Molecular Typing of Enterotoxigenic *Staphylococcus aureus* Isolated from Bovine Mastitis in Korea

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**ABSTRACT.** One hundred and sixty-six *Staphylococcus aureus* isolates from mastitic milk samples from different cows on 26 farms were investigated for staphylococcal enterotoxins (SEs) and toxic shock syndrome toxin-1 (TSST-1) by polymerase chain reaction (PCR) and reverse passive latex agglutination assay (RPLA). SEs and the TSST-1 gene were detected in thirty-seven isolates based on a multiplex PCR; SEA was detected in 32 isolates, SEB in 3 isolates, SEC in 1 isolate, and SEA and the TSST-1 gene in 1 isolate. Of the 37 enterotoxigenic isolates, thirty-three isolates were enterotoxigenic according to RPLA, where 29 isolates produced SEA, 3 isolates produced SEB, and 1 isolate produced SEC. The enterotoxin-producing *S. aureus* isolates were further characterized by pulsed-field gel electrophoresis (PFGE). A macrorestriction analysis revealed 11 PFGE patterns. Among the 33 enterotoxigenic *S. aureus* isolates, 45.4% exhibited the same PFGE pattern I. Accordingly, although the enterotoxin-producing *S. aureus* isolates from bovine mastitis were genetically diverse, 1 common genotype prevailed on the farms, indicating that PFGE pattern I isolates may be the most disseminated in Korea.

**KEY WORDS:** bovine mastitis, staphylococcal enterotoxins, *Staphylococcus aureus*.

Mastitis is an important mammary gland disease that is usually caused by bacterial infection. *Staphylococcus aureus* (*S. aureus*) is one of the major pathogens and thus of economic significance to dairy farms as it causes a reduction in milk quality and loss of production [4, 14]. Some of the *S. aureus* isolates from bovine milk have the ability to form different staphylococcal enterotoxins (SEs) and also toxic shock syndrome toxin-1 (TSST-1) [7, 10, 13]. Raw milk is a potential source of enterotoxigenic *S. aureus* in milk and milk products, especially in the case of defective pasteurization, and may also contribute to an increased udder pathogenicity of the organisms.

Many molecular epidemiological studies have already been conducted on enterotoxigenic *S. aureus* isolated from bovine milk, food, and humans [15, 17, 21]. Yet, despite this detailed genetic characterization of enterotoxigenic *S. aureus*, little is known of the enterotoxin-producing *S. aureus* isolates from cows in Korea.

Accordingly, the purpose of the current study was to investigate SEs and characterize the genotypes of enterotoxin-producing *S. aureus* isolates derived from mastitic milk from cows originating in different provinces in Korea.

A total of 166 *S. aureus* isolates were isolated from bovine mastitic milk samples (somatic cell counts (SCCs) were > 50 × 10^4 cells/ml) from 26 different farms in 8 provinces during the period from January to October 1998. The SCCs were determined with a Fossomatic milk cell counter (Milkoscan-4,000, FOSS Electric Co.). All the isolates were identified by a previously described method [16].

The bacterial strains used in the current study included standard strains of *S. aureus*, characterized as SEs (SEA, SEB, SEC, SED, and SEE), and TSST-1 producing strains, all of which were obtained from Dr. Bohach G. A. of the Idaho University, Moscow 83844, U.S.A. The types of SEs and TSST-1 produced by *S. aureus* were determined with an SET-RPLA kit (staphylococcal enterotoxin A, B, C, D detection kit by reverse passive latex agglutination; Oxoid, Basingstoke, Hampshire, UK) and the multiplex PCR method [21].

The isolation of chromosomal DNA and cleavage with a restriction enzyme were performed as described previously [9]. The macrorestriction analysis of the chromosomal DNA from the cultures was performed with the restriction enzyme SmaI (Promega Corp, Madison, U.S.A.), and the subsequent pulsed-field gel electrophoresis was performed with a 1% agarose gel in a GenePath system (Bio-Rad Laboratories Inc, Hercules, CA) in a 0.5 × Tris-borate-EDTA buffer at 14°C. After the PFGE, the gel was stained with ethidium bromide, washed with distilled water, and photographed under UV light. Lambda DNA (Bio-Rad) was used as the standard size markers, and the PFGE patterns were interpreted based on the criteria of Tenover et al. [20].

The SEs and TSST-1 production of the 166 *S. aureus* isolates is summarized in Table 1.

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The SEs and TSST-1 gene were detected in thirty-seven isolates based on a multiplex PCR, where SEA was detected in 32 isolates, SEB in 3 isolates and SEC in 1 isolate. In addition, SEA and the TSST-1 gene were both detected in 1 isolate. Table 1 also shows the discrepancy in enterotoxigenic type detection when results from SET-RPLA and the PCR method were compared.

Of the 37 enteroxigenic isolates, thirty-three isolates...
were enterotoxigenic according to SET-RPLA, where 29 isolates produced SEA, 3 isolates produced SEB, and 1 isolate produced SEC.

The enterotoxin studies were performed by PCR amplification of the respective genes. Previously, a correlation was found between PCR results and the detection of SEs when using commercial reversed-passive latex agglutination assays [1]. Nevertheless, the discrepancies in Table 1 may have been due to the fact that the SET-RPLA kit detects the enterotoxin itself, whereas the PCR method detects its gene. Under certain circumstances, SEs may not be produced or only produced to a certain level below the detection limit of SET-RPLA [2, 6]. The production of SEs and TSST-1 by S. aureus strains associated with bovine mastitis has already been described elsewhere [3, 9, 11]. The importance of the toxin formation by S. aureus in relation to udder pathogenesis remains unclear, but superantigenic toxins would seem to induce immunosuppression in dairy animals [4]. The predominance of specific enterotoxin types among S. aureus from bovine milk is variable. Genigeorgi’s review concluded that there is no prevailing type of SEs, apart from the strains isolated from foods involved in staphylococcal gastroenteritis, where SEA is the main type of SE recovered [8]. Previous data also indicate that SEA is mostly involved in outbreaks of staphylococcal food poisoning [5]. In the current study, most of the enterotoxigenic S. aureus isolates were type A, thereby constituting a health hazard to consumers, especially in the case of defective pasteurization, but the possible involvement of these toxins in other staphylococcal disease is not well documented.

Recently, a molecular typing method for PFGE has been applied extensively to differentiate among S. aureus strains [19, 22]. The 33 enterotoxigenic S. aureus isolates in the present study were further analyzed for their epidemiological relationship by using PFGE. The restriction patterns for all 33 isolates of S. aureus are shown in Fig. 1 and Table 2. Digestion of the chromosomal DNAs of the 33 enterotoxigenic S. aureus isolates with the restriction enzyme SmaI revealed 11 different restriction patterns (I-IX). All the SEA-producing isolates exhibited 9 PFGE patterns, whereas...
the 3 SEB-producing isolates exhibited 2 different PFGE patterns. Therefore, the current study demonstrated the possibility of different genotypes among enterotoxigenic S. aureus isolates from different cows, but, this heterogeneity was minor. Fifteen of the enterotoxigenic S. aureus isolates exhibited a PFGE pattern I, and pattern áT was found to prevail in eight out of the twelve farms, including farms in geographically separated regions of Korea. The SEA-producing S. aureus isolates from geographically distant locations showed a considerable genetic diversity, yet PFGE pattern áT was the most disseminated among the isolates. Thus, attention should be given to the dissemination of enterotoxigenic S. aureus PFGE pattern 1 isolates.

REFERENCES

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