NOTE *Bacteriology*

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

Suk-kyung LIM[1)], Yi-seok JOO[1)], Jin-san MOON[1)], Ae-ri LEE[1)], Hyang-mi NAM[1)], Sung-hwan WEE[1)] and Hong-bum KOH[2)]*

[1)]*National Veterinary Research and Quarantine Service, Ministry of Agriculture and Forestry, 480 Anyang, Kyonggido, 430–824 and* [2)]*Research Institute of Veterinary Medicine, Chonnam National University, 300, Gwangju 500–757, South Korea*

(Received 29 July 2003/Accepted 14 January 2004)

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*.

J. Vet. Med. Sci. 66(5): 581–584, 2004

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 collected from bovine mastitic milk samples (somatic cell counts (SCCs) were $> 50 \times 10^4$ cells/m*l*) 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].

* CORRESPONDENCE TO: KOH, H., Research Institute of Veterinary Medicine, Chonnam National University, 300, Gwangju, 500–757, South Korea.

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; Oxiod, 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 SmaáT (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.

SEs and the 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 entertoxigenic isolates, thirty-three isolates

Table 1. Assay of enterotoxins and toxic shock syndrome toxin-1 by reverse passive latex agglutination (RPLA) and multiplex-polymerase chain reaction for *Staphylococcus aureus* isolates from bovine mastitis in Korea

Strains	No. of strain	Staphylococcal enterotoxins and TSST-1										
		RPLA				Multiplex-PCR						
		A	B	C	D	A	B	C	D	E	TSST-1	TSST-1 + SEA
S. aureus isolates	166	29	3	1	–	32	3	1	–	–	–	1
Control												
S. aureus ATCC13565 (SEA)	1	1	–	–	–	1	–	–	–	–	–	–
S. aureus MNHOCH(SEB)	1	–	1	–	–	–	1	–	–	–	–	–
S. aureus MNDON(SEC)	1	–	–	1	–	–	–	1	–	–	–	–
S. aureus RN4220 pMIN403 (SEC-Bovine)	1	–	–	1	–	–	–	1	–	–	–	–
S. aureus FRI 472(SED)	1	–	–	–	1	–	–	–	1	–	–	–
S. aureus FRI326(SEE)	1	–	–	–	–	–	–	–	–	1	–	–
S. aureus FRIMN8(TSST-1)	1	–	–	–	–	–	–	–	–	–	1	–
S. aureus RN4220(No toxin)	1	–	–	–	–	–	–	–	–	–	–	–

–, Not detected.

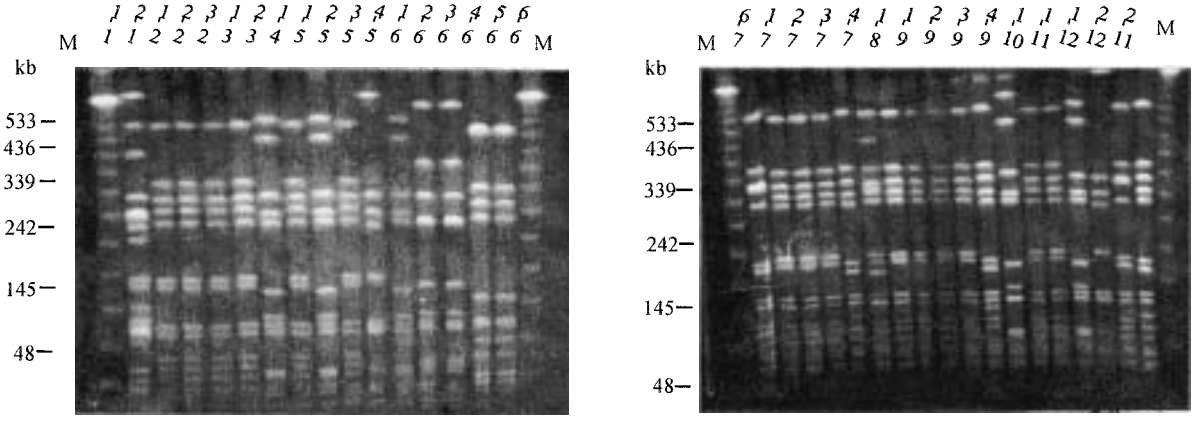

Fig. 1. Patterns of pulsed-field gel electrophoresis for *Sma*I digested genome DNA from enterotoxigenic *Staphylococcus aureus* isolates. M indicates the marker lambda ladder(kb).

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. aurues* 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 SmaáT revealed 11 different restriction patterns (I-IX). All the SEA-producing isolates exhibited 9 PFGE patterns, whereas

Table 2. PFGE patterns of enterotoxigenic *Staphylococcus aureus* isolates from bovine mastitic milk

SEs[a)]	Farm	Isolate	PFGE Pattern
	1	1 –1	II
		1 –2	I
	2	2 –1	I
		2 –2	I
		2 –3	III
	3	3 –1	IV
		3 –2	I
	4	4 –1	I
	5	5 –1	I
		5 –2	I
		5 –3	V
		5 –4	VI
	6	6 –1	I
		6 –2	I
SEA		6 –3	VII
		6 –4	VIII
		6 –5	II
		6 –6	III
		6 –7	IX
	7	7 –1	I
		7 –2	I
		7 –3	I
		7 –4	I
	8	8 –1	VII
	9	9 –1	I
		9 –2	VII
		9 –3	I
		9 –4	VIII
	10	10–1	VII
	11	11–1	X
SEB	12	12–1	XI
		12–2	XI
SEC	11	11–2	X

a) Staphylococcal enterotoxins.

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 more disseminated than others [22]. This phenomenon has two possible explanations: the pattern áT has an enhanced virulence compared to other types and/or the predominant isolates were transmitted between the study farms. The fact that the predominant genotypes were more resistant to neutrophil bactericidal activities than the rare genotypes, supports the existence of predominant *S. aureus* strains with enhanced virulence in the bovine mammary gland [19].

Furthermore, it is possible that transmission of infection occurred due to the movement of cows between the 8 farms, as the same clone was transmitted between cows on the same farm, indicating a persistent cow-to-cow spread of the infectious strains, possibly via milking machines or the hands of milkers.

The difference between the enterotoxin types that produced PFGE pattern X suggests that the acquisition of *ent* genes may have occurred by horizontal transmission (11–1, 11–2 isolates). The widespread transmission of the *ent* gene among 15 SEC-producing strains has already been studied [12], and this phenomenon may be a common mechanism for the spread of *ent* genes among *S. aureus* strains. But further investigation is needed to determine the transmission of the *ent* gene.

Consequently, although the SEs-producing isolates exhibited genetic diversity, PFGE patternáTwas the most disseminated among the isolates. Thus, attention should be given to the dissemination of enterotoxigenic *S. aureus* PFGE pattern I isolates.

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