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 mortality, morbidity and excess hospital costs. 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. However, reduced susceptibility to chlorhexidine in Staphylococcus aureus has been reported since the last century. 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.
gene is mainly located on multiresistance plasmids and can co-transmit with antimicrobial resistance genes, which may contribute to the survival of qacA/B-positive S. aureus, especially MRSA.\(^5\) Recently, a study suggested that qacA/B carriage might contribute to an increasing global dominance of CC22 and ST239 clones.\(^6\) It suggested that the antiseptic resistance genes could be associated with some S. aureus clones under particular conditions.

In China, there have been few reports of reduced susceptibility to chlorhexidine in S. aureus. This study aimed to describe the prevalence, in vitro chlorhexidine susceptibility and molecular characteristics of qacA/B-positive S. aureus isolates from clinical and hospital environmental samples in China.

**Materials and methods**

**Bacterial strains**

A total of 499 non-replicated S. aureus isolates were collected from patients and the clinical environment during 2008 to 2012. Among these, 145 MRSA and 178 MSSA isolates were cultured from clinical specimens from seven tertiary care hospitals located in distinct geographical areas in China, 70 MRSA isolates were cultured from swab samples from superficial sites of different patients admitted to a surgical ICU of a tertiary hospital in Beijing and 106 MRSA isolates were cultured from the ICU environment. The sampling methods and identification of MRSA have been described in our previous studies.\(^7\)

**Detection of antiseptic resistance genes and selection of control isolates**

DNA was extracted using Wizard\(^\text{®}\) Genomic DNA Purification Kit (Promega Corporation, Madison, WI, USA) according to the manufacturers’ instructions. The presence of the qacA/B genes was detected by PCR.\(^8\) Seventy-two of the qacA/B-negative S. aureus isolates from clinical specimens (15 MRSA and 13 MSSA), ICU patients’ superficial sites (39 MRSA) and a hospital environment (5 MRSA) were randomly selected as control isolates. The other antiseptic resistance genes in qacA/B-positive isolates and control isolates, including smr, qacG, qacH and qacJ, were detected by PCR as previously described.\(^9\)

**spa typing, MLST, SCCmec typing and PFGE**

The 26 qacA/B-positive isolates and 72 control isolates were characterized by spa typing. The purified spa PCR products were sequenced and the repeats were assigned by using the spa typing database web site (http://spaserver.ridom.de). MLST was performed and each allele sequence profile was submitted to the MLST database (http://saureus.mlst.net) to determine the ST. The MRSA isolates were characterized by SCCmec typing according to Milheirico et al.\(^10\) The 26 qacA/B-positive isolates were typed by PFGE with CHEF-Mapper apparatus. Cluster analysis was performed by using BioNumerics software version 6.0 (Applied Maths, Sint-Martens-Latem, Belgium). A similarity cut-off of 80% was used to define a PFGE cluster.

**Identification of virulence and antimicrobial resistance genes**

The presence of the virulence-associated sasX gene and sea-see, seq-sej, etp, etb, tst and pvl genes was detected by PCR for the qacA/B-positive isolates and the control isolates.\(^11\)–\(^13\) The mupA gene encoding mupirocin resistance and the norA gene encoding quinolone resistance were also detected by PCR.\(^9\)

**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).\(^14\) A 2-fold dilution series, from 32 to 0.0625 mg/L, was prepared from a 20% (w/v) chlorhexidine gluconate solution (Sigma) and the MICs were determined by the agar dilution method.\(^14\)

**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 \(\chi^2\) 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 Ji’nan 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, qacH 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 and 72 control isolates were characterized 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. aMSSA. 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>qacA/B gene</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></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></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></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></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></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></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></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></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></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></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></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></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></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></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 mupA gene. None of the seq, see, sej, etc 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 trimetoprim/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 different 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 accordence with previous studies reporting that more MRSA than MSSA carried qacA/B.9

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 China17 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.18 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 mupA and antiseptic resistance in MRSA from Korea.19 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


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