Original Article

Antimicrobial Resistance and Molecular Characteristics of Methicillin-Resistant *Staphylococcus aureus* Isolates from Child Patients of High-Risk Wards in Shenzhen, China

Yang Qin1,2, Feiqiu Wen2*, Yuejie Zheng2, Ruizhen Zhao2, Qinghua Hu1, and Renli Zhang3

1First Affiliated Hospital of Jinan University, Guangzhou; 2Shenzhen Children’s Hospital, Shenzhen; and 3Center for Disease Control and Prevention (CDC), Shenzhen, China

SUMMARY: Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are responsible for high rates of mortality and thus pose a substantial burden to public health worldwide. Here, we investigated the antimicrobial susceptibility and molecular characteristics of MRSA isolated from child patients at Shenzhen Children’s Hospital. We characterized 140 MRSA strains through antimicrobial susceptibility testing. We further performed spa typing, multilocus sequence typing (MLST), staphylococcal cassette chromosome mec (SCCmec) analysis, *pvl* gene analysis, and pulsed-field gel electrophoresis (PFGE). The analyzed MRSA strains were found to be sensitive to most non-β-lactam antimicrobial agents. Sequence type (ST) 59 was found to be the most common MLST lineage (54.3%). Most MRSA isolates belonged to the SCCmec IV (64.3%) and V (22.8%) types. The MRSA-ST59-SCCmec IV-t437 clone was the most predominant strain that infected 28.6% of all patients studied. Moreover, 50.7% of MRSA isolates were found to be *pvl*-positive. We report preliminary data on the prevalence and distribution of MRSA genotypes in Shenzhen Children’s Hospital. We characterized MRSA colonization dynamics in child patients in China, and our findings can serve as the basis for the development of strategies to prevent MRSA infection and transmission.

INTRODUCTION

*Staphylococcus aureus* is a common pathogen that causes various forms of infectious diseases in humans (1). Methicillin-resistant *S. aureus* (MRSA) has been reported to cause skin and soft tissue infections, pneumonia, foreign-body infections, endocarditis, septic arthritis, blood-stream infections, sepsis, and osteomyelitis in both hospital and community settings (2). The health burden caused by high MRSA infection rate has contributed to increase globally (3).

Transmission of MRSA infection has been associated with healthcare facilities, especially large tertiary-care facilities (4). Children infected with MRSA act as potential reservoirs for the subsequent spread of MRSA in the community (5). Furthermore, immunologically immature infants and newborns, especially those born prematurely or who require specialized care, are the most susceptible to MRSA infection (6).

The latent shift of MRSA clones at both the local and global levels is of great interest, primarily because a detailed understanding of MRSA epidemiology will be necessary to establish of public health interventions to control the spread of MRSA. Molecular typing techniques including spa typing, multilocus sequence typing (MLST), staphylococcal cassette chromosome mec (SCCmec) analysis, and pulsed-field gel electrophoresis (PFGE), have been used to characterize the epidemiology and differentiation of MRSA isolates worldwide (7). Previous studies using these techniques demonstrated that the most prevalent MRSA clones have unique geographical distributions. For example, MRSA clones USA300, sequence type 80 (ST80), ST59, and ST30, known to cause community-acquired infections (CA-infections), were reported in the USA (8), Europe, the Asia-Pacific region, and worldwide, respectively (9). Currently, the most widespread MRSA clone responsible for skin and soft tissue infections (SSTIs) in mainland China is ST59-SCCmec IV (10). However, the epidemiology of MRSA in Chinese children has not been well studied (11,12). In this study, we isolated and characterized 140 clinical MRSA strains isolated from child patients in Shenzhen Children’s Hospital, China. We aimed to identify the phenotypic and genotypic markers for these isolates, including antimicrobial resistance, spa type, SCCmec type, PFGE pattern, and sequence types of 7 unlinked housekeeping genes (*arcC, aroE, glpF, gmk, pta, tpi, and yqil*). Finally, we also aimed to determine the association of these MRSA isolates with the *pvl* gene, which encodes the cytoxin Panton-Valentine leukocidin (PVL). PVL is known to causes leukocyte destruction and is associated with increased of *S. aureus* virulence (13).

MATERIALS AND METHODS

Strain collection, MRSA confirmation, and clinical information: A total of 140 non-duplicate MRSA isolates were collected from Shenzhen Children’s Hospital neonatal department, pediatric intensive care
Detection of the \textit{pvl} gene: The \textit{pvl} gene was detected in all isolates using the forward and reverse primers, 5'-ACACATATGCAATAGTTATT-3' and 5'-AAAGCAATGCAATTGATGTA-3' (19). PCR amplification was performed according to the following profile: 1 cycle of 5 min at 94ºC, 35 cycles of 45 s at 94ºC, 45 s at 60ºC, 90 s at 72ºC, and a final extension step of 10 min at 72ºC using Ex Taq (Takara Bio, Shiga, Japan). Amplified fragments were sequenced and compared to the EF571841.1 sequence.

PFGE typing: PFGE has been considered the gold standard technique used in molecular epidemiology studies (20). For PFGE, chromosomal DNA was digested with the SmaI restriction enzyme at 14ºC as previously described and separated with a voltage of 6 volts/cm and an angle of 120º. Pulse increase from 5 s to 40 s was set as the switch time. A molecular weight standard was used for each gel to curate and normalize the DNA fragments. Optimization of 0.5% and band tolerance of 1.5% were selected during comparison of DNA profiles. Isolates were considered to be genetically related if the dice correlation coefficient was > 80%. Cluster analysis was performed using the unweighted pair group method using arithmetic mean (UPGMA). PFGE patterns were analyzed using Bionumerics version 6.6 (Applied-Maths, Sint-Martens-Latem, Belgium).

Statistical analysis: Statistical analyses were performed using SPSS (ver. 16; Chicago, IL, USA). Student’s \( t \)-test was used for normally distributed variables, while and the \( \chi^2 \) test was used for detecting the homogeneity of proportions for categorical data. All hypotheses were two-tailed. Results were considered statistically significant at \( P \)-value \( (P) < 0.05 \).

RESULTS

MRSA confirmation of isolates: \textit{S. aureus} identification was performed using a combination of phenotypic tests, including microscopic examination, measurement of coagulase production, catalase activity, and Vitek microbiology analysis. MRSA isolates were screened using cefoxitin discs. All isolates tested positive for both the \textit{mec}A and \textit{nuc} genes. A total of 140 non-duplicate MRSA isolates from 140 patients were selected for further antimicrobial susceptibility testing and molecular typing.

Antimicrobial susceptibility profiles: Results of antimicrobial susceptibility testing indicated that all 140 MRSA isolates were resistant to oxacillin and penicillin (Table 1) but susceptible to nitrofurantoin, quinupristin, vancomycin, levoflaxacin, and moxifloxacin. The majority of MRSA isolates were also resistant to erythromycin (77.8%), clindamycin (75.0%), and tetracycline (55.6%), while 7.4% and 3.7% were resistant to sulfamethoxazole/thrimethoprim and gentamicin, respectively. Only 3.7% and 7.4% of isolates exhibited intermediate susceptibility to ciprofloxacin and rifampin, respectively. Susceptibilities to other antimicrobials are presented in Table 1. We further selected 43 MRSA isolates, including 29 CA-MRSA strains and 14 healthcare-associated MRSA (HA-MRSA) strains, for further antibiotic susceptibility analysis (sulfamethoxazole/thrimethoprim, erythromycin, ciprofloxacin, clindamycin,
gentamicin, tetracycline, vancomycin, and linezolid). Results showed no significant differences in different isolate types among different antimicrobials (Table 2).

**Molecular characteristics of MRSA isolates:** spa typing identified 24 known spa types, out of which the most frequently detected were t437, t114, t5132, t324, t664, t116, t309, t441 and t034, which together account for 82.9% (116/140) of all spa-typed isolates. A total of 76 isolates (54.3%) were typed as t437; 11 isolates (7.9%) were typed as t114; and 6 isolates were typed as t324. t437 was the most frequently detected spa type in this cohort.

MLST test identified 23 distinct ST types. The most predominant ST type was ST59, which accounted for 54.3% (76/140) of all isolates, followed by ST1 (12.1%, 17/140). Eight isolates were ST45, 8 were ST338, 5 were ST398, and 5 were ST72. Three isolates were typed as ST88; only 2 were ST22, and 2 were ST25. The remaining isolates were identified as ST1, ST9, ST15, ST19, ST30, ST488, ST537, ST1507, ST2808, and ST3068, which corresponded to 1 isolate each. Notably, 4 STs that were characterized as ST2962, ST3185, ST3187, and ST3188, were observed in China for the first time. Thus, ST59 was the most frequently detected MLST type in this cohort.

Using the multiplex SCCmec typing method, 18 out of the 140 MRSA isolates were classified as non-typeable (NT). These isolates were distributed among t437 (6 isolates), t309 (4 isolates), and other spa types (8 isolates), including ST59 (6 isolates), ST1 (3 isolates), ST45 (1 isolate), and other of MLST types (8 isolates). Only SCCmec IV and SCCmec V clones were identified in the samples analyzed. A total of 90 isolates (64.3%) were identified as SCCmec IV, while 32 (22.9%) were SCCmec V.

The pvl gene was detected in 71 isolates (50.7%) that were SCCmec IV, SCCmec V, and non-typeable. A total of 35.6% (32/90) of SCCmec IV and 81.3% (26/32) of SCCmec V isolates were pvl-positive, 47.4% (36/76) of ST59 isolates carried the pvl gene. As shown in Table 1.

### Table 1. Antimicrobial resistance profiles of MRSA isolates from the child patients (n = 140)

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>R, %</th>
<th>L, %</th>
<th>S, %</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt;</th>
<th>MIC range</th>
</tr>
</thead>
<tbody>
<tr>
<td>oxacillin</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>4–4</td>
</tr>
<tr>
<td>nitrofurantoin</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>16</td>
<td>16</td>
<td>16–32</td>
</tr>
<tr>
<td>sulfamethoxazole/trimethoprim</td>
<td>7.4</td>
<td>0</td>
<td>92.6</td>
<td>12</td>
<td>12</td>
<td>10–320</td>
</tr>
<tr>
<td>erythromycin</td>
<td>77.8</td>
<td>3.7</td>
<td>96.3</td>
<td>0.5</td>
<td>1</td>
<td>0.5–2</td>
</tr>
<tr>
<td>ciprofloxacin</td>
<td>75.0</td>
<td>3.7</td>
<td>96.3</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5–2</td>
</tr>
<tr>
<td>clindamycin</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5–0.25</td>
</tr>
<tr>
<td>gentamicin</td>
<td>3.7</td>
<td>0</td>
<td>96.3</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5–0.25</td>
</tr>
<tr>
<td>tetracycline</td>
<td>75.6</td>
<td>0</td>
<td>24.4</td>
<td>16</td>
<td>16</td>
<td>1–16</td>
</tr>
<tr>
<td>vancomycin</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>0.5</td>
<td>1</td>
<td>0.5–1</td>
</tr>
<tr>
<td>levofloxacin</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>0.125</td>
<td>0.5</td>
<td>0.12–0.5</td>
</tr>
<tr>
<td>moxifloxacin</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25–0.25</td>
</tr>
</tbody>
</table>

R, resistant; I, intermediate; S, susceptible; MIC, minimum inhibitory concentration (µg/ml); MIC<sub>50</sub>, the minimal concentration of an antimicrobial agent necessary to inhibit the growth of 50% MRSA (µg/ml); MIC<sub>90</sub>, the minimal concentration of an antimicrobial agent necessary to inhibit the growth of 90% MRSA (µg/ml).

### Table 2. Antimicrobial susceptibility of partial methicillin-resistant S. aureus isolates

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Resistant (%)</th>
<th>CA (%)</th>
<th>HA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sulfamethoxazole/trimethoprim</td>
<td>2 (4.7)</td>
<td>2 (6.9)</td>
<td>0</td>
</tr>
<tr>
<td>erythromycin</td>
<td>36 (83.7)</td>
<td>24 (82.8)</td>
<td>12 (85.7)</td>
</tr>
<tr>
<td>ciprofloxacin</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>clindamycin</td>
<td>33 (76.7)</td>
<td>22 (75.9)</td>
<td>11 (78.6)</td>
</tr>
<tr>
<td>gentamicin</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>tetracycline</td>
<td>29 (67.4)</td>
<td>21 (72.4)</td>
<td>8 (57.1)</td>
</tr>
<tr>
<td>vancomycin</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>linezolid</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

CA, community-acquired; HA, healthcare-associated.

### Table 3. Characteristics of methicillin-resistant S. aureus isolates

<table>
<thead>
<tr>
<th>Origin</th>
<th>SCCmec type</th>
<th>IV (n = 90)</th>
<th>V (n = 32)</th>
<th>NT (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA-MRSA</td>
<td></td>
<td>63</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td>HA-MRSA</td>
<td></td>
<td>27</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>spa</td>
<td></td>
<td>t437 (48), t114 (11), t324 (6), t5132 (2), t116 (4), t441 (2), t034 (4), other (17)</td>
<td>t437 (22), t5132 (2), t441 (2), t034 (4), other (2)</td>
<td>t437 (6), t309 (4), other (8)</td>
</tr>
<tr>
<td>ST</td>
<td></td>
<td>ST59 (52), ST1 (14), ST45 (7), ST398 (1), ST72 (5), other (11)</td>
<td>ST59 (18), ST338 (8), ST398 (4), other (2)</td>
<td>ST59 (6), ST1 (3), ST45 (1), other (8)</td>
</tr>
<tr>
<td>PVL-positive (CA + HA)&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
<td>32 (21 + 11)</td>
<td>26 (20 + 6)</td>
<td>13 (10 + 3)</td>
</tr>
</tbody>
</table>

<sup>1</sup>: No. of CA-MRSA with PVL-positive and HA-MRSA with PVL-positive.
of MRSA, optimize treatment, and identify the modes of pathogenicity (22). Modern genotyping techniques, such as sequencing of protein A (spa type), MLST, SCCmec, and PFGE typing, allow the evolution of prevalent MRSA clones to be monitored (21,23). Shenzhen Children’s Hospital is the only pediatric hospital in Shenzhen, China that treats more than 6,000 outpatients daily on average. Therefore, detailed understanding of the antimicrobial susceptibility and molecular characteristics of MRSA isolates in this hospital can help to devise control measures against MRSA infection, allow the investigation of suspected MRSA outbreaks, and prevent MRSA nosocomial transmission in Shenzhen.

Results from the present study showed that children younger than 1 year old comprised the highest number of child patients with MRSA visited the Shenzhen Children’s Hospital. Immunologically immature infants and newborns, particularly those born prematurely or requiring specialized care, are especially susceptible to MRSA infections. Therefore, the high prevalence of MRSA infections detected in children under 1 year old in Shenzhen should be noted.

Antimicrobial susceptibility testing of the MRSA isolates in child patients at Shenzhen Children’s Hospital revealed high rates of β-lactam resistance but greater sensitivity to most non-β-lactam antimicrobials, such as nitrofurantoin, quinupristin, vancomycin, levofloxacin, and moxifloxacin. A few isolates were found to be sensitive to erythromycin, clindamycin, or tetracycline. This situation could have been caused by the widespread use of antibiotics (particularly β-lactam antibiotics) in China (24) These results suggest that antimicrobial use exerted a strong selective pressure that facilitated the horizontal transfer of antibiotics-resistant genes within the hospital. Compared with the results of a previous study (25), our findings showed lower levels of susceptibility to erythromycin, clindamycin, penicillin, and tetracycline and revealed no significant difference in antimicrobial susceptibility among genetically distinct MRSA isolates (Table 2). Although sulfamethoxazole/trimethoprim and clindamycin are the appropriate first-line therapeutic options for the treatment of skin and soft-tissue infections in Europe, wherein CA-MRSA are widespread (26), our results suggest that these antibiotics are not likely to be successful for effective treatment of MRSA infections in Shenzhen, in China.

As previously reported, the spa types of MRSA isolates vary geographically. spa type t041 is a common strain type found in southern Germany (27). spa type t002 is also common in Israel (28), while spa type t044 is widespread in European countries (29). Our results indicate that t437 was the predominant spa type among MRSA isolates from Shenzhen, south China, and accounts for 57.1% of all MRSA isolates, consistent with a previous study of isolates collected in Beijing, northern China (30).

The MLST types of MRSA isolates have also been reported to vary geographically. For example, ST59 is mostly found in the Asia-Pacific region, and ST30 was reported worldwide, including in the USA, western Pacific region, Europe, Hong Kong, and Japan. In mainland China, ST59-MRSA was the predominant MRSA isolate detected in children (11,12). In our study,
the predominant MLST types were determined to be ST59 and ST338, both of which belong to CC59, which has previously been reported in Hong Kong, Denmark, England, Germany, USA, and the Asia-Pacific region (31). In summary, we validated the presence of 140 MRSA strains from 140 child patients treated in Shenzhen, China and investigated their antimicrobial susceptibilities and molecular characteristics. Our results provide baseline information on antimicrobial resistance and the molecular characteristics of MRSA strains infecting child patients in Shenzhen, China. These MRSA isolates were found to be sensitive to most non-β-lactam antimicrobial agents. The MRSA-ST59-SCC mec IV-1437 clone was the most predominant strain detected among all samples. Our findings verified the MRSA colonization dynamics in children and can contribute to the design of strategies and prophylactic measures that aim to prevent the spread of MRSA.

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Conflict of interest None to declare.

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