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

Fateh Rahimi[1], Mohammad Katouli[2], Mohammad Reza Pourshafie[3*]

[1]Department of Microbiology, Faculty of Science, University of Isfahan, Iran
[2]Genecology Research Centre, Faculty of Science, Health and Education and Engineering, University of the Sunshine Coast, Queensland, Australia and
[3]Department of Microbiology, Pasteur Institute of Iran, Tehran, Iran

Running title: HA and CA-MRSA in Iran

* Corresponding author:

Mohammad Reza Pourshafie, Department of Microbiology, Pasteur Institute of Iran, Pasteur Ave, Tehran, 13164, Iran.

Email: pour@pasteur.ac.ir

Abstracts

Staphylococcus aureus is a leading cause of hospital-(HA) and the community-acquired (CA) infections worldwide. Recently, *S. aureus* strains resistant to methicillin (MRSA) have become established within both communities. We isolated 314 isolates of MRSA from hospitalized patients in a referral hospital (HA isolates) and its outpatient clinic (n=268) (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 structure of SCC*mec* elements and *ccr* types were 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 SCC*mec* type III and *ccr* type 3 were dominant (91%), but 20% of the CA-isolates belonging to less prevalent types, carried only SCC*mec* types IVa, c and *ccr* type 2. These isolates also carried *pvl* gene and contained SGA prophage type. Our results indicate that whilst the dominant clonal groups of HA- and CA-MRSA belong to SCC*mec* type III and carry *ccr* type 3 genes, several distinct but less prevalent types of CA-MRSA carrying SCC*mec* type IVa, c and type 2 *ccr* are also found in Tehran. These strains carry *pvl* genes and SGA prophage type, a characteristic that may be used as a marker for detection of CA–MRSA in this country.

Key words: CA-MRSA, HA-MRSA, SCC*mec* and *ccr* type, PhPlate, prophage

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 first methicillin resistance *S. aureus* (MRSA) strain in 1960 (Sakoulas and Moellering 2008), MRSA has become established as the most prevalent pathogen in hospitals worldwide (Stryjewski and Chamber 2008). MRSA strains have generally been confined to healthcare settings and predominantly affected individuals with co-morbidity or other specific risk factors, such as prolonged hospital stay and nursing home residency (Elston and Barlow 2009). Staphylococcal cassette chromosome *mec* (SCC*mec*) element has but other 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 MRSA (HA-MRSA). These strains, designated community-acquired MRSA (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 and Barlow 2009). CA-MRSA appear 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 and Barlow 2009). Furthermore, MRSA infections occurring in

the community among healthy individuals without risk factors are being reported with increasing frequency in different parts of the world necessitating a change in the approach to empirical antimicrobial therapy (Maltezou and Giamarellou 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 and Brüssow 2002). Prophage incorporation into *S. aureus* chromosome results in an increased ability of the bacteria to colonize the host tissues through ecological adaptation to human host by evasion from 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 and Salyers 2003; Pantůček et al. 2004).

The classification of temperate phages of *S. aureus* (*Siphoviridae*) into six phage types with human disease implications, have been proposed before (Rahimi et al. 2012; Rahimi et al. 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, staphylokinase and β-lysin) and SGL 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 *Myoviridae* family (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 SCC*mec* 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 (n= 1016) in a tertiary-care hospital that offers subspecialty care in central part of Tehran and its outpatient clinic (n=706) with staphylococcal infections. The information of each patient, including the sex and age, date and location of sampling (outpatient and inpatient), was collected from 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 included those that were admitted for 72 hrs.

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

Antibiotic susceptibility tests

According to the guidelines of Clinical and Laboratory Standards Institute (CLSI) (Clinical and Laboratory Standard Institute 2006), all *S. aureus* isolates were examined for susceptibility to oxacillin (1 µg), using disc diffusion method. E-test (AB, Biomerieux, Marcy l'Etoile, France) 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 disc diffusion method as described by 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-trimethoprime (1.25-23.75 μg), quinupristin – dalfopristin (15 μg), tetracycline (30 μg) and tobramycin (10 μg) (Mast Diagnostics, Merseyside, United Kingdom). Also, all isolate were tested for susceptibility to fusidic acid (10 μg) by disc diffusion assay (McLaws et al. 2011).

DNA extraction and PCR assay

High Pure PCR Template Preparation kit (Roche, Mannheim, Germany) was used to extract DNA according to manufacturer's guidelines with some modifications. For the measurement of DNA concentration, Nanodrop 1000 (NanoDrop, Wilmington, USA) was employed. One microliter of each extracted DNA was used as a template in PCR reaction. *nucA* and *mecA* genes PCR primers were according to Du *et al*. (Du et al. 2002). PCR conditions were as described previously (Rahimi et al. 2012).

SCC*mec* and *ccr* typing

A multiplex PCR typing assay was used for typing of SCC*mec* gene which contained 8 pairs of primers including the unique and specific primers for SCC*mec* types and subtypes I, II, III, IVa, IVb, IVc, IVd, and V (Zhang et al. 2005). Another multiplex PCR assay was used for characterization of *ccr* gene complexes which employed four sets of primers specific for each of the *ccr* genes i.e. *ccr*AB-β2, *ccr*AB-α2, *ccr*AB-α3, and *ccr*AB-α4 (Zhang et al. 2005).

The multiplex PCR mixture contained 10X PCR buffer, Taq DNA polymerase (0.5 U) (HT Biotechnology, Cambridge, United Kingdom), 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 Pantucek and colleagues (Pantůček et al. 2004), 3A, 11, 77, 187 and Twort-like phage genes (serogroups A (SGA), B (SGB), F (SGF, with 2 subtypes SGFa/b), L (SGL) and D (SGD)) were used for prophage typing as described previously (Rahimi et al. 2012; Rahimi et al. 2013a).

Detection of pvl gene

For detection of *pvl* gene encoding PVL toxin among MRSA isolates, specific primers were used as described by McClure *et al*. (2006). PCR mixture contained 10X PCR buffer, Taq DNA polymerase (0.5 U) (HT Biotechnology, Cambridge, United Kingdom), each primer (1.6 μM), MgCl2 (1.2 μM) and each of dNTPs (0.64 μM). The PCR cycles were according to McClure *et al*. (McClure et al. 2006).

Typing of MRSA isolates

A biochemical fingerprinting method (the PhPlate system), specifically developed for *S. aureus* strains (PhP-CS, PhPlate AB, Stockholm, Sweden), was used to type MRSA strains (Persson et al. 2006). The fingerprinting method was performed according to the guidelines of manufacturer. In summary, a loopful of a fresh bacterial culture was inoculated in 10mL of PhPlate growth medium containing 0.2% (w/v) proteose peptone, 0.05% (w/v) yeast extract, and 0.5% (w/v) NaCl and 0.011% (w/v) bromothymol blue. Aliquots of 175μL of each bacterial suspension (582 isolates) were inoculated into the 24 wells of each set by the aid of a multichannel pipette. Plates were then incubated at 37°C and scanned at intervals of 16, 40 and 64 h using HP Scanjet 4890 scanner (Talebi et al. 2007). After the final scan, the PhPlate software (PhPWin 4.2) was used to import the images and creates 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 correlation (similarity) coefficient. The obtained similarity

matrix was then clustered according to the unweighted pair group method with arithmetic averages (UPGMA) to get 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 as common biochemical phenotypes (C-BPT). The diversity of the bacterial populations was calculated as Simpson's index of diversity (Di) (Sneath and 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 outpatient clinic, 582 (33.8%) isolates were shown to be MRSA. Of these, 314 isolates came from hospitalized patients (HA-isolates) and the rest (268) came from 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 hours 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% showed resistance to erythromycin and ciprofloxacin respectively (Table 2). Furthermore, a high level of resistance (ranging between 83%-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 was significantly higher than CA-MRSA strains, except for penicillin, tetracycline and clindamycin where there was no difference 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 5 antibiotics 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) to oxacillin, with only 1% showing MIC≥24 µg/ml (Table 4). In contrast, 20% of CA-isolates had a low level resistance (MIC≥4 µg/ml) to oxacillin and 36% showing MIC≥128 µg/ml. In this study neither of HA-MRSA strains nor CA-MRSA isolates showed resistance to vancomycin.

Forty six percent of MRSA isolates were from wounds and they mainly belonged to SCC*mec*III type. This type was also common among the strains isolated from other sources (Table 5).

Prophage typing

With 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 proghage genes were detected in 53 (9.1%), 315 (54.1%), 582 (100%), 582 (100%) and 582 (100%) of the isolates, respectively. PCR reaction also showed that all isolates contained at least 1 prophage sero-group and 2 sub-groups.

SGF serotype was present in 100% of the MRSA isolates, also SGFa and SGFb was the dominant (100%) sub-types 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 sub-groups constituted 42.6% of the isolates. 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 SCC*mec* type III and were PCR positive with the *ccr*AB-α4 specific primers indicating the presence of type 3 *ccr*. Moreover, 53 isolates which showed low resistance to oxacillin (MIC=4 µg/ml) carried SCC*mec* type IV and type 2 *ccr.* These isolates belonged to SGA prophage type, carried *pvl* gene and were isolated from wound (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 *pvl* gene among the MRSA isolates, was limited to low level oxacillin resistance (MIC=4 µg/ml).

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 SCC*mec* type III and harbored type 3 *ccr*. On the other hand, all S- and C-PhP

types 15-18 (53 isolates), belonged to CA-group and carried SCC*mec* type IV, type 2 *ccr* and SGA prophage type (Table 7).

Discussion

This is the first report on the prevalence and typing of community-acquired MRSA strains and their clonal dissemination compared with HA-MRSA strains in Iran. We combined a high-resolution PhP typing, SCC*mec* and prophage typing to confirm the presence of certain clonal groups of *S. aureus* in the hospital. According to PhP typing results, a majority of the isolates belonged to 18 C-types with minor S-type found only amongst CA-MRSA isolates. The prevalence of CA-MRSA isolates have been shown 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 community-acquired 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.

The prevalence of HA-MRSA strains in Iran has been varied between 19.2% and 80% (Fatholahzadeh et al. 2008; Rahimi et al. 2009; Japoni et al. 2011; Rahimi et al. 2012; Javidnia et al. 2013; Rahimi et al. 2013a; Rahimi et al. 2013b). 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 hospitals as well as methodology used. Our findings confirmed the high diversity among the CA-MRSA isolates in Iran. Majority of these strains were isolated from wound infections which is a common form of *S. aureus* infection in the community (David and

Daum 2010). Nonetheless, the prevalence of sources from which these strains were isolated are 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 and Daum 2010). Apart from penicillin of the CA- strains in our study showed a high level of resistance to erythromycin similar to that found in 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 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 to 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 as there are no reports of MRSA resistance to linezolid and quinupristin – dalfopristin (Fatholahzadeh et al. 2008; Japoni et al. 2011; Rahimi et al. 2012; Javidnia et al. 2013; Rahimi et al. 2013a; Rahimi et al. 2013b). 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; Japoni et al. 2011; Rahimi et al. 2012; Javidnia et al. 2013; Rahimi et al. 2013a; Rahimi et al. 2013b), 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 SCC*mec* type III was almost 91% followed with 9.1% of SCC*mec* type IV, while Japooni and colleagues (Japoni et al. 2011) could isolate strains with SCC*mec* types II, III, IVa, IVc, IVd and V in the south of Iran. It might be due, in part, to higher number of outpatients in comparison to inpatients tested in that study, and also the differences in geographical regions the studies have been done.

Here, all MRSA isolates that shared SCC*mec* 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 SCC*mec* type IV strains may acquire resistance to none 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 necrotising pneumonia (Labandeira-Rey et al. 2007; Otter and 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 bacteremia (Alp et al. 2009). Interestingly, PVL was found in CA-MRSA strains and was absent in HA-MRSA isolates, but there are some reports emphasizing on prevalence of CA-MRSA strains without *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 from other countries (Pantůček et al.

2004; Workman et al. 2006; Rahimi et al. 2012; Rahimi et al. 2013a). In reports published by Pantuceck (Pantůček et al. 2004) in Czech Republic, Workman (Workman et al. 2006) in USA, and our group (Rahimi et al. 2012; Rahimi et al. 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 Czeck Republic, Iran, and USA, respectively. Different 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 having 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- of HA strains.

We also isolated 53 strains that showed different characteristics compare to other MRSA isolates. These isolates were susceptible to all antibiotics tested (except penicillin) with a low level of resistance to oxacillin in MIC test (MIC= 4 µg/ml). Moreover, they harbored SCC*mec* type IV and type 2 *ccr*, and also contained *pvl* gene. These findings are consistent with the definition of CA-MRSA isolates (David and 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 harbored 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 SCC*mec* type III was the dominant type in HA-MRSA and CA-MRSA isolates followed with SCC*mec* type IV (a or c). All CA-MRSA isolates which shared SCC*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 harboring SCC*mec* type IV can acquire resistance to other classes of antibiotics to survive in the hospital environment. This might be due, in part, to bacteria dissemination from community to hospital. High prevalence of SCC*mec* type III and type 3 *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 the previously report in Iran (Fatholahzadeh et al. 2008) suggesting an increase in the frequency of CA-MRSA infection in Tehran.

Conclusion

Our results illustrate the presence and persistence of highly resistant clonal group of HA- and CA-MRSA strains in Tehran with the possibility that hospital could be the reservoir for dissemination of these strains in the community. In addition to that, we found that CA-MRSA isolates contained specific prophage pattern 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*mec* type IV and *pvl* gene in CA-MRSA strains.

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Table 1. Distribution of *S. aureus* strains isolated from different samples and different wards.

	Urology	Oncology	Pediatrics	Ophthalmology	Respiratory	ICU	ENT	Surgery	Gynecology
Abscess	2	-	7	-	1	44	4	18	13
Blood	-	13	40	-	-	-	-	-	30
Ear	-	-	-	-	-	-	13	-	-
Eye	-	-	-	16	-	-	-	-	-
Nose	-	-	29	-	18	35	37	27	19
Sputum	-	15	12	-	63	13	-	-	-
CSF	-	29	-	-	-	17	-	37	43
Urine	261	18	84	-	-	-	-	-	90
Wound	88	174	44	-	14	74	-	232	48
Total	351	249	216	16	96	183	54	314	243

Abbreviations: ICU, Intensive care unit; ENT, Ear, Nose and Throat; CSF, Cerebrospinal fluid

Table 2. The rate of resistance of MRSA strains isolated in this study against the 18 antibiotics tested. Abbreviations of the antibiotics are as follows: 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.

Antimicrobial drug (disk concentration)	Source of MRSA		Total (%)	P value
	HA (%)	CA (%)		
P	314 (100)	268 (100)	582 (100)	
E	313 (99)	215 (80)	528 (91)	<0.0001
CIP	313 (99)	213 (79)	524 (90)	<0.0001
TN	302 (96)	210 (78)	512 (88)	<0.0001
T	308 (98)	198 (74)	506 (87)	
CD	304 (97)	196 (73)	500 (86)	
AN	307 (98)	192 (72)	499 (86)	<0.0001
K	312 (99)	171 (64)	483 (83)	<0.0001
SXT	292 (93)	139 (52)	431 (74)	<0.0001
RP	295 (94)	130 (49)	425 (73)	<0.0001
GM	279 (89)	134 (50)	413 (71)	<0.0001
MN	189 (60)	90 (34)	279 (48)	<0.0001
FC	35 (11)	18 (7)	53 (9)	0.0822
NI	29 (9)	6 (2)	35 (6)	0.0003
C	11 (4)	1 (0.4)	12 (2)	0.0084
VA*	0	0	0	
LZD	0	0	0	
SYN	0	0	0	

* Resistance was measured using E-test

546 Table 3. Antimicrobial resistance patterns for *Staphylococcus aureus* isolated from hospitals.

No. of strains resistant to:	HA-MRSA n=314 (%)	CA-MRSA n=268 (%)	Total n= 582 (%)
1 antibiotic	0	53 (20)	53 (9)
P	0	53 (20)	53 (9)
2 antibiotics	2 (0.6)	2 (0.7)	4 (0.7)
P, CIP	1 (0.3)	0	1 (0.2)
P, E	1 (0.3)	2 (0.7)	3 (0.5)
3 antibiotics	0	3 (1)	3 (0.5)
P, Cip, E	0	3 (1)	3 (0.5)
4 antibiotics	4 (1.3)	12 (5)	16 (2.7)
P, Cip, E, K	4 (1.3)	0	4 (0.7)
P, Cip, E, TN	0	12 (5)	12 (2)
5 antibiotics	1 (0.3)	2 (1)	3 (0.5)
P, Cip, E, K, T	1 (0.3)	0	1 (0.2)
P, Cip, E, TN, T	0	2 (1)	2 (0.3)
6 antibiotics	3	4	7
P, Cip, E, T, K, AN	3 (1)	0	3 (1)
P, Cip, E, TN, T, CD	0	4 (2)	4 (1)
7 antibiotics	2	21	23
P, Cip, E, T, CD, K, AN	2 (0.6)	0	2 (0.3)
P, Cip, E, TN, T, AN, CD	0	21 (8)	21 (4)
8 antibiotics	7	32	39
P, Cip, E, TN, T, K, AN, CD	0	32 (12)	32 (6)
P, Cip, E, T, TN, K, AN, CD	7 (2.2)	0	7 (1)
9 antibiotics	3	5	8
P, Cip, E, TN, T, K, AN, CD, RP	3 (1)	0	3 (1)
P, Cip, E, TN, T, K, AN, CD, SXT	0	5 (1.8)	5 (1)
10 antibiotics	13	0	13
P, Cip, E, TN, T, K, AN, CD, RP, SXT	13 (4.1)	0	13 (2)
11 antibiotics	90	44	134
P, Cip, E, TN, T, K, AN, CD, SXT, RP, GM	90 (29)	40 (14)	130 (22)
P, Cip, E, TN, T, K, AN, CD, SXT, GM, MN	0	4 (2)	4 (1)
12 antibiotics	154	72	226
P, Cip, E, TN, T, K, AN, CD, SXT, GM, MN, RP	154 (49)	72 (27)	226 (39)
13 antibiotics	6	12	18
P, Cip, E, TN, T, K, AN, CD, SXT, GM, RP, MN, FC	6 (2)	12 (5)	18 (3)
14 antibiotics	18	5	23
P, Cip, E, TN, T, K, AN, CD, SXT, GM, RP, MN, NI, FC	18 (6)	5 (2)	23 (4)
15 antibiotics	11	1	12
P, Cip, E, TN, T, K, AN, CD, SXT, GM, C, RP, MN, NI, FC	11 (4)	1 (0.4)	12 (2)

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Table 4. MICs range in different MRSA isolates. MRSA had MIC ≥4 µg/ml.

MIC (µg/ml)	256	128	96	64	32	24	4
CA-MRSA	18 (7%)	79 (29%)	38 (14%)	7 (3%)	17 (6%)	56 (21%)	53 (20%)
HA-MRSA	144 (45%)	93 (30%)	21 (7%)	23 (7%)	31 (10%)	2 (1%)	0

Table 5. Prevalence of different SCC*mec* types and their indicator among MRSA strains isolated from different samples.

Samples	SCC*mec* III	SCC*mec* IVa	SCC*mec* IVc	*pvl*	SGA prophage	Total
Abscess	18	-	-	-	-	18
Blood	16	4	1	5	5	21
Ear	4	-	-	-	-	4
Eye	9	-	-	-	-	9
Nose	43	-	-	-	-	43
Sputum	81	-	-	-	-	81
CSF	39	6	-	6	6	45
Urine	85	10	1	11	11	96
Wound	234	23	8	31	31	265

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

Phage patterns	Phage types					Frequency
	SGA	SGB	SGF	SGFa	SGFb	
1	+	+	+	+	+	34 (5.8%)
2	+	-	+	+	+	19 (3.3%)
3	-	+	+	+	+	281 (48.3%)
4	-	-	+	+	+	248 (42.6%)

600 Table 7. Prevalence of different PhP types among HA and CA-MRSA isolates.

PhP type	HA-MRSA					CA-MRSA					Total (%)
	SCC*mec*	*ccr*	*pvl*	SGA prophage type	No. of isolates (%)	SCC*mec*	*ccr*	*pvl*	SGA prophage type	No. of isolates (%)	
CT1	III	3	-	-	22 (7)	-	-	-	-	-	22 (4)
CT2	III	3	-	-	76 (24)	III	3	-	-	55 (20.5)	131 (23)
CT3	III	3	-	-	109 (35)	III	3	-	-	70 (26)	179 (31)
CT4	III	3	-	-	2 (1)	III	3	-	-	2 (1)	4 (0.6)
CT5	III	3	-	-	10 (3)	III	3	-	-	9 (3)	19 (3)
CT6	III	3	-	-	7 (2)	III	3	-	-	5 (2)	12 (2)
CT7	III	3	-	-	1 (0.5)	III	3	-	-	3 (1)	4 (0.6)
CT8	III	3	-	-	8 (2)	III	3	-	-	9 (3)	17 (3)
CT9	III	3	-	-	23 (7)	III	3	-	-	18 (7)	41 (7)
CT10	III	3	-	-	4 (1)	-	-	-	-	-	4 (0.6)
CT11	III	3	-	-	2 (1)	III	3	-	-	2 (1)	4 (0.6)
CT12	III	3	-	-	2 (1)	III	3	-	-	4 (1.5)	6 (1)
CT13	III	3	-	-	1 (0.5)	III	3	-	-	5 (2)	6 (1)
CT14	III	3	-	-	47 (15)	III	3	-	-	33 (12)	80 (14)
CT15	-	-	-	-	-	IVc	2	+	+	6 (2)	6 (1)
CT16	-	-	-	-	-	IVa	2	+	+	13 (5)	13 (2)
CT17	-	-	-	-	-	IVa	2	+	+	4 (1)	4 (0.6)
CT18	-	-	-	-	-	IVa	2	+	+	15 (6)	15 (2)
ST1	-	-	-	-	-	IVa	2	+	+	1 (0.4)	1 (0.2)
ST2	-	-	-	-	-	IVa	2	+	+	1 (0.4)	1 (0.2)
ST3	-	-	-	-	-	IVa	2	+	+	1 (0.4)	1 (0.2)
ST4	-	-	-	-	-	IVa	2	+	+	1 (0.4)	1 (0.2)
ST5	-	-	-	-	-	IVa	2	+	+	1 (0.4)	1 (0.2)
ST6	-	-	-	-	-	IVa	2	+	+	1 (0.4)	1 (0.2)
ST7	-	-	-	-	-	IVa	2	+	+	1 (0.4)	1 (0.2)
ST8	-	-	-	-	-	IVc	2	+	+	1 (0.4)	1 (0.2)
ST9	-	-	-	-	-	IVa	2	+	+	1 (0.4)	1 (0.2)
ST10	-	-	-	-	-	IVa	2	+	+	1 (0.4)	1 (0.2)
ST11	-	-	-	-	-	IVa	2	+	+	1 (0.4)	1 (0.2)
ST12	-	-	-	-	-	IVc	2	+	+	1 (0.4)	1 (0.2)
ST13	-	-	-	-	-	IVc	2	+	+	1 (0.4)	1 (0.2)
ST14	-	-	-	-	-	IVa	2	+	+	1 (0.4)	1 (0.2)
ST15	-	-	-	-	-	IVc	2	+	+	1 (0.4)	1 (0.2)

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