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Meta-Analysis of Biofilm Formation, Antibiotic Resistance Pattern, and Biofilm-Related Genes in *Pseudomonas aeruginosa* Isolated from Clinical Samples

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Resistant microorganisms such as *Pseudomonas aeruginosa* grow by developing biofilms in hospitals. We aimed to investigate the biofilm formation and the frequencies of biofilm-related genes and their associations with antibiotic resistance pattern in *P. aeruginosa* isolated from Iranians' clinical samples. This review was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. We conducted a systematic literature search in scientific databases using medical subject heading terms, including ''*Pseudomonas aeruginosa,*'' ''biofilm formation,'' ''biofilm-related genes,'' ''antibiotic resistance,'' and ''prevalence,'' to obtain related articles published from 1st January, 2000, to 30th March, 2019. The studies reporting the prevalence of biofilm formation, the frequencies of biofilm-related genes, and the antibiotic resistance pattern in *P. aeruginosa* retrieved from Iranian patients were included. Meta-analysis was performed using the Comprehensive Meta-Analysis software. The pooled rate of biofilm formation was calculated as 86.5% (95% confidence interval [CI]: 79–91.6). The combined frequencies of strong, moderate, and weak biofilms were 51% (95% CI: 37.4–64.4), 29.2% (95% CI: 20.9–39.1), and 25.4% (95% CI: 11.5–47.2), respectively. The pooled prevalence of *laslR*, *algD*, *algU*, *ppyR*, and *pelF* genes were 93.6% (95% CI: 88.1–96.6), 91.4% (95% CI: 80.8–96.4), 89.3% (95% CI: 85.2–92.3), 98.7% (95% CI: 96.5–99.6), and 93% (95% CI: 82.7–97.3), respectively. The highest combined antibiotic resistance rates of *P. aeruginosa* isolates were against piperacillin/tazobactam (90%). This study showed that biofilm formation was higher in multidrug-resistant (MDR) *P. aeruginosa* than non-MDRs. A significant correlation was observed between biofilm formation and antibiotic resistance in 50% of studies included in this review.

Keywords: antibiotic resistance pattern, biofilm formation, *Pseudomonas aeruginosa*

Introduction

Pseudomonas aeruginosa is a Gram-negative opportunistic microorganism causing serious infections in debilitated patients, including those with cystic fibrosis, AIDS, and chronic diseases, as well as burn patients and those hospitalized in intensive care unit (ICU).[1] Moreover, *P. aeruginosa* can lead to a broad spectrum of the urinary tract, burn wound, blood circulation, and respiratory tract infections.[2,3]

P. aeruginosa infection is a common nosocomial infection with high mortality and morbidity rates, especially among immunocompromised[4] and patients with burn wounds.[5] Extensive use of systemic antibiotics as well as surgical debridement have increased the risk of infections caused by Gram-negative bacteria such as *P. aeruginosa*.[6] A variety of antimicrobial mechanisms such as the expression of efflux pumps, suppression of enzyme production, and biofilm formation have rendered the treatment of bacterial infections problematic.[7] Thus, it is important to employ appropriate antibiotics to reduce mortality and hospitalization rates, as well as the economic burden associated with resistant Gram-negative bacterial infections.[8]

The antibiotic-resistant bacterial strains are mainly developed due to the inappropriate use of antibiotics leading to the propagation of antimicrobial mechanisms among bacteria.[9] Multidrug-resistant (MDR) *P. aeruginosa* infections are major health care concerns in today's world.[10,11] There

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are limited effective antibiotics against MDR *P. aeruginosa* leading to longer hospital stays and costs, as well as higher mortality and morbidity rates.[12]

P. aeruginosa can cause either chronic or acute infections depending on the host's health status and the life cycle adopted by the microorganism in the host's body.[13] The pooled prevalence of MDR *P. aeruginosa* has been estimated to be 58% in Iran,[14] constituting 10–20% of all nosocomial infections.[15]

Many bacteria, including *P. aeruginosa*, produce biofilms to survive in the host's body and harsh environments.[16] Biofilm formation allows cumulative bacterial growth by adhering to surfaces through self-secreted matrix extracellular polymeric substance (EPS). The EPS contains polysaccharides, proteins, and nucleic acids allowing the organism to thrive in difficult conditions such as undesirable pH, humidity, and temperature, as well as in exposition with antimicrobial agents.[17]

Biofilm formation is a complex phenomenon involving several internal signaling pathways and the expression of multiple biofilm-related genes such as *pel*, *psl*, and *alg*.[18] The difference between planktonic and biofilm persistence reduces the metabolic activity of biofilms, inactivates antimicrobial agents, and suppresses the expression of specific enzymes.[19,20]

Several genes are involved in biofilm formation by *P. aeruginosa.* The *pslA* gene encodes an exopolysaccharide involved in biofilm structure. Also, alginate is another polysaccharide encoded by *algD*, *algU*, and *algA* genes participating in the structure of biofilms and enhancing the adherence capability of *P. aeruginosa*.[21] Besides, *psl*, *pel*, and *ppyR* genes encode other exopolysaccharides involved in biofilm formation.[22] Biofilms are structurally categorized into weak, moderate, or potent, each presenting variable capabilities to protect bacteria against antimicrobial agents.[23]

The induction of virulence factors (such as type III enzyme secretion systems) and decreased permeability of bacterial membranes during biofilm formation confer resistance to bacteria against different classes of antibiotics.[24,25] So, biofilm formation allows *P. aeruginosa* to create chronic infections and persist in harsh environments such as hospitals.[26,27] Acknowledging the relationship between bacterial genotype and biofilm phenotype can help to effectively control severe infections caused by biofilm forming in *P. aeruginosa* strains.[16]

Regarding the significant role of *P. aeruginosa* in hospital-acquired infections and the lack of a comprehensive study on this issue, we aimed to investigate the combined prevalence of biofilm formation, biofilm subgroups (*i.e.*, potent, moderate, and weak), antibiotic resistance pattern, the combined prevalence of biofilm-related genes, and the association between biofilm formation and antibiotic resistance in *P. aeruginosa* strains isolated from Iranian patients.

Materials and Methods

The present meta-analysis was performed according to the guidelines of the Meta-analysis of Observational Studies in Epidemiology (MOOSE) and Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Supplementary Data).

We conducted a systematic literature search in Web of Science, Cochrane Library, Scopus, PubMed, and Google Scholar databases using the following medical subject heading terms: *Pseudomonas aeruginosa*, biofilm formation, biofilm-related genes, antibiotic resistance, and prevalence. The combinational search was conducted applying the following strategy (in the MEDLINE for example): ''((Biofilm formation) OR Biofilm production) AND *Pseudomonas aeruginosa*) AND Prevalence) AND Iran)).'' Studies published between 1st January, 2000, and 30th March, 2019, were retrieved. The articles reporting the prevalence of biofilm formation, the frequencies of biofilm-related genes, and the antibiotic resistance pattern of *P. aeruginosa* in Iranian patients were included. The references of all included studies were also checked for finding additional records.

Eligibility criteria

The major inclusion criteria included reporting the rate of biofilm formation, the prevalence of biofilm-related genes, and the antibiotic resistance pattern in *P. aeruginosa* isolated from Iranian patients' samples. Also, only studies in which the standard microtiter plate test had been used as the biofilm formation assay were included.[28] In this technique, the *P. aeruginosa* isolates were grown at 37°C overnight in Mueller Hinton Broth (MHB) containing 0.25% glucose. The cultures were diluted 1:100 in MHB medium. Sterile 96-well microtiter plates were inoculated with 125 μL of the bacterial suspension and incubated for 24 hours at 37°C without agitation. The wells were washed with 300 μL distilled water in triplicate and dried at room temperature. All wells were stained with 125 μL of 0.1% crystal violet solution for 10 minutes. Then, wells were washed thrice with distilled water. The wells were destained with 125 μL of 30% acetic acid solution. Finally, the optical density (OD) of each sample was measured at 570 nm by a spectrophotometer (Smart Spec plus Spectrophotometer Bio-RAD). The experiment was repeated in triplicate and the mean value of OD was calculated. Based on the optical density index (ODi) of the samples and on the mean of the OD of the negative control (ODc), the biofilm was classified as strong ($4\times ODc < ODi$), mod ($2\times ODc < ODi \leq 4\times ODc$), weak ($ODc < ODi \leq 2\times ODc$), and nonproducer of biofilm ($ODi < ODc$).

Also, for determining the antibiotic resistance rate, the studies should have used one of the standard susceptibility tests such as Broth dilution (either Macrobroth or Microbroth dilution) and disk diffusion methods according to the Clinical and Laboratory Standards Institute (CLSI).[29]

Reviews, editorials, congress and meeting abstracts, literatures in languages other than English, case reports, and letters to editors were excluded. Articles without full text, duplicate reports, and studies with unclear and missing data were also omitted.

Screening

Duplicates were initially identified and eliminated after entering all the recognized studies into a self-created database. After that, the articles were assessed by two reviewers (H.K.M. and M.H.F.) by screening titles, abstracts, topics, and finally full texts. At each level, the reviewers independently screened the articles and finally merged their

conclusions. Discrepancies were resolved by discussion before finalizing the records for the next level. In case of disagreements, a third assessor (A.K.) was assigned to make a decision. Finally, the studies were assessed for eligibility before the final selection.

Quality assessment

Methodological quality assessment of the studies was performed using a checklist for necessary items as outlined in the Critical Appraisal Skills Programmed checklists. For each article, a series of critical questions was asked. If the pertinent data were presented, the question was scored ''yes.'' If there was any doubt or no information in the study, that question was marked as ''no'' or ''can't tell.'' The studies were given a total rate of either ''strong,'' ''moderate,'' or ''weak,'' based on the number of questions scored ''yes.''[30] Finally, weak studies were removed from the study. Overall, the employed scoring system for quality assessment of quantitative (*i.e.*, cross-sectional surveys) included 10 questions. The scores were categorized as weak (0–4), moderate (6–8), and strong (>8)[31] (Supplementary Table S1).

Data extraction

A data extraction form was designed to extract the relevant characteristics of each study. The extracted information included the first authors' names, time of the study, year of publication, location, sample size, biofilm formation rate, the correlation between biofilm formation and antibiotic resistance, and the type of biofilm (*i.e.*, potent, moderate, and weak). Two of the authors (A.K. and K.M.) extracted the data.

Data analysis and statistical methods

Meta-analysis was performed using the Comprehensive Meta-Analysis software (Version 3.3.070). The rate of biofilm formation was calculated with 95% CI. The heterogeneity was assessed by the Cochrane Q and I^2 tests. Considering the heterogeneity indices, the random-effects model was used to calculate the pooled frequencies. Subgroup analyses were conducted based on the type of biofilm, biofilm-related genes, and antibiotic resistance rate. Quantitative Egger weighted regression test and Funnel plot were used to investigate publication bias. p-value of <0.05 was considered the statistical significance threshold.

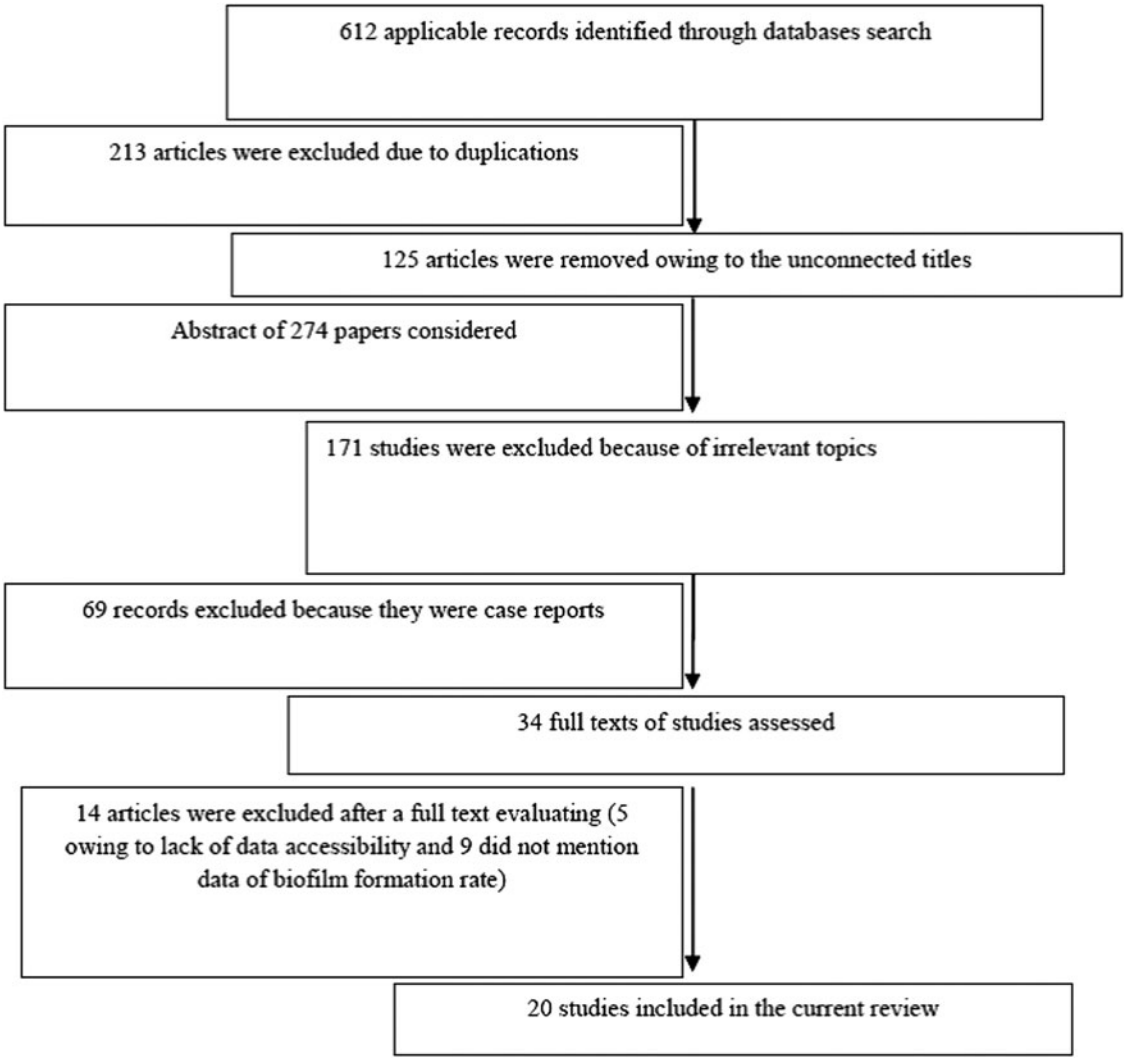

FIG. 1. Conceptual model of study search and selection.

Results

Study inclusion criteria and characteristics of the eligible studies

A total of 612 studies were retrieved and 34 full texts were reviewed. Twenty studies met our inclusion criteria (Fig. 1).

The finally included studies covered different regions of Iran, but most of them had been performed in Tehran. Overall, the biofilm formation rate varied from 43.5% to 99.5% in *P. aeruginosa* isolates from Iranian patients (Fig. 2, Table 1).

Overall effects

Heterogeneity analysis. The heterogeneity indices among the included studies were as $Q^2 = 206.3$, $I^2 = 90.7$, and $t = 3$ ($p = 0.006$). According to the observed heterogeneity indices, the random-effects model was used to combine the frequencies of biofilm formation.

Biofilm prevalence. The pooled rate of biofilm formation was calculated as 86.5% (95% CI: 79–91.6, Table 2).

The publication bias was evaluated using the Funnel plot (Fig. 3). Egger's linear regression test was used to further reveal any publication bias and possible asymmetrical data distribution in the selected studies. No publication bias was observed according to Egger's linear regression test ($p = 0.00$). Subgroup analysis based on the type of biofilm showed that the combined rates of potent, moderate, and weak biofilms were 51% (95% CI: 37.4–64.4), 29.2% (95% CI: 20.9–39.1), and 25.4% (95% CI: 11.5–47.2), respectively. On the other hand, 51%, 29.2%, and 25.4% of *P. aeruginosa* isolates were potent, moderate, and weak biofilm producers.

Biofilm-related genes. The pooled prevalence of *laslR*, *algD*, *algU*, *ppyR*, and *pelF* genes was 93.6% (95% CI: 88.1–96.6), 91.4% (95% CI: 80.8–96.4), 89.3% (95% CI: 85.2–92.3), 98.7% (95% CI: 96.5–99.6), and 93% (95%

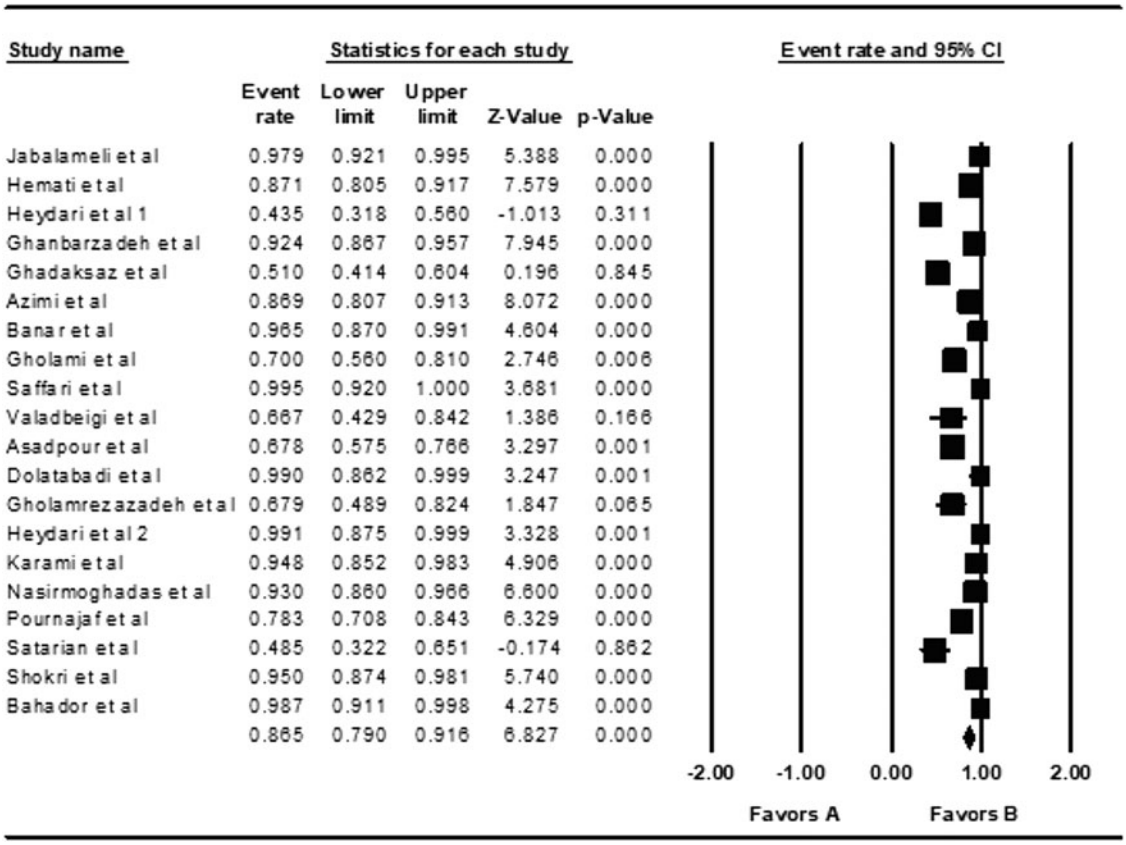

FIG. 2. Forest plot of the meta-analysis of prevalence of biofilm formation in *Pseudomonas aeruginosa* isolated from clinical samples. In the forest plot (a graphical display), the *X*-axis forms the effect size scale, plotted on the top of the plot. Each row, except the bottom one, represents a study's effect size estimate in the form of a point and a (95%) CI. This is the statistically correct way of representing the results of a single study, namely as an estimate of an interval in which the ''true'' effect (in the population) will most probably lie. Remember it is assumed that every study in the meta-analysis is a study of a complete probability sample of a specified population. If this assumption is not met in a study, no inference can be made from the ''sample'' to a population and hence, comparing the observed effect size with observations in other studies is not meaningful. The point estimate is represented in the forest plot by a smaller or a larger bullet. The relative size of these bullets represents a study's weight in the generation of the meta-analytic result. The plot presented in this figure is fictitious and constructed for illustration purposes: if CIs are entirely on the positive side of zero, in traditional terminology, these studies show a statistically significant positive effect. If CIs are entirely on the negative side of zero, in traditional terminology, these studies show a statistically significant negative effect. If CIs include zero, in traditional terminology, these studies show an effect that is not statistically significant. CI, confidence interval.

Table 1. Characteristics of Included Studies in This Review

First author	Time of study	Publication (year)	Location	Sample size	Biofilm rate	Correlation between biofilm and AB resistance	Biofilm type, n (%)		
							Strong	Moderate	Weak
Jabalameli[63]	2010	2012	Tehran	96	93 (97.9)	No reported	46 (49.46)	25 (26.88)	22 (23.65)
Hemati[48]	2012–2013	2014	Tehran	140	122 (87.1)	Yes	79 (64.75)	30 (24.59)	13 (10.65)
Heydari[64]	2011	2015	Tehran	62	27 (43.5)	No	18 (66.7)	—	9 (33.3)
Corehtash[65]	2013	2015	Tehran	144	133 (92.4)	Yes	—	—	—
Ghadaksaz[66]	2010–2012	2015	Tehran	104	53 (51)	No	—	—	—
Azimi[67]	2013–2014	2016	Tabriz	160	139 (87)	No reported	110 (79.13)	18 (12.94)	11 (7.91)
Banar[23]	2013–2014	2016	Tehran	57	55 (96.5)	No	17 (30.9)	26 (47.3)	12 (21.8)
Tabatabaei[68]	—	2017	—	50	35 (70)	Yes	35 (100)	—	—
Saffari[69]	2014–2015	2017	Tehran	92	92 (99.5)	Yes	—	11 (12)	81 (88)
Valadbeigi[70]	2015	2017	Ilam	18	12 (66.7)	Yes	—	—	—
Asadpour[71]	—	2018	Rasht	90	61 (67.8)	Yes	—	—	
Dolatabadi[72]	—	2018	Tehran	50	50 (99.5)	No reported	17 (33.33)	33 (66.66)	
Gholamrezazadeh[73]	2015	2018	Kerman	28	19 (68)	Yes	8 (42.1)	7 (36.84)	4 (21.05)
Heidari[74]	2016–2017	2018	Shiraz	56	56 (99.5)	No reported	—	—	—
Karami[75]	2016–2017	2018	Hamadan	58	55 (94.8)	Yes	—	—	—
Nasirmoghadas[76]	2015	2018	Isfahan	100	93 (93)	No	4 (4.3)	22 (23.65)	67 (72.04)
Pournajaf[21]	2016–2017	2018	Tehran	143	112 (78.3)	No reported	64 (57.1)	31 (27.6)	17 (15.2)
Satarian[77]	2008–2009	2018	Tehran	33	16 (48.5)	Yes	—	—	—
Shokri[78]	2013–2014	2018	Isfahan	80	76 (95)	No reported	—	—	—
Bahador[79]	2017	2019	Bandar Abbas	75	74 (98.7)	Yes	45 (60)	26 (34.3)	3 (4.3)

In this method, the sample size and event rate (prevalence) were used to calculate the combined biofilm produced. The rate of biofilm formation was calculated with 95% CI through random-effects model. p-value of <0.05 was considered the statistical significance threshold.

CI, confidence interval.

CI: 82.7–97.3), respectively. The frequencies of other biofilm-related genes have been demonstrated in Table 2. The combined prevalence of MDR *P. aeruginosa* isolates was obtained as 66.9% (95% CI: 42–84.9). As shown in Table 3, the highest pooled rates of antibiotic resistance were against piperacillin/tazobactam, gatifloxacin, ceftriaxone, and carbenicillin with rates of 90% (95% CI: 98–99.9), 87.2% (95% CI: 75–93.9), 80.9% (95% CI: 53.3–94), and 80.5% (95% CI: 32.4–97.3), respectively. Also, the lowest antibiotic resistance rates were against

Table 2. Overall Effects of Subgroup Analysis in *Pseudomonas aeruginosa* Isolated from Clinical Samples of Iranian Patients

Subgroups	No. of studies	Heterogeneity test			Egger's test		Random model		
		Prevalence (95% CI) (%)	Z	p	Q	p	I^2	T	p
MDR		66.9 (42–84.9)	1.34	0.00	284.8	0.00	96.1	0.81	0.43
Overall effect (biofilm)	20	86.5 (79–91.6)	6.8	0.00	206.3	0.00	90.7	3	0.006
Biofilm types									
Strong	11	51 (37.4–64.4)	0.13	0.00	108.9	0.00	90.8	0.85	0.41
Moderate	10	29.2 (20.9–39.1)	0.12	0.00	67.9	0.00	86.7	0.5	0.6
Weak	10	25.4 (11.5–47.2)	2.8	0.00	214	0.00	95.8	0.8	0.4
Genes related to biofilm formation									
rhlIR	1	83.6 (76.5–88.8)	7.1	1.00	0.00	0.00	0.00	—	—
lasIR	1	93.6 (88.1–96.6)	7.7	1.00	0.00	0.00	0.00	—	—
pslA	5	77.3 (59.8–88.7)	2.8	0.00	45.4	0.4	91.2	0.9	0.00
pelA	3	51.8 (45.9–57.6)	0.2	0.51	3.7	0.00	46.5	0.4	0.71
algD	7	91.4 (80.8–96.4)	5	0.00	42.8	0.00	86	2.4	0.05
algL	3	70.2 (64.6–75.3)	6.5	0.46	1.5	0.00	0.00	1.3	0.40
algU	4	89.3 (85.2–92.3)	11.2	0.17	4.9	0.00	38.9	4.9	0.03
lasB	2	55.9 (11.7–92.4)	0.20	0.8	33.7	0.00	97	—	—
ppyR	3	98.7 (96.5–99.6)	8	0.9	0.10	0.00	0.00	0.17	0.00
pslD	1	54.4 (41.5–66.1)	0.6	1	0.00	0.00	0.00	—	—
pelF	1	93 (82.7–97.3)	4.9	1	0.00	0.00	0.00	—	—

Biofilm formation was calculated using Comprehensive Meta-Analysis software as presented in Table 1. The heterogeneity was assessed by the Cochrane Q and I^2 tests. Considering the heterogeneity indices, the random-effects model was used to calculate the pooled frequencies. Usually, I^2 value <50% suggests significant heterogeneity in the reported effect sizes. Also, Egger's linear regression test was used to further reveal any publication bias and possible asymmetrical data distribution in the selected studies.

MDR, multidrug resistant.

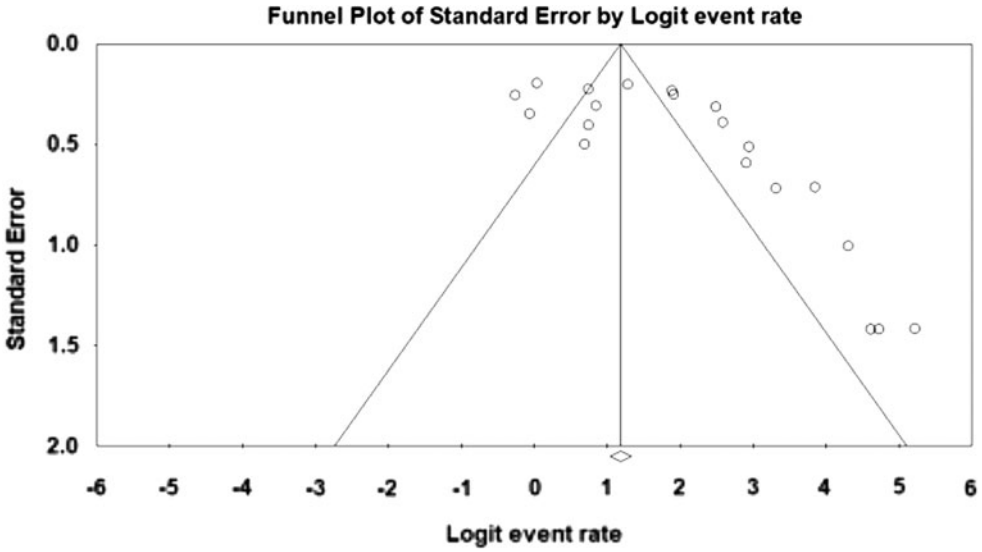

FIG. 3. Funnel plot of meta-analysis on the biofilm formation rate in *Pseudomonas aeruginosa* isolated from clinical samples. A funnel plot is a scatterplot study precision. It is used primarily as a visual assistance for discovering bias or systematic heterogeneity. A symmetric inverted funnel shape arises from a ''well-behaved'' data set, in which publication bias is unlikely. An asymmetric funnel indicates a relationship between publication bias and study precision. This suggests the possibility of either publication bias or a systematic difference between studies of higher and lower precision (typically ''small study effects'').

colistin, polymyxin B, and tigecycline with rates of 2.4% (95% CI: 0.3–16.6), 3.1% (95% CI: 0.5–16.6), and 5% (95% CI: 0.3–48.3), respectively. A correlation was observed between biofilm formation and antibiotic resistance in 10 out of 20 included studies in this review.

Discussion

Our study showed that the rate of biofilm formation by *P. aeruginosa* isolates from Iranian patients varied from 43.5% to 99.5% in different locations of Iran. Overall, the pooled ratio of biofilm formation was calculated as 86.5%.

TABLE 3. SUBGROUPS ANALYSIS FOR ANTIBIOTIC RESISTANCE IN *PSEUDOMONAS AERUGINOSA* ISOLATED FROM CLINICAL SAMPLES

		Heterogeneity test			*Egger's test*		*Random model*		
Subgroups	*No. of studies*	*Prevalence (95% CI) (%)*	Z	p	Q	p	I^2	T	p
Imipenem	15	48.1 (31.3–65.3)	0.21	0.00	314.9	0.00	95.5	1.4	0.18
Ciprofloxacin	15	49.3 (33.8–65)	0.08	0.00	295.8	0.00	95.2	0.02	0.98
Gentamicin	15	53.4 (36.3–69.7)	0.38	0.00	340.7	0.00	95.8	0.40	0.69
Amikacin	16	47.2 (31–64)	0.32	0.00	361.2	0.00	95.8	0.27	0.78
Ceftriaxone	4	80.9 (53.3–94)	2.1	0.00	31.4	0.00	90.4	1.7	0.22
Ceftazidime	15	54.6 (39.8–68.6)	0.6	0.00	297.3	0.00	95.2	0.05	0.95
Cefepime	8	63 (34.8–84.5)	0.34	0.00	0.90	0.00	204.1	0.39	0.70
Piperacillin/tazobactam	2	90 (98–99.9)	0.97	0.00	19.2	0.00	94.8	—	—
Levofloxacin	4	46.9 (7.9–90.6)	0.10	0.00	126.5	0.00	97.6	0.02	0.98
Aztreonam	11	51.3 (30.7–71.5)	0.11	0.00	271.1	0.00	96.3	2.7	0.02
Piperacillin	10	33.1 (16.6–55.2)	1.5	0.00	248.3	0.00	96.3	2.3	0.04
Tobramycin	8	64.4 (38.3–84.1)	1	0.00	161.5	0.00	95.6	0.1	0.90
Ticarcillin	4	38.2 (8.3–80.8)	0.49	0.00	73.3	0.00	95.9	0.4	0.7
Polymyxin B	6	3.1 (0.5–16.6)	3.6	0.00	49.6	0.00	89.9	2.7	0.05
Tigecycline	2	5 (0.3–48.3)	2	0.00	4.2	0.03	76.4	—	—
Colistin	7	2.4 (0.3–16.6)	3.4	0.00	74.3	0.00	91.9	4.9	0.008
Meropenem	9	62.7 (44.2–78.2)	1.35	0.00	132.9	0.00	93.9	0.81	0.49
Carbenicillin	2	80.5 (32.4–97.3)	1.29	0.00	26.2	0.00	96.1	—	—
Piperacillin/tazobactam	7	47 (15.4–81.4)	0.14	0.00	118.3	0.00	94.9	1.1	0.31
Trimethoprim-sulfamethoxazole	2	87.9 (67.1–97.1)	2.5	0.00	9.8	0.002	89.8	—	—
Tetracycline	2	72.6 (7.2–98.9)	0.53	0.00	54.3	0.00	98.1	—	—
Norfloxacin	3	13.4 (9.7–18.2)	9.9	0.00	0.002	0.00	0.00		
Gatifloxacin	2	87.2 (75–93.9)	4.5	0.00	3.9	0.00	74.9	—	—

In comparison, Abidi *et al.* reported that most of *P. aeruginosa* isolates from contact lenses of Karachi-Pakistan patients could form biofilms, in which the biolfilm production was significantly higher than MDR.[32] In a study by Senturk *et al.* in Turkey, 78% of *P. aeruginosa* isolates from patients with urinary tract infection produced biofilm.[33] In comparison with our study, several studies have also observed lower rates of biofilm formation by *P. aeruginosa*, including Prince *et al.* in the United States (28.6%)[34] and Kádár *et al.* in Hungary (23.3%).[35] In another report by Hou *et al.* in China, none of 29 *P. aeruginosa* isolates recovered from the Ophthalmology ward-produced biofilm.[36]

Several intracellular signaling pathways are involved in the induction of transcription factors that activate biofilm-related genes.[37] In our study, the pooled frequencies of *lasIR, algD, algU, ppyR, and pelF* genes were 93.6%, 91.4%, 89.3%, 98.7%, and 93%, respectively. In the study of Hou *et al.* in which no *P. aeruginosa* isolate produced biofilm, 31% of the isolates expressed the *pslA* gene.[31] Zaranza *et al.* reported the prevalence of *algD* gene as 39% in Brazil.[38] Another study conducted by Stehling *et al.* in Brazil reported the prevalence of *algD* and *algU* genes as 100% and 25%, respectively.[39] Mitov *et al.* in Bulgaria described that *algD* and *lasB* genes were expressed in 91.1% and 100% of *P. aeruginosa* isolates, respectively.[40] Also, Wolska and Szweda found that the *algD* gene was expressed in 93.5% of *P. aeruginosa* isolates.[41]

Biofilm formation is one of the several mechanisms participating in antibiotic resistance of *P. aeruginosa.* In our study, the combined prevalence of MDR *P. aeruginosa* was reported as 66.9%, of which the highest pooled antibiotic resistance was against piperacillin/tazobactam with a resistant rate of 90%. In addition, the lowest resistance rate was observed against colistin (2.4%). Gill *et al.* demonstrated that 50% of *P. aeruginosa* isolates from ICU were MDR with the maximum and minimum resistance rates against aminoglycosides (88%) and monobactams (2%), respectively.[42] The findings of Khan *et al.* from Pakistan showed that 30% of *P. aeruginosa* strains were MDR with the highest resistance rate against cefuroxime and cefixime (each with 100%) and the lowest resistance rate against amikacin (10%).[43] Gomila *et al.* in their study in a public hospital in Spain demonstrated that 21.4% of *P. aeruginosa* isolates were MDR.[44] In the recent study, maximum resistance was observed against ceftazidime and cefepime (nearly 90%), while all of the isolates were sensitive to colistin.[44] Du *et al.* further reported the highest resistance rate against ampicillin and chloramphenicol (100%) and the lowest against ceftazidime (38%).[45] The European Antimicrobial Resistance Surveillance Network (EARS-Net) in 2015 reported an increasing trend for resistance against piperacillin/tazobactam during 2011–2015, with the highest resistance related to piperacillin/tazobactam (36.1%) and levofloxacin (36.6%), and the lowest (1%) was against colistin in European hospitals. Similarly, resistance to piperacillin/tazobactam, levofloxacin, and colistin was reported as 27.1%, 29.5%, and 1.1%, respectively in the U.S. hospitals.[46,47] Overall, our findings were in accordance with previous reports showing relatively low resistance of *P. aeruginosa* strains against colistin. Therefore, this antibiotic can be appropriate to treat hospital infections caused by *P. aeruginosa*, especially in patients with the weak immune systems such as patients in the burn unit.

A correlation was found between biofilm formation and antibiotic resistance in 10 out of 20 studies included in this review. This observation indicates that biofilm formation increases the resistance rate and facilitates the chronicity of the infection. In some of these studies, it was reported that biofilms may directly confer resistance against specific antibiotics. In accordance, Banar *et al.* also noticed that although some *P. aeruginosa* strains were resistant to ceftazidime in biofilm form, they were susceptible to this antibiotic in the planktonic form.[23] Also, Hemati *et al.* reported a significant correlation between biofilm formation and the minimum inhibitory concentration (MIC) values of ceftazidime, meropenem, and amikacin.[48] MIC is the lowest concentration of a chemical, usually a drug, which prevents visible growth of a bacterium or bacteria.[49] Out of studies reviewed here, six observed higher rates of biofilm formation, especially the potent subtype, among MDR *P. aeruginosa* strains. Similarly, Zaranza *et al.* reported that biofilm formation protected bacteria against the host's immune system and antibiotics.[38] Others also showed that biofilm formation reduced the efficacy of administrated antibiotics.[50–52]

This study showed that biofilm formation was higher in MDR than non-MDR *P. aeruginosa* strains. Generally, a significant correlation was observed between biofilm formation and antibiotic resistance in *P. aeruginosa* retrieved from Iranian patients' clinical samples. Some studies did not highlight a correlation between biofilm formation and antibiotic resistance, suggesting the involvement of other resistance mechanisms such as efflux pumps, altered outer membrane permeability, toxin/antitoxin systems, and the expression of β-lactam resistance genes.[53] The increasing use of antibiotics and rising numbers of invasive procedures, together with the enhancement of intrinsic and acquired resistance mechanisms of *P. aeruginosa*, cause the evolution of MDR strains of *P. aeruginosa* in clinical centers.[54] The intrinsic resistance comprises reductions in membrane permeability, efflux mechanism pumping the antimicrobial agents outside the cell wall, and production of inactivation enzymes.[55] Mutational changes or the acquisition of resistance mechanisms through horizontal gene transfer during chemotherapy are the routine ways of acquired resistance.[56]

Biofilm formation can enhance antibiotic resistance in *P. aeruginosa*, which in turn leads to the chronicity and difficulty in the treatment of the infection, as well as longer hospital stay and higher therapeutic costs, especially in immunocompromised patients.[57] Persistence of infection and the emergence of resistance during antibiotic therapy have been shown to negatively affect patient outcomes. Carmeli and *et al.* showed that emergence of resistance (at least a fourfold increase in MIC compared to baseline) had significant effects on both mortality and length of hospital stay.[58] Also, Centers for Disease Control and Prevention (CDC) reported that emergence of resistance was related to an average adjusted increase of about 6 days in the length of hospital stay. Briefly, patients who suffered from infection with MDR isolates of *P. aeruginosa* usually have increased mortality and morbidity.[59]

This review from Iran is helping in knowing the antibiotic resistance pattern and consequently prescribing the suitable antimicrobial agents for the treatment of infection caused by *P. aueroginosa* in clinical settings. Also, data obtained regarding the combined prevalence of biofilm-related genes, and the association between biofilm formation and antibiotic resistance, can provide us with comprehensive information

in this area. Certainly, the information, in this case, can help us to take preventive measures.

It has been suggested that using effective antimicrobial agents such as plant extracts along with antibiotics can increase their efficacy by generating a synergistic effect.[60] Also, applying complementary pharmaceuticals alongside with antibiotics is recommended in patients with MDR *P. aeruginosa* infections to induce virulence genes and reinforce therapeutics' efficacies.[61]

Incorporating unpublished data was not possible in this review, which was the main limitation of our study. Also, in cases of missing data, we did not contact the authors of studies for further clarity.

In conclusion, this study showed that biofilm formation was higher in MDR *P. aeruginosa* than non-MDRs. Also, a significant correlation was observed between biofilm formation and antibiotic resistance. Regarding the multifaceted etiology of antibiotic resistance and a steady increase in its prevalence and spread worldwide, more frequent outbreaks of infections resulting from MDR isolates are expected.[62] Therefore, using multitargeted and combinational therapies (*i.e.* antimicrobial agents such as plant extracts along with antibiotics) is useful to increase the efficacy of drugs by generating synergistic effects against pathogens.

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Supplementary Material

Supplementary Data
Supplementary Table S1

References

1. Yetkin, G., B. Otlu, A. Cicek, C. Kuzucu, and R. Durmaz. 2006. Clinical, microbiologic, and epidemiologic characteristics of *Pseudomonas aeruginosa* infections in a University Hospital, Malatya, Turkey. Am. J. Infect. Control 34:188–192.
2. Yang, L., L. Jelsbak, R.L. Marvig, S. Damkiær, C.T. Workman, M.H. Rau, S.K. Hansen, A. Folkesson, H.K. Johansen, O. Ciofu, N. Høiby, M.O. Sommer, and S. Molin. 2011. Evolutionary dynamics of bacteria in a human host environment. Proc. Natl. Acad. Sci. 108:7481–7486.
3. Khaledi, A., D. Esmaeili, S.A. Jamehdar, S.-A. Esmaeili, A. Neshani, and A. Bahador. 2016. Expression of MFS efflux pumps among multidrug resistant *Acinetobacter baumannii* clinical isolates. Der. Pharm. Lett. 8:262–267.
4. Nelson, K., C. Weinel, I. Paulsen, R. Dodson, H. Hilbert, V. Martins dos Santos, D.E. Fouts, S.R. Gill, M. Pop, M. Holmes, L. Brinkac, M. Beanan, R.T. DeBoy, S. Daugherty, J. Kolonay, R. Madupu, W. Nelson, O. White, J. Peterson, H. Khouri, I. Hance, P. Chris Lee, E. Holtzapple, D. Scanlan, K. Tran, A. Moazzez, T. Utterback, M. Rizzo, K. Lee, D. Kosack, D. Moestl, H. Wedler, J. Lauber, D. Stjepandic, J. Hoheisel, M. Straetz, S. Heim, C. Kiewitz, J.A. Eisen, K.N. Timmis, A. Düsterhöft, B. Tümmler, and C.M. Fraser. 2002. Complete genome sequence and comparative analysis of the metabolically versatile *Pseudomonas putida* KT2440. Environ. Microbiol. 4:799–808.
5. Turner, K.H., J. Everett, U. Trivedi, K.P. Rumbaugh, and M. Whiteley. 2014. Requirements for *Pseudomonas aeruginosa* acute burn and chronic surgical wound infection. PLoS Genet. 10:e1004518.
6. Wanis, M., S.A. Walker, N. Daneman, M. Elligsen, L. Palmay, A. Simor, and R. Cartotto. 2016. Impact of hospital length of stay on the distribution of Gram negative bacteria and likelihood of isolating a resistant organism in a Canadian burn center. Burns 42:104–111.
7. Gholami, A., M. Barati, M. Vahdani, H. Vahdani, and M. Karimi. 2011. Pattern of empirical antibiotic administration in emergency department of an educational hospital in Tehran. Razi J. Med. Sci. 18:17–23.
8. Zilberberg, M.D., A.F. Shorr, S.T. Micek, C. Vazquez-Guillamet, and M.H. Kollef. 2014. Multi-drug resistance, inappropriate initial antibiotic therapy and mortality in Gram-negative severe sepsis and septic shock: a retrospective cohort study. Crit. Care 18:596.
9. Mehrad, B., N.M. Clark, G.G. Zhanel, and J.P. Lynch III. 2015. Antimicrobial resistance in hospital-acquired gram-negative bacterial infections. Chest 147:1413–1421.
10. Vaez, H., A. Salehi-Abargouei, and F. Khademi. 2017. Systematic review and meta-analysis of imipenem-resistant *Pseudomonas aeruginosa* prevalence in Iran. Germs 7:86.
11. Abbasi, A., S.M. Maddah, A. Mahboubi, A. Khaledi, H. Vazini, and D. Esmaeili. 2017. Investigate the inhibitory effects of Satureja khuzestanica essential oil against housekeeping fabD and exoA genes of *Pseudomonas aeruginosa* from hospital isolates using RT-PCR technique. Ann. Med. Health Sci. Res. 7:246–250.
12. Nakamura, A., K. Miyake, S. Misawa, Y. Kuno, T. Horii, S. Kondo, Y. Tabe, and A. Ohsaka. 2013. Meropenem as predictive risk factor for isolation of multidrug-resistant *Pseudomonas aeruginosa*. J. Hosp. Infect. 83:153–155.
13. Furukawa, S., S. Kuchma, and G. O'Toole. 2006. Keeping their options open: acute versus persistent infections. J. Bacteriol. 188:1211–1217.
14. Vaez, H., A. Salehi-Abargouei, Z.R. Ghalehnoo, and F. Khademi. 2018. Multidrug resistant *Pseudomonas aeruginosa* in Iran: a systematic review and metaanalysis. J. Global Infect. Dis. 10:212.
15. Fazeli, H., R. Akbari, S. Moghim, T. Narimani, M.R. Arabestani, and A.R. Ghoddousi. 2012. *Pseudomonas aeruginosa* infections in patients, hospital means, and personnel's specimens. J. Res. Med. Sci. 17:332.
16. Hall-Stoodley, L., J.W. Costerton, and P. Stoodley. 2004. Bacterial biofilms: from the natural environment to infectious diseases. Nat. Rev. Microbiol. 2:95.
17. Harmsen, M., L. Yang, S.J. Pamp, and T. Tolker-Nielsen. 2010. An update on *Pseudomonas aeruginosa* biofilm formation, tolerance, and dispersal. FEMS Immunol. Med. Microbiol. 59: 253–268.
18. Fazli, M., H. Almblad, M.L. Rybtke, M. Givskov, L. Eberl, and T. Tolker-Nielsen. 2014. Regulation of biofilm formation in Pseudomonas and Burkholderia species. Environ. Microbiol. 16:1961–1981.
19. Anderson, G., and G. O'Toole. 2008. Innate and induced resistance mechanisms of bacterial biofilms. In T. Romeo (ed.), Bacterial Biofilms. Current Topics in Microbiology and Immunology, vol 322. Springer, Berlin, Heidelberg, pp. 85–105.
20. Del Pozo, J., and R. Patel. 2007. The challenge of treating biofilm-associated bacterial infections. Clin. Pharmacol. Ther. 82:204–209.

21. Pournajaf, A., S. Razavi, G. Irajian, A. Ardebili, Y. Erfani, S. Solgi, S. Yaghoubi, A. Rasaeian, Y. Yahyapour, R. Kafshgari, S. Shoja, and R. Rajabnia. 2018. Integron types, antimicrobial resistance genes, virulence gene profile, alginate production and biofilm formation in Iranian cystic fibrosis *Pseudomonas aeruginosa* isolates. Infez. Med. 26:226–236.
22. Colvin, K.M., Y. Irie, C.S. Tart, R. Urbano, J.C. Whitney, C. Ryder, P.L. Howell, D.J. Wozniak, and M.R. Parsek. 2012. The Pel and Psl polysaccharides provide *Pseudomonas aeruginosa* structural redundancy within the biofilm matrix. Environ. Microbiol. 14:1913–1928.
23. Banar, M., M. Emaneini, M. Satarzadeh, N. Abdellahi, R. Beigverdi, W.B. van Leeuwen, and F. Jabalameli. 2016. Evaluation of mannosidase and trypsin enzymes effects on biofilm production of *Pseudomonas aeruginosa* isolated from burn wound infections. PLoS One 11:e0164622.
24. Berube, B.J., S.M. Rangel, and A.R. Hauser. 2016. *Pseudomonas aeruginosa*: breaking down barriers. Curr. Genet. 62:109–113.
25. Guilhen, C., N. Charbonnel, N. Parisot, N. Gueguen, A. Iltis, C. Forestier, and D. Balestrino. 2016. Transcriptional profiling of *Klebsiella pneumoniae* defines signatures for planktonic, sessile and biofilm-dispersed cells. BMC Genomics 17:237.
26. Karatan, E., and P. Watnick. 2009. Signals, regulatory networks, and materials that build and break bacterial biofilms. Microbiol. Mol. Biol. Rev. 73:310–347.
27. Davies, D.G., M.R. Parsek, J.P. Pearson, B.H. Iglewski, J.W. Costerton, and E.P. Greenberg. 1998. The involvement of cell-to-cell signals in the development of a bacterial biofilm. Science 280:295–298.
28. O'Toole, G.A. 2011. Microtiter dish biofilm formation assay. J. Vis. Exp. e2437.
29. Balouiri, M., M. Sadiki, and S.K. Ibnsouda. 2016. Methods for *in vitro* evaluating antimicrobial activity: a review. J. Pharm. Anal. 6:71–79.
30. CASP. 10 questions to help you make sense of qualitative research, 2006. Available at http://www.sph.nhs.uk/sph-files/caspappraisal-tools/?searchterm=casp (accessed October 1, 2012).
31. Boyle, M.H. 1998. Guidelines for evaluating prevalence studies. Evid. Based Mental Health 1:37–39.
32. Abidi, S.H., S.K. Sherwani, T.R. Siddiqui, A. Bashir, and S.U. Kazmi. 2013. Drug resistance profile and biofilm forming potential of *Pseudomonas aeruginosa* isolated from contact lenses in Karachi-Pakistan. BMC Ophthalmol. 13:57.
33. Senturk, S., S. Ulusoy, G. Bosgelmez-Tinaz, and A. Yagci. 2012. Quorum sensing and virulence of *Pseudomonas aeruginosa* during urinary tract infections. J. Infect. Dev. Ctries. 6:501–507.
34. Prince, A.A., J.D. Steiger, A.N. Khalid, L. Dogrhamji, C. Reger, S.E. Claire, A.G. Chiu, D.W. Kennedy, J.N. Palmer, and N.A. Cohen. 2008. Prevalence of biofilm-forming bacteria in chronic rhinosinusitis. Am. J. Rhinol. 22:239–245.
35. Kádár, B., M. Szász, K. Kristóf, N. Pesti, G. Krizsan, J. Szentandrássy, L. Rókusz, K. Nagy, and D. Szabó. 2010. *In vitro* activity of clarithromycin in combination with other antimicrobial agents against biofilm-forming *Pseudomonas aeruginosa* strains. Acta Microbiol. Immunol. Hung. 57:235–245.
36. Hou, W., X. Sun, Z. Wang, and Y. Zhang. 2012. Biofilm-forming capacity of *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* from ocular infections. Invest. Ophthalmol. Vis. Sci. 53:5624–5631.
37. Römling, U., and C. Balsalobre. 2012. Biofilm infections, their resilience to therapy and innovative treatment strategies. J. Intern. Med. 272:541–561.
38. Zaranza, A.V., F.C. Morais, M.S. do Carmo, and A. de Mendonça Marques, C. Andrade-Monteiro, T.F. Ferro, V. Monteiro-Neto, and P.d.M.S. Figueiredo. 2013. Antimicrobial susceptibility, biofilm production and adhesion to HEp-2 cells of *Pseudomonas aeruginosa* strains isolated from clinical samples. J. Biomater. Nanobiotechnol. 4:98.
39. Stehling, E.G., W.D.d. Silveira, and D.d.S. Leite. 2008. Study of biological characteristics of *Pseudomonas aeruginosa* strains isolated from patients with cystic fibrosis and from patients with extra-pulmonary infections. Braz. J. Infect. Dis. 12:86–88.
40. Mitov, I., T. Strateva, and B. Markova. 2010. Prevalence of virulence genes among bulgarian nosocomial and cystic fibrosis isolates of *Pseudomonas aeruginosa*. Braz. J. Microbiol. 41:588–595.
41. Wolska, K., and P. Szweda. 2009. Genetic features of clinical *Pseudomonas aeruginosa* strains. Pol. J. Microbiol. 58:255–260.
42. Gill, J., S. Arora, S. Khanna, and K.H. Kumar. 2016. Prevalence of multidrug-resistant, extensively drug-resistant, and pandrug-resistant *Pseudomonas aeruginosa* from a tertiary level intensive care unit. J. Global Infect. Dis. 8:155.
43. Khan, F., A. Khan, and S.U. Kazmi. 2014. Prevalence and susceptibility pattern of multi drug resistant clinical isolates of *Pseudomonas aeruginosa* in Karachi. Pak. J. Med. Sci. 30:951.
44. Gomila, M., M. del Carmen Gallegos, V. Fernández-Baca, A. Pareja, M. Pascual, P. Díaz-Antolín, E. García-Valdés, and J. Lalucat. 2013. Genetic diversity of clinical *Pseudomonas aeruginosa* isolates in a public hospital in Spain. BMC Microbiol. 13:138.
45. Du, Y., N. Mesaros, P. Nordmann, P. Plésiat, M. Rousset-Delvallez, J. Van Eldere, Y. Glupczynski, Y. VanLaethem, F. Jacobs, P. Lebecque, A. Malfroot, P.M. Tulkens, and F. Van Bambeke. 2007. *Pseudomonas aeruginosa*: resistance and therapeutic options at the turn of the new millennium. Clin. Microbiol. Infect. 13:560–578.
46. Dou, Y., J. Huan, F. Guo, Z. Zhou, and Y. Shi. 2017. *Pseudomonas aeruginosa* prevalence, antibiotic resistance and antimicrobial use in Chinese burn wards from 2007 to 2014. J. Int. Med. Res. 45:1124–1137.
47. ECDC E. 2013. Antimicrobial resistance surveillance in Europe 2012. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net) Stockholm.
48. Hemati, S., F. Azizi-Jalilian, I. Pakzad, M. Taherikalani, A. Maleki, S. Karimi, A. Monjezei, Z. Mahdavi, M.R. Fadavi, K. Sayehmiri, and N. Sadeghifard. 2014. The correlation between the presence of quorum sensing, toxin-antitoxin system genes and MIC values with ability of biofilm formation in clinical isolates of *Pseudomonas aeruginosa*. Iran. J. Microbiol. 6:133.
49. Lambert, R., and J. Pearson. 2000. Susceptibility testing: accurate and reproducible minimum inhibitory concentration (MIC) and non-inhibitory concentration (NIC) values. J. appl. Microbiol. 88:784–90.
50. Finnan, S., J.P. Morrissey, F. O'Gara, and E.F. Boyd. 2004. Genome diversity of *Pseudomonas aeruginosa* isolates from cystic fibrosis patients and the hospital environment. J. Clin. Microbiol. 42:5783–5792.
51. Wareham, D.W., and M.A. Curtis. 2007. A genotypic and phenotypic comparison of type III secretion profiles of *Pseudomonas aeruginosa* cystic fibrosis and bacteremia isolates. Int. J. Med. Microbiol. 297:227–234.

52. Parsek, M.R., and P.K. Singh. 2003. Bacterial biofilms: an emerging link to disease pathogenesis. Annu. Rev. Microbiol. 57:677–701.
53. Aslam, B., W. Wang, M.I. Arshad, M. Khurshid, S. Muzammil, M.H. Rasool, M.A. Nisar, R.F. Alvi, M.A. Aslam, M.U. Qamar, M.K.F. Salamat, and Z. Baloch. 2018. Antibiotic resistance: a rundown of a global crisis. Infect. Drug Resist. 11:1645.
54. Defez, C., P. Fabbro-Peray, N. Bouziges, A. Gouby, A. Mahamat, J. Daures, and A. Sotto. 2004. Risk factors for multidrug-resistant *Pseudomonas aeruginosa* nosocomial infection. J. Hosp. Infect. 57:209–216.
55. Hancock, R.E. 1998. Resistance mechanisms in *Pseudomonas aeruginosa* and other nonfermentative gram-negative bacteria. Clin. Infect. Dis. 27(Suppl_1):S93–S99.
56. Poole, K. 2011. *Pseudomonas aeruginosa*: resistance to the max. Front. Microbiol. 2:65.
57. Leekha, S., C.L. Terrell, and R.S. Edson. 2011. General principles of antimicrobial therapy. Mayo Clin. Proc. 86: 156–167.
58. Carmeli, Y., N. Troillet, A.W. Karchmer, and M.H. Samore. 1999. Health and economic outcomes of antibiotic resistance in *Pseudomonas aeruginosa*. Arch. Intern. Med. 159:1127–1132.
59. Hirsch, E.B., and V.H. Tam. 2010. Impact of multidrug-resistant *Pseudomonas aeruginosa* infection on patient outcomes. Expert Rev. Pharmacoecon. Outcomes Res. 10:441–451.
60. Sofowora, A., E. Ogunbodede, and A. Onayade. 2013. The role and place of medicinal plants in the strategies for disease prevention. Afr. J. Tradit. Complement. Altern. Med. 10:210–229.
61. Koo, H., R.N. Allan, R.P. Howlin, P. Stoodley, and L. Hall-Stoodley. 2017. Targeting microbial biofilms: current and prospective therapeutic strategies. Nat. Rev. Microbiol. 15:740.
62. Farahani, R.K., P. Ehsani, M. Ebrahimi-Rad, and A. Khaledi. 2018. Molecular detection, virulence genes, biofilm formation, and antibiotic resistance of *Salmonella enterica* serotype enteritidis isolated from poultry and clinical samples. Jundishapur J. Microbiol. DOI: 10.5812/jjm.69504 (In press).
63. Jabalameli, F., A. Mirsalehian, B. Khoramian, M. Aligholi, S.S. Khoramrooz, P. Asadollahi, M. Taherikalani, and M. Emaneini. 2012. Evaluation of biofilm production and characterization of genes encoding type III secretion system among *Pseudomonas aeruginosa* isolated from burn patients. Burns 38:1192–1197.
64. Heydari, S., and F. Eftekhar. 2015. Biofilm formation and β-lactamase production in burn isolates of *Pseudomonas aeruginosa*. Jundishapur J. Microbiol. 8:e15514.
65. Corehtash, Z.G., F.F. Ahmad Khorshidi, H. Akbari, and A.M. Aznaveh. 2015. Biofilm formation and virulence factors among *Pseudomonas aeruginosa* isolated from burn patients. Jundishapur J Microbiol. 8:e22345.
66. Ghadaksaz, A., A.A.I. Fooladi, H.M. Hosseini, and M. Amin. 2015. The prevalence of some Pseudomonas virulence genes related to biofilm formation and alginate production among clinical isolates. J. Appl. Biomed. 13:61–68.
67. Azimi, S., H.S. Kafil, H.B. Baghi, S. Shokrian, K. Najaf, M. Asgharzadeh, …. M. Aghazadeh. 2016. Presence of exoY, exoS, exoU and exoT genes, antibiotic resistance and biofilm production among *Pseudomonas aeruginosa* isolates in Northwest Iran. GMS Hyg. Infect. Control 11:Doc04.
68. Tabatabaei, M., and N. Sohrabi. 2017. Comparison of biofilm formation and antibiotic resistance pattern of *Pseudomonas aeruginosa* in human and environmental isolates. Microb. Pathog. 109:94–98.
69. Saffari, M., S. Karami, F. Firoozeh, and M. Sehat. 2017. Evaluation of biofilm-specific antimicrobial resistance genes in *Pseudomonas aeruginosa* isolates in Farabi Hospital. J. Med. Microbiol. 66:905–909.
70. Valadbeigi, H., N. Sadeghifard, and M.B. Salehi. 2017. Assessment of biofilm formation in *Pseudomonas aeruginosa* by antisense mazE-PNA. Microb. Pathog. 104:28–31.
71. Asadpour, L. 2018. Antimicrobial resistance, biofilm-forming ability and virulence potential of *Pseudomonas aeruginosa* isolated from burn patients in northern Iran. J. Global Antimicrob. Resist. 13:214–220.
72. Dolatabadi, S., H.N. Moghadam, and M. Mahdavi-Ourtakand. 2018. Evaluating the anti-biofilm and antibacterial effects of Juglans regia L. extracts against clinical isolates of *Pseudomonas aeruginosa*. Microb. Pathog. 118:285–289.
73. Gholamrezazadeh, M., M.R. Shakibaie, F. Monirzadeh, S. Masoumi, and Z. Hashemizadeh. 2018. Effect of nano-silver, nano-copper, deconex and benzalkonium chloride on biofilm formation and expression of transcription regulatory quorum sensing gene (rh1R) in drug-resistance *Pseudomonas aeruginosa* burn isolates. Burns 44:700–708.
74. Heidari, H., M. Hadadi, H.S. Ebrahim-Saraie, A. Mirzaei, A. Taji, S. Hosseini, and M. Motamedifar. 2018. Characterization of virulence factors, antimicrobial resistance patterns and biofilm formation of *Pseudomonas aeruginosa* and *Staphylococcus* spp. strains isolated from corneal infection. J. Fr. Ophtalmol. 41:823–829.
75. Karami, P., P. Mohajeri, R.Y. Mashouf, M. Karami, M.H. Yaghoobi, D. Dastan, and M.Y. Alikhani. 2018. Molecular characterization of clinical and environmental *Pseudomonas aeruginosa* isolated in a burn center. Saudi J. Biol. Sci. 26:1731–1736.
76. Nasirmoghadas, P., S. Yadegari, S. Moghim, B.N. Esfahani, H. Fazeli, F. Poursina, S.A. Hosseininassab, and H.G. Safaei. 2018. Evaluation of biofilm formation and frequency of multidrug-resistant and extended drug-resistant strain in *Pseudomonas aeruginosa* isolated from burn patients in Isfahan. Adv. Biomed. Res. 7:61.
77. Satarian, F., H.M. Hosseini, A. Ghadaksaz, M. Amin, and A.A. Fooladi. 2018. Multi-drug resistant clinical *Pseudomonas aeruginosas* inhibited by Ferula gummosa Boiss. Recent Pat. Antiinfect. Drug Discov. 13:89–99.
78. Shokri, D., M.R. Khorasgani, M. Mohkam, S.M. Fatemi, Y. Ghasemi, and A. Taheri-Kafrani. 2018. The inhibition effect of lactobacilli against growth and biofilm formation of *Pseudomonas aeruginosa*. Probiotics Antimicrob. Proteins 10:34–42.
79. Bahador, N., S. Shoja, F. Faridi, B. Dozandeh-Mobarrez, F.I. Qeshmi, S. Javadpour, and S. Mokhtary. 2019. Molecular detection of virulence factors and biofilm formation in *Pseudomonas aeruginosa* obtained from different clinical specimens in Bandar Abbas. Iran. J. Microbiol. 11:25–30.

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