J. Dairy Sci. 97:1–10
http://dx.doi.org/10.3168/jds.2014-7972

Genetic relatedness and virulence factors of bovine *Staphylococcus aureus* isolated from teat skin and milk

L. B. da Costa,* P. J. Rajala-Schultz,*[1] A. Hoet,*† K. S. Seo,‡ K. Fogt,‡ and B. S. Moon‡
*Department of Veterinary Preventive Medicine, and
†Division of Epidemiology, The Ohio State University, Columbus 43210
‡Department of Basic Sciences, Mississippi State University, Mississippi State 39762

ABSTRACT

The objective of this study was to assess the role of teat skin colonization in *Staphylococcus aureus* intramammary infections (IMI) by evaluating genetic relatedness of *Staph. aureus* isolates from milk and teat skin of dairy cows using pulsed-field gel electrophoresis and characterizing the isolates based on the carriage of virulence genes. Cows in 4 known *Staph. aureus*-positive herds were sampled and *Staph. aureus* was detected in 43 quarters of 20 cows, with 10 quarters positive in both milk and skin (20 isolates), 18 positive only in milk, and 15 only on teat skin. Quarters with teat skin colonized with *Staph. aureus* were 4.5 times more likely to be diagnosed with *Staph. aureus* IMI than quarters not colonized on teat skin. Three main clusters were identified by pulsed-field gel electrophoresis using a cutoff of 80% similarity. All 3 clusters included both milk and skin isolates. The majority of isolates (72%) belonged to one predominant cluster (B), with 60% of isolates in the cluster originating from milk and 40% from teat skin. Genotypic variability was observed within 10 pairs (formed by isolates originating from milk and teat skin of the same quarter), where isolates in 5 out of the 10 pairs belonged to the same cluster. Forty-two virulence factors were screened using PCR. Some virulence factors were carried more frequently by teat skin isolates than by milk isolates or isolates from quarters with high somatic cell counts. Isolates in the predominant cluster B carried virulence factors *clfA* and *clfB* significantly more often than isolates in the minor clusters, which may have assisted them in becoming predominant in the herds. The present findings suggest that teat skin colonization with *Staph. aureus* can be an important factor involved in *Staph. aureus* IMI.

Key words: *Staphylococcus aureus*, milk and teat skin, pulsed-field gel electrophoresis, virulence factor

INTRODUCTION

Staphylococcus aureus is a major pathogen that can cause a wide variety of diseases in humans and animals. In cattle, *Staph. aureus* is often associated with IMI and is considered a contagious pathogen that is mostly transmitted from cow to cow during the milking process. *Staphylococcus aureus* usually causes subclinical infections with negative effects on milk quality and production, which also increases the risk of culling as well as labor, treatment, and replacement costs. A solid understanding of the epidemiology of *Staph. aureus* infections (e.g., sources and transmission of the organism) is crucial for an effective control program.

Results from the published literature regarding the role of teat skin as a source of *Staph. aureus* IMI are conflicting, and involvement of teat skin in *Staph. aureus* epidemiology is not fully understood (Zadoks et al., 2002; Haveri et al., 2008; Capurro et al., 2010). The use of molecular techniques, such as pulsed-field gel electrophoresis (**PFGE**; Haveri et al., 2007), binary typing (Zadoks et al., 2000), and multilocus sequence typing (MLST; Enright et al., 2000), has brought new insight but also more questions regarding this issue. Zadoks et al. (2002) concluded that most *Staph. aureus* mastitis cases are caused by strains highly adapted to the mammary gland and different from skin isolates. However, another study suggested that most *Staph. aureus* isolates from teat skin and teat canal were genetically indistinguishable from those isolated from infected mammary glands (Haveri et al., 2008). It has also been reported that isolates from extramammary sites (such as vagina, muzzles or nares, and hock skin) were indistinguishable from isolates found in milk (Capurro et al., 2010; Mørk et al., 2012). It remains unclear whether isolates originating from different sources belong to the same strain and trigger similar inflammatory response in the mammary gland and if they carry same virulence factors that contribute to the severity of IMI.

A limited number of *Staph. aureus* strains are typically detected within a herd with one predominant strain causing the majority of IMI (Joo et al., 2001;

Received January 21, 2014.
Accepted July 21, 2014.
[1]Corresponding author: rajala-schultz.1@osu.edu

Table 1. Characteristics of the study herds (herd size and prevalence of bovine *Staphylococcus aureus* based on earlier sampling), number of cows and quarters sampled, and number of cows from which isolates originated, number of positive milk and teat skin samples, and average SCC value ($\times 10^3$ cells/mL) in quarters infected with *Staph. aureus* in the study herds

Herd ID	Herd size[1]	Herd prevalence (%)	No. of cows/quarters sampled	Source cows (no.)	Positive samples		
					Milk	Teat skin	SCC
1	140	15	15/60	8	5	15	601
2	201	8	15/60	4	11	6	3,714
3	97	7	12/48	4	5	2	7,410
4	128	6	15/60	4	7	2	4,183

[1]Includes both lactating and dry cows.

Tenhagen et al., 2007; Haveri et al., 2008). It has been suggested that low prevalence strains act similarly to environmental pathogens and could simply be colonizers of the skin and contaminants in the milk, and thus no or only a mild response would be detected in the mammary gland (Sommerhäuser et al., 2003; Fournier et al., 2008). It could be assumed that the predominant strains possess certain characteristics that have allowed them to become prevalent in a herd and to cause IMI. The capability of *Staph. aureus* to cause disease is related to several virulence factors that allow the organism to adhere to a surface, invade or avoid the immune system, and cause harmful effects to its host (Bien et al., 2011). *Staphylococcus aureus* is able to produce large array of toxins and other virulence factors that contribute to the manifestation and severity of staphylococcal infections and pathogenesis of mastitis (Sutra and Poutrel, 1994). There is a paucity of studies comparing carriage of virulence factors in bovine *Staph. aureus* isolates from different sources. The overall objective of the current study was to assess the association between teat skin colonization by *Staph. aureus* and *Staph. aureus* IMI by (1) evaluating genotypic relatedness of *Staph. aureus* isolates from milk and teat skin of dairy cows using PFGE, and (2) characterizing the isolates based on the carriage of virulence factors.

MATERIALS AND METHODS

Selection of Herds

Four Ohio dairy herds previously involved in other studies and known to have *Staph. aureus* IMI were included in the study. A whole-herd sampling, collecting composite milk samples from each lactating cow, had been conducted in these herds 2 to 6 mo before the cows were sampled for this study. Those milk culture results had revealed *Staph. aureus* IMI prevalence, on a cow level, of between 6 and 15% in these herds. Sample size calculation for the current study was based on an assumption that quarters colonized by *Staph. aureus* on teat skin would be 2 times more likely to have a *Staph. aureus* IMI than quarters not colonized (P. Rajala-Schultz, unpublished data). Based on this and on the results of the whole-herd sampling, 5 known *Staph. aureus*-positive cows, 5 cows with no previous *Staph. aureus*-positive cultures, and 5 cows with unknown status (not sampled in the herd testing) from each herd were randomly selected (www.random.org) to be enrolled in the study, except in herd 3, where 4, 4, and 4 cows were sampled, respectively. In total, 57 cows in their first to third lactation were sampled and included in the study. Information about herd size and *Staph. aureus* herd prevalence is presented in Table 1. All herds practiced pre- and postmilking teat dipping, treated clinical mastitis cases with antibiotics, applied blanket dry-cow therapy, and had their milking equipment regularly serviced and properly maintained.

Bacteriological Procedures and Identification of Staph. aureus

Milk. In total, 228 quarter milk samples were aseptically collected immediately before routine milking following procedures described by the National Mastitis Council (Hogan et al., 1999). Milk samples and teat skin swabs from each quarter were collected using disposable latex gloves that were changed between each animal. Milk samples were transported in ice to the laboratory, and kept frozen for up to 1 wk until further processing. Milk samples were thawed at room temperature and 10 μL of milk was plated on blood agar containing 5% sheep blood (Remel Inc., Lenexa, KS) and on *Staph. aureus* selective BBL Chromagar plates (BD Diagnostic Systems, Sparks, MD). Plates were incubated at 37°C and checked for growth at 24 and 48 h. *Staphylococcus aureus* was phenotypically identified based on colony morphology (rose to mauve colonies on Chromagar plates) and hemolysis, Gram stain, positive catalase test, and positive tube coagulase rabbit plasma test, which was read at 4 and 24 h. Colony counts on each plate were recorded and a sample with ≥1 cfu/10