, AMK, CIP, LVX, NOR, OFX, GAT	3	-	+	-
24	22-M	First 48 h	Moderate	+	NET, TOB, MEM, IPM, DOR, ATM, CAZ, FEP, CZA, TZP, PIP, C, CD, GN, AMK, CIP, LVX, NOR, OFX, GATCD	0.75	-	+	-
25	26-M	Third week	Moderate	-	NET, TOB, MEM, IPM, DOR, ATM, CAZ, CZA, TZP, PIP, FEP, GN, AMK, CIP, LVX, NOR, OFX, GAT	0.75	-	+	-
26	39-M	Second week	Strong	+	NET, TOB, MEM, IPM, DOR, ATM, CAZ, FEP, CZA, TZP, PIP, CIP, GN, AMK, LVX, NOR, OFX, GAT	0.75	+	-	-
27	43-F	First 48 h	Strong	+	NET, TOB, MEM, IPM, DOR, ATM, CAZ, FEP, CZA, TZP, PIP, CIP, GN, AMK, LVX, NOR, OFX, GAT	3	-	+	-
28	27-F	Fourth week	Strong	+	NET, TOB, MEM, IPM, DOR, ATM, CAZ, FEP, TZP, PIP, GN, FOX, AMK, CIP, LVX, NOR, OFX, GAT	0.75	+	-	-
29	41-M	Third week	Weak	+	NET, TOB, MEM, IPM, DOR, ATM, CAZ, FEP, TZP, PIP, CIP, GN, AMK, LVX, NOR, OFX, GAT	3	+	-	-
30	25-F	First 48 h	Moderate	+	NET, TOB, MEM, IPM, DOR, ATM, CAZ, TZP, PIP, GN, AMK, CIP, LVX, NOR, OFX, GAT	3	+	-	-
31	36-M	Second week	Strong	+	NET, TOB, MEM, IPM, DOR, ATM, CAZ, TZP, PIP, GN, AMK, CIP, LVX, NOR, OFX, GAT	2.5	+	-	-
32	33-M	Fourth week	Strong	+	NET, TOB, MEM, IPM, ATM, CAZ, TZP, PIP, GN, AMK, CIP, LVX, NOR, OFX, GAT	0.75	+	-	-
		First 48 h		-	NET, TOB, MEM, IPM, ATM, CAZ, TZP, PIP, CIP, GN, AMK, LVX, NOR, OFX, GAT	0.75		-	-
33	30-F	Fourth week	Moderate	+	TOB, MEM, IPM, ATM, CAZ, PIP, GN, TZP, GN, AMK, CIP, LVX, NOR, OFX, GAT	2.5	+	-	-
34	30-M	First 48 h	Strong	+	TOB, MEM, IPM, ATM, CAZ, PIP, GN, TZP, AMK, CIP, LVX, NOR, OFX, GAT	3	+	-	-
35	28-F	Second week	Weak	+	TOB, MEM, IPM, ATM, CAZ, PIP, GN, TZP, AMK, CIP, LVX, NOR, OFX, GAT	0.75	+	-	-

TABLE 1 (Continued)

ID	G	Duration of hospitalization	MTP	Congo red	ARPs	Colistin minimum inhibitory concentration (MIC)	Mu ltidrug-resistant (MDR)	Exte nsively drug-resistant (XDR)	Pan drug-resistant (PDR)
36	39-M	First 48 h	Weak	+	TOB, MEM, IPM, ATM, CAZ, PIP, GN, TZP, AMK, CIP, LVX, NOR, OFX, GAT	8(R)	+	-	-
37	47-M	Last of first week	Strong	+	TOB, MEM, IPM, ATM, CAZ, PIP, CIP, GN, TZP, AMK, LVX, NOR, OFX, GAT	0.75	+	-	-
38	46-M	Fourth week	-	-	TOB, MEM, IPM, ATM, CAZ, PIP, TZP, GN, AMK, CIP, LVX, NOR, OFX, GAT	2.5	+	-	-
39	45-F	First 48 h	-	-	TOB, MEM, IPM, ATM, CAZ, PIP, TZP, GN, AMK, CIP, LVX, NOR, OFX, GAT	3	+	-	-
40	35-M	Last of first week	Strong	+	TOB, MEM, IPM, ATM, CAZ, PIP, TZP, GN, AMK, CIP, LVX, NOR, OFX, GAT	1	+	-	-
41	42-M	Fourth week	-	-	TOB, MEM, IPM, ATM, CAZ, TZP, PIP, GN, AMK, CIP, LVX, NOR, OFX, GAT	2.5	+	-	-
42	39-F	First 48 h	Strong	-	TOB, MEM, IPM, ATM, CAZ, TZP, PIP, GN, AMK, CIP, LVX, NOR, GAT	8(R)	+	-	-
43	37-M	Fourth week	-	-	TOB, MEM, IPM, ATM, CAZ, TZP, PIP, GN, AMK, CIP, LVX, NOR, GAT	1	+	-	-
44	16-M	Last of first week	Strong	+	TOB, MEM, IPM, ATM, CAZ, TZP, PIP, GN, AMK, CIP, LVX, NOR, GAT	1.5	+	-	-
45	23-M	First 48 h	Moderate	+	TOB, MEM, IPM, ATM, CAZ, TZP, PIP, GN, AMK, CIP, LVX, NOR, GAT	1	+	-	-
46	26-M	Last of first week	Strong	+	TOB, MEM, IPM, ATM, CAZ, TZP, PIP, GN, AMK, CIP, LVX, NOR, GAT	1.5	+	-	-
47	45-F	Second week	-	-	TOB, MEM, IPM, ATM, CAZ, PIP, GN, AMK, CIP, LVX, NOR, GAT	0.75	+	-	-
48	67-M	First 48 h	Moderate	-	TOB, MEM, IPM, ATM, CAZ, PIP, GN, AMK, CIP, LVX, NOR, GAT	128(R)	+	-	-
49	54-F	Third week	Strong	+	TOB, MEM, IPM, ATM, CAZ, PIP, GN, AMK, CIP, LVX, NOR, GAT	2.5	+	-	-
50	33-M	Last of first week	Strong	+	TOB, MEM, ATM, CAZ, PIP, GN, AMK, CIP, LVX, NOR, GAT	3	+	-	-
51	65-M	Third week	-	-	TOB, MEM, ATM, CAZ, PIP, GN, AMK, CIP, LVX, NOR, GAT	1.5	+	-	-

(Continues)

TABLE 1 (Continued)

ID	G	Duration of hospitalization	MTP	Congo red	ARPs	Colistin minimum inhibitory concentration (MIC)	Mu ltidrug-resistant (MDR)	Exte nsively drug-resistant (XDR)	Pan drug-resistant (PDR)
52	43-M	Last of first week	Strong	+	TOB, MEM, ATM, CAZ, PIP, GN, CIP, LVX, NOR, GAT	1	+	-	-
53	55-F	First 48 h	-	-	MEM, ATM, CAZ, PIP, GN, CIP, LVX, NOR, GAT	128(R)	+	-	-
54	76-M	Fourth week	Strong	+	MEM, ATM, CAZ, PIP, GN, CIP, LVX, NOR, GAT	2.5	+	-	-
55	43-F	Third week	Weak	-	MEM, ATM, CAZ, PIP, GN, CIP, LVX, NOR, GAT	3	+	-	-
56	12-M	Second week	Strong	+	MEM, ATM, CAZ, PIP, GN, CIP, LVX, NOR, GAT	1	+	-	-
57	34-M	Last of first week	Weak	-	MEM, ATM, CAZ, PIP, GN, LVX, GAT	1.5	+	-	-
58	35-M	Last of first week	-	-	MEM, ATM, CAZ, PIP, GN, LVX, GAT	1	+	-	-
59	45-M	Fourth week	-	-	MEM, ATM, CAZ, PIP, GN, GAT	6 C	+	-	-
60	32-F	Second week	Moderate	+	MEM, ATM, CAZ, PIP, GN, GAT	128(R)	+	-	-
61	43-M	Last of first week	-	-	ATM, CAZ, PIP, GN, GAT	0.75	+	-	-
62	55-M	First 48 h	Strong	+	ATM, CAZ, PIP, GN, GAT	1	+	-	-
63	33-M	Fourth week	Weak	-	IPM, ATM, PIP, GN, GAT	3	+	-	-
64	21-M	Last of first week	Strong	+	IPM, ATM, PIP, GN, GAT	1	+	-	-
65	22-M	Last of first week	-	-	IPM, ATM, PIP, GAT	1	+	-	-
66	44-F	Fourth week	Strong	+	IPM, ATM, PIP, GAT	1	+	-	-
67	33-F	First 48 h	Weak	-	IPM, ATM, PIP	0.5	+	-	-
68	54-M	Fourth week	Weak	-	IPM, ATM, PIP	1	+	-	-
69	33-M	Last of first week	-	-	IPM, ATM, PIP	0.5	-	-	-
70	23-F	First 48 h	-	-	IPM, ATM, PIP	0.75	-	-	-
71	45-M	Last of first week	Strong	+	PIP	1	-	-	-
72	45-F	Fourth week	-	-	PIP	1	-	-	-
73	23-F	First 48 h	Strong	+	-	-	-	-	-
74	33-M	Fourth week	Weak	-	-	-	-	-	-
75	34-F	Last of first week	-	-	-	-	-	-	-
76	33-F	Fourth week	Strong	+	-	-	-	-	-
77	24-F	Last of first week	Weak	-	-	-	-	-	-

TABLE 1 (Continued)

ID	G	Duration of hospitalization	MTP	Congo red	ARPs	Colistin minimum inhibitory concentration (MIC)	Mu ltidrug-resistant (MDR)	Exte nsively drug-resistant (XDR)	Pan drug-resistant (PDR)
78	32-M	Last of first week	-	-	-	-	-	-	-
79	43-F	Fourth week	Strong	+	-	-	-	-	-
80	22-F	First 48 h	Moderate	+	-	-	-	-	-
81	23-M	Last of first week	-	-	-	-	-	-	-
82	44-F	Fourth week	Strong	+	-	-	-	-	-
83	33-F	First 48 h	Moderate	-	-	-	-	-	-
84	44-F	First 48 h	Strong	+	-	-	-	-	-
85	22-M	First 48 h	-	-	-	-	-	-	-
86	23-M	Last of first week	Weak	+	-	-	-	-	-
87	14-M	First 48 h	Moderate	+	-	-	-	-	-
88	17-F	Fourth week	-	-	-	-	-	-	-
89	65-M	First 48 h	Moderate	-	-	-	-	-	-
90	43-F	Fourth week	Strong	+	-	-	-	-	-
91	22-F	Fourth week	Strong	+	-	-	-	-	-
92	23-F	Last of first week	Moderate	+	-	-	-	-	-
93	43-M	Last of first week	Strong	+	-	-	-	-	-
94	34-M	First 48 h	Strong	+	-	-	-	-	-
95	43-M	Fourth week	-	-	-	-	-	-	-
96	22-M	Fourth week	-	-	-	-	-	-	-
97	33-F	Fourth week	-	-	-	-	-	-	-
98	43-F	First 48 h	Strong	+	-	-	-	-	-
99	43-M	Last of first week	-	-	-	-	-	-	-
100	22-M	Fourth week	Weak	+	-	-	-	-	-
101	13-F	First 48 h	Weak	+	-	-	-	-	-
102	23-M	Fourth week	Strong	+	-	-	-	-	-
103	34-M	Last of first week	Weak	+	-	-	-	-	-
104	22-F	Fourth week	Strong	+	-	-	-	-	-

(Continues)

TABLE 1 (Continued)

ID	G	Duration of hospitalization	MTP	Congo red	ARPs	Colistin minimum inhibitory concentration (MIC)	Mu ltidrug-resistant (MDR)	Exte nsively drug-resistant (XDR)	Pan drug-resistant (PDR)
105	44-M	First 48 h	Strong	+	-	-	-	-	-
106	33-F	Fourth week	Weak	+	-	-	-	-	-
107	33-F	First 48 h	Strong	+	-	-	-	-	-
108	34-F	Fourth week	Weak	+	-	-	-	-	-
109	23-F	Fourth week	Weak	+	-	-	-	-	-
110	43-M	First 48 h	Strong	+	-	-	-	-	-

Abbreviations: AMK, Amikacin; ATM, Aztreonam; C/T, Ceftolozane-Tazobactam; CAZ, Ceftazidime; CIP, Ciprofloxacin; CZA, Ceftazidime-Avibactam; DOR, Doripenem; FEP, Cefepime; GAT, Gatifloxacin; GN, Gentamicin; IPM, Imipenem; LVX, Levofloxacin; MEM, Meropenem; NET, Netilmicin; NOR, Norfloxacin; OFX, Ofloxacin; PIP, Piperacillin; TIM, Ticarcillin-Clavulanicacid; TOB,Tobramycin; TZP, Piperacillin-Tazobactam.

antimicrobial resistance using the chi-squared test. *p*-Values lower than 0.05 were assumed to be significant.

3 | RESULTS

One hundred and ten clinical *P. aeruginosa* isolates from burn wound infections were utilized (Figures 1 and 2). Molecular method was also used to validate *P. aeruginosa* isolates that were already identified biochemically. The patients were typically 35.32 years old (SD: 11.74 years) (Table 1). The highest rate of resistance to antibiotics for *P. aeruginosa* isolates was as follows: Piperacillin 68% ($n = 75$), Ceftazidime 59% ($n = 65$), Meropenem 56% ($n = 63$), Gentamycin 56% ($n = 63$), Gatifloxacin 56% ($n = 63$), but the lowest rates of resistance to antibiotics were related to Ticarcillin-Clavulanic acid 22% ($n = 25$), and Ceftolozane-Tazobactam 16% ($n = 6$) (Table 1, Figure 3). Afterwards, MICs were calculated using E test and just seven isolates were found to be resistant to colistin. For the 110 *P. aeruginosa* samples, colistin reference MICs were in the range of 6–128 mg/L. The MDR and XDR strains had a prevalence of 38% ($n = 42$), and 22% ($n = 25$), respectively. However, one strain was PDR. Table 1 shows a phenotypic schema of antibiotic prevalence, diversity, and resistance of the strains. Table 2 represents 74 patterns of combination, each with 13 antibiotics. Only one isolate could survive all the seven antibiotics representing 13 classes. The biofilm generation was analyzed using MTP and Congo red agar methods. In CRA, 66 isolates (67%) formed biofilms and black colonies, and 44 isolates (50%) formed red colonies. In MTP test, isolates were divided into strong, moderate, weak, and no biofilm-forming. The values of OD at 570 nm for positive and negative (TSB) controls were 0.525 ± 0.062 and 0.055 ± 0.009, respectively. The OD570 of the strains ranged from 0.125 ± 0.056 to $1.745 \pm 0.$ 054. As such, 84 isolates (76%) formed biofilms: 45 (40%) were strong, 19 (32%) were moderate, 20 (21%) were weak formers, and 26 (43%) were not biofilm forming. Among the 45 strong biofilm-producers, 2% ($n = 9$) and 42% ($n = 18$) were found to be XDR and MDR, respectively, while one isolate was PDR. In the moderate group, nine isolates (76%) were XDR and six isolates (76%) were MDR. Besides, in the weak group, three isolates (76%) were XDR but eight isolates (76%) were MDR (Table 1). Figure 4 demonstrates the relationship between biofilm generation capability and antibiotic resistance. We found that nonbiofilm group showed higher antibiotic resistance than biofilm producing strains (Figure 4). Statistically, no significant relation was observed between biofilm-forming capability and antibiotic resistance ($p = 0.80$); however, a significant relation existed between biofilm forming and XDR ($p = 0.04$).

4 | DISCUSSION

P. aeruginosa is a nosocomial pathogen with MDR that can cause fatal infections in critically unwell individuals. *P. aeruginosa* is known to be a major cause of wound infections. Compared to the previous study

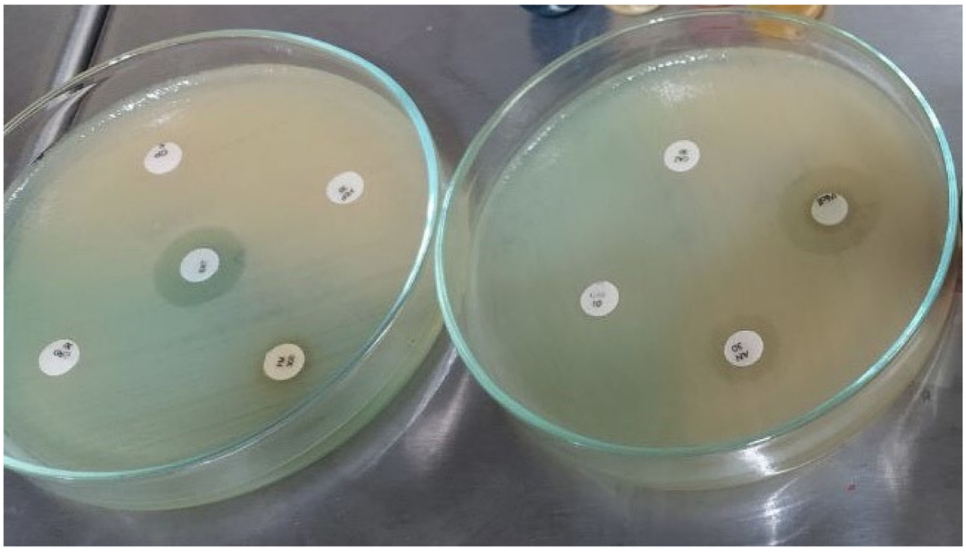

FIGURE 3 Antibiogram test.

on the frequency of this pathogen in burn wounds in southwest Iran,[20] we showed that *P. aeruginosa* frequency in burn wounds was 31%, which is possibly due to the differences in infection control measures. In recent years, drug resistance has been a great challenge to the infection treatment. In our study, MDR isolates had a high percentage (38%), which is in line with the results of a number of studies in Iran and other countries.[21,22] Multidrug antibiotic resistance lowers antibiotic ability to reduce infections. Over the last two decades, carbapenem has been at the front of antibiotic treatment for *P. aeruginosa* infections. However, carbapenems has gradually lost its efficiency as the isolates developed antibiotic resistance capability.[23] According to the results of antibiotic susceptibility test in this work, the rate of resistance to meropenem, imipenem, and doripenem was 57%, 47%, and 67%, respectively, which was in agreement with some other studies.[24–26] Besides, the rate of resistance of the strains to amikacin, gentamycin, tobramycin, aztreonam, piperacillin, meropenem, imipenem, ceftazidime, norfloxacin, and gatifloxacin was over 50%, which was in line with the results of Perez et al. and Del Barrio et al.[21,27] Despite its side effects (e.g., nephrotoxicity and neurotoxicity), colistin is the only choice for the treatment of the infections induced by XDR or MDR strains.[23] According to the antibiotic susceptibility test, most of the isolates were vulnerable to colistin, which is in concord with some previous results both in Iran and abroad.[28–30] Colistin remains the best antibiotic against MDR *P. aeruginosa* isolates. In the absence of alternative therapies, resistance to this antibiotic can thwart therapeutically measures. Furthermore, the findings of this study revealed that MDR *P. aeruginosa* strains were disseminated throughout various clinical wards in our hospital, indicating a lack of appropriate supervision on this issue at this hospital; thus, infection control measures should be implemented to prevent the transmission of *P. aeruginosa* strains. In biomedical papers, many classifications have been used to define multidrug resistant isolates of *P. aeruginosa*. MDR was characterized in the majority of studies as acquired resistance to at least one drug in three or more antimicrobial categories, primarily aminoglycosides, antipseudomonal penicillin, cephalosporins, carbapenems, and fluoroquinolones.[31,32] Given that the samples were collected from a burn unit, the high incidence of MDR patients in the current investigation may be rationalized. The presence of such high resistance *P. aeruginosa* is not unusual in our region, since Anvarinejad et al. and Sarhangi et al. previously demonstrated a high rate of MDR among isolates from burn patients and clinical isolates from Shiraz City, respectively.[33,34]

P. aeruginosa biofilm formation can result in the losing of antibacterial vulnerability and increasing of antibiotic concentrations in the treatment of infections induced by these isolates. Moreover, the biofilm matrix can protect bacteria from immune system cells and antibiotic agents. In this study, *P. aeruginosa* isolates could create biofilms, though with different degrees. As said before, biofilm formation capacity and resistance to all antibiotic agents were significantly inversely related ($p = 0.70$). In other words, biofilms had more density in sensitive strains than in resistant strains, which is also in compliance with some previous studies. However, some studies reported that MDR strains outperformed sensitive strains in terms of biofilm production.[35] Biofilm generation seems to act as a survival strategy for bacteria in case they face antibiotic agents, particularly in strains with an insufficient level of antibiotic resistance.

In sum, *P. aeruginosa* resistance to antimicrobial agents has largely challenged infection control. Higher resistance occurred when the isolates were transferred to the patients. Since less than 50% of *P. aeruginosa* samples generated strong biofilms, it is essential to observe hygienic care to inhibit *P. aeruginosa* transmission to the patients.

TABLE 2 Antibiotic resistance patterns among 110 *Pseudomonas aeruginosa* isolates count.

NET, TOB, MEM, DOR, ATM, CAZ, FEP, TIM, C/T, CZA, TZP, PIP, GN, AMK, LVX, NOR, OFX, GAT	8
NET, TOB, MEM, DOR, ATM, CAZ, FEP, TIM,C/T, CZA, TZP, PIP,E, GN, T,AMK, LVX, NOR, OFX, GAT	1
NET, TOB, MEM, DOR, ATM, CAZ, FEP, TIM, CZA, TZP, PIP, CD, E, T, C/T, GN, AMK, LVX, NOR, OFX, GATCD	2
NET, TOB, MEM, DOR, ATM, CAZ, FEP, TIM, CZA, TZP, PIP, CIP,C/T, GN, AMK, LVX, NOR, OFX, GAT	1
NET, TOB, MEM, DOR, ATM, CAZ, FEP, TIM, CZA, TZP, PIP, GN,T,C/T, AMK, LVX, NOR, OFX, GAT	1
NET, TOB, MEM, DOR, ATM, CAZ, FEP, TIM, PIP, TZP, CIP, CZA,C/T, GN, AMK, CIP, LVX, NOR, OFX, GAT	1
NET, TOB, MEM, DOR, ATM, CAZ, FEP, TIM, TZP, PIP, CZA, GN, AMK, CIP, LVX, NOR, OFX, GAT	2
TNET, TOB, MEM, IPM, DOR, ATM, CAZ, FEP, TIM, CZA, TZP, PIP, CD, GN, AMK, CIP, LVX, NOR, OFX, GATCD	2
NE T,TOB,MEM,IPM,DOR,ATM,CAZ,FEP,TIM,CZA,TZP,PIP,CIP, CD,GN,FOX,AMK,LVX,NOR,OFX,GATCD	1
NET,TOB,MEM,IPM,DOR,ATM,CAZ,FEP,TIM,CZA,PIP,SXT,C, CD,GN,TZP,AMK,CIP,LVX,NOR,OFX,GATCD	1
NET,TOB, MEM,IPM,DOR,ATM,CAZ,FEP,CZA,TZP,PIP,C,CD,GN,AMK, CIP,LVX,NOR,OFX,GATCD	3
NET, TOB, MEM, IPM, DOR, ATM, CAZ, FEP, CZA, TZP, PIP, CIP, GN, AMK, LVX, NOR, OFX, GAT	3
NET, TOB, MEM, IPM, DOR, ATM, CAZ, FEP, TZP, PIP, GN, FOX, AMK, CIP, LVX, NOR, OFX, GAT	4
NET, TOB, MEM, IPM, DOR, ATM, CAZ, FEP, TZP, PIP, CIP, GN, AMK, LVX, NOR, OFX, GAT	1
NET, TOB, MEM, IPM, DOR, ATM, CAZ, TZP, PIP, GN, AMK, CIP, LVX, NOR, OFX, GAT	2
NET, TOB, MEM, IPM, ATM, CAZ, TZP, PIP, CIP, GN, AMK, LVX, NOR, OFX, GAT	2
TOB, MEM, IPM, ATM, CAZ, TZP, PIP, GN, AMK, CIP, LVX, NOR, OFX, GAT	8
TOB, MEM, IPM, ATM, CAZ, TZP, PIP, GN, AMK, CIP, LVX, NOR, GAT	5
TOB, MEM, IPM, ATM, CAZ, PIP, GN, AMK, CIP, LVX, NOR, GAT	3
TOB, MEM, ATM, CAZ, PIP, GN, AMK, CIP, LVX, NOR, GAT	2
TOB, MEM, ATM, CAZ, PIP, GN, CIP, LVX, NOR, GAT	1
MEM, ATM, CAZ, PIP, GN, CIP, LVX, NOR, GAT	4
MEM, ATM, CAZ, PIP, GN, LVX, GAT	2
MEM, ATM, CAZ, PIP, GN, GAT	2
ATM, CAZ, PIP, GN, GAT	2
IPM, ATM, PIP, GN, GAT	2
IPM, ATM, PIP, GAT	2
IPM, ATM, PIP	4
PIP	2

Abbreviations: AMK, Amikacin; ATM, Aztreonam; C/T, Ceftolozane-Tazobactam; CAZ, Ceftazidime; CIP, Ciprofloxacin; CZA, Ceftazidime-Avibactam; DOR, Doripenem; FEP, Cefepime; GAT, Gatifloxacin; GN, Gentamicin; IPM, Imipenem; LVX, Levofloxacin; MEM, Meropenem; NET, Netilmicin; NOR, Norfloxacin; OFX, Ofloxacin; PIP, Piperacillin; TIM, Ticarcillin-Clavulanicacid; TOB,Tobramycin; TZP, Piperacillin-Tazobactam.

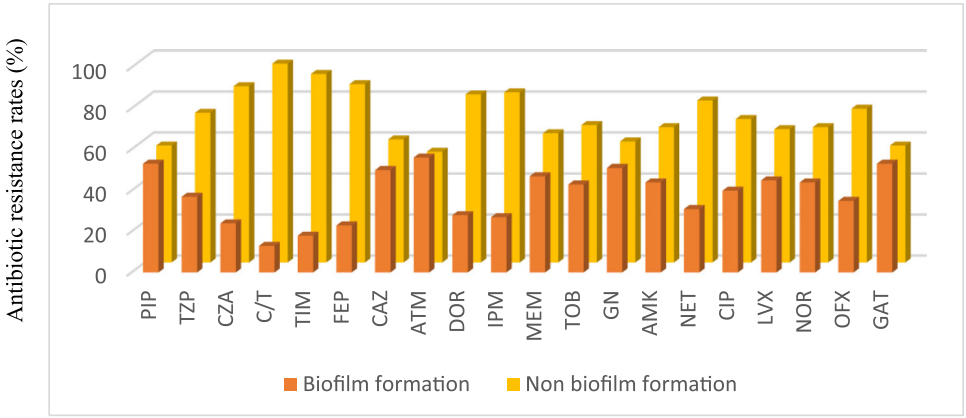

FIGURE 4 The frequency of antibiotic resistance in biofilm producer and nonbiofilm producer *Pseudomonas aeruginosa*.

5 | LIMITATION

If more time had been taken for the study, the number of specimens would have been higher, which would have determined the prevalence of MDR, XDR, and PDR with a more accurate and realistic probability.

AUTHOR CONTRIBUTIONS

Sousan Akrami: Conceptualization; investigation; methodology; writing—original draft; writing—review and editing. **Alireza Ekrami**: Resources; supervision. **Arshid Y. Avarvand**: Conceptualization; data curation; formal analysis; investigation.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All data are in article. However, all the datasets supporting the conclusions of this article are available. Additional data of this paper can be obtained upon request. You can contact the corresponding author, if you wish to ask for the data for this study.

TRANSPARENCY STATEMENT

The lead author Arshid Yousefi Avarvand affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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