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# Epidemiological and molecular characterization of community and hospital acquired *Staphylococcus aureus* strains prevailing in Shenyang, Northeastern China

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## ABSTRACT

In order to obtain adequate information for the treatment of methicillin resistant *Staphylococcus aureus* (MRSA) infections, it is crucial to identify trends in epidemiological and antimicrobial resistance patterns of local *S. aureus* strains. Community and hospital acquired *S. aureus* isolates ( $n=202$ ) were characterized using staphylococcal cassette chromosome *mec* (SCCmec) typing, pulse field gel electrophoresis (PFGE) analysis, *spa* typing and minimal inhibitory concentration (MIC) determination. The prevalence of the Panton-Valentine leukocidine (*pvl*) and several antibiotic resistance genes among the isolates were also detected by PCR. All of the *S. aureus* isolates were susceptible to vancomycin, daptomycin and linezolid. Three hospital isolates were resistant to teicoplanin while 14 showed intermediate resistance to teicoplanin. The resistance patterns of community-acquired MRSA (CA-MRSA) isolates to other antimicrobials were similar to those of hospital-acquired MRSA (HA-MRSA) isolates except for clindamycin and gentamicin. There was excellent correlation between phenotypes and genotypes in the determination of *S. aureus* resistance to erythromycin, gentamicin, and tetracycline. The SCCmec type II and SCCmec type IV were the predominant types detected in hospital and community isolates, respectively. The most frequently encountered *spa* types were t002 and t030 both in HA- and CA-MRSA isolates. Pulsotype A was the most predominant pulsotype identified among the isolates tested, followed by pulsotype B. Seventy-two hospital isolates (19 HA-MRSA and 53 HA-MSSA) and 10 CA-MRSA were positive for the *pvl* gene. This study shows that the combination of susceptibility testing and various molecular methods has provided useful information on the antibiotic resistance and molecular diversity of *S. aureus* in a specific region of China. The high proportion of *pvl* positive MSSA and MRSA isolates observed in this study indicates that adequate measures are needed to curtail the spread of those MRSA and MSSA clones prevailing both in hospital and the community.

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## Introduction

Methicillin resistant *Staphylococcus aureus* (MRSA) is one of the most prevalent nosocomial bacterial pathogens.<sup>1-5</sup> causing a wide variety of diseases such as skin and soft tissue infections, bloodstream infections, pneumonia, osteomyelitis and endocarditis, as well as toxin-mediated syndromes like toxic shock and food poisoning.<sup>1,4</sup> MRSA was first identified in England in 1961 after which it emerged worldwide.<sup>3</sup> MRSA is now responsible for infections with significant morbidity and mortality and it has developed resistance to multiple antimicrobial agents complicating the clinical treatment of infections.<sup>4-8</sup> Since the 1990s, MRSA colonization and infections have been increasingly reported among the general population/community and are referred to as community-acquired MRSA (CA-MRSA) strains.<sup>2</sup> CA-MRSA strains have been reported in numerous countries such as Australia, New Zealand, the United Kingdom, Canada and the United States.<sup>5</sup> In 2008, a study performed in a teaching hospital in Wenzhou, China described and compared the characteristics of hospital and community acquired *S. aureus* strains.<sup>9</sup> Isolates of CA-MRSA are distinct from those of hospital-acquired MRSA (HA-MRSA) in many characteristics which include susceptibility to antimicrobial agents, the type of genetic staphylococcal cassette chromosome *mec* (SCC*mec*) elements, and exotoxin gene repertoire.<sup>10</sup>

Currently, MRSA has become a notorious etiologic agent both in health care facilities and in the community in China, and several studies have shown that this tendency is increasing.<sup>2</sup> According to a study published in 2005, the mean incidence of MRSA across China was over 50% with the prevalence in Shanghai being over 80%.<sup>11</sup> It is, therefore, of paramount importance to understand the molecular epidemiology and to potentially predict the trends in antibiotic-resistance patterns of MRSA strains both in hospitals and within the community.

It appears that under the selective pressure caused by the extensive use of antibiotics, MRSA clones which carry resistance genes can better adapt to environmental change.<sup>6</sup> These resistant genes include the *erm* genes (*ermA*, *ermB*, *ermC*) encoding erythromycin resistance, the *tet* genes (*tetK*, *tetL*, *tetM*, *tetO*) encoding tetracycline resistance and the *aac(6)-aph(2'')*, *aph(3')-III* and *ant(4)-I* genes that encode gentamicin resistance.<sup>12,13</sup> Resistance determinants can be transposed from one strain to another, sometimes across the species barrier by conjugation, mobilization, phage-mediated transduction, and via as yet unknown mechanisms, resulting in the wide dissemination of antibiotic resistance among circulating staphylococcal strains. Therefore, there is a continuous requirement to determine the effectiveness of these antibiotics against *S. aureus* infections and the enactment of effective drug policies in China.

The aim of the present study was to investigate the local epidemiological and antimicrobial resistance patterns of *S. aureus* strains in Shenyang, which is considered necessary to establish empirical and specific therapy guidance for *S. aureus* treatment. We identified the antibiotic susceptibility patterns and the molecular characteristics of isolates using several methods, including SCC*mec* typing, pulsed-field gel

electrophoresis (PFGE) analysis, *spa* typing, assessment of several antibiotic resistance genes and minimum inhibitory concentration (MIC) determination. Since MSSA isolates carrying *pvl* have been reported in the hospital environment and are thought to evolve into MRSA strains through acquisition of a type of SCC*mec* element that harbors *mecA* gene,<sup>6,14</sup> the prevalence of Panton-Valentine leukocidine (*pvl*) gene<sup>9</sup> among the isolates was also assessed.

## Materials and methods

### *S. aureus* isolate collection

The Shengjing hospital, a 2045-bed tertiary care center located in Shenyang, is one of three affiliated hospitals of the China Medical University. The hospital serves patients mostly coming from three large provinces located in the northeast of China and admits over 70,000 patients annually.

The hospital-acquired *S. aureus* isolates were obtained from individual patients between January and October 2008 at the Shengjing Hospital of China Medical University in Shenyang from the following departments: internal medicine, nephrology, hematology, gastroenterology, endocrinology, intensive care unit, respiratory medicine, surgical department, surgery intensive care, neurosurgery, thoracic surgery, and pediatrics. In total, 180 hospital-acquired *S. aureus* were obtained from different clinical specimens, which included sputum ( $n=94$ ), pus ( $n=41$ ), blood ( $n=25$ ), urine ( $n=9$ ), prostatic fluid ( $n=4$ ), catheter tips ( $n=3$ ), drainage fluids ( $n=3$ ), and ascites ( $n=1$ ). The definition of hospital-acquired *S. aureus* infection was as follows: (1) an *S. aureus* infection identified at least 48 h after admission to a hospital; (2) a history of hospitalization, surgery, dialysis, or residence in a long-term care facility within one year of the positive culture date; (3) a permanent indwelling catheter or percutaneous medical device (e.g. tracheostomy tube, gastrostomy tube or Foley catheter) present at the time of culture; or (4) a known positive culture for *S. aureus* prior to the study period.<sup>15</sup> As a comparison, 2103 third-year students from China Medical University (located in the same region as Shengjing hospital) were screened for nasal carriage of *S. aureus* from whom 22 MRSA were identified. These MRSA strains were deemed to be of community origin since the students were all healthy and health care-related risk factors were absent.<sup>2</sup>

Only one isolate from each patient or student was used. Isolates were first cultured on sheep blood agar (Becton Dickinson, Cockeysville, MD) at 35 °C for 48 h. Phenotypic identification of *S. aureus* was initially based on colony morphology which was then confirmed using the Pos Combo type 41 MicroScan panels (Baxter Diagnostics, Inc., West Sacramento, CA). All the isolates were stored in a storage solution (80% glycerol, 20% brain heart infusion) at -80 °C.

### Antimicrobial susceptibility test

MICs were determined using the broth microdilution method according to the procedure recommended by the Clinical and Laboratory Standards Institute.<sup>16,17</sup> The antibiotics tested were penicillin G, oxacillin, tetracycline, erythromycin, and gentamicin (Sigma Chemical Co., St. Louis,

MO), ceftazidime (Fujisawa Pharmacy Co., Osaka, Japan), vancomycin (Sigma Chemical, St. Louis, MO), teicoplanin, clindamycin, daptomycin, linezolid, rifampicin, levofloxacin (Daichi Pharmaceutical Co., Tokyo, Japan), chloramphenicol and trimethoprim-sulfamethoxazole (SXT). The reference strain *S. aureus* ATCC 29213 was used as a control standard in each test.

#### DNA extraction

Genomic DNA was obtained from 2 mL overnight cultures of each isolate using a Biospin Bacteria Genomic DNA Extraction Kit (Biotech Biological Technology Co., Ltd) and by following the manufacturer's instructions. Five microlitres of lysostaphin (100 µg/mL) were added to digest the bacterial cell wall at 37 °C for 60 min.

#### PCR detection of the *mecA* gene and *SCCmec* typing

Methicillin resistant phenotypic identification of the *S. aureus* isolates was confirmed by the detection of the *mecA* gene. All isolates were subjected to *mecA* gene detection by PCR using primers mA1 and mA2 (Table 1). *SCCmec* typing was performed using PCR according to previous reports.<sup>18,19</sup> MRSA strains NCTC10442, N315, 85/2082, CA05 and WIS were used as reference standards for *SCCmec* typing (I, II, III, IV and V).

#### Detection of antimicrobial resistance genes and *pvl* gene

Antibiotic resistance genes associated with macrolide resistance (*ermA*, *ermB* and *ermC*), aminoglycoside resistance (*aac(6')*-*aph(2')*, *aph(3')*-III, *ant(4')*-I) and tetracycline resistance (*tetK*, *tetL*, *tetM*, *tetO*) were detected by PCR using previously published protocols and primers as shown in Table 1.<sup>12,20,21</sup> All *S. aureus* isolates were screened for the *pvl* toxin gene by PCR with primers *pvl-1* and *pvl-2* as described previously (Table 1).<sup>6,22</sup> MRSA strains MW2 was used as a reference standard for *pvl* gene identification.

#### *spa* typing

The *spa* gene encoding protein A of *S. aureus* was used for MRSA typing and amplified by PCR with primers 1113F and 1514R (Table 1).<sup>23</sup> Sequencing of the *spa* gene was performed by the Beijing Gene Institution (Beijing, China) using standard methods. The nucleotide sequences were analyzed using the RIDOM Staph-Type software (Ridom GmbH, Germany) to assign a *spa* type to the various isolates (<http://www.ridom.de/spaserver/>).

#### PFGE analysis

Chromosomal DNAs from the MRSA isolates were digested with *Sma*I and separated by PFGE using a Gene Path system (CHEF-DR, PULS WAVE.760; Bio-Rad, Hercules, USA) according to the manufacturer's instructions. A 48.5 kb ladder was used as a DNA size marker (Bio-Rad, cat. # LS:170-3635). Isolates were considered identical when their PFGE patterns contained the same number and sizes of fragments. PFGE patterns with fewer than 4 band differences between an existing genotype

were defined as subtypes of that genotype.<sup>2,24</sup> Genotypes were categorized alphabetically.

#### Statistical analysis

Statistical comparisons were applied to determine the significance of differences in resistance rates using Pearson's  $\chi^2$  test (SPSS version 12.0). *p*-Values of less than 0.05 were considered statistically significant.

## Results

### Antimicrobial susceptibility

Of the 180 hospital-acquired *S. aureus* isolates identified, 107 were MRSA and 73 were MSSA. Table 2 indicates that all isolates were susceptible to vancomycin, daptomycin, and linezolid. Two HA-MRSA (1.9%) and one HA-MSSA (1.4%) isolates were resistant to teicoplanin and 13 HA-MRSA (12.1%) isolates and one HA-MSSA (1.4%) isolate showed intermediate resistance to teicoplanin. There was no significant difference in the prevalence of teicoplanin resistance between HA-MRSA and HA-MSSA isolates ( $p > 0.05$ ). All of the CA-MRSA isolates were susceptible to vancomycin, daptomycin, linezolid, and teicoplanin. In addition to the antibiotics above, the resistant rates to penicillin G, ceftazidime, erythromycin, levofloxacin, tetracycline, gentamicin, clindamycin were higher for HA-MRSA isolates, at 98.1%, 98.1%, 97.2%, 96.2%, 93.4%, 91.6%, and 87.9%, respectively. In contrast, the resistant rates to rifampicin, chloramphenicol, and SXT were moderately low, at 45.8%, 9.3%, and 10.3%, respectively. The resistance patterns of CA-MRSA isolates to the antibiotics tested were similar to those of the HA-MRSA isolates for most of the antimicrobial agents except for clindamycin and gentamicin.

Compared to HA-MRSA isolates, the HA-MSSA isolates presented lower resistant rates to almost all antibiotics tested. HA-MSSA isolates showed resistance only to penicillin G, erythromycin, clindamycin, tetracycline and gentamicin to some degree. The predominant antibiotypes among the HA-MRSA isolates were resistant to penicillin G, oxacillin, ceftazidime, erythromycin, tetracycline, gentamicin, levofloxacin, and clindamycin. The predominant antibiotypes among the HA-MSSA isolates were those showing resistance to penicillin G, erythromycin, and clindamycin.

### Antimicrobial resistance genes

Table 3 shows that the *ermA* gene was frequently detected in both HA-MRSA (93/107, 86.9%) and HA-MSSA (59/73, 80.8%) isolates while the detection rates of the *ermB* and *ermC* genes were significantly higher in HA-MSSA isolates (51/73, 69.9%; 58/73, 79.5%) compared to HA-MRSA isolates (49/107, 45.8%; 50/107, 46.7%). The triple combination of *ermA*, *ermB* and *ermC* was present in 17.8% of HA-MRSA isolates and 49.3% of HA-MSSA isolates. In contrast, the CA-MRSA isolates only possessed the *ermA* gene, which was detected in 13 (59.1%) erythromycin-resistant isolates.

In this study, the HA-MRSA isolates were more likely to contain the *tetM* (91.6% versus 69.9%), *tetK* (45.8% versus 19.2%)

**Table 1 – Primers used in this study.**

Gene name	Primers	Nucleotide sequence (5'–3')	Size of product (bp)	Reference
<i>mecA</i>	mA1	5'-TGCTATCCACCCTCAAACAGG-3'	300	2
	mA2	5'-AACGTTGTAACCACCCCAAGA-3'		
<i>pvl</i>	pvl-F	5'-ATGCTGGACATGATCCAA-3'	970	22
	pvl-R	5'-AACTATCTCTGCCATATGGT-3'		
<i>ccr1-3</i>	ccrB <sub>2</sub> Ino	5'-ATTGCCTTGATAATAGCCTTCT-3'	700	19
	CA1-4	5'-AACCTATATCATCAATCAGTACGT-3'		
	pSJ2223 C	5'-TAAAGGCATCAATGCACAAACACT-3'		
	LG12H3B	5'-AGCTCAAAGCAAGCAATAGAAT-3'		
<i>ccrC</i>	SFCC4	5'-CCTTTATAGACTGGATTATTCAAAATAT-3'	520	18
	SFCC1	5'-CGTCTATTACAAGATGTTAAGGATAAT-3'		
<i>mecI-mecRI</i> (class A)	mI4	5'-CAAGTGAATTGAAACCGCCT-3'	1900	2
	mcR3	5'-GTCTCCACGTTAATTGCATT-3'		
<i>IS1272-mecA</i> (class B)	IS5	5'-AACGCCACTCATAACATATGGAA-3'	2000	
	mA6	5'-TATACCAAACCCGACAAC-3'		
<i>IS431mecL-mecA</i> (class C)	mA2	5'-AACGTTGTAACCACCCCAAGA-3'	2000	
<i>ermA</i>	IS2	5'-TGAGGTTATTTCAGATATTTTCGATGT-3'	645	20
	<i>ermA</i> -up	5'- TCTAAAAAGCATGTAAGAA-3'		
<i>ermB</i>	<i>ermA</i> -dn	5'- CTTCGATAGTTTATTAATATTAGT-3'	639	
	<i>ermB</i> -up	5'- GAAAAGGATCTCAACCAAATA-3'		
<i>ermC</i>	<i>ermB</i> -dn	5'- AGTAACGGTACTTAAATTGTTTAC-3'	642	
	<i>ermC</i> -up	5'- TCAAAACATAATATAGATAAA-3'		
<i>tetK</i>	<i>ermC</i> -dn	5'- GCTAATATTGTTTAAATCGTCAAT-3'	1159	12
	<i>tetK</i> -up	5'- TATTTTGGCTTTGTATTCTTTTCAT-3'		
<i>tetL</i>	<i>tetK</i> -dn	5'- GCTATACCTGTTCCCTCTGATAA-3'	1077	
	<i>tetL</i> -up	5'- ATAAATTGTTTCGGGTCGGTAAT-3'		
<i>tetO</i>	<i>tetL</i> -dn	5'- AACCGCCAACTAATGACAATGAT-3'	1723	
	<i>tetO</i> -up	5'- AACTTAGGCATTCGGCTCAC-3'		
<i>tetM</i>	<i>tetO</i> -dn	5'- TCCCACTGTTCCATATCGTCA-3'	1862	
	<i>tetM</i> -up	5'- AGTTTTAGCTCATGTTGATG-3'		
<i>ant(4')-I</i>	<i>tetM</i> -dn	5'-TCCGACTATTTGGACGACGG-3'	367	21
	<i>ant(4')-I-a</i>	5'-ATGGCTCTCTTGGTCGTCAG-3'		
<i>aph-(3')-III</i>	<i>ant(4')-I-b</i>	5'-TAAGCACACGTTCTGGCTG-3'	175	
	<i>aph-(3')-III-a</i>	5'-CGATGTGGATTGCGAAAAC-3'		
<i>aac(6)-aph(2'')</i>	<i>aph-(3')-III-b</i>	5'-CACCGAAATAACTAGAACCC-3'	279	
	<i>aac(6)-aph(2'')</i> -a	5'-CATTATACAGAGCCTTGGGA-3'		
<i>spa</i>	<i>aac(6)-aph(2'')</i> -b	5'-AGGTTCTCGTTATTCCTCGTA-3'	Variable	23
	1113F	5'-TAAAGACGATCCTTCGGTGAGC-3'		
	1514R	5'-CAGCAGTAGTGCCGTTTGCTT-3'		

**Table 2 – Antibiotic resistance profile of *S. aureus* (MSSA and MRSA) from Shenyang, China.**

Antimicrobials	Total no. of isolates (n = 202)								
	HA-MRSA (n = 107)			CA-MRSA (n = 22)			HA-MSSA (n = 73)		
	Resistant	Intermediate	Susceptible	Resistant	Intermediate	Susceptible	Resistant	Intermediate	Susceptible
PEN	105 (98.1)	0 (0.0)	2 (1.9)	22 (100.0)	0 (0.0)	0 (0.0)	67 (91.8)	0 (0.0)	6 (8.2)
OXA	107 (100.0)	0 (0.0)	0 (0.0)	22 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	73 (100.0)
FOX	105 (98.1)	0 (0.0)	2 (1.9)	22 (100.0)	0 (0.0)	0 (0.0)	1 (1.4)	0 (0.0)	72 (98.6)
VAN	0 (0.0)	0 (0.0)	107 (100.0)	0 (0.0)	0 (0.0)	22 (100.0)	0 (0.0)	0 (0.0)	73 (100.0)
TEC	2 (1.9)	13 (12.1)	92 (86.0)	0 (0.0)	0 (0.0)	22 (100.0)	1 (1.4)	1 (1.4)	71 (97.3)
CLI	94 (87.9)	1 (0.9)	12 (11.2)	6 (27.3)	0 (0.0)	16 (72.7)	38 (52.1)	0 (0.0)	35 (47.9)
RIF	49 (45.8)	1 (0.9)	57 (53.3)	7 (31.8)	0 (0.0)	15 (68.2)	1 (1.4)	0 (0.0)	72 (98.6)
GEN	98 (91.6)	2 (1.9)	7 (6.5)	3 (13.6)	0 (0.0)	19 (86.4)	19 (26.0)	0 (0.0)	54 (74.0)
LVX	103 (96.3)	0 (0.0)	4 (3.7)	18 (81.8)	0 (0.0)	4 (18.2)	6 (8.2)	1 (1.4)	66 (90.4)
CHL	10 (9.3)	0 (0.0)	97 (90.7)	0 (0.0)	0 (0.0)	22 (100.0)	2 (2.7)	1 (1.4)	70 (95.9)
ERY	104 (97.2)	0 (0.0)	3 (2.8)	20 (90.9)	0 (0.0)	2 (9.1)	56 (76.7)	1 (1.4)	16 (21.9)
SXT	11 (10.3)	0 (0.0)	96 (89.7)	2 (9.1)	0 (0.0)	20 (90.9)	2 (2.7)	2 (2.7)	69 (94.5)
LINE	0 (0.0)	0 (0.0)	107 (100.0)	0 (0.0)	0 (0.0)	22 (100.0)	0 (0.0)	0 (0.0)	73 (100.0)
DAPT	0 (0.0)	0 (0.0)	107 (100.0)	0 (0.0)	0 (0.0)	22 (100.0)	0 (0.0)	0 (0.0)	73 (100.0)
TCY	100 (93.5)	1 (0.9)	6 (5.6)	18 (81.8)	0 (0.0)	4 (18.2)	20 (27.4)	2 (2.7)	51 (69.9)

PEN, penicillin G; OXA, oxacillin; FOX, cefoxitin; VAN, vancomycin; TEC, teicoplanin; CLI, clindamycin; RIF, rifampicin; GEN, gentamicin; LVX, levofloxacin; CHL, chloramphenicol; ERY, erythromycin; SXT, trimethoprim-sulfamethoxazole; TCY, tetracycline; LINE, linezolid; DAPT, daptomycin.

Table 3 – Characterization of hospital isolates from Shenyang based on antibiotic susceptibility patterns and detection of antibiotic resistance genes.

	Erythromycin				Tetracycline				Gentamicin				No. of isolates (%)	
	Pattern (R or S)	Resistance genes			Pattern (R or S)	Resistance genes			Pattern (R or S)	Resistance genes				
		ermA	ermB	ermC		tetM	tetK	tetL		tetO	aac(6')-aph(2'')	aph(3')-III		ant(4)-I
HA-MRSA (n=107)	R	+	+	-	R	+	-	-	R	+	-	-	38 (35.5)	91 (85.0)
	R	+	-	+	R	+	-	-	R	+	+	-	33 (30.8)	6 (5.6)
	R	+	+	+	R	+	+	+	R	-	-	-	8 (7.5)	5 (4.7)
	R	+	-	-	R	+	-	+	R	-	-	-	8 (7.5)	5 (4.7)
	R	-	-	+	R	+	-	+	R	+	+	-	6 (5.6)	
	S	-	-	-	R	-	-	-	R	-	-	-	6 (5.6)	
	S	-	-	+	R	+	+	-	R	-	-	-	3 (2.8)	
CA-MRSA (n=22)	R	+	-	-	S	-	-	-	S	-	-	-	2 (1.9)	
	R	-	-	-	R	+	-	-	R	+	-	-	8 (36.4)	10 (45.5)
	S	-	-	-	R	-	-	-	R	-	-	-	11 (50.0)	2 (9.1)
	S	-	-	-	S	-	-	-	R	-	-	-	3 (13.6)	7 (31.8)
	S	-	-	-	S	-	-	-	S	-	-	-	3 (13.6)	3 (13.6)
HA-MSSA (n=73)	R	+	+	+	R	+	+	-	R	+	+	-	7 (9.6)	15 (20.5)
	R	+	-	+	S	+	-	-	R	-	-	-	22 (30.1)	44 (60.3)
	R	+	+	-	S	+	-	+	S	-	-	-	15 (20.5)	7 (9.6)
	S	+	+	+	S	-	-	-	S	+	+	-	15 (20.5)	7 (9.6)
	S	-	+	-	S	-	-	-	S	-	-	-	7 (9.6)	7 (9.6)

and *tetL* (11.2% versus 9.6%) genes and less likely to contain *tetO* (15.0% versus 30.1%) than the HA-MSSA isolates. Among the CA-MRSA isolates, 86.4% (19/22) were resistant to tetracycline. Only eight of these isolates possessed the *tetM* gene.

The aminoglycoside-resistance gene *aac(6')-aph(2'')* was identified most frequently in HA-MRSA isolates (102/107, 95.3%). The *aph(3')-III* gene was detected in six (5.6%) HA-MRSA and in seven (9.6%) HA-MSSA isolates but was not detected in any of the CA-MRSA isolates. Interestingly, the *ant(4)-I* gene was detected in two CA-MRSA isolates (9.1%) but was not detected in any of the hospital isolates.

#### Molecular characteristics of CA-MRSA and HA-S. aureus isolates

SCCmec types were identified in 107 HA-MRSA and 22 CA-MRSA isolates as shown in Table 4. Six HA-MRSA and one CA-MRSA isolate(s) could not be assigned SCCmec types using the established protocols. For the HA-MRSA isolates, the most frequently detected SCCmec types were SCCmec type II (43.0%, 46/107) and SCCmec type III (28.0%, 30/107), followed by SCCmec type V (13.1%, 14/107) and SCCmec type I (10.3%, 11/107). For the CA-MRSA, SCCmec type IV (68.2%; 15/22) and V (27.3%; 6/22) were the most frequently found.

The *spa* types were assigned by sequencing analysis of PCR products of the *spa* gene (Table 4). Nine *spa* types (t002, t030, t645, t2793, t287, t2310, t2094, t571 and t2592) were found in 180 of the hospital-acquired isolates and two (t002 and t030) were found in 22 of the CA-MRSA isolates. The most frequently encountered *spa* types were shown to be t002 and t030 both in HA-MRSA ( $n=57$ ;  $n=44$ ) and CA-MRSA isolates ( $n=19$ ;  $n=3$ ).

A total of seven pulsotypes were identified among the isolates and they were classified into seven major groups (A to G) according to their band patterns (data not shown). As shown in Table 4, the majority of the *S. aureus* isolates ( $n=77$ ), including both hospital- and community-acquired isolates, belonged to the pulsotype A cluster, followed by pulsotypes B, C and G.

#### Prevalence of the *pvl* gene

Of 202 *S. aureus* isolates tested, 72 HA isolates (e.g., 19 HA-MRSA and 53 HA-MSSA) and 10 CA-MRSA isolates were positive for the *pvl* gene. All of the 53 HA-MSSA isolates positive for *pvl* were obtained from pus while 19 of the *pvl* positive HA-MRSA isolates were obtained from 13 sputum and 6 ascite samples. Further statistical analysis showed that patients with *pvl* positive HA-MRSA isolates were older than *pvl* positive HA-MSSA carriers (65.8 year versus 11.9 year;  $p<0.05$ ). Two SCCmec types were identified among the 19 *pvl* positive HA-MRSA isolates, namely the SCCmec type II (10/107; 9.3%) and type V (3/107; 2.8%). Among the 22 CA-MRSA isolates, the *pvl* positive isolates were SCCmec type IV (6/22; 27.3%) and type V (3/22; 13.6%).

## Discussion

This study reveals that the HA-MRSA, HA-MSSA and CA-MRSA isolates differ significantly in antimicrobial susceptibility (Table 2). Considering the low resistance rates of HA-MRSA

and CA-MRSA to vancomycin, teicoplanin, chloramphenicol, daptomycin, linezolid and SXT, these antimicrobials may still be effective in therapy for *S. aureus* infections. In contrast, the high resistance rates of isolates to penicillin, oxacillin, cefoxitin, levofloxacin, erythromycin, tetracycline, strongly indicates that the use of these antibiotics should not be encouraged for treating patients with such *S. aureus* infections in the Shenyang region of China.

All *S. aureus* isolates were susceptible to vancomycin, daptomycin and linezolid. However two (1.9%) HA-MRSA and one (1.4%) HA-MSSA isolates were resistant to teicoplanin while 13 (12.1%) HA-MRSA and one (1.4%) HA-MSSA isolate showed intermediate resistance to this antibiotic. Glycopeptides including vancomycin and teicoplanin are of great importance in the treatment of patients with penicillinase-producing and methicillin-resistant *S. epidermidis* and *S. aureus* infections.<sup>25</sup> However, along with the increasing use of vancomycin and teicoplanin in clinical practice, there have been numerous reports on the emergence of MRSA with reduced susceptibility to glycopeptides in addition to teicoplanin therapy failure.<sup>26-28</sup> Since our study showed that teicoplanin resistance was present in HA-MRSA and HA-MSSA isolates, it would seem prudent to routinely screen both MRSA and MSSA isolates for reduced susceptibility to both vancomycin and teicoplanin. Our results also indicated that daptomycin and linezolid remain effective in treating those MRSA or MSSA strains exhibiting teicoplanin intermediate resistance.

As shown in Table 2, about 97.2%, 93.5%, 91.6% and 87.9% of the HA-MRSA isolates were resistant to erythromycin, tetracycline, gentamicin and clindamycin, respectively. In addition, 13.6% and 27.3% of CA-MRSA isolates were resistant to gentamicin and clindamycin. Compared to the HA-MRSA isolates, the CA-MRSA isolates were therefore more likely to be susceptible to gentamicin and clindamycin. Clindamycin is recommended by current guidelines as an option for the management of CA-MRSA infections.<sup>29</sup> Nevertheless, resistance to clindamycin was detected in 27.3% of the CA-MRSA in this study, a fact that should be taken into consideration in the future management of CA-MRSA. Most of the HA-MSSA and CA-MRSA isolates manifested susceptibility to several antimicrobials excluding the  $\beta$ -lactams (e.g. penicillin G, oxacillin, cefoxitin), and both CA-MRSA and HA-MSSA isolates showed low resistance to gentamicin. However, compared to CA-MRSA, HA-MSSA isolates were more likely to have high-level resistance to clindamycin. It may be that the HA-MSSA isolates demonstrate reduced susceptibility to clindamycin due to the selective pressures in the clinical setting.

Table 3 demonstrates that *ermA* was the most common gene encoding macrolides resistance in both the hospital and community acquired *S. aureus* isolates. The *ermB* and *ermC* genes were more prevalent in HA-MSSA than in HA-MRSA isolates. Other investigators have also found that *ermA* is the dominant *erm* gene in *S. aureus*.<sup>13</sup> Table 3 shows that three HA-MRSA and 21 HA-MSSA isolates were susceptible to erythromycin even though they possessed at least one of the three *erm* genes. Similarly, Sekiguchi et al.<sup>30</sup> found discordance among phenotypic erythromycin susceptibility and the presence of *erm* genes. They stated that this discordance might be attributed to a mutation in the coding or promoter region of the genes. We also noted that eight of CA-MRSA were

**Table 4 – Molecular characteristics of isolates from hospital and community settings and detection of virulence genes.**

Isolates	Pulsotype	No. of isolates	spa type	Antibiotic resistance profile <sup>a</sup>	pvl toxin gene	SCCmec type						
						I	II	III	IV	V	NT <sup>b</sup>	
HA-MRSA n = 107	A	51	t002	POFYGLET	–	8						
				POFYGLET	–		27					
				POFYGLET	+		10					
				POFGLET	–		3					
				POFYGLET	–		3					
	B	44	t030	POiE	–	3						
				POFYRGLHEST	–		3					
				POFYRGLET	–			24				
				POFCYGLET	–			3				
				POFRGLT	–			3				
	D	6	t002	POFYRGLET	–					8		
				POFRGLT	+					3		
	E	3	t2793	POFYGLET	+						3	
				POFCiYLHES	+						3	
F	3	t287	POFCiYLHES	+								
			POFGES	–				8				
CA-MRSA n = 22	A	19	t002	POFYLES	+				6			
				POFYGET	–				1			
				POFLTS	+						1	
				POFYGET	+						2	
				POFL	–						1	
	B	3	t030	POFGLETS	–					2		
				POFYGLETS	+					1		
	HA-MSSA n = 73	C	29	t645	PYE	+	NE <sup>c</sup>					
					PYT	–	NE					
P					+	NE						
PYE					+	NE						
G		8	t2793	PYE	+	NE						
				PYE	+	NE						
A		7	t2094	P	+	NE						
				PYGE	–	NE						
B	7	t2592	PE	+	NE							

<sup>a</sup> O, oxacillin; P, penicillin G; T, tetracycline; F, ceftioxin; S, trimethoprim-sulfamethoxazole; E, erythromycin; G, gentamicin; V, vancomycin; L, levofloxacin; R, rifampicin; C, Teicoplanin; Y, clindamycin; H, chloramphenicol; i, intermedial.

<sup>b</sup> NT, nontypable.

<sup>c</sup> NE, not determined.

resistant to erythromycin but did not possess any erythromycin resistance genes as determined by PCR (Table 3). This might be explained by the location of these genes on small plasmids, which were occasionally lost.<sup>20,31,32</sup> The most frequently identified gene implicated in aminoglycoside resistance in our study was *aac(6)-aph(2'')*, which was found in 95.3% of HA-MRSA, 45.5% of CA-MRSA and 39.7% of HA-MSSA isolates, respectively. The *Aph(3')-III* gene, infrequently found in *S. aureus*, was identified in 5.6% of HA-MRSA and 9.6% of HA-MSSA isolates, respectively. The *ant(4)-I* gene, another infrequently found gene in *S. aureus*, was detected in 9.1% of CA-MRSA isolates.

The use of several different molecular methods is currently necessary for the accurate typing of MRSA, both in outbreak situations and for international comparisons.<sup>33</sup> In this study, PFGE analysis indicated that the HA-MRSA isolates could be grouped into five different types (Pulsotype A, B, D, E and F), pulsotype A was the most predominant type (51/107; 47.7%), followed by pulsotype B (44/107; 41.1%). Concurrently, CA-MRSA isolates were grouped into two different types (Pulsotype A and B), 19 belonged to pulsotype A and three strains

belonged to pulsotype B. The *spa* typing revealed four *spa* types including t002, t030, t2793 and t287 among the HA-MRSA isolates, while two *spa* types of t002 and t030 were identified in CA-MRSA. The results demonstrated that the predominant *spa* types (t002 and t030) and pulsotypes (A and B) in community acquired MRSA were also predominant among hospital isolates, which suggested that they shared the same clonal origin. This also indicates that the cross-transmission of *S. aureus* strains between the community and hospital environment may have occurred which is in accordance with previous reports documenting increasing colonization and infection due to MRSA strains of community origin in hospitals and vice versa.<sup>34,35</sup>

SCCmec typing of HA-MRSA isolates revealed that 13.1% (14/107) strains carried SCCmec type V, which is mainly carried by CA-MRSA isolates<sup>36</sup> indicating that HA-MRSA and CA-MRSA isolates might not be distinguished merely by identifying their SCCmec genotypes. Ma et al.<sup>10</sup> reported that 49 of 51 strains (96.0%) isolated from both community- and healthcare-associated cases in Uruguay carried either SCCmec type IV or SCCmec type V elements. In recent years, there

have been numerous reports describing nosocomial outbreaks caused by strains harboring SCCmec type IV, which was originally identified in CA-MRSA strains.<sup>37,38</sup> Compared with SCCmec type I, II and III, SCCmec type IV and type V are small in size and are more prone to transfer between staphylococcal species, presumably via phage-associated transduction.<sup>2,18,36</sup> In this study, seven MRSA strains could not be assigned SCCmec types using the established protocols. Presently work is being undertaken to assign SCCmec types to those seven strains using recently reported primers.<sup>45</sup>

Worldwide pandemic MRSA clones have been reported as the New York/Japan clone (ST5-type II SCCmec), the pediatric clone (ST5-type IV SCCmec), the archaic clone (ST250-type I SCCmec), the Iberian clone (ST247-SCCmec type 1A), and the Brazilian/Hungarian clone (ST239-type III SCCmec) by Oliveira et al.<sup>39</sup> In the present study, we identified several different MRSA clones that were distinguishable based on SCCmec typing, *spa* typing, and distinct antimicrobial resistance profiles (Table 4). We conclude that *spa* types t030 and t002 were the two most common *spa* types identified in this study. Most of the t030 belong to the Brazilian ST239-MRSA-III epidemic clones while the t002 belong to both the New York/Japan ST5-MRSA-II and the pediatric ST5-MRSA-IV epidemic clones which are the most prevalent in Asian countries, especially China.<sup>11,40</sup>

It is known that the *pvl* gene locus is carried on bacteriophages, namely  $\phi$ PVL,  $\phi$ SLT,  $\phi$ Sa2mw and  $\phi$ 108PVL, which encode *pvl* components. *S. aureus* isolates producing PVL have been strongly associated with severe skin and soft-tissue infections (SSTIs), and occasionally, severe necrotizing pneumonia.<sup>6</sup> Some previous studies have described a low prevalence of *pvl* genes (<5%) in HA-MRSA,<sup>41,42</sup> while *pvl* positive CA-MRSA clones have spread globally.<sup>2,43</sup> In our study, the *pvl* gene was identified in 17.8% (19/107) of HA-MRSA and 45.5% (10/22) CA-MRSA isolates, respectively. In Taiwan, during 2005 the prevalence of *pvl* positive MRSA isolates was 23.3% in hospitals.<sup>44</sup> Yu et al. also reported the prevalence of the *pvl* gene among clinical isolates of *S. aureus* in a teaching hospital, and most of the *pvl* positive isolates were SCCmec type III.<sup>9</sup> Furthermore, in this study we also found a high prevalence of *pvl* positive MSSA isolates of hospital origin.

Overall, the present study suggests a bi-directional movement of *S. aureus* strains between healthcare and community settings in Shenyang, China. The spread of *pvl* positive MRSA clones with specific genotypes within hospitals and the community is a particular cause of concern and could have important implications for the implementation of effective infection control strategies and measures.

## Conclusions

This study has shown that the combination of susceptibility testing and various molecular methods provides useful information on the antibiotic resistance and molecular diversity of *S. aureus* in a region of Northeastern China. Although the number of *S. aureus* isolates available for our investigation together with relevant epidemiological data was limited, the high proportion of *pvl* positive MRSA and MSSA isolates observed in this study indicates that adequate measures are needed in

order to curtail and control the spread and establishment of such MRSA and MSSA isolates in Chinese health care institutions.

## Conflicts of interest

The authors declare no conflicts of interest.

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