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Staphylococcus aureus recovered from hospital personnel

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

3 **Running title:** Molecular characterization of *S. aureus*

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21

22 **Abstract**

23 Introduction

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

28 Aim

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

32 Methodology

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

39 Results

40 Thirty-one out of 276 (11%) hospital personnel were *S. aureus* carriers, whereas 11/276
41 (4%) carried MRSA. Fifty out of 59 (85%) of *S. aureus* isolates were resistant or
42 intermediate resistant to erythromycin. The dominant genotypes of MRSA recovered
43 from hospital personnel were ST398-t034-SCC*mec*IV/V, and ST630-t084/t2196,

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

46 Conclusion

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

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

57

58

59 **Introduction**

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

65 *S. aureus* can be transmitted via person to person or person to inanimate objects contact.

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

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

79 **Methods**

80 **Specimen collection**

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

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

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

97 **Identification of *S. aureus* isolates**

98 For identification at species level, all *S. aureus* isolates were subjected to partial 16S
99 rRNA gene sequencing using the primers and PCR conditions as described previously.
100 [7] Amplified PCR products were sequenced by Sangon Biotech (Shanghai, China).
101 Sequence similarity searches were carried out using BLAST tool (NCBI:
102 <https://www.ncbi.nlm.nih.gov/>) [8].

103 **Antimicrobial susceptibility testing**

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

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

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

118 **Determination of *pvl*, *ica* and enterotoxin genes**

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

124 **Multi-locus sequence typing**

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

130 ***spa* typing**

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

136 **Statistical analysis**

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

139 **Results**

140 ***S. aureus* isolates**

141 Over the 22 months period between 2017-2019, 535 gram-positive isolates were
142 recovered from a total of 368 samples taken from 276 hospital personnel in four tertiary
143 hospitals, including 196 isolates recovered from 108 hospital personnel in hospital I
144 (H-I); 101 isolates recovered from 43 hospital personnel in hospital II (H-II), 174
145 isolates recovered from 100 hospital personnel in hospital III (H-III) and 64 isolates
146 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 ceftiofur 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. *SCCmec* types were determined in 14 *mecA* positive *S. aureus*. Five
174 isolates harbored *SCCmec* type IV, 3 isolates carried *SCCmec* type V and 1 isolate
175 *SCCmec* type II. We were not able to assign *SCCmec* 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 enterotoxin genes**

180 *pvl*, *ica*, *tsst* and enterotoxin 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

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

193 ***spa* typing results**

194 *spa* typing of *S. aureus* isolates revealed that the isolates possessed diverse *spa* types ,
195 including t034 (n=12), t078 (n=6), t084 (n=6), t189 (n=2), t227(n=1), t289 (n=1), t346
196 (n=2), t437 (n=2), t491 (n=2), t701 (n=3), t796 (n=3), t954 (n=2), t1794(n=1), t2196
197 (n=4), t2279 (n=1), t8862 (n=3), t8945 (n=3), t11653(n=2) and t14014 (n=2) (Table
198 1,S1) (Fig 1).

199 **Discussion**

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

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

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

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

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

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

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

239 Tianjin is one of the 12 major cities in China that was included in previous China
240 National surveillance studies (CNSS) of clinical *S. aureus* in 2013 [4, 19]. Here, we
241 carried out a pilot study to provide details of *S. aureus* carriage among the hospital
242 personnel in four tertiary hospitals in Tianjin, China. The CNSS reported that MRSA
243 ST239-t030/t037-SCC*mec*III and ST5-t002/t570-SCC*mec*II were the predominant
244 genotypes causing infections in China [4, 19]. Moreover, the ST239-t030 was also
245 reported to be the dominant clinical MRSA clone in Tianjin [12]. In this study, no
246 MRSA ST239-t030 and ST5-t002/t570 genotypes were recovered from hospital
247 personnel. The dominant genotype of MRSA that were recovered from hospital
248 personnel in this study included ST398-t034-SCC*mec*IV/V, and ST630-t084/t2196,
249 which was not consistent with the major MRSA genotypes reported by the CNSS [4,
250 19].

251 In China, MRSA ST398 accounts for nearly 20% of skin and soft-tissue infections [20].
252 MRSA ST398-t034-SCC*mec*IV/V was one of the predominant genotypes among
253 hospital personnel in this study, thus posing a risk both to patients and the hospital
254 personnel. Furthermore, it has previously been reported that *pvl* positive MRSA ST398-
255 t034 was a cause of human infections in Sweden [21] and China [20]. In this study, two
256 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]. Three 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

279 examine the cross-transmission.

280 To our knowledge, this is the first detailed molecular characterization of MRSA and
281 MSSA recovered from hospital personnel in Tianjin, China. Whether our findings do
282 represent the issue in other parts of China remains to be addressed. In our study, we did
283 find that the predominant genotype of MRSA recovered from hospital personnel in
284 Tianjin was different from the main genotypes responsible for infections in China,
285 whereas, the dominant genotype of MSSA isolated from hospital personnel was
286 consistent with the main MSSA genotypes recovered from the clinic. Due to their
287 previous association with hospital infections, the *S. aureus* clones identified in this
288 study may well pose a health threat to patients, their family members as well as the
289 hospital personnel. Therefore, it is necessary to adapt a regular National screening
290 program for hospital personnel to better identify such clones and associated risks they
291 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

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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

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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	<i>spa</i> types	SCC <i>mec</i> types	<i>mecA</i>	MIC (FOX) μ g/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 from hospital personnel in four tertiary hospitals in Tianjin, North China

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

	<i>seu</i>	2 (14.3)	0 (0)	6.65	<0.05
Other toxic factors	<i>tsst</i>	2 (14.3)	0 (0)	6.65	<0.05
	<i>pvl</i>	3 (21.4)	4 (8.9)	1.6	>0.05
	<i>ica</i>	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.