INTRODUCTION

*Staphylococcus aureus* is the most important nosocomial pathogen and also the common cause of skin and soft tissue infection in the community (Alp et al., 2009). Whilst antibiotics are still used efficiently to control infections by these bacteria, there have been an increasing number of reports on the emergence of multiple-drug-resistant (MDR) *S. aureus*, especially among hospitalized patients (Rahimi et al., 2013a). Since the introduction of the first meticillin-resistant *S. aureus* (MRSA) strain in 1960 (Sakoulas & Moellering, 2008), MRSA has become established as the most prevalent pathogen in hospitals worldwide (Stryjewski & Chambers, 2008). MRSA strains have generally been confined to healthcare settings and predominantly affect individuals with comorbidities or other specific risk factors, such as prolonged hospital stay and nursing-home residency (Elston & Barlow, 2009). The staphylococcal cassette chromosome mec (SCCmec) element has additional characteristics such as bacterial virulence and transmissibility (Novick et al., 2010).

There are many reports indicating the emergence of MRSA infections in individuals in the community with no history of healthcare (Zetola et al., 2005). These infections have been attributed to new strains of MRSA, genetically and phenotypically distinct from the typical MDR hospital-acquired (HA)-MRSA. These strains, designated community-acquired (CA)-MRSA, whilst universally resistant to beta-lactam antibiotics, are typically susceptible to other anti-staphylococcal agents and often encode Panton–Valentine leukocidin (PVL), an exotoxin and a virulence factor (Vandenesch et al., 2003; Elston & Barlow, 2009). CA-MRSA appears to be associated with increased transmission and hospitalization, skin and soft tissue infection such as furuncles, cellulitis and skin abscesses, and, rarely, severe diseases such as necrotizing pneumonia (Elston & Barlow, 2009). Furthermore, MRSA infections occurring in the community among healthy individuals without risk factors are being reported with increasing frequency in

Characteristics of hospital- and community-acquired meticillin-resistant *Staphylococcus aureus* in Tehran, Iran

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*Staphylococcus aureus* is a leading cause of hospital-acquired (HA) and community-acquired (CA) infections worldwide. Recently, *S. aureus* strains resistant to meticillin (MRSA) have become established within both communities. We isolated 314 isolates of MRSA from hospitalized patients in a referral hospital (HA isolates) and 268 isolates from its outpatient clinic (CA isolates) in Tehran, Iran, between February 2008 and December 2010. These isolates were tested for their susceptibility to 17 antibiotics and typed using the PhPlate system. The diversity in the structures of staphylococcal cassette chromosome mec (SCCmec) elements and ccr types was also detected using a multiplex-PCR assay and isolates were examined for the presence of different classes of prophages. Whilst all isolates were resistant to penicillin, the HA isolates were significantly more resistant to all other antibiotics tested than the CA isolates. Isolates carrying only SCCmec type III and ccr type 3 were dominant (91 %), but 20 % of the CA isolates belonging to less prevalent types carried only SCCmec types IVa, c and ccr type 2. These isolates also carried *pvl* genes and contained SGA prophage type. Our results indicate that whilst the dominant clonal groups of HA- and CA-MRSA belong to SCCmec type III and carry ccr type 3 genes, several distinct but less prevalent types of CA-MRSA carrying SCCmec type IVa, c and type 2 ccr are also found in Tehran. These strains carry *pvl* genes and the SGA prophage type, a characteristic that might be used as a marker for detection of CA-MRSA in this country.

Abbreviations: CA, community-acquired; HA, hospital-acquired; MDR, multiple drug resistant; MRSA, meticillin resistant *Staphylococcus aureus*; PVL, Panton–Valentine leukocidin; SCCmec, staphylococcal cassette chromosome mec; TSST-1, toxic shock syndrome toxin-1.
different parts of the world, necessitating a change in the approach to empirical antimicrobial therapy (Maltezou & Giamarello, 2006; Bassetti et al., 2010).

Bacteriophages can, through horizontal gene transfer and lysogenic phage conversion, convert a non-virulent strain of *Staphylococcus* to a virulent one (Boyd & Brüssow, 2002). Phage incorporation into the *S. aureus* chromosome results in an increased ability of the bacterium to colonize the host tissues through ecological adaptation to human hosts by evasion of the immune system and production of virulence factors such as enterotoxins, staphylokinase, β-lysin, lipase, exfoliative toxin A, toxic shock syndrome toxin-1 (TSST-1) and PVL (Wilson & Salyers, 2003; Pantůček et al., 2004).

The classification of temperate phages of *S. aureus* (Siphoviridae) into six phage types with human disease implications has been proposed (Rahimi et al., 2012, 2013a). These groups include SGA (encoding PVL), SGB (encoding exfoliative toxin A, TSST-1 and lipase), SGF with two subtypes (SGFa/b) (encoding enterotoxins A, G, K and P, exfoliative toxin A, TSST-1 and lipase), SGF with two subtypes (SGFa/b) (encoding enterotoxins A, G, K and P, exfoliative toxin A, TSST-1 and lipase), SGF with two subtypes SGFa/b), L (SGL) and D (SGD) on the basis of their lytic activity, morphology and serological properties. Moreover, SGD (Twort-like phages) phage type is related to lytic phages and is a member of the family Myoviridae (Pantůček et al., 2004; Workman et al., 2006).

In this study we aimed to characterize CA-MRSA and HA-MRSA isolates from patients in a referral hospital in Tehran, Iran, and its outpatient clinic with respect to the structure of SCCmec elements and ccr types as well as the presence of different classes of prophages.

**METHODS**

**Sample collection and isolation of *S. aureus***. Between February 2008 and December 2010, a total of 1722 isolates of *S. aureus* were initially isolated from hospitalized patients with staphyloccocal infections in a tertiary-care hospital in central Tehran that offers subspecialty care (*n*=1016) and its outpatient clinic (*n*=706). The information on each patient, including sex, age, date and location of sampling (outpatient or inpatient), was collected from the laboratory information system. For inpatient isolates, the number of days from admission to culture procurement was determined by subtracting the admission date from the procurement date. The frequency of *S. aureus* strains isolated from different sources is shown in Table 1. The inclusion criteria for the hospitalized patients specified those who were inpatients for at least 72 h.

All isolates were identified at the genus level after an initial isolation on blood agar (Merck), using biochemical tests such as growth at 10–15% NaCl, positive catalase and negative oxidase reactions, mannitol fermentation, DNase and coagulase tests (Kateet et al., 2010), and confirmed as *S. aureus* using species-specific primers for the *nucA* gene (see below). *S. aureus* (ATCC 29213) and *Staphylococcus epidermidis* (ATCC 35984) were used as positive and negative controls respectively.

**Antibiotic susceptibility tests**. According to the guidelines of the Clinical and Laboratory Standard Institute (CLSI, 2006), all *S. aureus* isolates were examined for susceptibility to oxacillin (1 μg), using the disc diffusion method. E-testing was used to determine the MICs for oxacillin and vancomycin of all identified MRSA isolates, according to the manufacturer’s instructions. Sixteen common antibiotics were employed to determine the susceptibility of the MRSA isolates by the disc diffusion method as described by the CLSI (2006). These included amikacin (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), clindamycin (2 μg), erythromycin (15 μg), gentamicin (10 μg), kanamycin (30 μg), linezolid (30 μg), minocycline (30 μg), nitrofurantoin (300 μg), penicillin (5 μg), rifampicin (2 μg), sulphamethoxazole–trimethoprim (1.25–23.75 μg), quinupristin–dalfopristin (15 μg), tetracycline (30 μg) and tobramycin (10 μg) (Mast Diagnostics). Also, all isolates were tested for susceptibility to fusidic acid (10 μg) by the disc diffusion assay (McLaws et al., 2011).

**DNA extraction and PCR assay**. The High Pure PCR Template Preparation kit (Roche) was used to extract DNA according to the manufacturer’s guidelines with some modifications. For the measurement of DNA concentration, Nanodrop 1000 (Nanodrop) was employed. One microlitre of each extracted DNA was used as a PCR template. PCR primers for *nucA* and *mecA* genes were as described previously by Du et al. (2002). PCR conditions were as described previously (Rahimi et al., 2012).

**SCCmec and ccr typing**. A multiplex PCR typing assay that contained eight pairs of primers including the unique and specific primers for SCCmec types and subtypes I, II, III, IVa, IVb, IVc, Vd and V was used for typing of the SCCmec gene (Zhang et al., 2005). Another multiplex PCR assay was used for characterization of ccr gene complexes employing four sets of primers specific for each of the ccr genes, i.e. ccr-AB2, ccr-AB-22, ccr-AB-x3 and ccr-AB-x4 (Zhang et al., 2005).

The multiplex PCR mixture contained 10× PCR buffer, Taq DNA polymerase (0.5 U) (HT Biotechnology), each primer (1.6 μM), MgCl2 (1.2 μM) and each of dNTPs (0.64 μM). The PCR cycles were as previously described (Zhang et al., 2005).

**Prophage typing**. According to Pantůček et al. (2004), 3A, 11, 77, 187 and Twort-like phage genes [serogroups A (SGA), B (SGB), F (SGF), with two subtypes SGFa/b), L (SGL) and D (SGD)] were used for prophage typing as described previously (Rahimi et al., 2012, 2013a).

**Detection of *pvl* genes**. For detection of *pvl* genes encoding PVL toxin among MRSA isolates, specific primers were used as described by McClure et al. (2006). PCR mixtures contained 10× PCR buffer, Taq DNA polymerase (0.5 U) (HT Biotechnology), each primer (1.6 μM), MgCl2 (1.2 μM) and each of dNTPs (0.64 μM). The PCR cycles were carried out as described by McClure et al. (2006).

**Typing of MRSA isolates**. A biochemical fingerprinting method (the PhenePlate system), specifically developed for *S. aureus* strains (PhP-CS, PhPPlate), was used to type MRSA strains (Persson et al., 2006). The fingerprinting method was performed following the manufacturer’s guidelines. Briefly, a loop of a fresh bacterial culture was inoculated in 10 ml of PhPlate growth medium containing 0.2% (w/v) proteose peptone, 0.05% (w/v) yeast extract, 0.5% (w/v) NaCl and 0.011% (w/v) bromothymol blue. Aliquots of 175 μl of each bacterial suspension (582 isolates) were inoculated into the 24 wells of each set using a multichannel pipette. Plates were then incubated at 37 °C and scanned at intervals of 16, 40 and 64 h using an HP Scanjet 4890 scanner (Talebli et al., 2007). After the final scan, the PhPlate software (PhPWin 4.2) was used to import the images and create absorbance data for each reading according to the manufacturer’s instructions. The mean values of all three readings were compared pair-wise, and the similarity among the strains was determined as a correlation (similarity) coefficient. The similarity matrix thus obtained was then clustered according to the unweighted pair group method with arithmetic averages (UPGMA) to obtain a dendrogram.
An identity level of 0.975 was established based on the reproducibility of the system after testing 20 isolates in duplicate. Strains showing similarities to each other above this level were considered as identical and named common biochemical phenotypes (C-BPT). The diversity of the bacterial populations was calculated as Simpson’s index of diversity (Di) (Sneath & Sokal, 1973; Talebi et al., 2007).

Statistical analysis. Data were tested for univariate comparisons of categorical results by Fisher’s exact test using GraphPad Prism 5.0 (GraphPad Software). Differences with P values below 0.05 were considered statistically significant.

RESULTS

Prevalence of MRSA isolates
Of the 1722 S. aureus isolates collected from hospitalized patients and clinic outpatients, 582 isolates (33.8 %) were shown to be MRSA. Of these, 314 were HA isolates and the rest (268) came from the outpatient clinic and were regarded as CA isolates. CA-MRSA strains were defined as all MRSA isolates obtained from outpatients and also hospitalized patients isolated within 72 h after admission to the hospital.

Antibiotic resistance patterns of MRSA isolates
All MRSA isolates (CA and HA) were resistant to penicillin whereas 91 and 90 %, respectively, showed resistance to erythromycin and ciprofloxacin (Table 2). Furthermore, a high level of resistance (ranging from 83 to 88 %) was found against tobramycin, tetracycline, clindamycin, amikacin and kanamycin (Table 2). All MRSA isolates were susceptible to quinupristin–dalfopristin, linezolid and vancomycin, with low resistance against chloramphenicol, nitrofurantoin and fusidic acid. The rate of antibiotic resistance among HA-MRSA strains was significantly higher than among CA-MRSA strains, except for penicillin, tetracycline and clindamycin, where there were no differences between the two groups (Table 2).

In all, 529 (91 %) of the 582 MRSA strains were resistant to 2–15 antibiotics (Table 3). The highest number of MRSA isolates susceptible to all antibiotics tested belonged to CA-MRSA strains (n=53), compared with HA-MRSA strains (n=0). In contrast, HA-MRSA isolates showed resistance to five or more antibiotics (n=308; 98 %), which was significantly higher (P<0.0001) than that found in CA-MRSA isolates (n=198; 74 %) (Table 3).

The MIC of both CA-MRSA and HA-MRSA strains was determined using the E-test. The results showed that 75 % of HA isolates had a high-level resistance (MIC ≥128 µg ml⁻¹) to oxacillin, with only 1 % showing MIC ≥24 µg ml⁻¹ (Table 4). In contrast, 20 % of CA isolates had a low-level resistance (MIC ≥4 µg ml⁻¹) to oxacillin and 36 % had an MIC ≥128 µg ml⁻¹. In this study, neither HA-MRSA strains nor CA-MRSA isolates showed resistance to vancomycin.

Forty-six per cent of MRSA isolates were from wounds and they mainly belonged to SCCmecIII type. This type was also common among the strains isolated from other sources (Table 5).

Prophage typing

With the exception of the Twort-like (SGD) and SGL, all types of prophages were detected using single PCR (Table 6). SGA, SGB, SGF, SGFa and SGFb prophage genes were detected in 53 (9.1 %), 315 (54.1 %), 582 (100 %), 582 (100 %) and 582 (100 %) of the isolates, respectively. PCR also showed that all isolates contained at least one prophage serogroup and two subgroups. The SGF serotype was present in 100 % of the MRSA isolates; SGFa and SGFb were the dominant (100 %) subtypes among the isolates. Four different patterns were identified among the MRSA isolates, with pattern 3 being the dominant pattern (48.3 %) (Table 6). Pattern 4 with SGF prophage and its two subgroups constituted 42.6 % of the isolates. The lowest frequency of phages was SGA (9.1 %) and pattern 2,
including SGA, SGF, SGFa and SGFb, with 3.3% of the isolates (Table 6).

**SCCmec and ccr typing**

In all, 529 MRSA isolates carried SCCmec type III and were PCR positive with the ccrAB-2a-specific primers indicating the presence of type 3 ccr. Moreover, 53 isolates that showed low resistance to oxacillin (MIC = 4 µg ml⁻¹) carried SCCmec type IV and type 2 ccr. These isolates belonged to SGA prophage type, carried pvl gene and were isolated from wounds (58.5%), urine (20.8%), CSF (11.3%) and blood (9.4%), respectively (Table 5). Furthermore, they showed susceptibility to all antibiotics tested except for penicillin. The presence of the pvl gene among the MRSA isolates was limited to low-level oxacillin resistance (MIC = 4 µg ml⁻¹).

**Typing of MRSA isolates**

Typing of 582 isolates showed the presence of 33 PhP types consisting of 18 common (C) (n=567) and 15 single (S) PhP types (Table 7). The HA-MRSA isolates (n=314) belonged to 14 C-PhP types, whereas CA-MRSA strains were more diverse and consisted of 15 S- and 16 C-PhP types.

All isolates belonging to C-PhP types 1–14 amongst CA- and HA-MRSA isolates (n=529 isolates) carried SCCmec type III and harboured type 3 ccr. On the other hand, all S- and C-PhP types 15–18 (53 isolates) belonged to CA-group and carried SCCmec type IV, type 2 ccr and SGA prophage type (Table 7).

### DISCUSSION

This is the first report on the prevalence and typing of CA-MRSA strains and their clonal dissemination compared with HA-MRSA strains in Iran. We combined a high-resolution PhP typing assay, SCCmec and prophage typing to confirm the presence of certain clonal groups of S. aureus in the hospital. According to the PhP typing results, a majority of the isolates belonged to 18 C-types with minor S-types found only amongst CA-MRSA isolates. The prevalence of CA-MRSA isolates has been reported in different studies using different typing methods (Trindade et al., 2005; Rossney et al., 2007; Cercenado et al., 2008; Chua et al., 2008; Wu et al., 2010; Brennan et al., 2012; Mediavilla et al., 2012). In accordance with our findings, these CA isolates belonged to clones that are different from HA-MRSA isolates and only showed resistance to beta-lactam antibiotics, with susceptibility to other classes of antibiotics.

Prevalence estimates of HA-MRSA strains in Iran vary from 19.2% to 80% (Fatholahzadeh et al., 2008; Rahimi et al., 2009, 2012, 2013a, b; Japoni et al., 2011; Javidnia et al., 2013). This huge difference in prevalence of HA-MRSA isolates in Iran could be due in part to the number of patients studied and the geographical locations of

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**Table 2. Rate of resistance of MRSA strains isolated in this study against the 18 antibiotics tested**

<table>
<thead>
<tr>
<th>Antimicrobial drug (disc concentration)</th>
<th>Source of MRSA</th>
<th>Total (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HA (%)</td>
<td>CA (%)</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>314 (100)</td>
<td>268 (100)</td>
<td>582 (100)</td>
</tr>
<tr>
<td>E</td>
<td>313 (99)</td>
<td>215 (80)</td>
<td>528 (91)</td>
</tr>
<tr>
<td>CIP</td>
<td>313 (99)</td>
<td>213 (79)</td>
<td>524 (90)</td>
</tr>
<tr>
<td>TN</td>
<td>302 (96)</td>
<td>210 (78)</td>
<td>512 (88)</td>
</tr>
<tr>
<td>T</td>
<td>308 (98)</td>
<td>198 (74)</td>
<td>506 (87)</td>
</tr>
<tr>
<td>CD</td>
<td>304 (97)</td>
<td>196 (73)</td>
<td>500 (86)</td>
</tr>
<tr>
<td>AN</td>
<td>307 (98)</td>
<td>192 (72)</td>
<td>499 (86)</td>
</tr>
<tr>
<td>K</td>
<td>312 (99)</td>
<td>171 (64)</td>
<td>483 (83)</td>
</tr>
<tr>
<td>SXT</td>
<td>292 (93)</td>
<td>139 (52)</td>
<td>431 (74)</td>
</tr>
<tr>
<td>RP</td>
<td>295 (94)</td>
<td>130 (49)</td>
<td>425 (73)</td>
</tr>
<tr>
<td>GM</td>
<td>279 (89)</td>
<td>134 (50)</td>
<td>413 (71)</td>
</tr>
<tr>
<td>MN</td>
<td>189 (60)</td>
<td>90 (34)</td>
<td>279 (48)</td>
</tr>
<tr>
<td>FC</td>
<td>35 (11)</td>
<td>18 (7)</td>
<td>53 (9)</td>
</tr>
<tr>
<td>NI</td>
<td>29 (9)</td>
<td>6 (2)</td>
<td>35 (6)</td>
</tr>
<tr>
<td>C</td>
<td>11 (4)</td>
<td>1 (0.4)</td>
<td>12 (2)</td>
</tr>
<tr>
<td>VA*</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>LZD</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SYN</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Resistance was measured using the E-test.

*Resistance was measured using the E-test.

*, No significant difference. P, penicillin; E, erythromycin; CIP, ciprofloxacin; TN, tobramycin; T, tetracycline; CD, clindamycin; AN, amikacin; K, kanamycin; SXT, cotrimoxazole; RA, rifampicin; GM, gentamicin; MN, minocycline; FC, fusidic acid; NI, nitrofurantoin; C, chloramphenicol; VA, vancomycin; SYN, quinupristin–dalfopristin; LZD, linezolid.

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hospitals as well as the methodology used. Our findings confirmed the high diversity among the CA-MRSA isolates in Iran. The majority of these strains were isolated from wound infections, which is a common form of \textit{S. aureus} infection in the community (David & Daum, 2010). Nonetheless, the prevalence of sources from which these strains were isolated is similar to those reported worldwide (Naimi et al., 2003; Huang et al., 2006).

In contrast to HA-MRSA isolates, CA-MRSA strains were more susceptible to different classes of antibiotics, which is consistent with reports published elsewhere (Trindade et al., 2005; Huang et al., 2006; David & Daum, 2010). Apart from penicillin resistance, the CA- strains in our study showed a high level of resistance to erythromycin similar to that found in the USA (Huang et al., 2006) but much higher than that seen in other studies (Charlebois et al., 2002; Baggett et al., 2003). We also found that the rate

Table 3. Antimicrobial resistance patterns for \textit{S. aureus} isolated from hospitals

Data are expressed as number (%) of resistant strains.

<table>
<thead>
<tr>
<th>No. of strains resistant to:</th>
<th>HA-MRSA $n=314$</th>
<th>CA-MRSA $n=268$</th>
<th>Total $n=582$</th>
</tr>
</thead>
<tbody>
<tr>
<td>One antibiotic</td>
<td>0</td>
<td>53 (20)</td>
<td>53 (9)</td>
</tr>
<tr>
<td>Two antibiotics</td>
<td>2 (0.6)</td>
<td>2 (0.7)</td>
<td>4 (0.7)</td>
</tr>
<tr>
<td>Three antibiotics</td>
<td>1 (0.3)</td>
<td>0</td>
<td>1 (0.2)</td>
</tr>
<tr>
<td>Four antibiotics</td>
<td>3 (1)</td>
<td>0</td>
<td>3 (0.5)</td>
</tr>
</tbody>
</table>

Table 4. MIC range in different MRSA isolates

MRSA had MIC $\geq 4 \mu g \text{ ml}^{-1}$. Data expressed as $n$ (%).

<table>
<thead>
<tr>
<th>MIC ((\mu g \text{ ml}^{-1}))</th>
<th>256</th>
<th>128</th>
<th>96</th>
<th>64</th>
<th>32</th>
<th>24</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA-MRSA</td>
<td>18 (7)</td>
<td>79 (29)</td>
<td>38 (14)</td>
<td>7 (3)</td>
<td>17 (6)</td>
<td>56 (21)</td>
<td>53 (20)</td>
</tr>
<tr>
<td>HA-MRSA</td>
<td>144 (45)</td>
<td>93 (30)</td>
<td>21 (7)</td>
<td>23 (7)</td>
<td>31 (10)</td>
<td>2 (1)</td>
<td>0</td>
</tr>
</tbody>
</table>
of resistance to ciprofloxacin among our isolates was unusually higher than that found in other studies (Huang et al., 2006; Chung et al., 2008), which could be due to the use of this antibiotic in Iran. We also found a much higher rate of resistance to clindamycin among our CA-MRSA isolates compared with the USA (Huang et al., 2006) and Korea (Chung et al., 2008) suggesting that clindamycin may not be a drug of choice for treatment of MRSA infections in Iran.

In this research, both the HA- and CA-MRSA isolates were susceptible to vancomycin, linezolid and quinupristin–dalfopristin. These antibiotics could therefore be the most effective antibiotics against infections caused by MRSA strains if the prevalence of MRSA strains increases in Iran because there are no reports of MRSA resistance to linezolid and quinupristin–dalfopristin (Fatholahzadeh et al., 2008; Javidnia et al., 2011; Japoni et al., 2012, 2013a, b). Whilst the lack of resistance to vancomycin in our study was consistent with other studies in Iran and elsewhere (Chung et al., 2008; Fatholahzadeh et al., 2008; Rahimi et al., 2009, 2012, 2013a, b; Japoni et al., 2011; Javidnia et al., 2013), Thompson and colleagues reported a prevalence of 13% of vancomycin-resistant S. aureus (VRSA) and vancomycin intermediate S. aureus (VISA) isolates found in community sewage-treatment plants in Australia (Thompson et al., 2013).

Here we also showed that the frequency of SCCmec type III was almost 91% followed by 9.1% of SCCmec type IV, while Japoni et al. (2011) were able to isolate strains with SCCmec types II, III, IVa, IVc, IVd and V in the south of Iran. It might be due, in part, to a higher number of outpatients in comparison with inpatients tested in that study, and also the differences in the geographical regions where studies were carried out.

Here, all MRSA isolates that shared SCCmec type IV (a or c) showed susceptibility to all classes of antibiotics except for penicillin. This is contrary to other studies (Davis et al., 2006), suggesting that SCCmec type IV strains may acquire resistance to non-beta-lactam antibiotics in order to survive in the hospital environment or through exposure to these antibiotics. It might also be due in part to their new distribution from community to hospital.

PVL is a bacteriophage-encoded virulence factor of S. aureus that has been linked to furuncles, cutaneous abscesses, severe necrotic skin infections and severe necrotizing pneumonia (Labandeira-Rey et al., 2007; Otter & French, 2011). In our study, 9.1% of the MRSA isolates were PVL positive. While PVL is associated with an increased incidence of the above-mentioned S. aureus infections, the result is not surprising, since other investigators have suggested that PVL is not an important virulence factor in the pathogenesis of staphylococcal bacteraemia (Alp et al., 2009). Interestingly, PVL was found in CA-MRSA strains and was absent in HA-MRSA isolates, but there are some reports highlighting the prevalence of CA-MRSA strains without the pvl gene (Lina et al., 1999).

In our study, four different prophage patterns were detected. Different prophage patterns have been already observed among the MRSA strains isolated in other countries (Pantuček et al., 2004; Workman et al., 2006; Rahimi et al., 2012, 2013a). In reports published by Pantuček et al. (2004) in the Czech Republic, Workman et al. (2006) in the USA, and our group (Rahimi et al., 2012, 2013a) in Iran, 9, 10, 8 and 4 prophage patterns have been identified, respectively. Also, different dominant patterns of prophage including SGA of human source, SGF and SGFb of human source and SGA of costal water source have been reported in the Czech Republic, Iran, and the USA, respectively. Different

### Table 5. Prevalence of different SCCmec types and their indicator among MRSA strains isolated from different samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>SCCmec III</th>
<th>SCCmec IVa</th>
<th>SCCmec IVc</th>
<th>pvl</th>
<th>SGA prophage</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abscess</td>
<td>18</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>18</td>
</tr>
<tr>
<td>Blood</td>
<td>16</td>
<td>4</td>
<td>1</td>
<td>5</td>
<td>5</td>
<td>21</td>
</tr>
<tr>
<td>Ear</td>
<td>4</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>4</td>
</tr>
<tr>
<td>Eye</td>
<td>9</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>9</td>
</tr>
<tr>
<td>Nose</td>
<td>43</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>43</td>
</tr>
<tr>
<td>Sputum</td>
<td>81</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>81</td>
</tr>
<tr>
<td>CSF</td>
<td>39</td>
<td>6</td>
<td>–</td>
<td>6</td>
<td>6</td>
<td>45</td>
</tr>
<tr>
<td>Urine</td>
<td>85</td>
<td>10</td>
<td>1</td>
<td>11</td>
<td>11</td>
<td>96</td>
</tr>
<tr>
<td>Wound</td>
<td>234</td>
<td>23</td>
<td>8</td>
<td>31</td>
<td>31</td>
<td>265</td>
</tr>
</tbody>
</table>

–, No SCCmec type IV (a or c), pvl gene and SGA prophage found.

### Table 6. The frequency of prophage patterns among MRSA isolates

<table>
<thead>
<tr>
<th>Phage pattern</th>
<th>Phage type</th>
<th>No. of isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SGA</td>
<td>SGB</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
ecological settings and locations of these studies may be the causes of the differences observed. In the current study, 51 and 32% of the sewage and clinical isolates showed SGF, SGFa, SGFb and SGB prophage patterns, respectively, which were similar to the patterns reported in our previous study (Rahimi et al., 2013a). Therefore, the circulation of MRSA clonal types in STPs isolated from different hospitals and in community is suggested. It has to be noted that all isolates with SGA prophage type (prophage patterns 1 and 2) belonged to single types whereas the MRSA strains lacking SGA (i.e. prophage patterns 3 and 4) belonged to common types. These results suggest that the presence of SGA prophage type among MRSA strains in this country may be correlated with the source as CA or HA strains.

We also isolated 53 strains that showed different characteristics compared with other MRSA isolates. These isolates were susceptible to all antibiotics tested (except penicillin) with a low level of resistance to oxacillin (MIC=4 μg ml⁻¹). Moreover, they harboured SCCmeC type IV and type 2 ccr, and also contained the pvl gene. These findings are consistent with the definition of CA-MRSA isolates (David & Daum, 2010; Mediavilla et al., 2012). In addition, we also found a new characteristic in CA-MRSA strains tested. Prophage type SGA, which is responsible for phage encoded pvl gene, was common among CA-MRSA isolates. This gene was detected in all 53 MRSA isolates mentioned above, and none of the other isolates harboured this type of prophage type. In addition, the susceptibility of these isolates to different classes of antibiotics tested was consistent with a report from South Korea (Chung et al., 2008), but is in contrast to another study from Korea (Lee et al., 2004).

Similar to other studies undertaken in Iran (Fatholahzadeh et al., 2008; Japoni et al., 2011), our findings showed that

<table>
<thead>
<tr>
<th>PhP type</th>
<th>No. of isolates (%)</th>
<th>Total no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCCmek ccr pvl SGA prophage type</td>
<td>HA-MRSA</td>
<td>CA-MRSA</td>
</tr>
<tr>
<td>CT1 III 3 – –</td>
<td>22 (7)</td>
<td>–</td>
</tr>
<tr>
<td>CT2 III 3 – –</td>
<td>76 (24)</td>
<td>–</td>
</tr>
<tr>
<td>CT3 III 3 – –</td>
<td>109 (35)</td>
<td>22 (26)</td>
</tr>
<tr>
<td>CT4 III 3 – –</td>
<td>2 (1)</td>
<td>2 (1)</td>
</tr>
<tr>
<td>CT5 III 3 – –</td>
<td>10 (3)</td>
<td>9 (3)</td>
</tr>
<tr>
<td>CT6 III 3 – –</td>
<td>7 (2)</td>
<td>5 (2)</td>
</tr>
<tr>
<td>CT7 III 3 – –</td>
<td>1 (0.5)</td>
<td>3 (1)</td>
</tr>
<tr>
<td>CT8 III 3 – –</td>
<td>8 (2)</td>
<td>9 (3)</td>
</tr>
<tr>
<td>CT9 III 3 – –</td>
<td>23 (7)</td>
<td>18 (7)</td>
</tr>
<tr>
<td>CT10 III 3 – –</td>
<td>4 (1)</td>
<td>–</td>
</tr>
<tr>
<td>CT11 III 3 – –</td>
<td>2 (1)</td>
<td>2 (1)</td>
</tr>
<tr>
<td>CT12 III 3 – –</td>
<td>2 (1)</td>
<td>4 (1.5)</td>
</tr>
<tr>
<td>CT13 III 3 – –</td>
<td>1 (0.5)</td>
<td>5 (2)</td>
</tr>
<tr>
<td>CT14 III 3 – –</td>
<td>47 (15)</td>
<td>33 (12)</td>
</tr>
<tr>
<td>CT15 – – – –</td>
<td>–</td>
<td>IVc 2 + +</td>
</tr>
<tr>
<td>CT16 – – – –</td>
<td>–</td>
<td>IVa 2 + +</td>
</tr>
<tr>
<td>CT17 – – – –</td>
<td>–</td>
<td>IVa 2 + +</td>
</tr>
<tr>
<td>CT18 – – – –</td>
<td>–</td>
<td>IVa 2 + +</td>
</tr>
<tr>
<td>ST1 – – – –</td>
<td>–</td>
<td>IVa 2 + +</td>
</tr>
<tr>
<td>ST2 – – – –</td>
<td>–</td>
<td>IVa 2 + +</td>
</tr>
<tr>
<td>ST3 – – – –</td>
<td>–</td>
<td>IVa 2 + +</td>
</tr>
<tr>
<td>ST4 – – – –</td>
<td>–</td>
<td>IVa 2 + +</td>
</tr>
<tr>
<td>ST5 – – – –</td>
<td>–</td>
<td>IVa 2 + +</td>
</tr>
<tr>
<td>ST6 – – – –</td>
<td>–</td>
<td>IVa 2 + +</td>
</tr>
<tr>
<td>ST7 – – – –</td>
<td>–</td>
<td>IVa 2 + +</td>
</tr>
<tr>
<td>ST8 – – – –</td>
<td>–</td>
<td>IVc 2 + +</td>
</tr>
<tr>
<td>ST9 – – – –</td>
<td>–</td>
<td>IVa 2 + +</td>
</tr>
<tr>
<td>ST10 – – – –</td>
<td>–</td>
<td>IVa 2 + +</td>
</tr>
<tr>
<td>ST11 – – – –</td>
<td>–</td>
<td>IVa 2 + +</td>
</tr>
<tr>
<td>ST12 – – – –</td>
<td>–</td>
<td>IVc 2 + +</td>
</tr>
<tr>
<td>ST13 – – – –</td>
<td>–</td>
<td>IVc 2 + +</td>
</tr>
<tr>
<td>ST14 – – – –</td>
<td>–</td>
<td>IVa 2 + +</td>
</tr>
<tr>
<td>ST15 – – – –</td>
<td>–</td>
<td>IVc 2 + +</td>
</tr>
</tbody>
</table>
SCC\textit{mec} type III was the dominant type in HA-MRSA and CA-MRSA isolates followed by SCC\textit{mec} type IV (a or c). All CA-MRSA isolates that shared SCC\textit{mec} type IV (a or c) showed susceptibility to all classes of antibiotics except for penicillin. This is contrary to other reports suggesting that strains harbouring SCC\textit{mec} type IV can acquire resistance to other classes of antibiotics to survive in the hospital environment. This might be due, in part, to bacterial dissemination from community to hospital. High prevalence of SCC\textit{mec} type III and type 3 \textit{ccr} as indicators of hospital acquired MRSA in sewage strains suggests the clinical origin of these isolates. Nonetheless, the frequency of CA-MRSA in our study was higher than previously reported in Iran (Fatholahzadeh et al., 2008), suggesting an increase in the frequency of CA-MRSA infection in Tehran.

**CONCLUSIONS**

Our results illustrate the presence and persistence of highly resistant clonal groups of HA- and CA-MRSA strains in Tehran with the possibility that hospitals could be the reservoir for dissemination of these strains in the community. In addition, we found that CA-MRSA isolates contained specific prophage patterns that differed from the clinical isolates reported in this country and elsewhere. We suggest that the presence of SGA prophage type could also be another characteristic in addition to SCC\textit{mec} type IV and \textit{pvl} gene in CA-MRSA strains.

**ACKNOWLEDGEMENTS**

This research was funded by a grant from Ministry of Health of Iran, Deputy of Research and Innovation.

**REFERENCES**


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