Epidemiology of *Pseudomonas aeruginosa* in cystic fibrosis patients in Iran: A systematic review and meta-analysis

Ali Alavi Foumani[1], Tofigh Yaghubi Kalurazi[2], Farzaneh Mohammadzadeh Rostami[3], Hadi Sedigh Ebrahim-Saraie[4], Ali Nazari-Alam[5], Mehrdad Halaji[3]

[1]Inflammatory Lung Disease Research Center, Department of Internal Medicine, Razi Hospital, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran;
[2]Department of Health, Nutrition and Infectious Diseases, School of Medicine Guilan University of Medical Sciences, Rasht, Iran;
[3]Department of Microbiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran;
[4]Razi Clinical Research Development Center, Razi Hospital, Guilan University of Medical Sciences, Rasht, Iran;
[5]Department of Microbiology, School of Medicine, Kashan University of Medical Sciences, Kashan, Iran

SUMMARY

The present study aims to investigate the prevalence of *Pseudomonas aeruginosa* in Iranian Cystic Fibrosis (CF) patients. We conducted a systematic search on this topic in Web of Science, PubMed, Embase, Scopus, and Google Scholar electronic databases to the end of July 2019. Then, 14 articles with eligible criteria were selected for data extraction and analysis by Comprehensive Meta-Analysis Software. The pooled prevalence of *P. aeruginosa* was 40.6% (95% CI: 32.4%-49.4%) ranging from 32.4% to 49.4%. There was a significant heterogeneity among the studies ($\chi 2=21.02$; $p<0.001$; $I^2=86.07\%$). The funnel plot for publication bias showed no evidence of asymmetry. Based on the results of Begg's and Egger's test no significant publication bias was observed. The study demonstrated a relative prevalence of *P. aeruginosa* among CF patients in Iran. Due to the rapid spread and infection severity of *P. aeruginosa* and other opportunistic pathogens, efforts are required to identify risk factors, reservoirs, transmission routes and source of infection.

Keywords: *Pseudomonas aeruginosa*, cystic fibrosis, Iran, meta-analysis.

INTRODUCTION

Cystic fibrosis (CF) is an autosomal recessive disorder described as a triad of chronic obstructive pulmonary disease, exocrine pancreatic insufficiency, and elevation of sodium and chloride concentration in sweat [1]. CF is caused by the presence of mutations in a gene called Cystic Fibrosis Transmembrane Conductance Regulator (CFTR), located on the long arm of chromosome 7. Identified in 1989, CFTR is a cAMP-dependent chloride necessary for normal ion transport across epithelial cells [2, 3]. The CFTR gene mutations in CF patients are a major cause of mortality and morbidity mainly determined by recurrent and chronic respiratory tract infections [4]. Because of the inherited nature of this disorder, familiar anamnesis plays an important role in risk assessment. CF is most commonly observed in white people of Northern European descent but it is seen in all races. Neutrophilic inflammation and pulmonary infection lower breathing tract are associated with increased structural lung disorder

Corresponding author
Mehrdad Halaji
E-mail: Mehrdad.md69@gmail.com

and long-term disability of pulmonary features in children with cystic fibrosis. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are the two most common bacterial species associated with chronic lung infections in cystic fibrosis (CF) [5-9].
P. aeruginosa is a Gram-negative, opportunistic, and nosocomial pathogen, widespread throughout the environment. This pathogen can cause serious human infections and is the most prevalent respiratory pathogen in CF patients [10]. Nearly half of all CF patients and the 60% of adult CF patients have *P. aeruginosa*. Despite the decreased number of chronically-infected patients, it is clinically difficult to eradicate *P. aeruginosa* from the respiratory tract [11, 12]. The main reason of its pathogenic activity is the high level of intrinsic resistance to antibiotics. According to the literature, there is no comprehensive data regarding the prevalence of *P. aeruginosa* in CF patients in Iran. Therefore, the present study aimed to investigate the prevalence of *P. aeruginosa* in Iranian CF patients through a meta-analytic approach.

MATERIALS AND METHODS

Search strategies
The study was designed in accordance to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Supplementary).
A systematic literature search was conducted using a number of electronic databases including Web of Science, PubMed, Embase, Scopus, and Google Scholar to classify Iranian studies published up to the end of 2019. The Medical Subject Headings (MeSH), Non-MeSH terms and keywords such as "*Pseudomonas aeruginosa*" OR "*P. aeruginosa*" AND "cystic fibrosis" OR "CF" in combination with «Iran" were searched in the title, abstract and keywords fields.

Selection criteria
Two reviewers checked the search results in the databases with the related keywords independently and analyzed the titles, abstracts, and full texts to apply eligibility for inclusion according to inclusion criteria, and any discrepancies were resolved through consensus.
The searches were limited to articles published in English or Persian language with English abstract which indexed in the Web of Science, PubMed, Embase, and Scopus.
The studies with the following inclusion criteria were included:
a) use of standard methods for *P. aeruginosa* isolation;
b) availability of data on prevalence of *P. aeruginosa* among clinical sample in CF patients.

Studies that did not investigate *P. aeruginosa* among CF patients, review articles, case report, articles available only in abstract form, duplicate reports and studies which the results of *P. aeruginosa* was unclear in them were excluded.

Quality assessment
The quality assessment of the study was also judged independently by two authors using a checklist provided by the Joanna Briggs Institute (JBI) [13] and disagreements were resolved by consensus. Items associated with title and abstract, introduction, methods, results, discussion, and other data were investigated, and a score was assigned to each item.

Data extraction
The following items were extracted from all the selected studies: the name of first author, the time of performing and the location of the study, publication date, characterization of population studies, sample size, source of isolation and the frequency or prevalence of *P. aeruginosa*.

Statistical analysis
Meta-analysis was performed using "Comprehensive Meta-Analysis (CMA)" software version 2.2 (Biostat, Englewood, NJ). The pooled prevalence of *P. aeruginosa*, with 95% confidence intervals (95%CI) was estimate by the random-effects model. All eligible information was pooled and analyzed based on random-effects model due to the potential heterogeneity. Statistical heterogeneity groups were calculated using Cochrane Q-test ($p<0.05$ was considered statistically significant) and I-squared (I2) index. The possibility of publication bias was checked by Begg's rank correlation test, and Egger's weighted regression tests in combination with a funnel plot were used ($p<0.05$ was considered statistically significant). Possible sources of heterogeneity were measured by sensitivity analysis, meta-regression and subgroup analysis based on the location of the study [14].

RESULTS

Database search and characterization of studies

The database search yielded 150 citations. Among them, 133 were removed by index, title and abstract screening and 17 were retrieved in full text. Of these 17 reviewed studies, results of three studies were unclear, and were excluded upon a full text search. Finally, 14 studies accorded with eligibility criteria were subjected to meta-analysis [5, 15-28]. Out of 14 included studies, five studies reported the prevalence of *P. aeruginosa* on hospitalized patients. The searching procedure for selection of eligible studies is presented in Figure 1. The full results of the articles, sample size, prevalence of *P. aeruginosa*, source of samples and characterization of patient are shown in Table 1.

Meta-analysis

Fourteen articles evaluated the prevalence of *P. aeruginosa* in Iranian patients. From these studies, the estimated pooled prevalence of *P. aeruginosa* was 40.6% (95% CI: 32.4%-49.4%) (Figure 2). There was a significant heterogeneity among the 14 studies (χ^2=21.02; p<0.001; I^2=86.1%). The symmetric funnel plot showed no evidence of publication bias (Figure 3). Furthermore, Begg's and Egger's tests were achieved to quantitatively assess the publication biases. Based on the results of Begg's test (z=1.64, p=0.05) and Egger's test (t=1.48, p=0.16) a significant publication bias was not observed.

Subgroup analysis

The results of subgroup analysis based on region shown that pooled prevalence of *P. aeruginosa* was 66.7% (95% CI: 54%-77%), 32% (95% CI: 23%-43%), 20% (95% CI: 14%-30%) and 42% (95% CI: 32%-51%) among Bushehr (South of Iran), Isfahan (Center of Iran), Mashhad (Northeast of Iran) and Tehran (North of Iran), respectively (Figure 1).

Sensitivity analysis and meta-regression

Meta-regression results showed a non-significant decrease of the prevalence rates of *P. aeruginosa* among CF patients, coefficients: -0.08383 (95% CI: -0.19437- 0.02672, p=0.13) (Figure 4). Moreover, the sensitivity analyses were performed by excluding one study at a time to consider the impact

Table 1 - Characteristics of studies included in the meta-analysis.

Study	*Publication year*	*Years of study*	*Location*	*Sample size CF*	*P. aeruginosa isolation frequency*	*Source of sample*	*Characterization of isolates*	*References*
Eftekhar	2003	-	Tehran	64	21	Sputum	-	[16]
Khodadad	2006	-	Tehran	30	13	Sputum	-	[20]
Tajbakhsh	2008	-	Bushehr	63	42	Sputum	-	[24]
Eftekhar	2009	2004-2005	Tehran	46	31	Sputum	-	[17]
Khanbabaeea	2012	2004-2010	Tehran	129	50	Sputum	Hospitalized	[19]
Khalilzadeh	2012	2006-2010	Tehran	23	10	Sputum	Hospitalized	[5]
Fard M	2012	2009-2010	Isfahan	27	7	Throat		[27]
Fazeli	2013	2003-2008	Isfahan	59	21	Sputum	-	[26]
Douraghi	2014	2011-2012	Tehran	100	40	Sputum	-	[15]
Vali	2014	2011-2012	Tehran	52	21	Sputum	Hospitalized	[25]
Nobandegani	2016	2011-2012	Tehran	172	52	Sputum	Hospitalized	[22]
Nodoushan	2017	2013-2015	Tehran	59	42	Sputum	-	[18]
Sharifi	2018	2016-2017	Mashhad	100	21	-	Hospitalized	[23]
Aghamohammadi	2019	2014-2015	Tehran	174	40	Sputum	-	[28]

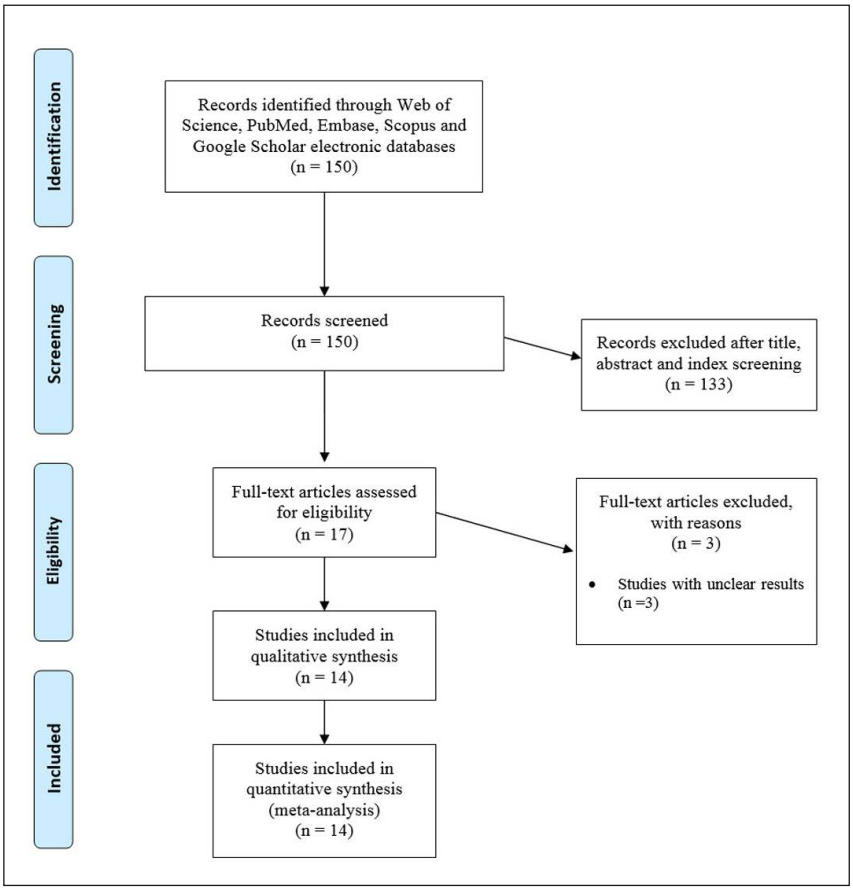

Figure 1 - Flow chart of study selection for inclusion in the systematic review.

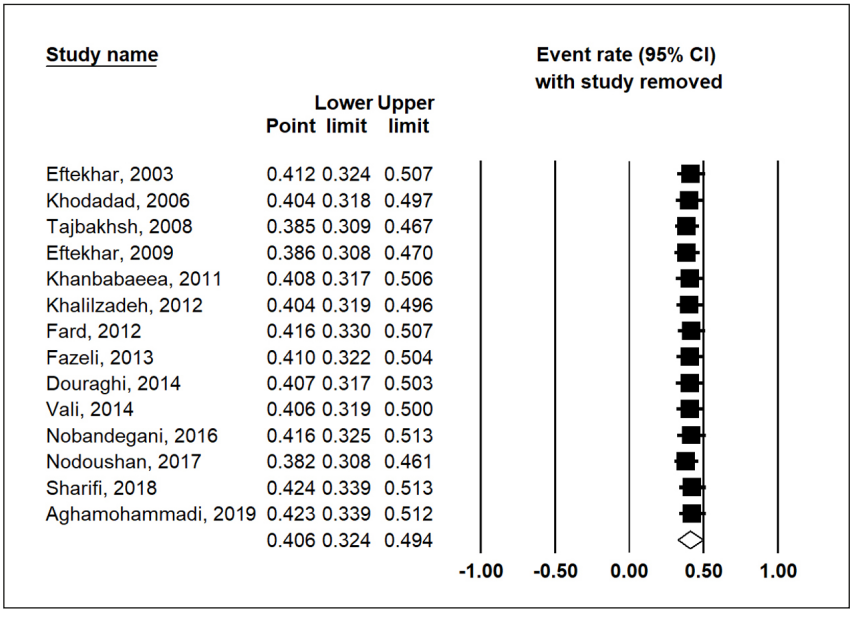

Figure 2 - Forest plot of the meta-analysis of *P. aeruginosa* prevalence in humans.

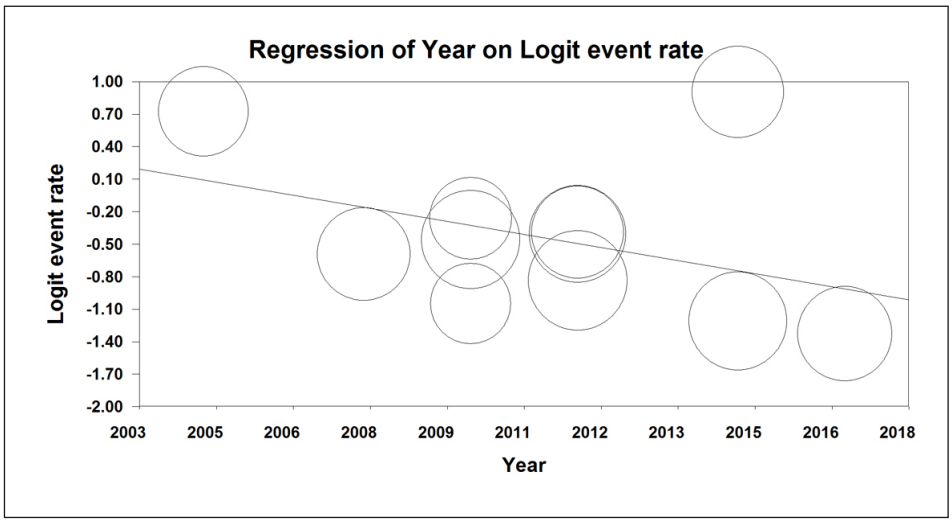

Figure 3 - Funnel plot of publication bias for the included studies.

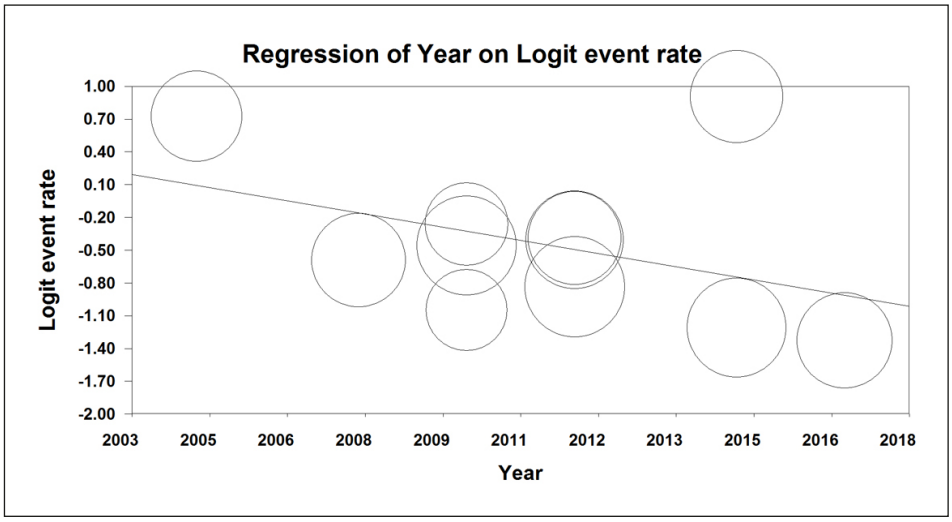

Figure 4 - Meta-regression of the log-event rates by the year.

of each study on the summary results and between-study heterogeneity (Figure 5).

DISCUSSION

Previously, several studies have reported the emergence and increased incidence of *P. aeruginosa* isolates and the related infections associated with increased morbidity and mortality in CF patients [29]. Although many studies have investigated the prevalence of *P. aeruginosa* isolated from CF patients, there is no comprehensive information regarding the prevalence of *P. aeruginosa* in Iran [30, 31]. Obtaining such data can contribute to prevent chronic airway infections caused by the *P. aeruginosa* isolates [32]. In the present study, the pooled prevalence of *P. aeruginosa* among Iranian CF patients was found to be 40.6% (95% CI: 32.4%-49.4%). The described prevalence in the current study is in agreement with that reported among CF patients in the Netherlands (57%), the United States (52.5%), and Australia (61.5%) [33-35]. The reported variations can be attributed to the differences in the studied population, sample size, detection method, and the stage of infection [36]. According to results of meta-regression, the rate of *P. aeruginosa* decreased, though not significantly, among the CF population each year. The decreasing trends of *P. aeruginosa* isolation over these

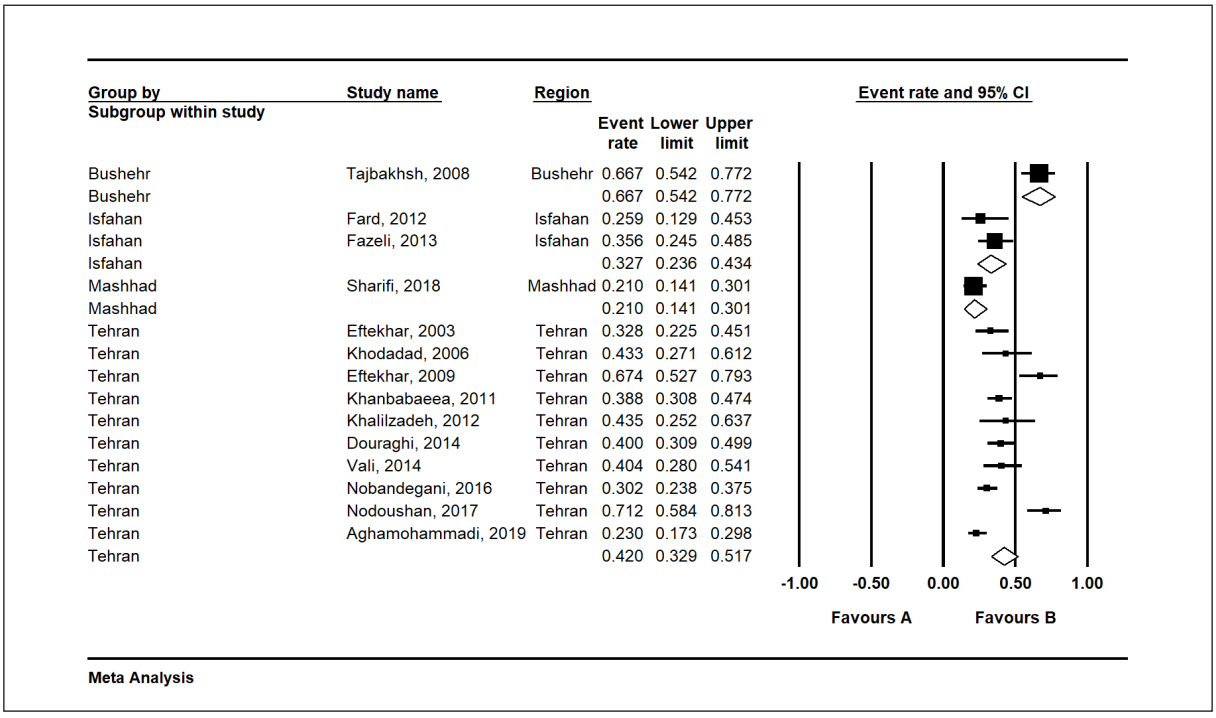

Figure 5 - Forest plot of pooled estimated prevalence of *P. aeruginosa* in subgroup analysis based on location of studies.

years might be due to the replacement of other opportunistic pathogens such as *S. aureus*, *Acinetobacter baumannii*, and *Stenotrophomonas maltophilia* [37]. In accordance with our results, Razvi et al. reported that the prevalence of *P. aeruginosa* infection among CF patients in United States decreased from 60.4% in 1995 to 56.1% in 2005 [38].

Razvi et al. further observed that the prevalence of *S. maltophilia* increased from 4.0% in 1996 to 12.4% in 2005 [38]. In this regard, numerous studies have reported increased rates of *S. maltophilia* infection in CF patients [39-42]. Furthermore, in a cohort study, Crull et al. revealed a reduction in the prevalence of both chronic and mucoid *P. aeruginosa* infection over an 11-year observation period [43].

Understanding the epidemiology and factors associated with *P. aeruginosa* infection may improve care in CF adults [43]. We were not able to assess the risk factors associated with the development of *P. aeruginosa* infection; however, this evaluation might help prevent *P. aeruginosa* infection and ultimately improve the quality of life [35, 44].

In a prospective cohort study conducted by Rosenfeld et al., CFTR genotype, female gender, age at diagnosis, and pancreatic enzyme levels were reported as risk factors for the initial *P. aeruginosa* infection in children [45]. Moreover, to control and prevent *P. aeruginosa* infections among CF patients, it is necessary to determine whether exists a persistence of the same strain or a reinfection due to a new strain. In this connection, previous studies reported that the majority of CF patients were colonized by a unique genotype of *P. aeruginosa* [46-48]. However, other reports have detected co-infections with multiple *P. aeruginosa* strains in these patients [49-51]. Moreover, the role of the environment as a source of *P. aeruginosa* infection in CF patients is challenging to prove and remains a matter of controversy [52].

The main limitation of our study was that most of the included studies were limited to few regions of Iran, so the results may not reflect the actual epidemiology in Iran.

CONCLUSIONS

In conclusion, the current study showed a remarkable prevalence of *P. aeruginosa* among CF

patients in Iran. Due to the rapid spread and major severity of *P. aeruginosa* and other opportunistic pathogens, it is necessary to identify risk factors, reservoirs, and transmission routes so as to successfully control the infections among Iranian CF patients.

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

Funding support
Self-funding.

REFERENCES

[1] 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 i*solates. *Infez Med.* 2018; 26 (3), 226-36.
[2] Dalcin PdTR, Rampon G, Pasin LR, et al. Adherence to treatment in patients with cystic fibrosis. *Jornal Brasileiro de Pneumologia.* 2007; 33 (6), 663-70.
[3] Grossman S, Grossman LC. Pathophysiology of cystic fibrosis implications for critical care nurses. *Crit Care Nurse.* 2005; 25 (4), 46-51.
[4] Fallahi G, Najafi M, Farhmand F, et al. The clinical and laboratory manifestations of Iranian patients with cystic fibrosis. *Turk J Ped.* 2010; 52 (2), 132-8.
[5] Khalilzadeh S, Boloursaz M, Baghaie N, et al. Microbial colonization and drug resistance in patients with cystic fibrosis. *J Compreh Ped* 2012; 3 (1), 25-8.
[6] Murray TS, Egan M, Kazmierczak BI. *Pseudomonas aeruginosa* chronic colonization in cystic fibrosis patients. *Curr Opin Ped.* 2007; 19 (1), 83-8.
[7] Mott LS, Park J, Murray CP, et al. Progression of early structural lung disease in young children with cystic fibrosis assessed using CT. *Thorax.* 2012; 67 (6), 509-16.
[8] Stick SM, Brennan S, Murray C, et al. Bronchiectasis in infants and preschool children diagnosed with cystic fibrosis after newborn screening. *J Pediatr.* 2009; 155 (5), 623-8.
[9] Ramsey KA, Foong RE, Grdosic J, et al. Multiple-breath washout outcomes are sensitive to inflammation and infection in children with cystic fibrosis. *Ann Am Thorac Soc.* 2017; 14 (9), 1436-42.
[10] Farajzadeh Sheikh A, Shahin M, Shokoohizadeh L, et al. Molecular epidemiology of colistin-resistant *Pseudomonas aeruginosa* producing NDM-1 from hospitalized patients in Iran. *Iran J Basic Med Sci.* 2019; 22 (1), 38-42.
[11] Rumbaugh KP, Hamood AN, Griswold JA. Analysis of *Pseudomonas aeruginosa* clinical isolates for possible variations within the virulence genes exotoxin A and exoenzyme S. *J Surg Res.* 1999; 82 (1), 95-105.
[12] Faghri J, Nouri S, Jalalifar S, Zalipoor M, Halaji M. Investigation of antimicrobial susceptibility, class I and II integrons among *Pseudomonas aeruginosa* isolates from hospitalized patients in Isfahan, Iran. *BMC Res Notes.* 2018; 11 (1), 806.
[13] Munn Z, Moola S, Lisy K, Riitano D, Tufanaru C. Methodological guidance for systematic reviews of observational epidemiological studies reporting prevalence and cumulative incidence data. *Int J Evid Based Healthc.* 2015; 13 (3), 147-53.
[14] Zeng X, Zhang Y, Kwong JS, et al. The methodological quality assessment tools for preclinical and clinical studies, systematic review and meta-analysis, and clinical practice guideline: a systematic review. *J Evid Based Med.* 2015; 8 (1), 2-10.
[15] Douraghi M, Ghasemi F, Dallal MS, Rahbar M, Rahimiforoushani A. Molecular identification of *Pseudomonas aeruginosa* recovered from cystic fibrosis patients. *J Prev Med Hyg.* 2014; 55 (2), 50.
[16] Eftekhar F, Hosseinkhan N, Asgharzadeh A, Tabatabaii A. Genetic profiling of *Pseudomonas aeruginosa* isolates from Iranian patients with cystic fibrosis using RAPD-PCR and PFGE. *Iran J Basic Med Sci.* 2009; 12 (3), 126-32.
[17] Eftekhar F, Rostamizadeh F, Khodadad A, Henry D, Speert DP. Isolation and genetic fingerprinting of *Pseudomonas aeruginosa* from Iranian patients with cystic fibrosis using RAPD-PCR. *Iranian Journal of Biotechnology.* 2003; 1 (2), 95-100.
[18] Jafari Nodoushan A, Golzar A, Hassanzad M, Sayedi SJ, Velayati A. Low bone mineral density and associated factors in patients with cystic fibrosis: A cross-sectional study. *Int J Ped*. 2017; 5 (7), 5237-44.
[19] Khanbabaee G, Akbarizadeh M, Sayyari A, et al. A survey on pulmonary pathogens and their antibiotic susceptibility among cystic fibrosis patients. *Braz J Infect Dis.* 2012; 16 (2), 122-8.
[20] Khodadad A, Najafi M, Ashtiani M, et al. Pulmonary pseudomonas colonization in cystic fibrosis. *Tanaffos.* 2006; 5 (2), 41-8.
[21] Modaresi M, Faghihinia J, Baharzadeh F. Cystic fibrosis prevalence among a group of high-risk Iranian children. *J Isfahan Med School.* 2012; 30 (180).
[22] Nobandegani NM, Mahmoudi S, Pourakbari B, et al. Antimicrobial susceptibility of microorganisms isolated from sputum culture of patients with cystic fibrosis: *Methicillin-resistant Staphylococcus aureus* as a serious concern. *Microb Pathog*. 2016; 100, 201-4.
[23] Sharifi MN, Kianifar HR, Bagheri S, Sayedi SJ. Microbiology of upper respiratory tract pathogens in cystic fibrosis patients. *Acta Medica Iranica.* 2018; 56 (7), 450-6.
[24] Tajbakhsh S, Hogardt M, Heesemann J, Grzonka C, Adler K. Detection of *Pseudomonas aeruginosa* in sputum samples by modified fluorescent in situ hybridization. *African J Biotechnol.* 2008; 7 (5).
[25] Vali P, Shahcheraghi F, Seyfipour M, et al. Pheno-

typic and genetic characterization of carbapenemase and ESBLs producing gram-negative bacteria (GNB) isolated from patients with cystic fibrosis (CF) in Tehran hospitals. *J Clin Diagn Res.* 2014; 8 (1), 26.
]26] Fazeli H, Akbari R, Moghim S, Esfahani BN. Phenotypic characterization and PCR-Ribotypic profile of *Pseudomonas aeruginosa* isolated from cystic fibrosis patients in Iran. *Adv Biomed Res.* 2013; 2 (18).
[27] Forozsh FM, Irajian G, Moslehi TZ, et al. Drug resistance pattern of *Pseudomonas aeruginosa* strains isolated from cystic fibrosis patients at Isfahan AL Zahra hospital, Iran (2009-2010). *Iran J Microbiol.* 2012; 4 (2), 94-7.
[28] Aghamohammadi A, Keivanfar M, Navaei S, et al. First cystic fibrosis patient registry annual data report-cystic fibrosis foundation of Iran. *Acta Medica Iranica.* 2019; 57 (1), 33-41.
[29] Knudsen PK, Olesen HV, Hoiby N, et al. Differences in prevalence and treatment of Pseudomonas aeruginosa in cystic fibrosis centres in Denmark, Norway and Sweden. *J Cyst Fibros.* 2009; 8 (2), 135-42.
[30] 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.
[31] Faraji F, Mahzounieh M, Ebrahimi A, et al. Molecular detection of virulence genes in *Pseudomonas aeruginosa* isolated from children with cystic fibrosis and burn wounds in Iran. *Microb Pathog.* 2016; 99, 1-4.
[32] Vongthilath R, Richaud Thiriez B, Dehillotte C, et al. Clinical and microbiological characteristics of cystic fibrosis adults never colonized by *Pseudomonas aeruginosa*: Analysis of the French CF registry. *PLoS One.* 2019; 14 (1): e0210201.
[33] van Mansfeld R, Willems R, Brimicombe R, et al. *Pseudomonas aeruginosa* genotype prevalence in Dutch cystic fibrosis patients and age dependency of colonization by various *P. aeruginosa* sequence types. *J Clin Microbiol.* 2009; 47 (12), 4096-101.
[34] Registry CFFP. Annual data report to the center directors. 2008
[35] Abdul Wahab A, Hammoudeh M, Allangawi M, Al-Khalaf F, Chandra P. Bone mineral density in cystic fibrosis patients with the CFTR I1234V mutation in a large kindred family is associated with pancreatic sufficiency. *Int J Rheumatol.* 2014; 2014: 465395.
[36] Govan JR, Brown AR, Jones AM. Evolving epidemiology of *Pseudomonas aeruginosa* and the *Burkholderia cepacia complex* in cystic fibrosis lung infection. *Future Microbiol.* 2007; 2 (2): 153-64.
[37] Esposito A, Pompilio A, Bettua C, et al. Evolution of *Stenotrophomonas maltophilia* in cystic fibrosis lung over chronic infection: a genomic and phenotypic population study. *Front Microbiol.* 2017; 8, 1590.
[38] Razvi S, Quittell L, Sewall A, et al. Respiratory microbiology of patients with cystic fibrosis in the United States, 1995 to 2005. *Chest.* 2009; 136 (6), 1554-60.
[39] Ballestero S, Virseda I, Escobar H, Suarez L, Baquero F. *Stenotrophomonas maltophilia* in cystic fibrosis patients. *Eu J Clin Microbiol Infect Dis.* 1995; 14 (8), 728-9.
[40] Denton M, Todd NJ, Kerr KG, Hawkey PM, Littlewood JM. Molecular epidemiology of *Stenotrophomonas maltophilia* isolated from clinical specimens from patients with cystic fibrosis and associated environmental samples. *J Clin Microbiol.* 1998; 36 (7), 1953-8.
[41] Marchac V, Equi A, Le Bihan-Benjamin C, Hodson M, Bush A. Case-control study of *Stenotrophomonas maltophilia* acquisition in cystic fibrosis patients. *Eur Resp J.* 2004; 23 (1), 98-102.
[42] Talmaciu I, Varlotta L, Mortensen J, Schidlow DV. Risk factors for emergence of *Stenotrophomonas maltophilia* in cystic fibrosis. *Pediatr. Pulmonol.* 2000; 30 (1), 10-5.
[43] Crull MR, Ramos KJ, Caldwell E, et al. Change in *Pseudomonas aeruginosa* prevalence in cystic fibrosis adults over time. *BMC Pulm Med.* 2016; 16 (1), 176.
[44] Lipuma JJ. The changing microbial epidemiology in cystic fibrosis. *Clin Microbiol Rev.* 2010; 23 (2), 299-323.
[45] Rosenfeld M, Emerson J, McNamara S, et al. Risk factors for age at initial Pseudomonas acquisition in the cystic fibrosis epic observational cohort. *J Cyst Fibros.* 2012; 11 (5), 446-53.
[46] Logan C, Habington A, Lennon G, et al. Genetic relatedness of *Pseudomonas aeruginosa* isolates among a paediatric cystic fibrosis patient cohort in Ireland. *J Med Microbiol.* 2012; 61 (Pt 1), 64-70.
[47] Speert DP, Campbell ME, Henry DA, et al. Epidemiology of *Pseudomonas aeruginosa* in cystic fibrosis in British Columbia, Canada. *Am J Respir Crit Care Med.* 2002; 166 (7), 988-93.
[48] Leone I, Chirillo MG, Raso T, Zucca M, Savoia D. Phenotypic and genotypic characterization of *Pseudomonas aeruginosa* from cystic fibrosis patients. *Eur J Clin Microbiol Infect Dis.* 2008; 27 (11), 1093-9.
[49] Mowat E, Paterson S, Fothergill JL, et al. *Pseudomonas aeruginosa* population diversity and turnover in cystic fibrosis chronic infections. *Am J Respir Crit Care Med.* 2011; 183 (12), 1674-9.
[50] Workentine ML, Sibley CD, Glezerson B, et al. Phenotypic heterogeneity of *Pseudomonas aeruginosa* populations in a cystic fibrosis patient. *PLoS One.* 2013; 8 (4), e60225.
[51] Finnan S, Morrissey JP, O'Gara F, Boyd EF. Genome diversity of *Pseudomonas aeruginosa* isolates from cystic fibrosis patients and the hospital environment. *J Clin Microbiol.* 2004; 42 (12), 5783-92.
[52] Van Daele SG, Franckx H, Verhelst R, et al. Epidemiology of *Pseudomonas aeruginosa* in a cystic fibrosis rehabilitation centre. *Eur Respir J.* 2005; 25 (3), 474-81.