Metallo-beta-Lactamase-producing *Pseudomonas aeruginosa* in Iran: a systematic review and meta-analysis

Hamid Vaez[1], Farzad Khademi[2], Amin Salehi-Abargouei[3,4] Amirhossein Sahebkar[5,6,7]

[1]Department of Microbiology, School of Medicine, Zabol University of Medical Sciences, Zabol, Iran;
[2]Department of Microbiology, School of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran;
[3]Nutrition and Food Security Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran;
[4]Department of Nutrition, Faculty of Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran;
[5]Neurogenic Inflammation Research Center, Mashhad University of Medical Sciences, Mashhad, Iran;
[6]Biotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran;
[7]School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran.

SUMMARY

Metallo-beta-lactamase (MBL)-producing *Pseudomonas aeruginosa* is considered to be a serious threat to human health worldwide. Limited information is available concerning the prevalence of MBL-producing *P. aeruginosa* in Iran. The aim of the present study was to investigate the prevalence of MBL-producing *P. aeruginosa* in different parts of Iran. We searched major electronic databases including PubMed, ISI Web of Science, Scopus and Google Scholar as well as two Iranian search engines using appropriate keywords. After applying inclusion and exclusion criteria, related papers were recruited for the study. The prevalence of MBL-producing *P. aeruginosa* in Iranian population was about 32.4 percent. Our findings also revealed that the highest prevalence of MBL-producing *P. aeruginosa* was in Isfahan with 60% (95% CI: 0.27-0.86). In addition, in Iranian population the most reported MBL gene was *bla*VIM and *bla*IMP, with frequencies of 19% (95% CI: 0.15-0.23) and 11% (95% CI: 0.08-0.14), respectively. Based on our findings, in the majority of Iranian hospitals, the prevalence of MBL-producing *P. aeruginosa* is alarmingly high necessitating the need for designing appropriate infection control programs.

Keywords: Metallo-beta-lactamase, *Pseudomonas aeruginosa*, Imipenem resistance.

INTRODUCTION

Pseudomonas aeruginosa is known as a non-fermenting Gram-negative bacillus that is motile, catalase- and oxidase-positive. *P. aeruginosa* is an opportunistic pathogen widely distributed in the hospital environments and accounts for severe diseases such as urinary tract infections (UTIs), bloodstream infections (BSIs), pneumonia and wound infections, especially in immunocompromised patients [1]. Besides, *P. aeruginosa* is clinically important since it possesses several virulence factors that can cause serious health problems [2]. Treatment of *P. aeruginosa* infections is considered as a major problem for physician due to a wide variety of antibiotic resistance mechanisms documented in this species, particularly production of extended-spectrum-beta-lactamases (ESBL) and metallo-beta-lactamases (MBL) enzymes. The ESBL and MBL production enables bacteria to hydrolyze extended-spectrum cephalosporins and carbapenems, conferring resistance against ceftazidime, ceftriaxone, cefotaxime and carbapenems [3]. Four classes of beta-lactamase enzymes including class A, B, C and D have been recognized. Based on the molecular structure and protein homology, these classes are divided into different subclasses. Molecular study of class A (VEB, PER, GES, SHV, PSE, BEL and CTX-M, C

Corresponding author
Farzad Khademi
E-mail: k_farzad@yahoo.com

(AmpC) and D (OXA-type-enzymes) revealed that these enzymes use a catalytically active serine residue for the inactivation of beta-lactam antibiotics, whereas class B beta-lactamases (IMP, VIM, GIM, SPM, NDM) are metallo-beta-lactamases and require zinc [4, 5]. Apart from simultaneous resistance against different antibiotics, the most important feature of multi-drug resistant strains is that these isolates have developed a wide array of strategies to evade the killing activity of the antibiotics, including the horizontal gene transfer among species [6].

Determination of the prevalence of MBL-producing isolates is crucial to guide infection control policies and appropriate use of antibiotics; therefore, the aim of the present study was to investigate the relative frequency (RF) of MBL-producing *P. aeruginosa* in different parts of Iran. To this end, we performed a systematic literature review to find published data on the prevalence of MBL-producing *P. aeruginosa* in different provinces of Iran.

MATERIALS AND METHODS

Search strategy

We searched major electronic databases including PubMed, ISI Web of Science, Scopus and Google Scholar, as well as two Iranian search engines including Iranian Scientific Information (www.sid.ir) and Magiran (www.magiran.com). In addition, citations of articles were manually searched to find relevant missing articles. Following keywords in combination were used; "*Pseudomonas aeruginosa*" AND "Iran" AND/OR "MBL-producing *P. aeruginosa*" AND "Iran" AND/OR "multi-drug resistance *P. aeruginosa*" AND "Iran". Our study was restricted to the original articles/brief reports published in Persian or English and reporting the prevalence of MBL-producing *P. aeruginosa* by phenotypic and molecular (PCR) methods.

Inclusion criteria

Inclusion criteria used in the present study were as follows:

- *P. aeruginosa* isolates were collected from patients referred to Iranian hospitals.
- *P. aeruginosa* strains were collected from clinical samples.
- Phenotypic methods (imipenem/meropenem alone, and in combination with EDTA) according to CLSI guidelines were used to detect prevalence of MBL-producing isolates of *P. aeruginosa,* because these are approved methods and are widely available.
- Cross-sectional descriptive studies (analyzed data from a population).

Exclusion criteria

Articles were excluded if:

- Samples were partially or totally taken from archive of antibiotic-resistant strains of *P. aeruginosa.*
- Samples were collected from different parts of Iran, and we were unable to attribute findings to distinct provinces.
- Studies in which repetitive samples were used.
- Studies with unclear materials and methods regarding the origin of samples, duplicate publications, and studies with incomplete results.

Data collection

According to defined criteria, data were collected and cross-checked based on titles, abstracts and full texts. Relative frequency of MBL-producing *P. aeruginosa*, name of the first author, time of study, and reported MBL resistance genes were extracted from studies. Disagreements were resolved by discussion between the authors.

Statistical analysis

Total number of participants and the number of samples with MBL-producing *P. aeruginosa* were used to calculate logit event rate and its confidence interval to be used for meta-analysis [7]. The DerSimonial and Laird random-effects model was used to derive the summary estimate. The I-squared and Cochran's Q test were used to assess heterogeneity between studies [7]. We used subgroup analysis to explore the prevalence rates according to province in which the study was conducted.

To explore the extent to which the overall calculations might depend on a specific study, sensitivity analysis was done. Publication bias was evaluated by inspecting Begg's funnel plots and asymmetry tests including Egger's regression asymmetry test and Begg's adjusted rank correlation test [7]. Statistical analyses were performed using the Comprehensive Meta-analysis software (Version 2.2).

Quality assessment

We used Joanna Briggs Institute (JBI) checklist to evaluate the quality of obtained papers [8]. According to this checklist several items such as population size, research objectives, sample collection method and statistical analysis approach are evaluated. One score was assigned to each parameter and study was included if at least seven scores achieved.

RESULTS

Totally, we found 2,552 records by applying the aforementioned search strategies (Figure 1). After title and abstract screening and evaluation, 1800 articles were excluded. Ultimately, based on applied exclusion criteria, 26 articles with full text describing the prevalence of MBL-producing *P. aeruginosa* were selected for this systematic review and meta-analysis (Table 1) [9-34].

Most of studies were performed in Tehran (7) and Isfahan (4), followed by Ahvaz (3), Shiraz (3), Kermanshah (2), Mashhad (2), Zanjan (2), Zahedan (1), Markazi (1) and Urmia (1). Based on our findings, the prevalence of MBL-producing *P. aeruginosa* in the Iranian population was about 32.4% (95% CI: 24.4-41.5) (Figure 2). The heterogeneity between studies was significant (Cochran's Q test, Q statistic = 517.036, P value<0.001, I^2=95.16%). Although a slight asymmetry was seen in the funnel plot (Figure 3), asymmetry tests did not show

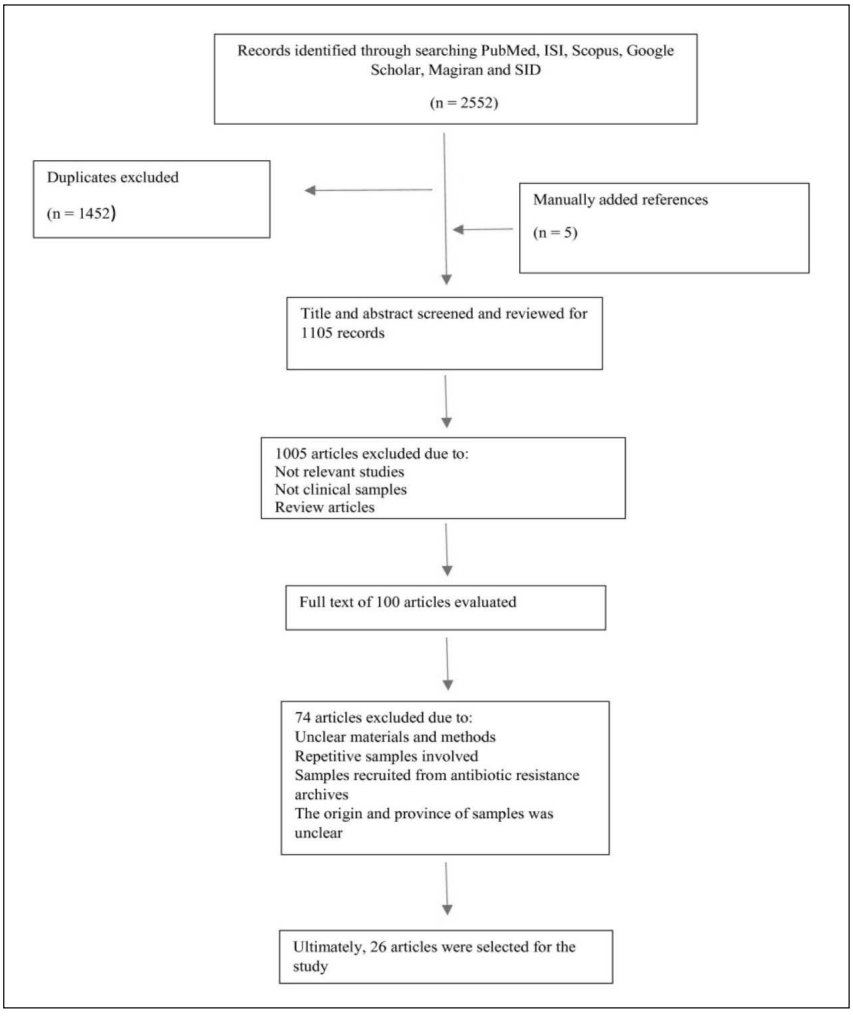

Figure 1 - Flow diagram showing literature review progress All'interno della figura, eliminarne il titolo.

any evidence of publication bias (Begg's test, P value=0.208, Egger's test, P value=0.338).

Our findings also revealed that the highest prevalence of MBL-producing *P. aeruginosa* was in Isfahan (60%, 95% CI: 0.27-0.86), while the lowest prevalence was reported from Zahedan province (4.7%, 95% CI: 0.02-0.08) (Table 1). In addition, in Iranian population the most frequently reported

Table 1 - Characteristics of studies involved in the systematic review and meta-analysis
Abbreviation: W, wound; B, blood culture; U, urinary tract infection; Sp, sputum; BW, burn wound; CDDT, combined double disk test.

First author	*Time of study*	*Province*	*Total samples*	*Type of samples*	*Laboratory method*	*MBL proportion (%)*	*Ref.*
Khosravi	2005-006	Ahvaz	100	W, B, U	Etest, PCR	8	23
Mosavian	2011-012	Ahvaz	236	W, B, U, Sp	CDDT, PCR	46.6	26
Sheikh	2011-2012	Ahvaz	223	W, B, U, Sp	CDDT, PCR	45.7	15
Total						30.6	
Rastegar	2013	Tehran	255	BW	CDDT, PCR	0	24
Fallah	2012	Tehran	100	BW	CDDT, PCR	48	14
Saderi	2008	Tehran	100	BW	CDDT, PCR	65	30
Hakemivala	2012	Tehran	47	BW	CDDT, PCR	27.7	18
Aghamiri	2011-012	Tehran	212	W, B, U, Sp	CDDT, PCR	33	10
Bejestani	2011-012	Tehran	90	W, B, U, Sp	CDDT, PCR	3.3	12
Sadredinamin	2014-015	Tehran	100	BW	CDDT, PCR	81	31
Total						31.2	
Doosti	2011-012	Zanjan	70	W, B, U, Sp	CDDT, PCR	51.4	13
Hemati	2012-013	Zanjan	120	W, B, U, Sp	CDDT, PCR	29.2	19
Total						39.6	
Sarhangi	2011-012	Shiraz	240	W, B, U, Sp	CDDT, PCR	7.9	33
Japoni	2009-010	Shiraz	270	BW	Etest	22.2	20
Rostampour	2012-013	Shiraz	42	BW	CDDT, PCR	61.9	28
Total						25.1	
Fazeli	2008-009	Isfahan	79	BW	CDDT, PCR	51.9	16
Khorvash	2012-013	Isfahan	48	W, B, U, Sp	CDDT, PCR	43.8	22
Saffari	2014-015	Isfahan	150	BW	CDDT, PCR	96	32
Sedighi	2012-013	Isfahan	106	W, B, U, Sp	CDDT, PCR	24.5	34
Total						60.7	
Nakhaei	2014	Mashhad	70	W, B, U, Sp	CDDT, PCR	35.7	27
Mirbagheri	2011-012	Mashhad	131	W, B, U, Sp	CDDT, PCR	49.6	25
Total						43.2	
Akya	2011-012	Kermanshah	60	W, B, U, Sp	CDDT, PCR	48.3	11
Abiri	2012	Kermanshah	225	W, B, U, Sp	CDDT, PCR	20	9
Total						32.3	
Ghamgosha	2012-013	Zahedan	191	U, Sp, W	Etest, PCR	4.7	17
Jazani	2010	Urmia	100	W, B, U, Sp	CDDT	7	21
Sadeghi	2011-012	Markazi	108	W, B, U, Sp	CDDT, PCR	18.5	29

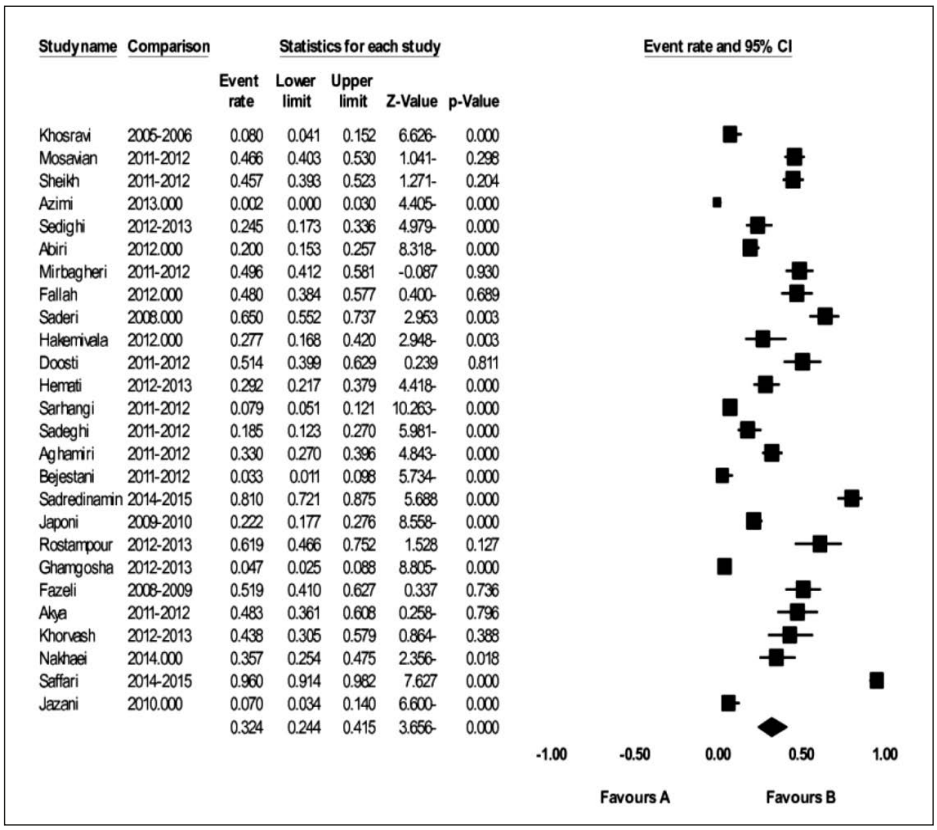

Figure 2 - Meta-analysis examining the overall prevalence of MBL-producing *P. aeruginosa* on studies conducted in Iran. The analysis revealed that the overall prevalence was about 32.4% (95%CI: 24.4-41.5). The analysis was conducted using random-effects model.

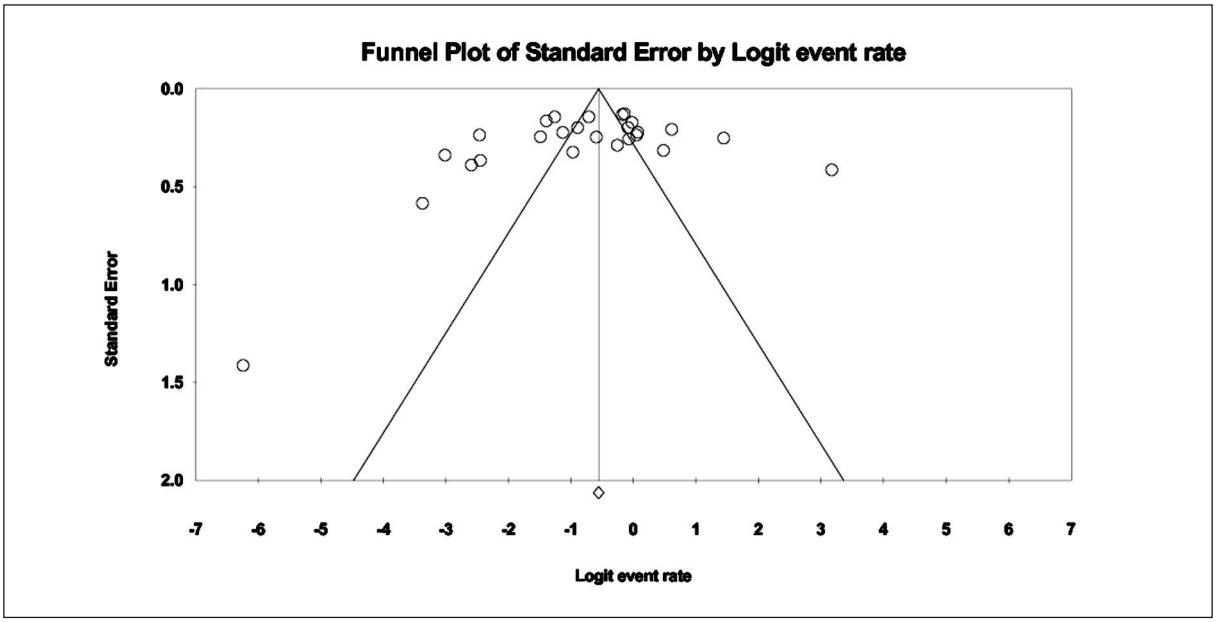

Figure 3 - Funnel plot with pseudo 95% confidence interval demonstrating the effect sizes derived from each study (logit event rate) against their corresponding standard errors (SEs).

MBL genes were *bla*VIM and *bla*IMP, with 19% (95% CI: 0.15-0.23) and 11% (95% CI: 0.08-0.14), respectively (Table 2). The highest prevalence rates of *bla*VIM and *bla*IMP were seen in Mashhad (50%, 95% CI: 0.41-0.58) and Isfahan (31.3%, 95%CI: 0.15-0.37), respectively.

DISCUSSION

The importance of *P. aeruginosa* in causing infections in the Iranian hospitals has been particularly highlighted in recent years and antibiotic resistance rates against this microorganism have been reported from different parts of the country. We conducted this study to provide a systematic evaluation of the prevalence of MBL-producing *P. aeruginosa* in Iran.

In this study, we collected data on the prevalence of MBL-producing *P. aeruginosa* in hospitals of different cities of Iran. Several independent studies have reported the substantial impact of MBL-producing *P. aeruginosa* on patients' outcomes [35-37]. For example, the study conducted by Lautenbach et al. revealed that carbapenem-resistant *P. aeruginosa* strains are associated with a high mortality rate and hospitalization [35]. A study conducted in three German hospitals also showed that the mortality rate is significantly higher in patients infected with MBL-pro-

Table 2 - Prevalence of MBL-genes based on different provinces.
Abbreviation; NA, not applicable.

First author	*Province (Ref.)*	*MBL-genes (proportion %)*				
		VIM	*IMP*	*SPM*	*KHM*	*SIM*
Khosravi	Ahvaz (23)	8	0.5	NA	NA	NA
Sheikh	Ahvaz (15)	0.4	11.7	0	NA	NA
Mosavian	Ahvaz (26)	0.8	28.4	0	NA	NA
Rastegar	Tehran (24)	2	1.6	0	NA	NA
Saderi	Tehran (30)	13	0.5	NA	NA	NA
Aghamiri	Tehran (10)	33	9.4	NA	NA	NA
Fallah	Tehran (14)	0.5	6	NA	NA	NA
Hakemivala	Tehran (18)	0.1	2.1	0	NA	NA
Bejestani	Tehran (12)	0.5	3.3	NA	NA	NA
Sadredin	Tehran (31)	0	13	NA	NA	NA
Hemati	Zanjan (19)	NA	23.3	16.7	NA	4.2
Doosti	Zanjan (13)	32	14.3	NA	NA	NA
Saffari	Isfahan (32)	21	NA	NA	NA	NA
Fazeli	Isfahan (16)	43	NA	NA	NA	NA
Sedighi	Isfahan (34)	0.5	NA	NA	NA	NA
Khorvash	Isfahan (22)	14.6	31.3	0	NA	NA
Sadeghi	Markazi (29)	38	2.8	0	NA	NA
Ghamgosha	Zahedan (17)	3.7	0.3	0	NA	NA
Nakhaei	Mashhad (27)	11.4	NA	NA	NA	NA
Mirbagheri	Mashhad (25)	50	NA	NA	NA	NA
Akya	Kermanshah (11)	8.3	NA	NA	NA	NA
Abiri	Kermanshah (9)	0.9	15.1	NA	NA	NA
Sarhangi	Shiraz (33)	4.2	3.3	0	NA	0
Rostampou	Shiraz (28)	NA	11.9	0	9.5	NA

ducing *P. aeruginosa* [37]. The present analysis revealed that the prevalence of MBL-producing *P. aeruginosa* in Iranian hospitals is alarmingly high, with an overall estimated prevalence of 32.4%. This prevalence is particularly higher than those reported from Swedish hospitals. For instance, the study by Erlandsson et al. in Swedish ICUs demonstrated that the prevalence of MBL-producing *P. aeruginosa* was less than 1% [38]. The results of study conducted by Liakopoulos et al. in Greece showed that 28% of investigated isolates were MBL-producing [39]. Another study performed in 23 Korean hospitals during 2005 revealed that 10.8% of investigated *P. aeruginosa* isolates were MBL-producing [40]. Nevertheless, our findings indicate a lower prevalence compared with some regional countries; for example, the results of investigation conducted by Kaleem et al. in Pakistan showed that the prevalence of MBL-producing *P. aeruginosa* was 78% [41]. Also, Hashem et al. demonstrated that the prevalence of MBL-producing *P. aeruginosa* was 64% in Egypt [42]. According to our findings extracted from 26 articles, the prevalence of MBL-producing *P. aeruginosa* was 32.4% and was more than 30% in many Iranian cities (Table 1). With a provincial perspective, our meta-analysis suggested that Isfahan had the highest prevalence (60%), followed by Mashhad (43%). The lowest prevalence of resistance was reported from Zahedan (4.7%) (Table 1).

Diversity in the prevalence of MBL-producing *P. aeruginosa* in different regions is attributable to irregular and varied use of antibiotics, low quality of personal hygiene, and inadequate environmental cleaning and infection control policies [43]. According to our findings, *bla*VIM is the most widely investigated gene in *P. aeruginosa* strains isolated from patients admitted to Iranian hospitals (reported in 22 studies) followed by IMP (reported in 18 studies) (Table 2). The highest reported prevalences for *bla*VIM, and *bla*IMP genes were 19% (95% CI: 0.15-0.23) and 11% (95% CI: 0.08-0.14), respectively. The highest prevalence of these genes was reported from Mashhad (50% for *bla*VIM) and Isfahan (31.3% for *bla*IMP).

Verona integron-encoded metallo-β-lactamases (VIM) are one of the most important beta- lactamases identified in *P. aeruginosa*. VIM-producing isolates of *P. aeruginosa* have been reported worldwide, including in European countries such as Italy, France, Greece, Belgium, Germany, Sweden, and some countries of Asia such as Japan, South Korea and Malaysia [44-51].

MBL genes are usually located in transferable genetic elements such as integrons and plasmids along with other antibiotic resistance genes. Therefore, dissemination of strains harboring MBL genes are of crucial importance, and appropriate measures should be taken into consideration by infection control programs [52].

KHM and SIM are novel MBLs which were first identified in *Citrobacter freundii* isolated from patients with urinary tract infection in Japan and from *Acinetobacter baumannii* isolated from patients with urinary tract infection in Korea, respectively [53, 54]. Noteworthy, these novel MBL enzymes have been reported from Shiraz and Zanjan provinces by Rostampour et al. and Hemati et al., indicating rapid dissemination of MBL genes [19, 28].

It has been documented that implementation of rational antibiotic use guidelines is integral to prevent dissemination of antibiotic resistance strains. Adequate dosing regimen according to pharmacokinetics-pharmacodynamics parameters and renal function, avoidance of arbitrary antibiotic administration, use of definitive regimens instead of empirical therapy when results of antibiotic susceptibility tests are available and adequate documentation of administration are key elements to achieve a proper pattern of antibiotic use [55, 56].

There are some limitations that should be taken into account while interpreting the results of the present meta-analysis. The results obtained by phenotypic and genotypic methods may not be the same due to different specificity and sensivity values of applied methods. Moreover, data was not available from all regions of Iran; thus, our findings cannot fully represent the prevalence of MBL-producing *P. aeruginosa* in Iran.

CONCLUSION

Based on the present findings, the prevalence of MBL-producing *P. aeruginosa* is alarmingly high in the majority of Iranian hospitals. This should be considered as a serious threat to healthcare settings. Constant monitoring of MBL-producing

P. aeruginosa could guide antibiotic therapy and improve the patients' outcomes, including hospitalization, mortality and morbidity.

Acknowledgments
The authors are grateful to the director and principal of Zabol University of medical sciences for their support.

Funding
No funding

Ethical approval
Not required

Conflict of interest
None to declare

REFERENCES

[1] Vaez H., Faghri J., Nasr Esfahani B., Moghim S., Fazeli H., Sedighi M. Antibiotic resistance patterns and genetic diversity in clinical isolates of *Pseudomonas aeruginosa* isolated from patients of a referral hospital, Isfahan, Iran. *Jundishapur. J. Microbiol*. 8, 1-6, 2015.
[2] Pereira S.G., Rosa A.C., Cardoso O. Virulence factors as predictive tools for drug resistance in *Pseudomonas aeruginosa*. *Virulence* 6, 679-683, 2015.
[3] Strateva T., Yordanov D. *Pseudomonas aeruginosa* - a phenomenon of bacterial resistance. *J. Med. Microbiol*. 58, 1133-1148, 2009.
[4] Poole K. *Pseudomonas aeruginosa*: resistance to the max. *Front. Microbiol*. 2, 1-13, 2011.
[5] Walther-Rasmussen J., Hoiby N. Class A carbapenemases. *J. Antimicrob. Chemother*. 60, 470-482, 2007.
[6] Esposito S., De Simone G. Update on the main MDR pathogens: prevalence and treatment options. *Infez. Med*. 25, 301-310, 2017.
[7] Borenstein M., Hedges L.V., Higgins J.P.T., Rothstein H.R. Introduction to Meta-Analysis. 1-421, 2009.
[8] Sterne J.A., Egger M., Smith G.D.Systematic reviews in health care: Investigating and dealing with publication and other biases in meta-analysis. *Brit. Med J*. 14, 323,101-105, 2001
[9] Abiri R., Mohammadi P., Shavani N., Rezaei M. Detection and genetic characterization of metallo-beta-Lactamase IMP-1 and VIM-2 in *Pseudomonas aeruginosa* strains from different hospitals in Kermanshah, Iran. *Jundishapur. J. Microbiol*. 8, 1-5, 2015.
[10] Aghamiri S., Amirmozafari N., Fallah Mehrabadi J., Fouladtan B., Samadi Kafil H. Antibiotic resistance pattern and evaluation of metallo-beta lactamase genes including bla-imp and bla-vim types in *Pseudomonas aeruginosa* isolated from patients in Tehran hospitals. *ISRN Microbiol*. 1-6, 2014.
[11] Akya A., Salimi A., Nomanpour B., Ahmadi K. Prevalence and clonal dissemination of metallo-beta-lactamase-producing *Pseudomonas aeruginosa* in Kermanshah. *Jundishapur. J. Microbiol*. 8, 1-5, 2015.
[12] Bejestani F.M, Hakemivala M., Momtaheni R., Bejestani O.B., Gholami M. The frequency of imp and vim genes among *Pseudomonas aeruginosa* isolates from children's medical center of Tehran. *Arch. Clin. Infect. Dis*. 10, 1-4, 2015.
[13] Doosti M., Ramazani A., Garshasbi M. Identification and characterization of metallo-beta-lactamases producing *Pseudomonas aeruginosa* clinical isolates in University Hospital from Zanjan Province, Iran. *Iran. Biomed. J*. 17, 129-33, 2013.
[14] Fallah F., Borhan R.S, Hashemi A. Detection of bla(IMP) and bla(VIM) metallo-beta-lactamases genes among *Pseudomonas aeruginosa* strains. *Int. J. Burns Trauma* 3, 122-124, 2013.
[15] Farajzadeh Sheikh A., Rostami S., Jolodar A., Tabatabaiefar M.A., Khorvash F., Saki A. Detection of metallo-beta lactamases among carbapenem-resistant *Pseudomonas aeruginosa*. *Jundishapur. J. Microbiol*. 7, 1-6, 2014.
[16] Fazeli H., Moslehi Z., Irajian G., Salehi M. Determination of drug resistance patterns and detection of bla-VIM gene in *Pseudomonas aeruginosa* strains Isolated from burned patients in the Emam Mosa Kazem hospital, Esfahan, Iran (2008-9). *Iran. J. Med. Microbiol*. 3, 1-8, 2010.
[17] Ghamgosha M., Shahrekizahedani S., Kafilzadeh F., Bameri Z., Taheri R.A., Farnoosh G. Metallo-beta-Lactamase VIM-1, SPM-1, and IMP-1 Genes among clinical *Pseudomonas aeruginosa* species isolated in Zahedan, Iran. *Jundishapur. J. Microbiol*. 8, 1-5, 2015.
[18]. Hakemi Vala M., Hallajzadeh M., Hashemi A., Goudarzi H., Tarhani M., Sattarzadeh Tabrizi M. Detection of Ambler class A, B and D β-lactamases among *Pseudomonas aeruginosa* and *Acinetobacter baumannii* clinical isolates from burn patients. *Ann. Burns Fire Disasters* 27, 8-13, 2014.
[19] Hemati F., Sourouri-Zanjani R., Haghi F., Zeighami H. Determination of antibiotic resistance profile and frequency of metallo-beta-lactamase in *Pseudomonas aeruginosa* isolates. Zanjan University Medical Sciences Journal 22, 77-85, 2014.
[20] Japoni A., Anvarinejad M., Farshad S., Giammanco G.M, Rafaatpour N., Alipour E. Antibiotic susceptibility patterns and molecular epidemiology of metallo-beta-lactamase producing *Pseudomonas aeruginosa* strains isolated from burn patients. *Iran. Red. Crescent. Med. J*. 16, 1-6, 2014.
[21] Jazani N.H., Zahedi A., Garebagi N. Phenotypic detection of metallo-β-lactamase producing *Pseudomonas aeruginosa* isolated from Urmia hospitals. *African J. Microbiol. Res*. 6, 1387-1392, 2012.

[22] Khorvash F., Yazdani M.R., Shabani S., Shabani S., Alizadeh H., Soudi A.A. Detection of different types of Metallo-β-Lactamases among *Pseudomonas aeruginosa* isolates obtained from Intensive Care Unit. *J. Med. Microbiol. Infec. Dis*. 2, 84-90, 2014.
[23] Khosravi A.D., Mihani F. Detection of metallo-beta-lactamase-producing *Pseudomonas aeruginosa* strains isolated from burn patients in Ahwaz, Iran. *Diagn. Microbiol. Infect. Dis*. 60, 125-128, 2008.
[24] Lari A.R., Azimi L., Soroush S., Taherikalani M. Low prevalence of metallo-beta-lactamase in *Pseudomonas aeruginosa* isolated from a tertiary burn care center in Tehran. *Int. J. Immunopathol. Pharmacol*. 28, 384-389, 2015.
[25] Mirbagheri S.Z., Meshkat Z., Naderinasab M., Rostami S., Nabavinia M.S., Rahmati M. Study on imipenem resistance and prevalence of blaVIM1 and blaVIM2 metallo-beta lactamases among clinical isolates of *Pseudomonas aeruginosa* from Mashhad, Northeast of Iran. *Iran. J. Microbiol*. 7, 72-78, 2015.
[26] Moosavian M., Rahimzadeh M. Molecular detection of metallo-beta-lactamase genes, bla IMP-1, bla VIM-2 and bla SPM-1 in imipenem resistant *Pseudomonas aeruginosa* isolated from clinical specimens in teaching hospitals of Ahvaz, Iran. *Iran. J. Microbiol*. 7, 1-6, 2015.
[27] Nakhaei Moghaddam M., Hasanabady M.H. Detection of Metallo-beta-lactamase bla-VIM1 gene in clinical isolates of *Pseudomonas aeruginosa* in Mashhad. *J. Shahid. Sadoughi. Univ. Med. Sc.* 24, 74-82, 2016.
[28] RostamPour S., Gorzin A.A., Motamedi G. Frequency of blaKHM-1, blaIMP-1,2 and bla-SPM-1 genes in clinical isolates of metallo β-lactamase producing *Pseudomonas aeruginosa* in hospitalized burned patients in Ghotbeddin Shirazi Hospital. *Journal Qazvin University of Medical Sciences* 19, 21-29, 2015.
[29] Sadeghi A., Raahimi B., Shojapour M. Molecular detection of metallo-β-lactamase genes blaVIM1, blaVIM-2, bla, IMP-1bla, IMP-2 and blaSPM *Pseudomonas aeruginosa* isolated from hospitalized patients in Markazi province by Duplex-PCR. *African. J. Microbiol. Res*. 6, 2965-2969, 2012.
[30] Saderi H., Lotfalipour H., Owlia P., Salimi H. Detection of metallo-β-Lactamase producing *Pseudomonas aeruginosa* isolated from burn patients in Tehran, Iran. *Lab. Medicine* 41, 609-612, 2010.
[31] Sadredinamin M., Hashemi A., Goudarzi H., Tarashi S., Nojookambari N.Y., Taki A. Detection of blaIMP, blaVIM and OprD genes among *Pseudomonas aeruginosa* isolated from burn patients. *J. Mazandaran Univ .Med Sci*. 26, 181-186, 2016.
[32] Saffari M., Firoozeh F., Pourbabaee M., Zibaei M. Evaluation of metallo-beta-lactamase-production and carriage of bla-VIM Genes in *Pseudomonas aeruginosa* isolated from burn wound infections in Isfahan. *Arch. Trauma Res*. 5, 1-5, 2016.
[33] Sarhangi M., Motamedifar M., Sarvari J. Dissemination of *Pseudomonas aeruginosa* producing blaIMP1, blaVIM2, blaSIM1, blaSPM1 in Shiraz, Iran. *Jundishapur. J. Microbiol*. 6, 1-5, 2013.
[34] Sedighi M., Vaez H., Moghoofeie M., Hadifar S., Oryan G., Faghri J. Molecular detection of metallo-beta-lactamase gene blaVIM-1 in imipenem-resistant *Pseudomonas aeruginosa* strains isolated from hospitalized patients in the hospitals of Isfahan. *Adv. Biomed. Res*. 4, 1-6, 2015.
[35] Lautenbach E., Synnestvedt M., Weiner M.G., Bilker W.B., Schein J. Imipenem resistance in *Pseudomonas aeruginosa*: emergence, epidemiology, and impact on clinical and economic outcomes. *Infect. Control. Hosp. Epidemiol*. 31, 47-53, 2010.
[36] Liu Q., Li X, Li W., Du X., He J.Q., Tao C. Influence of carbapenem resistance on mortality of patients with *Pseudomonas aeruginosa* infection: a meta-analysis. *Sci. Rep*. 5, 1-10, 2015.
[37] Willmann M., Kuebart I., Marschal M., Schroppel K., Vogel W., Flesch I. Effect of metallo-beta-lactamase production and multidrug resistance on clinical outcomes in patients with *Pseudomonas aeruginosa* bloodstream infection: a retrospective cohort study. *BMC Infect. Dis*. 13, 1-9, 2013.
[38] Erlandsson M., Gill H., Nordlinder D., Giske C.G., Jonas D., Nilsson L.E. Antibiotic susceptibility patterns and clones of *Pseudomonas aeruginosa* in Swedish ICUs. *Scand. J. Infect. Dis.* 40, 487-494, 2008.
[39] Liakopoulos A., Mavroidi A., Katsifas E.A., Theodosiou A., Karagouni A.D., Miriagou V. Carbapenemase-producing *Pseudomonas aeruginosa* from central Greece: molecular epidemiology and genetic analysis of class I integrons. *BMC Infect. Dis*. 13, 1-7, 2013.
[40] Lee K., Park A.J., Kim M.Y., Lee H.J., Cho J.H., Kang J.O. Metallo-beta-lactamase-producing *Pseudomonas* spp. in Korea: high prevalence of isolates with VIM-2 type and emergence of isolates with IMP-1 type. *Yonsei. Med. J*. 50, 335-339, 2009.
[41] Kaleem F., Usman J., Hassan A., Khan A. Frequency and susceptibility pattern of metallo-beta-lactamase producers in a hospital in Pakistan. *J. Infect. Dev. Ctries*. 4, 810-813, 2010.
[42] Hashem H., Hanora A., Abdalla S., Shaeky A., Saad A. Dissemination of metallo-beta-lactamase in *Pseudomonas aeruginosa* isolates in Egypt: mutation in blaVIM-4. *APMIS* 125, 499-505, 2017.
[43] Harris A.D., McGregor J.C., Furuno J.P. What infection control interventions should be undertaken to control multidrug-resistant gram-negative bacteria? *Clin. Infect. Dis*. 43, 57-61, 2006.
[44] Pollini S., Antonelli A., Venturelli C., Maradei S., Veggetti A., Bracco S. Acquisition of plasmid-borne blaIMP-19 gene by a VIM-1-positive *Pseudomonas aeruginosa* of the sequence type 235 epidemic lineage. *J. Antimicrob. Chemother*. 68, 722-724, 2013.

[45] Corvec S., Poirel L., Decousser J.W., Allouch P.Y., Drugeon H., Nordmann P. Emergence of carbapenem-hydrolysing metallo-beta-lactamase VIM-1 in *Pseudomonas aeruginosa* isolates in France. *Clin. Microbiol. Infec*t. 12, 941-942, 2006.
[46] Tsakris A., Pournaras S., Woodford N., Palepou M.F., Babini G.S., Douboyas J. Outbreak of infections caused by *Pseudomonas aeruginosa* producing VIM-1 carbapenemase in Greece. *J. Clin. Microbiol.* 38, 1290-1292,2000.
[47] Castanheira M., Deshpande L.M., Costello A., Davies T.A., Jones R.N. Epidemiology and carbapenem resistance mechanisms of carbapenem-non-susceptible *Pseudomonas aeruginosa* collected during 2009-11 in 14 European and Mediterranean countries. *J. Antimicrob. Chemother*. 69, 1804-1814, 2014.
[48] Walsh T.R, Toleman M.A, Poirel L., Nordmann P. Metallo-beta-lactamases: the quiet before the storm? *Clin. Microbiol. Rev*. 18, 306-325, 2005.
[49] Yatsuyanagi J., Saito S., Harata S., Suzuki N., Ito Y., Amano K. Class 1 integron containing metallo-beta-lactamase gene blaVIM-2 in *Pseudomonas aeruginosa* clinical strains isolated in Japan. *Antimicrob. Agents. Chemother.* 48, 626-628, 2004.
[50] Lee K., Lim J.B., Yum J.H., Yong D., Chong Y., Kim J.M. bla(VIM-2) cassette-containing novel integrons in metallo-beta-lactamase-producing *Pseudomonas aeruginosa* and *Pseudomonas putida* isolates disseminated in a Korean hospital. *Antimicrob. Agents. Chemother*. 46, 1053-1058 , 2002.
[51] Khosravi Y., Tee Tay S., Vadivelu J. Metallo-beta-lactamase-producing imipenem-resistant *Pseudomonas aeruginosa* clinical isolates in a university teaching hospital in Malaysia: detection of IMP-7 and first identification of IMP-4, VIM-2, and VIM-11. *Diagn. Microbiol. Infect. Dis*. 67, 294-296, 2010.
[52] Vaez H., Moghim S., Nasr Esfahani B., Ghasemian Safaei H. Clonal relatedness among imipenem-resistant *Pseudomonas aeruginosa* isolated from ICU-Hospitalized Patients. *Crit. Care. Res. Pract.* 1-5, 2015.
[53] Sekiguchi J., Morita K., Kitao T., Watanabe N., Okazaki M., Miyoshi-Akiyama T. KHM-1, a novel plasmid-mediated metallo-beta-lactamase from a *Citrobacter freundii* clinical isolate. *Antimicrob. Agents. Chemother*. 52, 4194-4197, 2008.
[54] Lee K,. Yum J.H., Yong D., Lee H.M., Kim H.D., Docquier JD. Novel acquired metallo-beta-lactamase gene, bla(SIM-1), in a class 1 integron from *Acinetobacter baumannii* clinical isolates from Korea. *Antimicrob. Agents .Chemother*. 49, 4485-4491, 2005.
[55] Metan G., Kaynar L., Yozgat N. et al. A change for the antibacterial treatment policy to decrease carbapenem consumption at a haematopoietic stem cell transplantation centre. *Infez. Med*. 25, 33-37, 2017.
[56] Viceconte G., Maraolo A. E., Iula V. D., Catania M. R.a, Tosone G., Orlando R. Appropriateness of antibiotic prescription for targeted therapy of infections caused by multidrug-resistant bacteria: assessment of the most common improper uses in a tertiary hospital in southern Italy. *Infez. Med.* 25, 224-233, 2017.