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Prevalence and characterization of *Staphylococcus aureus* isolated from goat milk powder processing plants

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ABSTRACT

Staphylococcus aureus is a major concern for the food processing industry because of its virulence factors. The aim of this study was to determine the prevalence of *S. aureus* strains in goat milk powder processing plants, and to characterize these strains by antimicrobial susceptibility testing, pulsed-field gel electrophoresis (PFGE) and PCR. PCR detected genes encoding staphylococcal enterotoxin (*sea* to *set*), staphylococcal enterotoxin-like (*selj* to *selv*), toxic-shock syndrome toxin-1 (*tst*), exfoliative toxin (*eta* and *etb*), Paton-Valentine Leukocidin (*pvl*), and methicillin resistance (*mec*A). A total of 910 samples including 62 raw goat milk samples from a milking station and 848 samples from seven different sampling sites in four goat milk powder plants were collected. Out of 910 samples, 95 (10.4%) samples, including 34 (54.8%) of 62 milking station samples and 61 (7.2%) of 848 milk powder processing plant samples, were positive for *S. aureus*. 63.2% of 95 isolates contained one or more virulence genes. The five most predominant virulence genes were *pvl* (29.5%), *sec* (23.2%), *ser* (16.8%), *tst* (14.7%), and *seb* (12.6%). 90.5% of strains were resistant to at least one antibiotic. Resistance was most frequently observed to trimethoprim/sulfamethoxazole (89.5%), erythromycin (30.5%), tetracycline (22.1%), ampicillin (16.8%), chloramphenicol (15.8%), and rifampicin (9.5%). A total of 44 PFGE patterns were generated among all the isolates. PFGE demonstrated that some isolates with the same PFGE patterns came from different goat milk powder processing stages. Our findings indicate that *S. aureus* has spread among different goat milk powder producing locations. In addition, cross-contamination of *S. aureus* exists in goat milk powder processing stages. The presence of *S. aureus* strains in goat milk powder processing stages poses a potential threat to public health.

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1. Introduction

Staphylococcus aureus is a notorious pathogenic microorganism that causes foodborne poisoning and infections both in humans and animals (Wang et al., 2012). Some *S. aureus* strains can express a large number of virulence factors including staphylococcal enterotoxins (SEs), staphylococcal enterotoxin-like (SEls), exfoliative toxin A and B (*eta* and *etb*) and toxic shock syndrome toxin-1 (*tst*) genes. SEs or SEls are well known as a major cause of food poisoning (Argudin, Mendoza, & Rodicio, 2010). Due to its ubiquitous nature, *S. aureus* commonly exists in food processing environments, including dairy production plants (Jorgensen, Mork, Hogasen, & Rorvik, 2005). Previous studies have also reported that raw milk is a potential reservoir for *S. aureus* (Fusco & Quero, 2014), and this bacterium has also been isolated from the environments and workers of dairy farms (Lim et al., 2013).

Due to its low sensitization and nutrition, goat milk is mostly being used as a raw material for infant formula. However, milk powder is easily contaminated with *S. aureus* (Wang et al., 2012). Although pasteurization can kill *S. aureus* cells, enterotoxins produced by *S. aureus* can still cause foodborne diseases in humans (Yamashita et al., 2003). In addition, studies have reported that some *S. aureus* strains persist in powdered infant formula (Wang et al., 2012). These may increase the risk of food poisoning for consumers.

Previous studies on foodborne pathogens in dairy processing focused on *Listeria monocytogenes* and *Escherichia coli* O157:H7 (Almeida et al., 2013; Barancelli et al., 2014; Cagri-Mehmetoglu et al., 2011). However, reports on *S. aureus* in goat milk powder

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processing plants are relatively scanty. Therefore, the objective of this work was to determine the prevalence of *S. aureus* strains in raw goat milk and goat milk powder processing plants, and to characterize these strains by determining antimicrobial susceptibility, virulence genes, and pulsed-field gel electrophoresis (PFGE) profiles.

2. Material and methods

2.1. Sample collection and isolation of S. aureus

From September 2012 to March 2013, a total of 848 samples from seven different sampling sites in four goat milk powder plants were collected. There were 195 samples from plant A, 240 samples from plant B, 241 samples from plant C, and 172 samples from plant D. Seven different sampling sites including tank milk, pre-spray drying areas, spray drying areas, powder-packaging room, ground and wall, workers, and final products were considered. Pre-spray drying areas included air filter, stabilization tank, milk clarifier and balance tank. Spray drying areas included spray dryer, spray drying tower, fluidized bed, cooling bed and vibrating sieve. The goat milk powder samples analyzed in this study included semi finished (100 g or 100 mL/sample) and finished (100 g/sample) products in four goat milk powder processing plants. The swab samples were collected from equipment, ground, wall, and workers during processing and packaging in four goat milk powder processing plants. In addition, a part of raw milk in four goat milk powder plants were collected from one goat milking station, there were 62 raw milk samples from the goat milking station. Isolation and identification of *S. aureus* were performed as described previously by Wang et al. (2012). Briefly, 25 g of milk powder or 25 mL of milk sample was placed into a sterile triangle glass bottle containing 225 mL of buffered peptone water (BPW, Beijing Land Bridge Technology Ltd., Beijing, China), and all swabs were placed into a sterile 50 mL flat bottom tube containing 30 mL of trypticase soy broth (TSB) (Beijing Land Bridge Technology Co. Ltd., Beijing, China). The solution was incubated at 37 °C in an air bath with shaking at 100 rpm for 24 h. After pre-enrichment, 3 mL aliquot was transferred into a sterile 50 mL flat bottom tube containing 30 mL of trypticase soy broth (TSB, Beijing Land Bridge Technology Ltd., Beijing, China) containing 7.5% NaCl. After 18–24 h incubation at 35 °C, a loopful culture was inoculated onto Baird–Parker agar (BPA, Beijing Land Bridge Technology Ltd., Beijing, China) plates with 5% egg yolk and tellurite. Following incubation at 35 °C for 24 h, one or two presumptive coagulase-positive colonies per sample (black colonies surrounded by 2–5 mm clear zones) were transferred to Trypticase soy agar (TSA, Beijing Land Bridge Technology Ltd., Beijing, China) plates for further purification. Colonies were then confirmed as *S. aureus* by PCR (Hema PCR system 9600, Zhuhai, China) detection of the thermonuclease gene (*nuc, S. aureus* specific) (Brakstad, Aasbakk, & Maeland, 1992). All isolates were stored at −80 °C until use.

2.2. DNA extraction and PCR for virulence and mecA genes

DNA of each isolate was obtained by boiling method. Briefly, all strains recovered on TSA plate at 37 °C overnight. Approximately, 10–20 colonies were obtained with pre-moistened cotton swabs and suspended into 700 μl distilled water. The mixture was boiled at 100 °C for 20 min. After centrifugation at 13,000 rmp for 5 min, supernatants were transferred to a new 1.5 mL collection tube. All the DNA templates stored at −40 °C until use with PCR amplification.

All isolates were screened for virulence and *mecA* genes by PCR amplification (Hema PCR system 9600, Zhuhai, China). The information of all primers used for PCR is shown in Table 1, and the references of all primers were shown in supplementary materials. The primers were synthesized by TaKaRa Biotechnology Co., Ltd (Dalian, China). The genes encoding enterotoxins (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *ser*, *ses*, and *set*), enterotoxin-like (*selj*, *selk*, *sell*, *selm*, *seln*, *selo*, *selp*, *selq*, *selu*, *and selv*), toxic-shock syndrome toxin-1 (*tst*), exfoliative toxin genes (*eta* and *etb*), Paton-Valentine Leukocidin (*pvl*), and methicillin resistance (*mec*A) were detected by PCR. PCR products were visualized by UV transillumination (Universal Hood II, Bio-Rad, Italy) after electrophoresis on 1.0% (w/v) agarose gels (Hydragene) with 0.5 mg/ml ethidium bromide (Sigma) in 0.5 × TBE buffer by a DYY-6C electrophoresis system (Beijing Liuyi Instrument Factory, Beijing).

2.3. Pulsed-field gel electrophoresis (PFGE)

PFGE was performed for *S. aureus* isolates according to a standard protocol developed by Pulse Net for *S. aureus* (McDougal et al., 2003). Briefly, agarose-embedded DNA was digested with 50 U of *Sma*I for 3 h in a water bath at 30 °C. DNA fragments were separated by electrophoresis in 0.5 × TBE buffer at 14 °C for 21 h on a CHEFIII Mapper electrophoresis system (Bio-Rad Laboratories, Richmond, CA, USA) with pulse time of 5–40 s. The gels were stained with ethidium bromide and images were taken under UV transillumination. The images were analyzed with BioNumerics Software (Applied Maths, Kortrijk, Belgium) by using Dice coefficients and unweighted pair group method with arithmetic averages (UPGMA) to achieve dendrograms with an optimization value of 0.5% and a 1.5% band position tolerance. *Salmonella* serotype Branderup strain H9812 digested with *Xba*I was used as a molecular size marker.

2.4. Antimicrobial susceptibility testing

According to the agar dilution method described by Clinical Laboratory Standards Institute (CLSI, 2012), a total of 14 antibiotics, including erythromycin (ERY, ≥8 μg/mL), oxacillin (OXA, ≥4 μg/mL), cefoxitin (FOX, ≥8 μg/mL), cefoperazone (CFP, ≥64 μg/mL), vancomycin (VAN, ≥32 μg/mL), tetracycline (TET, ≥16 μg/mL), chloramphenicol (CHL, ≥32 μg/mL), trimethoprim/sulfamethoxazole (SXT, ≥8/152 μg/mL), ciprofloxacin (CIP, ≥4 μg/mL), amikacin (AMK, ≥32 μg/mL), ampicillin (AMP, ≥32 μg/mL), rifampicin (RIF, ≥4 μg/mL), gatifloxacin (GAT, ≥2 μg/mL), and amoxicillin-clavulanic acid (AMC, ≥8/4 μg/mL) were tested. *S. aureus* ATCC 29213 and *E. coli* ATCC 25922 were used as quality control in each run.

2.5. Statistical analysis

The Chi-square ($\chi 2$) tests were performed with SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, USA) for Windows and a probability value of less than 5% was considered to be significant.

3. Results

3.1. Prevalence of S. aureus

Out of 910 samples, 95 (10.4%) samples were positive for *S. aureus*, including 34 (54.8%) of 62 milking station samples and 61 (7.2%) of 848 milk powder processing plant samples. Among the 61 *S. aureus*-positive milk powder plant samples, 13 (6.7%; 13/195) samples were from plant A, followed by 12 (5.0%; 12/240) samples from plant B, 30 (12.4%; 30/241) samples from plant C, and 6 (3.5%; 6/172) samples from plant D. In addition, 16 *S. aureus*-positive samples were found in spray drying areas, followed by 13 samples

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