Molecular characterization of vancomycin-intermediate Staphylococcus aureus isolates from Tehran

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ABSTRACT

Objective: To determine the prevalence and some genetic characteristics of clinical isolates of Staphylococcus aureus (S. aureus) with reduced susceptibility to vancomycin.

Methods: A total of 414 isolates of S. aureus were collected from clinical specimens from hospitals in Tehran. Vancomycin-intermediate S. aureus (VISA) was determined by brain heart infusion agar containing 4 μg/mL vancomycin screening plate and confirmed via E-test. VISA isolates were analysed by vanA, vanB, mecA, staphylococcal cassette chromosome mec types, surface protein A (Spa) types and agr specific groups.

Results: Brain heart infusion agar containing 4 μg/mL vancomycin screening tests revealed that 17.14% (n = 71) of S. aureus isolates were VISA phenotype. Ten of the 71 isolates were confirmed by E-test method (minimal inhibitory concentration was 4 to 8 μg/mL). All VISA isolates were susceptible to linezolid and 6 isolates (60%) were methicillin-resistant S. aureus. Five isolates belonged to agr Group II, 4 belonged to agr Group I and 1 belonged to agr Group III. Spa type 0103, and staphylococcal cassette chromosome mec Type III were dominant among VISA isolates.

Conclusions: This study provides further evidence of the global dissemination of VISA isolates and emphasizes to vancomycin susceptibility testing prior to antibiotic therapy.

1. Introduction

Staphylococcus aureus (S. aureus) is an important bacterium responsible for community and hospital acquired infections. Most of S. aureus infections are caused by methicillin-resistant S. aureus (MRSA) isolates, which the glycopeptide antibiotic vancomycin is considered the effective antimicrobial for these infections. Unfortunately, widespread empirical use of vancomycin has led to emergence of strains with reduced susceptibility to vancomycin. Most infections caused by clinical isolates intermediate S. aureus (VISA) strains occur in patients with serious underlying diseases such as diabetes and malignancy. Other diseases such as endocarditis or infection of a prosthetic joint with a high bacterial load may also predispose an individual to the development of VISA infection during glycopeptide therapy[1-3].

Because of the difficulty of testing methods, the exact prevalence of VISA and heterogeneous VISA (hVISA) remains uncertain[1,4-6]. According to the Clinical and Laboratory Standards Institute guidelines, the minimum inhibitory concentration (MIC) of vancomycin for susceptible, intermediate, or resistant strains is 2 μg/mL, 4–8 μg/mL or 16 μg/mL, respectively[7]. A subpopulation of cells in hVISA strains with MIC of 4 μg/mL for vancomycin could not detected via reference methods such as broth microdilution, agar dilution and standard E-test methods[4]. The population analysis profile-area under the curve (PAP-AUC) method is the gold standard for detection of hVISA, but it is labor-intensive, costly and also impractical to perform for a large number of isolates[4,8,9]. Riederer et al. in 2011 reported that brain heart infusion (BHI) agar supplemented
with $3$ or $4 \, \mu g/mL$ vancomycin is a useful alternative screening method for detecting hVISA and VISA respectively\cite{10}. The sensitivity and specificity of their methods were $100\%$ and $94.6\%$ for BHI-supplemented with $3 \, \mu g/mL$ vancomycin, and $100\%$ and $99.2\%$ for BHI-supplemented with $4 \, \mu g/mL$ vancomycin\cite{10}.

After the first hVISA and VISA strains appeared in 1996 in Japan, reduced susceptibility to vancomycin in clinical isolates of \textit{S. aureus} have been reported in many parts of the worlds, however, there are only a few reports of hVISA and VISA strains from Iran\cite{3-6,11,12}. Therefore in this study, we detected prevalence of VISA strains among clinical isolates of \textit{S. aureus} collected in teaching hospitals over a 3 years period. Our study may also provide genetics information of VISA strains including spa types, \textit{mecA} gene, staphylococcal cassette chromosome \textit{mec} (SCC\textit{mec}) types and \textit{agr} specificity groups.

### 2. Materials and methods

#### 2.1. Bacterial strains

A total of 414 non-consecutive clinical isolates of \textit{S. aureus} which were collected during 2009 to 2012 from teaching hospitals in Tehran, were screened for vancomycin susceptibility. All isolates were identified by conventional bacteriological methods (Gram-positive cocci, catalase-positive, mannitol-fermenting, slide and tube coagulase-positive and deoxyribonuclease-positive). All \textit{S. aureus} strains were stored at $-70^\circ C$ in BHI broth containing $20\%$ (v/v) glycerol.

#### 2.2. Detection of VISA

All \textit{S. aureus} isolates were screened for VISA strains on BHI agar containing $4 \, \mu g/mL$ of vancomycin (BHI-4V) as previously described\cite{10}. The growth of one or more colonies after $48$ h was considered positive. A positive isolate on BHI-4V screening plates was further analysed by the MICs using E-test (AB Biodisk, Solna, Sweden). The isolate was considered VISA if the MIC of vancomycin was $4$ to $8 \, \mu g/mL$. MIC of isolates which displayed a VISA profile on the E-test was further confirmed by the agar dilution method\cite{7}.

#### 2.3. Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed as recommended by the Clinical Laboratory Standards Institute using disk diffusion method for all isolates. Antimicrobial disks (Mast, UK) tested included oxacillin (1 \mu g), gentamicin (10 \mu g), amoxicillin (30 \mu g), ciprofloxacin (5 \mu g), tetracycline (30 \mu g), co-trimoxazol (1.25 \mu g + 23.75 \mu g), erythromycin (15 \mu g), rifampin (5 \mu g), clindamycin (10 \mu g) and linezolid (30 \mu g). \textit{S. aureus ATCC} 25923 was used as a control strain\cite{7}.

### 2.4. PCR for detection of \textit{mecA}, \textit{vanA} and \textit{vanB} genes

Genomic DNA of VISA isolates were extracted using DNA extraction kit (GeneAll, Korea). Lysostaphin at the final concentration of $20 \, \mu g/mL$ in lysis buffer [Tris-Hcl (50 mmol/L), 1\% sodium dodecyl sulfonate (w/v) and ethylene diamine tetraacetic acid (100 mmol/L)] was used. The DNA was used as the template in all PCRs experiments. All VISA strains were analyzed for the \textit{mecA}, \textit{vanA} and \textit{vanB} genes. PCR Red Master Mix (Ambicon, Denmark) was used for all PCR reactions in an Eppendorf thermal cycler (Mastercycler® gradient, Germany). Amplification program consisted of initial denaturation at $94^\circ C$ for $5$ min, $30$ cycles of denaturation at $94^\circ C$ for $60$ s, annealing at $55^\circ C$ for $60$ s for \textit{mecA}, $57^\circ C$ for \textit{vanA} and $52^\circ C$ for \textit{vanB}, extension at $72^\circ C$ for $60$ s and a final step of $72^\circ C$ for $5$ min. The PCR products were analyzed by electrophoresis in a 1.4\% agarose gel and stained with gel red (Biotium, USA)\cite{13,14}. The primers and size of the expected amplification products were listed in Table 1.

| Targets | Primers | Sequence\textsuperscript{*} | Product size (bp) | References |
|---------|---------|-----------------------------|-------------------|------------|
| \textit{agr}\textsuperscript{groups} | Pan F | ATGCACATGTTGTCAGCATG | 439 | \cite{15} |
| \textit{mecA} | F | GTGTAATTGCTTACATAGTGTCGAT | 572 | \cite{10} |
| \textit{vanA} | F | CATTATGAGAAAGTAAAACCGTAGA | 406 | \cite{10} |
| \textit{vanB} | F | GGCGGAGAATGTTGGGATAGAG | 588 | \cite{10} |

\textsuperscript{*} Sequence of primer as synthesized \textsuperscript{5′} to \textsuperscript{3′}.

#### 2.5. Multiplex PCR for SCC\textit{mec} typing

All VISA strains were analyzed for SCC\textit{mec} using multiplex PCR. The primers and size of the PCR products were listed in Table 1. The cycling parameters were as follows: an activation
step at 95 °C for 5 min, followed by 30 cycles of initial denaturation at 94 °C for 30 s, 57 °C for 1.5 min and 72 °C for 1.5 min, and a final extension at 72 °C for 10 min[13].

2.6. Duplex PCR for agr typing

The agr specificity groups for VISA strains were determined by two duplex PCR[15]. A forward primer, pan-agr, according to conserved sequences from the agrB gene, was used in all reactions. Four reverse primers, each specific for amplification of a single agr group based on nucleotide polymorphism of agrD or agrC genes, were used. The primers and size of the PCR products were listed in Table 1. The cycling parameters were as follows: 94 °C for 5 min followed by 30 cycles at 94 °C for 1 min, 50 °C for 30 s and 72 °C for 1 min, and a final extension at 72 °C for 7 min.

2.7. Spa typing

All VISA strains were analyzed by staphylococcal protein A (spa) typing as previously described[16]. The short sequence repeat X region of the spa gene was amplified using the primers F ( 5′-AGACGATCCTTGCGTGAC-3′) and R ( 5′-GCTTTTGCATGTCATTTACTG-3″). PCR cycling conditions were as follows: an initial denaturation at 94 °C for 3 min; 30 cycles at 94 °C for 30 s, at 55 °C for 30 s, and at 72 °C for 1 min, and a final extension at 72 °C for 10 min. Amplified PCR products were purified with QIAquick gel extraction kit. Purified PCR products were sequenced commercially by an ABI 3730XL DNA analyzer (Applied Biosystems) in both directions. Spa sequences were determined using BioNumerics v7.1 (Applied Maths) software and the SpaServer website.

3. Results

3.1. Bacterial isolates

A total of 414 non-duplicates S. aureus isolates included in this study. The isolates recovered from tracheal aspiration (n = 210, 50.7%), blood (n = 71, 17.1%), wound (n = 49, 11.8%), bronchoalveolar lavages (n = 24, 5.7%), catheter (n = 16, 3.8%), urine (n = 14, 3.3%), sputum (n = 10, 2.4%) or others (n = 20, 4.8%).

3.2. Detection of VISA

A total of 71 (17.14%) isolates were grown on BHI-4V plates. The E-tests of vancomycin MIC distribution among those 71 isolates were 2 μg/mL for 51 isolates, 3 μg/mL for 10 isolates, 4 μg/mL for 5 isolates, 6 μg/mL for 2 isolates and 8 μg/mL for 3 isolates. The E-test results were confirmed with repeat testing. The agar dilution of vancomycin MIC for those 10 VISA isolates was concordantly positive. The antimicrobial susceptibility, MICs, isolation source of VISA stains and clinical characteristics of patients with VISA infection were shown in Tables 2 and 3.

3.3. Antibiotic susceptibility testing

The disk diffusion test was performed for all 414 S. aureus (Table 3). All isolates were susceptible to linezolid. The antimicrobial resistance of VISA isolates were 60.0% for oxacillin, 70.0% for erythromycin and tetracycline, 80.0% for amoxicillin and clindamycin, 40.0% for ciprofloxacin, 50.0% for co-trimoxazole and 30.0% for rifampin. The antimicrobial susceptibility assay revealed that non-VISA isolates (Table 4) were mostly resistant to oxacillin (61.1%), tetracycline (46.6%)
and erythromycin (41.6%). Of 343 vancomycin-susceptible *Staphylococcus aureus* isolates (no growth on BHI-4V, Table 4), the highest resistance was observed for gentamicin (55.9%) followed by amoxicillin (53.3%), oxacillin (51.3%) and tetracycline (47.5%).

### Table 4

**In vitro antibiotic resistance pattern of 414 *S. aureus* isolates to 9 antimicrobial agents [n (%)].**

| Antibiotics      | No growth on BHI-4V (n = 343) | Growth on BHI-4V (n = 71) |
|------------------|---------------------------------|---------------------------|
| Oxacillin        | 176 (51.3)                      | 37 (61.6)                 |
| Ciprofloxacin    | 97 (28.2)                       | 19 (31.6)                 |
|ERYThromycin      | 114 (33.2)                      | 25 (41.6)                 |
| Tetracycline     | 163 (47.5)                      | 28 (46.6)                 |
| Amoxicillin      | 183 (53.3)                      | 22 (36.6)                 |
| Gentamicin       | 192 (55.9)                      | 18 (30.0)                 |
| Clindamycin      | 136 (39.6)                      | 11 (18.3)                 |
| Co-trimoxazol    | 55 (16.0)                       | 14 (23.3)                 |
| Rifampin         | 56 (16.3)                       | 13 (21.6)                 |
| Linezolid        | 0%                              | 0%                        |

#### 3.4. Identification of mecA, vanA, vanB, agr groups and SCCmec genes

All of the 10 VISA isolates were evaluated for the mecA gene using PCR. The mecA gene was found in six (60%) of 10 VISA strains and vanA and vanB genes were not found in any of the VISA strains. The presence of agr specificity groups in VISA isolates was determined by PCR. Most of VISA isolates belonged to agr Group II (50%), followed by agr Group I (40%) and agr Group III (10%). All of the VISA strains with resistance to methicillin (VISA-MRSA) were examined by multiplex PCR for SCCmec types. One isolate was found to be SCCmec-Type I and five isolates were Type III (Table 5).

#### 3.5. The spa typing

Seven spa types (t030, t230, t037, t586, t1149, t2467 and t12925) were identified in the 10 VISA isolates (Table 4). The most prevalent spa type was t030 (50%). One new repeat sequences was found and it was assigned (t12925) (spaServer: ridom.de).

### Table 5

**Genetic characteristics of VISA isolates.**

| Isolates | Date (mo/day/yr) of isolation | agr group | mecA | vanA and vanB | SCCmec | spa type | spa repeat |
|----------|------------------------------|-----------|------|---------------|--------|----------|------------|
| TMU 1    | 3/11/09                      | I         | +    | -             | III    | t030     | 15:12:16:02:24:24 |
| TMU 2    | 6/15/10                      | II        | -    | -             | III    | t037     | 15:12:16:02:25:17:24 |
| TMU 3    | 5/21/11                      | I         | +    | -             | III    | t030     | 15:12:16:02:24:24 |
| TMU 4    | 7/17/11                      | II        | -    | -             | I      | t2467    | 11:10:34:22:25:25 |
| TMU 5    | 9/14/11                      | II        | +    | -             | III    | t030     | 15:12:16:02:24:24 |
| TMU 6    | 2/17/12                      | II        | +    | -             | III    | t030     | 15:12:16:02:24:24 |
| TMU 7    | 2/19/12                      | II        | +    | -             | III    | t030     | 15:12:16:02:24:24 |
| TMU 8    | 3/01/12                      | I         | -    | -             | II     | t1149    | 08:16:34:24:34:17:17 |
| TMU 9    | 3/28/12                      | I         | +    | -             | III    | t12925   | 26:23:13:23:31:29:17:25:17:25:28 |
| TMU 10   | 4/17/12                      | II        | +    | -             | -      | -        | -          |

The spa types were listed based on the Ridom SpaServer website.

#### 4. Discussion

In this study, 414 non-repetitive *S. aureus* isolates were analyzed to determine the prevalence of VISA isolates. The result showed that VISA clinical isolates of Tehran hospitals were relatively low (2.41%). The prevalence of VISA in previous study from Iran was a slightly higher (2.9%) than our results[11]. In Asia, prevalence of VISA in Turkey (2.4%) was similar to our data, but in China (0.5%), Japan (0.24%), and Korea (0.09%) were lower than our results[17-20]. It also found that VISA strains increased from 2009 (one isolate) to 2012 (five isolates) (Table 5).

While the majority of detected VISA isolates were MRSA strains, in this study we showed that 2.06% of methicillin sensitive *S. aureus* (MSSA) isolates were VISA[14,21,22]. The high occurrence of VISA in MRSA strains was reported by Hu et al.[18] and Sun et al.[23], whereas in this study, we showed that the occurrence of VISA among MRSA (2.72%) and MSSA (2.06%) strains were approximately the same.

The occurrence of VISA strains among MRSA and MSSA isolates indicates a potential for development of vancomycin resistant *S. aureus* isolates and it is an important to pay an attention to detect of VISA in both MSSA and MRSA population.

Most VISA isolates in this study were identified from wound infection (four isolates), followed by trachea (three isolates), blood (two isolates) and drainage (one isolate). Therefore, diverse clinical specimens should be considered for isolating VISA strains.

Linezolid was fully active on VISA and all *S. aureus* in this study. Since the number of VISA isolates was low, we did not compare the rate of resistance between VISA strains and other isolates, but according to Table 3, VISA strains was found more resistant to multiple antibiotics, including erythromycin, tetracycline, clindamycin, rifampin and co-trimoxazole.

We used BHI-4V plate for screening VISA isolates. BHI-4V plate screening method was suggested by Riederer et al., as an alternative to PAP-AUC for detection of VISA isolates[10]. A total of 71 isolates were grown on BHI-4V plates in this study and by using E-test, we found only 10 isolates with vancomycin MIC between 4 and 8 μg/mL. The study was performed according to Chung et al. and 18 of...
the 33 isolates that were grown on BHI-4V were confirmed as hVISA via the PAP-AUC method and 15 were identified as VISA[20]. Hence, we suggest that it is an important to evaluate the results of BHI-4V via the PAP-AUC method for discriminating VISA and hVISA isolates. In this study, we confirmed VISA isolates via E-test.

The SCCmec typing revealed that most prevalent genotype among our VISA strains was SCCmec Type III and one isolate was SCCmec Type I. In the previous study from Iran, these SCCmec genotypes for VISA strains were also reported[11]. SCCmec Type III and SCCmec Type I are related to nosocomial infections. The majority of previous studies have indicated that VISA strains were more associated with SCCmec Type II, yet the study of Havaei et al.[12], and Hsueh et al.[24], from Taiwan showed that SCCmec Type III was predominant. The SCCmec Type III is the main SCCmec genotype in Iranian MRSA isolates[25-28]. Given this, the association between reduced vancomycin susceptibility and SCCmec genotype is more likely dependent to predominant SCCmec gene cassette types in each country. On the other hand, while agr Group II has been related with reduced vancomycin susceptibility, in the present study, 40% of VISA strains belonged to agr Group I. All VISA strains in the study of Hsueh et al. were agr Group I and these agr types are common in Iran and in Taiwan[24,29-32].

Molecular characterisation of all VISA isolates showed seven different spa types and four of them have been reported elsewhere; two of them (t586 and t2467) are new in Iran, and one of them is new allele (t12925). While six spa types were detected only once, the spa t030 was dominant and accounted for 40% of all our VISA isolates. The spa t030 was detected in 100% isolates during 2006–2008 in Ankara and also in Turkey, 70.3% of MRSA isolates during 2011 was t030[33]. The high prevalence (80.1%) of spa t030 was also reported in Chinese MRSA isolates in 2013,[34]. Three of four spa t030 strains in this study were SCCmec Type III. The spa t030 is also highly associated with the SCCmec Type III in China and Turkey. SCCmec Type III–spa t030 clone was the most common MRSA clone in Turkey during the 6 years of the study period and it is also represents a major public health problem in China[33,34]. As Ridom SpaServer, spa t030 is more frequently associated with ST 239 and ST 249, and related to CC8/239.

Vancomycin treatment failure was common in MRSA infections and was more pronounced in patients infected with VISA isolates[35,36]. A total of 10 patients with VISA infections, with a mean age of 52.7 years comprising 7 men and 3 women, were included in this study. The medical records regarding demographics, underlying diseases, history of exposure to antimicrobials and outcomes were shown in Table 3. One patient with VISA-MSSA bacteraemia after an initial failure ceftriaxone therapy was treated with rifampin. The other patient with VISA-MSSA central venous line infection after an initial failure vancomycin therapy were treated with gentamicin. Ventilator associated pneumonia with VISA-MRSA isolate was also treated with rifampin after an initial failure vancomycin therapy. Unfortunately, the outcome for seven other patients with VISA infection was hospital mortality due to underlying diseases and/or persistent infections and others factors (i.e., age and immune status and so on).

In conclusion, both MSSA and MRSA isolates with reduced vancomycin susceptibility infections are associated with higher rates of treatment failure and mortality. Therefore, early recognition via MIC susceptibility testing by E-test or dilution methods will help to select antimicrobial therapy and the clinical management of S. aureus infections.

**Conflict of interest statement**

We declare that we have no conflict of interest.

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**References**

[1] Kim ES, Bae IG, Cho JE, Choi YJ, Kim IH, Kang GS, et al. Clinical and molecular characterization of invasive heteroresistant vancomycin-intermediate *Staphylococcus aureus* infections in Korean Hospitals. *J Clin Microbiol* 2016; 54(3): 760-3.

[2] Huang SH, Chen YC, Chuang YC, Chiu SK, Fung CP, Lu PL, et al. Prevalence of vancomycin-intermediate *Staphylococcus aureus* (VISA) and heterogeneous VISA among methicillin-resistant *S. aureus* with high vancomycin minimal inhibitory concentrations in Taiwan: a multicenter surveillance study, 2012–2013. *J Microbiol Infectol Immun* 2015; doi: 10.1016/j.jmii.2015.07.003.

[3] Spagnolo AM, Orlando P, Panatto D, Amicizia D, Perdelli F, Cristina ML. *Staphylococcus aureus* with reduced susceptibility to vancomycin in healthcare settings. *J Prev Med Hyg* 2014; 55(4): 137-44.

[4] Zhang S, Sun X, Chang W, Dai Y, Ma X. Systematic review and meta-analysis of the epidemiology of vancomycin-intermediate and heterogeneous vancomycin-intermediate *Staphylococcus aureus* isolates. *PLoS One* 2015; 10(8): e0136082.

[5] Howden BP, Davies JK, Johnson PD, Stinear TP, Grayson ML. Reduced vancomycin susceptibility in *Staphylococcus aureus*, including vancomycin-intermediate and heterogeneous vancomycin-intermediate strains: resistance mechanisms, laboratory detection, and clinical implications. *Clin Microbiol Rev* 2010; 23: 99-139.

[6] van Hal SJ, Wehrhahn MC, Barbagiannakos T, Mercer J, Chen D, Paterson DL, et al. Performance of various testing methodologies for detection of heteroresistant vancomycin-intermediate *Staphylococcus aureus* in bloodstream isolates. *J Clin Microbiol* 2011; 49: 1489-94.

[7] Clinical and Laboratory Standards Institute. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard-tenth edition*. CLSI document M07–A10. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.

[8] Mirza HC, Sancak B, Gürt D. The prevalence of vancomycin-intermediate *Staphylococcus aureus* and heterogeneous VISA among methicillin-resistant strains isolated from pediatric population in a Turkish university hospital. *Microb Drug Resist* 2015; 21(5): 537-44.

[9] Khatib R, Riederer K, Shemes S, Musta AC, Szpunar S. Correlation of methicillin-resistant *Staphylococcus aureus* vancomycin minimal inhibitory concentration results by Etest and broth microdilution
methods with population analysis profile: lack of Etest overestimation of the MIC. *Eur J Clin Microbiol Infect Dis* 2013; 32(6): 803-6.

Riederer K, Shemes S, Chase P, Musta A, Mar A, Khatib R. Detection of intermediately vancomycin-susceptible and heterogeneous *Staphylococcus aureus* isolates: comparison of Etest an agar screening methods. *J Clin Microbiol* 2011; 49: 2147-50.

Aligholi M, Emaneini M, Jabalameli F, Shahsavan S, Dabiri H, Sedaght H, Havaei SA, Azimian A, Fazeli H, Naderi M, Ghazvini K, Samiee SM, et al. Genetic characterization of methicillin resistant and sensitive, vancomycin intermediate *Staphylococcus aureus* strains isolated from different Iranian hospitals. *ISRN Microbiol* 2012; 2012: 215275.

Chung G, Cha J, Han S, Poujol D, Ladero C, Tuszynski SJ, et al. Nationwide surveillance study of vancomycin intermediate *Staphylococcus aureus* strains in Korean hospitals from 2001 to 2006. *J Microbiol Biotechnol* 2010; 20(3): 637-42.

Richter SS, Satola SW, Crispell EK, Heilmann KP, Dohrn CL, Riahi F, et al. Detection of *Staphylococcus aureus* isolates with heterogeneous intermediate-level resistance to vancomycin in the United States. *J Clin Microbiol* 2011; 49: 4203-7.

Sun W, Chen H, Liu Y, Zhao C, Nichols WW, Chen M, et al. Prevalence and characterization of heterogeneous vancomycin-intermediate *Staphylococcus aureus* isolates from 14 cities in China. *Antimicrob Agents Chemother* 2009; 53: 3642-9.

Hsuieh PR, Lee SY, Perng CL, Chang TY, Lu JJ. Clonal dissemination of metillin-resistant and vancomycin-intermediate *Staphylococcus aureus* in a Taiwanese hospital. *Int J Antimicrob Agents* 2010; 36(4): 307-12.

Fatholahzadeh B, Emaneini M, Gilbert G, Udo E, Aligholi M, Modarressi MH, et al. Staphylococcal cassette chromosome mec (SCCmec) analysis and antimicrobial susceptibility patterns of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates in Tehran. *Iran. Microb Drug Resist* 2008; 14(3): 217-20.

Namvar AE, Afsahr M, Asghari B, Rastegar Lari A. Characterisation of SCCmec elements in methicillin-resistant *Staphylococcus aureus* isolated from burn patients. *Burns* 2014; 40(4): 708-12.