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Antimicrobial susceptibility, virulence determinant carriage and molecular characteristics of *Staphylococcus aureus* isolates associated with skin and soft tissue infections

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ABSTRACT

A better understanding of the antimicrobial susceptibility, carriage of virulence determinants and molecular characteristics of *Staphylococcus aureus* isolates associated with skin and soft tissue infections (SSTIs) may provide further insights related to clinical outcomes with these infections. From January 2012 to September 2013, a total of 128 non-duplicate *S. aureus* isolates were recovered from patients with SSTIs. All 128 *S. aureus* SSTI isolates carried at least five virulence genes tested. Virulence genes detected among at least 70% of all tested isolates included *hld* (100%), *hla* (95.3%), *icaA* (96.9%), *clf* (99.2%), *sdrC* (79.7%), *sdrD* (70.3%), and *sdrE* (72.7%). The prevalence of MRSA isolates with 10 virulence genes tested (54.4%, 31/56) was significantly higher than that among MSSA isolates (35.2%, 25/71) ($p < 0.05$). The positive rates of *seb*, *sen*, *sem*, *sdrE* and *pvl* among MRSA isolates were significantly higher than among MSSA isolates ($p < 0.05$). ST7 and ST630 accounting for 10.9% were found to be the predominant STs. The most prevalent *spa* type was t091 (8.6%). MRSA-ST59-SCC*mec* IV was the most common clone (12.3%) among MRSA isolates whereas among MSSA isolates the dominant clone was MSSA-ST7 (15.5%). Six main clonal complexes (CCs) were found, including CC5 (52.3%), CC7 (11.7%), CC59 (8.6%), CC88 (6.3%), CC398 (4.7%), and CC121 (3.1%). A higher carriage of *seb* and *sec* was found among CC59 isolates. In comparison to CC5 and CC7 isolates, those with the highest carriage rates (>80.0%) of *sdrC* and *sdrD*, CC59 isolates had lower prevalence of these two virulence genes. All CC59 isolates were susceptible to gentamicin and trimethoprim/sulfamethoxazole, while CC5 and CC7 isolates had resistance rates to these two antimicrobials of 25.4% and 20.9%, and 40.0% and 40.0%, respectively. The resistance rates for tetracycline, clindamycin, and erythromycin among CC5 isolates were lower than among CC7 and CC59 isolates. In conclusion, the molecular

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typing of *S. aureus* SSTI isolates in the present study showed considerable heterogeneity. ST7 and ST630 became prevailing clones. Different *S. aureus* clones causing SSTIs were associated with specific antimicrobial resistance and virulence gene profiles.

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Introduction

Staphylococcus aureus, particularly methicillin-resistant *S. aureus* (MRSA), is an important human pathogen responsible for many infectious diseases including skin and soft tissue infections (SSTIs), foreign-body infections, pneumonia, septic arthritis, endocarditis, osteomyelitis, sepsis, and bloodstream infections in both hospital and community settings.[1] The ability of this clinically important pathogen to successfully persist within the hosts is largely due to the carriage of a battery of virulence factors which promote adhesion, acquisition of nutrients, and evasion of host immunologic responses.[2,3] Some *S. aureus* isolates also produce one or more additional exoproteins, such as toxic shock syndrome toxin-1 (TSST-1), staphylococcal enterotoxins (SEs), exfoliative toxins (ETs), and leukocidins.[2–4] Recently, Panton-Valentine leukocidin (*pvl*) encoded by two contiguous and cotranscribed genes (*lukS-PV* and *lukF-PV*) is an important virulence factor for community-acquired MRSA (CA-MRSA) affecting individuals without apparent risk factors for hospital acquisition.[5,6] *S. aureus* is the most common bacterial pathogen identified from SSTIs.[7] SSTIs caused by MRSA is associated with a high incidence of treatment failure and recurrence.[8] A better understanding of the antimicrobial susceptibility, carriage of virulence determinants, and molecular characteristics of *S. aureus* isolates associated with SSTIs may provide further insights related to clinical outcomes of these infections. Molecular typing has proved to be an important tool to investigate MRSA epidemiology. Pulsed-field gel electrophoresis (PFGE) patterns, SCC*mec* typing, *spa* typing, and multi-locus sequence typing (MLST) have been proven useful for monitoring the evolutionary process of pandemic MRSA clones.[1] In China, ST239-MRSA-III is a predominant MRSA clone among adults, while ST59-MRSA-IV is the most prevalent clone among children.[9,10] In a previous study we investigated the molecular typing of *S. aureus* isolated from patients with SSTIs at our hospital from December 2002 to June 2008 and found that ST239, ST1018, ST59, ST7, and ST88 were the most prevalent sequence types.[11] A shift of important clones has been observed in several studies.[12–14] A report from China found a rapid change of MRSA over a 15-year period at a tertiary care hospital, when the ST239-MRSA-III-t037 clone was replaced by the emerging ST239-MRSA-III-t030 clone.[15] Understanding the shift of important clones at the local and international levels is of great significance. To understand the shift of *S. aureus* clones associated with SSTIs, the present study aimed to investigate the antimicrobial susceptibility, carriage of virulence determinants, and molecular characteristics of *S. aureus* isolates associated with SSTIs at the hospital in 2012–2013.

Materials and methods

Collection of clinical isolates and S. aureus confirmation

From January 2012 to September 2013, a total of 128 non-duplicate *S. aureus* isolates (single isolate per patient) were collected at The First Affiliated Hospital of Wenzhou Medical University, China from pus samples of hospitalized patients with SSTIs. Lesions requiring incision and drainage or with spontaneously draining purulent fluid, carbuncles, furuncles, boils, cellulitis with purulent drainage, chronic ulcer, and deep wounds were included. *S. aureus* isolates from patients with SSTIs with clinical signs and symptoms of infection such as increased white blood cell counts, fever, local redness, swelling, and exudate were considered invasive isolates and included for investigation. Isolates were identified as *S. aureus* using Gram stain, positive catalase and coagulase test results, and Vitek microbiology analyzer (bioMérieu, Marcy l'Etoile, France). *S. aureus* ATCC25923 was used as a control strain.

Ethics statement

This study was approved by the Institutional Ethics Review Board of The First Affiliated Hospital of Wenzhou Medical University. All patients provided written informed consent for this study. The written informed consents were also obtained from the next of kin, caretakers, or guardians on behalf of the minors/children enrolled.

Antimicrobial susceptibility testing

S. aureus susceptibility to penicillin (10 units), erythromycin (15 μg), clindamycin (2 μg), rifampicin (5 μg), tetracycline(30 μg), linezolid (30 μg), mupirocin (5 μg), quinupristin/dalfopristin (15 μg), trimethoprim/sulfamethoxazole (1.25/23.75 μg), gentamicin (10 μg), ciprofloxacin (5 μg), Chloramphenicol (30 μg), and nitrofurantoin (300 μg) were determined using disc diffusion test recommended by the Clinical and Laboratory Standards Institute (CLSI).[16] All discs were obtained from Oxoid Ltd. Vancomycin MICs for *S. aureus* isolates were determined by agar dilution method. Interpretive standards for the antimicrobial susceptibility test and D-test for tested *S. aureus* isolates were in accordance with the guidelines provided by CLSI.[16] Susceptibility of *S. aureus* to mupirocin was determined by disc diffusion, with a zone diameter ≥14 mm on a 5 μg disc indicating susceptibility as described previously.[17,18] *S. aureus* ATCC 25923 and *Escherichia coli* ATCC25922 were used as reference strains for antimicrobial susceptibility testing.

DNA extraction

S. aureus isolates tested were cultured on blood agar overnight at 35 °C. Then, three to four bacterial colonies were suspended and incubated in 150 μL sterile distilled water with lysostaphin (1 mg/mL) (Sangon, China) at 37 °C for 1 h. Finally, DNA was extracted following the instructions of the Genomic DNA Extraction kit (Sangon, China). The extracted DNA was stored at −20 °C and prepared for PCR detection.

Identification of MRSA isolates and pvl detection

A multiplex PCR protocol was used for simultaneous amplification of *mecA*, 16S rRNA, and *pvl* genes as described previously.[19] MRSA isolates harbouring *mecA* were confirmed using MRSA N315 and *pvl*-positive MRSA isolate identified in our previous study as positive control strains.[20]

Detection of virulence genes

Virulence genes, including toxins (*sea*, *seb*, *sec*, *sed*, *seg*, *seh*, *sei*, sej, *seo*, *sen*, *sem*, *edin*, *hla*, *hlb*, *hld*, *hlg*, *tst*, *eta*, *etb*), adhesins (*clfA*, *cna*, *sdrC*, *sdrD*, and *sdrE*), *icaA* and *arcA* were identified using PCR assays with primers and conditions previously described.[21,22] PCR primers used for PCR assays were shown in Table 1.

S. aureus isolates harbouring virulence genes determined in our previous study were used as positive control strains for detecting virulence genes.[20]

SCC*mec* typing

SCC*mec* typing of MRSA isolates was performed using a battery of multiplex PCRs as described previously.[23] MRSA isolates with unanticipated fragments or lacking fragments by multiplex PCR were defined as non-typeable (NT). MRSA NCTC 10442 (SCC*mec*I), MRSA N315 (SCC*mec* II), MRSA 85/2082 (SCC*mec* III), MRSA JCSC 4744 (SCC*mec* IV), and MRSA WZ153 (SCC*mec* V) were used as control strains for SCC*mec* typing.

spa typing

The *spa* variable repeat region from each *S. aureus* isolate was amplified using simplex PCR oligonucleotide primers as previously described.[24,25] Following their purification and sequencing, *spa* types were assigned using the *spa* database website (http://www.ridom.de/spaserver).

Multi-locus sequence typing (MLST)

MLST typing of *S. aureus* isolates was performed using amplification of internal fragments of the seven housekeeping genes of *S. aureus* as described previously.[26] Following purification and sequencing of these genes, the sequences were compared with the existing sequences available on the MLST website for *S. aureus* (http://saureus.mlst.net), and STs were determined according to the allelic profiles. Novel STs were deposited in the MLST database (http://saureus.mlst.net/).

Statistical analysis

Differences between groups were assessed by using the chi-square test. The software SPSS 13.0 was used to perform calculations. *p*-Values of <0.05 were considered statistically significant.

Results and discussion

Antimicrobial susceptibility

Among 128 *S. aureus* isolates, 57 (44.5%) were identified as MRSA determined by cefoxitin disc diffusion test and were positive for *mecA*. The MRSA prevalence in the present study was lower than the 54.1% reported between December 2002 and June 2008.[11] The resistance rates for *S. aureus*, MRSA, and MSSA isolates to antimicrobials are listed in Table 2. All isolates tested were susceptible to vancomycin, linezolid, dalfopristin/quinupristin, and nitrofurantoin. Of 128 *S. aureus* isolates, 72.7% (93/128) with resistance to three or more classes of antimicrobial agents tested were defined as multidrug-resistant isolates. Only two isolates tested were susceptible to all antimicrobial agents tested. Twenty-three (18.0%) isolates were only resistant to penicillin. Ten (7.8%) isolates were concomitantly resistant to two antimicrobial agents tested (penicillin and another antimicrobial agent). Resistance rates of *S. aureus*, MRSA, and MSSA isolates to penicillin, clindamycin, and gentamicin were above 60%, whereas to tetracycline, gentamicin, ciprofloxacin, trimethoprim/sulfamethoxazole, chloramphenicol and rifampicin were less than 40%. Three isolates were positive for D-test, indicating that resistance of these isolates to clindamycin was inducible. Only one MRSA isolate was resistant to mupirocin as it exhibited no zone of inhibition.

Virulence gene profiling

The invasiveness of *S. aureus* largely depends on the carriage of a battery of virulence factors.[2,3] All *S. aureus* SSTI isolates in the present study harboured at least five virulence genes tested. Frequencies of virulence genes are shown in Table 3. Virulence genes were detected among at least 70% of all tested isolates included *hld* (100%), *hla* (95.3%), *icaA* (96.9%), *clf* (99.2%), *sdrC* (79.7%), *sdrD* (70.3%), and *sdrE* (72.7%). Less than 10% of the isolates tested carried *eta* (7.0%), *sed* (6.3%), *seh* (7.0%), *tst* (4.7%), and edin (5.5%). Multiple isolates harboured *pvl* (12.5%), *sea* (35.9%), *seb* (14.8%), *sec* (21.1%), *sei* (21.1%), *seg* (26.6%), *sem* (30.5%), *sen* (31.3%), *seo* (27.3%), *hlb* (22.7%), *hlg* (18.8%), and *cna* (32.0%). All *S. aureus* isolates tested were negative for *sej*, *etb*, and *arcA*. Fifty-six (43.75%, 56/128) isolates harboured more than 10 tested virulence genes, among which two isolates harboured 16 genes, seven isolates 14 genes, 13 isolates 13 genes, six isolates 12 genes, 10 isolates 11 genes, and 18 isolates 10 genes. Of MRSA isolates, 54.4% (31/56) harboured more than 10 tested virulence genes, which was significantly higher than that among MSSA isolates (35.2%, 25/71) ($p < 0.05$).

Staphylococcal enterotoxins (SEs), including five major classical antigenic types of SEs (SEA to SEE) and newly identified SEs are the cause of food poisoning in humans.[27]

Table 1 – PCR primers used for PCR assays.

PCR product	Primer description	Primer sequence	Refs.
sea	*sea-Up*	TTGCAGGGAACAGCTTTAGGCAATC	21
	sea-Dn	TGGTGTACCACCCGCACATTGA	
seb	*seb-Up*	GACATGATGCCTGCACCAGGAGA	21
	seb-Dn	AACAAATCGTTAAAAACGGCGACACAG	
sec	*sec-Up*	CCCTACGCCAGATGAGTTGCACA	21
	sec-Dn	CGCCTGGTGCAGGCATCATATC	
sed	*sed-Up*	GAAAGTGAGCAAGTTGGATAGATTGCGGCTAG	21
	sed-Dn	CCGCGCTGTATTTTTCCTCCGAGAG	
see	*see-Up*	TGCCCTAACGTTGACAACAAGTCCA	21
	see-Dn	TCCGTGTAAATAATGCCTTGCCTGAA	
seg	*seg-Up*	TGCTCAACCCGATCCTAAATTAGACGA	21
	seg-Dn	CCTCTTCCTTCAACAGGTGGAGACG	
seh	*seh-Up*	CATTCACATCATATGCGAAAGCAGAAG	21
	seh-Dn	GCACCAATCACCCTTTCCTGTGC	
sei	*sei-Up*	TGGAGGGGCCACTTTATCAGGA	21
	sei-Dn	TCCATATTCTTTGCCTTTACCAGTG	
sej	*sej-Up*	CTCCCTGACGTTAACACTACTAATAACCC	21
	sej-Dn	TATGGTGGAGTAACACTGCATCAAAA	
sem	*sem-Up*	CTATTAATCTTTGGGTTAATGGAGAAC	22
	sem-Dn	TTCAGTTTCGACAGTTTTGTTGTCAT	
sen	*sen-Up*	ATGAGATTGTTCTACATAGCTGCAAT	22
	sen-Dn	AACTCTGCTCCCACTGAAC	
seo	*seo-Up*	AGTTTGTGTAAGAAGTCAAGTGTAGA	22
	seo-Dn	ATCTTTAAATTCAGCAGATATTCCATCTAAC	
tst	*tst-Up*	AGCCCTGCTTTTACAAAAGGGGAAAA	21
	tst-Dn	CCAATAACCACCCGTTTTATCGCTTG	
eta	*eta-Up*	CGCTGCGGACATTCCTACATGG	21
	eta-Dn	TACATGCCCGCCACTTGCTTGT	
etb	*etb-Up*	GAAGCAGCCAAAAACCCATCGAA	21
	etb-Dn	TGTTGTCCGCCTTTACCACTGTGAA	
hla	*hla-Up*	CTGATTACTATCCAAGAAATTCGATTG	22
	hla-Dn	CTTTCCAGCCTACTTTTTTATCAGT	
hlb	*hlb-Up*	GTGCACTTACTGACAATAGTGC	22
	hlb-Dn	GTTGATGAGTAGCTACCTTCAGT	
hld	*hld-Up*	AAGAATTTTTATCTTAATTAAGGAAGGAGTG	22
	hld-Dn	TTAGTGAATTTGTTCACTGTGTCGA	
hlg	*hlg-Up*	GTCAYAGAGTCCATAATGCATTTAA	22
	hlg-Dn	CACCAAATGTATAGCCTAAAGTG	
sdrC	*sdrC-Up*	CGCATGGCAGTGAATACTGTTGCAGC	21
	sdrC-Dn	GAAGTATCAGGGGTGAAACTATCCACAAATTG	
sdrD	*sdrD-Up*	CCACTGGAAATAAAGTTGAAGTTTCAACTGCC	21
	sdrD-Dn	CCTGATTTAACTTTGTCATCAACTGTAATTTGTG	
sdrE	*sdrE-Up*	GCAGCAGCGCATGACGGTAAAG	21
	sdrE-Dn	GTCGCCACCGCCAGTGTCATTA	
cna	*cna-Up*	TTCACAAGCTTGGTATCAAGAGCATGG	21
	cna-Dn	GAGTGCCTTCCCAAACCTTTTGAGC	
clfA	*clfA-Up*	ATTGGCGTGGCTTCAGTGCTTG	21
	clfA-Dn	GCTTGATTGAGTTGTTGCCGGTGT	
arcA	*arcA-Up*	CACGTAACTTGCTAGAACGAG	21
	arcA-Dn	GAGCCAGAAGTACGCGAG	
icaA	*icaA-Up*	TCAGACACTTGCTGGCGCAGTC	21
	icaA-Dn	TCACGATTCTCTCCCTCTCTGCCATT	

Additionally, SEs are also associated with other diseases such as allergy sensitization, asthma, chronic obstructive pulmonary disease, scarlet fever, glomerulonephritis, and vasculitis.[28–31] The genes encoding these SEs but *sej* were found among *S. aureus* SSTI isolates, with different carriage proportions ranging from 6.3% to 35.9% in this investigation. In particular, the positive rates for *seb*, *sen*, and *sem* among MRSA isolates were significantly higher than among MSSA isolates ($p < 0.05$). The Sdr proteins encoded by the tandemly arrayed *sdrC*, *sdrD*, and *sdrE* are microbial surface components which recognize adhesive matrix molecules and have different roles in *S. aureus* pathogenicity.[32] Strong correlations between *S. aureus* invasiveness and the presence of one of the allelic variants of the *sdrE* gene, as well as carriage of the *sdrD* gene and bone infections caused by *S. aureus*, have been reported previously.[33,34] Our previous study showed that 95.5% (85/89) of *S. aureus* isolates responsible for bloodstream infection harboured at least one *sdr* locus and 84.3% (75/89) possessed more

Table 2 – Antimicrobial resistance profiles of MRSA, MSSA, and *S. aureus* isolates.

	MRSA (*n* = 57) R (%)	MSSA (*n* = 71) R (%)	*S. aureus* (*n* = 128) R (%)
Tetracycline	36.8	28.2	32.0
Gentamicin	31.6	14.1	21.9
Penicillin	96.5	97.2	96.9
Oxacillin	100	0.0	44.5
Clindamycin	71.9	66.2	68.8
Erythromycin	71.9	67.6	69.5
Ciprofloxacin	36.8	15.5	25.0
Linezolid	0	0	0
Rifampicin	10.5	0	4.7
Trimethoprim/ sulfamethoxazole	28.1	19.7	23.4
Nitrofurantoin	0	0	0
Cefaclor	40.4	1.4	18.8
Chloroamphenicol	15.8	5.6	10.2
Imipenem	17.5	0	7.8
Mupirocin	1.8	0	0.8
Dalfopristin/ quinupristin	0	0	0
Vancomycin	0	0	0

Table 3 – The frequencies of virulence genes among *S. aureus*, MRSA and MSSA isolates.

	S. aureus (*n* = 128) (%)	MRSA (*n* = 57) (%)	MSSA (*n* = 71) (%)	*p*-values[a]
sea	35.9	42.1	31.0	>0.05
seb	14.8	21.1	9.9	<0.05
sec	21.1	21.1	21.1	
sed	6.3	1.8	9.9	>0.05
seg	26.6	31.6	22.5	>0.05
seh	7.0	3.5	9.9	>0.05
sei	21.1	21.1	21.1	
sej	0	0	0	
sen	31.3	43.9	21.1	<0.05
sem	30.5	43.9	19.7	<0.05
seo	27.3	26.3	28.2	>0.05
tst	4.7	5.3	4.2	>0.05
eta	7.0	1.8	11.3	>0.05
etb	0	0	0	
clfA	99.2	98.2	100	>0.05
cna	32.0	26.3	36.6	>0.05
sdrC	79.7	89.5	71.8	>0.05
sdrD	70.3	70.2	70.4	>0.05
sdrE	72.7	80.7	66.2	<0.05
icaA	96.9	96.5	97.2	>0.05
arcA	0	0	0	
pvl	12.5	15.8	9.9	<0.05
hla	95.3	100	91.5	>0.05
hlb	22.7	26.3	19.7	>0.05
hld	100	100	100	
hlg	18.8	17.5	19.7	>0.05
edin	5.5	0	9.9	>0.05

[a] MRSA group was compared with MSSA group.

than two *sdr* loci.[35] Similarly, 120 (93.8%) of 128 SSTI isolates in the present study were found to harbour at least one *sdr* locus (*sdrC*, *sdrD*, *or sdrE*), with 18 (14.1%), 39 (30.5%), and 63 (49.2%) harbouring one, two, or three of these loci, respectively. The positive rate of *sdrE* among MRSA isolates was significantly higher than among MSSA isolates. *S. aureus* isolates producing TSST-1 encoded by the *tst* gene have been associated with toxic shock syndrome, staphylococcal scarlet fever, and neonatal toxic shock-like exanthematous diseases.[3] However, the present study found that only 4.7% of *S. aureus* SSTI isolates harboured *tst*. Our previous study found that the prevalence of *hlb* among *S. aureus* isolates associated with bloodstream infection was 67.4%.[35] However, *hlb* was only identified among 22.7% of *S. aureus* SSTI isolates, while the positivity rates of *hla* and *hld* were 95.3% and 100%, respectively.

pvl has been closely associated with CA-MRSA infections and there is a strong epidemiological association between carriage of *pvl* genes and successful CA-MRSA lineages.[1,36] Infections caused by *pvl*-positive *S. aureus* isolates are predominantly represented by skin and soft-tissue infection.[5,6] Among *S. aureus* isolates causing SSTI in our hospital between December 2002 and June 2008, the overall positivity rates of *pvl* genes 23.4% (26/111), and among MRSA and MSSA isolates the rates were, 21.7% (13/60) and 25.5% (13/51), respectively.[11] Compared with our previous study, the overall positivity rates of *pvl* genes in the present study (12.5%) was lower, as were the rates among MRSA (15.8%) and MSSA (9.9%) isolates, indicating that there is a decreased trend in the prevalence of *pvl* genes among *S. aureus* SSTI isolates at our hospital.

Molecular typing

Molecular typing of *S. aureus* isolates tested are shown in Table 4. Among 57 MRSA isolates, 24, 14, 13, and three harboured SCC*mec* types III, IV, II, and V, respectively. Three isolates were classified as non-typeable.

A total of 28 STs were identified among 128 *S. aureus* isolates. ST7 and ST630 accounting for 10.9% (14/128 each) were found to be the predominant STs, followed by ST59 (8.6%, 11/128), ST5 (6.3%, 8/128), ST88 (6.3%, 8/128), ST1 (5.5%, 7/128), ST965 (5.5%, 7/128), ST398 (4.7%, 6/128 each), ST25 (4.7%, 6/128), and ST188 (3.9%, 5/128). ST239, ST6, and ST121 accounted for four isolates each. ST1463 and ST1821 accounted for three isolates each. ST8 and ST1349 accounted for two isolates each. The remaining STs including ST12, ST15, ST118, ST692, ST789, ST1281, ST1920, ST2259, ST2832, ST2833, and ST72 were identified in only one isolate. The STs of nine isolates were not identified. Two novel STs characterized as ST2832 and 2833 were identified in two MRSA isolates and have been deposited in the MLST database (http://saureus.mlst.net/). Sixteen PVL-positive isolates were distributed among nine different STs including ST88 (five isolates), ST59 (three isolates), and ST121 (two isolates). ST239 and ST5 were the most dominant STs in China.[9,37] Interestingly, in the present study, these two predominant STs were found to be minor clones, while ST630 and ST7, seldom noted in Chinese isolates previously, were the major clones among *S. aureus* SSTI isolates. Another study from China found ST398 accounting for 17.1% (28/164) as the most common ST among

Table 4 – Molecular characteristics of *S. aureus* SSTIs isolates.

CC (no.)	STs (no.)	*spa* types (no.)	MRSA (no.)	MSSA (no.)	SCC*mec* (no.)
5 (60)	ST630 (14)	*t*377 (7)	2	5	III (2)
		*t*4047 (2)		2	
		*t*030 (1)	1		III
		*t*4549 (1)		1	
		*t*4047 (2)	2		II (1), IV (1)
		*t*5554 (1)		1	
	ST5 (8)	*t*2460 (2)	2		II (2)
		*t*311 (2)	2		II (2)
		*t*4352 (1)	1		II
		*t*002 (1)		1	
		*t*548 (1)		1	
		*t*535 (1)		1	
	ST965 (7)	*t*062 (7)	7		III (4), II (2), NT (1)
	ST25 (6)	*t*349 (2)		2	
		*t*227 (2)	2		III (2)
		*t*8170 (1)		1	
		*t*078 (1)		1	
	ST188 (5)	*t*189 (4)	1	3	II
		*t*1858 (1)	1		III
	ST239 (4)	*t*030 (3)	3		III (3)
		*t*2270 (1)	1		III (1)
	ST6 (4)	*t*701 (3)		3	
		*t*10519 (1)	1		III
	ST1462 (3)	*t*189 (3)		3	
	ST1821 (3)	*t*377 (1)		1	
		*t*2196 (1)		1	
		*t*4549 (1)	1		II
	ST8 (2)	*t*008 (1)		1	
		*t*377 (1)		1	
	ST15 (1)	*t*062		1	
	ST118 (1)	*t*189 (1)		1	
	ST1920 (1)	*t*286 (1)		1	
	ST72 (1)	*t*148		1	
	ST1 (7)	*t*127 (7)	2	5	III (2)
7 (15)	ST7 (14)	*t*091 (8)	2	6	III (2)
		*t*796 (5)		5	
		*t*2828 (1)	1		IV
	ST789 (1)	*t*091 (1)	1		III
59 (11)	ST59 (11)	*t*437 (8)	7	1	IV (6), V (1)
		*t*163 (3)	2	1	IV (1), NT (1)
88 (8)	ST88 (8)	*t*5348 (3)	1	2	IV
		*t*1764 (1)	1		II
		*t*5351 (1)		1	
		*t*2788 (1)		1	
		*t*7637	1		V
		*t*1376	1		IV
398 (6)	ST398 (6)	*t*571 (4)	1	3	IV
		*t*011 (1)		1	
		*t*034 (1)		1	
121 (4)	ST121 (4)	*t*159 (3)	2	1	III (1), IV (1)
		*t*2091 (1)		1	

S. aureus SSTI isolates.[38] In contrast to our previous study where ST239 was the most prevalent ST accounting for 21.6% (24/111) of *S. aureus* SSTI isolates,[11] this ST accounted for only 3.1% (4/128) in the present study. Interestingly, ST630 not found in our previous study turned out to be the predominant ST in the present study, while ST1018, the second most prevalent ST in our previous study, was not found.

Fifty-two *spa* types were identified among the 128 isolates. The most prevalent *spa* type was *t*091 (8.6%, 11/128), followed by *t*062 (7.0%, 9/128), *t*377 (7.0%, 9/128), *t*437 (7.0%, 9/128), *t*189 (6.3%, 8/128), *t*127 (4.7%, 6/128), *t*796 (3.9%, 4/128), *t*571 (3.1%, 3/128), *t*4047 (3.1%, 3/128), and *t*030 (3.1%, 3/128). Other types identified included *t*159, *t*163, *t*5348, and *t*701 (three isolates each). In previous reports of Chinese isolates, *t*30 and *t*37, typically associated with ST239, were the most prevalent *spa* types.[9,15] The proportions of *t*37 and *t*30 were extremely low, as well as the proportions of ST239 and ST5 in the present study.

Fifty-seven MRSA isolates were distributed in different clones. 12.3% (7/57) of MRSA isolates belonged to MRSA-ST59-SCC*mec* IV-t 437/163, which was the most common clone in the present study. Likewise, in a study from China ST59-MRSA-IVa-t437 was found to be the predominant clone among CA-MRSA isolates associated with SSTIs in children.[39] Our previous study conducted between December 2002 and June 2008 found that ST239-MRSA-SCC*mec* III accounting for 30.2% (19/63) was the most prevalent clone among MRSA SSTI isolates, followed by ST1018-MRSA-SCC*mec*III accounting for 15.9% (10/63).[11] However, only three MRSA isolates were ST239-MRSA-SCC*mec* III and there was no ST1018-MRSA-SCC*mec*III isolate in the present study. Among 71 MSSA isolates, the dominant clone was MSSA-ST7 (15.5%, 11/71), followed by MSSA-ST630 (12.7%, 9/71), MSSA-ST398 and MSSA-ST1 (7.0%, 5/71 each), and MSSA-ST25 and MSSA-ST88 (5.6%, 4/71 each). MRSA-ST398 isolates are usually associated with infections in both animals and humans.[40,41] ST398 MSSA isolates of human origin are usually linked to t571.[42] In the present study, four of six ST-398 *S. aureus* isolates, including three MSSA and one MRSA isolate, were linked to t571.

Taken together, our data showed that *S. aureus* isolates associated with SSTIs were genetically diverse and the main clones associated with SSTIs are going through a rapid shift in our hospital.

Comparison of antimicrobial resistance and molecular typing among the major clonal complexes (CCs)

Clustering analysis by eBURST v3 showed that six clonal complexes (CCs) were found (Table 4), including CC5 (52.3%, 67/128), CC7 (11.7%, 15/128), CC59 (8.6%, 11/128), CC88 (6.3%, 8/128), and CC398 (4.7%, 6/128). The distribution of some virulence genes, especially enterotoxin genes, were correlated with different MRSA lineages.[43,44] In the present study, a higher carriage of *seb* and *sec* was found among CC59 isolates, while a higher carriage of enterotoxin genes were found among CC5 isolates (Table 5). All CC7 and CC59 isolates were not carrying *seh* and *seo*, while 11.8% and 34.2% of CC5 isolates were found to carry these two genes, respectively. Although the proportions of *seb* and *sec* among CC5 and CC7 isolates were low, the positivity rates of these two enterotoxin genes among CC59 isolates were 54.5% (*seb*) and 63.6% (*sec*). The prevalence of *cna* among CC5 isolates was 38.8%, while this virulence gene was identified in none of the CC7 and CC59 isolates. Compared with the higher carriage rates (>80.0%) of *sdrC* and *sdrD* among CC5 and CC7 isolates, the positivity rates of two *sdr* loci were significantly lower among CC59 isolates, especially *sdrD* prevalence of 9.1%. Interestingly, all CC59 isolates carried *sdrE*, while only 26.7% of CC7 isolates were found to carry *sdrE*. The carriage rates of *pvl* and *hlb* among CC59 isolates were significantly higher than those rates among CC5 and CC7 isolates ($p < 0.05$). These differences in carriage rates of virulence genes among different CC isolates suggested that different *S. aureus* lineages associated with SSTIs have specific patterns of virulence genes.

The predominant MRSA clones in China were associated with specific antimicrobial resistance profiles.[45] In the present study, although 81.8% of CC59 isolates were MRSA, all isolates were susceptible to gentamicin, rifampicin, and trimethoprim/sulfamethoxazole (Table 5). However, the resistance rates for gentamicin and trimethoprim/sulfamethoxazole were respectively 25.4% and 20.9% among CC5 isolates, and 40.0% and 40.0% among CC7 isolates (Table 5). The resistance rates for tetracycline, clindamycin, and erythromycin among CC5 isolates were relatively lower relative to CC7 and CC59 isolates (Table 5). As the prevalence of MRSA among CC7 isolates was lower than CC5 and CC59 isolates, the resistance rates of some antimicrobial agents among CC7 isolates were lower than other CC isolates, such as ciprofloxacin and chloramphenicol. However, only one isolate with resistance to mupirocin in this investigation belonged to CC7.

Table 5 – Specific antimicrobial resistance and virulence gene profiles of major CCs.

	CC5 ($n = 67$) (%)	CC7 ($n = 15$) (%)	CC59 ($n = 11$) (%)
Virulence genes			
sea	37.3	23.3	27.3
seb	7.5	13.3	54.5
sec	19.4	0	63.6
sed	10.4	6.7	0
seg	34.3	0	27.3
seh	11.8	0	0
sei	29.8	6.7	0
sen	44.8	6.7	27.3
sem	40.3	6.7	9.1
seo	34.3	0	0
tst	7.5	0	0
eta	9	6.7	9.1
clfA	100	100	100
cna	38.8	0	0
sdrC	89.6	93.3	54.5
sdrD	82.1	100	9.1
sdrE	82.1	26.7	100
icaA	95.5	100	100
pvl	4.5	0	27.3
hla	91.0	93.3	100
hlb	26.9	0	45.5
hld	100	100	100
hlg	10.4	13.3	27.3
edin	4.5	0	0
Antimicrobial agents			
MRSA	43.3	26.7	81.8
Tetracycline	25.4	66.7	45.5
Gentamycin	25.4	40.0	0
Penicillin	97	93.3	100
Clindamycin	59.7	73.3	81.8
Erythromycin	62.7	73.3	81.8
Ciprofloxacin	35.8	6.7	18.2
Rifampicin	7.5	0	0
Trimethoprim/ sulfamethoxazole	20.9	40	0
Chloroamphenicol	9.0	0	27.3
Mupirocin	0	6.7	0

In conclusion, the molecular characteristic of *S. aureus* SSTI isolates in the present study showed considerable heterogeneity and ST7 and ST630 were the prevalent clones. Different *S. aureus* clones causing SSTIs were associated with specific antimicrobial resistance and virulence gene profiles.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

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