INTRODUCTION

Coagulase-positive *Staphylococcus aureus* (CPS) is a common pathogen associated with serious community-related and hospital-acquired diseases, and has long been considered a major public health problem (Pesavento, Ducci, Comodo, & Nostro, 2007). Bakery products are classified based on their water activity (aw) value; cream-filled cakes, one of the most common bakery products consumed worldwide, have an intermediate moisture content with an aw value ranging from 0.6 to 0.85 (Smith & Simpson, 1995). Since most bacteria require a high aw for growth, bacterial problems are limited to bakery products with a relatively high moisture content; thus, moisture-filled bakery products have been implicated in outbreaks of foodborne illness, thereby posing safety concerns (Smith, Daifas, El-Khoury, Koukuotsis, & El-Khoury, 2004). In particular, staphylococcal food poisoning (SFP) is a type of intoxication that results from the consumption of foods containing sufficient amounts of one (or more) preformed enterotoxins. Symptoms of SFP include a rapid onset (2–8 hr), with nausea, violent vomiting, and abdominal cramping with or without diarrhea (Argudín, Mendoza, & Rodicio, 2010).

Many of the known *S. aureus* virulence factors can be described as toxins, which are generally defined as poisonous substances. Thus, the distinction from other virulence factors, that is, molecules that increase the potential of a pathogen to cause disease in a broader sense, is that toxins are secreted by the producing organism and interfere directly with the host (Otto, 2014). *S. aureus* is a major human pathogen bacteria that produces various types of toxic proteins such as enterotoxins, toxic shock syndrome toxin 1 (TSST-1), and

Abstract

We determined the toxin gene profile, toxin production, antibiotic resistance coagulase serotype, and genetic diversity of 42 coagulase-positive *Staphylococcus aureus* (CPS) isolates collected from 1,464 cream-filled bakery products in Korea. Among the CPS isolates, 37 (88.1%) produced enterotoxin genes in combination with another toxin; 26 (61.9%) of the strains were positive for *sea*, 1 (2.4%) for *sea-seb*, and 4 (9.5%) for *sea-sec*. Among the strains showing antibiotic resistance, 28 (66.7%) showed resistance to only one antibiotic, whereas nine (21.4%) showed resistance to multiple antibiotics: 4 (9.5%) strains were both mecA-positive and oxacillin-resistant. Most strains are resistant to at least one antibiotic—benzyl penicillin. The CPS isolates were classified into eight coagulase serotypes. This information will be valuable for assessing the capability risks of CPS food poisoning, contributing a better known of the epidemiology result associated with CPS contamination in bakery products.

**KEYWORDS**

antibiotic resistance, coagulase serotype, cream-filled bakery products, *Staphylococcus aureus*, toxin gene
exfoliative toxin (ET) all of which can cause food poisoning (Balaban & Rasooly, 2000; Jung et al., 2005). Several types of staphylococcal enterotoxin (SE) have been identified by serological, biochemical analyses, and molecular genetic diversity over the years, including SEA, SEB, SEC, and SED (Sharma, Rees, & Dodd, 2000). S. aureus infection results in different symptoms, which largely depend on the type of toxin secreted; therefore, it is important to be able to reliably and accurately distinguish the type of toxins produced from strains isolated from food to ensure proper treatment and monitor the trends of foodborne illness.

Staphylocoagulase (SC) is an extracellular protein produced by S. aureus and has been regarded as a hallmark for the identification of S. aureus, arguably the most virulent and clinically important pathogenic species. SC can be classified into eight types of antigenic coagulases (I–VIII). Serotyping methods were developed to identify SC types based on the inhibition of clotting activity with type-specific antisera and have been widely used for epidemiological studies of S. aureus, especially in Japan. Coagulase types VII accounting for about 70% of all outbreaks, III (12%), II (11%), and VI (3%) are, respectively, the first, second, third, and fourth most predominant types of coagulases responsible for food poisoning incidents throughout Japan (Oda, 1998; Shimizu et al., 2000).

Staphylococcus aureus is also a frequent cause of human infections, and strains that show antimicrobial resistance, particularly multidrug resistance especially methicillin and vancomycin, have become a major global public health problem in recent. The excessive and inappropriate use of antimicrobial agents is responsible for the emergence and rapid spread of resistant bacterial strains (Kunin, 1993; Fallah, Saei-Dehkordi, Rahnama, Tahmasby, & Mahzoumeh, 2012). In particular, methicillin-resistant S. aureus (MRSA) has increasingly been reported to dominate S. aureus infections and is rising in prevalence, becoming a cause of great clinical concern worldwide. The resistant phenotype of MRSA strains is related to the acquisition of a set of genes that are induced and expressed during β-lactam exposure; all MRSA strains harbor the mecA gene, encoding penicillin-binding protein 2a (PBP2a) as the key resistance enzyme for virtually all β-lactam antibiotics (Ábrók, Lázár, Szécsényi, Déák, & Urbán, 2018; Zhan & Zhu, 2018). Accordingly, polymerase chain reaction (PCR)-based detection of the highly conserved mecA gene is the gold standard for the determination of methicillin resistance in staphylococci. Moreover, Mup (encoding pseudomonic acid A), a topical antimicrobial agent produced by *Pseudomonas fluorescens*, has been used to eradicate MRSA from the nasal carriage and staphylococci catheter colonization (Sareyyüpoğlu, Ozyurt, Haznedaroğlu, & Ardiç, 2008). An alternative strain-typing method, which is available commercially, is the DiversiLab typing system (DL; biomérieux, Inc., Durham, NC, USA), which uses the presence of DNA repetitive elements in the organism’s genome to determine the genetic relatedness of bacterial and fungal isolates. Cream products more prone to have problems of food poisoning associated with *S. aureus*. However, little studies have been conducted on the characteristics of *S. aureus* isolated from cream-filled bakery products. The aim of the present study was to characterize different *S. aureus* isolates collected from cream-filled bakeries in Korea. This characterization was based on the ability of the isolates to produce and express SEs. We further determined the antibiotic susceptibility profiles of the isolates to monitor the presence and prevalence of MRSA strains in cream-filled bakery products, and the presence of other virulence factors. Furthermore, the relationship between MRSA and their ability to produce virulence factors was evaluated.

## MATERIAL AND METHOD

### 2.1 Bacterial isolates and identification

Forty-two *S. aureus* strains were isolated from 1,464 samples cream-filled bakery collected in bakery markets in Korea. In brief, each 25 g sample was enriched with 225 ml of tryptic soy broth with 10% NaCl (Merck, Darmstadt, Germany) at 35°C for 24 hr and then streaked on 10-μl baird-parker agar plates (Merck). The typical five colony was conducted coagulase test on baird parker RPF agar (bioMérieux, Marcy L’Etoile, France). Incubation at 35°C 24 hr, a typical colony was selected on blood agar, which was analyzed by a catalase test, gram staining, and biochemical test with the Vitek 2 (bioMérieux, Marcy L’Etoile, France) for identification.

### 2.2 Detection of SEs toxin by the reverse passive latex agglutination (RPLA) assay

Positive *S. aureus* strains were analyzed with a SEs detection kit using RPLA (SET-RPLA; Denka Seiken, Tokyo, Japan) according to the manufacturer’s instructions.

### 2.3 Detection of *Staphylococcus aureus* toxigenic genes by PCR

Chromosome DNA was extracted with an ultraclean™ microbial DNA isolation kit (MO Bio Laboratories Inc., CA, USA) according to the manufacturer’s instructions. The samples were then subjected to gradient PCR (Biometra, Gottingen, Germany) with primer pairs listed in Table 1 (Bioneer, Cheongwon, Chungbuk, Korea). PCR was performed AccuPower PCR Premix (Bioneer, Daejon, Korea) containing 35 ng/μl of template DNA and 10 pmol of primer in total 20-μl reaction mixture. PCR was performed 35 cycles of 95°C for 60 s, 55°C for 60 s, and 72°C for 60 s, which was preceded by a 300 s denaturation step at 95°C and 300 s extension step at 72°C. PCR products were separated by electrophoresis on a 2% seakem agarose gel (Takara Bio, Otsu, Japan) with ethidium bromide solution (1 μg/ml) at 100 V for 30 min and visualized under UV transilluminator.

### 2.4 Antibiotic susceptibility testing

Antibiotic test was performed with the Vitek 2 (bioMérieux, Marcy L’Etoile, France) according to the manufacturer’s instructions. The AST-P601 *S. aureus* card (bioMérieux, Marcy L’Etoile, France) used for contained benzyl penicillin, cefoxitin screen, ciprofloxacin,
clindamycin, erythromycin, gentamicin, inducible clindamycin resistance, linezolid, mupirocin, nitrofurantoin, oxacillin, quinupristin/dalfopristin, rifampicin, telithromycin, tetracycline, trimethoprim/sulfamethoxazole, tigecycline, and vancomycin. The result was interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2011). Strains *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were included as quality control strains in the antibiotic susceptibility testing (AST) test.

### 2.5 | Identification of MRSA

*Staphylococcus aureus* 42 strains were resistant to oxacillin, and then PCR was used to confirm that these strains also harbored the *mecA* and *mupA* gene for identification of MRSA strains (Table 1). PCR was performed 10 cycles of amplification (94°C for 30 s, 64°C for 30 s, and 72°C for 45 s) and 25 cycles of amplification (94°C for 45 s, 50°C for 45 s, and 72°C for 1 min), which was preceded by a 5 min denaturation step at 94°C and followed by a 10 min extension step at 72°C. Each PCR was conducted in triplicate, and the amplified products were separated by electrophoresis as described above.

### 2.6 | Identification of coagulase serotype by PCR

Serological coagulase typing was performed using PCR (Table 2) essentially as described above with the following cycling parameters: PCR was performed over 30 cycles of 94°C for 30 s, 52°C for 30 s, and 72°C for 1 min, which was preceded by a 5 min denaturation step at 95°C and followed by a 7 min extension step at 72°C. Each PCR was conducted in triplicate, and the amplified products were separated by electrophoresis as described above.

### 2.7 | Rep-PCR (DiversiLab) genetic typing system

*Staphylococcus aureus* strains were cultured on blood agar for 24 hr at 35°C. Genomic DNA was extracted as described above, and the DNA was diluted of 35 ng/μg. Rep-PCR was performed using the DiversiLab Staphylococcus kit (Bacterial BarCodes, Inc., Houston, TX, USA) according to the manufacturer’s product insert. PCR was performed on a gradient using the following parameters: 94°C for 2 min and then 35 cycles of amplification (94°C for 30 s, 45°C for 30 s, and 70°C for 90 s), with 70°C for 3 min. Analysis of rep-PCR products was implemented using the DiversiLab (Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). The DNA fingerprint patterns were automatically downloaded onto a secure laboratory designated DiversiLab program. Agreement between methods was assessed at different rep-PCR SI cutoffs, including 80%, 85%, and 90%, as generated by the DiversiLab software, and the relatedness was determined by cluster analysis according to the guidelines provided by the manufacturer.

### 3 | RESULT AND DISCUSSION

#### 3.1 | SEs production

Coagulase-positive *S. aureus* strains are readily killed by pasteurization or cooking; however, the microorganism’s enterotoxins tend to be heat-stable and can survive even at the high temperatures (121°C) used to process low-acid canned foods (Stewart, Cole, & Schaffner, 2003). Among the 42 CPS strains isolated from cream-filled bakery products, RPLA analysis showed that a total of 10 strains (23.8%) produced one or more SEs: 19.0%, 2.4%, and 2.4% of the strains produced SEA, SEA + SEB, and SEA + SEC, respectively (Table 3). In RPLA, the most commonly employed and commercially available enterotoxin identification method, the enterotoxins are identified by specific antibodies (Sharma et al., 2000). For further confirmation, we also amplified the enterotoxin-encoding genes in the CPS strains; *sea* was the predominant gene, detected in 26 strains (61.9%, Table 3). Overall, 11.9% (5/42) of the *S. aureus* strains harbored the

### Table 1 | Oligonucleotide primers used of staphylococcal toxin genes

| Target | Oligonucleotide sequence | Amplicon size (bp) | References |
|--------|--------------------------|--------------------|------------|
| *sea*  | TTGGAAACGGTTAAAAACGAA    | 127                | Johnson et al. (1991) |
|        | GAACCTTCCCAATAAAAACA     |                    |            |
| *seb*  | TCGCATCAAACTGACAACAG     | 478                | Johnson et al. (1991) |
|        | GCAGGTACTCTTATAAGTGCTGC  |                    |            |
| *sec*  | CTCAGAGAAGCTAGATAAAGCCTAGG | 257              | Johnson et al. (1991) |
|        | TCAAAATCGGATTTAAGATTCC   |                    |            |
| *sed*  | CTAGTTTGGAATATCTCTTTAAGC | 317                | Johnson et al. (1991) |
|        | TTAATCATATATCTTATAAGGTAAACATC |          |            |
| *mupA* | TATATTATCGAGTGAAGGGTTGG  | 456                | Anthony, Connor, Power, and French (1999) |
|        | AATTTAATCCACATTTGTTCGTTAA |                  |            |
| *mecA* | GGTAGAAATGACGTGACGTCGATAA| 310                | Pérez-Roth, Claverie-Martin, Villar, and Mendez-Alvarez (2001) |
|        | CCAATCCACATTTGTTCGTTAA   |                    |            |
targeted classical SEs genes (sea, seb, and sec), including sea-sec (9.5%, 4/42) and sea-seb (2.4%, 1/42). Complete agreement of the results between the RPLA and PCR identification methods was obtained for nine of the 42 strains (21.4%). This level is much lower than the agreement rate of 93.9% reported by Kerouanton et al. (2007). Therefore, it is necessary to consider both the phenotype and genotype to best distinguish the enterotoxin of *S. aureus*.

In Korea, SEA is the most frequent SE type; however, other SEs, including novel SEs, have previously been reported to be detected together with SEA (Cha et al., 2006; Hyeon et al., 2013). The SEA toxin type has also been associated with several *S. aureus* outbreaks linked to bakery products (Stewart et al., 2003). However, various toxins have been detected in such outbreaks, and the major toxin type varies widely in different countries. Therefore, SE identification could provide information on the origin of the foodborne pathogen, and their genetic characterization and geographical characterization. But new pathogenic factors are being reported and so further studies on novel clones with unknown genetic backgrounds and their relation are needed (Kerouanton et al., 2007), including specific investigations for different types of foods and countries.

Foodborne outbreaks associated with cream-filled bakery products are frequently attributed to inadequate refrigeration during manufacturing or storage (Bryan, 1976). Therefore, there is a need to carefully manage the production of such products from the manufacturing stage to the sales and distribution stage up to consumption.

### 3.2 AST and MRSA identification

Antimicrobial resistance analysis showed that 37 of the 42 strains were resistant to more than one antibiotic tested. The highest resistance rate was found to P (88.1%), followed by OX (9.5%), GM (4.8%), TE (4.8%), and E (2.4%). There was no antibiotic resistance to VA detected in any strain (Table 4).

Overall, nine (21.4%) multidrug-resistant strains were detected. In particular, the OX-resistant MRSA strains were resistant to three or five antibiotics (Table 5). PCR confirmation and the PBP2’ RPLAS reaction of these candidate MRSA strains identified six strains (14.2%) with the *mecA* gene. One strain harbored the *mecA* and *mupA* genes simultaneously. However, four strains were OX- and multi-resistant strains. Two strains were susceptible to both OX and OXSF antibiotics (Table 6).
In accordance with the CLSI guidelines, the mecA-positive and OX-resistant strains were concluded to be MRSA. Four strains were found to be positive for mecA and were consistently resistant to OX. All MRSA strains were resistant to all β-lactams tested, including penicillin. Regarding the involvement of MRSA in food poisoning, Jones, Kellum, Porter, Bell, and Schaffner (2002) reported the first outbreak of gastrointestinal illness caused by community-acquired MRSA. Food is also an important source for the transfer of antimicrobial resistance, which can occur by means of residues of antibacterials, through the transfer of resistant foodborne pathogens, or through the ingestion of resistant strains of the original food microflora and ultimate resistance transfer to pathogenic microorganisms (Hennekinne, Buyser, & Dragacci, 2012). Therefore, studies on the antimicrobial resistance of microorganisms isolated from foods are very important and can be used as basic data to prevent community infection. Moreover, further study is necessary to test the hypothesis that MRSA can be cross-contaminated between humans and foods, emphasizing the importance of improving hygiene in food production practices as a countermeasure to limit the spread of antimicrobial-resistant organisms and foods.

3.3 | Identification of coagulase serotype by PCR

Serological coagulase typing was performed using the PCR coa genes of eight different serotypes (Table 7). The serological coagulase types of the 42 isolates were classified into seven categories according to the toxin genes: coagulase type V strains 47.6% (n = 20) encoded sea (n = 12) or sea-sec (n = 2), type IV 14.3% (n = 6) encoded sea (n = 5), and type VII 14.3% (n = 6) encoded sea (n = 3), sea + seb (n = 1), and sea + sec (n = 1). Coagulase type VII strains have been the predominant types responsible for food poisoning since the 1980s. Coagulase VII, which accounted for about 75.6% of the total number of outbreaks, was the predominant type, and the second most prevalent type was coagulase IV (Cha et al., 2006). Coagulase types VII (70%), III (12%), II (11%), and VI (3%) are most predominant types of coagulases responsible for food poisoning incidents throughout Japan (Shimizu et al., 2000). Another report showed that the four predominant coagulase genotypes of Staphylococcus aureus (I, II, VII, and VIII) were more common than isolates harboring the six rare coagulase types (III, IV, V, VI, IX, and X (Moon et al., 2007). In the present study, the V type was the most dominant, although VII and IV, which are genotypes related to food poisoning problems, were also isolated. These results differ from the common coagulase type of S. aureus known to cause food poisoning, but warrant further study. In addition, it is necessary to further examine the characteristics of isolated S. aureus and relate these characteristics to determine the causes of food poisoning.

3.4 | Test method: rep-PCR DiversiLab microbial typing system

Typing by DiversiLab revealed that the six MRSA isolates were genetically diverse with a 95% similarity. In particular, the homology of strains was very high (98.6%), and strains resistant to OX showed a homology of 99.9% or more (Figure 1). Moreover, these results confirm that DiversiLab, although considered to be

**TABLE 4** Antimicrobial resistance of Staphylococcus aureus strains isolated from cream-filled bakery products

| Antimicrobial agent | Resistance (%) | Intermediate (%) | Susceptible (%) |
|---------------------|----------------|------------------|-----------------|
| P                   | 88.1           | 0                | 11.9           |
| OX                  | 9.5            | 0                | 90.5           |
| GM                  | 4.8            | 9.5              | 85.7           |
| CIP                 | 0              | 0                | 100            |
| E                   | 2.4            | 2.4              | 95.2           |
| TEL                 | 0              | 0                | 100            |
| CM                  | 0              | 0                | 100            |
| QDA                 | 0              | 0                | 100            |
| LNZ                 | 0              | 0                | 100            |
| TEC                 | 0              | 0                | 100            |
| VA                  | 0              | 0                | 100            |
| TE                  | 4.8            | 0                | 95.2           |
| TGC                 | 0              | 0                | 100            |
| FT                  | 0              | 0                | 100            |
| MUP                 | 0              | 0                | 100            |
| RA                  | 0              | 0                | 100            |
| SXT                 | 0              | 0                | 100            |

Note. CIP: ciprofloxacin; CM: clindamycin; E: erythromycin; FT: nitrofurantoin; GM: gentamicin; LNZ: linezolid; MUP: mupirocin; OX: oxacillin; P: benzyl penicillin; QDA: quinupristin/dalfopristin; RA: rifampicin; SXT: trimethoprim/sulfamethoxazole; TE: tetracycline; TEC: teicoplanin; TEL: telithromycin; TGC: tigecycline; VA: vancomycin.
Cream-filled breads are one of the most common bakery products consumed worldwide. However, these products are particularly susceptible to physical, chemical, and microbiological hazards during storage.

Table 5: Multiple drug resistance of Staphylococcus aureus isolates from cream-filled bakery products

| No. of drugs | Resistant patterns | No. of resistant strains | Total (%) |
|--------------|--------------------|--------------------------|-----------|
| 5            | P OXSF OX E ICR   | 1                        | 1 (2.4)   |
| 3            | P OXSF OX         | 3                        | 3 (7.1)   |
| 2            | P TE              | 2                        | 5 (11.9)  |
| P GM         |                    | 2                        |           |
| P ICR        |                    | 1                        |           |
| 1            | P                  | 28                       | 28 (66.7) |
| 0            |                    | 5                        | 5 (11.9)  |

Note: E: erythromycin; ICR: inducible clindamycin resistance; OX: oxacillin; OXSF: cefoxitin screen; P: benzyl penicillin; TE: tetracycline.

Table 6: List of MRSA strains isolated from cream-filled bakery products

Table 7: Coagulase types of Staphylococcus aureus strains identified by PCR

| Coagulase serotype | I (%) | II (%) | III (%) | IV (%) | V (%) | VI (%) | VII (%) | VIII (%) | N* (%) |
|--------------------|-------|--------|---------|--------|-------|--------|---------|----------|--------|
| 0 (0)              |       | 3 (7.1)| 1 (4.8) | 6 (14.3)| 20 (47.6)| 2 (4.7)| 6 (14.3)| 1 (2.4)  | 3 (7.1) |

Note: N*: not detected.
at the outlet, which is exacerbated by the general lack of overview related to food safety (Smith et al., 2004). Food safety are complex issues involving many inter-related variables parameter, including moisture, aw, pH, preservatives, microbial ecology of the food nutrient matrix, packaging, and storage temperature. Overall, bakery products with a particularly high moisture content have been implicated in several outbreaks of foodborne disease. Therefore, continuing education in food hygiene and safety for bakery personnel, food handlers, and, indeed, the general public is also a critical component in the battle against foodborne disease. In our study, we showed the presence of resistant strains of S. aureus in bakery products sold across Korea and identified SEA as the predominant enterotoxin. These results confirmed potential risks of S. aureus contamination and useful for will be assessment of the in cream-filled bakery products: profiling of the toxin phenotype, genotype, antimicrobial resistance, coagulase type, and genetic diversity.

**ETHICAL APPROVAL AND CONSENT TO PARTICIPATE**

Ethics approval and consent to participate is not applicable to this manuscript, since it does not report on or involve the use of any animal or human data or tissues.

**ACKNOWLEDGMENTS**

This research was supported by Main Research Program of the Korea Food Research Institute (KFRI) funded by the Ministry of Science, ICT & Future Planning.

**CONFLICT OF INTEREST**

The authors declare that they do not have any conflict of interest.

**ORCID**

Yong Sun Cho
https://orcid.org/0000-0001-9972-1454

Myung Ki Lee
https://orcid.org/0000-0002-5615-2455

Sun Hye Hwang
https://orcid.org/0000-0003-4851-4855

**REFERENCES**

Ábrók, M., Lázár, A., Szécsényi, M., Deák, J., & Urbán, E. (2018). Combination of MALDI-TOF MS and PB2’ latex agglutination assay for rapid MRSA detection. *Journal of Microbiology Methods*, 144, 122–124. https://doi.org/10.1016/j.mimet.2017.11.021

Anthony, R. M., Connor, A. M., Power, E. G. M., & French, G. L. (1999). Use of the polymerase chain reaction for rapid detection of high-level mupirocin resistance in staphylococci. *European Journal of Clinical Microbiology and Infectious Diseases*, 18, 30–34. https://doi.org/10.1007/s100960050222

Argudín, M. A., Mendoza, M. C., & Rodicio, M. R. (2010). Food poisoning and *Staphylococcus aureus* enterotoxins. *Toxins*, 2, 1751–1773. https://doi.org/10.3390/toxins2071751

Balaban, N., & Rasooly, A. (2000). *Staphylococcus* enterotoxins. *International Journal of Food Microbiology*, 61, 1–10. https://doi.org/10.1016/S0168-1605(00)00377-9

Bryan, F. (1976). Public health aspects of cream filled pastries: A review. *Journal of Milk Food Technology*, 39, 289–296. https://doi.org/10.4315/0022-2747-39.4.289

Cha, J. O., Lee, J. K., Jang, Y. H., Yoo, J. I., Park, Y. K., Kim, B. S., & Lee, Y. S. (2006). Molecular analysis of S. aureus isolates associated with staphylococcal food poisoning in South Korea. *Journal of Applied Microbiology*, 101, 864–871.

Clinical and Laboratory Standards Institute (2011). *Performance standards for antimicrobial susceptibility testing*; 19th informational supplement. CLSI document M100-S19. Clinical and Laboratory Standards Institute, Wayne, PA.

Fallah, A. A., Saei-Dehkordi, S. S., Rahnama, M., Tahmasby, H., & Mahzoumeh, M. (2012). Prevalence and antimicrobial resistance patterns of Listeria species isolated from poultry products marketed in Iran. *Food Control*, 28, 327–332. https://doi.org/10.1016/j.foodcont.2012.05.014

Hennekinne, J. A., De Buyser, M. L., & Dragacci, S. (2012). *Staphylococcus aureus* and its food poisoning toxins: Characterization and outbreak investigation. *FEMS Microbiology Reviews*, 36, 815–836.

Hyeon, J. Y., Chung, G. T., Bing, S. H., Kwon, K. S., Lee, H. H., Kim, S. J., Jeon, S. E., Kang, Y. H., & Kim, J. (2013). A foodborne outbreak of *Staphylococcus aureus* associated with fried chicken in Republic of Korea. *Journal of Microbiology and Biotechnology*, 23, 85–87.

Johnson, W. M., Tyler, S. D., Ewan, E. P., Ashton, F. E., Pollard, D. R., & Rozee, K. R. (1991). Detection of genes for enterotoxins, exfoliative toxins, and toxic shock syndrome toxin 1 in *Staphylococcus aureus* by the polymerase chain reaction. *Journal of Clinical Microbiology*, 24, 426–430.

Jones, T. F., Kellum, M. E., Porter, S. S., Bell, M., & Schaffner, W. (2002). An outbreak of community-acquired foodborne illness caused by methicillin-resistant *Staphylococcus aureus*. *Emergent Infectious Diseases*, 8, 82–84. https://doi.org/10.3201/eid0801.010174

Jung, H. J., Cho, J. I., Park, S. H., Ha, S. D., Lee, K. H., Kim, C. H., Song, E. S., Chung, D. H., Kim, M. G., Kim, K. Y., & Kim, K. S. (2005). Genotypic and phenotypic characteristics of *S. aureus* isolated from lettuces and raw milk. *Korean Journal of Food Science and Technology*, 37, 134–141.

Kerouanton, A., Hennekinne, J. A., Lettrete, C., Petit, L., Chenèau, O., Brisabois, A., & De Buyser, M. L. (2007). Characterization of *Staphylococcus aureus* strains associated with food poisoning outbreaks in France. *International Journal of Food Microbiology*, 115, 369–375. https://doi.org/10.1016/j.ijfoodmicro.2006.10.050

Kunin, C. M. (1993). Resistance to antimicrobial drugs—A worldwide calamity. *Annals of Internal Medicine*, 118, 557–561. https://doi.org/10.7326/0003-4819-118-7-19930410-00011

Moon, J. S., Lee, A. R., Kang, H. M., Lee, E. S., Joo, Y. S., Park, Y. H., Kim, M. N., & Koo, H. C. (2007). Antibiogram and coagulase diversity in staphylococcal enterotoxin-producing *Staphylococcus aureus* from bovine mastitis. *Journal of Dairy Science*, 90, 1716–1724. https://doi.org/10.3168/jds.2006-512

Oda, T. (1998). A review of staphylococcal food poisoning in Japan. *Journal of Food Hygiene Society of Japan*, 39, 179–185.

Otto, M. (2014). Current opinion in microbiology *Staphylococcus aureus* toxins. *Current Opinion in Microbiology*, 17, 32–37. https://doi.org/10.1016/j.mimb.2013.11.004

Perez-Roth, E., Claverie-Martin, F., Villar, J., & Mendez-Alvarez, S. (2001). Multiplex PCR for simultaneous identification of *Staphylococcus aureus* and detection of methicillin and mupirocin resistance. *Journal of Clinical Microbiology*, 39, 4037–4041. https://doi.org/10.1128/JCM.39.11.4037-4041.2001

Pesavento, G., Ducci, B., Comodo, N., & Nostro, A. L. (2007). Antimicrobial resistance profile of *Staphylococcus aureus* isolated from raw meat: A research for methicillin resistant *Staphylococcus*...
aureus (MRSA). Food Control, 18, 196–200. https://doi.org/10.1016/j.
foodcont.2005.09.013
Sakai, F., Takemoto, A., Watanabe, S., Aoyama, K., Ohkubo, T., Yanahira,
S., Igarashi, H., Kozaki, S., Hiramatsu, K., & Ito, T. (2008). Multiplex
PCRs for assignment of Staphylocoagulase types and subtypes of
type VI Staphylocoagulase. Journal of Microbiology Methods, 75, 312-
317. https://doi.org/10.1016/j.mimet.2008.07.003
Sareyyüpoğlu, B., Ozyurt, M., Haznedaroğlu, T., & Ardiç, N. (2008). Detection of
methicillin and mupirocin resistance in staphylococcal hospital isolates with a touchdown
multiplex polymerase chain reaction. Folia Microbiologica (Praha), 53, 363–367. https://doi.
org/10.1007/s12223-008-0056-4
Sharma, N. K., Rees, C. E. D., & Dodd, C. E. R. (2000). Development of a
single-reaction multiplex PCR toxin typing assay for Staphylococcus
aureus strains. Applied Environmental Microbiology, 66, 1347–1353.
https://doi.org/10.1128/AEM.66.4.1347-1353.2000
Shimizu, A., Fujita, M., Igarashi, H., Takagi, M., Nagase, N., Sasaki, A., &
Kawano, J. (2000). Characterization of Staphylococcus aureus coagu-
lase type VII isolates from staphylococcal food poisoning outbreaks
(1980–1995) in Tokyo, Japan by pulsed-field gel electrophoresis. Journal of Clinical Microbiology, 38, 3746–3749.
Smith, J. P., & Simpson, B. K. (1995). Modified atmosphere packaging
of bakery and pasta products. In J. M. Farber, & K. L. Dodds (Eds.),
Principles of modified atmosphere and sous vide product packaging (p.
207). Lancaster, UK: Technomic EdszPublishing Company.
Smith, J. P., Daifas, D. P., El-Khoury, W., Koukuotsis, J., & El-Khoury, A.
(2004). Shelf life and safety concerns of bakery products—A review.
Critical Reviews in Food Science and Nutrition, 44, 19–55. https://doi.
org/10.1080/10408690490263774
Stewart, C. M., Cole, M. B., & Schaffner, D. W. (2003). Managing the risk
of staphylococcal food poisoning from cream-filled baked goods to
meet a food safety objective. Journal of Food Protection, 66, 1310–
1325. https://doi.org/10.4315/0362-028X-66.7.1310
Tenover, F. C., Gay, E. A., Frye, S., Eelis, S. J., Healy, M., & McGowan Jr, J.
E. (2009). Comparison of typing results obtained for methicillin-re-
sistant Staphylococcus aureus isolates with the DiversiLab system and
pulsed-field gel electrophoresis. Journal of Clinical Microbiology, 47,
2452–2457. https://doi.org/10.1128/JCM.00476-09
Zhan, X. Y., & Zhu, Q. Y. (2018). Evolution of methicillin-resistant
Staphylococcus aureus: Evidence of positive selection in a penicillin-
binding protein (PBP) 2a coding gene mecA. Infection, Genetics and
Evolution, 59, 16–22. https://doi.org/10.1016/j.meegid.2018.01.018

How to cite this article: Cho YS, Lee MK, Hwang SH. Toxin
gene profiles, genetic diversity, antimicrobial resistance, and
coagulase type of Staphylococcus aureus from cream-filled
bakery products. Food Sci Nutr. 2019;7:1727–1734. https://doi.
org/10.1002/fsn3.1011