

Presence of enterotoxigenic *Staphylococcus aureus* in bulk tank milk from Argentine dairy farms

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ABSTRACT

Staphylococcus aureus is the most prevalent bovine mastitis pathogen in Argentina. The ability of this organism to produce enterotoxins is linked to staphylococcal food poisoning. Staphylococcal enterotoxins are low molecular weight proteins, highly resistant to heat and proteolytic enzyme activity. The aim of this study was to determine the ability to produce enterotoxins and types of enterotoxins A through E produced among 94 *S. aureus* isolated from bulk tank milk in Argentina by enzyme-linked immunosorbent assay. Eleven isolates (11.7 %) produced enterotoxins. Seven of them (7.4 %) produced enterotoxin C, two (2.1 %) enterotoxin D, one (1.1 %) enterotoxin B and one (1.1 %) enterotoxins C-D-E. None of the isolates produced enterotoxins A or E alone. Since presence of staphylococcal enterotoxins constitute a potential risk to public health, these findings underscore the need to control *S. aureus* bovine mastitis and to limit bacterial multiplication in bulk tank milk.

Key words: enterotoxins, bulk tank milk, mastitis, *Staphylococcus aureus*

RESUMEN

Presencia de *Staphylococcus aureus* enterotoxigénico en leche de tanque de frío de tambos de Argentina. *Staphylococcus aureus* es el patógeno causante de mastitis más prevalente en Argentina. Las enterotoxinas producidas por este organismo constituyen una de las causas más importantes de intoxicación alimentaria en seres humanos. Las enterotoxinas estafilocócicas son proteínas de bajo peso molecular, termoestables y resistentes a enzimas proteolíticas. El objetivo del presente trabajo fue determinar por enzimoimmunoensayo la presencia de enterotoxinas A-E y establecer su tipo en 94 aislamientos de *Staphylococcus aureus* obtenidos de leche de tanque de frío de tambos de Argentina. Se identificaron 11 % aislamientos enterotoxigénicos (11,7 %); siete (7,4 %) produjeron enterotoxina C, dos produjeron enterotoxina D (2,1 %), uno produjo enterotoxina B (1,1 %) y uno produjo enterotoxinas C-D-E (1,1 %). No se detectaron aislamientos que produjeran enterotoxinas A o E solamente. Estos hallazgos indican la necesidad de implementar un eficaz control de la mastitis bovina para disminuir la presencia de *S. aureus* en leche de tanque y evitar riesgos potenciales para la salud pública.

Palabras clave: Enterotoxinas, leche de tanque, mastitis, *Staphylococcus aureus*

Staphylococcus aureus is the most prevalent bovine mastitis pathogen worldwide (14) and one of the leading causes of foodborne disease in humans (7). This organism is capable of producing a large number of secreted and cell-surface-associated virulence factors that contribute to its ability to cause infection and disease; however, staphylococcal food poisoning relies on staphylococcal enterotoxin (SE) production (7). Twenty different types of SEs have been described, SEA-SEE and SEG-SEU; however, seven types including SEA, SEB, SEC₁, SEC₂, SEC₃, SED and SEE are the most frequently associated with food poisoning, while from the newly described types only SEH, SEG and SEI have been implicated in gastroenteric disease (1, 7).

Many different foods have been implicated in staphylococcal food poisoning. Among them, several dairy prod-

ucts have been involved in outbreaks in different countries (7). There is only scarce information on staphylococcal food poisoning in Argentina since most small scale food poisoning outbreaks go unreported as they do not require medical assistance (8). *S. aureus* isolates associated with food poisoning from different Argentine provinces have been recently characterized by phenotypic and genotypic methods; although the food source from which organisms were isolated was not mentioned (9). The main source of dairy products contamination are food handlers through manual contact or via the respiratory tract; however, contamination of raw milk due to animal carriage or infections cannot be neglected (7, 14). Infected mammary glands from lactating cows are considered the most important bulk tank milk contamination source by *S. aureus* (14). The aim of this study was to assess the occurrence of entero-

toxigenic *S. aureus* isolated from bulk tank milk samples from farms located in different dairy areas of Argentina.

Ninety four *S. aureus* isolates were obtained from bulk tank milk samples submitted to the Milk Quality and Mastitis Laboratory at the Rafaela Experiment Station, INTA. These isolates were collected over a period of 27 months, from April 2005 to July 2007 and belonged to 47 dairy farms from the Western Area of Santa Fe Province, 24 from the Eastern Area of Córdoba Province, 10 from the South-eastern Area of Entre Ríos Province and 13 from Buenos Aires catchment Area. Only one isolate from each dairy farm was included in the study. One hundred µl of 1:10 milk dilution were seeded on Columbia agar base (Britania, Buenos Aires Argentina) with 5 % calf blood and incubated at 37 °C for 48 h. Greyish-white or golden-yellow colonies 3 to 5 mm diameter displaying broad partial and narrow complete hemolysis zones were further identified to species level by standard procedures (12). Briefly, colonies were tested for cell morphology after Gram staining, catalase production, clumping factor and coagulase production using rabbit plasma, acetoin production and selective growth on P agar with 7 µg/ml acriflavine. Isolates yielding dubious phenotypic tests were not included in the study, while those identified as *S. aureus* were preserved by freezing at -80 °C in brain heart infusion (Britania, Buenos Aires Argentina) with 15 % glycerol prior to enterotoxin detection. Isolates were recovered from frozen stocks and cultured in TECRA® *Staphylococcus* growth medium (TECRA Int. Pty Ltd, French Forest, NSW, Australia). In a first step, culture supernatants of the 94 isolates were extracted and tested for enterotoxin production SEA through SEE with a sandwich ELISA (Tecra® VIA™, Staphylococcal Enterotoxin, Australia) without discrimination of the SE type, according to the manufacturer's instructions. Test sensitivity for enterotoxin detection was 1 ng/ml for each toxin. In a second step, those isolates yielding enterotoxins were further tested for identification of enterotoxin type by an ELISA including specific antibodies against SEA, SEB, SEC, SED and SEE (Tecra® VIA™, Staphylococcal Enterotoxin ID, Australia) according to the manufacturer's instructions. Positive and negative controls were provided by the manufacturer.

Staphylococcus aureus is the most prevalent cause of bovine intramammary infection in Argentina (14) and its prevalence in bulk tank milk has been reported (2). All isolates included in this study were Gram-positive cocci, catalase-positive, produced coagulase, grew in P agar and produced acetoin, while only five were clumping factor-negative. Eleven isolates (11.7 %) produced enterotoxins. Among these, 10 yielded only one enterotoxin type, while the remainder produced three types. Frequency and distribution of enterotoxin types among isolates is shown in Table 1. Similar percentages of classic enterotoxin-producing strains (12.9 % and 13.8 %) were found among 70 *S. aureus* isolated from bulk tank milk in the Czech Republic (15) and from 160 bovine mastitic milk isolates from California herds (3), respectively. Conversely, higher percentages of enterotoxin-producing strains (22.1 % and 23.6 %) were found among *S. aureus* isolated from 220 bulk tank milk samples from different regions of Norway (5) and from 696 mastitic cow milk samples from Korean dairy herds (10), respectively.

Enterotoxin C was the most frequently detected SE in the present study, which was in accordance with a previous study carried out on *S. aureus* strains isolated from bulk tank milk in Norway (5); while SEB was the predominant type for isolates from the same origin obtained in the Czech Republic (15). Frequency of enterotoxin types among *S. aureus* isolates has been shown to vary not only according to the origin (i.e. human, food, animals), but also within isolates from the same origin (1, 6, 11). Several reports suggest that prevalence of SEs among bovine mastitis isolates is determined by management and environmental factors that can favour the spread of certain types in distinct geographical areas, since similar SE patterns have been found in genetically closely related isolates (1, 5, 11, 13).

A survey carried out in Argentina showed a high proportion of enterotoxigenic strains among *S. aureus* isolated from food and food handlers (4). Although the predominant SE type was not determined in the former survey, a recent study showed that most *S. aureus* from food origin isolated in Argentina (55.7 %) carried the *sea* gene and were capable of producing SEA (9). Differences in SE type

Table 1. Enterotoxin production by *Staphylococcus aureus* isolated from bulk tank milk in Argentina.

Province	Number of isolates				Negative
	SEB	SEC	SED	SEC-SED-SEE	
Santa Fe	1	2	2	1	41
Córdoba	0	3	0	0	21
Buenos Aires	0	2	0	0	11
Entre Ríos	0	0	0	0	10
Total	1 (1.1 %)	7 (7.4 %)	2 (2.1 %)	1 (1.1 %)	83

prevalence compared with the present study likely reflect the distinct origin of the isolates. Since raw milk consumption is prohibited in Argentina, it can be considered that food handlers are the most usual contamination source leading to food poisoning. Nevertheless, since these toxins resist heat treatment, the present findings indicate a potential public health hazard and underscore the need to establish both effective bovine mastitis control programs and proper milk cooling methods to limit *S. aureus* presence and multiplication in bulk tank milk.

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