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Relationships among superantigen toxin gene profiles, genotypes, and pathogenic characteristics of *Staphylococcus aureus* isolates from bovine mastitis

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ABSTRACT

Staphylococcus aureus is one of the major etiological agents of bovine mastitis, harboring a wide variety of staphylococcal superantigen (SAg) toxin genes. The SAg toxin genes are reported to be closely associated with the pathogenicity of the *Staph. aureus* causing the bovine mastitis. This study was conducted to investigate SAg toxin gene profiles and to assess the relationships among SAg toxin genes, genotypes of *Staph. aureus*, and their pathogenic properties. A total of 327 quarter milk samples were collected from bovine mastitis cases for isolation and identification of pathogens. In total, 35 isolates were identified as *Staph. aureus*, and the prevalence of *Staph. aureus* in milk samples was 13.6% (35/256). Polymerase chain reaction (PCR) and randomly amplified polymorphic DNA (RAPD) assays were used to detect the SAg toxin genes and to genotype *Staph. aureus* strains isolated from milk samples of bovine mastitis in 10 dairy herds located in Ningxia, China, respectively. The results showed that among the *Staph. aureus* isolates (n = 35), 71.4% (n = 25) of isolates carried at least one SAg toxin gene. In total, 18 SAg genes and 21 different gene combination patterns were detected among these isolates. The most common SAg genes in *Staph. aureus* isolates were *sei*, *sen*, and *seu* (44.0% each), followed by *seo*, *tst*, and *etB* (28.0% each), *etA* (24.0%), *sem* and *sep* (16.0% each), *seb*, *sec*, *sed*, and *sek* (12.0% each), and *sea* and *seh* genes (8.0% each); the *seg*, *sej*, and *ser* genes were present in 4.0% of the isolates. Three gene combinations were found to be related to mobile genetic elements that carried 2 or more genes. The egc-cluster of the *seg-sei-sem-sen-seo* genes, located on the pathogenicity island Type I υSaβ, was detected in 16% of isolates. Interestingly, we observed 6 RAPD genotypes (I to VI) in *Staph. aureus* isolates, and 2 of these genotypes were strongly associated with the severity of bovine mastitis; there was a close relationship between the RAPD genotypes and SAg genes. Isolates of RAPD type III were more frequently associated with clinical and subclinical mastitis, whereas strains of type VI were mostly related to subclinical mastitis. In addition, SAg genes were related to severity of bovine mastitis. We conclude that an obvious relationship exists among RAPD genotypes, SAg toxin genes, and severity of bovine mastitis.
Key words: *Staphylococcus aureus*, bovine mastitis, superantigen toxin genes, PCR

INTRODUCTION

Staphylococcus aureus is a global pathogen causing several serious diseases in humans and animals. This microorganism is also one of the most common etiological agents of bovine mastitis, which results in huge economic losses to the dairy industry due to the reduced milk quality and production (Deb et al., 2013; Gomes and Henriques, 2016). Importantly, the enterotoxins produced by *Staph. aureus* are common causes of food poisoning globally (Li et al., 2015; Sabouni et al., 2014). *Staphylococcus aureus* has a strong capability to produce a wide variety of enterotoxins, including toxic shock syndrome toxin 1 (**TSST-1**), Panton-Valentine leukocidin (**PVL**), exfoliative toxins A and B (ETA and ETB), and staphylococcal enterotoxins (SE) including classical enterotoxins (SEA to SEE) with proven emetic activity and newly described enterotoxins, such as staphylococcal-like proteins (SEIL, SEIQ, SEG to SER) without emetic activity. To date, 23 serologically different staphylococcal enterotoxin and staphylococ-

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cal-like proteins have been recognized (Pinheiro et al., 2015; Podkowik et al., 2016). Collectively, these toxins are known as staphylococcal superantigens (**SAg**) because of their superantigenic activity. These SAg toxin genes have been reported to be associated with the virulence factors in bovine IMI, and these toxins may have an important role in the pathogenesis of bovine mastitis (Aman and Adhikari, 2014; Reyes-Robles et al., 2016). Recent studies have reported that SAg toxin genes are associated with mobile genetic elements such as plasmids, pathogenicity islands, and prophages, suggesting the horizontal transfer of these genes among the bacteria (Malachowa and DeLeo, 2010; Alibayov et al., 2014). In addition, staphylococcal food poisoning, resulting from the consumption of contaminated food with staphylococcal enterotoxins, is one of the most common food-borne illnesses (Suzuki et al., 2015; Corredor Arias et al., 2016).

Considerable genetic heterogeneity in natural populations of *Staph. aureus* has been documented, and a small number of clonal types not only cause the majority of bovine IMI but also have a wide geographical distribution (Silva et al., 2013; Lundberg et al., 2014). As different pathogenic properties of the pathogens require different strategies toward the prevention and treatment of the disease, research on the genotypes of *Staph. aureus* causing bovine IMI and their pathogenic properties is of particular clinical importance.

Although a large number of virulence factors have been found in *Staph. aureus* isolates from bovine IMI, very few studies have investigated the relationships between subtypes of *Staph. aureus* isolates from bovine IMI and their pathogenic properties. Thus, the present study was carried out to determine SAg toxin genes profiles and randomly amplified polymorphic DNA (**RAPD**) genotypes of *Staph. aureus* isolates and to analyze the relationships among SAg toxin genes, genotypes, and pathogenic characteristics of *Staph. aureus* isolates from bovine mastitis in the dairy herds of Ningxia province of China.

MATERIALS AND METHODS

Bacterial Isolates

Previously isolated *Staph. aureus* isolates (n = 35) from cases of bovine mastitis (Wang et al., 2016) were used in the present study. A total of 327 quarter milk samples were collected from cows with clinical and subclinical mastitis between March and October 2013, which came from 10 herds in 4 geographic regions (Yinchuan, Wuzhong, Shizuishan, and Zhongwei) of Ningxia province (Figure 1), located in northwest China. The majority of cows in the study herds were Chinese Holstein and were housed in freestall barns with straw, sawdust, or sand as bedding. The average size of the herds was 253 cows, and their average yield was 8,540 kg of milk per lactation. A total of 2,527 cows were involved in the epidemiological investigation of bovine mastitis. Among them, healthy cows accounted for 50.3% (1,272/2,527), the prevalence of clinical mastitis was 13.2% (333/2,527), and the incidence of subclinical mastitis at the cow and quarter level were 36.5% (922/2,527) and 17.8% (1,794/10,108), respectively. Bacterial isolates were identified based on morphological, biochemical, and molecular methods as described previously (Wang et al., 2016). Briefly, after incubation on blood agar, the isolates produced grayish-white or yellow colonies with distinct zones of hemolysis. They were gram-positive cocci and produced catalase, coagulase, and DNase. All the isolates were confirmed by using the PCR amplification of 16S rRNA gene specific to *Staph. aureus*.

DNA Isolation and Purification

Each *Staph. aureus* isolate was grown overnight at 37°C in brain heart infusion broth (Oxoid, Basingstoke, UK). The genomic DNA of the isolate was extracted and purified using TIANamp Bacteria DNA Kit (Tiangen Biotech, Beijing, China) according to the manufacturer's instruction.

Detection of SAg Toxin Genes

The presence of genes encoding enterotoxins (*se*), TSST-1(*tst*), Panton-Valentine leukocidin (*PVL*), and exfoliative toxins (*etA* and *etB*) in the entire set of *Staph. aureus* isolates (n = 35) was tested by PCR assays. The SAg toxin genes, primer sequences, and the expected size of PCR products are shown in Table 1. The reaction mixture (25 μL) for each PCR contained 50 ng of genomic DNA, 20 pmol of each primer, and 12.5 μL of 2× Taq PCR MasterMix (Tiangen Biotech). Amplification was carried out with the following cycling conditions: a predenaturation step at 94°C for 3 min, followed by 30 cycles of amplification (denaturation at 94°C for 30 s, annealing for 30 s at specific temperature, and extension at 72°C for 1 min), and ending with a final extension at 72°C for 5 min. All PCR-amplified products were characterized by 0.8% agarose gel electrophoresis in 1× Tris-acetate-EDTA buffer, followed by staining with Gel Red and visualization under UV light. The *Staph. aureus* strain ATCC29213 was used as the positive control and the negative control (only sterile water) was used in each PCR reaction.