**J. Dairy Sci. 90:1716–1724**
**doi:10.3168/jds.2006-512**

# Antibiogram and Coagulase Diversity in Staphylococcal Enterotoxin-Producing *Staphylococcus aureus* from Bovine Mastitis

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## ABSTRACT

We investigated antibiogram and coagulase gene diversity in staphylococcal enterotoxin (StE)-producing *Staphylococcus aureus* isolated from raw milk samples of cows infected with mastitis from 140 dairy farms in Korea between 1997 and 2004. Of the 696 *Staph. aureus* isolates collected in this study, 164 isolates (23.6%) produced one or more staphylococcal enterotoxins (A to D), and 19 isolates (2.7%) were methicillin-resistant. The percentage of StE-producing *Staph. aureus* (SES) isolates resistant to methicillin, kanamycin, neomycin, amikacin, and tetracycline was greater than that of non-SES. Ten coagulase genotype patterns were observed, including 4 main types comprising I (25.4%), II (13.9%), VII (13.2%), and VIII (17.8%). More than 4 *Staph. aureus* types were isolated from each of 82 dairy farms in different geographic locations, and only 1 coagulase genotype pattern was observed in 39 of the herds (47.6%). There was no significant correlation between coagulase genotypes harbored by *Staph. aureus* and their specific StE type. The percentage of isolates producing major StE types (A, B, AC, and ABCD) and being resistant to cephalothin and methicillin was greater among the *Staph. aureus* isolates with the 4 predominant coagulase genotypes (I, II, VII, and VIII) than among the isolates harboring the 6 rare coagulase types (III, IV, V, VI, IX, and X). Based on coagulase gene polymorphisms, our data indicate that a broad distribution of identical or closely related enterotoxin-producing *Staph. aureus* strains seem to contribute to bovine mastitis in the Republic of Korea.

**Key words:** bovine mastitis, *Staphylococcus aureus*, enterotoxin, coagulase polymorphism

## INTRODUCTION

Bovine mastitis is a major disease that affects the dairy industry, and *Staphylococcus aureus* is one of the most frequently isolated pathogens from both subclinical and chronic infections (Watts, 1988; Bramley, 1992). Some *Staph. aureus* isolates from bovine milk carry different staphylococcal enterotoxins (**StE**) or toxic shock syndrome toxin-1 (Kenny et al., 1993; Matsunaga et al., 1993). These toxins are responsible for food poisoning outbreaks and toxigenic syndrome in humans respectively (Llewelyn and Cohen, 2002); they may also contribute to the persistence of *Staph. aureus* in bovine mammary glands and increased udder pathogenicity (Ferens et al., 1998).

Several reports (Larsen et al., 2000; Stephan et al., 2001; da Silva et al., 2005) have noted that the production of StE in *Staph. aureus* isolated from bovine mastitis may be determined by environmental and management factors in each geographical area. This genetic variability in StE production contributed to the emergence of distinct epidemiological profiles that were dependent on predominant strains within a herd. It indicates the necessity to identify such strains or subtypes before applying specific measures of mastitis control (Larsen et al., 2000; Stephan et al., 2001; da Silva et al., 2005). In addition, the staphylococcal enterotoxin C (SEC)-producing strains have been isolated frequently from bovine mastitis in northeast Switzerland (Stephan et al., 2001), Brazil (da Silva et al., 2005), and Japan (Katsuda et al., 2005). Recently, the occurrence of new types of StE (SEG to SER and SEU) has been reported, but the relationship between these new StE and bovine mastitis has not been established (Katsuda et al., 2005).

Many molecular epidemiological studies have already been conducted on enterotoxigenic *Staph. aureus* isolated from bovine milk, food, and humans (Tsen and Chen, 1992; Cremonesi et al., 2005; Boerema et al., 2006). Polymerase chain reaction and PCR-RFLP analysis of the 3′ end of the gene encoding staphylococcal coagulase (*coa*) have been proposed as methods for typing *Staph. aureus* isolates for epidemiological study. It was previously described that varying numbers (3 to 9) of 81-bp tandem repeats in the *coa* gene determined sequence analysis (Lange et al., 1999; Scherrer et al.,

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Received August 5, 2006.
Accepted November 6, 2006.
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2004). Amplified DNA fragments of different sizes can be further discriminated by digestion with *Alu*I (Goh et al., 1992). This PCR-based genotyping method has provided detailed epidemiological information about *Staph. aureus* (Schwarzkopf and Karch, 1994; Hookey et al., 1998).

In the last few years, the results of epidemiologic research based on the polymorphism of the *coa* gene in different countries indicate that a few *Staph. aureus* subtypes are responsible for most cases of bovine mastitis, and that these clones have a broad geographic distribution (Fitzgerald et al., 1997; Annemüller et al., 1999; Lange et al., 1999; da Silva and da Silva, 2006). Despite this detailed genetic characterization of StE-producing *Staph. aureus* (**SES**) in other countries, little is currently known of SES isolates from bovine milk in the Republic of Korea. An understanding of this epidemiological relationship is also necessary for the design of more effective mastitis control programs against enterotoxigenic *Staph. aureus*. Based on the previous experience in epidemiological investigation by PCR-RFLP analysis of the *coa* gene of *Staph. aureus* isolated from bovine mastitis in our study (Moon et al., 2003, 2007; Lim et al., 2004), we characterized subtypes and antibiograms of SES isolates derived from bovine mastitis milk collected from different provinces of the Republic of Korea by PCR-based subtying of the *coa* gene.

## MATERIALS AND METHODS

### *Milk Sampling*

One hundred forty herds located in the Republic of Korea (Table 1) with mastitis problems (bulk milk SCC greater than 200,000 cells/mL) were selected for possible further study from August 1997 to December 2004. The SCC was measured by using a Milkoscan 4000 (Foss Electric Co., Hillerød, Denmark). Milk samples were collected using aseptic technique from individual quarters of the cows that were suspected to have subclinical mastitis as detected by high SCC and clinical observation, and the samples were cultured according to standard protocols suggested by the National Mastitis Council (Harmon et al., 1990).

### *Isolation and Identification of Staph. aureus and Enterotoxin Production*

*Staphylococcus aureus* was isolated from milk samples in dairy herds according to the protocols of the National Mastitis Council (Harmon et al., 1990). An aliquot of 10 μL from each sample was spread over blood agar plates (Bacto-Agar, Difco, Detroit, MI) containing 5% washed sheep erythrocytes and incubated at 37°C for 24 h. Colonies suspected of being staphylococci

**Table 1.** Distribution of staphylococcal enterotoxin (StE)-producing or methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from mastitic milk of dairy cows from 1997 to 2004 in the Republic of Korea[1]

| | Region | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Gyeonggi | | Gangwon | | Chung cheong | | Jeolla | | Gyeong sang | | Total (all isolates) | | Total MRSA | | Total StE-producing isolates[2] | |
| Interval | Herds | Isolates | Herds | Isolates | Herds | Isolates | Herds | Isolates | Herds | Isolates | Herds | Isolates | Herds | Isolates | Herds | Isolates |
| 1997–1998 | 9 | 56 | 1 | 6 | 11 | 99 | 3 | 14 | 1 | 4 | 25 | 179 | 0 | 0 | 19 | 62 |
| 1999–2000 | 22 | 89 | 6 | 11 | 13 | 47 | 8 | 26 | 3 | 6 | 52 | 179 | 10 | 17 | 28 | 57 |
| 2001–2002 | 10 | 24 | 0 | 0 | 5 | 16 | 4 | 8 | 1 | 1 | 20 | 49 | 0 | 0 | 11 | 11 |
| 2003–2004 | 20 | 135 | 11 | 80 | 11 | 73 | 0 | 0 | 1 | 1 | 43 | 289 | 1 | 2 | 25 | 34 |
| Total | 61 | 304 | 18 | 97 | 40 | 235 | 15 | 48 | 6 | 12 | 140 | 696 | 11 | 19 | 83 | 164 |

[1] Among the total herds examined at this study, only the number of herds with the isolation of *Staph. aureus* isolates is indicated here.

[2] Only the production of StE from A to D (SEA to SED) was examined using reverse passive latex agglutination (SET-RPLA kit, Oxoid, Ltd., Basingstoke, UK) according to the kit manufacturer's instructions.