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Molecular characterization of methicillin-resistant and susceptible *Staphylococcus aureus* recovered from hospital personnel

Running title: Molecular characterization of *S. aureus*

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Abstract

Introduction

Methicillin resistant *Staphylococcus aureus* (MRSA) is one of the major causes of hospital acquired infections. Over the past two decades MRSA has become 'epidemic' in many hospitals worldwide. However, little is known about the genetic background of *S. aureus* recovered from hospital personnel in China.

Aim

The aim of this study was to determine the genetic diversity of MRSA and methicillin susceptible *S. aureus* (MSSA) recovered from hospital personnel in Tianjin, North China.

Methodology

Three hundred and sixty-eight hand or nasal swabs were collected from 276 hospital personnel in four tertiary hospitals in Tianjin, North China between November 2017 and March 2019. In total, 535 gram-positive bacteria were isolated, of which 59 were identified as *S. aureus*. Staphylococcal cassette chromosome *mec* (SCC*mec*) typing, multi-locus sequence typing (MLST) and *spa* typing were performed to determine molecular characteristics of *S. aureus*.

Results

Thirty-one out of 276 (11%) hospital personnel were *S. aureus* carriers, whereas 11/276 (4%) carried MRSA. Fifty out of 59 (85%) of *S. aureus* isolates were resistant or intermediate resistant to erythromycin. The dominant genotypes of MRSA recovered from hospital personnel were ST398-t034-SCC*mec*IV/V, and ST630-t084/t2196,

whereas major genotypes of MSSA included ST15-t078/t084/t346/t796/t8862/ t8945/t11653 and ST398-t189/t034/ t078/t084/t14014.

Conclusion

Although, the predominant genotypes of MRSA recovered from hospital personnel in this study were different from those main genotypes that have previously been reported to cause infections in Tianjin and in other geographic areas of China, the MRSA ST398-t034 genotype has previously been reported to be associated with livestock globally. The dominant MSSA genotypes recovered from hospital personnel were consistent with those previously reported MSSA genotypes recovered from the clinic. The diversity of *S. aureus* genotypes warrantee further surveillance and genomic studies to better understand the relatedness of these bacteria with those recovered from patients and community.

Key words: *Staphylococcus aureus*, hospital personnel, *spa* typing, MLST

Introduction

Associated with healthcare, community and livestock methicillin resistant *S. aureus* (MRSA) is a major public health concern worldwide. 29%-35% of clinical isolates recovered in healthcare settings in the US and Europe are MRSA responsible for mild to life threatening infections [1,2]. Additionally, this bacterium has developed resistance to multiple antibiotics subsequently limiting treatment options.

S. aureus can be transmitted via person to person or person to inanimate objects contact. Due to their occupation, hospital personnel have been implicated in the transmission of MRSA to vulnerable patients and acted as a vector for such transmission between the patients and hospital environments [3]. The Chinese National Surveillance study (CNSS) carried out in 2013 found that ST239-t030/t037-SCC*mec* III and ST5-t002/t570-SCC*mec* II were predominant MRSA genotypes responsible for clinical infections in China, whereas ST7-t091/t796, ST188-t189 and ST398-t571/t034 were the major genotypes of methicillin-susceptible *S. aureus* (MSSA) causing clinical infections in China [4]. However, little is known about the genotypes circulating among hospital personnel in China, hence it has been challenging to find common interfaces between major MRSA/MSSA clones recovered from patients and hospital personnel.

In this study we report the antibiotic susceptibility, molecular characterization and genetic diversity of MRSA and MSSA recovered from hospital personnel in Tianjin, North China.

Methods

Specimen collection

A total of 368 samples were collected from four hospital (Nankai hospital, Tianjin Medical University General Hospital, Tianjin Central Hospital of Gynecology Obstetrics and Tianjin Baodi hospital) personnel (n=276) between November 2017 and March 2019.

Cotton swabs (Yangsheng Biotech, China) were used to collect samples from anterior nares of hospital personnel. The cotton swab was gently inserted into one of the nostrils up to 1 cm and was rotated 3 times to sample the inner surfaces [5]. Hand samples were collected using sterile PBS buffer – soaked cotton swabs. Swab- based samples were taken from palmar side of both hands [6]. All samples were transferred back to the lab within 1 h of sampling. The research protocol and informed consent was approved by the Ethics committee of Tianjin Science and Technology Commission (approval No TMUaMEC2017017). All research was performed in accordance with the relevant guidelines and regulations. Informed consent was obtained from all participants.

All specimens were inoculated onto mannitol salt agar (MSA, Oxoid, Basingstoke, UK), and incubated aerobically at 37℃ for 24 to 72 hours. Colonies with typical *S. aureus* characteristics were purified using nutrient agar (NA, Oxoid, Basingstoke, UK).

Identification of *S. aureus* isolates

For identification at species level, all *S. aureus* isolates were subjected to partial 16S rRNA gene sequencing using the primers and PCR conditions as described previously. [7] Amplified PCR products were sequenced by Sangon Biotech (Shanghai, China). Sequence similarity searches were carried out using BLAST tool (NCBI: https://www.ncbi.nlm.nih.gov/) [8].

Antimicrobial susceptibility testing

A panel of 9 antibiotics were selected to determine the antimicrobial susceptibility of all *S. aureus* isolates, including cefoxitin (FOX/30 µg), chloramphenicol (C/30 µg), clindamycin (DA/2 µg), erythromycin (E/15 µg), gentamicin (CN/10 µg), penicillin (PG/10 unit), rifampin (RD/5 µg), teicoplanin (TEC/30 µg), and tetracycline (T/30 µg). In addition, the minimum inhibitory concentrations (MICs) for cefoxitin were determined using E-test (Biomerieux, Basingtoke, UK) The results were interpreted as susceptible, intermediate resistant, or resistant according to the recommendations of Clinical and Laboratory Standards Institute (CLSI: 24th edition) [9].

Determination of *mecA* gene and SCC*mec* types

Genomic DNA of all *S. aureus* isolates were prepared using commercial DNA extraction kit (Solarbio Co. Ltd, China) according to the manufacturer's instructions. *mecA* gene was determined using the PCR protocol and primers as described by Kondo et al [10]. SCC*mec* types were determined for all MRSA isolates using a combination of *mec* and *ccr* gene complexes [10].

Determination of *pvl, ica* and enterotoxin genes

pvl gene was determined for all *S. aureus* as described previously [11]. Biofilm production was determined using *ica*R forward and reverse primers [12]. Seventeen enterotoxin genes (*sea, seb*, *sec, sed, see, seg, seh, sei*, *sej, sek*, *sel, sem*, *sen*, *seo, sep*, *ser*, *seu*) and the *tsst* gene were detected for 59 *S. aureus* by using a protocol as described previously [13].

Multi-locus sequence typing

Multilocus sequence typing (MLST) was used to determine the sequence types of all *S. aureus* isolates (https://pubmlst.org/saureus/info/primers.shtml). The amplicons were sequenced by Sangon biotech (Shanghai, China). The sequence types were assigned by comparing the combination of seven alleles to those in the *S. aureus* database (https://pubmlst.org /saureus/).

spa typing

The x- region of the protein A (*spa*) gene of all *S. aureus* isolates was amplified using *spa* F (5'- AGACGATCCTTCGGTGAGC-3') and *spa* R (5'-GCTTTTGCAATG TCATTTACTG-3') primers. Amplified PCR products were sequenced by Sangon Biotech (Shanghai, China). The *spa* types were then determined using the BioNumerics software *spa* typing tool (Applied Maths, Belgium) [14].

Statistical analysis

The χ^2 test was used to analyze the quantitative variables. A *P*-value < 0.05 was considered statistically significant.

Results

S. aureus isolates

Over the 22 months period between 2017-2019, 535 gram-positive isolates were recovered from a total of 368 samples taken from 276 hospital personnel in four tertiary hospitals, including 196 isolates recovered from 108 hospital personnel in hospital I (H-I); 101 isolates recovered from 43 hospital personnel in hospital II (H-II), 174 isolates recovered from 100 hospital personnel in hospital III (H-III) and 64 isolates were recovered from 25 hospital personnel in hospital IV (H-IV). Fifty-nine (11%)

147 isolates were identified as *S. aureus*, 338 isolates were determined to be coagulase
148 negative staphylococci, and 138 isolates were identified as non-staphylococcal isolates,
149 including those from *Bacillus*, *Micrococcus*, and *Enterococcus* genus. 34 *S. aureus*
150 were recovered from hospital personnel in H-II, 21 from hospital personnel in H- I, 2
151 from hospital personnel in H-III and 2 from hospital personnel in H- IV (Table 1).
152 Thirty-one out of 276 (11%) hospital personnel carried *S. aureus*, whereas eleven out
153 of 276 (4%) were MRSA carriers. MRSA carriers were from five departments of 4
154 tertiary hospitals, including Gastroenterology department (GD), Emergency unit (ER),
155 Medical Examination Center (MEC), Obstetrics and Gynecology department (OG) and
156 Hepatobiliary Surgery department (HPS). Hospital personnel who carried *S. aureus*
157 were from eight departments of 4 tertiary hospitals, including Gastroenterology
158 department (GD), Dermatology department (DT), Emergency unit (ER), Hepatobiliary
159 Surgery department (HPS), Medical Examination Center (MEC), Obstetrics and
160 Gynecology department (OG), Thoracic Surgical department (TS), and Ultrasonic room
161 (UR). No *S. aureus* was recovered from personnel in the Endocrine, Rehabilitation,
162 Chinese medicine, Urology, Oncology departments and Clinical laboratory.
163 **Antimicrobial susceptibility test**
164 Forty-five (76%) *S. aureus* were resistant to penicillin, followed by 24 (41%) *S. aureus*
165 were resistant to erythromycin, 20 (34%) to clindamycin, 14 (24%) to cefoxitin, 7 (12%)
166 to teicoplanin, 3 (5%) to tetracycline, 3 (5%) to gentamicin, 2 (3%) to chloramphenicol,
167 and 1(2%) to rifampicin. In addition, 26 (44%) *S. aureus* showed intermediate
168 resistance towards erythromycin, and only 9 (15%) isolates were fully susceptible to

169 erythromycin (Tables S1). Fifty-one (86%) isolates were resistant to at least 2
170 antibiotics. MICs to cefoxitin varied ranging from 1.5 to 8 μg/ml (Table 1).

171 **_mecA_ gene and _SCCmec_ typing results of _S. aureus_**

172 _mecA_ gene was determined for all _S. aureus_ isolates (n=59), of which 14 (24%) were
173 _mecA_ positive. SCC_mec_ types were determined in 14 _mecA_ positive _S. aureus_. Five
174 isolates harbored SCC_mec_ type IV, 3 isolates carried SCC_mec_ type V and 1 isolate
175 SCC_mec_ type II. We were not able to assign SCC_mec_ types to four of the isolates due
176 to the lack of _mec_ complex. In these isolates we were only able to identify type 2 _ccr_
177 complex. In addition, we were not able to identify either _mec_ and _ccr_ gene complexes
178 in one _S. aureus_ isolate (Table 1).

179 **Determination of _pvl, ica, tsst_ and enteroxotin genes**

180 _pvl, ica, tsst_ and enteroxotin genes were determined for 59 _S. aureus_ isolates. 7 (12%)
181 out of 59 isolates were _pvl_ gene positive, including 3 MRSA and 4 MSSA. Of 59 isolates,
182 the _ica_ gene was detected in 5 (8%) isolates, including 2 MRSA and 3 MSSA. The toxic
183 shock syndrome toxin gene was detected in 2 (3.4%) MRSA isolates. In addition,
184 determination of enterotoxin genes resulted to the following: _seg_ (n=47, 80.0%), _sen_
185 (n=33, 55.9%), _seb_ (n=19, 32.2%), _sei_(n=14, 23.7%), _seo_ (n=13, 22%), _sem_ (n=12,
186 20.3%), _ser_ (n=11, 18.6%), _see_ (n=7, 11.9%), _sea_ (n=3, 5.1%),_sed_ (n=2, 3.4%), _seu_
187 (n=2, 3.4%), _seh_ (n=1,1.7%).*sel* (n=1, 1.7%), _sek_ (n=1, 1.7%), _seq_ (n=1, 1.7%), _sec_
188 (n=0) and _sej_(n=0) (Table 2).

189 **MLST typing**

190 Multi-locus sequence types were determined for 49 _S. aureus_ isolates, including ST5

(n=5), ST6 (n=3), ST7(n=3), ST15 (n=10), ST25 (n=5), ST59 (n=2), ST88 (n=3), ST188 (n=2), ST398 (n=10), ST630 (n=5) and ST943 (n=1) (Table 1).

spa typing results

spa typing of *S. aureus* isolates revealed that the isolates possessed diverse *spa* types , including t034 (n=12), t078 (n=6), t084 (n=6), t189 (n=2), t227(n=1), t289 (n=1), t346 (n=2), t437 (n=2), t491 (n=2), t701 (n=3), t796 (n=3), t954 (n=2), t1794(n=1), t2196 (n=4), t2279 (n=1), t8862 (n=3), t8945 (n=3), t11653(n=2) and t14014 (n=2) (Table 1,S1) (Fig 1).

Discussion

S. aureus is a major nosocomial pathogen associated with mild to life-threatening infections. It has been evidenced that the carriage of *S. aureus* plays an important role in the pathogenesis of infections [1]. Hospital personnel play important role in transmission of healthcare associated pathogens [3]. In this study, we report the antibiotic susceptibility, molecular characterisation and genetic diversity of MRSA/MSSA recovered from four tertiary hospital personnel in Tianjin, China.

In this study, 31/276 (11%) hospital personnel carried *S. aureus*, and eleven (4%) were carriers of MRSA, which is consistent with the average MRSA carriage rates of healthcare workers [3].

Fourteen (24%) *S. aureus* isolates were resistant to cefoxitin, 45 (76%) isolates were resistant to penicillin, 24 (41%) isolates were resistant to erythromycin, 20 (34%) isolates were resistant to clindamycin, and only one isolate (2%) was resistant to rifampicin, which was lower than the rates reported for hospital-associated *S. aureus*

isolates by others [7, 15]. In addition, it is worth to note that 44% of *S. aureus* showed intermediate resistance to erythromycin, and only 9 (15%) isolates were fully susceptible to erythromycin. It has been reported that erythromycin was one of the most frequently prescribed antibiotics in China between 2004 to 2012[16]. However, recent reports suggest that its annual use continues to increase [16]. The misuse and over prescription of erythromycin may have contributed to the unusual high levels of erythromycin resistance and intermediate resistance in *S. aureus* that were recovered from hospital personnel in this study. Fifty-seven (97%) *S. aureus* were resistant to at least one antibiotic, and only two isolates (3%) were fully susceptible to all 9 antibiotics tested.

SCC*mec* type I, II and III were reported to be hospital associated, whereas SCC*mec* types IV and V have been associated with the community [17]. In this study, the majority (n=8) of *S. aureus* carried community associated SCC*mec* elements, and one *S. aureus* harboured type II SCC*mec*. Four *S. aureus* carried SCC due to harbouring *ccr* but lacked the *mec* gene complex. In addition, one *S. aureus* was identified to carry Pseudo (ψ) SCC due to lack of both the *mec* gene complex and *ccr* gene complex [18]. Our data demonstrate the complex diversity of SCC*mec* and SCC elements in *S. aureus* isolates recovered from hospital personnel.

The prevalence of SE genes in clinical *S. aureus* was reported to descent in the following order: *ser>sek>sem>sei>sen>seg>seu>sej>sed>seo>sec>sel>seq> seb>tsst> sea>seh>see* [13]. In contrast, in this study *seg* (80.0%), *sen* (55.9%) and *seb* (32.2%) genes were the most prevalent SE genes in *S. aureus* isolates recovered from hospital

personnel. Contrary to previous studies that detected no *see* gene in clinical *S. aureus* isolates [13], the *see* gene was detected in 7 isolates in this study (Table 2). The abundance of SEs in *S. aureus* that were recovered from hospital personnel in this study is rather worrying finding.

Tianjin is one of the 12 major cities in China that was included in previous China National surveillance studies (CNSS) of clinical *S. aureus* in 2013 [4, 19]. Here, we carried out a pilot study to provide details of *S. aureus* carriage among the hospital personnel in four tertiary hospitals in Tianjin, China. The CNSS reported that MRSA ST239-t030/t037-SCC*mec*III and ST5-t002/t570-SCC*mec*II were the predominant genotypes causing infections in China [4, 19]. Moreover, the ST239-t030 was also reported to be the dominant clinical MRSA clone in Tianjin [12]. In this study, no MRSA ST239-t030 and ST5-t002/t570 genotypes were recovered from hospital personnel. The dominant genotype of MRSA that were recovered from hospital personnel in this study included ST398-t034-SCC*mec*IV/V, and ST630-t084/t2196, which was not consistent with the major MRSA genotypes reported by the CNSS [4, 19].

In China, MRSA ST398 accounts for nearly 20% of skin and soft-tissue infections [20]. MRSA ST398-t034-SCC*mec*IV/V was one of the predominant genotypes among hospital personnel in this study, thus posing a risk both to patients and the hospital personnel. Furthermore, it has previously been reported that *pvl* positive MRSA ST398-t034 was a cause of human infections in Sweden [21] and China [20]. In this study, two out of 10 (17%) *S. aureus* ST398-t034 recovered from hospital personnel harboured the

257 *pvl* gene, including one MRSA and one MSSA ST398-t034 isolates respectively.

258 In this study, no MRSA ST239 was recovered from hospital personnel, however, we
259 identified four MRSA ST630-t2196/t084. ST630 and ST239 belong to clonal complex
260 (CC) 8 [4], however, ST630 is a variant of ST239 clone and possesses changes
261 within the *arcC* and *aroE* locuses [22]. To this end, MRSA ST630-t4549 was reported
262 to cause endocarditis and bacteremia [20,21]. Moreover, *pvl* positive MRSA ST59-t437
263 found in our study is a well-known community associated MRSA that was first reported
264 in the USA in 2005 [23], but has since emerged worldwide as a life-threatening
265 pathogen [24].

266 He et al., reported that ST7-t091/t796, ST188-t189 and ST398-t571/t034 were the main
267 genotypes of MSSA that cause bacteremia in China, followed by ST15-t084 [4]. In this
268 study, 45 (76%) MSSA isolated from hospital personnel belonged to two major
269 genotypes: ST15-t078/t084/t346/t796/t8862/t8945/t11653 (n=9), and ST398-
270 t189/t034/t078/t084/t14014 (n=6). Our findings were consistent with data reported for
271 MSSA (ST398-t034 and ST15-t084) that have previously been isolated from clinical
272 specimens [12]. Tree MSSA ST88-t034/t14014 were recovered in this study. MSSA
273 ST88 was reported to be the most common clone that causes soft-tissue and blood
274 infections [20], and thus the prevalence of MSSA ST88 among hospital personnel in
275 this study may pose a potential risk for patients to acquiring *S. aureus* infections while
276 in the hospital.

277 This study has a number of limitations: only samples recovered from the hospital
278 personal were included. No samples from patients or community were included to

examine the cross-transmission.

To our knowledge, this is the first detailed molecular characterization of MRSA and MSSA recovered from hospital personnel in Tianjin, China. Whether our findings do represent the issue in other parts of China remains to be addressed. In our study, we did find that the predominant genotype of MRSA recovered from hospital personnel in Tianjin was different from the main genotypes responsible for infections in China, whereas, the dominant genotype of MSSA isolated from hospital personnel was consistent with the main MSSA genotypes recovered from the clinic. Due to their previous association with hospital infections, the *S. aureus* clones identified in this study may well pose a health threat to patients, their family members as well as the hospital personnel. Therefore, it is necessary to adapt a regular National screening program for hospital personnel to better identify such clones and associated risks they pose.

Author statement

Authors and contributors

ZX: conceptualization, methodology, software, validation, resources, data curation, writing-original draft preparation visualization, supervision, project administration and funding. XDL, DT: formal analysis, investigation. ZYS, LQG, CXD: investigation. NJT: writing-review and editing, HVM: conceptualization, methodology, writing-review and editing. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare that there are no conflicts of interest

Funding information

This work was supported by the Tianjin Municipal Science and Technology Commission [17JCYBJC43000]

Ethical approval

The research protocol and informed consent was approved by the Ethics committee of Tianjin science and technology commission (approval No TMUaMEC2017017).

Consent for publication

No applicable

Acknowledgement

No applicable

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Table 1 Molecular characterization and antimicrobial susceptibility of methicillin-resistant *Staphylococcus aureus* (MRSA) recovered from four tertiary hospitals in Tianjin, North China

No	Hospital	Sources	Personnel ID	Sites	CC	ST	*spa* types	SCC*mec* types	*mecA*	MIC (FOX) μg/ml	Antibiotic resistance pattern
1	A	ER	525	H	CC15	15	t084	V	+	**8**	FOX-PG-E(I)
2	A	ER	515	N	CC25	25	t227	Pseudo (ψ) SCC	+	**2**	FOX-PG-CN-TEC
3	A	OG	3	H	CC59	59	t437	II	+	**2**	FOX-PG-E-TEC-DA
4	A	HPS	21	H		59	t437	IV	+	**6**	FOX-PG-E-T-DA
5	B	HPS	23	N	CC398	398	t034	IV	+	**2**	FOX-PG-E-DA
6	B	HPS	10	N		398	t034	IV	+	**8**	FOX-E-DA
7	B	HPS	23	N		398	t034	V	+	**1.5**	FOX-PG-E-DA
8	C	OG	74	H		398	t034	V	+	**3**	FOX

9	C	OG	43	H		UT	t034	IV	+	**6**	FOX-PG-E
10	D	MEC	22	N	CC8	630	t084	IV	+	**6**	FOX-E-T-DA
11	B	HPS	1	H		630	t2196	SCC	+	**8**	FOX-PG-TEC-RD-E(I)
12	B	HPS	1	H		630	t2196	SCC	+	**3**	FOX-PG-TEC-E(I)
13	B	HPS	1	H		630	t2196	SCC	+	**3**	FOX-PG-TEC-E(I)
14	A	GD	405	N	CC7	943	t289	SCC	+	**3**	FOX-PG-T-TEC-E(I)

A: hospital 1; B: hospital 2; C: hospital 3; D: hospital 4; UT: un-typable, I: intermediate resistance, H: hands, N: anterior nares

DT: Dermatology department, ER: Emergency room, GD: gastroenterology department, HPS: Hepatobiliary Surgery department, MEC: Medical examination center, OG: Obstetrics and gynecology department, TS: Thoracic surgical department, UR: Ultrasonic room.

C: chloramphenicol (30 μg), CN: gentamicin, (10 μg), DA: clindamycin (2 μg), E: erythromycin (15 μg), FOX: cefoxitin (30 μg), PG: penicillin (10 unit), RD: rifampin, (5 μg), T: tetracycline (30 μg), TEC: teicoplanin (30 μg)

Table 2 Detection of the staphylococcal enterotoxin genes in *S. aureus* isolates recovered form hospital personnel in four tertiary hospitals in Tianjin, North China

SE gene		No of positive isolates			
	Total (n=59)	MRSA (n=14)	MSSA (n=45)	χ^2	*P*
Classic SE genes	*sea*	0 (0)	3 (6.7)	0.98	>0.05
	seb	6 (42.9)	13 (28.9)	0.95	>0.05
	sec	0 (0)	0 (0)	-	-
	sed	0 (0)	2 (4.4)	0.64	>0.05
	see	1 (7.1)	6 (13.3)	0.39	>0.05
Non-classic SE genes:	*seg*	8 (57.1)	39 (86.7)	5.74	**<0.05**
	seh	1 (7.1)	0 (0)	3.27	>0.05
	sei	6 (42.9)	8 (17.8)	3.71	>0.05
	sej	0 (0)	0 (0)	-	-
	sek	1 (7.1)	0 (0)	3.26	>0.05
	sel	0 (0)	1 (2.2)	0.32	>0.05
	sem	0 (0)	12 (26.7)	6	**<0.05**
	sen	6 (42.9)	27 (60)	1.27	>0.05
	seo	1 (7.1)	12 (26.7)	2.36	>0.05
	seq	1 (7.1)	0 (0)	2.49	>0.05
	ser	1 (7.1)	10 (22.2)	1.6	>0.05

	seu	2 (14.3)	0 (0)	6.65	**<0.05**
Other toxic factors	*tsst*	2 (14.3)	0 (0)	6.65	**<0.05**
	pvl	3 (21.4)	4 (8.9)	1.6	>0.05
	ica	2 (14.3)	3 (6.7)	0.8	>0.05

Figure 1 Minimum spanning tree based on *spa* types of *S. aureus*

Each colour indicates an individual *spa* type; each circle represents one *spa* type. The pieces of section in each circle indicate the number of isolates.