Investigation of *Staphylococcus aureus* positive for Staphylococcal enterotoxin S and T genes

Yusuke SATO'O1–3,6), Katsuhiko OMOE2,3), Yasuko AIKAWA2), Mayuko KANO2), Hisaya K. ONO2–5), Dong-Liang HU5), Akio NAKANE4) and Motoyuki SUGAI1,7)*

1)Department of Bacteriology, Hiroshima University Graduate School of Biomedical & Health Sciences, Hiroshima-shi, Hiroshima 734-8551, Japan
2)Department of Veterinary Medicine, Faculty of Agriculture, Iwate University, Ueda 3-18-8, Morioka, Iwate 020-8550, Japan
3)The United Graduate School of Veterinary Sciences, Gifu University, Gifu, Gifu 501-1193, Japan
4)Department of Microbiology and Immunology, Hiroshima University School of Medicine, 5 Zaifu-cho, Hiroshima, Aomori 036-8560, Japan
5)Laboratory of Zoonoses, Kitasato University School of Veterinary Medicine, Towada, Aomori 034-8628, Japan
6)Present address: Division of bacteriology, Department of infection and immunity, School of Medicine, Jichi Medical University, Shimotsuke, Tochigi 323-0498, Japan
7)Present address: Antimicrobial Resistance Research Center, National Institute of Infectious Diseases (NIID), Murayama, Tokyo 189-0002, Japan

**ABSTRACT.** *Staphylococcus aureus* produces staphylococcal enterotoxins (SEs) and causes food poisoning. It is known that almost all SE-encoding genes are present on various types of mobile genetic elements and can mobilize among *S. aureus* populations. Further, plasmids comprise one of SE gene carriers. Previously, we reported novel SEs, SES and SET, harbored by the plasmid pF5 from Fukuoka5. In the present study, we analyzed the distribution of these SEs in various *S. aureus* isolates in Japan. We used 526 *S. aureus* strains and found 311 strains positive for at least one SE/SE-like toxin gene, but only two strains (Fukuoka5 and Hiroshima3) were positive for ses and set among the specimens. We analyzed two plasmids (pF5 and pH3) from these strains and found that they were different. Whereas these plasmids partially shared similar sequences involved in the ser/self/set/ser gene cluster, other sequences were different. A comparison of these plasmids with those deposited in the NCBI database revealed that only one plasmid had the ser/self/set/ser cluster with a stop mutation in set similar to that in pH3. In addition, the chromosomes of Fukuoka5 and Hiroshima3, positive for ses and set, were classified into different genotypes. Despite the low rate of gene positivity for these SEs, it is suggested that there is diversity in plasmids and strains carrying these two SEs. Consequently, regarding the entire feature of SE prevalence, we improved the multiplex PCR detection method for the SE superfamily to obtain further insight.

**KEY WORDS:** plasmid, *Staphylococcus aureus*, staphylococcal enterotoxin, staphylococcal food poisoning

Staphylococcal food poisoning (SFP) is a food-borne disease caused by *Staphylococcus aureus*. It is a toxin-mediated disease, not an infectious disease. Heat- and proteolysis-stable protein toxins, namely staphylococcal enterotoxins (SEs), are its causative agents [1, 3]. Since the 1990s, many new toxins have been reported, and until now, more than 20 SEs and SE-like toxins (SEls) have been reported. The identification of new SEs continues and SFP outbreaks are being reported worldwide; moreover, SFP continues to be an important issue in public health and food safety. Thus, an understanding of all features of enterotoxigenic *S. aureus* and SEs is important for the prevention and control of SFP.

It has been reported that almost all SE and SEI genes reside on various mobile genetic elements, such as prophages, *S. aureus* pathogenicity islands, genomic islands, and plasmids [12]. Among these SE/SEI genes, sed, self, ser, ses, and set are known to reside on plasmids. The history of the identification of these toxins is as follows; Bayles et al. described that sed genes are harbored by pIB485 [2], and Zhang et al. described that self was harbored adjacent to sed on pIB485 [30]. On pF5 from food...
poisoning isolates, Omoe et al. reported a novel ser and Ono et al. described ses and set [16, 18].

The horizontal transfer of S. aureus plasmids contributes to the evolution of S. aureus [5, 6, 8, 11, 12, 14, 19, 28, 29]. Concerning SEs and SFPs, the transfer of plasmids harboring SE genes results in a recipient producing SEs, ultimately causing food poisoning outbreaks. This can threaten food safety and public health. Therefore, it is important to survey enterotoxigenic plasmids and S. aureus. However, to date, there is little information about these, and especially SES (staphylococcal enterotoxin S) and SET (staphylococcal enterotoxin T). Although we previously reported the vomit-inducing activity, superantigenic activity, and mRNA expression of these SEs, a genetic characterization of these enterotoxigenic plasmids has not been conducted. In the present study, to unveil the features of these newly identified SEs, we carried out an epidemiological analysis, comparison of plasmids, and molecular typing of strains carrying these plasmids.

**MATERIALS AND METHODS**

**Bacterial strains, growth conditions, and SE/SE1 genotyping**

In total, 526 S. aureus strains were used in this study for SES and SET gene detection. Forty-two strains were isolated from staphylococcal food poisoning outbreaks and 329 nasal swab isolates, as described in our previous study [22]. Seven methicillin-resistant Staphylococcus aureus (MRSA) isolates, 134 bovine mastitis isolates, and 14 mouse skin isolates were used in this study for the first time. All strains were collected in Japan. S. aureus was cultured overnight at 37°C in Soybean-casein-digest broth (Nissui, Tokyo, Japan) under shaking conditions and subjected to SES and SET detection. Lyse-n-Go (Thermo-Fisher Scientific, Waltham, MA, USA) was used for DNA extraction. New primers for the detection of ses, SES1 (5′-TCGGGATATATGCGGGCAGTTGAA-3′) and SES2 (5′-GGTCTAACTCTTGATTTGAATGGTTCT-3′), and primers to detect set, SET1 (5′-GGTCTGATTATATGCGGGCAGTTGAA-3′) and SET2 (5′-GGTCTGATTATATGCGGGCAGTTGAA-3′), were used. PCR conditions were described in our previous report [17].

**Purification and shotgun sequence of S. aureus plasmids harboring ses and set**

Purification of plasmids from S. aureus strains positive for ses and set was carried out as described previously [16, 18]. The TOPO shotgun cloning kit (Invitrogen, Carlsbad, CA, USA), pCR4®Blunt-TOPO® (Invitrogen) and One shot® TOPO10 electrocomp™ E. coli (Invitrogen) were used for preparation of the shotgun library. White colonies were cultured in LB broth (Sigma Aldrich, St. Louis, MO, USA) containing 100 µg/ml ampicillin (Wako Pure Chemical Industries, Osaka, Japan) and cultured at 37°C under shaking conditions overnight. The cultured cells were subjected to plasmid extraction using the QIAprep® miniprep (Qiagen, Hilden, Germany) system. Nucleotide sequences of the shotgun library were obtained using an automatic DNA sequencer ABI3100Avant (Applied Biosystems, Foster City, CA, USA) and assembled using AGCT software, ver. 4.0 (Genetyx, Tokyo, Japan). Gaps of contigs were closed by primer walking with primers designed at contig ends.

**Sequence analysis**

Open reading frames (ORFs) were identified using ORF Finder (http://www.ncbi.nlm.nih.gov/orf32/orf.html) and in silico Molecular Cloning software (In Silico Biology, Inc., Yokohama, Japan), and ORFs were annotated with the Basic Local Alignment Search Tool (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi) and through a search of the DNA Data Bank of Japan (DDJB; http://blast.ddbj.nig.ac.jp/top-j.html). A comparison of plasmid sequences was performed using the Genome Matcher software (Version 3.012. [15]). Plasmid SAP047A was used for genomic comparison (No. GQ900405). RASTA was used for toxin-antitoxin detection [23].

**Genotyping methods**

agr typing of ses- and set-positive strains was carried out using previously reported primers [10]. The genotyping data of SE/SE1, coagulase, MLST, and genomic elements were already collected in our previous study [22].

**Modification of multiplex PCR for SE/SE1s**

To improve our previous multiplex PCR [17], the following modifications were used. To detect ses and set, SES1 and SES2 were added to previously reported multiplex PCR set 1, and SET1 and SET2 were added to multiplex PCR set 3. Further, to avoid non-specific bands, new primers detecting femB, femB3 (5′-CACATGGTACAGCATC-3′) and femB4 (5′-TGGTTGCATTGATCTT-3′), were used. A QIAGEN Multiplex PCR kit (Qiagen) was used to set up multiplex PCR. PCR was carried out in a 50 µl reaction volume, and all primer sets contained 0.4 µM of each primer as the final concentration. The PCR conditions were as follows. After 95°C for 15 min, 35 thermal cycles (94°C 30 sec, 57°C 90 sec, 72°C 90 sec) were carried out. After the cycles, a 72°C, 10 min final extension was carried out. Then, agarose gel electrophoresis was performed with 0.5× TBE buffer and a 3% agarose gel. To validate the modified PCR, we used 10 S. aureus genomic DNA samples (196E, S6, FRI-361, FRI-326, FRI-569, N315, Mu50, MW2, Fukuoka5, and RN4220). All strains except for Fukuoka5 were used as controls with our previous PCR method [17]. Genomic DNA was extracted as described previously [21]. After setup with the QIAGEN Multiplex PCR kit, KOD Multi & Epi (Toyobo, Osaka, Japan) was also used for multiplex PCR to confirm that primer sets worked well with another enzyme. This PCR was carried out in a 10 µl reaction volume with 0.2 U enzyme, and all primer sets contained 0.3 µM of each primer as the final concentration. The PCR conditions were as follows. After 94°C for 2 min, 25 thermal cycles (94°C 10 sec, 57°C 30 sec, 68°C 30 sec) were carried out.
RESULTS

Prevalence of ses and set in a variety of S. aureus populations

By using PCR with the novel primers specific for ses and set, we analyzed their prevalence in a variety of S. aureus isolates. More than half of these were enterotoxigenic. Of the 526 strains analyzed, 311 (59.1%) were positive for at least one SE/SEl gene tested, and all food poisoning isolates, all MRSA isolates, 226 nasal swab isolates, and 36 bovine mastitis isolates were positive for one or more SE/SEl gene. In contrast, 103 nasal swab isolates, 98 bovine mastitis isolates, and all 14 mouse skin isolates were negative for all SE/SEl genes.

Focusing on plasmid-associated enterotoxins, sed, selj, and ser were not rare in S. aureus (sed: 25 strains; selj: 15 strains; ser: 15 strains), whereas ses and set were rare (Table 1). Only two isolates tested positive for ses and set. These strains that were positive for both ses and set were isolated from different food poisoning outbreaks in Hiroshima prefecture (Hiroshima3) and Fukuoka prefecture (Fukuoka5) in Japan. The SE genotype of Fukuoka5 has already been reported in our previous paper [18], whereas that of Hiroshima3 has not yet been reported. Hiroshima3 was also positive for other plasmid-associated SE/SEl genes, selj and ser, as well as ses and set. Concerning the enterotoxin profile, 12 strains carried only sed, 13 strains carried sed, selj, and ser, and two strains mentioned previously herein carried selj, ser, ses, and set as plasmid-associated toxin genes. Some strains harbored SEs/SEls as chromosomally associated SE/SEls in some combination.

Analysis of two plasmids harboring ses and set

Our previous study delineated the partial sequence of pF5 and confirmed that ses and set were present on pF5 [20]. However, in Hiroshima3, it was not clear whether these genes were present in the genome or plasmid. Therefore, we extracted plasmids from Fukuoka5 and Hiroshima3 and determined their complete sequences. Both Fukuoka5 and Hiroshima3 carried a single plasmid, namely pF5 and pH3, respectively. Both plasmids shared the coding sequences of four plasmid-associated SEs/SEls (SER, SElJ, sed, selj), whereas that of Hiroshima3 showed a low GC % (28.9% and 30.5% in pF5 and pH3, respectively). These two plasmids also shared partially similar sequences and carried similar ser/selj/set/ses clusters (ORF1-4 of pF5 and ORF1-4 of pH3), as shown Fig. 1. However, ORF3 in pH3 (encoding SET) was 204 bp, which was shorter than ORF3 in pF5, whereas ORF1, 2, and 4 of pF5 and pH3 were identical (Tables 2 and 3). These results were determined to be due to the deletion of T (position: 2,523 bp in pH3) and a subsequent frameshift mutation inside set. A further detailed comparative analysis is shown in Fig. 2. In addition to the ser/set/ses cluster, there were other similar ORFs (ORF5-14 and ORF32-33 in pH3) indicated as a blue rectangle near this cluster (the second and third maps in Fig. 2). Of these, many proteins were predicted to encode hypothetical proteins, whereas the others were predicted to encode replication proteins, DNA-binding proteins, and alcohol dehydrogenase. This region around the ser/set/ses cluster was conserved in both plasmids, but the other region was not. The two plasmids had different heavy metal resistance operons. pF5 had a cadmium resistance operon (ORF17-ORF18) and arsenic resistance operon (ORF33-ORF35), whereas pH3 had a mercury resistance operon (ORF19-ORF25), as shown in Tables 2, 3, and Fig. 1. In addition, pH3 had a penicillin resistance operon (ORF27-29), whereas pF5 had no antibiotic resistance operons. Furthermore, neither plasmid carried any type II toxin-antitoxin or tra genes.

Subsequently, we compared our two plasmids and another plasmid that was positive for ses and set. To date (Oct, 2020), the plasmid SAP047A isolated from human clinical blood in the USA had been the only plasmid deposited in the NCBI database. The ser/set/ses cluster in this plasmid was similar to that of our two plasmids. Of note, the same stop mutation in set was found in plasmids SAP047A and pH3. In addition, the penicillin resistance operon (similar to that in pH3) and cadmium resistance operon (similar to that in pF5) were found in plasmid SAP047A. In addition, the sequences around selj and ser of the two plasmids were almost identical (approximately 98%) to that of pIB485, which carried ser, selj, and sed, although the sequence of ser was partial.

Table 1. Staphylococcal enterotoxin/staphylococcal enterotoxin like toxin (SE/SEl) distribution in each population

|                  | SFP*,** (n=42) | MRSA (n=7) | Nasal swab* (n=329) | Bovine mastitis (n=134) | Mouse skin (n=14) | Total |
|------------------|----------------|------------|---------------------|-------------------------|------------------|-------|
| Any SE/SEI       |                |            |                     |                         |                  |       |
| se/sel positive  | 42             | 7          | 226                 | 36                      | 0                | 311   |
| se/sel negative  | 0              | 0          | 103                 | 98                      | 14               | 215   |
| SES/SET          |                |            |                     |                         |                  |       |
| ses positive     | 2              | 0          | 0                   | 0                       | 0                | 2     |
| set Positive     | 2              | 0          | 0                   | 0                       | 0                | 2     |

*SE/SEl detection without SES and SET in these isolates was done in our previous paper [22]. **Staphylococcal food poisoning.
These results indicated that enterotoxin-associated plasmids shared similar sequences, including enterotoxin cassettes.

Next, a comparison by molecular typing methods for chromosomes, not plasmids, was carried out. The results are shown in Table 4. These two strains had different genetic backgrounds. Fukuoka5 was classified into agrI, CoaIII, and Sequence type (ST) 8 based on agr typing, Coa typing, and MLST, respectively. Conversely, Hiroshima3 was classified into agrII, CoaII, and an ST5 single locus variant. None of the strains had S. aureus pathogenicity islands. This result indicated that there were two types of genetically distinct isolates positive for ses and set.

Modified multiplex PCR

Lastly, we tried to improve the multiplex PCR for SE/SEI genotyping. Novel primers for detecting ses, set, and femB were included. The primers for ses and those for set were added to primer sets 1 and 3, respectively. Novel primers for femB, instead of previous primers,
Table 2. Open reading frame (ORF) map of pF5

| ORF No. | Start | Stop  | Length | Direction | Products                          |
|---------|-------|-------|--------|-----------|-----------------------------------|
| ORF1    | 1     | 780   | 780    | +         | SER                               |
| ORF2    | 861   | 1,667 | 807    | -         | SEJ                               |
| ORF3    | 1,993 | 2,643 | 651    | -         | SET                               |
| ORF4    | 3,053 | 3,826 | 774    | -         | SES                               |
| ORF5    | 4,426 | 4,719 | 294    | +         | Hypothetical protein              |
| ORF6    | 4,794 | 6,728 | 1,935  | +         | Hypothetical protein              |
| ORF7    | 6,732 | 7,043 | 312    | +         | Hypothetical protein              |
| ORF8    | 7,052 | 7,681 | 630    | -         | Putative ABC transporter          |
| ORF9    | 7,919 | 8,308 | 390    | +         | Hypothetical protein              |
| ORF10   | 8,296 | 8,574 | 279    | +         | Hypothetical protein              |
| ORF11   | 8,642 | 9,187 | 546    | +         | Hypothetical protein              |
| ORF12   | 9,338 | 9,556 | 219    | +         | Hypothetical protein              |
| ORF13   | 9,578 | 10,306| 729    | +         | Replication protein               |
| ORF14   | 10,393| 10,752| 360    | +         | DNA-binding protein               |
| ORF15   | 11,612| 12,403| 792    | -         | Replication associated protein    |
| ORF16   | 12,826| 13,770| 945    | +         | Replication initiation protein    |
| ORF17   | 15,392| 16,009| 618    | +         | Cadmium resistance transporter    |
| ORF18   | 16,028| 16,375| 348    | +         | Cadmium efflux regulator         |
| ORF19   | 17,681| 17,983| 303    | +         | Hypothetical protein              |
| ORF20   | 20,445| 21,161| 717    | -         | Replication initiation protein    |
| ORF21   | 21,393| 21,878| 486    | +         | Putative transposase             |
| ORF22   | 22,211| 25,858| 3,648  | -         | Surface protein                   |
| ORF23   | 26,162| 26,836| 675    | +         | Putative transposase             |
| ORF24   | 27,924| 28,418| 492    | +         | Hypothetical protein              |
| ORF25   | 28,492| 29,100| 609    | +         | Hypothetical protein              |
| ORF26   | 30,078| 30,578| 501    | +         | Putative acetyltransferase       |
| ORF27   | 30,841| 32,553| 1,713  | +         | Oligoendopeptidase F             |
| ORF28   | 32,550| 33,845| 1,296  | +         | Putative ABC transporter          |
| ORF29   | 33,846| 34,781| 936    | +         | Putative nucleotide binding protein|
| ORF30   | 34,992| 35,705| 714    | +         | Hypothetical protein              |
| ORF31   | 35,692| 36,909| 1,218  | +         | Membrane transporter              |
| ORF32   | 37,372| 37,938| 567    | +         | Hypothetical protein              |
| ORF33   | 38,345| 38,656| 312    | +         | Arsenic reductase                 |
| ORF34   | 38,674| 39,963| 1,290  | +         | Arsenic efflux pump protein       |
| ORF35   | 39,963| 40,277| 315    | +         | Arsenical resistance operon repressor|
| ORF36   | 40,338| 40,937| 600    | -         | Sin recombinase                   |
| ORF37   | 41,205| 41,624| 420    | -         | Hypothetical protein              |
| ORF38   | 41,748| 42,719| 972    | +         | Alcohol dehydrogenase            |

Fig. 2. Comparative analysis of the similarity among the three plasmids, pF5, pH3, and plasmid SAP047A. The genome matcher program was used. Arrows indicate open reading frames (ORFs), and their orientation is the ORF direction. Sequence homology (%) is shown by the color scale. If there was 100% homology, the sequences are connected by red lines. Moreover, if there was less than 70% homology, there are no lines. The color range is red (100%) to blue (70%). Green and blue highlights indicate the ser/self/ser/self cluster and similarly shared ORFs, respectively. The scale bar indicates 5 kb. The accession numbers for pF5, pH3, and plasmid SAP047A are AB765928, AB765929, and GQ900405, respectively.
were used to avoid nonspecific bands. To confirm that PCR was performed properly, 10 S. aureus strains, which were known to be of various SE/SEl genotypes, were subjected to multiplex PCR. The results are shown in Fig. 3. All PCR products corresponding to every gene, including ses and set, were obtained, and the sizes of all products were consistent with the predicted sizes. This result confirmed that the modified multiplex PCR performed well. In addition, it was also confirmed that these primer sets worked well with another PCR enzyme (Supplementary Fig. 1).

**DISCUSSION**

Plasmids comprise an important factor associated with evolving processes in *Staphylococcus*. The majority of *S. aureus* strains has one or more plasmids and their plasmids carry various virulence factors [9, 12]. These virulence factors include antibiotic resistance genes, heavy metal resistance genes, and toxin genes. The SE/SEl family is a plasmid-related toxin. The horizontal gene transfer of these plasmids is responsible for acquiring enterotoxigenicity and might render recipient cells food poisoning pathogens. In the present study, we focused on and analyzed newly described plasmid-encoding SEs, ses and set, and delineated their detailed genetic background.

Although Ono et al. reported a partial sequence of plasmid pF5 from Fukuoka5 [20], the whole nucleotide sequence of an ses/set-positive plasmid isolated in Japan was not available. In this study, we conducted complete genome sequencing of two plasmids in Japan, including pF5, and identified that these two plasmids were typical enterotoxigenic plasmids in *Staphylococcus*. *S. aureus* plasmids can be divided into three types [12]. According to this classification, both plasmids sequenced in this study were type II plasmids because of the intermediate length, resistance genes, and lack of tra responsible for conjugation. Although some reports have shown that some plasmids carry the toxin-antitoxin system responsible for plasmid maintenance, our plasmids did not. As described previously herein, it seems that self is associated with ser as a cassette in the same plasmids, whereas sed is independent of them. Although only two strains were identified and analyzed in this study, it seems that ses and set form a similar homologous cassette in those two strains; that is, two nearly identical gene sequences are aligned in the same direction. The recombination of these three enterotoxin cassettes might cause divergence of the enterotoxigenic plasmid in *S. aureus*.

*S. aureus* strains positive for ses and set were rare (only two strains from food poisoning), compared with those harboring other SEs/SEls, especially the plasmid-encoding SE/SEl genes sed, selj, and ser. Consistent with our reports, other groups reported *S. aureus* positive for these three genes [4, 7, 26]. In contrast, only two of 526 strains were positive for ses and set and carried plasmids (approximately 0.5%). A recent study by Vu et al. analyzed the distribution of several superantigens in *S. aureus* from diabetic foot ulcers and demonstrated that the positivity rates of ses and set were relatively lower than those of other SE genes, similar to our present result [27]. We speculate that few *S. aureus* strains are positive for ses and set. There might be some explanations for this;
specifically, our sequenced plasmids did not have any tra and toxin-antitoxin systems. tra mediates plasmid spreading among the S. aureus population [13]. The toxin–antitoxin system confers stability to the plasmid in cells [24]. A lack of these genes in plasmids appears to be a disadvantage for plasmid distribution in the S. aureus population.

Moreover, Fukuoka5 and Hiroshima3 are classified into different backgrounds, clonal complexes (CC) 8 and CC5, respectively. However, these CCs are well known SE-plasmid associated CCs. Our recent studies indicated that sed, self, and ser were strongly associated with the specific lineages CC5 and CC8 in SFP outbreaks [25]. Two strains positive for ses and set were also classified into these groups, indicating the lineage specificity of these SEs. With respect to plasmids associated with another toxin, exfoliative toxin type B, recent reports indicated that strains carrying the ETB (exfoliative toxin B) plasmid from impetigo patients could be classified into a specific clonal complex, CC121 [20]. McCarthy et al. suggested a lineage-associated plasmid [13]. Since each CC carries a unique restriction-modification system, each CC tends to accept unique MGEs, including plasmids, and shows their own MGE profiles.

This is the first study to shed light on two new plasmid-encoding SEs, ses and set. To date, these toxins have been assed in only a few studies as a part of molecular epidemiology [27]. We found that there is variability in the plasmids and strains carrying these plasmids, although ses and set are thought to be rare in the S. aureus population. This indicates that the plasmids and strains do not represent the spread of a single clone. However, it remains unclear how much variation exists. Considering staphylococcal enterotoxins E, its positive rate is known to vary regionally, being less common in Japan and more common in Europe and USA [1, 22, 25]. That is why the positive rate of these novel SEs in other countries might show a different trend. Because we used isolates only in Japan, differences in prevalence rates by region are still unknown. In other words, the features of these plasmids and these newly identified SEs remain to be elucidated. Therefore, further studies are needed to understand this. Our new modified multiplex PCR is easy to use and will facilitate future studies, because reactions including our primers, used to detect these SEs, can be performed well with at least two different PCR kits.

POTENTIAL CONFLICTS OF INTEREST. The authors have nothing to disclose.

ACKNOWLEDGMENTS. This study was supported by a grant-in-aid for scientific research from the Japan Society for the Promotion of Science (grants 20580338 and 23380177), a Health Science Research Grant from the Ministry of Health, Labor and Welfare, a Japan Research Program on Emerging and Re-emerging Infectious Diseases from the Japan Agency for Medical Research and Development (AMED) under grant number JP19fk0108061. We thank Masami Morimatsu (Institute for Genetic Medicine, Hokkaido University) and Norio Yamagishi (Department of Veterinary Sciences, Obihiro University of Agriculture and Veterinary Medicine) for kindly providing S. aureus strains.

REFERENCES

1. Argudín, M. A. , Mendoza, M. C. and Rodicio, M. R. 2010. Food poisoning and Staphylococcus aureus enterotoxins. Toxins (Basel) 2 : 1751–1773. [Medline] [CrossRef]
2. Bayles, K. W. and Lando, J. J. 1989. Genetic and molecular analyses of the gene encoding staphylococcal enterotoxin D. J. Bacteriol. 171 : 4799–4806. [Medline] [CrossRef]
3. Bergdoll, M. S. 1983. Enterotoxins. Staphylococci and Staphylococcal Infections, pp. 559–598. (Easton, C. S. F. and Adlam, C, eds.), Academic Press, London.
4. Chiang, Y. C., Liao, W. W., Fan, C. M., Pai, W. Y., Chiou, C. S. and Tsen, H. Y. 2007. Novel multiplex PCR detection of Staphylococcal enterotoxins (SEs) N, O, P, Q, R, U, and survey of SE types in Staphylococcus aureus isolates from food-poisoning cases in Taiwan. Int. J. Food Microbiol. 121 : 66–73. [Medline] [CrossRef]
5. Crupper, S. S., Worrell, V., Stewart, G. C. and Lando, J. J. 1999. Cloning and expression of cadD, a new cadmium resistance gene of Staphylococcus aureus. J. Bacteriol. 181 : 4071–4075. [Medline] [CrossRef]
6. Ji, G. and Silver, S. 1992. Regulation and expression of the arsenic resistance operon from Staphylococcus aureus plasmid p258. J. Bacteriol. 174 : 3684–3694. [Medline] [CrossRef]
7. Hwang, S. Y., Kim, S. H., Jang, E. J., Kwon, N. H., Park, Y. K., Koo, H. C., Jung, W. K., Kim, J. M. and Park, Y. H. 2007. Novel multiplex PCR for the detection of the Staphylococcus aureus superantigen and its application to raw meat isolates in Korea. Int. J. Food Microbiol. 117 : 99–105. [Medline] [CrossRef]
8. Laddaga, R. A., Chu, L., Misra, T. K. and Silver, S. 1987. Nucleotide sequence and expression of the mercurial-resistance operon from Staphylococcus aureus plasmid p258. Proc. Natl. Acad. Sci. USA 84 : 5106–5110. [Medline] [CrossRef]
9. Lindsay, J. A. 2010. Genomic variation and evolution of Staphylococcus aureus. Int. J. Med. Microbiol. 300 : 98–103. [Medline] [CrossRef]
10. Lina, G., Bouttie, F., Tristan, A., Bes, M., Etienne, J., Vandenesch, F. 2003. Bacterial competition for human nasal cavity colonization: role of Staphylococcal agr alleles. Appl. Environ. Microbiol. 69 : 18–23. [Medline] [CrossRef]
11. Massidda, O., Mingoia, M., Fadda, D., Whalen, M. B., Montanari, M. P. and Varaldo, P. E. 2006. Analysis of the beta-lactamase plasmid of borderline methicillin-susceptible Staphylococcus aureus: focus on bla complex genes and cadmium resistance determinants cadD and cadX. Plasmid 55 : 114–127. [Medline] [CrossRef]
12. Malachowa, N. and DeLeo, F. R. 2010. Mobile genetic elements of Staphylococcus aureus. Cell. Mol. Life Sci. 67 : 3057–3071. [Medline] [CrossRef]
13. McCarthy, A. J. and Lindsay, J. A. 2012. The distribution of plasmids that carry virulence and resistance genes in Staphylococcus aureus is lineage associated. BMC Microbiol. 12 : 104. [Medline] [CrossRef]
14. Morton, T. M., Johnston, J. L., Patterson, J. and Archer, G. L. 1995. Characterization of a conjugative staphylococcal mupirocin resistance plasmid. Antimicrob. Agents Chemother. 39 : 1272–1280. [Medline] [CrossRef]
15. Ohtsubo, Y., Ikeda-Ohtsubo, W., Nagata, Y. and Tsuda, M. 2008. GenomeMatcher: a graphical user interface for DNA sequence comparison. BMC
Bioinformatics 9: 376. [Medline]  [CrossRef]

16. Omoe, K., Hu, D. L., Takahashi-Omoe, H., Nakane, A. and Shinagawa, K. 2003. Identification and characterization of a new staphylococcal enterotoxin-related putative toxin encoded by two kinds of plasmids. Infect. Immun. 71: 6088–6094. [Medline]  [CrossRef]

17. Omoe, K., Hu, D. L., Takahashi-Omoe, H., Nakane, A. and Shinagawa, K. 2005. Comprehensive analysis of classical and newly described staphylococcal superantigenic toxin genes in Staphylococcus aureus isolates. FEMS Microbiol. Lett. 246: 191–198. [Medline]  [CrossRef]

18. Ono, H. K., Omoe, K., Imanishi, K., Iwakabe, Y., Hu, D. L., Kato, H., Saito, N., Nakane, A., Uchiyama, T. and Shinagawa, K. 2008. Identification and characterization of two novel staphylococcal enterotoxins, types S and T. Infect. Immun. 76: 4999–5005. [Medline]  [CrossRef]

19. Paulsen, I. T., Brown, M. H. and Skurray, R. A. 1998. Characterization of the earliest known Staphylococcus aureus plasmid encoding a multidrug efflux system. J. Bacteriol. 180: 3477–3479. [Medline]  [CrossRef]

20. Růžičková, V., Pantůček, R., Petráš, P., Machová, I., Kostýlková, K. and Doškař, J. 2012. Major clonal lineages in impetigo Staphylococcus aureus strains isolated in Czech and Slovak maternity hospitals. Int. J. Med. Microbiol. 302: 237–241. [Medline]  [CrossRef]

21. Sato’o, Y., Omoe, K., Ono, H. K., Nakane, A. and Hu, D. L. 2013. A novel comprehensive analysis method for Staphylococcus aureus pathogenicity islands. Microbiol. Immunol. 57: 91–99. [Medline]  [CrossRef]

22. Sato’o, Y., Omoe, K., Naito, I., Ono, H. K., Nakane, A., Sugai, M., Yamagishi, N. and Hu, D. L. 2014. Molecular epidemiology and identification of a Staphylococcus aureus clone causing food poisoning outbreaks in Japan. J. Clin. Microbiol. 52: 2637–2640. [Medline]  [CrossRef]

23. Sevin, E. W. and Barloy-Hubler, F. 2007. RASTA-Bacteria: a web-based tool for identifying toxin-antitoxin loci in prokaryotes. Genome Biol. 8: R155. [Medline]  [CrossRef]

24. Schuster, C. F. and Bertram, R. 2016. Toxin-antitoxin systems of Staphylococcus aureus. Toxins (Basel) 8: E140. [Medline]  [CrossRef]

25. Suzuki, Y., Omoe, K., Hu, D. L., Sato’o, Y., Ono, H. K., Monma, C., Arai, T., Konishi, N., Kato, R., Hirai, A., Nakama, A., Kai, A. and Kamata, Y. 2014. Molecular epidemiological characterization of Staphylococcus aureus isolates originating from food poisoning outbreaks that occurred in Tokyo, Japan. Microbiol. Immunol. 58: 570–580. [Medline]  [CrossRef]

26. Varshney, A. K., Mediavilla, J. R., Robiou, N., Guh, A., Wang, X., Gialanella, P., Levi, M. H., Kreiswirth, B. N. and Fries, B. C. 2009. Diverse enterotoxin gene profiles among clonal complexes of Staphylococcus aureus isolates from the Bronx, New York. Appl. Environ. Microbiol. 75: 6839–6849. [Medline]  [CrossRef]

27. Weiss, A. A., Murphy, S. D. and Silver, S. 1977. Mercury and organomercurial resistances determined by plasmids in Staphylococcus aureus. J. Bacteriol. 132: 197–208. [Medline]  [CrossRef]

28. Yamaguchi, T., Hayashi, T., Takami, H., Ohnishi, M., Murata, T., Nakayama, K., Asakawa, K., Ohara, M., Komatsuzawa, H. and Sugai, M. 2001. Complete nucleotide sequence of a Staphylococcus aureus exfoliative toxin B plasmid and identification of a novel ADP-ribose transferase, EDIN-C. Infect. Immun. 69: 7760–7771. [Medline]  [CrossRef]

29. Zhang, S., Iandolo, J. J. and Stewart, G. C. 1998. The enterotoxin D plasmid of Staphylococcus aureus encodes a second enterotoxin determinant (sej). FEMS Microbiol. Lett. 168: 227–233. [Medline]  [CrossRef]