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Prevalence and persistence of coagulase-negative *Staphylococcus* species in three dairy research herds

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ABSTRACT

Coagulase-negative *Staphylococcus* species (CNS) were isolated from 11.3% (1407 of 12,412) of mammary quarter milk samples obtained from cows in three dairy research herds in 2005. Approximately 27% (383/1407) of CNS was identified to the species level. The species distribution among those CNS identified from all herds was *Staphylococcus chromogenes* (48%), *Staphylococcus hyicus* (26%), *Staphylococcus epidermidis* (10%), *Staphylococcus simulans* (7%), *Staphylococcus warneri* (2%), *Staphylococcus hominis* (2%), *Staphylococcus saprophyticus* (1%), *Staphylococcus xylosus* (1%), *Staphylococcus haemolyticus* (<1%), *Staphylococcus sciuri* (<1%), and *Staphylococcus intermedius* (<1%). *Staphylococcus-chromogenes* was the predominant CNS isolated from all three herds; however, differences were seen in the prevalence of other CNS species. A total of 158 CNS (*S. chromogenes* $n = 66$, *S. hyicus* $n = 38$, *S. epidermidis* $n = 37$, *S. simulans* $n = 10$, and *S. warneri* $n = 7$) were analyzed by pulsed-field gel electrophoresis (PFGE). The majority (33/41) of CNS isolated from the same mammary quarter on more than one occasion had the same PFGE pattern indicating persistence of the same infection over time. When all PFGE patterns for each CNS were analyzed, no common pulsotype was seen among the three herds indicating that CNS are quite diverse. Composite milk somatic cell count (SCC) data were obtained ±14 d of when CNS were isolated. Average milk SCC (5.32 $\log_{10}$/ml) for cows in which CNS was the only bacteria isolated was significantly higher than the average milk SCC (4.90 $\log_{10}$/ml) from cows with quarter milk samples that were bacteriologically negative.

1. Introduction

Coagulase-negative *Staphylococcus* species (CNS) are isolated frequently from bovine milk; however, the importance of CNS intramammary infections (IMI) has not been clearly delineated. The literature often refers to CNS as minor mastitis pathogens, which suggests that CNS are non-pathogenic or mildly pathogenic. Studies have shown that CNS isolated from bovine mammary secretions consists of several species including *Staphylococcus chromogenes*, *Staphylococcus hyicus*, *Staphylococcus simulans*, *Staphylococcus epidermidis*, *Staphylococcus warneri*, *Staphylococcus sciuri* and *Staphylococcus xylosus* (Harmon and Langlois, 1989; Matthews et al., 1990, 1992). The designation CNS is used to include all staphylococci isolated from bovine milk that are not *Staphylococcus aureus* (Oliver et al., 2004b) including coagulase-positive strains of *S. hyicus*. CNS are isolated frequently from aseptically collected bovine milk and are generally associated with a mild inflammatory response in the mammary gland. CNS are often the most prevalent organisms isolated from milk samples in herds using effective mastitis control procedures. The most commonly isolated CNS are part of the normal skin flora and include *S. simulans*, *S. hyicus*, *S. chromogenes*, and *S. epidermidis* (Oliver et al., 2004a). *Staphylococcus xylosus*, *Staphylococcus*

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saprophyticus, *S. sciuri* and *Staphylococcus cohnii* are found free-living in the environment. CNS appear to be opportunistic and infect the teat canal and mammary gland from skin sources or from the environment (Oliver et al., 2004a). The prevalence of CNS from various herds has been reported to range from 3% to 30% of quarters, and involve 27–55% of cows (Harmon and Langlois, 1995). The prevalence of CNS in mammary secretions of primigravid heifers during the prepartum period has been reported to be as high as 50–60% of mammary quarters (Oliver and Jayarao, 1997; Oliver et al., 2004a). In three dairy research herds in Tennessee, we reported that 22% of mammary quarters of lactating cows were infected with CNS (Oliver and Jayarao, 1997). The last study that identified CNS from these three farms was reported over 15 years ago. At that time, 105 CNS isolates were identified and the prevalence was as follows: *S. chromogenes* (40%), *S. simulans* (13.3%), *S. hyicus* (13.3%), *S. xylosus* (8.6%), *S. sciuri* (5.7%), *S. warneri* (4.7%), *S. epidermidis* (4.7%), *Staphylococcus hominis* (3.8%), *Staphylococcus haemolyticus* (3.8%), *Staphylococcus capitis* (<1%), and *S. saprophyticus* (<1%) (Matthews et al., 1990). Objectives of the present study were to determine the prevalence of CNS from the same three dairy research herds of The University of Tennessee and to determine if there was a change in prevalence over the 15-year period.

2. Materials and methods

2.1. Quarter milk samples

Quarter milk samples for microbiological evaluation were collected prior to milking following procedures recommended by the National Mastitis Council (Oliver et al., 2004b) and described by Oliver et al. (1994) from three dairy research herds of The University of Tennessee; Middle Tennessee Research and Education Center (MTREC), Dairy Research and Education Center (DREC) and the East Tennessee Research and Education Center (ETREC). Samples are routinely collected at these farms from cows before calving, during early lactation, near drying off, during routine herd surveys, when cows leave the herd, and from cows with clinical mastitis. Before sample collection, teats of cows were dipped with a pre-milking teat disinfectant, cleaned thoroughly and dried with individual disposable paper towels, and teat ends were sanitized with swabs containing 70% isopropyl alcohol. Following collection, milk samples were frozen and maintained at −20 °C until analysis.

2.2. Bacterial identification

Milk samples were examined following procedures recommended by the National Mastitis Council (Oliver et al., 2004b) and as described by Oliver et al. (1994). Briefly, foremilk samples (10 μl) from each mammary quarter were plated onto one quadrant of a trypticase soy agar plate supplemented with 5% defibrinated sheep blood (Becton Dickinson and Company, Franklin Lakes, NJ, USA). Plates were incubated at 37 °C and bacterial growth was observed at 24-h intervals for 3 d. Bacteria on primary culture medium were identified tentatively according to colony morphologic features, hemolytic characteristics, and catalase test. Isolates identified presumptively as staphylococci were tested for coagulase by the tube coagulase method. CNS isolated from quarter milk samples were chosen randomly and identified to the species level by the API Staph System (bioMerieux Inc., Hazelwood, MO, USA). Additional tests to identify CNS included: β-glucosidase, β-glucuronidase, β-galactosidase and turanose. Only CNS from mammary secretions containing >5000 colony forming units/ml were identified to the species level.

2.3. Somatic cell count (SCC)

SCC or somatic cell scores (SCS) were obtained from Dairy Herd Improvement Association (DHIA) records for the selected cows. The SCC or SCS were from composite sample data obtained ±14 d of when CNS were isolated. Average $\log_{10}$ SCC of non-infected cows was compared with the average $\log_{10}$ SCC of cows infected with CNS only. Comparisons were made for each herd and with all three herds grouped together.

2.4. Statistical analysis

SCC data were transformed to $\log_{10}$ before analysis using SAS v 9.1 (SAS Institute Inc., Cary, NC, USA). Analysis of variance was used to compare $\log_{10}$ SCC between cows infected with CNS only and uninfected cows by mixed model (Proc Mixed, SAS, 2004) as follows:

$$y = m + \text{CNS} + \text{herd} + \text{cow} + \text{lactation} + \beta\text{DIM} + e$$

where y = dependent variable $\log_{10}$ SCC, μ = intercept, e = residual error of model, and CNS = group of positive or negative culture for CNS, analyzed as a fixed effect.

Random effects were analyzed for herd (herd identification), cow within herd (cow identification) and lactation (three groups: group 1 for first lactation, group 2 for second lactation and group 3 for >2 lactations). Days in milk (DIM) was analyzed as a covariant in the model. Least squares means were separated using Tukey's significant difference test, and statistical significance was set at $P < 0.05$.

2.5. Pulsed-field gel electrophoresis (PFGE)

PFGE was performed on select CNS isolates as described by McDougal et al. (2003). A single bacterial colony was inoculated into Brain Heart Infusion broth (Becton Dickinson Microbiology System, Cockeysville, MD, USA) and incubated with shaking overnight at 35–37 °C. Turbidity of the bacterial cell suspension was adjusted with saline to an optical density of 0.90–1.1 using a spectrophotometer at 610 nm. An aliquot (200 μl) of each bacterial suspension was transferred to a 1.5 ml microcentrifuge tube, centrifuged at 12,000 × *g* for 2–4 min, and the supernatant discarded. The pellet was resuspended in 300 μl of Tris–EDTA (TE) buffer, (10 mM Tris–HCl, 1 mM EDTA [pH 8.0]) and equilibrated in a 37 °C water bath for 10 min. After equilibration, 4 μl of lysostaphin (1 mg/ml stock in 20 mM sodium acetate [pH 4.5]) were added to each tube and