Epidemiology of VIM-1-imipenem resistant *Pseudomonas aeruginosa* in Iran: A systematic review and meta-analysis

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Background: *Pseudomonas aeruginosa* is an opportunistic human pathogen which causes serious problems, especially in people who have immunodeficiency. Metallo beta-lactamase (MBL) resistance in this bacterium has led some difficulties in treating bacterial infections. MBLs are being reported with increasing frequency worldwide. The aim of the present systematic review and meta-analysis was to collect data about the relative frequency (RF) of VIM-1-imipenem resistant *P. aeruginosa* (VIM-1-IRPA) in different regions of Iran and report an overall prevalence if possible. **Materials and Methods:** PubMed, ISI web of science, Scopus and Google Scholar were searched using following key terms: "*P. aeruginosa*," "imipenem," "VIM-1" and "Iran" were. Articles/abstracts, which used clinical specimens and had done polymerase chain reaction to detect the VIM-1 gene of MBL genes, were included in this review. STATA SE version 11.2 (StataCorp, College Station, TX, USA) was used for statistical analysis. **Results:** Out of 5457 results found, 10 articles were eligible to be included in our systematic review and meta-analysis. These studies were carried out in Tehran, Isfahan, Kurdistan, Ahvaz, Markazi and Northwest of Iran (Orumieh and Tabriz). Pooled estimation of 1972 *P. aeruginosa* samples showed that 13% (95% confidence interval = 10.5-16.5%]) of strains were VIM-1 positive. VIM-1-IRPA RF in different studies varied from 0% to 19.5% in Isfahan and Markazi provinces, respectively. We found a moderate heterogeneity (Chochran Q-test, $P = 0.032$, I-squared = 50.7%) of VIM-1-IRPA RF among studies. **Conclusion:** According to the results of this study VIM-1-IRPA RF in Iran is in low-level Prevention strategies to reduce the prevalence rates of VIM-1 positive strains in Iran are needed.

Key words: Imipenem resistant *Pseudomonas aeruginosa*, Iran, systematic review, VIM-1

INTRODUCTION

Pseudomonas aeruginosa is a clinically significant Gram-negative rod-shaped bacterium that may be selected and propagated within the hospital environment. Antimicrobial resistance in this species is becoming a growing concern and limits therapeutic alternatives. Carbapenems are commonly used as last-resort drugs for the treatment of infections caused by multidrug-resistant *P. aeruginosa* isolates. However, intensive use of carbapenems in the treatment of nosocomial *P. aeruginosa* infections has facilitated the emergence of mechanisms that confer resistance to carbapenems, such as diminished permeability, overexpression of the intrinsic efflux systems, and production of carbapenemases.[1,2]

The class B metallo-beta-lactamases (MBLs), which require zinc ion as a metal co-factor for carbapenemase activity, are able to confer resistance to carbapenem antibiotics in a wide variety of Gram-negative bacteria.[3-6] Acquisition of class B MBLs constitutes a growing family of carbapenem-hydrolyzing b-lactamases among *P. aeruginosa* strains.[1] *P. aeruginosa* strains producing acquired MBLs have mostly been reported as sporadic isolates or as causing small nosocomial outbreaks.[7-9] These enzymes efficiently hydrolyze all bl-lactam compounds except aztreonam, and in most cases their genes reside within class 1 integrons of various compositions of gene cassettes.[1,10] MBL enzymes are a cause of concern because they are able to hydrolyze most beta-lactams, including imipenem and meropenem, drugs considered of reserve for the treatment of Gram-negative multidrug-resistant strains.[11,12] In addition, MBLs are encoded on genes linked to mobile elements, a condition that facilitates their spread among different bacterial species and genera.[11,13] Lately, there has been a dramatic increase in the detection and spread of acquired and transferable families of these metalloenzymes (IMP, VIM, SPM, GIM, SIM and AIM enzymes).[14] MBL producing *P. aeruginosa* bacteria are slowly but steadily increasing within hospitals, causing outbreaks and/or hyperendemic situations in several

centers, mostly in the Far East and south of Europe.[1,7,8,15] Studies have identified the risk factors for MBL acquisition[16,17] as well as the outcome of *P. aeruginosa* infections caused by MBL producers.[18] The increasing prevalence of nosocomial infections produced by MBL-possessing *P. aeruginosa* strains severely compromises the selection of appropriate treatments and is, therefore, associated with significant morbidity and mortality.[19] Previous studies have shown that this epidemic occurrence is frequently due to the clonal spread of VIM-type MBLs, but also due to horizontal transmission of these enzymes between unrelated clonal strains.[1,6,20] In several European countries, also the increase of MBL production in *P. aeruginosa* is due primarily to the spread of VIM-type MBLs, suggesting a large reservoir of the respective blaVIM gene cassettes.[7,8,15]

Although there are many reports from different cities of Iran, the average rate of VIM-1-imipenem resistant *P. aeruginosa* (VIM-1-IRPA) in Iranian hospitals is still unknown. In the present study, we aimed to systematically review published data about the prevalence rate of VIM-1-IRPA, as detected by the polymerase chain reaction (PCR) amplification of the VIM-1 gene from different parts of Iran and provide an overal relative frequency (RF) for Iran using meta-analysis.

MATERIALS AND METHODS

Search strategy

PubMed, ISI web of science, Scopus and Google Scholar were searched (from October 2012 to November 2013) using following keywords: "*P. aeruginosa*", "*P. aeruginosa*", "imipenem," "metallobrta lactamase," "MBLs," "VIM-1 gene." No limitation was used while searching databases. References list of all studies also was reviewed for any other related publication. All these steps were done by two authors (ASA and MS) and any disagreements with article selection were resolved through discussion, and a third author (JF) was available to resolve the disagreement.

Inclusion criteria

Retrieved articles were selected if:
1. In this articles *P. aeruginosa* samples were collected from Iranian hospitals because this review study is limited to Iran country and the purpose of this study was to measure the prevalence of this resistant strains in Iran only,
2. Clinical specimens were taken from patients. If there were personnel specimens as well, personnel results were not included for analysis because Personnel samples are samples transferred from patients with repetitive strain and therefore not valid. Furthermore, all of the studies that have participated in this survey have been conducted on clinical samples from patients,
3. PCR method was incorporated to detect VIM-1 gene. Phenotypic results were not included because:

   a. Phenotypic methods had variable sensitivities and specificities in various studies,
   b. Phenotypic methods were affected by many factors such as PH of culture media, incubation period of isolates, commercial discs, media used in different studies and also personnel's skills,
   c. Breakpoints of phenotypic methods may change over time and make the interpretation of previous results more difficult,
4. All articles were carried out in previous years and recent articles (up to 2013) involved in this review study.

Exclusion criteria

Studies were excluded from our systematic review if:
1. Samples were partially/totally selected from IRPA collections,
2. Method for detecting IRPA strains could not be discovered from the paper,
3. The origin of samples was not clear, meaning that the reviewer(s) could not find out which region or population (i.e., inpatients, personnel, or outpatients) the specimens were gathered from.

In the case of duplicate publication, studies with more sample size or with more detailed results was chosen for our systematic review.

Data collection

Two independent reviewers (ASA, MS) extracted the data about first author's last name, publication date, sample size, number of participants with VIM-1-IRPA, its RF, and research location.

Statistical analysis

The Number of total participants and the number of participants with VIM-1-IRPA were used to calculating RF that was then converted to log RF and its standard error (SE) for meta-analysis.[21] Overall effect was derived using random effects model, which takes between-study variation into account.[22] Statistical heterogeneity between studies was evaluated using Cochran's Q-test and I-squared.[21] Sensitivity analysis was used to explore the extent to which inferences might depend on a particular study or a number of publications. Publication bias was evaluated by looking over Begg's funnel plots.[23] Formal statistical assessment of funnel plot asymmetry was done by Egger's regression asymmetry test and Begg's adjusted rank correlation test.[22] All Statistical analyses were conducted using STATA version 11.2 (StataCorp, College Station, TX, USA). $P < 0.05$ were considered as statistically significant.

RESULTS

Out of 5457 retrieved articles, 36 studies matched our inclusion criteria, out of which 10 (abstract with full-text
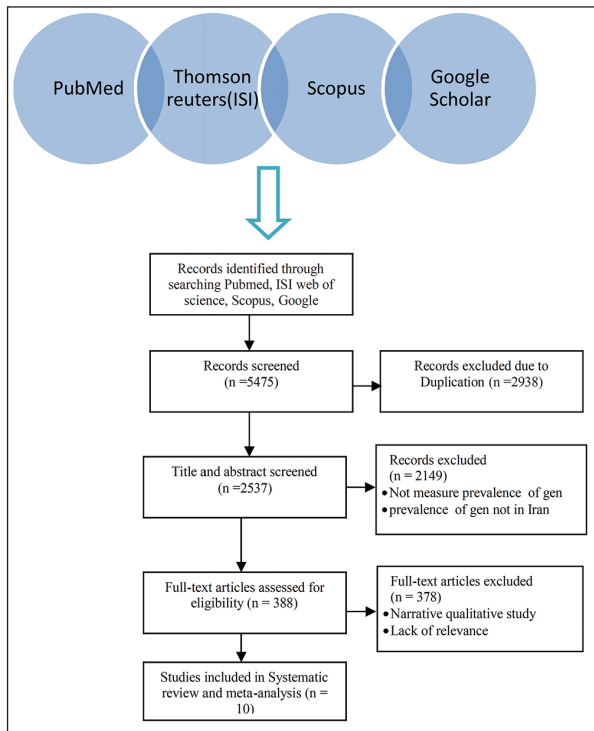
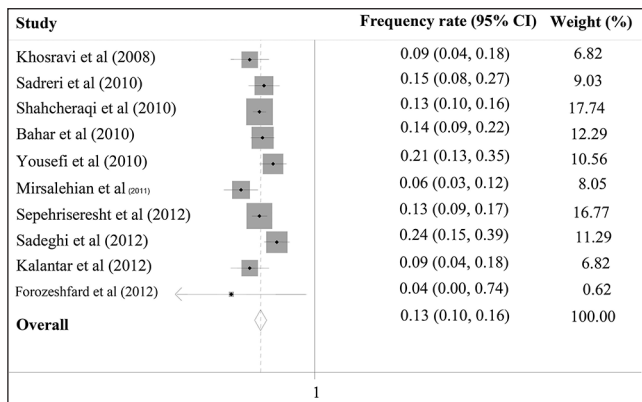**Figure 1:** Flowchart of study selection process

Figure 2: Forest plot illustrating weighted relative frequency using random effects model for assessing overall prevalence of positive VIM-1 *Pseudomonas aeruginosa* samples in Iranian hospitalized patients
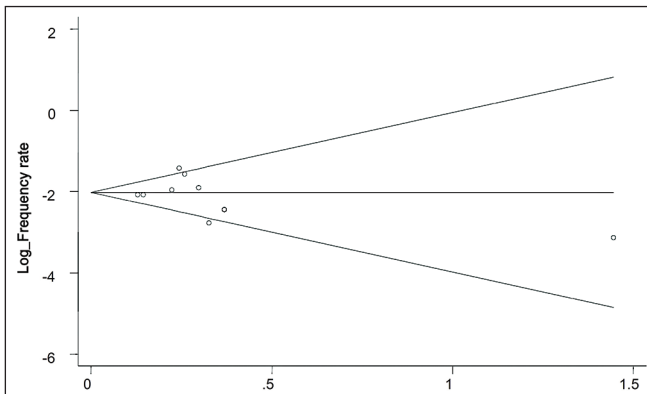

Figure 3: Begg's funnel plots (with pseudo 95% confidence intervals) of the relative frequencies (RFs) versus the standard errors of the Framingham risks in studies that evaluated the positive VIM-1 in *Pseudomonas aeruginosa* samples in Iranian hospitalized patients

Table 1: Charachteristics of studies included in the systematic review and meta-analysis

Study	Year of publication	City of data collection	Sample size	VIM	RF of VIM (%)
Khosravi and Mihani[27]	2008	Ahvaz	100	8	8
Forozsh *et al*.[25]	2012	Isfahan	11	0	0
Kalantar *et al*.[26]	2012	Kurdistan	100	8	8
Sadeghi *et al*.[29]	2012	Markazi	108	21	19.5
Yousefi *et al*.[33]	2010	Northwest (Orumieh and Tabriz)	104	18	17.3
Sepehriseresht *et al*.[31]	2012	Tehran	483	51	18.2
Saderi *et al*.[30]	2010		100	13	13
Shahcheraghi *et al*.[32]	2010		610	68	11.1
Mirsalehian *et al*.[28]	2011		170	10	5.9
Bahar *et al*.[24]	2010		186	23	12.3

RF = Relative frequency

articles) were selected for analysis [Table 1].[24-33] In studies that we investigate samples include blood, urea, wound, eye infection, respiratory system, surgery and burning specimens that take from patients and hospitalization patientin male/female and over ages. Overally participants from 10 eligible studies were included in our meta-analysis [Figure 1]. Our analysis showed that the prevalence rate of positive VIM-1 strains of *P. aeruginosa* is about 13% in Iranian population (95% confidence interval: 0-19.5) [Figure 2]. Between study heterogeneity was moderate (Cochrane Q-test, $P = 0.032$, I-squared = 50.7).

Sensitivity analysis showed that none of the included studies can significantly change the overall prevalence. Although there was a slight asymmetry in the Begg's funnel plot, asymmetry tests did not show any evidence about publication bias (Egger's test, $P = 0.570$, Begg's test, $P = 0.571$) [Figure 3].

DISCUSSION

Results of our study was evaluation 10 articles that prevalence of VIM-1 gene in IRPA strains in studies of Khosravi Mihan, ForozeshFard, Kalantar *et al.*, Sadeghi *et al.*, Yousefi *et al.*, Sepehriseresht *et al.*, Saderi *et al.*, Shahcheraghi *et al.*, Mirsalehian *et al.* and Bahar *et al.* was 8%, 0%, 8%, 19.5%, 17.3%, 18.2%, 13%, 11.1%, 5.9% and 12.3%, respectively.

During the recent years, assays for detection of VIM-MBL gene in *P. aeruginosa* became popular among Iranian researchers. Based on these studies, we reported the cumulative prevalence of VIM-1 IRPA in Iran country.

We tried to compare our study with several studies in the countries of American and European. In Italy, 3 studies have

been done in this context. According to Francesco Luzzaro *et al.*, (2003) the VIM-1 gene only detect in 1 isolate from 506 isolates of IRPA. Cristiana Lagatolla was conducted 2 studies. In the first study on 89 IRPA isolates, 54 (84%) isolates had VIM-1 gene (2004) second study reported that 86 strains between 174 IRPA had VIM-1 gene (2006). In Brazil, 3 studies were conducted. According to Sader *et al.*, (2005), 183 *P. aeruginosa* isolates has been investigating that VIM-1 gene does not detect by molecular methods finally. In other study by Fernanda *et al.* (2009), 31 *P. aeruginosa* isolates were considered but no exsist VIM-1 gene in these isolates. Also, Franco *et al.*,[14] show that no exist this gene in 238 isolates of *P. aeruginosa* in his study. Two studies were conducted in Spain. According to the survey by Carvalho *et al.*, (2005), 27 isolates of *P. aeruginosa* investigated that VIM-1 gene not identified in these strains. Also, in another study on 236 *P. aeruginosa* isolates by Gutierrez *et al.*, (2007), not detect VIM-1 gene.

According to our study, the mean prevalence of VIM-1-IRPA in Iran was 13%.

Mean prevalence of VIM-1 IRPA in Iran is moderately higher than Brazil and Spain but lower than Italy.

These findings show high presence of VIM-1 gene in Iranian *P. aeruginosa* isolates. In a total perspective, Iran has a higher prevalence VIM-1 IRPA compared some countries. This finding indicates that physicians may face difficulties in treatment of MBL (such as VIM-1) producing *P. aeruginosa* infections.

This study has some limitations. First, it cannot fully represent Iran because there were no data on VIM-IRPA from many parts of the country for example: South and southern of Iran. However, as described above, this is preferred to mix the results from different phenotypic methods with genotypic ones. Second, due to limited access to in-press articles and theses, some studies might have been missed, which is also suggested by statistical analysis.

CONCLUSION

According to the results of this study, VIM-1-IRPA RF in Iran is in a low level, but the prevalence of this gene is in high rate in some studies and likely to rise; thus, measures should be taken to keep the emergence and transmission of these strains to a minimum. Prevention strategies to reduce the prevalence rates of VIM-1 positive strains in Iran are needed. Basically, we need guidelines to prevent the spread of these strains in hospitals that administrate by doctors and staff.

AUTHORS' CONTRIBUTION

MS contributed to the conception and design of the work; the acquisition, analysis, and interpretation of data for the work. ASA contributed in data analysis, Drafting the work and revising it critically for important intellectual content. JF contributed to final approval of the version to be published. GO contributed in the revising the draft and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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