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High-Level Vancomycin-Resistant *Staphylococcus aureus* (VRSA) in Iran: A Systematic Review

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ABSTRACT

Background: *Staphylococcus aureus* is a major human pathogen worldwide. Vancomycin has been used for decades to treat multidrug resistant *S. aureus.* Ten years has passed since the first report of vancomycin resistant *S. aureus* (VRSA). The objective of this systematic review was to determine the total number of VRSA isolates that have been reported from Iran.

Methods: Search terms reflected "Iran", "vancomycin" and "*S. aureus*" were searched in the ISI web of knowledge, PubMed, SciVerse, and Google scholar. Also two Persian scientific databases and 13 recent national congresses were investigated. Articles / abstracts working on *S. aureus* in Iran, evaluating vancomycin MIC and / or PCR of *vanA/B* were included in this systematic review.

Results: Out of the 3484 records found in mentioned resources, 13 related studies were included in the final analysis. The result showed that at least 24 VRSA isolates which have been reported from Iran up to September 2012.

Conclusion: It seems that many Iranian researchers did not follow a specific guideline for reporting and confirming VRSA. Establishing an Iranian reference center where studies on VRSA can be registered, evaluated and confirmed is strongly recommended.

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Introduction

Staphylococcus aureus is undoubtedly one of the most hazardous agents among bacterial pathogens and nowadays has become a major threat both in hospital and community settings. About 1% of all admitted patients in U.S. hospitals are infected with *S. aureus*. In other words, approximately 400,000 infections are being reported annually in the U.S. (1).

Moreover, antibiotic resistance has complicated treatment procedure.

The resistance causes extra treatment costs and also longer length of stay; it may result in treatment failure as well (2).

Before the discovery of antibiotics, mortality rate of *S. aureus* strains was more than 75 % (3). After penicillin was discovered, soon resistant strains appeared and restricted physicians' choices for treating staphylococcal in fections. Then methicillin was used as the new antibiotic therapy against this pathogen. Shortly after the introduction of methicillin, *S. aureus* acquired resistance and the first methicillin resistant *S. aureus* (MRSA) strains were reported in 1961 in London but this was partly ignored at that time (4).

In the later decades, resistance rate among *S. aureus* strains increased dramatically and MRSA became a worldwide hazard (5).

For instance, in the past ten years, the prevalence of MRSA in many hospitals is reaching 50% (5, 6). Thus, the high prevalence of MRSA forced clinicians to use vancomycin, which is a glycopeptide antibiotic discovered before methicillin, as the first-line of treatment against M- RSA despite its side effects (7).

Sixty years has passed since the discovery of vancomycin but vancomycin resistant *S. aureus* (VRSA) appeared only in a limited number of cases and just in the last ten years.

A brief history of vancomycin is shown in Figure 1 (8-13). In the past 20 years, *vanA*-me diated vancomycin resistance has been desc ribed in detail (14).

Briefly, the resistance ge- ne is located on a transposon called Tn*1546*. This transposon contains a set of genes (including *vanA*) which encode enzymes that replace the C-terminal D-Ala-D-Ala residues of the peptidoglycan precursor with D-Ala-D-Lac. Vancomycin binds to normal precursors by forming hydrogen bonds between its peptide portion and the D-Ala-D-Ala dipeptide. The structural change in the precursor leads to loss of vital hydrogen bonds and extreme reduction in affinity of vancomycin to these cell wall precursors (14, 15).

By phenotypic approach, as described in clinical and laboratory standards institute (CLSI), VRSA is defined as an isolate with minimum inhibitory concentration (MIC) of vancomycin greater than or equal to 16 $\mu g.mL^{-1}$ as determined with broth microdilution (16). However there are some reports regarding the genotype-negative phenotype-positive VRSA (i.e. *va nA/B* negative but within resistance range according to MIC) (17, 18).

Considering the fact mentioned above, we aimed to find the total number of VRSA isolates reported in Iran, defined by the phenotypic and/or genotypic approach.

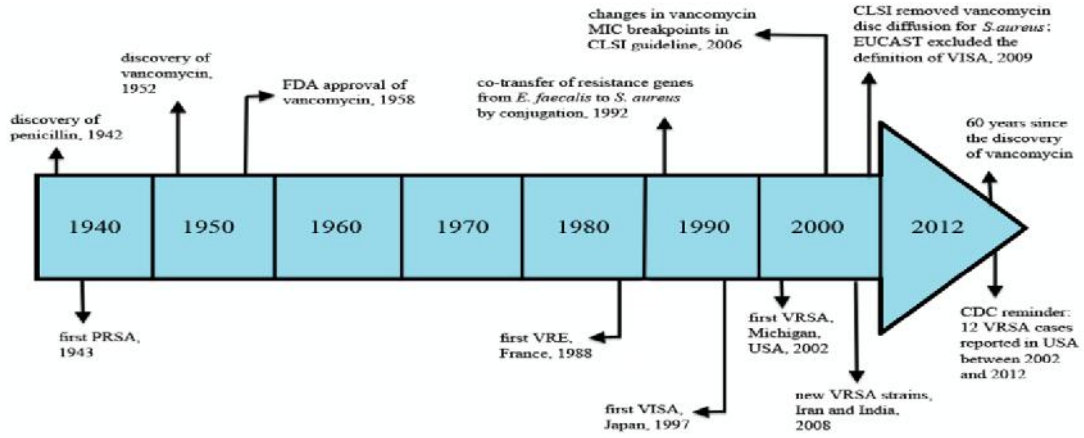

Figure 1. A brief history of vancomycin resistance

PRSA: penicillin resistant *S. aureus* / FDA: food and drug administration / VRE: vancomycin resistant *Enterococcus* / VISA: vancomycin intermediate *S. aureus* / VRSA: vancomycin resistant *S. aureus* / CLSI: clinical and laboratory standards institute/ EUCAST: European committee on antimicrobial susceptibility testing / CDC: centers for disease control and prevention

Methods

Searching in references

Searched time periods and search engines

All articles that were indexed prior to September 2012 in the ISI web of knowledge, SciVerse, PubMed, Google Scholar, Scientific Information Database (SID) and IranMedex search engines were searched.

Searching for English articles

All articles that contained the words "Iran", "vancomycin" and "*Staphylococcus aureus*" were searched in the ISI web of knowledge, PubMed and SciVerse. Google scholar was also searched with keywords of "vancomycin", "*Staphylococcus aureus*", "Iran" and "minimum inhibitory concentration".

Searching for Farsi articles

IranMedex and Scientific Information Database (SID) were searched for the keywords of "vancomycin" and "*aureus*" in both English and Farsi.

Supplementary search with "Google domain search" option

All academic Farsi websites (.ac.ir) were searched with Google domain search for the words "vancomycin", "*aureus*", "MIC" and "minimum". Searching in the full-text of Farsi articles in SID website (sid.ir) was done by Google domain search with the same keywords used for SID.

Updating the results

After completing the search in November 2011, the results of English engines were updated using Google Scholar Alert with the same strategy used in Google Scholar. Farsi results were updated weekly using Google domain search in Persian websites (.ir) with keywords of "*aureus*" and "vancomycin".

Searching in the abstract books of congresses

Abstract books of microbiology congresses in recent years (1st-5th clinical microbiology, 4th laboratory and clinic, infections and anti-

biotic resistance, rational usage of antibiotics, 10th-13th microbiology and 1st medical bacteriology) were investigated.

Contacting experts

Iranian researchers in the field of staphylococci were asked if they were working on resistance to vancomycin. If the answer was positive, they were asked to send supplementary information.

Defining the resistance to vancomycin

According to CLSI (2012), all strains with vancomycin "broth microdilution" MIC of ≥ 16 µg.mL^{-1} are defined as VRSA (16). However, in Iran (based on the current restrictions) this method is rarely used. So, we considered all strains with MIC ≥ 16 µg.mL^{-1} that were determined by either of the existing methods (i.e. broth macro- and microdilution, agar dilution and E-test) as VRSA.

Omitting disc diffusion results

From 2009 and forward, CLSI has excluded the zone diameters of vancomycin for *S. aureus* (16). All strains identified as VRSA only by the disc diffusion method (not MIC) were excluded.

Inclusion and Exclusion criteria

All the studies working on *S. aureus* in Iran, evaluating vancomycin MIC and/or PCR of *vanA/B* were included in our analysis. Exclusion criteria are shown in Figure 2.

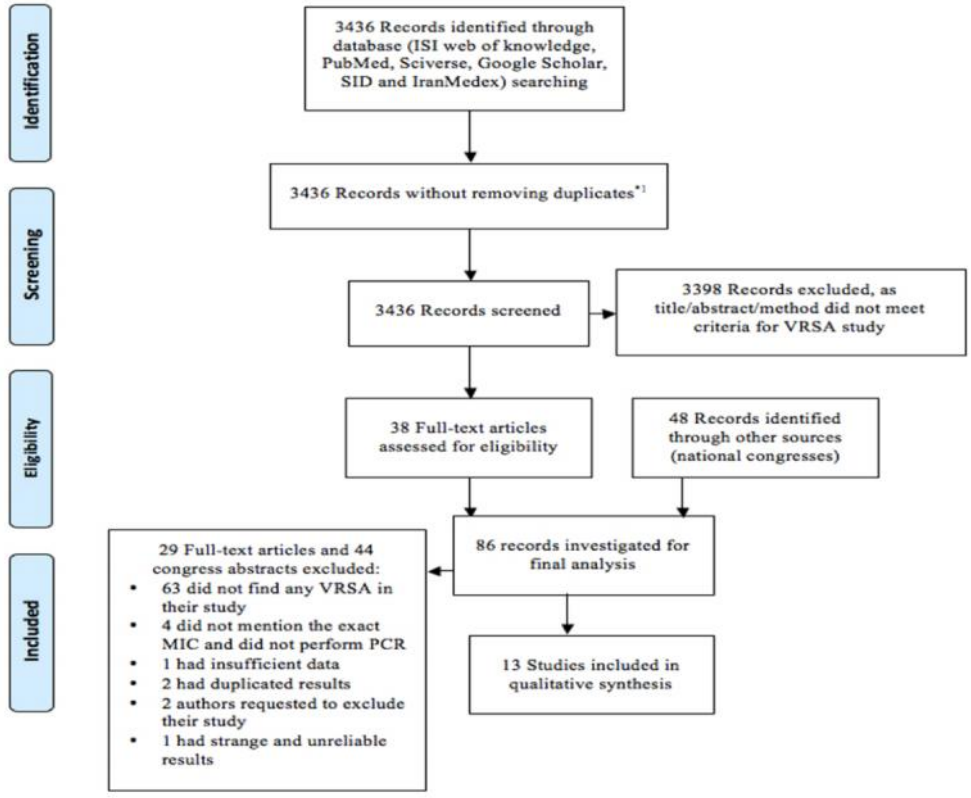

Figure 2. Flow chart showing the selection process and exclusion criteria

*1: Duplicate results could not possibly be removed because:
(A) The exported bibliographic data exported from Google Scholar were not identical to those of PubMed and the reference managing software did not recognize "duplicates" properly; (B) The Sciverse engine by itself had many duplicate records; (C) The bibliographic data was not exportable from Farsi search engines. Nevertheless, the authors made sure that duplicate data was not included for the final synthesis.

Designing results summary file

First and second author read the articles and summarized the results based on a previously designed format in excel. If they did not agree on the interpretation of the results, fourth author (referee) was asked to determine the correct interpretation.

Results

Out of the 3436 reviewed articles, 9 related studies were chosen for final analysis. In addition, we found 48 related studies in national congresses from which 4 abstracts were added to the final analysis *(Figure 2).*

The final analysis revealed that to date, at least 24 VRSA isolates have been reported from Iran *(Table 1).* (18-30) Clinical information for five of these isolates was also available *(Table 2)* (22, 23, 28-30).

Table 1. Detailed information about VRSA isolates reported in Iran

VRSA Number	City	Year of Publication/ Presentation	MIC (µg/mL), method	*vanA/B* PCR, result of PCR	Reference
1-4	Tehran	2005	>256 Ω, Σ	NA¶	Saderi *et al* [19]
5	Tehran	2005	128 Ω, Σ	NA	Saderi *et al* [19]
6	Isfahan	2007	256 Σ	NA	Mostafavizadeh *et al* [20]
7	Isfahan	2007	32 Σ	NA	Mostafavizadeh *et al* [20]
8-9	Karaj	2008	>128 Π	NA	Faghri *et al* [21]
10	Tehran	2008	>256 Ω, Σ	NA	Saderi *et al* [22]
11*	Tehran	2008	512 Ψ	*vanA*, (+)	Aligholi *et al* [23]
12 N	Sari	2010	32 Ψ **	NA	Ghasemian *et al* [24]
13 N	Sari	2010	16 Ψ **	NA	Ghasemian *et al* [24]
14N-15 N	Gorgan	2011	>256 Σ	*vanA*, (–)	Rahimi-alang *et al* [18]
16-17	Ghaemshahr, Sari	2011	25 Ψ **	NA	Alikhani *et al* [25]
18	Tabriz	2011	?	*vanA*, (+)	Sheikh Moniri *et al* [26]
19 N	Khorramabad	2011	16 Ω	NA	Hosain Zadegan *et al* [27]
20	Rasht	2012	128Ω	*vanA*, *vanB* ***	Anvari *et al* [28]
21	Rasht	2012	256 Ω	*vanA*, *vanB* ***	Anvari *et al* [28]
22	Rasht	2012	256 Ω	*vanA*, *vanB* ***	Anvari *et al* [28]
23	Tehran	2012	512 Ψ	*vanA*, (+)	Dezfulian *et al* [29]
24	Mashhad	2012	512 Σ	*vanA*, (+)	Azimian *et al* [30]

Ψ: Broth microdilution; Ω: Agar dilution; Σ: E-test; Π: Broth macrodilution;
¶ NA: Not available; N: Nasal sample
* Two VRSA strains were mentioned in the study, but later one of them found to be mixed with *Enterococci* and was therefore excluded from this review (Dr. Mohammad Emaneini, department of microbiology, school of medicine, Tehran University of Medical Sciences, personal communication).
** Personal communication
*** Authors used primers nearly similar to the ones used by Clark *et al* (31) but in this study, *vanA* and *vanB* primer bands were 474 and 800 bp, respectively.

Table 2. Clinical information available from some of VRSA reported cases in Iran

City	Date	Age	Gender	Isolation site of VRSA	MIC (µg/mL)	*van*A PCR done, result of PCR	Reference
Tehran	2008	36	female	pus of wound	>256	NA*	Saderi *et al* (22)
Tehran	2008	67	male	post-heart surgery wound	512	yes, (+)	Aligholi *et al* (23)
Rasht	2012	25	male	pus	128	yes, (+)	Anvari *et al* (28)
Rasht	2012	79	male	pus	256	yes, (+)	Anvari *et al* (28)
Rasht	2012	60	male	pus	256	yes, (+)	Anvari *et al* (28)
Tehran	2012	51	female	abscess	512	yes, (+)	Dezfulian *et al* (29)
Mashhad	2012	26	male	bronchial aspirate	512	yes, (+)	Azimian *et al* (30)

* NA: Not available

Discussion

The total number of VRSA in the world is claimed to be fewer than twenty (32). At least three of these resistant strains are reported from Iran (23, 29, 30). Nevertheless, our results show that a greater number of VRSA strains have been reported from Iran and the resistance of *S. aureus* to vancomycin is actually worse than estimations and expectations. This underestimation may be due to the fact that almost all studies in Iran did not include a molecular approach for vancomycin resistance. Consequently, from international perspectives, the reported resistant strains would not be accepted as VRSA (33). Also, most of these studies did not completely follow a specific guideline such as the one recommended by centers for disease control and prevention (CDC) (34).

Health care workers can carry resistant strains of *S. aureus* in their noses. In this study we found 2 articles reported health care workers who carried VRSA strains in their noses. This can pose a threat to patients, especially those who undergo operations and the ones who need extensive care (35). Nasal carriage of VRSA should be taken seriously because the bacteria may spread by contact.

In conclusion, the number of VRSA reported in Iran is extremely high. Following the CDC guideline, performing molecular techniques and validating PCR results in an independent outside laboratory is recommended. We also suggest establishing an Iranian reference center where studies on VRSA can be registered, evaluated and confirmed.

Acknowledgement

None declared.

Conflict of Interest

None declared conflicts of interest.

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