Correlation between biofilm formation and antibiotic resistance in *Pseudomonas aeruginosa*: a meta-analysis

Mehdi Hadadi-Fishani[1], Azad Khaledi[2,3], Zahra Sadat Fatemi-Nasab[2,3]

[1]Department of Medical Bacteriology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran; [2]Infectious Diseases Research Center, Faculty of Medicine, Kashan University of Medical Sciences, Kashan, Iran; [3]Department of Microbiology and Immunology, Faculty of Medicine, Kashan University of Medical Sciences, Kashan, Iran

SUMMARY

Biofilm formation is one of the important resistance mechanisms in *Pseudomonas aeruginosa*. This study aimed to consider the correlation between biofilm formation and antibiotic resistance in *Pseudomonas aeruginosa* through a systematic review and meta-analysis. This study was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) strategies. Scientific databases were searched by MeSH terms and keywords such as "*Pseudomonas aeruginosa*", "biofilm formation", "antibiotic resistance", "prevalence" AND "Iran", to obtain articles published from 1st January 2016 to 30th November 2019. Studies recording biofilm formation and antibiotic resistance in *P. aeruginosa* recovered from clinical samples of Iranian patients were included. Data analysis was performed using CMA software. The combined biofilm formation rate was reported as 87.6% (95% CI: 80-92.5). The heterogeneity index among the selected articles was Q2=96.5, I^2=85.5, and t=0.26 (*p=0.16*). The pooled occurrences of strong, moderate and weak biofilms were 47.7% (95% CI: 28.7-67.3), 30.2% (95% CI: 19.4-43.8), and 27.4% (95% CI: 8.8-59.8), respectively. The pooled prevalence of MDR *P. aeruginosa* strains was as follows: 62.5% (95% CI: 40-77.2). The highest combined rates of antibiotic resistance were against ceftriaxone and tobramycin with the rates of 79.2.9% (95% CI: 54.2-96.2) and 64.4% (95% CI: 36.3-92), respectively. Also, the lowermost antibiotic resistance rates were against colistin and polymyxin B, with the prevalence of 2.1% (95% CI: 0.2-18.1), and 3% (95% CI: 0.5-17.3), respectively. More than half of the studies included in the present review showed a significant correlation between biofilm formation and antibiotic resistance pattern.

Keywords: antibiotic resistance, biofilm formation, correlation, *Pseudomonas aeruginosa*, meta-analysis.

INTRODUCTION

Pseudomonas aeruginosa is a Gram-negative opportunistic bacterium producing severe infections in weakened patients such those hospitalized in intensive care unit (ICU), people suffering from cystic fibrosis, AIDS, and burn patients [1]. A wide range of infections including the urinary tract infection (UTI), cystic fibrosis, burn wound infection, septicemia, and respiratory tract infections are caused by *P. aeruginosa* [2].

Multi Drug Resistant *Pseudomonas aeruginosa* (MDRPA) is referred to isolates with resistant to at least three diverse classes of antibiotics including aminoglycosides, carbapenems, antipseudomonal penicillins, quinolones, and cephalosporins [3, 4]. Infections result from MDR isolates are main health care problem for health care systems [5, 6]. Owing to intrinsic and acquired resistance of *P.*

Corresponding author
Azad Khaledi
E-mail: azadkh99@gmail.com

aeruginosa against a broad range of antibacterial agents, treatment of infections resulted from *P. aeruginosa* is challenging [7, 8]. Infections resulting from *P. aeruginosa* are chiefly challenging due to its inherent resistance to many antimicrobial agents and its capability of acquiring resistance to all effective antibiotics [9, 10]. Regarding the limited active antimicrobial agents against MDR *P. aeruginosa,* burden of hospitalization and therapeutic costs are more significant [11, 12]. Several antibacterial mechanisms include suppression of enzyme production, overexpression of efflux pumps, and biofilm formation has described for resistance of this microorganism that caused concern in clinical settings [13].

Biofilm formation is a key strategy used by *P. aeruginosa* to survive in harsh environment such as exposure to antibiotics agents and host immune responses [14]. Biofilms are sessile populations of microorganisms which are enclosed by the self-secreted extracellular polysaccharide matrix, or slime. Biofilm are usually more resistant to antibiotics in comparison to planktonic cells [15]. The National Heart, Lung, and Blood Institute reported that up to 80% of all bacterial infections are associated to biofilm formation [16].

Therefore, concerning the noteworthy role of *P. aeruginosa* in nosocomial infection, this study aimed to investigate the correlation between biofilm formation and antibiotic resistance in *P. aeruginosa* through a systematic review and meta-analysis.

MATERIALS AND METHODS

This study was conducted following Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) strategies. Scientific databases (Web of Science, Cochrane Library, Scopus, PubMed, and Google Scholar databases, etc.) searched by MeSH terms and keywords such as "*Pseudomonas aeruginosa*", "biofilm formation", "antibiotic resistance", "prevalence" AND "Iran", were used to get articles published from 1th January 2016 and 30th November 2019. The studies recording the biofilm formation, and the antibiotic resistance in *P. aeruginosa* recovered from clinical samples of Iranian patients were selected. The references section of all selected articles was also checked for finding additional studies.

Inclusion criteria

The cross-sectional studies reporting the biofilm formation rate and the antibiotic resistance in *P. aeruginosa* isolated from clinical samples of Iranian patients were included. Moreover, only articles in which standard microtiter plate test had been used for biofilm formation assessment were selected [17].

Also, for characterizing the antibiotic resistance pattern, only those studies that had used standard susceptibility tests such as macrobroth or microbroth dilution, or disk diffusion methods based on the Clinical & Laboratory Standards Institute were included (CLSI) [18].

Exclusion criteria

Letters to editors, reviews (systematic, meta-analysis, and narrative), editorials, conferences and meeting abstracts, case reports, and studies in languages other than English were excluded. Papers without full text, duplicates, and articles with unclear and missing information were not considered, too. As well, the two reviewers autonomously screened the studies and lastly merged their searches. Inconsistencies between them were resolved through discussion before settling the articles for the next level.

Extraction of data

An information extraction form was used to extract the related features of each record. These data were: the first authors' names, time of the study, biofilm formation rate, the correlation between biofilm formation and antibiotic resistance, year of publication, location, sample size, and the type of biofilm (i.e. strong, moderate, and weak).

Data Analysis

Data-analysis was done by the Comprehensive Meta-Analysis software. The biofilm formation was considered with 95% confidence interval (CI). Cochrane Q and I^2 tests were used for assessing the heterogeneity. In view of the heterogeneity, the random-effects model was applied to calculate the combined frequencies. Subgroup analyses were performed for the type of biofilm, and antibiotic resistance pattern. Publication bias was checked via Quantitative Egger weighted regression test and Funnel plot. P-value<0.05 was considered statistically significant.

RESULTS

Study inclusion criteria and characteristics of the eligible studies

A total of 612 studies were recovered, 15 studies met our inclusion criteria (Figure 1).

Generally, as shown in Figure 2 and Table 1, the biofilm formation rate varied from 48.5% to 99.5% in *P. aeruginosa* isolates from clinical samples from Iranian patients.

Overall effects

The heterogeneity index among the selected articles were as Q2=96.5, I^2=85.5, and t=0.26 (p=0.16). Random-effects model was used to pool the frequencies of biofilm formation among the selected articles in the current meta-analysis owing to observing heterogeneity.

The combined biofilm formation was reported as 87.6% (95% CI: 80-92.5), (Table 2). As presented in Figure 3, the publication bias was assessed by the Funnel plot. Also, Egger's linear regression test was applied to detect any potential publication bias and possible asymmetrical data distribution in the included articles. But, no publication bias was seen regarding Egger's linear regression test (p=0.16). As well, 47.7% (95% CI: 28.7-67.3), 30.2% (95% CI: 19.4-43.8), and 27.4% (95% CI: 8.8-59.8) of *P. aeruginosa* isolates were strong, moderate, and weak biofilm producers, respectively.

The pooled prevalence of MDR *P. aeruginosa* strains was achieved as 62.5% (95% CI: 40-77.2).

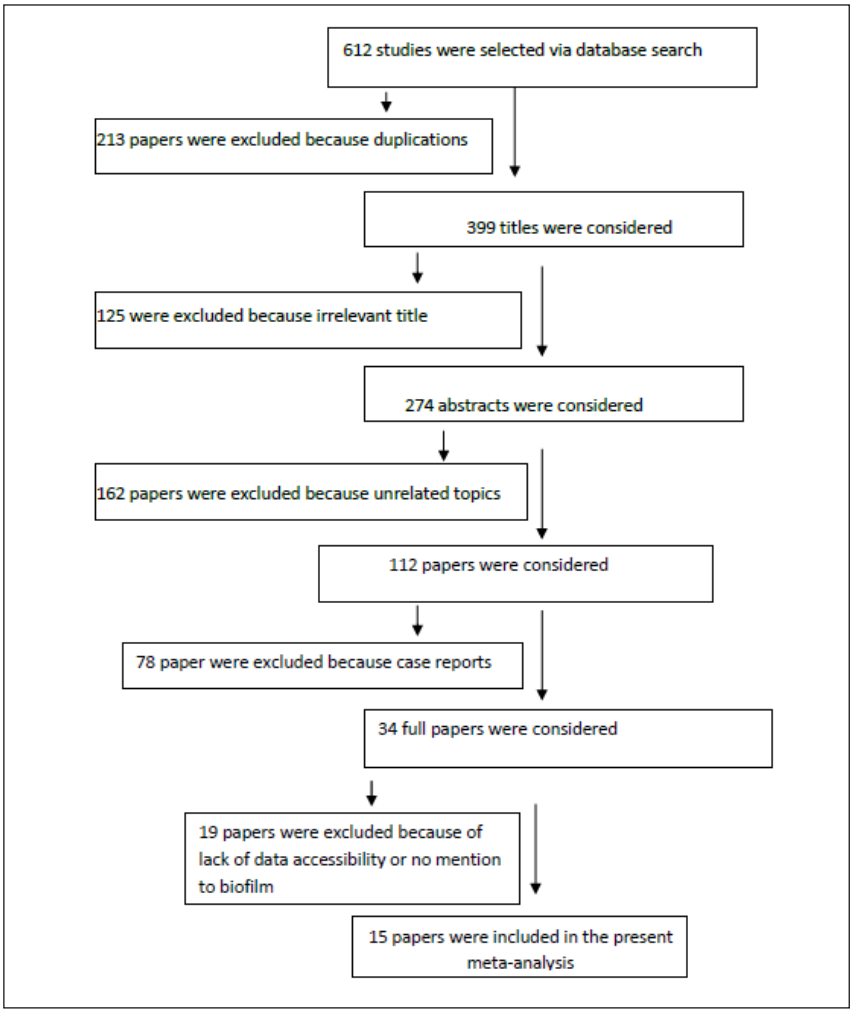

Figure 1 - Chart of selection studies for the present meta-analysis.

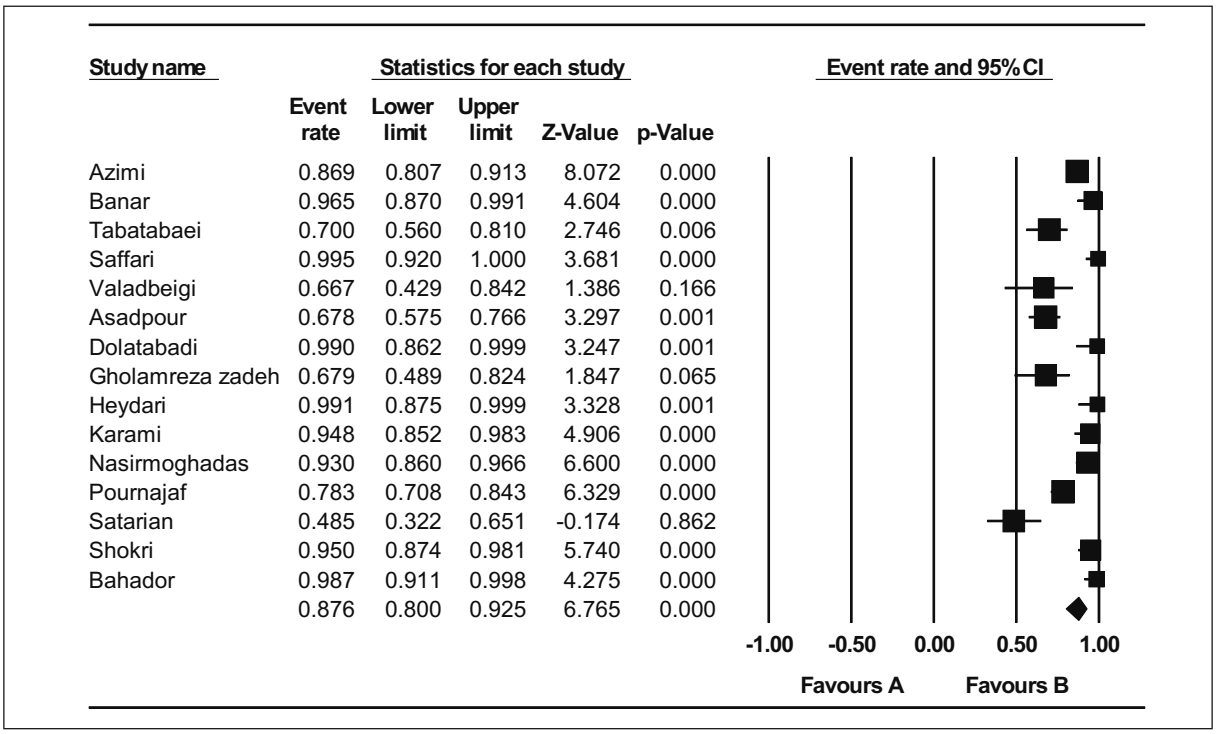

Figure 2 - Forest plot of the meta-analysis of combined biofilm formation in *P. aeruginosa* isolates.

Table 3 showed that the highest combined rates of antibiotic resistance were against ceftriaxone and tobramycin with the rates of 79.2.9% (95% CI: 54.2-96.2), and 64.4% (95% CI: 36.3-92), respectively. Also, the lowermost antibiotic resistance rates were against colistin and polymyxin B with the prevalence of 2.1% (95% CI: 0.2-18.1), and 3% (95% CI: 0.5-17.3), respectively. A correlation was seen between biofilm formation and antibiotic resistance in 8 out of 15 included articles in the current review.

DISCUSSION

Biofilm formation has increased antibiotic resistance and leads the use of higher concentrations of antibiotics in the treatment of infections caused by MDR *P. aeruginosa* isolates [19]. Overall, the current study showed that the biofilm formation rate of *P. aeruginosa* isolates of clinical samples from Iranian patients varied from 48.5% to 99.5%. In general, the combined ratio of biofilm formation was reported as 87.6%. As well, 47.7%, 30.2%, and 27.4% of *P. aeruginosa* isolates were strong, moderate, and weak biofilm producers, respectively. Accordingly, our findings were in agreement with the data published in previous studies where 40-100% of isolates were biofilm producers [20, 21]. As in a study conducted by Karami et al., 73% of both environmental and clinical isolates were biofilm producers [22]. Also, others reported the high biofilm formation that reveals the importance of biofilm formation by *P. aeruginosa* in most infections [23, 24]. In accordance with the present results, another study reported that 96.2% of isolates (both MDR, and Non-MDR) of clinical and environmental isolates had the ability to create the biofilm. The same study reported that 58.6% of MDR clinical isolates were producers of strong biofilm. It showed a significant correlation between MDR form and biofilm formation [25]. In contrast to the current findings, previous studies from different regions of the world showed a lower prevalence of biofilm formation, and consequently no association between biofilm producing and antibiotic resistance [26-28]. This issue possibly attributed to other mechanisms (presence of purines, plasmid acquisition, chromosomal mutation, and

Table 1 - Features of selected articles in the present study.

First author	*Time of study*	*Publication (year)*	*Location*	*Sample size*	*Biofilm rate*	*Correlation between biofilm and AB resistance*	*Biofilm type N (%)*		
							Strong	*Moderate*	*Weak*
Azimi	2013-2014	2016	Tabriz	160	139 (87%)	No	110 (79.13%)	18 (12.94%)	11(7.91%)
Banar	2013-2014	2016	Tehran	57	55 (96.5%)	No	17 (30.9%)	26 (47.3%)	12 (21.8%)
Tabatabaei	–	2017	–	50	35 (70%)	Yes	35 (100%)	–	–
Saffari	2014-2015	2017	Tehran	92	92 (99.5%)	Yes	–	11 (12%)	81 (88%)
Valadbeigi	2015	2017	Ilam	18	12 (66.7%)	Yes	–	–	–
Asadpour	–	2018	Rasht	90	61 (67.8%)	Yes	–	–	
Dolatabadi	–	2018	Tehran	50	50 (99.5%)	No reported	17 (33.33%)	33 (66.66%)	–
Gholamreza zadeh	2015	2018	Kerman	28	19 (68%)	Yes	8 (42.1%)	7 (36.84%)	4 (21.05%)
Heydari	2016-2017	2018	Shiraz	56	56 (99.5%)	No reported	–	–	–
Karami	2016-2017	2018	Hamadan	58	55 (94.8%)	Yes	–	–	–
Nasirmoghadas	2015	2018	Isfahan	100	93 (93%)	No	4 (4.3%)	22 (23.65%)	67 (72.04%)
Pournajaf	2016-2017	2018	Tehran	143	112 (78.3%)	No reported	64 (57.1%)	31 (27.6%)	17 (15.2%)
Satarian	2008-2009	2018	Tehran	33	16 (48.5%)	Yes	–	–	–
Shokri	2013-2014	2018	Isfahan	80	76 (95%)	No reported	–	–	–
Bahador	2017	2019	Bandar Abbas	75	74 (98.7%)	yes	45 (60%)	26 (34.3%)	3 (4.3%)

Table 2 - Overall effects of subgroups in *P. aeruginosa* isolates.

Subgroups	*Number of studies*	*Heterogeneity test*			*Egger's test*		*Random model*		
		Prevalence (95% CI) (%)	*Z*	*P*	*Q*	*P*	I^2	*T*	*P*
MDR	7	62.5 (40-77.2)	2.27	0.00	254	0.00	97.1	0.12	0.66
Biofilm	15	87.6 (80-92.5)	6.1	0.00	96.5	0.00	85.5	0.26	0.16
Biofilm types									
Strong	8	47.7 (28.7-67.3)	1.11	0.00	201	0.00	92	0.23	0.53
Moderate	8	30.2 (19.4-43.8)	0.13	0.00	77.3	0.00	89.3	0.61	0.34
Weak	7	27.4 (8.8-59.8)	0.23	0.00	178.4	0.00	93.1	0.05	0.11

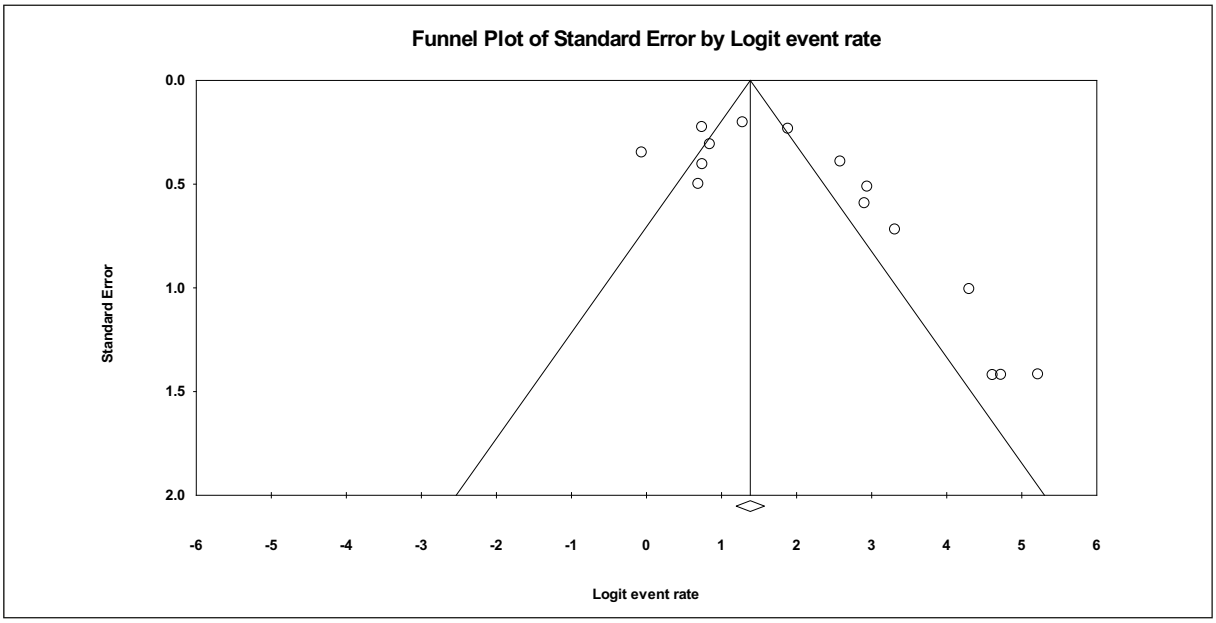

Figure 3 - Funnel plot of meta-analysis on the biofilm formation rate in *P. aeruginosa* isolated from Iranian patients.

Table 3 - Overall effects of antibiotic resistance in *P. aeruginosa* isolates.

Subgroups	*Number of studies*	*Heterogeneity test*			*Egger's test*		*Random model*		
		Prevalence (95% CI) (%)	*Z*	*P*	*Q*	*P*	I^2	*T*	*P*
Imipenem	13	46 (29.3-71.7)	0.32	0.00	219	0.00	94.3	0.13	0.19
Ciprofloxacin	13	51.1 (33.8-65)	0.08	0.00	218	0.00	98.1	0.04	0.56
Gentamicin	12	52.5 (29.9-70.1)	0.56	0.00	320	0.00	90.3	0.7	0.16
Amikacin	14	50 (45-55)	0.36	0.00	289	0.00	99.2	099	0.73
Ceftriaxone	3	79.2.9 (54.2-96.2)	3.4	0.00	34.3	0.00	86.4	1.9	0.001
Ceftazidime	12	57.4 (37.1-78.3)	1.7	0.00	311.8	0.00	87.9	0.06	0.17
Cefepime	6	60.5 (36.4-82.7)	0.36	0.00	0.67	0.00	256	0.76	0.64
Levofloxacin	3	46.9 (7.9-90.6)	0.10	0.00	126.5	0.00	97.6	0.02	0.98
Aztreonam	8	49.4 (33.1-76.3)	0.15	0.00	301	0.00	95.6	2.9	0.08
Piperacillin	7	30.5 (17.9-60.3)	1.8	0.00	134	0.00	88	3.1	0.00
Tobramycin	5	64.4 (36.3-92)	6	0.00	221	0.00	98.2	0.1	0.45
Ticarcillin	2	39 (11-82.9)	0.54	0.00	67.4	0.00	98.4	0.6	2.1
Polymyxin B	4	3 (0.5-17.3)	3	0.00	50	0.00	75	3.6	0.05
Tigecycline	2	6.8 (0. 6-51.5)	4.6	0.00	5.7	0.08	80.1	–	–
Colistin	5	2.1 (0.2-18.1)	3.9	0.00	74	0.00	90.9	6	0. 07
Meropenem	7	61.5 (46-82.1)	3.1	0.00	136	0.00	90	0.67	0.61
Piperacillin/ tazobactam	5	47 (16.3-77.8)	5.9	0.00	160	0.00	96	5.1	0.81

efflux pumps) involved in resistance against antibiotics [29].

P. aeruginosa is one of the major microorganisms accounting for drug-resistant hospital acquired infections [30]. The growing use of antibiotics and increasing use of invasive procedures, together with the development of intrinsic and acquired resistance mechanisms cause the evolution of MDR *P. aeruginosa* isolates in clinical locations [31].

In the present review, the pooled prevalence of MDR *P. aeruginosa* strains was achieved as 62.5%. A correlation was seen between biofilm formation and antibiotic resistance in more than 50% of included studies in the current review. As well, the highest combined rates of antibiotic resistance were against ceftriaxone and tobramycin with the rates of 79.2.9% and 64.4%, respectively. Also, the lowermost antibiotic resistance rates were against colistin and polymyxin B with the prevalence of 2.1% and 3%, respectively.

As all we know, carbapenems are the selective choice against MDR isolates; but in a few past decades the increasing frequency of *P. aeruginosa* carbapenem-resistant isolates has become a global concern [32]. Similarly, in our study, the rate of resistance against imipenem and meropenem was up to 50%, contrary with resistance to colistin and polymyxin B.

In the present study the resistance rate against colistin was 2%. Accordingly, in 2015, the European Antimicrobial Resistance Surveillance Network (EARS-Net) reported the lowest resistance rate (1%-1.1%) against colistin in the United States and Europeans hospitals [33, 34]. So, this antibiotic alongside polymyxin B (resistance rate of 3%) is the best choice for treating infections caused by *P. aeruginosa*.

This comprehensive meta-analysis from Iran can provide a comprehensive information in this area in knowing the antibiotic resistance pattern in clinical settings such as hospitals. Surely, this information can help us to take preventive measures.

CONCLUSION

In summary, this study reports a significant correlation between biofilm formation and antibiotic resistance. Antimicrobial resistance in *P. aeruginosa* is increasing worldwide. Therefore, it is suggested to use of alternative antimicrobial compounds such as plant extracts in combination with antibiotics or alone to increase the effectiveness of drugs by creating synergistic effects against MDR *P. aeruginosa* isolates.

Acknowledgements
None.

Conflicts of interest
The authors declare that they have no competing interests.

Funding
This study did not receive any financial support.

REFERENCES

[1] Yetkin G, Otlu B, Cicek A, et al. Clinical, microbiologic, and epidemiologic characteristics of *Pseudomonas aeruginosa* infections in a University Hospital, Malatya, Turkey. *Am J Infect Control.* 2006; 34 (4), 188-92.

[2] Yang L, Jelsbak L, Marvig RL, et al. Evolutionary dynamics of bacteria in a human host environment. *Proc Natl Acad Sci.* 2011; 108 (18), 7481-6.

[3] Falagas ME, Koletsi PK, Bliziotis IA. The diversity of definitions of multidrug-resistant (MDR) and pandrug-resistant (PDR) *Acinetobacter baumannii* and *Pseudomonas aeruginosa. J Med Microbiol.* 2006; 55 (12), 1619-29.

[4] Khaledi A, Esmaeili D, Jamehdar SA, et al. Expression of MFS efflux pumps among multidrug resistant *Acinetobacter baumannii* clinical isolates. *Der Pharm Lett.* 2016; 8, 262-7.

[5] Vaez H, Salehi-Abargouei A, Khademi F. Systematic review and meta-analysis of imipenem-resistant *Pseudomonas aeruginosa* prevalence in Iran. *Germs.* 2017; 7 (2), 86.

[6] Farahani RK, Ehsani P, Ebrahimi-Rad M, Khaledi A. Molecular detection, virulence genes, biofilm formation, and antibiotic resistance of Salmonella enterica serotype enteritidis isolated from poultry and clinical samples. *Jundishapur J Microbiol.* 2018; (In Press).

[7] Lila G, Mulliqi-Osmani G, Bajrami R, et al. The prevalence and resistance patterns of *Pseudomonas aeruginosa* in a tertiary care hospital in Kosovo. *Infez Med.* 2017; 25 (1), 21-6.

[8] Pournajaf A, Razavi S, Irajian G, et al. Integron types, antimicrobial resistance genes, virulence gene profile, alginate production and biofilm formation in Iranian cystic fibrosis *Pseudomonas aeruginosa* isolates. *Infez Med.* 2018; 26 (3), 226-36.

[9] Srifuengfung S, Tiensasitorn C, Yungyuen T, Dhiraputra C. Prevalence and antimicrobial susceptibility of *Pseudomonas aeruginosa* mucoid and non-mucoid type. *Southeast Asian J Trop Med. Public Health.* 2004; 35 (4), 893.

[10] Esmaeili D, Daymad SF, Neshani A, et al. Alerting prevalence of MBLs producing *Pseudomonas aeruginosa* isolates. *Gene Rep.* 2019; 16, 100460.
[11] Nakamura A, Miyake K, Misawa S, et al. Meropenem as predictive risk factor for isolation of multidrug-resistant *Pseudomonas aeruginosa*. *J Hosp Infect*. 2013; 83 (2), 153-5.
[12] Riccardi N, Rotulo GA, Favilli F, et al. *Pseudomonas aeruginosa* severe skin infection in a toddler with X-linked agammaglobulinemia due to a novel BTK mutation. *Infez Med.* 2019; 27 (1), 73-6.
[13] Gholami A, Barati M, Vahdani M, et al. Pattern of empirical antibiotic administration in emergency department of an educational hospital in Tehran. *Razi J Med Sci.* 2011; 18 (82), 17-23.
[14] El-Ganiny AM, Shaker GH, Aboelazm AA, El-Dash HA. Prevention of bacterial biofilm formation on soft contact lenses using natural compounds. *J Ophthalmic Inflamm Infect.* 2017; 7 (1), 11.
[15] Davey ME, O'toole GA. Microbial biofilms: from ecology to molecular genetics. *Microbiol Mol Biol Rev.* 2000; 64 (4), 847-67.
[16] Hassan A, Usman J, Kaleem F, et al. Evaluation of different detection methods of biofilm formation in the clinical isolates. *Braz J Infect Dis.* 2011; 15 (4), 305-11.
[17] O'Toole GA. Microtiter dish biofilm formation assay. *J Vis Exp.* 2011; (47), e2437.
[18] Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. *J PharmAnal.* 2016; 6 (2), 71-9.
[19] Shi H, Trinh Q, Xu W, et al. A universal primer multiplex PCR method for typing of toxinogenic Pseudomonas aeruginosa. *Appl Microbiol Biotechnol.* 2012; 95 (6), 1579-87.
[20] Gurung J, Khyriem AB, Banik A, et al. Association of biofilm production with multidrug resistance among clinical isolates of Acinetobacter baumannii and Pseudomonas aeruginosa from intensive care unit. *Indian J Crit Care Med.* 2013; 17 (4), 214.
[21] de Almeida KdCF, Calomino MA, Deutsch G, et al. Molecular characterization of multidrug-resistant (MDR) *Pseudomonas aeruginosa* isolated in a burn center. *Burns.* 2017; 43 (1), 137-43.
[22] Karami P, Mohajeri P, Mashouf RY, et al. Molecular characterization of clinical and environmental *Pseudomonas aeruginosa* isolated in a burn center. *Saudi J Biol Sci.* 2018.
[23] Vasiljević Z, Jovĉić B, Ćirković I, Đukić S. An examination of potential differences in biofilm production among different genotypes of *Pseudomonas aeruginosa*. *Arch Biol Sci.* 2014; 66 (1), 117-21.
[24] Senturk S, Ulusoy S, Bosgelmez-Tinaz G, Yagci A. Quorum sensing and virulence of Pseudomonas aeruginosa during urinary tract infections. *J Infect Devel Count.* 2012; 6 (06), 501-7.
[25] Karami P, Khaledi A, Mashoof RY, et al. The correlation between biofilm formation capability and antibiotic resistance pattern in *Pseudomonas aeruginosa*. *Gene Rep.* 2019; 100561.
[26] Prince AA, Steiger JD, Khalid AN, et al. Prevalence of biofilm-forming bacteria in chronic rhinosinusitis. *Am J Rhinol.* 2008; 22 (3), 239-45.
[27] Kádár B, Szász M, Kristóf K, et al. In vitro activity of clarithromycin in combination with other antimicrobial agents against biofilm-forming Pseudomonas aeruginosa strains. *Acta Microbiol Immunol Hung.* 2010; 57 (3), 235-45.
[28] Hou W, Sun X, Wang Z, Zhang Y. Biofilm-forming capacity of *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* from ocular infections. *Invest Ophthalmol Visual Sci.* 2012; 53 (9), 5624-31.
[29] Chen J, Su Z, Liu Y, et al. Identification and characterization of class 1 integrons among Pseudomonas aeruginosa isolates from patients in Zhenjiang, China. *Int J Infect Dis.* 2009; 13 (6), 717-21.
[30] Hassuna NA, Mohamed AHI, Abo-Eleuoon SM, Rizk HA-WA. High prevalence of multidrug resistant *Pseudomonas aeruginosa. Arch Clin Microbiol.* 2015; 6 (4).
[31] Porras-Gómez M, Vega-Baudrit J, Núñez-Corrales S. Overview of multidrug-resistant *Pseudomonas aeruginosa* and novel therapeutic approaches. *J Biomaterials Nanobiotechnol.* 2012; 3 (04), 519.
[32] Rossolini GM, Mantengoli E, Docquier J, et al. Epidemiology of infections caused by multiresistant gram-negatives: ESBLs, MBLs, panresistant strains. *Microbiol Bologna.* 2007; 30 (3), 332.
[33] Dou Y, Huan J, Guo F, et al. Pseudomonas aeruginosa prevalence, antibiotic resistance and antimicrobial use in Chinese burn wards from 2007 to 2014. *J Int Med Res.* 2017; 45 (3), 1124-37.
[34] ECDC E. Antimicrobial resistance surveillance in Europe 2012. *Ann Rep Eur Antimicrob Resist Surveil Net.* 2013.