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Full Length Research Paper

Molecular analysis and antibiotic resistance investigation of *Staphylococcus aureus* isolates associated with staphylococcal food poisoning and nosocomial infections

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Enterotoxins (SEs) generated by *Staphylococcus aureus* were considered to be the main reason for staphylococcal food-poisoning (SFP). The aim of this research is to investigate the distribution difference of enterotoxin genes in strains isolated from food, food poisoning, inpatients, respectively. Multiplex polymerase chain reaction (PCR) was employed to detect SEs and relationship between antibiotic resistance and living conditions of *S. aureus* has also been analyzed. Pulsed-Field Gel Electrophoresis (PFGE) was further applied for the determination of the genetic relationships between *S. aureus* isolates. It was striking to found that positive detection ratio of multi-enterotoxin genes and newly found enterotoxin genes in inpatients' isolates were higher than the other two (*P*<0.01). SEG, SEI, SEK, SEQ genes were mainly presented in isolates from inpatients while, SEA, SEE, SHE, SEQ genes mainly presented in isolates from food samples and SEA, SEP, SEC genes mainly found in strains from food poisoning. The most important fact was that percentages of isolates from inpatient resistant to oxacillin were 71.43%, which was significantly higher than the percentages (2.33% and 2.38%) of the other two original isolates. The similarity among these isolates was above 70% and PFGE was agreed to be a useful discriminating typing method for *S. aureus* isolates.

Key words: *Staphylococcus aureus*, enterotoxin genes, antibiotic resistance.

INTRODUCTION

Staphylococcus aureus is an important enterotoxin-producing causative agents accounting for food poisoning, which can also be the pathogen responsible for some other diseases, such as bacteraemia, endocarditis, pneumonia and toxic shock syndrome and so on (Hallin et al., 2007; Hu et al., 2008; Hsieh et al., 2008). It has been described previously that most *S. aureus* strains produce one or more groups of specific exoproteins, which include staphylococcal enterotoxins (SE), staphylococcal exofoliative toxins (ET), and toxic shock syndrome toxin 1 (TSST-1) (Balaban et al., 2000; Thomas et al., 2007). According to their biological activities and structural features, these toxins were classified as members of the pyrogenic toxinsuperantigen family, which associated with the pathogenic of *S. aureus* for the diseases described above (McCormick et al., 2001; Holtfreter et al., 2005; Hu et al., 2008). Based on their antigenicity, the SE which cause staphylococcal food poisoning was original classified as the classical staphylococcal enterotoxins (SEA to SEE) (Boerema et al., 2006). With the studying of SEs keep on going, a number of new types of SEs have been identified, such as SEG, SEH, SEI, SEJ, SEK, SEL, SEM, SEN, SEO, SEP, SEQ, SElR and SEU (Letertre et al., 2003; Omoe et al., 2003; Omoe et al., 2004). At the same time, it has been found that multiple super antigenic toxin genes could be possessed by one *S. aureus* strain.

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Abbreviations: Pulsed-field gel electrophoresis (PFGE); **SFP,** staphylococcal food-poisoning; **SEs,** Enterotoxins; **PCR,** polymerase chain reaction; **PFGE,** Pulsed-Field Gel Electrophoresis; **ET,** exofoliative toxins; **MRSA,** methicillin-resistant *Staphylococcus aureus*.

Table 1. Enterotoxin genotypes of *S. aureus* reference strains.

Bacteria	Strains	Enterotoxin genotype	Source
S. aureus	PHLS/ST0005	SEA+	Control laboratory of Bellinzona-Switzerlang, Switzerland
S. aureus	PHLS/ST0016	SEB+	
S. aureus	PHLS/ST0015	SEC+	
S. aureus	92-6211B	SED+,SEJ+	
S. aureus	ATCC27664	SEE+	
S. aureus	PHLS/ST0004	SEG+,SEI+	
S. aureus	95-2806	SEH+	
S. aureus	MW2	SEK+, SEQ+	Laboratories special for *S. aureus* study
S. aureus	N315	SEL+, SEP+	
S. aureus	MU50	SEI+, SEO+	
S. aureus	FRI-1169	SEO+, SER+	

(Omoe et al., 2002; Becker et al., 2004; Løvseth et al., 2004; Chen et al., 2004; Chiang et al., 2006; Hwang et al., 2007). What's more, different isolates may possess not only different SEs, but also different numbers of SEs, which may be related to the isolates' living conditions. As a result, gene-typing in *S. aureus* could be done according to the SEs they got. In our opinion, the different of virulence between strains possessed different SEs was also need to be analysis. It has also been known that some enterotoxin genes are associated with mobile genetic elements such as pathogenicity islands, which may transferred horizontally between staphylococcal strains. Consequently, it was possibility that enterotoxin genes have played an important role in the evolution of *S. aureus* as a pathogen (Baba et al., 2002; Yarwood et al., 2002). So gene-typing based on SEs could also be useful for evolutionary analysis of the pathogenicity of *S. aureus*, as well as for diagnostic and epidemiological purposes. On the other hand, *S. aureus* evolved resistance to antimicrobial drugs, such as penicillin, methicillin, oxacillin and so on, which made the treatment of *S. aureus* infections difficult. The situation was so serious for resistance spread quickly with the use of antibiotic increased. Recently, new study showed that hospital-acquired infections with *S. aureus*, especially methicillin-resistant *Staphylococcus aureus* (MRSA) infections, are a major cause of illness and death and impose serious economic costs on patients and hospitals. The evolution and spread of MRSA was suggested to be from hospital to other hospitals and then into the community (Klein et al., 2007). So much attention needed to be paid to the control of antibiotic-resistant *S. aureus*.

During this research, an established multiple polymerase chain reaction (PCR) have been applied for investigation of the distribution of enterotoxin genes in different *S. aureus* isolates. Totally 269 *S. aureus* strains which separated from food sample, food poisoning, and inpatients respectively, have been employed. And the differences of anti-biotic resistance among different original isolates have also been analyzed. In addition, pulsed-field gel electrophoresis has been applied for studying the homology among isolates from different samples.

MATERIALS AND METHODS

Bacterial strains and culture media

In this study, a total of Two hundred and eighty *S. aureus* samples were used. As shown in (Table 1), among these strains, eleven *S. aureus* reference strains were obtained from control laboratory of Bellinzona-Switzerlang, Switzerland and laboratories special for *S. aureus* study, China respectively. Eighty-seven *S. aureus* isolates were obtained from suspected food samples such as fresh milk and meat. Eighty-four isolates were collected from food poisoning outbreaks diagnosed by local government laboratories in Zhejiang, China; *S. aureus* isolates were isolated from patient feces, patient vomit, or the foods involved, and collected from the laboratories. Isolates from clinical samples were provided by other special laboratories for *S. aureus* study. Baird Parker selective media which added with Egg Yolk Tellurite solution was employed for isolation. After that, all strains were checked for purity and identified as *S. aureus* by their ability to coagulate citrated rabbit plasma (tube coagulase test). Then the isolates were further confirmed by biochemical reactions through GEN-PROBE.

DNA extraction and purification

Bacterial colonies were harvested from plates with a sterile loop and suspended in 1 ml sterile TE buffer (Tris–HCl 10 mmol, EDTA 1 mmol; pH 8.0). After that, genomic DNA extraction Kit (Qiagen GmbH, Hilden, Germany) was used for genomic DNA isolation. According to the manufacturer's protocol, optical density of each suspension was checked and adjusted by adding sterile TE buffer. Total DNA of *S. aureus* was then purified with the DNA purification kit (Qiagen) according to the manufacturer's instructions. The concentration of DNA solution was determined by referring to A260 values. Eluted DNA was then stored at −20°C.

Primers designation

The nucleotide sequences of all PCR primers used in this study are listed in Table 2. It also shows the size of amplified products respectively. The primer sets used to detect SEJ, SEK, SEL, SEM,

Table 2. Nucleotide sequences of primers used in this study.

Gene	Primer	Oligonucleotides sequence(5'-3')	PCR product(bp)	PCR set	Reference
SEA	SEA-3	CCTTTGGAAACGGTTAAAACG	127	1	[9]
	SEA-4	TCTGAACCTTCCCATCAAAAAC			
SEB	SEB-1	TCGCATCAAACTGACAAACG	477	3	[9]
	SEB-4	GCAGGTACTCTATAAGTGCCTGC			
SEC	SEC-3	CTCAAGAACTAGACATAAAAGCTAGG	271	3	[9]
	SEC-4	TCAAAATCGGATTAACATTATCC			
SED	SED-3	CTAGTTTGGTAATATCTCCTTTAAACG	319	3	[9]
	SED-4	TTAATGCTATATCTTATAGGGTAAACATC			
SEE	SEE-3	CAGTACCTATAGATAAAGTTAAAACAAGC	178	3	[9]
	SEE-2	TAACTTACCGTGGACCCTTC			
SEG	SEG-1	AAGTAGACATTTTTGGCGTTCC	287	4	[10]
	SEG-2	AGAACCATCAAACTCGTATAGC			
SEH	SEH-1	GTCTATATGGAGGTACAACACT	213	4	[10]
	SEH-2	GACCTTTACTTATTTCGCTGTC			
SEI	SEI-1	GGTGATATTGGTGTAGGTAAC	454	4	[10]
	SEI-2	ATCCATATTCTTTGCCTTTACCAG			
SEJ	SEJ-1	ATAGCATCAGAACTGTTGTTCCG	152	4	[8]
	SEJ-2	CTTTCTGAATTTTACCACCAAAGG			
SEK	SEK-1	TAGGTGTCTCTAATAATGCCA	293	5	[8]
	SEK-2	TAGATATTCGTTAGTAGCTG			
SEL	SEL-1	TAACGGCGATGTAGGTCCAGG	383	6	[8]
	SEL-2	CATCTATTTCTTGTGCGGTAAC			
SEM	SEM-1	GGATAATTCGACAGTAACAG	379	5	[8]
	SEM-2	TCCTGCATTAAATCCAGAAC			
SEN	SEN-1	TATGTTAATGCTGAAGTAGAC	282	6	[8]
	SEN-2	ATTTCCAAAATACAGTCCATA			
SEO	SEO-1	TGTGTAAGAAGTCAAGTGTAG	214	5	[8]
	SEO-2	TCTTTAGAAATCGCTGATGA			
SEP	SEP-3	TGATTTATTAGTAGACCTTGG	396	4	[8]
	SEP-4	ATAACCAACCGAATCACCAG			
SEQ	SEQ-1	AATCTCTGGGTCAATGGTAAGC	122	2	[8]
	SEQ-2	TTGTATTCGTTTTGTAGGTATTTTCG			
SER	SER-1	GGATAAAGCGGTAATAGCAG	166	6	[8]
	SER-4	GTATTCCAAACACATCTAAC			
Tst-1	Tst-3	AAGCCCTTTGTTGCTTGCG	447	5	[9]
	Tst-6	ATCGAACTTTGGCCCATACTTT			
femA	femA1	AAAAAAGCACATAACAAGCG	134	4, 5	[11]
	femA2	GATAAAGAAGAAACCAGCAG			
femB	femB1	TTACAGAGTTAACTGTTACC	651	3, 6	[12]
	FemB2	ATACAAATCCAGCACGCTCT			

SEN, SEO, SEP, SEQ and SER were designed according to published nucleotide sequences (Omoe et al., 2005). The primer sets used to detect TST-1 and SEA to SEE and the primer sets employed to detect SEG, SEH and SEI were designed by Omoe et al. (2002), respectively. These primer sets were designed to anneal to unique regions and generate amplicons that would allow identification of each se gene based on the molecular weight of its PCR product (Table 2). Primers that are specific to *S. aureus* to amplify femA and femB genes were used as an internal positive control (Ma et al., 2002). Combination of uniplex and multiplex PCR system was constructed. 10×primer master mixes (containing 2 μM each primer) of six sets (Set 1; SEA: Set 2; SEQ: Set3; SEB, SEC, SED, SEE, femB: femA: Set4; SEG, SEH, SEI, SEJ, SEP, femA: Set 5; SEK, SEM,SEO, TST-1, femA: Set6; SELL, SELN, SELR, femB) were prepared. All the primers were synthesized by bio-tec company (Shanghai, China).

Establishment of uniplex PCR and multiplex PCR

Each primer pair listed in table2 was used in uniplex PCR to evaluate the specificity of the primer and optimize the amplification

conditions. According to the instructions of PCR kit (Qiagen), the reaction carried out in 50 µl volumes containing 0.4 µM of each primer, 2 mM MgCl2, 200 µM each of dNTP, (Takara), 0.5U of TaKaRa EX Taq DNA polymerase (Takara), and 5 µl of 10×buffer (Takara). PCR amplification was performed simultaneously for 35 cycles: 30 s of denaturation at 94°C, 30 s of annealing at 55°C, and 60 s of extension at 72°C ending with a final extension at 72°C for 10 min. QIAGEN Multiplex PCR Kit was applied for multiplex PCR establishment. According to the protocol, each reaction mixture consisted of 25 µl of 2×QIAGEN Multiplex PCR Master Mix, 0.2 µM of each primer, and 10-100 ng of template DNA. After denaturation of DNA at 95°C for 15 min, 35 cycles of amplification (95°C for 30 s, 57°C for 90 s, and 72°C for 90 s) and a final extension at 72°C for 10 min was followed. Products were visualized on an agarose gel by using standard techniques.

Distribution of enterotoxin genes in different isolates

Genome DNA extracted from two hundred and sixty-nine *S. aureus* strains which separated from different samples were applied for multiplex PCR analysis. The distribution of enterotoxin genes in different strains was investigated by this method.

Antibiotic susceptibility

Vitek System (bioMe´rieux, Vitek, Inc., Hazelwood, Mo.) was employed for determination of MICs of selected antimicrobial agents. Eleven antibiotics were tested: cephazolin, erythromycin, gentamicin, levofloxacinnitrofurantoin, oxacillin, penicillin G, rifampicin, tetracycline, bactrim, vancomycin. MIC data were interpreted based on the instructions given by NCCLS.

Pulsed-field gel electrophoresis (PFGE)

According to the GenePath Reagent kit (Bio-Rad) recommendation, Genomic DNA was treated with lysozyme and proteinase K and then prepared in agarose plugs. After that,the DNA was digested with the 30 U restriction endonuclease SmaI. The DNA segments generated were separated in a 1% SeaKem Gold gel and were run in Tris-borate-EDTA buffer on the pulsed-field apparatus (Gene Path System; Bio-Rad) at 6.0 V/cm for 21 h, with pulse times ranging from 5 to 40 s. Gel images were analyzed by BioNumerics (Applied Maths, Kortrijk, Belgium), and the percentage similarity of profiles was calculated by the Dice coefficient. The unweighted-pair group method with arithmetic averages was used for clustering.

RESULTS

Distribution of enterotoxin genes in *S. aureus* isolates

In this study, 269 *S. aureus* strains were applied for enterotoxin gene distribution analysis. As positive control, femA and femB genes could be amplified from all of the tested isolates. Among the 87 isolates that separated from food samples, 86.2% isolates were diagnosed as positive for SE genes and multi-enterotoxin genes could be detected in 57.5% strains. On the other hand, the positive detection ratio of classic and newly found enterotoxin genes was 62.1% and 63.2% respectively. Of the 84 isolates that originated in food poisoning, SE genes were diagnosed in 73.8% isolates, multi-enterotoxin genes could be detected in 50.0% strains and the positive detection ratio of classic and newly found enterotoxin genes was 50.0% and 57.1% respectively. 98 isolates from inpatients possessed 84.7% and 75.5%positive detective ratio for enterotoxin genes and multi-enterotoxin genes. Classic and newly found enterotoxin genes could be diagnosed from 50.0% and 57.1% strains respectively. Another mathematic analysis result showed that the types of enterotoxin genes presented in isolates from food sample, food poisoning and patients were 17, 13 and 18. In conclusion, the positive detection ratio of multi-enterotoxin genes and newly found enterotoxin genes in inpatients' isolates were higher than the other two (P<0.01) It was also could be found form (Figure 1). That SEG, SEI, SEK, SEQ genes mainly presented in isolates from inpatients. Otherwise, SEA, SEE, SHE, SEQ genes mainly presented in isolates from food samples and SEA, SEP, SEC genes mainly found in strains from food poisoning.

Antibiotic susceptibility

During this study, the susceptibilities of these *S. aureus* strains to 11 antibiotics were determined and shown in (Table 3). It was found that all the isolates from inpatients had antibiotic resistance. However, the percentage of *S. aureus* strains separated from food and food poisoning which had antibiotic resistance was 59.77 and 84.52%. As a result, it suggested that the susceptibility of these strains to different antibiotic was associated with its origin. After making comparison of multi-antibiotic resistance ratio of different isolates, we found that the percentage of strains from inpatients was 90.82% which was remarkably higher than isolates from food and food poisoning. For the ratios of *S. aureus* strains separated from food and food poisoning which had multi-antibiotic resistance were 31.03 and 30.95%. The resistance of different original *S. aureus* strains to different antibiotic was also found to be related with its origin. *S. aureus* isolates from inpatients showed resistance to all eleven antibiotics. While, the strains isolated from food samples only showed resistance to rifampicin, vancomycin, levofloxacin and nitrofurantoin. Otherwise, the isolates separated from food poisoning samples only showed resistance to rifampicin, vancomycin and nitrofurantoin. *S. aureus* strains showed different resistance to different antibiotic. Although isolates from inpatients showed resistance to all antibiotics used in this study, the percentage of strains resistant to rifampicin, vancomycin, nitrofurantoin and bactrim was 2.04, 10.20, 13.27 and 1.02% respectively, however, ratios of the isolates resistant to other seven antibiotics was significantly higher, which were all above 70%. On the other hand, *S. aureus* strains separated from food and food poisoning only showed high resistance to Penicillin G. The ratios of which were 77.93 and 76.19%. The emergence of MRSA strains has become a

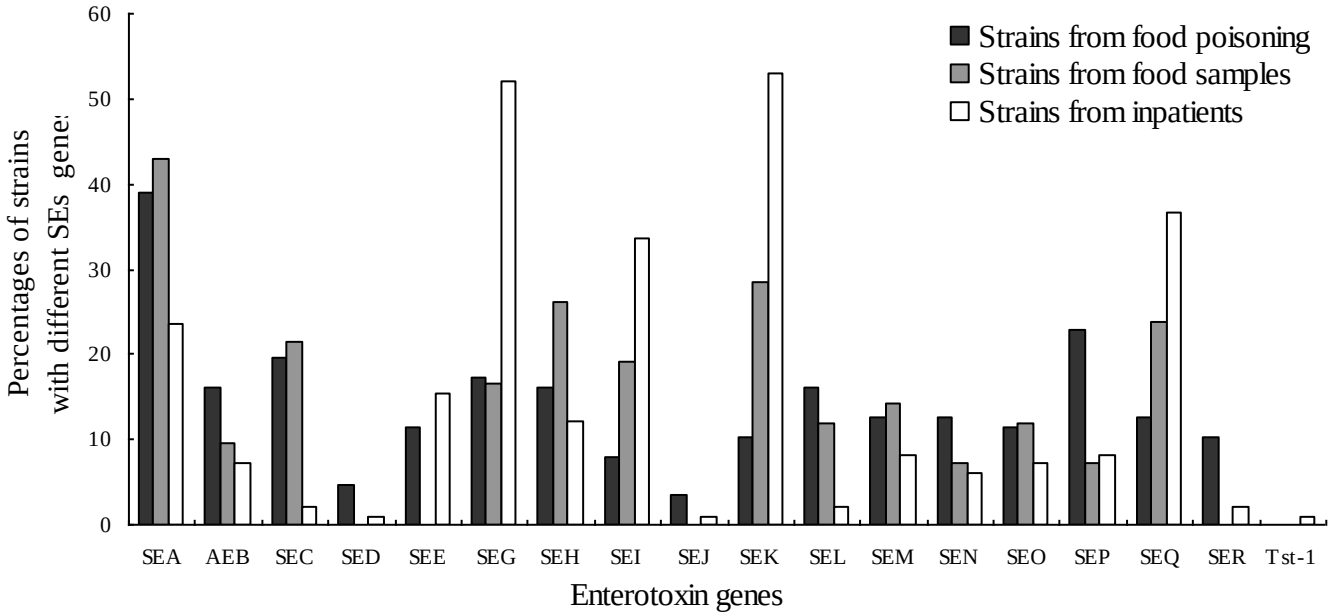

Figure 1. Percentages of different original strains with different SEs genes.

Table 3. Susceptibility of different original isolates to different antibiotics.

Isolates' origin Antibiotic	Food (%)	Food poisoning (%)	Patients (%)
Cephazolin	2.33	3.57	70.41
Erythromycin	37.21	41.67	78.57
Gentamicin	8.14	10.71	66.33
Levofloxacin	0	3.57	58.16
Nitrofurantoin	0	0	2.04
Oxacillin	2.33	2.38	71.43
Penicillin G	77.93	76.19	97.96
Rifampicin	0	0	10.20
Tetracycline	19.77	21.90	72.45
Bactrim	11.63	4.76	13.27
Vancomycin	0	0	1.02

major concern, which caused the treatment of *S. aureus* infection more difficult. The data showed that percentage of isolates from inpatients that resistant to oxacillin was 71.43%, which was also significantly higher than the percentages (2.33 and 2.38%) of the other two original isolates.

Molecular typing of *S. aureus* isolates by PFGE

During this research, 24 isolates from food samples, 23 isolates from food poisoning and 15 isolates from inpatients were all applied for typing by PFGE. Chromosomal DNA of *S. aureus* isolates were digested by Smal I, and produced 15-20 fragments. It was appeared to be 58 patterns and the overall similarity was between 60-100% (Figure 2). According to the PFGE result, patterns' similarity between strains which had same origin was comparable high. The patterns of isolates from inpatients grouped into three clusters, the similarity among which was above 70%. What's more, 73.3% isolates' patterns were in the same cluster and the similarity among them was above 75%. Other wise, 91.7% isolates from food samples were grouped into the same cluster and the similarity was above 70%. The other two strains' patterns were grouped into another cluster and the similarity was above 91%. The patterns of isolates from food poisoning could be grouped into four clusters; the similarity among them was above 70%. Only 13.0% food poisoning original isolates' patterns show similarity above 70% with patterns of strains from inpatients. On the other hand, the similarity between patterns of strains from food samples

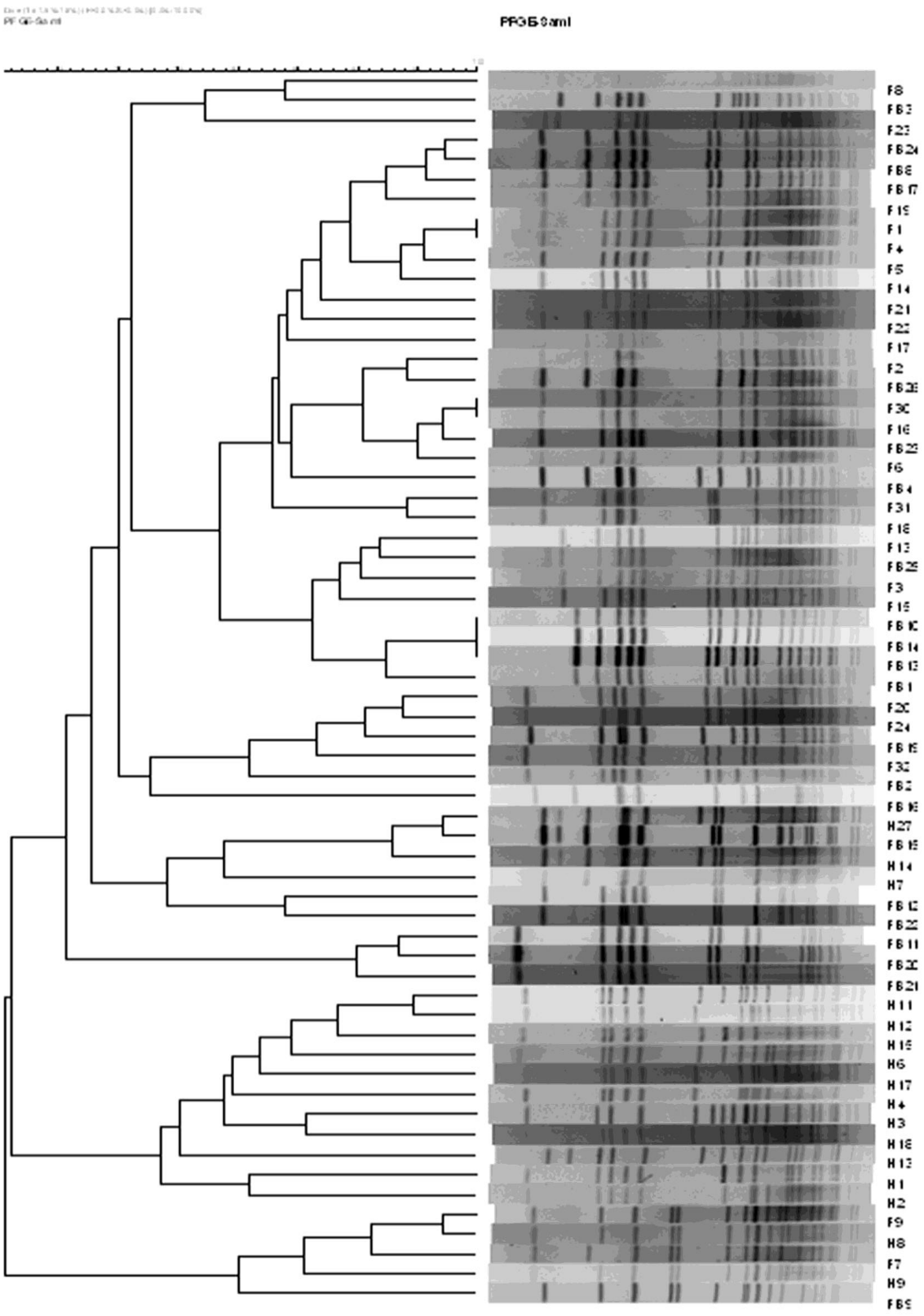

Figure 2. Dendrogram of PFGE macro restriction patterns from sixty two different original isolates. The scale indicates percent similarity. F: Strains isolated from food samples; FB: Strains isolated from food poisoning; H: Strains isolated from inpatients.

and food poisoning was significantly higher. 73.9% food poisoning original isolates' patterns showed similarity above 70% with isolates' from food samples. This study confirms that PFGE is a useful discriminating typing method for *S. aureus* isolates.

DISCUSSION

As far as we know, this research is the first comprehensive investigation of the relationship between *S. aureus* enterotoxins genotypes and the isolates' living conditions in China. Multiplex PCR which have been developed previously was applied for this study. The distribution of classical and newly described enterotoxins genes which was totally 18 types has been analyzed. The result was so interesting that there were so many enterotoxins genotypes presented in 269 isolates from three different origins. The ratios of SE genes and multi-enterotoxin genes detection in isolates from inpatients was 84.7 and 75.5% which was much higher than isolates from food samples and food poisoning. However, the ratios of classic and newly found enterotoxin genes detection in isolates from inpatients was 50.0 and 57.1% which was nearly the same or even less than that from food and food poisoning. It has been known that almost all super antigenic toxin genes are associated with mobile genetic elements such as genomic islands (Becker et al., 2004; Nashev et al., 2007). So it was strongly suggested that it should be easier for enterotoxins genes transfer in hospital conditions. And the evolution of new types of SEs may be quicker and frequenter in isolates from conditions outside. What' more, results further showed that SEG, SEI, SEK, SEQ genes mainly presented in isolates from inpatients. Otherwise, SEA, SEE, SHE, SEQ genes mainly presented in isolates from food samples and SEA, SEP, SEC genes mainly found in strains from food poisoning. It was also indicated that the distribution of enterotoxins genes were associated with the strains origin. The virulence' differences among strains possess different enterotoxins genes should be studied in further exploration. Consequently, this PCR-based super-antigenic toxin gene detection system could be performed easily in commonly equipped clinical laboratories and it was useful in SEs identification and genotyping, which may give some clue on the origin of strains responsible for *S. aureus* infections. *S. aureus* can adapt rapidly to the selective pressure of antibiotics. Antibiotic resistance and multi- antibiotic resistance has been developed in *S. aureus*, which made the treatment of *S. aureus* infections more difficult. Especially, the emergence and spread of methicillin-resistant S. aureus (MRSA) has already been the researching focus (Ma et al., 2002; Deurenberg et al., 2007). In this study, we have also investigated the difference of antibiotic resistance in different original isolates. In our opinion, this research can provide useful data for *S. aureus* infections control and prevention. Totally 11 antibiotics have been employed. And results showed that the antibiotic resistance difference among these 269 strains was significantly. Isolates from inpatients showed resistance to all the antibiotics used in this study, however, the other two was not. The percentage of strains from inpatients which possessed multi antibiotic resistance was 90.82%, which was also much higher than isolates from food (31.03%) and food poisoning (30.95%). As a result, it suggested that the susceptibility of these strains to different antibiotic was associated with its origin. It was so interesting to find that strains isolated from food samples only showed resistance to rifampicin, vancomycin, levofloxacin and nitrofurantoin. Otherwise, the isolates separated from food poisoning samples only showed resistance to rifampicin, vancomycin and nitrofurantoin, which gave some suggestions on the treatment of *S. aureus* associated food poisoning here. At the same time, for strains from patients show resistance to all antibiotics, and percentage of isolates from inpatients that resistant to oxacillin was 71.43%, which was also significantly higher than the percentages (2.33 and 2.38%) of the other two original isolates, this strongly suggested that the antibiotics have been abused in hospitals here. However, the finding showed that the percentages of these strains resistant to rifampicin, vancomycin, nitrofurantoin and bactrim was 2.04, 10.20, 13.27 and 1.02% respectively, which may also give some advices on the treatment of nosocomial infection response for *S. aureus*.

Totally 62 isolates from three origin were employed for PFGE pattern analysis, which has been reported to be the most discriminatory method for the genomic typing of *S. aureu* spp. The restriction enzyme Smal I was chosen for digestion of DNA as described before (Cha et al., 2006). According to the result, chromosomal DNA of *S. aureus* isolates produced 15-20 fragments were after digestion and 58 patterns were appeared, similarity among which was between 60-100%. It further confirmed that PFGE was useful in genomic typing of *S. aureu* spp. The patterns of isolates from inpatients grouped into three clusters, the similarity among which was above 70 and 73.3% isolates' patterns were in the same cluster, the similarity among which was above 75%. The other two original isolates showed nearly the same result, which suggested that homology among strains separated from the same sample was high. Another interesting finding was that homology of PFGE patterns between isolates from inpatients were far from the other two original samples, however, homology of PFGE patterns between isolates from food and food poisoning was near. As we thought the living conditions of isolates from food and food poisoning was similar, which accorded with the PFGE result properly.

In conclusion, distribution of enterotoxins genes in different isolates was associated with its original environment. It will be useful for identification of the origin of strains accounting for food poisoning. The difference of antibiotics resistance among them has also been analyzed.

Multi-antibiotics resistance ratios were high in isolates

from inpatients. In order to control *S. aureu* infections effectively, more attention needs to be paid for antibiotic abusing in hospital here. PFGE was confirmed to be another highly valuable technique for tracing *S. aureus* strains associated with SFP.

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