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Review Article

Prevalence of metallo-β-lactamase-encoding genes among carbapenem-resistant *Pseudomonas aeruginosa* strains isolated from burn patients in Iran

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Abstract

Carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) has been considered a major cause of infection and mortality in burn patients, especially in developing countries such as Iran. One of the most common mechanisms of carbapenem resistance is production of metallo-β-lactamases [(MBLs), including Verona Integron-encoded Metallo-beta-lactamase (VIM), imipenemase (IMP), São Paulo metalo-beta-lactamase (SPM), German imipenemase (GIM), New Delhi metallo-beta-lactamase (NDM), Dutch imipenemase (DIM), AIM, Seoul imipenemase (SIM), KHM, *Serratia* metallo-β-lactamase (SMB), Tripoli metallo-β-lactamase (TMB), and Florence imipenemase (FIM)]. Limited information is available on the prevalence of CRPA and MBLs in Iranian burn units. We performed a systematic search by using different electronic databases, including Medline (via PubMed), Embase, Web of Science, and Iranian Database. Of 586 articles published from January 2000 to December 2016, 14 studies reporting the incidence of CRPA and MBLs as detected by molecular methods in burn patients were included in this review. The meta-analyses showed that the prevalence of CRPA, IMP, and VIM was 76.8% (95% CI 67.5-84.1), 13.1% (95% CI 4.7-31.5), and 21.4% (95% CI 14.6-30.1), respectively, in Iranian burn centers and remaining MBLs types have not yet been detected. There was a high prevalence of MBLs and CRPA in Iranian burn centers. Therefore, these measurements should be applied nationally and rigorous infection control measures and antimicrobial stewardship will be the major pillars to control multidrug resistant microorganisms, such as CRPA.

Keywords: MBLs. CRPA. Burn. Iran.

INTRODUCTION

Pseudomonas aeruginosa is a major cause of life-threatening infections in burn patients worldwide[1,2]. It has a remarkable capacity to develop resistance to multiple classes of antimicrobial agents, and consequently, is considered a multidrug resistant (MDR) pathogen[3]. Carbapenems are considered first-line agents to treat severe cases of *P. aeruginosa* infections[4]. In spite of this, carbapenem-resistant *P. aeruginosa* (CRPA) has been increasing in recent years, which is associated with high mortality, morbidity, long hospital stays, and increased costs[1,5,6]. One of the most common mechanisms of carbapenem resistance is production of metallo-β-lactamases (MBLs)[6]. MBL enzymes are also able to hydrolyze penicillin and cephalosporins[7]. Twelve different types of MBLs (VIM, IMP, SPM, GIM, NDM, DIM, AIM, SIM, KHM, SMB, TMB, and FIM) have been identified so far[3]. These genes are usually encoded by mobile genetic elements, such as plasmids, transposons, and integrons, that allow them to disseminate horizontally among Gram-negative bacteria, posing a global challenge for all countries[3,6]. MBL-producing *P. aeruginosa* was originally *discovered* in 1991 in Japan. Since then, it has been described in different parts of the world[3,5,6,8]. The first MBL-positive strains of *P. aeruginosa* were described in an Iranian burn center in 2008 and are currently recognized as one of the crucial hospital-acquired infectious agents[5]. In Iranian burn centers, the prevalence of CRPA increased from 41% in 2008 to 57.4% in 2016[1,5]. To date, there are several reports from two different Iranian burn centers on the prevalence of MBLs among CRPA[5,9-12]. However, most of these studies only reported local information, and no systematic study has yet been performed. The aim of this surveillance review was to assess the prevalence of MBLs among CRPA in Iranian burn centers using a systematic review and meta-analysis according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement.

METHODS

Search strategies

We carried out a literature search of Medline (via PubMed), Embase, Web of Science, and Iranian Database from January 2000 to December 2016 using the following terms: *Pseudomonas aeruginosa* or *P. aeruginosa* and *Metallo-β-lactamases* or *MBLs* and *burn patients* in combination with Iran. Cross-sectional or cohort studies that reported the prevalence of MBLs in burn patients were considered. The titles and abstracts for possible inclusion in

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the reviews were screened by two independent reviewers. Reviews were restricted to studies published in English and Persian languages and revealed the prevalence or incidence of CRPA and MBLs genes in burn patients. Eligible articles were selected based on three stages: title, abstracts, and full-text publication. Studies with the following characteristics were included: studies conducted only on burn patients and reporting the frequency of carbapenem resistance and MBLs genes, detection of MBLs genes [Verona Integron-encoded Metallo-beta-lactamase (VIM), imipenemase (IMP), São Paulo metalo-beta-lactamase (SPM), German imipenemase (GIM), New Delhi metallo-beta-lactamase (NDM), Dutch imipenemase (DIM), AIM, Seoul imipenemase (SIM), KHM, *Serratia* metallo-β-lactamase (SMB), Tripoli metallo-β-lactamase (TMB), and Florence imipenemase (FIM)], by molecular methods [polymerase chain reaction (PCR)]. Studies that had one or more of the following characteristics were excluded: studies using phenotypic methods, duplicate and overlapping studies, studies published in languages other than English or Persian, studies with other than burn patients, studies that did not report CRPA and MBLs prevalence, nonhuman studies, review articles, congress abstracts, meta-analyses, or systematic reviews as well as articles available only in abstract form.

Data extraction and definitions

The following data were extracted from each study: name of first author, year of publication, year of study, study setting, number of cases investigated, study methods, sample size, and prevalence of CRPA and MBLs genes. Furthermore, to minimize the potential bias caused by an inadequate sample size, articles with less than 50 subjects were omitted. Two reviewers independently extracted all data from included studies, and a third reviewer reviewed results. Disagreements between the reviewers were resolved by consensus.

Quality assessment of studies

Two reviewers using a checklist provided by the Joanna Briggs Institute assessed the study quality independently[13].

Statistical analysis

Meta-analysis was performed by Comprehensive Meta-Analysis (Biostat V2.2) software. We reported the amount of residual heterogeneity using the I^2 statistic and Cochran's Q statistic to test the heterogeneity between inquiries. In order to assess any possible publication bias, Begg's rank correlation and Egger's weighted regression methods in combination with a funnel plot were used ($p < 0.05$ was considered indicative of statistically significant publication bias).

RESULTS

Our literature search yielded 586 studies, and of these, 14 based on the mentioned criteria were included in the meta-analysis (**Table 1**). **Figure 1** shows the study selection process and reasons for exclusion. Based on the 14 selected articles, the pooled prevalence of CRPA was 76.8% (95% CI 67.5-84.1). The pooled prevalence of IMP and VIM among CRPA was 13.1% (95% CI 4.7-31.5) and 21.4% (95% CI 14.6-30.1), respectively, as shown in **Table 2**. Heterogeneities between studies ($I^2 = 95$,

TABLE 1: Characteristics of studies included in the meta-analysis.

Study/*	Time of stud	Published time	Province	Sample size	CRPA	Detection method (PCR)	
						IMP (n)	VIM (n)
Khosravi[5]	2005–2006	2008	Ahwaz	100	41	0	8
Bahar[9]	2007–2008	2009	Zanjan	186	115	0	32
Saderi[12]	2008	2010	Tehran	100	69	0	13
Fazeli[14]	2008–2009	2010	Isfahan	79	74	NT	34
Sepehriseresht[15]	2008–2009	2012	Tehran	483	272	33	51
Mirsalehian[10]	2010	2010	Tehran	170	90	0	10
Neyestanaki[11]	2011–2012	2014	Tehran	214	100	3	55
Fallah[16]	2012	2013	Tehran	83	83	6	0
Akhavan[17]	2013	2014	Yazd	54	35	9	5
Lari[18]	2013	2015	Tehran	255	161	5	7
Radan[19]	2013–2014	2015	Isfahan	150	144	107	NT
Safari[20]	2014–2015	2016	Isfahan	150	144	NT	35
Sadredinamin[21]	2015–2016	2016	Tehran	100	95	13	0
Tarashi[22]	2016	2016	Tehran	309	278	30	52

CRPA: carbapenem-resistant *Pseudomona aeruginosa;* **PCR:** polymerase chain reaction; **IMP**: imipenemase; **VIM:** Verona Integron-encoded metallo-beta-lactamase; **NT:** not detected. *Author and respectve reference.

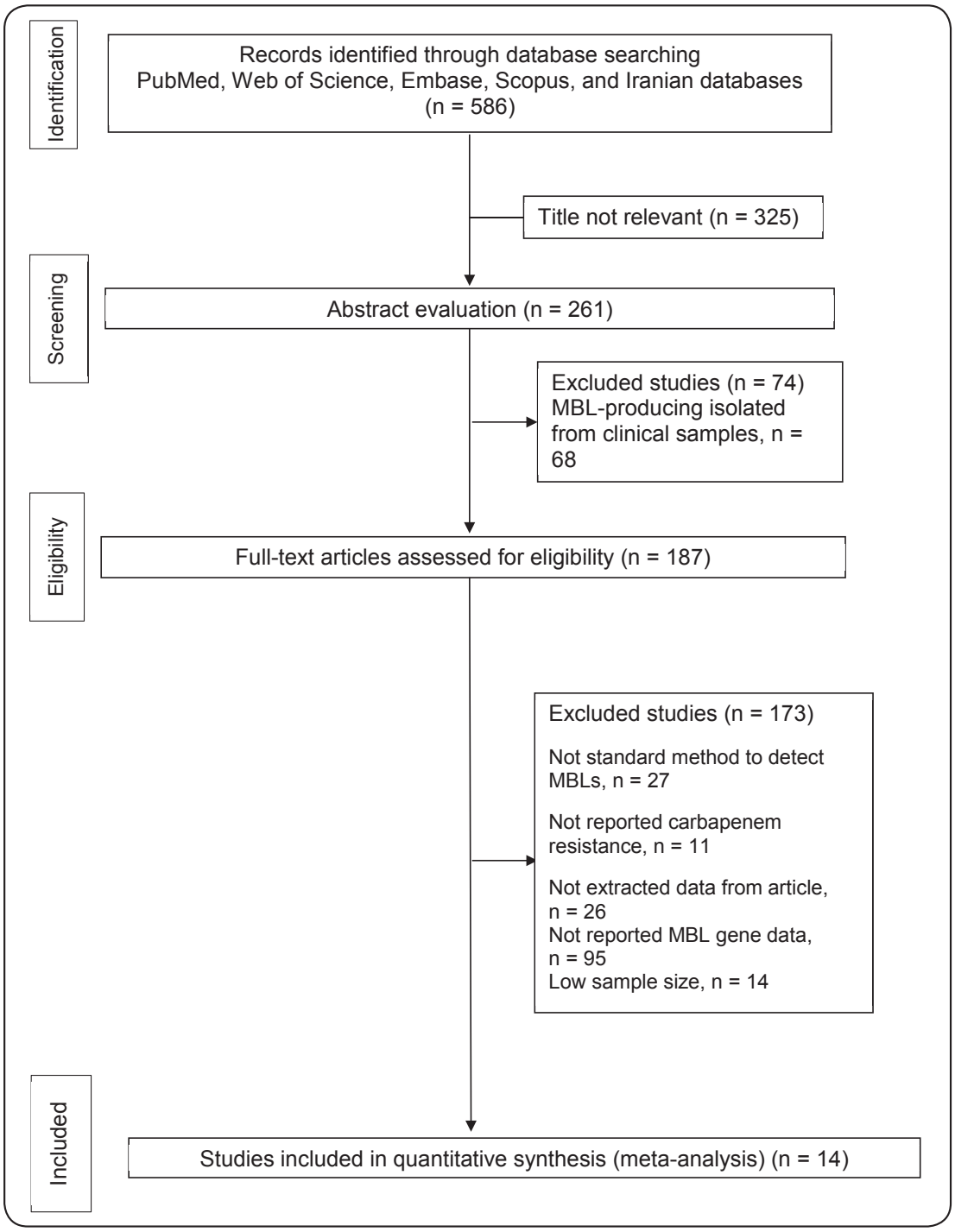

FIGURE 1: Summary of the literature search and study selection. **MBLs:** metallo-β-lactamases.

TABLE 2: Meta-analysis of CRPA and MBLs prevalence among burn patients in Iran.

Subgroup	Study (n)	Prevalence	n/N	Heterogeneity test, I2	Heterogeneity test, P-value	Begg's test	Egger's test
CRPA*	14	76.8 (67.5–84.1)	1,701/2,450	95	< .001	0.02	0.004
IMP	8	13.1 (4.7–31.5)	206/1,168	97	< .001	0.5	0.3
VIM	11	21.4 (14.6–30.1)	302/1,379	91	< .001	0.4	0.6

CRPA: carbapenem-resistant *Pseudomona aeruginosa;* **MBLs:** metallo-β-lactamases; **IMP**: imipenemase; **VIM:** Verona Integron-encoded metallo-beta-lactamase; **n:** number of events (CRPA, IMP, and VIM); **N:** total number of *Pseudomona aeruginosa* isolated from burn patients.

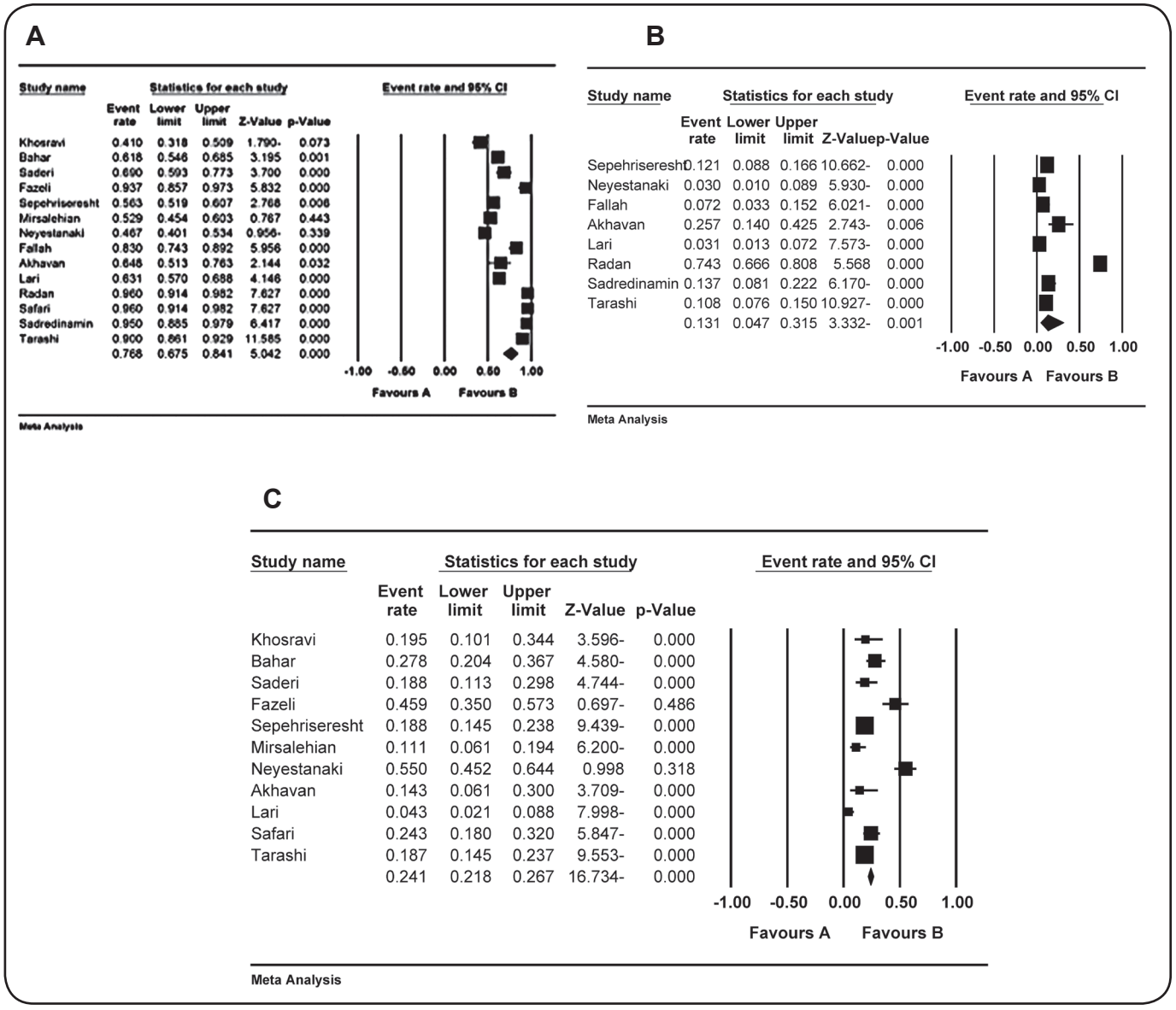

FIGURE 2: Forest plot of the studies included in this meta-analysis. **(A)** The prevalence of CRPA in burn patients. **(B)** and **(C)** The prevalence of IMP and VIM genes among CRPA, respectively. **CRPA:** carbapenem-resistant *Pseudomona aeruginosa;* **IMP**: imipenemase; **VIM:** Verona Integron-encoded metallo-beta-lactamase.

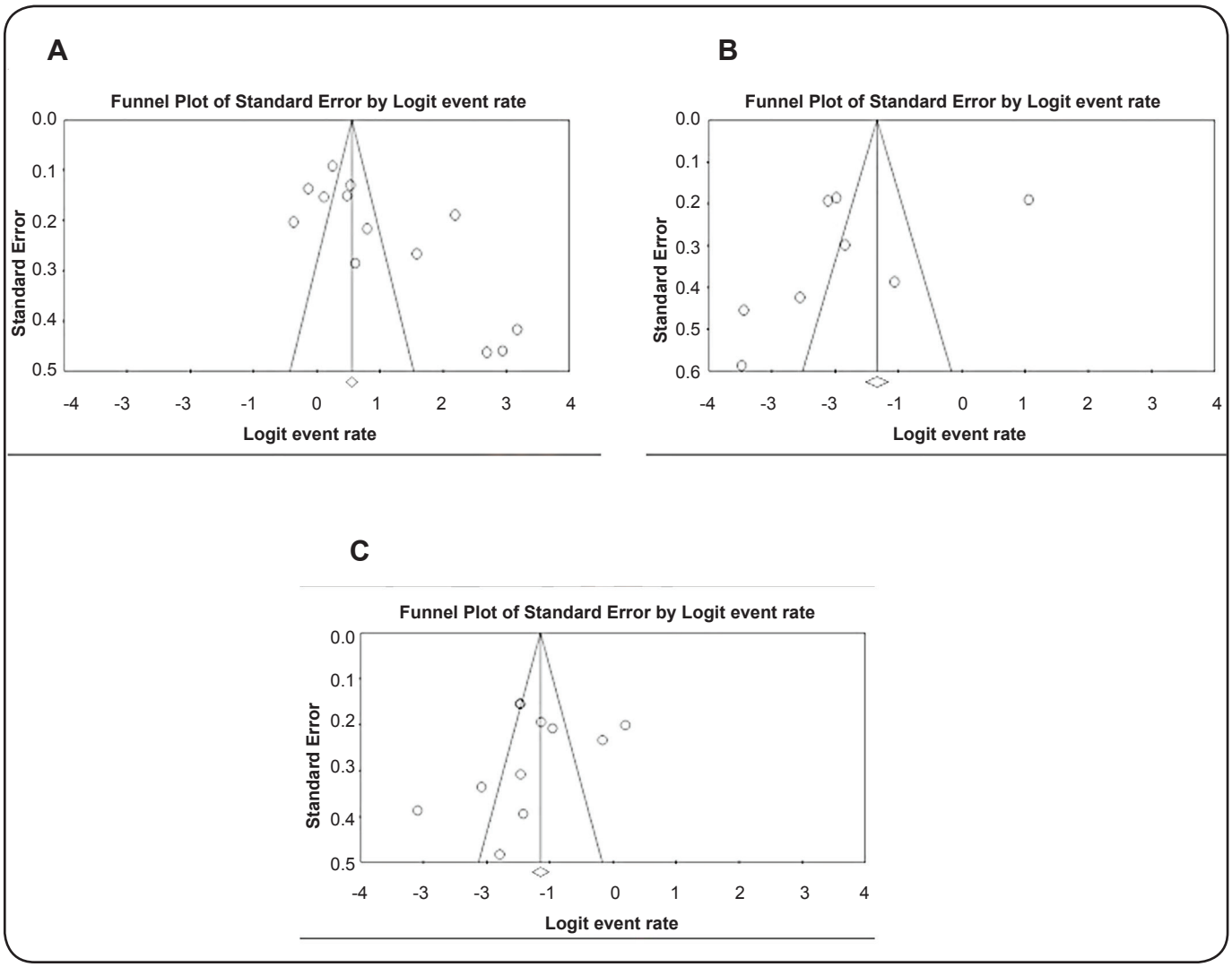

FIGURE 3: Funnel plot of publication bias for the included studies. **(A)** The prevalence of CRPA in burn patients. **(B)** and **(C)** The prevalence of IMP and VIM genes among CRPA, respectively. Asymmetric shape of funnel plots suggests bias in this meta-analysis. **CRPA:** carbapenem-resistant *Pseudomona aeruginosa;* **IMP**: imipenemase; **VIM:** Verona Integron-encoded metallo-beta-lactamase.

$p < 0.001$ for CRPA; $I^2 = 97$, $p < 0.001$ for IMP; and $I^2 = 91$, $p < 0.001$ for VIM) were found, so the random effect model was used for meta-analysis. **Figure 2** shows forest plots for the prevalence rate of CRPA, IMP, and VIM. As it is shown in **Table 2**, some evidence of publication bias was observed by Begg's rank correlation test ($p = 0.02$ for CRPA), but it was not observed for IMP and VIM genes ($p = 0.5$ for IMP and $p = 0.4$ for VIM). Some evidence of publication bias was detected by Egger's weighted regression ($p = 0.004$ for CRPA), but it was not detected for IMP and VIM genes ($p = 0.3$ for IMP and $p = 0.6$ for VIM). Asymmetric shapes of the funnel plots (**Figure 3**) show some evidence of publication bias among the evaluated papers.

DISCUSSION

To our knowledge, the current study was the first comprehensive systematic review addressing the prevalence of CRPA and MBLs genes in burn units in Iran. Based on the meta-analysis results, the pooled prevalence of CRPA, IMP, and VIM genes in burn patients was 76.8% (95% CI 67.5-84.1), 13.1% (95% CI 4.7-31.5), and 21.4% (95% CI 14.6-30.1), respectively[5,9-12,14-22]. Our analyses showed that the IMP and VIM genes were more prevalent in Iranian burn centers and remaining MBLs types have not yet been found. Our findings are similar to previous reports by other authors[23-25]. According to the analysis, it seems that the prevalence of CRPA and MBLs genes in Iranian burn units is higher than in industrialized countries, such as Norway and Sweden[26]. Several factors may explain this situation. First, most countries in Europe have an effective program of prevention and control of nosocomial infections[27]. While in developing countries, such as Iran, the hospital infection control committee may exist on paper, in practice, they barely exist[28]. Unfortunately, in Iran, infection control teams are inexperienced, inadequate, not well trained, and a clinical microbiologist is not included[29,30]. Hand hygiene is one of the simplest, most effective, and highly recommended infection control measures, but compliance with it is quite

poor among Iranian healthcare workers[30,31]. Heavy workload (patient overcrowding), limited infrastructures, inadequate healthcare worker to patient ratios, behavioral aspects, skin irritation by hand hygiene products, and lack of salary are the major reasons for noncompliance[30]. Second, wards with a high bed occupancy rate are a major cause of poor attention to infection control protocols in Iranian burn units[29,30]. Third, lack of isolation rooms is another common problem in Iranian burn hospitals; thereby, patients infected with MBL-producing *P. aeruginosa* mingle with other patients in multi-bed rooms[30]. Fourth, the use of personal protective equipment (PPEs) such as gowns, gloves, and mask is a simple method for infection controls[29]. In low-income countries, such as Iran, the use of PPEs is restricted owing to inadequate resources. In contrast, in the Netherlands, all hospitals are equipped with appropriate PPEs[29]. Fifth, in developing countries, such as Iran, inappropriate use of antibiotic and empiric therapies because of the lack of a good microbiological laboratory capacity is prevalent. Consequently, the emergence of resistant bacteria, such as CRPA, is accelerating in Iranian burn centers[30]. Unfortunately, in Iranian burn hospitals, routine detection of MBL production in *P. aeruginosa* is not performed. Therefore, we suggest that it is essential for carbapenem-resistant isolates to be screened for MBLs. Finally, lack of systems to monitor antibiotics prescribed by physicians and lack of a national stewardship have remained as a major challenge in Iranian burn units[32]. The present review had some limitations. First, the studies could not fully indicate the prevalence of MBLs among burn patients in Iran because the magnitude of MBLs infections was not yet determined in different areas of the country. Second, only published articles were considered in the present meta-analysis; hence, as in any other meta-analysis, the potential for publication bias should be considered. Third, heterogeneity was observed among the included studies. In conclusion, the prevalence of MBLs and CRPA was high in Iranian burn centers. Thereby, reducing antibiotic overuse, adherence to hand hygiene, early detection of MBLs producer isolates, education and training in antibiotic prescribing, environmental cleaning, contact precautions, and active surveillance are recommended strategies for prevention and spread of these strains.

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Conflict of interest

The authors declare that there is no conflicts of interest.

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