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ORIGINAL ARTICLE

Comparison of community and healthcare-associated MRSA in Iran

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ABSTRACT

Background: To characterize and compare the epidemiological and microbiological aspects of community and healthcare-associated MRSA (CA-MRSA, and HA-MRSA) cases in Iran, this prospective cohort study was conducted from January to December 2008 in seven hospitals.

Patients and methods: Staphylococci were isolated from 109 hospitalized patients. MRSA isolates were classified into HA-MRSA and CA-MRSA based on clinical features. Antibacterial susceptibility patterns of the isolates to eight antibiotics routinely used to treat infected patients were determined according to standard agar dilution methods. Staphylococcal Cassette Chromosome *mec* (*SCCmec*) type of isolates and their correlation with antimicrobial susceptibility patterns in CA and HC isolates were determined.

Results: Of 109 isolates, 15(13.7%) were community-associated and 94 (86.3%) were healthcare-associated MRSA. The most frequent *SCCmec* types in the studied hospitals were SCC *mec* type I (56.9%) and type II (22%). Relatively high resistance (>60%) of the MRSA to the seven tested antibiotics including: ciprofloxacin, trimethoprim-sulfamethoxazole, clindamycin, rifampin, erythromycin, tetracycline and doxycycline were noticed.

Conclusion: To our knowledge, this is the first time that the analysis of *SCCmec* type is carried out in Iran according to the clinical criteria. Difference in the prevalence of HC-MRSA and CA-MRSA based on the clinical and epidemiological features may indicate the need for revisiting the classification of MRSA. The high prevalence of multi-drug resistant MRSA could be as a result of the excessive use of antibiotics in the hospitals. Therefore, periodical assessment of antibacterial susceptibility patterns of the MRSA strains is warranted.

Keywords: *Community acquired MRSA; Healthcare-associated MRSA; Antibacterial susceptibility patterns; Staphylococcal cassette chromosome mec*.

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INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) was initially introduced in 1961 (1) and the first documented outbreak of infection was described in 1968 (2). Progressively, MRSA is becoming more prevalent in healthcare settings. According to Center for Disease Control (CDC) data, the proportion of the infections that are antibacterial resistant has been growing. In this regards, MRSA infections accounted for 22% of staphylococcal infections in 1995 while this rate

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increased up to 63% in 2004 (3). The recent data from the National Nosocomial Infection Surveillance (NNIS) system demonstrate a steady increase in the incidence of nosocomial infections caused by MRSA among ICU patients over time. MRSA now accounts for >60% of *S. aureus* isolates in US hospital ICUs (4).

For long times it was believed that MRSA strain was absolutely causative agent for nosocomial infections but during recent years many reports worldwide showed it as an emerging significant community-acquired pathogen (5-10).

Some features can help distinguish between community and nosocomial MRSA strains; (1) absence of hospital-associated risk factors; (2) susceptibility to most antibiotics other than β-lactams; (3) presence of fourth type of *SCCmec* (the element that contains the methicillin resistance determinant), in contrast to the types I-III which are typical of nosocomial MRSA strains; (4) the presence of genes encoding for toxins such as pantone-valentine leukocidin and the many staphylococcal enterotoxins (11-14).

A limited number of studies in Iran have attempted to determine *SCCmec* types in MRSA strains and to our knowledge there is no report from Iran regarding the differentiation of MRSA types according to epidemiologic and antibiotic susceptibility criteria. The objectives of this study therefore, were to determine the demographic and clinical features of the patients infected with community and health care-associated MRSA in two major university hospitals in Iran, and to identify the microbiological and molecular features of CA-MRSA and HA-MRSA.

PATIENTS and METHODS

This multicenter, prospective trial was conducted in seven hospitals in two major cites of Iran; Tehran, the capital in the north and Shiraz, a major city in the south of Iran. All the selected hospitals provide services to both in-patients and out-patients and for adults and children. During January to December 2008, 109 MRSA strains were isolated from the sterile body sites of the patients in the hospitals. Purified samples were sent to the reference laboratory in Infectious Pediatrics Research Center (Mofid hospital, Tehran). Demographic data of the enrolled patients were collected via medical records and sometimes by interview. Informed written consents have been received from all patients who enrolled in the study and approved by the authorized board of ethics at Shiraz and Tehran Universities of Medical Sciences.

Criteria for classification of CA and HA-MRSA: Classification as a health care-associated infection was based on: 1) the isolation of MRSA from a clinical specimen at least 48 hours after hospital admission, 2) history of hospitalization, dialysis, surgery or residence in a long-term care facility within the last year, 3) presence of a permanent indwelling catheter such as tracheostomy tube, or percutaneous medical device at the time of culture, 4) previous detection of MRSA from a clinical specimen (14,15). Any case with none of the above-mentioned criteria was considered as community-associated MRSA.

Based on the patients' demographic data, underlying clinical diseases, and previous history of antibiotic therapy, those who seemed to be colonized without any clinical infectious sites compatible with cultured MRSA were excluded from the study.

Antimicrobial susceptibility testing: The isolates were identified as *S. aureus* based on morphology, gram stain, catalase test, coagulase and DNase activities. Levels of minimum inhibitory concentration (MIC) corresponding to oxacillin and eight antibiotics including; ciprofloxacin, clindamycin, erythromycin, trimethoprim-sulfamethoxazole, rifampin, tetracycline, doxycycline and vancomycin which are frequently prescribed in our clinics were determined by agar dilution plates, as recommended by National

Committee for Clinical Laboratory Standards (NCCLS) (16). American Typing Culture Collection (ATCC 25923) of *S. aureus* was used as a control strain in antibacterial susceptibility testing. Following overnight incubation, the MICs breakpoints were interpreted according to the NCCLS recommendation (table 1). All antibiotics were purchased from Sigma (St. Louis, USA). Tests to determine inducible clindamycin resistance (D zone test) were not performed.

Table 1. Minimum inhibitory concentration break points of the tested antibiotics

Antibiotic	Sensitive	Intermediate	Resistance
Ciprofloxacin	≤1†	2	≥4
Co-trimoxazol	≤256	-	≥512
Oxacillin	≤2	-	≥4
Clindamycin	≤0.5	-	≥4
Rifampin	≤1	2	≥4
Tetracycline	≤4	8	≥16
Erythromycin	≤0.5	1-4	≥8
Doxycycline	≤4	16	≥16
Vancomycin	≤2	4-8	≥16

†All units are as µg/ml

Detection of SCCmec type by multiplex PCR: DNA was extracted from all MRSA strains by DNA extracted kit (Cinna gen, Iran). Amplification of target sites including *SCC mec* typing and mec gene detection were performed by multiplex PCR assay according to pervious description method (16). American Typing Culture Collections (ATCC), 25923 and 51153, were used as a *mecA* negative and positive, respectively.

Analysis of data was performed using SPSS statistical software (version 15, SPSS Inc., Chicago, USA). Student *t* test was used for continuous data. Prevalence was calculated using standard equations. The Fisher's exact test was used to test for significant associations between categorical variables. P≤0.05 was considered as significant.

RESULTS

Totally 109 isolates of *S. aureus* were isolated from patients in 7 participating hospitals in two different cities of Iran. These hospitals are the major referral centers in those cities. Of the received 198 samples, identified in local hospitals as MRSA using disk diffusion method, 109 (57%) isolates were confirmed as MRSA in the reference laboratory using MIC standard method. According to epidemiological criteria, 94 (87%) and 15 (13%) isolates were classified as HA- and CA-MRSA, respectively. There were 78 males (11 in CA-MRSA and 67 in HA-MRSA group) and 31 females (4 in CA-MRSA and 27 in HA-MRSA group) enrolled in the study, and the mean age was 43 years in this cohort (age range: 2 months to 76 years). CA-MRSA patients were younger than HA-MRSA counterparts (mean age, 35 years vs. 44 years, *P*=0.034).

Table 2. Prevalence of community and health-care associated MRSA based on the type of infection

Infection type	Number(%)MRSA cases		Total (%)
	CA*	HA	
Skin and soft tissue	3(20)	25(26)	28(26)
Bacteremia and sepsis	3(20)	15(16)	18(17)
Pneumonia	4(26)	15(16)	19(18)
Urinary tract infections	0(0)	5(6)	5(4)
Others†	5(34)	34(36)	39(35)
Total (%)	15 (100)	94 (100)	109 (100)

* **CA**: Community-associated, **HA**: Health care associated
† Including bone, peritoneal, CSF and joint infections

Type of infection varied among patients. Of 109 patients with MRSA diseases, skin and soft-tissue infections were more prevalent 28 (26%) followed by invasive infections 18 (17%) (table 2). Based on multiplex PCR assay, SSC *mec* type isolates consisted of type I; 62(56.9%), type II; 24(22%), type III ;11(10.1%) and type IV;12(11.1%). There was strong correlation between the type of SSC*mec* and epidemiological criteria for differentiation of community and health care-associated MRSA. Five patients who were classified as CA-MRSA according to the conventional standards had SCC*mec* type other than type IV while 3 of them

were found in this study to have at least one point in favor of hospital origin of MRSA. Antibacterial susceptibility patterns were determined and the results were classified based on both SCC*mec* type and epidemiologic differentiation (tables 3 and 4). Generally, it was revealed that CA-MRSA isolates were more susceptible to multiple antimicrobial agents than HA-MRSA isolates.

Table 3. Antibiotic resistance profiles of 109 MRSA strains according to epidemiological classification of MRSA

Antimicrobial agents	Number (%) Resistance			*P*-value
	HA-MRSA	CA-MRSA	Total	
Ciprofloxacin	68(72)	5(33)	73(67)	0.003
Trimethoprim-sulfamethoxazole	60(63)	4(26)	64(58)	0.007
Clindamycin	73(77)	8(53)	81(74)	0.045
Rifampin	63(67)	5(33)	68(62)	0.012
Tetracycline	78(83)	6(40)	84(77)	0.00
Erythromycin	73(77)	9(40)	79(72)	0.002
Doxycycline	58(90)	4(57)	62(87)	0.011

Table 4. Antibiotic resistance profiles of 109 MRSA strains according to *mec* type classification of MRSA

Antimicrobial agents	Number (%) Resistance			*P*-value
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Ciprofloxacin	68(72)	5(33)	73(67)	0.003
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Doxycycline	58(90)	4(57)	62(87)	0.011

Overall, SCC*mec* type I strains showed greater resistance than other types especially SCCmec-IV to the antibiotics including clindamycin, erythromycin, ciprofloxacin, rifampin, tetracycline, doxycycline and trimethoprim-sulfamethoxazole ($p<0.001$). Antibacterial susceptibilities of MRSA isolates were as follows: 43.2% to doxycycline, 41.3% to trimethoprim-sulfamethoxazole, 37.6% to rifampin, 33.1% to ciprofloxacin, 27.5% to erythromycin, 25.6% to clindamycin and 22.9% to tetracycline. Vancomycin was the most effective antibiotic against all of the MRSA strains, followed by trimethoprim-sulfamethoxazole which was effective against 41% of the isolates. Meanwhile, 21(19%) isolates exhibited resistance to all other tested antibiotics. The least effective antibiotic was revealed to be doxycycline with 13% efficacy on MRSA.

DISCUSSION

Serious infections due to methicillin resistant *S. aureus* such as bacteraemia, osteomyelitis, and sepsis are more prevalent in the hospital settings, but more importantly many cases of MRSA infections can be seen among previously healthy individuals with no exposure to health care setting, hence, these communities associated MRSA have become more important in daily practice (2,5,6). To our knowledge, this is the first prospective comparative study of CA-MRSA and HA-MRSA cases in Iran, in which a molecular examination of SCC*mec* type and epidemiologic differentiation were used (17).

Overall, patients with CA-MRSA (defined as MRSA infections identified in patients who lack established MRSA risk factors) were significantly younger compared with HA-MRSA counterparts ($p=0.034$). This finding is consistent with those of the previous studies in other countries in which CA-MRSA was more prevalent than HA-MRSA among children (18,19). Of growing concern is the emergence of MRSA in patients with no health-care contact or apparent risk factors. The incidence of the CA-MRSA infection in the present study was 13% of all MRSA strains, which is comparable with reports from other countries (14,20). This finding confirmed the fact that transmission of *S.aureus* from community to the hospitals and vice versa could happen frequently. This situation could gradually cause the loss of a clear cut border between the two populations (CA, and HA). Hence,

if left uncontrolled we would expect the progressive emerging of resistant isolates in a community acquired staphylococcus infections.

Another point worth mentioning is the discrepancy between the laboratory methodologies for the detection of antibacterial susceptibility patterns. Of 190 staphylococcus samples, only 109 were confirmed by standard method as MRSA. Disc diffusion method (Kirby-Bauer) is a rapid and cost-effective way for determination of resistance profile. However, standard disc of antibiotics and procedure such as preparation of 0.5 Mc Farland are needed to ensure the correct interpretation of disc diffusion results. Unfortunately the wrong results in disc diffusion method may cause annually a great load of budget on our health system with current limited resources. The reutilized application of broth microdilution, although expensive, is recommended over disc diffusion method because it can help reduce the improper antibiotic use and save on the related costs consequently. At least a routine internal quality control testing with a range of control strains should be carried out as a major part of the quality assurance process since it facilitates monitoring of the performance of the test (21).

In the present study, notably, types I, II and III were shown to belong to the hospital origin with multiple antibiotic resistance patterns. The type IV of SCC*mec* has been shown to be a typical of CA-MRSA (22). In consistent with other reports, type I SCC *mec* was more prevalent in the present study and exhibited greater multi-resistance to the antibiotics, compared to the other three types (23,24).

In addition to the molecular typing of MRSA, the major finding of the present study is determination of local antibiotic resistance profile in CA-MRSA and HA-MRSA. Clinicians usually rely on vancomycin for the management of serious invasive MRSA infections. But according to many studies, to treat CA-MRSA many other options such as doxycycline, flouroquinolones, rifampin, doxycycline with acceptable coverage can be used (25-28). According to the present investigation the most effective antibiotic in our setting against MRSA strains, in addition to vancomycin, was doxycycline with only a coverage rate of 43.12%. Therefore, the availability of limited number of effective antibiotics against MRSA could restrict the alternative choices to treat the patients. This may in future cause the emergence of vancomycin intermediate resistance *S.aureus* (VISA) stains. In other words, with the rapid emergence of CA-MRSA that causes skin and soft tissue infections in community, it seems that our available antibacterial drugs may soon meet the same fate as previous drugs such as penicillin with regard to their ability for treating skin infections (29,30).

Strong association between epidemiologic features and SCC*mec* type IV that has different characteristics from HA-MRSA could suggest that type IV may be driven from methicillin-susceptible *S. aureus* in community. Community-associated MRSA were more likely to have unique susceptibility pattern to multiple antimicrobial classes and have specific molecular features (based on SCC*mec*) compared with health care-associated isolates. These findings further support the idea from other studies (11) that most community-associated MRSA infections are not due to health care exposures. However, if in the present study a lager sample size was studied, more disproportion between epidemiologic criteria and genetic characteristics in the two types of MRSA would have been generated, accordingly. Community associated MRSA, now progressively entering the hospital settings may change to a primary infection in near future. Thus, in daily practice definite determination of features such as antimicrobial sensitivity regardless of epidemiologic criteria could be essential part of patients management.

The current study has several limitations. Determination of pulse field gel electrophoresis in HA- and CA-MRSA along with side analysis of antibiotic resistance patterns and concomitant

epidemiological typing of the isolates could generate stronger results.

In conclusion, to prevent the emergence of antibiotic resistance, periodical antibiotics susceptibility surveillance is recommended. The results of this study could be publicized for the clinicians and health care staff.

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