Prevalence of Methicillin-Resistant *Staphylococcus aureus* Nasal Colonization among Taiwanese Children in 2005 and 2006

Yhu-Chering Huang,1,3* Kao-Pin Hwang,2,3 Po-Yen Chen,4 Chih-Jung Chen,1,3 and Tzou-Yien Lin1,3

*Division of Pediatric Infectious Diseases, Chang Gung Children’s Hospital and Chang Gung Memorial Hospital at Linko1 and Kaohsiung;2 Taiwan; College of Medicine, Chang Gung University, Taoyuan, Taiwan3; and Department of Pediatrics, Taichung Veterans General Hospital, Taichung, Taiwan4*

Received 15 June 2007/Returned for modification 20 August 2007/Accepted 4 October 2007

From July 2005 to October 2006, a total of 3,046 children, of ages between 2 months and 5 years, presented for a well-child health care visit to one of three medical centers, which are located in the northern, central, and southern parts of Taiwan, and were surveyed for nasal carriage of methicillin-resistant *Staphylococcus aureus* (MRSA). The overall prevalences of *S. aureus* and MRSA nasal carriage among the children were 23% and 7.3%, respectively (18% and 4.8% in the central region, 25% and 6.7% in the southern region, and 27% and 9.5% in the northern region). Of the 212 MRSA isolates (96%) available for analysis, a total of 10 pulsed-field gel electrophoresis (PFGE) patterns with two major patterns (C [61%] and D [28%]) were identified. One hundred forty-nine isolates (70%) contained type IV staphylococcal cassette chromosome mec (SCCmec) DNA, and 55 isolates (26%) contained SCCmec IV. The presence of Panton-Valentine Leukocidin (PVL) genes was detected in 60 isolates (28%). Most MRSA isolates belonged to one of two major clones, characterized as sequence type 59 (ST59)/PFGE C/SCCmec IV/absence of PVL genes (59%) and ST59/PFGE D/SCCmec Vf/presence of PVL genes (25%). We concluded that between 2005 and 2006, 7.3% of healthy Taiwanese children were colonized by MRSA in nares. MRSA harbored in healthy children indicates an accelerated spread in the community.

Recent reports indicate that community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) infections are increasing worldwide and may now involve persons without risk factors predisposing them for acquisition (2, 11–14, 20, 24). Asymptomatic CA-MRSA colonization has been documented in healthy children attending the emergency departments and outpatient clinics of children’s hospitals (6, 19, 25, 27, 28).

Carriage of *S. aureus*, including MRSA, is well known to be a significant risk factor for subsequent infection (7, 29), and the anterior nares are the most consistent sites of colonization. The presence of *S. aureus* nasal colonization can provide an indication of a high risk for subsequent infection.

In Taiwan, previous reports (1, 3, 9, 15, 17, 23, 31) have indicated that during the period from 1997 to 2003, MRSA accounted for 9.8% to 36% of CA *S. aureus* infections in children without risk factors and the MRSA colonization rate in the general population ranged from 1.9% to ~3% for school children and 5.3% for healthy children presented for health care visits to 10.8% for health care workers and 13.6% for contacts of CA-MRSA infection. It is noteworthy, however, that most of these studies were conducted in the northern part of Taiwan and no island-wide survey has yet been conducted to elucidate this issue. To estimate the extent of MRSA in the community in Taiwan and to assess if there is an increasing trend of MRSA nasal colonization in healthy children during the past 5 years, we conducted this island-wide survey between 2005 and 2006. All collected MRSA isolates were also further characterized by molecular methods.

**MATERIALS AND METHODS**

This study was approved by the Institutional Review Board of the Chang Gung Memorial Hospital. From July 2005 to October 2006, all children of ages between 2 months and 5 years who presented for a well-child health care visit to any one of three medical centers in Taiwan were invited to participate in this study. The three medical centers involved were the Chang Gung Children’s Hospitals at Linko (hospital A) and Kaohsiung (hospital C) and Taichung Veterans General Hospital (hospital B), which are situated, respectively, in northern, southern, and central parts of Taiwan. In each hospital, around 80 subjects were recruited for study for each month, and the ages of the subjects were evenly distributed in seven separate age ranges, which included >2 to 6 months, >6 to 12 months, >12 to 18 months, >18 to 24 months, >2 to 3 years, >3 to 4 years, and >4 to 5 years. A culture from the anterior nares for the detection of MRSA was obtained from each subject after written consent was obtained from their parents/guardians.

Survey specimens for culture were obtained with a cotton swab, placed in the transport medium (Venturi Transystem; Copan Innovation Ltd., Limerick, Ireland), and then brought to and processed in the microbiological laboratories within 4 hours of the sampling. All *S. aureus* isolates were sent to Chang Gung Memorial Hospital at Linko for microbiological characterization. Identification of MRSA was confirmed according to Clinical and Laboratory Standards Institute 2005 guidelines (5). Pulsed-field gel electrophoresis (PFGE) with Smal digestion was used in this study to fingerprint the MRSA isolates and was performed according to procedures described previously (3, 16, 18). The genotypes were designated in alphabetical order, as in our previous studies (3, 15–18); any new genotype, if identified, was designated consecutively. PFGE patterns with fewer than four band differences from an existing genotype were defined as subtypes of that genotype.

SCCmec typing of isolates was done using a multiplex PCR strategy described previously (26). Control strains for SCCmec types I, II, III, and IVa, kindly provided by Keiichi Hirata, were as follows: type I, NCTI10442; type II, N315; type III, 85/2082; and type IVa, JCSC4744. SCCmec typing for type Vf was determined by using a particular primer described elsewhere (1), and strain TSGH-17, kindly provided by Chi-Chien Wang, was used as a control. However, the SCCmec typing method for type Vf yielded inconsistent results; thus, an
alternative method was used. The appearance of an amplicon with only two bands (414 bp and 243 bp) in the multiplex PCR analysis may have indicated that the isolate contained SCC\textit{mec} \textit{Vc}. To confirm their identities, a novel pair of primers, ccr\textit{C}-FF (5'-CAC TTA AYC CAT TGA CAC AG-3') and ccr\textit{C}-SR (5'-AAA GAT TGA GGA ATA AGA CT-3'), was designed according to the published sequence (GenBank accession no. AY894416 of the \textit{ccrC} gene of a Taiwanese strain, \textit{S. aureus} TSGH-17. Amplification of a specific 1,081-bp DNA fragment, which was subjected to further sequence analysis for some representative isolates in preliminary experiments, confirmed that the isolates contained SCC\textit{mec} \textit{Vc}.

The presence of Panton-Valentine leukocidin (PVL) genes was determined by a PCR strategy described previously (22). Some isolates of representative PFGE patterns were selected and underwent multilocus sequence typing (MLST) as described elsewhere (8). The allelic profiles were assigned through comparison of the sequences at each locus with those of the known alleles in the MLST database and were defined as sequence types accordingly.

RESULTS

During the study period, 1,279 subjects were recruited from hospital A, 1,011 subjects from hospital B (from July 2005 to June 2006), and 756 subjects from hospital C (from October 2005 to June 2006). All the children enrolled are Taiwanese. The number of subjects enrolled in each age group ranged from 430 for children of ages >6 to 12 months to 443 for children of ages >2 to 3 years. Of the total of 3,046 subjects enrolled in this study, 713 (23%) were colonized with \textit{S. aureus}. Of the 713 isolates, 221 (31%) were demonstrated to be MRSA. The details of the nasal MRSA colonization prevalence for subjects in the different parts of Taiwan are shown in Table 1. The MRSA colonization rate in northern Taiwan was significantly higher than that in the central (\(P < 0.001\)) and southern (\(P < 0.039\)) parts of Taiwan. The nasal MRSA colonization prevalences for the subjects in each age group were 8.4% for the children of ages >2 to 6 months and 6.3%, 3.2%, 3.9%, 9.0%, 9.5%, and 10.1% for children of ages >6 to 12 months, >12 to 18 months, >18 to 24 months, >2 to 3 years, >3 to 4 years, and >4 to 5 years, respectively. For those less than 18 months of age, the carriage rate decreased with increasing age (\(P = 0.0011\); Mantel-Haenszel test for trend), while for those older than 12 months of age, the carriage rate increased with increasing age (\(P < 0.0001\)).

Of the 221 MRSA isolates, 212 isolates were available for analysis. All of these 212 isolates were sensitive to vancomycin and teicoplanin. All but two of the isolates identified from hospital A were resistant to penicillin. Most isolates were resistant to erythromycin and clindamycin but sensitive to trimethoprim-sulfamethoxazole (SXT) and doxycycline. The detailed susceptibility distribution of various antibiotics for the isolates is shown in Table 2. No significant difference in antibiotic susceptibility patterns was noted among the isolates from the three different regions of Taiwan.

Table 3 illustrates the detailed distribution of PFGE patterns, SCC\textit{mec} types, and the presence/absence of PVL genes among these isolates. A total of 10 PFGE patterns were identified. Patterns C and D were the two most common patterns and accounted for 62% and 28% of the isolates analyzed, respectively. The distribution of PFGE patterns among the three regions showed a trend for a difference (\(P = 0.09\) by a log-likelihood contingency test). Four types (types II, III, IV, and V\textsubscript{T}) of SCC\textit{mec} genes were identified among the isolates, with type IV (70%) being the predominant type, followed by type V\textsubscript{T} (26%). The distribution of SCC\textit{mec} types among the three regions was significantly different (\(P = 0.03\)). Four isolates of the AF PFGE pattern were untypeable by the methods used in this study. PVL genes were present in 60 isolates (28%). Twenty-five isolates underwent MLST, and eight sequence types were identified. Sequence type 59 (ST59) was the most common type and accounted for 9 of 10 PFGE type C isolates, 4 of 6 PFGE type D isolates, and the isolate of PFGE type AN. The other two isolates of PFGE type D were ST338, which is a single-locus variant of ST59 (a single nucleotide difference in the \textit{gmk} locus). The remaining isolate of PFGE type C belonged to a new sequence type, which is a single-locus variant of ST59 (a single nucleotide difference in the \textit{pta} locus). One isolate of PFGE type F also belonged to a new sequence type, which is also a single-locus variant of ST9 (a single nucleotide difference in the \textit{gmk} locus). The detailed association of PFGE patterns with sequence types and SCC\textit{mec} types and the presence of the PVL gene of these isolates are shown in Table 4. The MRSA isolates characterized by ST59/PFGE type C/SCC\textit{mec} IV/absence of PVL genes and ST59/PFGE type D/SCC\textit{mec} V\textsubscript{T}/presence of PVL genes were the two most common clones and accounted for 59% and 25% of the isolates analyzed, respectively.

<table>
<thead>
<tr>
<th>Area of Taiwan</th>
<th>No. of subjects</th>
<th>No. (%) with \textit{S. aureus}\textsuperscript{a}</th>
<th>No. (%) with MRSA\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern</td>
<td>1,279</td>
<td>344 (26.9)</td>
<td>121 (9.5)</td>
</tr>
<tr>
<td>Central</td>
<td>1,011</td>
<td>180 (17.8)</td>
<td>49 (4.8)</td>
</tr>
<tr>
<td>Southern</td>
<td>756</td>
<td>189 (25)</td>
<td>51 (6.7)</td>
</tr>
<tr>
<td>Total</td>
<td>3,046</td>
<td>713 (23.4)</td>
<td>221 (7.3)</td>
</tr>
</tbody>
</table>

\textsuperscript{a} The rate of carriage of \textit{S. aureus} among children in the central region was significantly lower than those among children in the northern and southern regions (\(P < 0.001\)).

\textsuperscript{b} The rate of carriage of MRSA among children in the northern region was significantly higher than those among children in the central (\(P < 0.001\)) and southern (\(P = 0.039\)) regions.
noted in certain areas of the United States recently (6). Creech studies; however, an increasing trend in this regard has been
For healthy children, the nasal colonization rates ranged from
1999 to 2001 and 2002, national
onization prevalence estimates were 32.4% and 0.8%, respectively.
ning trend of nasal MRSA colonization prevalence might ac-
CA-MRSA is also being increasingly reported, the MRSA col-
patients or clinic and demographic characteristics (e.g., age and
gender) were reported to be associated with an increased risk
with MRSA acquisition were not analyzed and compared be-
tween the children with and without CA-MRSA colonization,
though all the children were healthy and presented for health
care visits. Living with a family member who works in a hospi-
tal or clinic and demographic characteristics (e.g., age and
gender) were reported to be associated with an increased risk
of MRSA colonization (6, 21, 25). Second, the persistence of
MRSA colonization (6, 21, 25). Second, the persistence of
MRSA nasal colonization prevalence.

**DISCUSSION**

Results from this study indicate that the national prevalence
of nasal MRSA colonization among otherwise healthy children
in Taiwan was 7.3% during the period from July 2005 to Oc-
tober 2006 inclusively, with values ranging from 4.8% in the
central region of Taiwan to 9.5% in the northern region of
Taiwan. Compared with those among the healthy children dur-
during the period of 2001 to 2002 (1, 17, 23) (Table 5), though the
study population was different for these studies, the nasal
MRSA colonization prevalence among healthy children in Tai-
wan increased significantly, from 1.9% in 2001 to 9.5% (P <
0.001) by chi-square test) during the period of 2005 to 2006 for
northern Taiwan and significantly from 3.3% to 6.7% for
southern Taiwan (P < 0.001 by chi-square test). This increas-
ting trend of nasal MRSA colonization prevalence might ac-
account for the increasing incidence of CA-MRSA infection in
children in Taiwan (3, 9, 31). In the United States, where
CA-MRSA is also being increasingly reported, the MRSA col-

**TABLE 3. Distribution of PFGE patterns, SCCmec types, and presence of PVL genes among 212 colonizing MRSA isolates**

<table>
<thead>
<tr>
<th>Area of Taiwan (n*)</th>
<th>PfGE pattern</th>
<th>SCCmec type</th>
<th>Presence of PVL genes</th>
<th>Absence of PVL genes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>C</td>
<td>D</td>
<td>Other</td>
</tr>
<tr>
<td>Northern (120)</td>
<td>1 (0.8)</td>
<td>66 (55)</td>
<td>40 (33)</td>
<td>13 (11)</td>
</tr>
<tr>
<td>Central (42)</td>
<td>2 (4.8)</td>
<td>28 (67)</td>
<td>10 (24)</td>
<td>2 (4.8)</td>
</tr>
<tr>
<td>Southern (50)</td>
<td>0</td>
<td>37 (74)</td>
<td>9 (18)</td>
<td>4 (8)</td>
</tr>
<tr>
<td>Total (212)</td>
<td>3 (1.4)</td>
<td>131 (62)</td>
<td>59 (28)</td>
<td>19 (9.0)</td>
</tr>
</tbody>
</table>

a n, no. of isolates.
b Four isolates were untypeable. The distributions of SCCmec types were significantly different among the three regions (P = 0.03).

genes (e.g., PVL), and contain SCCmec DNA (10). In contrast, the CA-MRSA clinical isolates in Tai-
were multiresistant and shared two common PFGE pat-
terns (patterns D and C in this study) (1, 3, 4, 30). In the current
study, more than 90% of the MRSA colonization iso-
lates were multiresistant to erythromycin and clindamycin but
sensitive to SXT and doxycycline. In addition, most coloniza-
isolates shared common molecular characteristics, and more than 80% of the isolates belonged to one of two major
clones, characterized by ST59/PFGE type C/SCCmec IV/ab-
ence of PVL genes or ST59/PFGE type D/SCCmec V*presence of PVL genes. However, among the clinical isolates, the
cloned characterized by ST59/PFGE type D/SCCmec V*presence of PVL genes was the dominant clone (1, 3, 30), while
among the colonized isolates, the clone characterized by ST59/
PFGE type C/SCCmec IV/absence of PVL genes was domi-
nant. It seemed that PVL genes, reported to be a virulence
factor associated with necrotizing pneumonia and abscesses
(22), may be associated with the ability of a PVL-positive clone
to cause infection.

There existed several limitations in the current study. First,
the demographic characteristics and the risk factors associated
with MRSA acquisition were not analyzed and compared be-
tween the children with and without CA-MRSA colonization,
though all the children were healthy and presented for health
care visits. Living with a family member who works in a hospi-
tal or clinic and demographic characteristics (e.g., age and
gender) were reported to be associated with an increased risk
of MRSA colonization (6, 21, 25). Second, the persistence of
MRSA carriage in the subjects could not be determined and
the incidence of subsequent MRSA infection in the subjects
could not be measured in this cross-sectional analysis of
MRSA nasal colonization prevalence.

In summary, 7.3% of healthy children in Taiwan were colo-
nized by MRSA in the nases during the period from 2005 to
2006. MRSA carriage in the children may accelerate the
spread in the community. Two major CA-MRSA clones were

**TABLE 4. Association of PFGE patterns with MLST, SCCmec types, and presence of PVL genes for 212 MRSA isolates**

<table>
<thead>
<tr>
<th>PFGE pattern</th>
<th>No. of subtypes</th>
<th>SCCmec type</th>
<th>Status of PVL genes</th>
<th>Sequence type</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (3)</td>
<td>3</td>
<td>III (2), IV (1)</td>
<td>Absent (2), Absent (2)</td>
<td>ST239</td>
</tr>
<tr>
<td>C (131)</td>
<td>25</td>
<td>IV (129), V*</td>
<td>Absent (129), Absent (129)</td>
<td>ST59, new*</td>
</tr>
<tr>
<td>D (59)</td>
<td>13</td>
<td>V* (53), IV (6)</td>
<td>Present (57), Absent (2)</td>
<td>ST59, ST338*</td>
</tr>
<tr>
<td>F (3)</td>
<td>2</td>
<td>II (2), V* (1)</td>
<td>Absent (2), Absent (2)</td>
<td>ST59</td>
</tr>
<tr>
<td>AF (4)</td>
<td>1</td>
<td>Untypeable</td>
<td>Absent (1), Absent (1)</td>
<td>ST59</td>
</tr>
<tr>
<td>AK (2)</td>
<td>2</td>
<td>IV</td>
<td>Absent (2), Absent (2)</td>
<td>ST5908</td>
</tr>
<tr>
<td>AN (3)</td>
<td>2</td>
<td>IV</td>
<td>Absent (2), Absent (2)</td>
<td>ST59</td>
</tr>
<tr>
<td>AQ (5)</td>
<td>3</td>
<td>IV</td>
<td>Absent (2), Absent (2)</td>
<td>ST5908</td>
</tr>
<tr>
<td>AR (1)</td>
<td>1</td>
<td>IV</td>
<td>Absent (1), Absent (1)</td>
<td>ST15</td>
</tr>
<tr>
<td>BA (1)</td>
<td>1</td>
<td>IV</td>
<td>Absent (1), Absent (1)</td>
<td>ST5</td>
</tr>
</tbody>
</table>

a n, no. of isolates.
b Each of the three sequence types marked with an asterisk is a single-locus
variant of ST59, but they differ from each other.
identified and would appear to have spread island-wide. Further studies are needed to determine the host factors of colonization and to develop strategies to disrupt transmission of CA-MRSA to susceptible hosts.

ACKNOWLEDGMENTS

This study was supported by a grant from National Science Counseling of Executive Yuan of Taiwan (NSC95-2314-B182A-145).

We have no financial relationships relevant to this article to disclose.

REFERENCES


