Characteristics of qacA/B-positive Staphylococcus aureus isolated from patients and a hospital environment in China

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Objectives: This study was designed to demonstrate the characteristics of qacA/B-positive Staphylococcus aureus in China.

Methods: One hundred and forty-five MRSA and 178 MSSA from clinical specimens from seven hospitals in different regions of China, 70 MRSA from superficial sites of patients and 106 MRSA from environmental samples from an ICU were collected and screened for the presence of the qacA/B gene. The qacA/B-positive isolates and 72 randomly selected qacA/B-negative control isolates were further characterized by MLST, spa typing and detection of toxin genes, as well as antimicrobial and chlorhexidine susceptibility. SCCmec typing was conducted for MRSA. PFGE was conducted for qacA/B-positive isolates.

Results: Twenty-five (7.8%) of the 321 MRSA isolates harboured qacA/B, including 11 isolates from clinical specimens (7.6%), 12 isolates from patients’ superficial sites (17.1%) and 2 isolates from an ICU environment (1.9%). Ten and five qacA/B-positive MRSA were identified as ST239-t030-MRSA-III and ST239-t037-MRSA-III, respectively. Six PFGE clusters and five singletons were identified among the 25 qacA/B-positive MRSA. Only one (0.6%) of the 178 MSSA isolates harboured qacA/B. qacA/B carriage in MRSA was statistically associated with spa-t037 and the presence of mupA. Compared with qacA/B-negative MRSA, the qacA/B-positive MRSA exhibited a lower susceptibility to chlorhexidine and higher resistance rates to clindamycin and trimethoprim/sulfamethoxazole.

Conclusions: Carriage of qacA/B, although it had a low prevalence, might be the main reason for declining susceptibility to chlorhexidine in MRSA from Chinese patients and is probably associated with spa-t037 and the presence of the mupA gene.

Keywords: S. aureus, chlorhexidine resistance, antimicrobial resistance, toxin genes

Introduction

MRSA infection has been shown to be associated with a high morbidity, mortality and excess hospital costs.1 Fortunately, the use of antiseptic agents in hospital helps to control the spread and acquisition of this notorious pathogen. As an important antiseptic agent, chlorhexidine has been widely used since 1954.2 However, reduced susceptibility to chlorhexidine in Staphylococcus aureus has been reported since the last century.3 It is generally accepted that one of the important mechanisms conferring resistance to chlorhexidine in S. aureus is the qacA/B gene, which encodes proton-motive force-dependent export pumps.4 The qacA/B
In vitro antimicrobial and chlorhexidine susceptibility

For the qacA/B-positive and control isolates, susceptibility to 14 antibiotics was determined by the agar disc diffusion method according to the CLSI guidelines (M07-A9). A 2-fold dilution series, from 32 to 0.0625 mg/L, was prepared fresh from a 20% (w/v) chlorhexidine gluconate solution (Sigma) and the MICs were determined by the agar dilution method.

Statistical analysis

Statistical analysis was carried out using SPSS 19.0 for Windows (SPSS Inc., Chicago, IL, USA). The ORs and 95% CIs for various factors in terms of qacA/B carriage were analysed by univariate and multivariate logistic regression. The Pearson’s χ² test or Fisher’s exact test was used to determine the differences in antimicrobial resistance between qacA/B-positive and qacA/B-negative isolates. A P value of < 0.05 was considered statistically significant. All tests of significance were two-tailed.

Results

Prevalence of antiseptic genes and in vitro chlorhexidine susceptibility

Among the 321 MRSA isolates, 25 isolates (7.8%) harboured qacA/B, including 11 isolates from clinical specimens (7.6%), 12 isolates from ICU patients’ superficial sites (17.1%) and 2 isolates from a hospital environment (1.9%). The prevalence of qacA/B in MRSA isolates from the three different sources was significantly different (P = 0.001), but the prevalence of qacA/B in clinical MRSA isolates from the seven hospitals was not significantly different (P = 0.655). Only one MSSA isolate (0.6%) from a blood sample of a patient in a hospital in Jinhua was identified as qacA/B-positive. Ten qacA/B-positive MRSA isolates had chlorhexidine MICs > 8 mg/L (Table S1, available as Supplementary data at JAC Online). None of the qacA/B-positive and qacA/B-negative isolates harboured smr, qacG or qacJ. Only one MRSA with a chlorhexidine MIC of 2 mg/L was qacG positive.

Molecular typing of qacA/B-positive and qacA/B-negative isolates

Twenty-five qacA/B-positive MRSA isolates were classified into three MLST types: ST239, ST5 and ST1289 (Table S2). Fifteen, five and two MRSA isolates were identified as t030, t037 and t002, respectively. Twenty-one of the qacA/B-positive MRSA isolates were identified as SCCmec type I (n = 2, 8.0%), II (n = 2, 8.0%) and III (n = 17, 68.0%), respectively. PFGE analysis revealed a high diversity among the 26 qacA/B-positive S. aureus from China. One major cluster (including eight isolates), five minor clusters (fewer than four isolates per cluster) and six singletons with unique PFGE patterns were identified (Figure 1). For the 59 selected qacA/B-negative MRSA isolates, a total of five MLST types and nine spa types were identified. Thirteen qacA/B-negative MSSA were assigned to 9 ST types and 12 spa types (Table S3).

Comparison of molecular and phenotypic characteristics between qacA/B-positive and qacA/B-negative MRSA

As shown in Table 1, there was no significant association between ST239 or spa-t030 and qacA/B-positive MRSA, but spa-t037 strains...
Figure 1. Dendrographic analysis of PFGE (SmaI) of 26 qacA/B-positive S. aureus isolates. All the qacA/B-positive S. aureus isolates were susceptible to vancomycin, teicoplanin, linezolid and quinupristin/dalfopristin, for which the results are not provided. *MSSA. ERY, erythromycin; CLI, clindamycin; RIF, rifampicin; CIP, ciprofloxacin; GEN, gentamicin; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline; LVX, levofloxacin; CTX, cefotaxime; AMK, amikacin. A black square indicates resistance to an antimicrobial.

Table 1. Comparison of ST, spa types, the presence of virulence and resistance genes and chlorhexidine MICs between qacA/B-positive and qacA/B-negative MRSA

<table>
<thead>
<tr>
<th>Variable</th>
<th>positive (n=25)</th>
<th>negative (n=59)</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST239</td>
<td>22 (88.0)</td>
<td>50 (84.7)</td>
<td>1.32 (0.33–5.35)</td>
<td>0.697</td>
</tr>
<tr>
<td>spa-t030</td>
<td>15 (60.0)</td>
<td>44 (74.6)</td>
<td>0.51 (0.19–1.38)</td>
<td>0.185</td>
</tr>
<tr>
<td>spa-t037</td>
<td>5 (20.0)</td>
<td>3 (5.1)</td>
<td>4.67 (1.02–21.33)</td>
<td>0.047</td>
</tr>
<tr>
<td>norA</td>
<td>24 (96.0)</td>
<td>57 (96.6)</td>
<td>0.84 (0.07–9.73)</td>
<td>0.891</td>
</tr>
<tr>
<td>mupA</td>
<td>6 (24.0)</td>
<td>2 (3.4)</td>
<td>9.00 (1.67–48.41)</td>
<td>0.010</td>
</tr>
<tr>
<td>sea</td>
<td>19 (76.0)</td>
<td>56 (94.9)</td>
<td>0.17 (0.04–0.75)</td>
<td>0.019</td>
</tr>
<tr>
<td>seb</td>
<td>7 (28.0)</td>
<td>31 (52.5)</td>
<td>0.35 (0.13–0.97)</td>
<td>0.043</td>
</tr>
<tr>
<td>sec</td>
<td>3 (12.0)</td>
<td>16 (27.1)</td>
<td>0.37 (0.10–1.39)</td>
<td>0.141</td>
</tr>
<tr>
<td>seg</td>
<td>3 (12.0)</td>
<td>3 (5.1)</td>
<td>2.54 (0.48–13.58)</td>
<td>0.274</td>
</tr>
<tr>
<td>seh</td>
<td>8 (32.0)</td>
<td>37 (62.7)</td>
<td>0.28 (0.10–0.75)</td>
<td>0.012</td>
</tr>
<tr>
<td>sei</td>
<td>3 (12.0)</td>
<td>3 (5.1)</td>
<td>2.54 (0.48–13.58)</td>
<td>0.274</td>
</tr>
<tr>
<td>tst</td>
<td>4 (16.0)</td>
<td>13 (22.0)</td>
<td>0.67 (0.20–2.31)</td>
<td>0.531</td>
</tr>
<tr>
<td>sasX</td>
<td>4 (16.0)</td>
<td>2 (3.4)</td>
<td>5.43 (0.92–1.08)</td>
<td>0.061</td>
</tr>
<tr>
<td>Chlorhexidine (MIC ≥4 mg/L)</td>
<td>22 (88.0)</td>
<td>27 (45.8)</td>
<td>8.69 (2.34–32.23)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

P values in bold font indicate P<0.05.
ORs and CIs in bold font indicate that the CI does not span 1.
were more likely to harbour qacA/B. Compared with the control isolates, more qacA/B-positive MRSA isolates carried the mupirocin resistance gene mupA. None of the sed, see, sej, eta and etb genes was found in qacA/B-positive or qacA/B-negative MRSA. Multivariate analysis showed that qacA/B-positive MRSA were significantly associated with spa-t037 (OR = 6.43; 95% CI = 1.37–30.21; \( P = 0.018 \)) and the presence of mupA (OR = 11.57; 95% CI = 2.10–63.65; \( P = 0.005 \)).

The qacA/B-positive MRSA isolates were more likely to exhibit a reduced susceptibility to chlorhexidine (MIC ≥ 4 mg/L) than the qacA/B-negative MRSA isolates (\( P = 0.001 \)). The qacA/B-positive MRSA also exhibited a higher resistance rate to clindamycin and trimethoprim/sulfamethoxazole (Figure S1).

**Discussion**

According to previous studies, there have been geographical differences in the distribution of qacA/B in MRSA worldwide.\(^9,15\) In this study, we evaluated the prevalence of qacA/B in S. aureus from distinct geographical areas and different sources in China. We found that there was a higher prevalence of qacA/B in MRSA from the superficial sites of ICU patients than from clinical specimens. Only one MSSA strain carrying qacA/B was found. This finding was in accordance with previous studies reporting that more MRSA than MSSA carried qacA/B.\(^8\)

PFGE analysis showed that there was a high diversity among the qacA/B-positive MRSA from China, which is consistent with studies in other countries.\(^9,16\) but we found a statistical association between the carriage of qacA/B and spa-t037, which had not previously been reported. spa-t037 was shown to be the predominant MRSA type in Shanghai in the east of China\(^17\) and was found to account for 14.3% of the MRSA from blood sources across China.\(^18\) However, due to the lack of data on chlorhexidine exposure, we could not determine in this study whether the qacA/B-positive MRSA might confer a selective advantage for t037 strains in response to chlorhexidine.

Generally, the qacA/B gene was located on transmissible plasmids, such as pSK1 or pSK107, which often encoded other antimicrobial resistance genes.\(^9,19\) Our study found a statistical correlation between the carriage of mupA and qacA/B. This is consistent with another study, which identified the coexistence of mupirocin and antiseptic resistance in MRSA from Korea.\(^20\) Further studies are needed to ascertain the mechanism underlying this finding, for example whether the mupA and qacA/B genes were co-located on the same plasmid.

Currently, whether the presence of qacA/B is the main reason for chlorhexidine resistance has not been definitely determined. Some reports have shown that the presence of qacA/B did not cause a significant increase in chlorhexidine MIC or MBC in vitro.\(^15\) In this study, we witnessed a significant correlation between qacA/B carriage and reduced susceptibility to chlorhexidine. As the other antiseptic genes were rarely found, this suggested that the qacA/B gene was the main reason for the reduced chlorhexidine susceptibility in our isolates.

In conclusion, we observed a reduced susceptibility of S. aureus isolates to chlorhexidine and presented detailed molecular and phenotypic characteristics of qacA/B-positive S. aureus isolates in China. Further work is required to study how to reduce the spread of qacA/B-positive S. aureus, especially in ICU patients.

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**Supplementary data**

Tables S1 to S3 and Figure S1 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

**Author contributions**


**References**


