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OCCURRENCE OF *STAPHYLOCOCCUS AUREUS* IN RAW MILK PRODUCED IN DAIRY FARMS IN SÃO PAULO STATE, BRAZIL

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ABSTRACT

The objective of the present study was to evaluate the occurrence of *Staphylococcus aureus* in milk produced in 37 farms located in the regions of Ribeirão Preto and São Carlos, state of São Paulo, Brazil. Two-hundred and eight samples of milk from individual cows showing subclinical mastitis, and 37 samples of bulk tank milk were analyzed. *S. aureus* strains were detected in 18 (7.3%) milk samples: 14 (6.7%) from samples of individual cows, and 4 (10.8%) from bulk tank milk. Two individual milk samples (14.3%) and two bulk milk samples contained enterotoxigenic *S. aureus*. PFGE analysis revealed the genetic heterogeneity of the strains isolated from raw milk, which presented to 13 *S. aureus* patterns. Results confirmed the potential transmission of staphylococcal food poisoning to consumers via milk of cows affected by subclinical mastitis, mainly when raw milk is ingested.

Key words: *Staphylococcus aureus*, raw milk, bovine, mastitis, enterotoxin

INTRODUCTION

Raw milk is an ideal growth medium for several microorganisms. Milk and its derivates are considered vehicles of *Staphylococcus aureus* for infection in humans (19). *S. aureus* is an important foodborne pathogen and causes a wide variety of diseases in humans and animals, ranging in severity from a mild skin infection to more severe diseases, such as pneumonia and septicemia (12). In dairy cattle, *S. aureus* is frequently associated with subclinical mastitis (1) and may contaminate milk and other dairy products (4).

S. aureus produces several staphylococcal virulence factors, including enterotoxins (SEA to SEE and SEG to SEQ), and other toxins, such as exfoliative toxin A and B, and toxic shock syndrome toxin (TSST-1) (8). Staphylococcal food poisoning (SFP) is recognized as a cause of foodborne diseases (10).

In Brazil, although the correct number of SFP outbreaks is unknown, previous studies indicated that raw milk, as well as dairy products manufactured from raw milk, play important roles in human outbreaks (18).

Mammary glands infected by *S. aureus* are the main cause

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of milk contamination (9). Contaminated milking equipment and the hands of the milkers are also common vehicles of transmission (7). Although pasteurization kills *S. aureus* cells, thermostable SEs generally retain their biological activity. SFP risk related to pasteurized milk products may be minimized by adequate chilling of raw milk until heat treatment, followed by rigorous efforts to prevent recontamination (10).

The increase in milk production observed in Brazil in recent years has resulted in improvements in several management techniques for dairy cattle, and has been followed by investments in the improvement of the microbiological quality of milk on dairy farms. However, little is known about the presence of mastitis-causing pathogenic bacteria in refrigerated raw milk from dairy cows.

The aim of present study was to investigate the occurrence of *S. aureus* and presence of enterotoxins in strains isolated in raw milk from individual dairy cows with subclinical mastitis and in bulk tank milk from dairy farms located in São Paulo, Brazil.

MATERIAL AND METHODS

Sampling

The study was conducted in 17 dairy farms located in the region of São Carlos and 20 farms in Ribeirão Preto, from February 2005 to March 2006. On each farm, individual milk samples were obtained from all cows showing signs of subclinical mastitis, as defined by the California Mastitis Test (CMT) (15). Triplicate samples (100 mL) were collected aseptically from the individual receptors of the milking machine during the first milking in the morning. At the end of the milking procedure, triplicate bulk tank milk samples (100 mL) were also collected in sterile glass flasks. Two hundred eight samples of milk from individual cows and thirty-seven samples of bulk tank milk were aseptically collected, and transported to the laboratory in coolers with ice (4-8^{o}C), and analyzed the same day.

Isolation and identification of *S. aureus*

Bacteriological methods used for isolation and identification of *S. aureus* were performed according to Silva *et al.* (16). Triplicate samples of milk were combined into one sample which was then submitted for analysis. Samples were plated on Baird Parker agar (Oxoid) supplemented with egg yolk and tellurite emulsion (1%) (Oxoid). Colonies suggestive colonies of *S. aureus* were submitted for the following tests: coagulase, catalase, DNAse, acetoin production and maltose fermentation (without 1 gas production) (3). Typical colonies were gray to black (potassium tellurite reaction) and were surrounded by clear zones (egg yolk reaction). Atypical colonies were gray to black, but did not show the clear zone (4).

Detection of enterotoxigenic *S. aureus* strains

S. aureus strains from typical and atypical colonies were tested for the production of enterotoxins A, B, C, D and TSST-1 by means of reverse passive latex agglutination (RPLA) (Oxoid), according to Igarashi et al. (1986). RPLA kits were used according to the manufacturer's instructions, without quantification. Agglutination was visually scored against a dark background after 24 hours of incubation at room temperature (17).

Molecular identification of *S. aureus* strains

All the *S. aureus* strains were subjected to chromosomal DNA and restriction endonuclease digestion according to McDougal *et al.* (13). Electrophoresis was carried out with 1% chromosomal grade agarose gel (Bio-Rad Laboratories, Munich, Germany) (size 13 cm × 0.55 cm) in 0.5× Tris-buffered saline and stained with ethidium bromide, using the CHEF-DR III pulsed-field electrophoresis system (Bio-Rad Laboratories). A 0.1–200 kb ladder (Low Range PFGE Marker, BioLabs, Schwalbach, Germany) and a 50–1000 kb ladder (Lambda Ladder PFGE Marker, BioLabs) were used as standards.

RESULTS AND DISCUSSION

Occurrence of *S. aureus* in raw milk of dairy farms in the state of São Paulo analyzed in this study are presented in Table 1. *S. aureus* was found in 18 (7.3%) milk samples. In

individual milk samples, *S. aureus* strains were found in 14 (6.7%) of the samples. Strains isolated from two (14.3%) samples were enterotoxigenic. From the 37 bulk milk samples, 4 (10.8%) were positive for *S. aureus*, and strains from 2 (50.0%) of them were enterotoxigenic. In a survey conducted by Brabes *et al*. (3) on dairy farms from the states of São Paulo and Minas Gerais, the mean incidence of *S. aureus* in individual milks from cows with mastitis was approximately 10.40%, which is much higher than the frequency observed in this study. However, a comparison of the results of the present study and those reported by other authors is difficult, because the occurrence of *S. aureus* as a causative agent of subclinical mastitis varies according to the area, handling practices of the animals, and hygienic conditions during milking, among other factors (9, 19).

Table 1. Occurrence of *S. aureus* in raw milk samples from the regions of São Carlos and Ribeirão Preto, state of São Paulo, Brazil, collected from February of 2005 to March of 2006.

Origin of milk samples	Number of samples analyzed	Number of samples showing *S. aureus* (%)	Number of toxigenic *S. aureus* (%) out of *S. aureus* isolates
Region of São Carlos:			
Individual cow	93	3 (3.2)	0 (0)
Bulk milk	17	2 (11.8)	1 (50.0)
Region of Ribeirão Preto:			
Individual cow	115	11 (9.6)	2 (18.2)
Bulk milk	20	2 (10.0)	1 (50.0)
All Regions:			
Individual cow	208	14 (6.7)	2 (14.3)
Bulk milk	37	4 (10.8)	2 (50.0)
Total	245	18 (7.3)	4 (22.2)

In the present study, only 4 (22.2%) *S. aureus* isolates were toxin producers. Although 30-50% of *S. aureus* isolates have the ability to produce one or more enterotoxins (8), results of this study are higher than those reported in Brazil by Araujo *et al*. (2), who observed only one enterotoxigenic (0.5%) *S. aureus* strain in raw milk collected from tanks. Sá *et al*. (14) did not detect TSST-1 in individual milk samples collected from cows affected by mastitis. However, the authors reported that nine milk samples (4.39%) contained enterotoxigenic strains, as follows: enterotoxin D in one (0.49%) sample, enterotoxin C in three samples (1.46%), and enterotoxin B in other three other (1.46%) samples, which is consistent with the data reported here.

Cardoso *et al*. (5) described for the first time the production of TSST-1 toxin in *S. aureus* isolated from subclinical mastitis cases in Brazil. These authors described how the co-production of different kinds of toxins may increase the toxigenic effects of these antigens, suggesting that TSST-1 is an important factor in the etiology of mastitis. However, in the present study there was no apparent correlation between the severity of mastitis and toxin detection in milk.

Molecular characterization of *S. aureus* strains by means of PFGE is shown in Table 2. Thirteen different patterns were observed from the 18 *S. aureus* strains isolated from individual and bulk milk samples. Strains SA2 (individual milk sample)

and SA3 (bulk milk sample) were isolated from the same dairy farm and showed genotypic similarity, suggesting the transference of the microorganism from infected animals to bulk milk, as described previously (1, 4, 10). Likewise, strains SA1 (individual milk sample) and SA6 (bulk milk sample), which were isolated from different farms (numbered 15 and 23) located in the same region (Ribeirão Preto), showed similar bands, demonstrating their genetic similarity. *S. aureus* dissemination in farms (number 22 and 23) may be due to the exchange or trade of animals between farms or due to hygienic failures during milk collection, once both farms delivered their milk to the same milk cooperative. Strains SA8, SA9, SA10 (individual milk sample) and SA11 (bulk milk sample), collected from the same dairy farm, were all similar, hence indicating that the lack of genetic diversity among *S. aureus* isolates from this farm may be related to a possible endemic characteristic of intramammary infections in this herd.

Table 2. Characterization of the *S. aureus* strains by PFGE and respective toxin production.

***S. aureus* strain number**	**PFGE type**	**Toxin(s)**	**Farm**	**Origin of sample**
Region of São Carlos:				
SA2	II	-	3	Individual cow
SA3	II	-	3	Bulk milk
SA4	III	-	5	Individual cow
SA7	V	TSST-1	17	Bulk milk
SA12	VII	-	19	Individual cow
Region of Ribeirão Preto:				
SA1	I	-	22	Individual cow
SA5	IV	-	23	Individual cow
SA6	I	TSST-1	23	Bulk milk
SA8	VI	-	29	Individual cow
SA9	VI	-	29	Individual cow
SA10	VI	-	29	Individual cow
SA11	VI	-	29	Bulk tank
SA13	VIII	B,C	36	Individual cow
SA14	IX	-	37	Individual cow
SA15	X	-	35	Individual cow
SA16	XI	-	35	Individual cow
SA17	XII	TSST-1	31	Individual cow
SA18	XIII	-	31	Individual cow

The large number of patterns observed indicate that there is great genetic heterogeneity among *S. aureus* strains isolated from raw milk on the farms studied. High variability in phenotypic and genotypic patterns may be due the diversity of locations in which *S. aureus* may be found. *S. aureus* may be isolated from the milk of cows affected by clinical and subclinical mastitis, from the surface of the teats, teat cups of the milking machine, and from the milkers (11).

Results of this trial suggest a relative wide geographical distribution of a number of *S. aureus* strains isolated from raw milk collected from individual cows with subclinical mastitis and from bulk tanks on farms located in São Paulo. Because *S. aureus* is a common causative agent of mastitis in Brazil (6), results of this study indicate a potential route of SFP transmission to consumers via contaminated milk obtained from cows affected by subclinical mastitis (19). Moreover, the fact that raw, unprocessed milk is largely consumed by the Brazilian population warrants concern about the persistence of *S. aureus* on dairy farms.

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