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Original Article

Methicillin-resistant *Staphylococcus aureus* nasal carriage in international medical conference attendees

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Abstract *Background:* Carriage of methicillin-resistant *Staphylococcus aureus* (MRSA) is associated with its transmission. International travels and massive gatherings may accelerate such transmission. MRSA carriage was surveyed among the attendees of two international medical conferences held in Taipei in 2010.
Methods: A total of 209 attendees from 23 countries were recruited. Nasal specimens were collected from each volunteer and subjected to polymerase chain reaction (PCR) detection for MRSA. Molecular analysis, including pulsed-field gel electrophoresis, multilocus sequence typing (MLST), typing of staphylococcal cassette chromosome *mec* (SCC*mec*) and staphylococcal protein A (*spa*) genes, and detection of Panton-Valentine leukocidin (PVL) and *sasX* genes, was performed.
Results: MRSA carriage was detected in 10 (4.8%) attendees from Vietnam (3/8, 37.5%), Korea (2/6, 33.3%), Japan (2/41, 4.9%), Philippines (2/52, 3.8%), and Bangladesh (1/4, 25.0%). The proportion of MRSA colonizers was significantly higher in the local hospital group compared to those from the other groups (3/17 vs. 7/192, $p < 0.05$). Six MRSA isolates were available for molecular analysis. They all carried a type IV SCC*mec* gene. Five pulsotypes were identified; four genotypes, respectively, were identified by MLST and *spa* typing. None of the isolates carried either PVL or *sasX* genes. None of common molecular characteristics was shared by

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isolates from different countries. Most of these isolates were local endemic community clone in each country.
Conclusions: As healthcare workers, a certain proportion of international medical conference attendees harbored MRSA in their nares, mostly local endemic community clones in each country, which has the potential of spread among attendees.

Introduction

Infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) have long been a global concern.[1] MRSA is usually considered a hospital pathogen, but community-acquired infection in patients without potential risk factors also is increasing.[1] MRSA causing the community-associated infection, so-called CA-MRSA, is recognized as a novel pathogen that is genetically different from those caused the healthcare-associated infection.[2] Prevailing CA-MRSA clones may vary in different continents or even countries.[3] However, Asian clones of CA-MRSA were recently identified in several European countries, whereas infections caused by USA300 (ST8) CA-MRSA, prevailing in the North America, also have been reported from several Asian countries.[3] Prior travel history or ethnic background associated with countries where the clones were endemic was found to be important contributing factors.[3]

International travel has been shown to contribute to the spread of some transmissible diseases as well as multidrug-resistant (MDR) clones of bacteria among different countries.[4] International massive gatherings, such as Olympic Games, World Cup of various sport competitions, assorted international festivals, etc., may offer an amplification factor for the infection transmission through international travel.[5] The pattern of global-to-local-to-global mobilization is apparently an efficient model for swapping and spreading of all sorts of objects, including infectious diseases.[6] Among such massive gatherings, many international medical conferences that held annually or periodically with various frequencies may represent an ignored field that warrants more attention.

Attendees of international medical conferences are usually healthcare workers (HCWs). They are under similar risks occurred to those attending other international massive gatherings. Nevertheless, they also are facing different challenges carried with peer colleagues from many medical institutions around the world. MRSA is one of the transmissible pathogens known to be attributable to the MRSA carriage, especially among HCWs that are at the interface between hospitals and communities.[7] Previous surveys for MRSA carriages among HCWs were usually for the investigation of outbreaks or endemics. The average MRSA carriage rate was 4.6%, ranging from 0 to 59%, in a pooled data of 33,318 screened HCWs from 127 investigations.[7] Another recent systematic review on MRSA carriage among HCWs in non-outbreak settings revealed a lower pooled MRSA colonization rate of 1.8% for all HCWs or 0—13% among physicians.[8] It is known that different sampling sites/methods and detection methods may lead to different results. Curiosity therefore remains whether significant difference may exist if the MRSA carriage of HCWs from different countries is simultaneously surveyed and compared.

In 2010, two separate international (Asian) conferences were held by the Taiwan Pediatric Association in Taipei, Taiwan. A survey for MRSA carriage was conducted to elucidate whether the carriage rate and the bacterial clones may vary among the international attendees from different countries simultaneously.

Materials and methods

Study design

The study was approved by the Institutional Review Board (99—0699B) of Chang Gung Memorial Hospital, Linkou. All international participants of the 6th Annual Conference of Asian Society of Pediatric Research and the 5th Asian Congress of Pediatric Infectious Diseases, which were held in April and September, 2010, respectively, in Taipei, Taiwan, were eligible and were invited to participate in the study. A total of 209 attendees from 23 countries, were enrolled (Table 1). In April, 87 attendees from 17 countries were enrolled, and in September, 122 attendees from 19

Table 1 Nasal carriage rate of methicillin-resistant *Staphylococcus aureus* (MRSA) among international conference attendees from different countries.

Country	Subject no.	MRSA no.	(%)
Philippines	52	2	(3.8)
Japan	41	2	(4.9)
Vietnam	8	3	(37.5)
Korea	6	2	(33.3)
Bangladesh	4	1	(25.0)
Thailand	19	0	
China	16	0	
Indonesia	14	0	
Malaysia	14	0	
Sri Lanka	8	0	
Hong Kong	4	0	
Singapore	4	0	
Others[a]	19	0	

[a] The number of study subjects from other countries included 3 each from Australia, India, and Saudi Arabia, 2 each from Mauritius and the United States, and 1 each from Canada, Denmark, France, Iraq, Swiss, and the United Kingdom.

countries were enrolled. A written informed consent and a questionnaire, including the demographic information and institutions, were obtained from each participant. No personal identifiers were included in the dataset for analysis.

Microbiological examination and antimicrobial susceptibility testing

Nasal swab specimens were collected by using dry Copan Transystem Liquid Stuart swabs (Venturi Transystem; Copan Diagnostics, Corona, CA). For each subject, both anterior nares were sampled simultaneously with two swabs. The swabs were then transported at room temperature and processed within 4 h.

To detect the presence of MRSA, a commercial kit (BD GeneOhmTM Staph SR Assay; Becton Dickinson, NJ, USA) using polymerase chain reaction (PCR) methods was performed according to the manufacturer's instruction. For those with positive PCR results, the paired swabs were then subjected for traditional microbial culture methods to produce MRSA colonies for the subsequent molecular characterization. *S. aureus* isolates were identified by standard methods.

Methicillin resistance was determined by the cefoxitin disc diffusion method recommended by the Clinical and Laboratory Standards Institute.[9] Susceptibilities of the isolates to the other 9 antibiotics (clindamycin, doxycyclin, erythromycin, fusidic acid, linezolid, penicillin, teicoplanin, trimethoprim/sulfamethoxazole, and vancomycin) were also determined by the disc diffusion method suggested by the CLSI.[9]

Genotyping and molecular characterization

Pulsed-field gel electrophoresis (PFGE) with *Sma*I digestion was used to fingerprint all MRSA isolates and the results analyzed as described previously.[10] PFGE patterns with less than 4-band differences from an existing pulsotype were defined as subtypes of that pulsotype. A BioNumerics Fingerprint types and Cluster Analysis software (Applied Maths, Austin, TX) was also applied for cluster analysis using the Dice coefficient and the unweighted pair-group method. Two representative MRSA isolates from the local attendees (Taiwan) were used as comparison.[11]

Multilocus sequence typing (MLST) was performed as described elsewhere.[12] Typing of staphylococcal cassette chromosome mec (SCC*mec*) and staphylococcal protein A (*spa*) genes was performed by published methods.[13,14] The presence of Panton-Valentine leukocidin (PVL) and *sasX* genes was also determined by PCR assays.[15,16]

Statistical analysis

The χ^2 test or Fisher exact test, as appropriate, was used for statistical analysis. Statistical significance was defined as $p < 0.05$.

Results

Most attendees were clinicians (84.2%) and aged between 31 and 60 years (89.5%) (Table 2). Among them, MRSA was detected by PCR in 10 (4.8%) attendees from five countries (Table 1). Four (4.6%) of the attendees with positive findings were from the 87 volunteers in April; three of them were from Vietnam, and the remaining one was from the Philippines. The other six (4.9%; two each from Japan and Korea and one each from the Philippines and Bangladesh) were from the 122 volunteers in September. A significantly higher carriage was noted among attendees from Vietnam (37.5%) and Korea (33.3%) although the overall numbers of attendees from the two countries were small. Comparison of demographic characteristics between the study subjects with and without the MRSA colonization is demonstrated in Table 2. Most of the demographic features were rather comparable between the two groups. However, among the various groups of healthcare units, the proportion of MRSA colonizers was significantly higher in the local hospital group compared to those from the other groups (3/17 vs. 7/192, $p < 0.05$).

Table 2 Comparison of demographic features between the study subjects with and without the carriage of methicillin-resistant *Staphylococcus aureus*.

Characteristics	No. (%) of subjects				*P*-value
	Colonizer ($n = 10$)		Non-colonizer ($n = 199$)		
Male gender	4	(40.0)	105	(52.8)	0.430
Age (yrs)					
<30	0	(0)	12	(5.7)	0.424
31–40	2	(20.0)	62	(31.2)	0.455
41–50	6	(60.0)	68	(34.2)	0.096
51–60	2	(20.0)	47	(24.6)	0.792
>60	0	(0)	10	(50.)	0.468
Medical career (yrs)					
<5	1	(10.0)	19	(9.5)	0.962
6–10	0	(0)	41	(20.6)	0.109
11–20	4	(40.0)	78	(39.2)	0.959
21–30	4	(40.0)	40	(20.1)	0.132
>30	1	(10.0)	20	(10.1)	0.996
Clinician	10	(100)	166	(83.4)	0.161
Healthcare units					
Medical center	6	(60.0)	118	(59.3)	0.965
Regional hospital	1	(10.0)	31	(15.6)	0.663
Local hospital	3	(30.0)	14	(7.0)	0.010
Clinics	0	(0)	19	(9.5)	0.305
Others	0	(0)	17	(8.5)	0.335

Among the 10 PCR-detected MRSA colonizing cases, only six isolates of MRSA (from three countries) were identified from the corresponding nasal cultures (Fig. 1). All six isolates were resistant to penicillin but were susceptible to fusidic acid, linezolid, teicoplanin, trimethoprim/sulfamethoxazole, and vancomycin. All but one isolate from Vietnam were susceptible to doxycyclin. Only the two isolates from Japan were susceptible to erythromycin. Clindamycin susceptibility was also found in two isolates from Japan and Korea, respectively. Susceptibility to both erythromycin and clindamycin were found in one isolate from Japan. Three (50.0%) of the isolates could be categorized as MDR bacteria that are non-susceptible to at least one agent in 3 or more drug categories.[17]

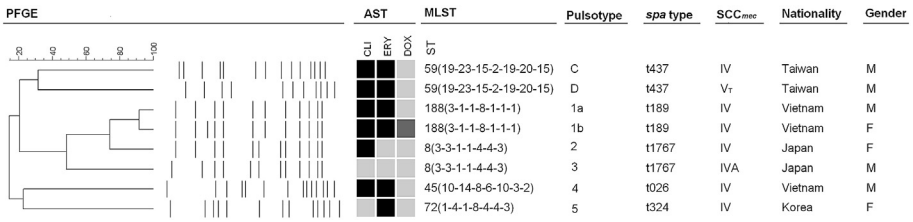

Figure 1. Pulsed-field gel electrophoresis (PFGE) pattern-based dendrogram, antimicrobial susceptibility testing (AST), multilocus sequence typing (MLST), staphylococcal cassette chromosome *mec* (SCC*mec*) and staphylococcal protein A (*spa*) genotypings of the 6 methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from international conference attendees. Two representative MRSA isolates from the local attendee (Taiwan) were used as comparison.[14] All isolates were susceptible to fusidic acid, linezolid, teicoplanin, trimethoprim/sulfamethoxazole, and vancomycin, and were resistant to penicillin. AST: black, resistant; dark grey, intermediate resistant; grey, susceptible; CLI, clindamycin; ERY, erythromycin; DOX, doxycyclin. Gender: M, male; F, female.

Molecular analysis revealed that the six isolates all carried type IV, including one type IVA, SCC*mec*. As shown in Fig. 1, five pulsotypes were identified, and only two of the three isolates from Vietnam shared a similar pulsotype. All pulsotypes were different from the predominant pulsotypes C and D from Taiwan with less than 30% of similarity. MLST analysis and *spa* typing both revealed four different types among the six isolates. The four STs were also completely different from the predominant ST59 found in Taiwan. PVL or *sasX* genes were not found in any of the MRSA isolates. None of common molecular characteristics was shared by isolates from different countries. Most of these isolates were local endemic community clone in each country, such as ST72-SCC*mec* IV-PVL-negative in Korea, ST188-SCC*mec* IV-PVL-negative and ST45-SCCmec IV-PVL-negative in Vietnam.

Discussion

Although the number of participants in the present study was not very large, the MRSA carriage rate (4.8%) was found to be similar to those reported previously among HCWs from a much larger population.[7] Furthermore, we also found a significantly higher carriage rate among participants from some countries (e.g., 37.5% from Vietnam and 33.3% from Korea). A previous report from Vietnam showed a much lower carriage (7.9%) in non-medical settings.[18] Another report from Korea also revealed a much lower carriage (7.8%) in HCWs.[19] However, the figures still seem to be much higher than the average rate (<5%) of MRSA carriage from other reports.[7,8] Despite the low participant numbers, this study is still able to reflect the relative difference among the countries. On the other hand, although the participant numbers were relatively higher (>10) in Thailand, China, Indonesia, and Malaysia, where the prevalence of MRSA was also high (28–53%),[3] no MRSA isolates were identified. The results may be explained by the reported low MRSA carriage rates (0–6%) among the countries.[20–23]

Genotyping analysis revealed that the majority of the MRSA isolates were "domestic" clones prevalent among each of the original countries. For example, the ST72-SCC*mec* IV/PVL-negative MRSA identified from a Korean attendee is the most predominant community MRSA clone in Korea, subsequently penetrating into healthcare settings.[3] The ST8 MRSA found in the two Japanese attendees also has emerged and become the major genotype in the community of Japan since 2003.[3,24] It has been reported that the most predominant clone of healthcare-associated MRSA is ST5 in Japan.[25] It is not known why community strains, rather than healthcare strains, were identified. However, the predominance of CA-MRSA clones as the nasal carriage among HCWs is not uncommon in previous reports.[11,26] Since the number of Japanese participants in this study was rather limited, the actual situation regarding the major clones of MRSA carriage among HCWs in Japan may need to be revealed by future studies. Interestingly, a recent report from a multicenter, prospective cohort study indicated that ST8 MRSA has emerged in Korea, causing bacteremia in both healthcare-associated and hospital-acquired settings.[27]Another recent report from Spain also indicated the emergence of ST72 MRSA which had been rare previously in Europe.[28] The process for such infiltration may not be traceable and may probably involve multiple sources and passages. However, the attendance of international conference may potentially provide an excellent opportunity for these originally "domestic" MRSA clones to be exchanged or transmitted in these countries.

Reports on molecular characteristics of MRSA have been rare from Vietnam. Recently, a study indicated that the most predominant clonal complexes (CC) are CC59, CC188, and CC45 among the *S. aureus* isolates from both community and clinical settings in Vietnam.[29] However, it remains uncertain whether the two strains, ST45 and ST188, identified from Vietnamese attendees in the present study had been common in Vietnam previously. In Taiwan, ST45 MRSA was relatively rare[30] and was firstly identified during an outbreak investigation in 2006.[31] Later in a study conducted in 2012, we found that 50% of the MRSA colonizing isolates were ST45 among the residents and staff, particularly foreign health care workers, from 14 nursing homes in Taiwan.[32] The ST45 MRSA appears to be a successful MRSA clone that is able to efficiently disseminate and constitute in various healthcare settings.

On the other hand, the two isolates of ST188-SCC*mec* IV-t189 MRSA happened to share a similar PFGE pattern (PFGE pattern AX) with another two bacteremic isolates we identified from Taiwan in 2010.[33] The clone, ST188-SCC*mec* IV-t189, actually has been increasingly described across countries in the Asia-Pacific region, including Australia[34] and China.[35] ST188-t189 MRSA isolates with other SCC*mec* types also have been reported, including ST188-SCC*mec*

III-t189 in Korea,[36] ST188-SCC*mec* V-t189 in Malaysia[37] and Hong Kong.[38] ST188-t189 also presented in methicillin-susceptible *S. aureus* isolates from Taiwan[26] and China.[35] ST188-t189 *S. aureus*, with or without methicillin resistance, appears to have been widespread among Asian countries. Similar to the situation found in ST45 MRSA described above, it remains unknown how the clones were transmitted across countries and continents. International travel may somehow contribute to this effect as it has been shown to play one of the major roles in the transmission of some multidrug-resistant microbial clones as well as transmissible diseases among countries.[4]

Unavoidably, there are some limitations to this study. Firstly, the total sampling number was rather low, so as to the isolate number. This problem may be inevitable for such studies under voluntary basis within a limited time frame during a specific event. Also both conferences were for pediatricians and may not represent the whole HCWs for the specific country. However, the aim of this study was not to determine the respective carriage rates among HCWs from different countries. Besides, we found a similar carriage rate (4.8%) to those published previously.[7] The results may to some extent exclude the possibility of sampling bias. Secondly, more detailed background information, such as the name of the hospital/city where each attendee was related to, should be added to the questionnaire. Without such information, we were not able to know the finding that two Vietnamese isolates shared a similar pulsotype was merely coincident or they had some epidemiological relatedness. Thirdly, the present study can be optimized to detect the MRSA carriage at two time points, i.e., at both arrival and departure. By comparing the paired results, it may be more clear whether the MRSA clones from different hosts may have exchanged or cross-transmitted during the period of the international conference.

International massive gatherings, such as the international medical conferences described herein, may further amplify the opportunity for the transmission of carriage or infection through international travel.[5] Mass gathering medicine has been recognized as an important field of modern medicine with global concerns by the World Health Organization and many countries.[39] However, researches looking into health issues associated with mass gatherings are still at an early stage.[40]

In conclusion, MRSA carriage rate among the international medical conference attendees was within the range of MRSA carriage among HCWs in non-outbreak settings. Most of these isolates were "domestic" community clones from the original countries of the attendees. The potential transmission or exchange of MRSA among the attendees needs further studies.

Conflicts of interest

All authors have no conflicts of interest.

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