

# Distribution of *sasX*, *pvl*, and *qacA/B* genes in epidemic methicillin-resistant *Staphylococcus aureus* strains isolated from East China

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**Background:** Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major nosocomial pathogen. Various virulence and antiseptic-resistant factors increase the pathogenicity of MRSA strains and allow for increased infection rates.

**Purpose:** The purpose of this study was to investigate the prevalence and distribution of virulence-associated and antiseptic-resistant genes from epidemic MRSA strains isolated from East China.

**Methods:** A newly designed multiplex PCR assay was used to assess whether the virulence-associated genes *sasX* and *pvl* and the chlorhexidine tolerance gene *qacA/B* were present in 189 clinical isolates of MRSA. Multilocus sequence typing (MLST) and Staphylococcal protein A (*spa*) typing of these isolates were also performed. The frequency of these genes in isolates with epidemic sequence types (STs) was investigated.

**Results:** Twenty STs and 36 *spa* types with five epidemic clones (ST5-t311, ST59-t437, ST5-t002, ST239-t030, and ST239-t037) were identified. The prevalence of *sasX*, *pvl*, and *qacA/B* in all isolates was 5.8%, 10.1%, and 20.1%, respectively. The prevalences of these genes in isolates with ST5, ST59, ST239, and other ST genetic backgrounds were all significantly different ( $P < 0.001$ ). Isolates that had the highest frequency of *sasX*, *pvl*, or *qacA/B* were ST239 (33.3%), ST59 (28.9%), and ST5 (34.1%), respectively. The gene distribution pattern from all of the isolates showed that *sasX-pvl-qacA/B+*, *sasX-pvl+qacA/B-*, and *sasX+pvl-qacA/B-* were closely associated with epidemic clones ST5-t311, ST59-t437, and ST239-t037, respectively.

**Conclusion:** There are significant differences in the prevalence of virulence-associated and antiseptic-resistant genes in epidemic MRSA strains. Using this information, more effective control and prevention strategies for nosocomial MRSA infections can be developed.

**Keywords:** MRSA, MLST, virulence genes, *sasX*, *pvl*, *qacA/B*, multiplex PCR

## Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as one of the most important nosocomial pathogens, causing considerable morbidity and mortality in hospitals.<sup>1</sup> Information regarding the molecular characteristics and distribution of virulence determinants and antiseptic-resistant genes of MRSA is essential for controlling and treating the diseases caused by this medically important pathogen. The novel *S. aureus* cell wall-anchored protein gene, *sasX*, is a critical factor for promoting nasal colonization, immune evasion, and virulence.<sup>2</sup> The recent spread of *sasX* from sequence type (ST) 239 to invasive clones belonging to other STs suggests that this ST shift may be a driving force behind the Asian MRSA epidemic.<sup>2</sup> In addition, an association between the presence of Panton-Valentine leukocidin (PVL), an *S. aureus*

toxin that causes neutrophil lysis, and the onset of invasive disease in communities has emerged worldwide. However, in a study by Shallcross et al, the PVL encoding gene, *pvl*, was consistently associated with skin and soft tissue infections and rarely associated with invasive disease.<sup>3</sup> Additional data are needed to reconcile the differences between these studies.

Chlorhexidine gluconate use in the prevention and decolonization strategies is being increasingly employed to control MRSA transmission, but increased tolerance to these treatments has been reported.<sup>4</sup> Decontamination with chlorhexidine gluconate is only effective for patients colonized with strains that lack the antiseptic-resistant genes *qacA/B*.<sup>5</sup> *qacA* and *qacB* genes are closely related, and their sequences only differ by seven nucleotides. Therefore, in this study, the presence of these genes was reported as a single gene.

The presence of both virulence factors and antiseptic resistance genes within a bacterial strain has been previously reported in Gram-negative and Gram-positive bacteria.<sup>6,7</sup> Quickly identifying these attributes in bacterial strains can have a major impact on treatment and decontamination strategies. In this study, we described a multiplex PCR assay capable of detecting three genes involved in either MRSA virulence or antiseptic resistance, *sasX*, *pvl*, and *qacA/B* simultaneously. The prevalence and distribution of these genes among epidemic clones from 189 MRSA strains isolated from Eastern China was also determined.

## Materials and methods

### MRSA isolates

One hundred and eighty-nine clinical MRSA isolates were collected from two tertiary care hospitals and two secondary hospitals located in Eastern China between January 2016 and May 2017. Clinical sources of the isolates included the respiratory tract (n=76), skin and soft tissue (n=31), blood cultures (n=40), sterile body fluid (n=24), and urinary tract (n=18). Bacterial identification and oxacillin resistance were performed using the Vitek 2 Compact Automated Microbiology System (BioMérieux, Durham, NC, USA) in a clinical microbiology laboratory accredited by the College of

American Pathologists and certified by the Clinical Laboratory Improvement Act.

### DNA extraction

All isolates were cultured on blood agar and incubated overnight at 37°C. DNA was isolated using a QIAamp DNA Mini Kit (Qiagen, Germantown, MD, USA) according to the manufacturer's instructions. The isolated DNA was used as the template for all PCRs.

### Gene typing

Multilocus sequence typing (MLST) was performed on all isolates by sequencing the internal fragments of seven house-keeping genes (*arcC*, *aroE*, *glpF*, *gmK*, *pta*, *tpi*, and *yqiL*). The sequence profile and ST of each allele were determined according to the MLST database (<http://saureus.mlst.net>).<sup>8</sup> The allelic profiles were assigned by comparing the sequences at each locus with those of the known alleles in the *S. aureus* MLST database and then were defined as the STs. Staphylococcal protein A (*spa*) typing involved PCR amplification, and subsequent sequencing of the highly variable X region in *spa* using the previously described primers *spa*-1113f and *spa*-1514r.<sup>9</sup> These *spa* sequences were analyzed and assigned a unique *spa* type using the *spa* database (<http://spa.ridom.de/>).<sup>10</sup>

### Multiplex PCR assay for *sasX*, *pvl*, and *qacA/B* detection

The specific primers used for the identification of *sasX*, *pvl*, and *qacA/B* genes were listed in Table 1. Three genes were detected by multiplex PCR assay simultaneously in a single tube. This amplification was performed using 12.5 µL Qiagen Multiplex PCR Master kit (Qiagen); 1.5, 1.0, or 0.5 µL of each upstream or downstream primer for *sasX*, *pvl*, and *qacA/B*, respectively; 2 µL of template DNA; and enough distilled water for a final volume of 25 µL. No-template control, sterile water instead of template DNA was incorporated in each run under the following conditions: 5 minutes of denaturation at 94°C, followed by 35 cycles for 30 seconds

**Table 1** Primers used in this study

Primer	Nucleotide sequence (5' to 3')	Product size (bp)	Reference
<i>sasX</i>	F:AGAATTAGAAGTACGTCTAAATGC R:GCTGATTATGTAATGACTCAAATG	522	2
<i>pvl</i>	F:GTGCCAGACAATGAATTACCC R:TTCATGAGTTTTCCAGTTCACCTTC	80	9
<i>qacA/B</i>	F:GCTGCATTTATGACAATGTTTG R:AATCCACCTACTAAAGCAG	321	12

at 94°C, 30 seconds at 56°C, and 30 seconds at 72°C, with a final 10 minutes elongation step at 72°C. The PCR products were analyzed on a 1.5% agarose gel and further confirmed by DNA sequencing. Sequence comparisons with publicly available sequence data were performed using BLAST analyses (<http://www.ncbi.nlm.nih.gov/BLAST/>).<sup>11</sup>

## Validation of multiplex PCR

Conventional single PCR assays were also performed for *sasX*, *pvl*, and *qacA/B*, as previously described.<sup>2,9,12</sup> The results from the single and multiplex PCR methods were compared, and 80 of the 189 clinical MRSA isolates were validated by both the conventional and multiplex PCR assays.

## Statistical analysis

Statistical analyses were performed using the SPSS version 16.0 statistical software package (SPSS Inc., Chicago, IL, USA). The descriptive data were presented as percentages for the categorical data. Pearson's chi-square test or Fisher's exact test were used to determine whether differences in the frequency of *sasX*, *pvl*, and *qacA/B* exist among isolates with different ST types. A *P*-value of  $\leq 0.05$  was considered statistically significant.

## Results

### Genotyping of MRSA isolates

Among the 189 MRSA isolates studied, 20 different STs and 36 *spa* types were identified. The three major STs were ST5 (88, 46.6%), ST59 (45, 23.8%), and ST239 (18, 9.5%). The remaining STs had frequencies ranging from 0.5% to 3.7% and were ST398, ST630, ST22, ST1, ST965, ST7, ST88, ST944, ST764, ST6, ST338, ST1611, ST2580, ST3355, ST3360, ST3361, and a new ST type. In addition, the most common *spa* types were t311 (71, 37.6%), t437 (28, 14.8%), t002 (14, 7.4%), t030 (10, 5.3%), t163 (8, 4.2%), and t037 (7, 3.7%). The remaining 30 *spa* types accounted for 27.0% of the total isolates, with none of the types accounting for more than 3.2%. The most common genotype was ST5-t311 (66, 34.9%), followed by ST59-t437 (22, 11.6%), ST5-t002 (14, 7.4%), ST239-t030 (10, 5.3%), and ST239-t037 (6, 3.2%).

### Validation of results of the multiplex PCR assay with clinical isolates

Comparisons between the data from the single and the multiplex PCR assays showed that 80 clinical isolates were completely concordant (100%). Of these 80 isolates, two harbored *sasX*, seven harbored *pvl*, 19 harbored *qacA/B*,

three harbored both *sasX* and *qacA/B*, and 49 were negative for all three genes.

## Prevalence and distribution of virulence determinants and antiseptic-resistant genes

Of the 189 clinical isolates of MRSA, the *sasX*, *pvl*, and *qacA/B* genes were found in 11 (5.8%), 19 (10.1%), and 38 (20.1%) of the isolates, respectively. The *sasX* and *qacA/B* genes were detected concomitantly in five (2.6%) of the isolates. Similarly, *sasX* and *pvl* were both found in one (0.5%) of the isolates. No isolates that harbored all three genes simultaneously were identified. Isolates were sorted into four groups, based on gene typing: ST5, ST59, ST239, and other ST types (which excluded the ST5, ST59, and ST239 groups), and further grouping analysis was performed. The results showed that the incidence rates of *sasX*, *pvl*, and *qacA/B* were all significantly different in isolates with ST5, ST59, ST239, and other ST genetic backgrounds ( $P < 0.001$ ). The *sasX* gene occurred with a maximum frequency in ST239 (33.3%), *pvl* had the highest frequency in ST59 (28.9%), and *qacA/B* occurred with the highest frequency in ST5 (34.1%). The *sasX* gene was not detected among 45 ST59 isolates, *pvl* was not detected among 18 ST239 isolates, and was found in only one (1.1%) of the 88 ST5 isolates. The prevalence of the three genes from the MRSA isolates harboring different ST types is summarized in Table 2.

Five gene distribution patterns, based on the presence of *sasX*, *pvl*, and *qacA/B* in the isolates, were determined (Table 3). These patterns included *sasX-pvl-qacA/B+* (33, 17.5%), *sasX-pvl+qacA/B-* (18, 9.5%), *sasX+pvl-qacA/B-* (5, 2.6%), *sasX+pvl-qacA/B+* (5, 2.6%), and *sasX+pvl+qacA/B-* (1, 0.5%). Furthermore, high incidence

**Table 2** Prevalence of three genes from MRSA isolates harboring different ST types

ST type (n)	Number (%) of genes positive for		
	<i>sasX</i>	<i>pvl</i>	<i>qacA/B</i>
ST5 (88)	2 (2.3%)	1 (1.1%)	30 (34.1%)*
ST59 (45)	0	13 (28.9%)*	2 (4.4%)
ST239 (18)	6 (33.3%)*	0	2 (11.1%)
Others <sup>#</sup> (38)	3 (7.9%)	5 (13.2%)	4 (10.5%)
Total (189)	11 (5.8%)	19 (10.1%)	38 (20.1%)

**Notes:** Data are expressed as n (%), unless otherwise indicated. \*Including ST398, ST630, ST22, ST1, ST965, ST7, ST88, ST944, ST764, ST6, ST338, ST1611, ST2580, ST3355, ST3360, ST3361, and a new ST type. \* $P \leq 0.001$ .

**Abbreviations:** MRSA, methicillin-resistant *Staphylococcus aureus*; ST, sequence type.

**Table 3** Distribution patterns of the three genes from MRSA isolates

Gene patterns	Number of isolates (%)	ST- <i>spa</i> type (n)
<i>sasX</i> + <i>pvl</i> + <i>qacA/B</i> -	1 (0.5%)	ST22-t309 (1)
<i>sasX</i> + <i>pvl</i> - <i>qacA/B</i> +	5 (2.6%)	ST239-t037 (2); ST5-t311 (1); ST3361-t311 (1); ST5-t437 (1)
<i>sasX</i> + <i>pvl</i> - <i>qacA/B</i> -	5 (2.6%)	ST239-t037 (4); ST965-t062 (1)
<i>sasX</i> - <i>pvl</i> + <i>qacA/B</i> -	18 (9.5%)	ST59-t437 (13); ST22-t309 (3); ST59-t8391 (1); ST338-t437 (1)
<i>sasX</i> - <i>pvl</i> - <i>qacA/B</i> +	33 (17.5%)	ST5-t311 (25); ST5-t421 (1); ST5-t037 (1); ST5-t2460 (1); ST59-t311 (1); ST59-t2380 (1); ST630-t4549 (1); ST3360-t311 (1); T965-t062 (1)

**Note:** Data are expressed as n (%), unless otherwise indicated.

**Abbreviations:** MRSA, methicillin-resistant *Staphylococcus aureus*; ST, sequence type.

of *sasX*-*pvl*-*qacA/B*+, *sasX*-*pvl*+*qacA/B*-, and *sasX*+*pvl*-*qacA/B*- was found in ST5-t311, ST59-t437 and ST239-t037 strains, respectively (75.8%, 72.2%, and 80.0%, respectively). The *sasX*, *pvl*, and *qacA/B* genes were not found in any of the remaining 127 isolates.

## Discussion

MRSA is considered the most significant multidrug-resistant organism that causes infections, and the global spread of MRSA has become a major problem worldwide.<sup>13,14</sup> Analysis of the distribution of virulence determinants and antiseptic-resistant genes of MRSA clones is valuable for understanding the evolution and dissemination of MRSA in different geographic regions. In this study, a multiplex PCR assay that targets the *sasX* and *pvl* virulence genes and the chlorhexidine-resistant gene, *qacA/B*, was evaluated using clinical isolates. Results of the present study showed that the multiplex PCR assay was accurate, simple, and reliable. To our knowledge, this is the first study demonstrating the simultaneous amplification and subsequent analyses of the presence of *sasX*, *pvl*, and *qacA/B* in MRSA isolates.

In previous reports, from other groups, the prevalence of the *sasX* gene in MRSA isolates revealed significant regional differences, with incidence rates ranging from 0% to 39%.<sup>2,15-18</sup> These studies also showed that the ST239 strains carried the *sasX* gene with the highest frequencies (up to 99.1%).<sup>17</sup> In addition, Li et al observed the recent spread of *sasX* from ST239 to invasive clones belonging to other STs.<sup>2</sup> Our results indicate that the *sasX* gene frequency (5.8%) of MRSA in East China is lower than that previously found at three different teaching hospitals in China (21%–39%, from 807 strains) and in Chinese children (10.7%, from 243 MRSA strains).<sup>2,15</sup> Our study also demonstrated that ST239 is dominant in the *sasX*-positive isolates and that the spread of *sasX* to other STs such as ST5, ST22, ST965, and ST3361 occurs. This study is the first to observe the presence of

*sasX* in ST22, ST965, and ST3361 isolates. The *sasX* gene has not been detected among the prevalent clone of community isolate ST59, indicating that *sasX*-positive clones spread predominantly within the hospital setting. Moreover, our study showed that the ST59 strains carried the *pvl* gene with the highest frequencies (28.9%). Meanwhile, *pvl* has not been detected among ST239 isolates, indicating that *pvl*-positive clones spread predominantly within the community setting. This finding was similar to the results from previous studies.<sup>3,19</sup>

Chlorhexidine gluconate, a water-soluble cationic bisbiguanide, has been widely used as an antiseptic agent for infection control. The correlation between the presence of *qacA/B* and chlorhexidine resistance has not been definitely determined.<sup>20</sup> However, the *qacA/B* carrier rate was found to be higher in *S. aureus* isolates with higher chlorhexidine minimal bactericidal concentrations.<sup>20</sup> In this study, we found that the highest incidence of *qacA/B* was in ST5 clone (34.1%), which was the predominant clone in the MRSA isolates from this region.<sup>21</sup> This finding was different from Batra et al's study which showed that carriage of *qacA/B* in an outbreak of ST239 MRSA strains was higher than that of other ST types.<sup>5</sup> Survival of these strains in a hospital environment must be considered since the high prevalence of the *qacA/B* gene might be due to either selective pressure imposed by the use of quaternary ammonium compounds (QACs) or cross-resistance and co-resistance between QACs and antibiotics.<sup>22</sup> The *qacA/B* genes are typically located on a transposon of a transmissible multidrug-resistant plasmid such as pSK1.5.<sup>23</sup> Therefore, surveillance of antiseptic-resistant MRSA could provide important information on the control of nosocomial infections. This is the first study to simultaneously report the prevalence of *sasX*, *pvl*, and *qacA/B* in Chinese MRSA strains. We found significant differences in the prevalence of these genes in specific ST types. The ST239 isolate type had the highest prevalence of *sasX*, isolates resembling ST59 had the highest rates

of *pvl*, and ST5-type isolates had a higher incidence of *qacA/B* than other ST types. Even so, understanding the distributional differences of virulence determinants and antiseptic-resistant genes in MRSA isolates is still limited and warrants continued intensive study.

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## Author contributions

HK designed the experiments and wrote the manuscript. LF, RJ, and JT performed research and analyzed data. All the authors have read, critically revised, and approved the final manuscript.

## Disclosure

The authors report no conflicts of interest in this work.

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