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Full Length Research Paper

Molecular subtyping of multiresistant community-associated methicillin-resistant *Staphylococcus aureus* isolates in Riyadh, Saudi Arabia

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A total of 550 subjects from the community and 190 subjects from health care-related facilities in Riyadh, Saudi Arabia were evaluated for the prevalence of nasal *Staphylococcus aureus* colonization and to identify risk factors associated with *S. aureus* and methicillin-resistant *S. aureus* (MRSA) colonization. Among the community subjects, 1% had nasal MRSA colonization. Subjects from health care-related facilities had a lower *S. aureus* colonization rate (4.2%) than community subjects (1.2%), but had a significantly higher rate of colonization with MRSA (1.65%). Age (P < 0.001) was a significant risk factor for *S. aureus* colonization, with subjects under age 21 years or between 52 and 61 years showing higher rates of colonization. Recent gastrointestinal disease (P = 0.010) and hospital admission (P = 0.011) were risk factors for nasal MRSA colonization. Comparison of hospital MRSA isolates with the colonization strains by staphylococcal cassette chromosome *mec* (SCC*mec*) gene typing and pulsed-field gel electrophoresis (PFGE) typing revealed that, most MRSA strains carried in the community were SCC*mec* type IV and that most clinical hospital isolates were type III, while health care facility-related carriage isolates were mainly SCC*mec* type III and type IV. Two new variant SCC*mec* types were identified. Six clusters of PFGE patterns were distinguished: two mainly comprised health care facility-related MRSA strains, three mainly comprised community MRSA strains and one comprised mixed community and health care facility-related MRSA strains. In conclusion, a high prevalence of MRSA colonization was observed among people with no relationship to the hospital setting. The high level of multiple-drug resistance among community MRSA strains in association with the previously reported excessive use of antibiotics highlights the importance of the problem of antibiotic selective pressure. Our results indicate that, both the colonial spread of MRSA and the transmission of hospital isolates contribute to the high MRSA in the community.

Key words: *Staphylococcus aureus,* methicillin-resistance, molecular subtyping.

INTRODUCTION

Meticillinv-resistant *Staphylococcus aureus* (MRSA) is a key nosocomial pathogen, which is becoming an important public-health problem. New strains of *S. aureus* displaying unique combinations of virulence factors and resistance traits have been associated with high morbidity and mortality in the community (Nicola et al., 2005). However, in recent years MRSA has been seen with increasing frequency in the community worldwide (Miller and Kaplan, 2009). Meticillin resistance first appeared among nosocomial isolates of *S. aureus* in 1961 (Jevons, 1961). Since that time, meticillin-resistant *S. aureus* (MRSA) has become widespread in hospitals and intensive care units around the world. MRSA is now one of the most common causes of bacterial nosocomial infections, accounting for 40 to 70% of the *S. aureus* infections in intensive care units (Sham et al., 1999). In the past, acquisition of MRSA colonisation or infection was generally considered to be restricted to the nosocomial setting (Yamasaki et al., 2005). However, in the past decade new strains of MRSA have emerged in the community, causing aggressive infections in young, or else healthy people. Suppurative skin infections

including epidemics of furunculosis and severe necrotising pneumonias are the most well-known clinical syndromes caused by these new strains. The increasing prevalence of community acquired MRSA in multiple countries and the substantial morbidity and mortality associated with these infections suggest that, community-acquired MRSA will continue to develop into a challenging public-health problem (Nicola et al., 2005).

It was estimated that, MRSA strains accounted 64 and 32% from hospital and non-hospital acquired *S. aureus* isolates (Ho et al., 1999). Community-acquired MRSA (C-MRSA) in the US and Australia had a staphylococcal cassette chromosome *mec* (SCC*mec*) type (type IV) (Okuma et al., 2002) different from those of the health care setting-associated MRSA (H-MRSA) strains, whose SCC*mec* types were mainly types I to III (Hiramatsu et al., 2001, Ito et al., 2001).

Although, a previous study revealed that, SCC*mec* types III and IIIA were the main types of clinical MRSA strains (Aires et al., 2003). The SCC*mec* types of C-MRSA strains in Riyadh hospital have not been studied, due to the very high prevalence MRSA and a high extent of multidrug resistance with a high extent of antibiotic use among hospitals MRSA isolates, leading to community base threat infections (Goetz et al., 1999; Ho et al., 1999; Lin et al., 2000).

The remarkable ability of the bacterium to acquire antibiotic-resistance mechanisms and advantageous pathogenic determinants has contributed to its emergence in both nosocomial and community settings. However, because of different selective pressures, several notable differences exist between nosocomial isolates and community-acquired strains (Nicola et al., 2005). The current study evaluated the yoke of *S. aureus* resistance in the community, the potential risk factors for nasal *S. aureus* and MRSA colonization and the molecular characteristics for subtybing of MRSA isolates from the community and clinical settings.

MATERIALS AND METHODS

Population study

The study was carried out with 550 community residents of Riyadh, KSA, serving as subjects. Other study participant was associated with health care facilities, including all subjects living in a nursing home, hemodialysis patients treated. In an outpatients setting at hemodialysis unit, volunteers from acute-care wards, patients hospitalized in acute-care wards. A total of 740 subjects underwent the same nasal specimen acquisition procedure and completed the questionnaire as part of the study. MRSA isolates from clinical specimens of hospitalized patients in Riyadh Hospital were concurrently collected during the study period for molecular typing.

Microbiological study

All study participants underwent swabbing of the nasal vestibule of both nares with a sterile swab (CultureSwab Transport System; Difco, Detroit, Mich.). The swab specimen was streaked onto two mannitol salt agar plates (Difco, Sparks, Md.), one of which was supplemented with oxacillin (4 µg/ml). These inoculated plates were incubated at 37°C for 48 h, after which morphological and gram stain examinations were conducted. Colonies of interest were selected for further inoculation onto sheep blood agar plates (Becton Dickinson Microbiology Systems, Cockeysville, Md.) at 37°C overnight. The coagulase test (Coagulase Plasma System; Difco) was used to identify *S. aureus*. Methicillin-susceptible *S. aureus* (MSSA) was preliminarily detected by its characteristic growth on mannitol salt agar and the absence of growth in the presence of oxacillin, while growth on both agar plates was presumed to indicate the presence of MRSA. All isolates were inoculated onto Mueller-Hinton agar (Becton Dickinson Microbiology Systems) containing 6 µg of oxacillin per ml and 4% NaCl to confirm methicillin resistance (Okuma et al., 2002).

Antimicrobial susceptibility testing

All *S. aureus* isolates were tested for their susceptibilities to oxacillin, erythromycin, clindamycin, trimethoprim-sulfamethoxazole, vancomycin, rifampin, tetracycline, ofloxacin and gentamicin by Kirby-Bauer disk diffusion method (Ruth et al., 2006). The susceptibilities of the isolates to moxifloxacin were tested with Mueller-Hinton agar containing 2 µg/ml of moxifloxacin at 37°C for 24 h. E-test strips were used to confirm the isolates resistance to moxifloxacin (Joyce et al., 1992).

Multiplex PCR for SCC*mec* typing

Multiplex PCR for SCC*mec* typing was performed by the method of Oliveira and de Lencastre (Rings et al., 1998). The *mecA* gene and seven different loci (loci A to H) along the *mecA* gene cassette were selected for amplification by PCR. The primer sets specific for these loci are tabulated in Table 1.

Genomic fingerprinting by PFGE

Total DNA was prepared and pulsed-field gel electrophoresis (PFGE) was performed as described previously (Macfarlane et al., 1999). The restriction enzyme SmaI was used at the temperature proposed by the manufacturer. The band patterns were visually compared and classified as indistinguishable (no differences), closely related (clonal variants, one to three band differences), possibly related (four to six band differences) and unrelated (more than six band differences) by the use of previously described criteria (Trividic et al., 2002). Isolates with banding patterns that differed from the main pattern by up to three bands were considered to represent subtypes of the main type.

To identify PFGE polymorphisms, each sample was analyzed by using molecular analyst fingerprinting, fingerprinting plus and fingerprinting DST software (Bio-Rad Laboratories, Richmond, Calif.). The grouping method was performed to deduce a dendrogram from the matrix by the unweighted pair group method with arithmetic averages clustering technique after calculation of similarities by using the Pearson correlation coefficient between each pair of organisms and the PFGE patterns were distinguished at the 70% similarity level.

Statistical analysis

Comparison of categorical variables and percentages between groups was done by the Pearson chi-square test or Fisher's exact test, as appropriate. Relative risk and 95% confidence intervals (CIs) were also calculated. Multivariate analysis was performed by

Table 1. The primer sets specific for the *mecA* gene and seven different loci (loci A to H) along the mecA gene cassette are as follows:

For type I-specific locus A, primers
Forward 5'-TTCGAGTTGCTGATGAAGAAGG-3' and reverse 5'-ATTTACCACAAGGACTACCAGC-3';
For type II-specific locus B, primers
Forward 5'ATTCATCTGCCATTGGTGATGC-3' and reverse 5'-CGAATGAAGTGAAAGAAAGTGG
For type II- and III-specific locus C, primers
Forward 5'-ATCAAGACTTGCATTCAGGC-3' and reverse 5'-GCGGTTTCAATTCACTTGTC
For type I-, II-, and IV-specific locus D, primers
Forward 5'-CATCCTATGATAGCTTGGTC-3' and reverse 5'-CTAAATCATAGCCATGACCG-3'
For type III-specific locus E, primers
Forward 5'-GTGATTGTTCGAGATATGTGG-3' and reverse 5'-CGCTTTATCTGTATCTATCGC-3'
For type III-specific locus F, primers
Forward 5'-TTCTTAAGTACACGCTGAATCG-3' and reverse 5'-GTCACAGTAATTCCATCAATGC-3'
For nonspecific locus G, primers
Forward 5'-CAGGTCTCTTCAGATCTACG-3' and R5'-GAGCCATAAACACCAATAGCC-3'
For nonspecific locus H, primers
Forward 5'-CAGGTCTCTTCAGATCTACG-3' and reverse 5'-GAAGAATGGGGAAAGCTTCAC-3'
For the specific *mecA* gene, primers
Forward 5'-TCCAGATTACAACTTCACCAGG-3' and reverse 5'-CCACTTCATATCTTGTAACG-3'

using a stepwise logistic regression model. The threshold for a significant difference was designated a *P* value of <0.05. Factors associated with *S. aureus* or MRSA colonization with *P* values <0.05 were further studied by using a logistical regression model.

RESULTS

Isolation of *S. aureus*

A total of 740 subjects, including 550 community residents and 393 subjects from health care facility-related settings, were recruited for the study. Among the 538 of 740 (24.1%) subjects with *S. aureus* colonization, 91 (16.9%) were colonized with isolates which were resistant to oxacillin, yielding an MRSA colonization rate of 4.1% (91 of 2,231). The demographic and clinical characteristics and the rates of *S. aureus* and MRSA colonization for the 550 community subjects and 190 subjects in health care facility-related settings are shown in Table 2.

Comparison of the data for the 550 community residents and the 190 subjects recruited from health care facility-related settings revealed that, the nasal *S. aureus* colonization rates of community residents (community subject *S. aureus* colonization [C-SA]) were significantly higher than those of subjects from health care facility-related settings (health care facility-related patient *S. aureus* colonization [H-SA]) (25.2 and 19.1%, respectively). On the contrary, the percentage of subjects colonized with C-MRSA isolates was significantly (P = 0.002) lower than the percentage of subjects colonized with H-MRSA isolates (3.5 and 6.9%, respectively). Subjects colonized with *S. aureus* had significantly higher MRSA colonization rates than health care facility-related subjects (P < 0.001) (Table 2). Among the 550 community subjects, 463 (25.2%) had C-SA. Among these subjects, 314 were students and 149 were community residents. Sixty-four (13.8%) of 463 isolates were C-MRSA, including 33 from students and 31 from other residents, yielding a C-MRSA colonization rate in the community of 3.5%. The C-SA rate was significantly higher (P < 0.001) among students than among other community residents. By analysis of the rate of C-MRSA colonization among the subjects with C-SA, the rate of C-MRSA colonization and C-SA was significantly higher (P = 0.003) among other residents than among students. No significant difference in MRSA colonization rates was found between

Table 2. Rates of *S. aureus* and MRSA colonization in the community and health care facility-related subject groups.

	Community subjects (*n* = (550)		Health care facility related subjects (*n* = 190)				Total
	Residents	Students	Hemodialysis	Chronic-care facility	Acute-care wards	Health care worker	
No. of subjects	212	292	55	71	53	57	740
Age (yr [range])	5-75	3-19	24-69	9-81	16-82	16-60	
% Males/% females	33.7/60.3	36.4/57.6	33.7/52.3	60/40	41.7/48.2	4.3/951.7	39.3/60.8
% of subjects with:							
DM	2.4	0	21	24	3.3	1.7	2.5
Hypertension	5.8	0.1	30.6	53	5.8	1.4	6.1
Chronic liver disease	2.0	0.3	20	25	2.9	2.9	3.0
Renal disease	0.6	0	56	2	0	0	1.2
Pulmonary disease	0.5	0.2	0	45	1.2	1.2	2.1
Gastrointestinal disease	10.6	1.9	4.7	16	5.8	13.7	6.8
Nasal disease[a]	20.2	15.2	3.5	0	4.3	18.7	15.9
Recent surgery[b]	1.1	0.6	0	0	0	0.7	0.7
Recent admission[b]	1.2	0.9	0	65	71	1.4	8.5
Recent visits for OPD[c]	31.3	34.2	100	0	8.7	32.4	33.2
Recent medication[a]	30.0	29.3	100	99	98.6	26.6	37.3
Recent antibiotic uses[a]	2.2	0.7	0	3	7.2	10.8	2.2
S. aureus colonization	16.5	281.8	15.3	15	21.2	19.3	24.1
MRSA colonization	3.6	3.3	5.1	11	4.1	5	3.1

other residents and students (Table 3).

The H-MRSA rates ranged from 5 to 11% (average, 6.9%). The highest MRSA colonization rate was seen among subjects recruited from chronic-care facilities (11%). No significant differences in the rates of H-SA (P = 0.230) and H-MRSA colonization (P = 0.169) between patients from a chronic-care facility, a hemodialysis center and acute-care wards and health care workers were observed. However, among the subjects with H-SA, the ratio of H-MRSA colonization/H-SA was significantly higher among patients than among health care workers (P = 0.042) (Table 3).

Antimicrobial susceptibility

All *S. aureus* isolates were susceptible to vancomycin, while 91 (16.9%) isolates were MRSA. Resistance to erythromycin, clindamycin, tetracycline and gentamicin was found in 55.8, 37.5, 60.4 and 19.5% of the isolates, respectively. Less than 5% of isolates were resistant to trimethoprim-sulfamethoxazole, rifampin, ofloxacin and moxifloxacin. For C-MSSA and C-MRSA colonization isolates, the rates of resistance to erythromycin (48.1 and 90.6%, respectively), clindamycin (25.8 and 90.6%, respectively), trimethoprim-sulfamethoxazole (12.8 and 35.9%, respectively), tetracycline (53.1 and 95.3%, respectively), ofloxacin (1 and 12.5%, respectively) and gentamicin (8.8 and 64.1%, respectively) were significantly different (Table 4).

Molecular study

SCC*mec* gene typing of nasal colonization

Table 3. Rates of colonization with *S. aureus* and MRSA between the community and health care facility-related subjects.

Colonizer	No. (%) of community subjects				Health care facility-related subjects						
					Patients (n = 254)						
	Residents (*n* = 212)	Student (*n* = 292)	Subtotal	P value[a]	Hemodialysis (*n* = 55)	Chronic-care Facility (*n* = 71)	Acute-care wards (*n* = 53)	Health care workers (n = 57)	Subtotal	P value[b]	P value[c]
S. aureus	49 (9.5)	134 (21.8)	463(15.2)	<0.001	13 (11.3)	15 (15)	16(19.2)	31 (16.3)	75 (14.1)	0.230	0.010
MRSA	21 (3.6)	32 (3.3)	44(3.5)	0.527	5 (3.9)	11 (15)	4(5.8)	7 (5)	27 (6.9)	0.255	0.002
S. aureus[d]	20.8	10.5	13.8	0.003	38.5	323.3	25	22.6	36	0.032	<0.001

Table 4. Antimicrobial susceptibilities of MRSA isolates from community and hospital-related colonizers and hospitalized patients.

Drug	% Resistant			P value[a]
	Community colonizers (*n* = 53)	Hospital-related colonizers (*n* = 24)	Clinical hospital strains (*n* = 16)	
Erythromycin	81.1	91.3	92	0.191
Clindamycin	79.6	96.3	100	0.231
TMP-SMX[b]	31.9	51.6	84.1	<0.0001
Vancomycin	0	0	0	
Rifampin	2.9	21.6	31.2	0.0002
Tetracycline	85.1	81.2	92	0.4883
Ofloxacin	12.5	55.6	87.4	<0.0001
Gentamicin	54.1	80.9	82.4	0.1248
Moxifloxacin	1.1	32.3	31.2	<0.0001

[a] P value by Fisher's exact test for the significant difference in drug resistance among community colonizers and clinical hospital str ains. [b] TMP-SMX, trimethoprim sulfamethoxazole.

isolates and clinical isolates showed 55 MRSA nasal colonization strains, 17 MRSA isolates from clinical specimens collected from hospitalized patients. All of the MRSA isolates had the *mecA* gene. The results of SCC*mec* typing of the nasal colonization isolates from the subjects in the community, health care facility-related subjects and clinical isolates from patients are shown in Table 5. A total of seven different SCC*mec* types were identified by multiplex PCR. In addition to previously described SCC*mec* types, two new variants were detected. Positive amplification of loci with locus-specific PCR primers revealed that, one variant belonged to SCC*mec* type III, while the other appeared to be either a type I or a type III variant (Figure 1). SCC*mec* type IV was the most common among the community colonizers (87.5%). The SCC*mec* type III group (type III, type IIIA and type III variants) (51.85%) and SCC*mec* type IV (40.7%) were the two most common types among the health care facility-related colonization isolates. Among the clinical isolates from hospitalized patients, all except one belonged to the SCC*mec* type III group (10 isolates of type III, 4 isolates of type IIIA and 2 isolates of the type III variant) .some isolates were found to be a new type III variant. These isolates were amplified with

Table 5. SCC*mec* types of MRSA isolates from community and hospital-related colonized subjects and from hospitalized patients.

SCC*mec* type	No. of isolates		
	Community colonizers (*n* = 53)	Hospital-related colonizers (*n* = 24)	Clinical hospital strains (*n* = 16)
I	1	1	0
II	0	1	0
III	1	7	9
IIIA	0	2	4
IV	55	9	1
Type III variant[a]	3	3	2
Not typed[b]	1	0	0
New variant[c]	1	0	0

[a]The new type III variant was defined when specific *mecA* type III loci E and F were present (31);[b]one isolate that was not typed was positive only for the *mecA* gene, with no PCR product obtained with the SCC*mec* gene tested; [c]an isolate contained the specific A, D, E and F loci, which were type I or type III specific and was designated the new type variant.

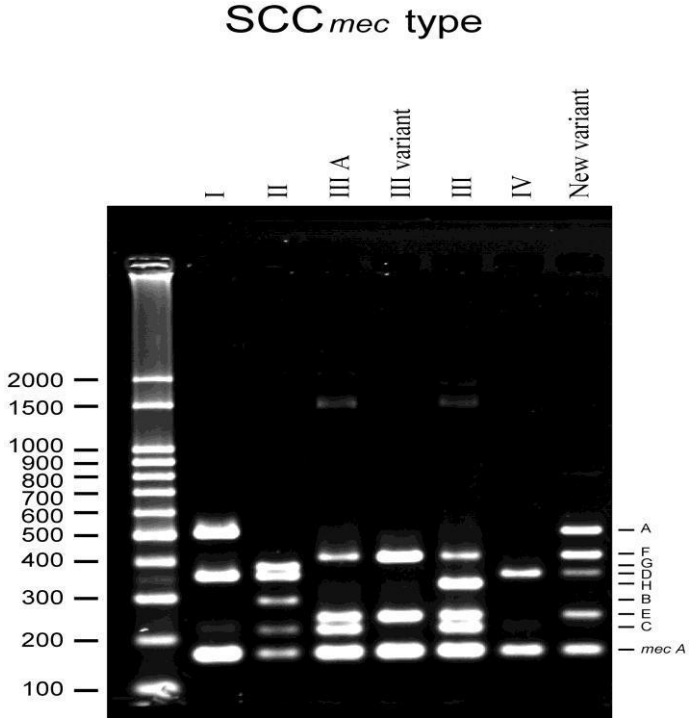

Figure 1. Identification of SCC*mec* types by multiplex PCR. The lane on the left contains a molecular weight marker. Loci specific to a SCC*mec* type by PCR are as follows: A, type I; B, type II; C, types II and III; D, types I, II and IV, E, type III; F, type III; G and H, nonspecific for a SCC*mec* type.

mecA-specific locus E and F primer sets, which were specific for the type III *mecA* gene. One isolate was designated a new type variant and multiplex PCR revealed that, this isolate contained the A, D, E and F loci, which were either type I or type III specific.

PFGE analysis of C-MRSA and H-MRSA isolates

A wide diversity of pulsotypes was found among the isolates from the different subject populations, as shown in Figure 1. Six clusters that included 51 (47.2%) isolates

were distinguished at the 70% similarity level and their band patterns showed that, they differed from each other by less than three bands. Except for one isolate from a community resident (isolate A27), all isolates of MRSA cluster I were from health care facility-related subjects or hospitalized patients. Nine (52.9%) of the 17 MRSA clinical hospital isolates from cluster VI or I were from patients at Pingtong Hospital. The isolates in clusters II, III and V were all community isolates, indicating that, C-MRSA isolates were easily distinguishable from the clinical hospital strains and the health care facility-related isolates. Clusters I and VI comprised community, health care facility-related and clinical hospital isolates, which indicates that, some of the isolates colonizing community and health care facility-related subjects were clonally related to clinical hospital isolates. The rates of drug resistance were significantly lower among MRSA isolates from subjects without risk factors than among isolates from subjects who had any predisposing risk factor or who were health care workers, as follows: rifampin, 2.4 and 18.8%, respectively; ofloxacin, 9.5 and 30.4%, respectively; moxifloxacin, 2.4 and 17.4%, respectively. For the other antimicrobial agents tested, no differences in resistance rates were found between subjects with and without risk factors (rates of resistance to clindamycin, 92.9 and 82.6%, respectively; rates of resistance to erythromycin, 88.1 and 92.8%, respectively; rates of resistance to tetracycline, 95.2% and 89.9%, respectively; rates of resistance to gentamicin, 64.3% and 71.0%, respectively).

DISCUSSION

Community-acquired MRSA infections have raised concerns worldwide (Moodley et al., 2009). Determination of whether community MRSA colonization originated from health care setting-related cases or by spread through horizontal transmission within the community may influence how this problem will be addressed.

In this study, subjects whose activities involved contact with a health care facility had a significantly higher rate of MRSA colonization than community subjects, even though the health care-facility related subjects had a lower overall *S. aureus* colonization rate than their community counterparts. Age was found to be the most significant factor for *S. aureus* colonization in this study. Recent admission to a hospital and gastrointestinal diseases were the most important factors associated with MRSA colonization among community subjects and is consistent with the findings of previous reports suggesting that, C-MRSA might originate from contact with a hospital environment (Charlebois et al., 2002; Goetz et al., 1999; Stacey et al., 1998), but is contrary to the findings of an earlier report (Moreno et al., 1995) that did not show any significant risk factors differentiating patients with C-MRSA and MSSA infections.

The rate of resistance to all the antimicrobials tested except vancomycin among our MRSA nasal isolates (over 10%) was higher than that in a previous study of urban poor individuals from San Francisco, where erythromycin and ciprofloxacin were the only two drugs to which rates of resistance were >10% (Po-Liang et al., 2005). The rates of multiple-drug resistance among our MRSA isolates were also higher than those presented in other reports (Centers for Disease Control and Prevention, 1999; Gross-Schulman et al., 1998; Ma et al., 2002), in which most clinical C-MRSA isolates were susceptible to various antibiotics except beta-lactams. The rate of resistance to clindamycin (92.9%) among the C-MRSA isolates from subjects without risk factors in this study was also higher than that in a study from the United States (Herold et al., 1998). The high rate of resistance to clindamycin among our community MRSA isolates (90.6%) was similar to the rate of resistance among clinical MRSA isolates in Taiwan (94.2%) (Hsueh et al., 2002), indicating that, clindamycin resistance is quite common among community and health care facility-related MRSA isolates.

This study found that, recent receipt of medical services was the major factor associated with MRSA colonization as well as the high level of multiple-drug resistance in MRSA nasal isolates. These findings may be explained by the high rate of antibiotic use in the Taiwan community (Liu et al., 1999) in which antimicrobial activity in urine was detected in 55.2% subjects on arrival at an emergency department and in 7.6% of high school students. Another study found that, the proportion of patient visits resulting in antimicrobial therapy in primary care units was 13.4% in Taiwan and that 31.3% of patients with a diagnosis of the common cold received antibiotic treatment (Chang et al., 2001).

We identified four factors which support the occurrence of transmission of MRSA outside the hospital setting. First, there was a high rate (3.41%) of MRSA colonization among community residents who did not have health care setting-related predisposing factors. Second, MRSA isolates from community residents with colonization had antibiograms which were different from those of the clinical hospital strains with regard to trimethoprim-sulfamethoxazole, rifampin, ofloxacin and moxifloxacin resistance. Third, most of the MRSA isolates responsible for colonization of community subjects were of SCC*mec* type IV, whereas most of the clinical hospital strains were of SCC*mec* type III. Fourth, molecular typing of MRSA isolates by PFGE revealed that, three clusters of MRSA isolates were mainly from colonized community residents and no clinical hospital MRSA isolates were in the clusters mainly formed by colonizing community strains.

With regard to the significant difference in trimethoprim-sulfamethoxazole, rifampin, ofloxacin and moxifloxacin resistance between community colonizers and clinical hospital strains, the MRSA isolates from health care facility-related colonizers had antimicrobial resistance

rates between the rates for the other two groups (Table 5). High proportions of the MRSA isolates from health care facility-related colonizers were of SCC*mec* type IV and type III and these were predominantly community colonizing strains and clinical hospital strains, respectively. The mixed characteristics of the health care facility-related colonizing strains and PFGE clusters I, III and IV among both community and heath care facility-related isolates suggest that, MRSA colonization among health care facility-related subjects may be a route of transmission from hospitals to the community.

In summary, the implications of MRSA colonization, infection and treatment should be explained to the patient and close relatives who assist with the patient's bodily care. Although, routes of MRSA transmission from the hospital setting to the community exist, the molecular evidence of the presence of colonizing strains in the community and the high rate of MRSA colonization among people without a relationship to the hospital setting suggest that, further measures to control antibiotic usage to reduce selective pressure for antibiotic resistance are urgently needed in the community as well as in hospitals. The risk factor assessment used in this study had several limitations. The proportion of study participants with recent antibiotic usage might have been underestimated because many patients might not have recognized that they had taken antibiotics, especially when the drugs were prescribed in a clinic as opposed to a hospital. The tracing of a 3-month medical history in this study was intended to limit the recall bias that might result from the review of a longer period.

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