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Short communication: Occurrence of methicillin-resistant *Staphylococcus aureus* and coagulase-negative staphylococci in dairy goat herds in Ohio, United States

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ABSTRACT

In light of the scarcity of information about the occurrence and epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) and coagulase-negative staphylococci (MRCNS) in small ruminants in general, and particularly dairy goats, we launched this limited-scope study. The findings reported here show the detection of MRSA and MRCNS in goat milk and teat skin samples from dairy goat herds in the state of Ohio. A total of 120 milk samples and 120 teat-swab samples were collected from 5 farms. After conventional isolation and phenotypic characterization of the staphylococci colonies, bacterial isolates were tested by PCR assay targeting the genes *nuc* to identify *Staphylococcus aureus* and *mecA* to detect MRSA and MRCNS. The clonal complexes of MRSA isolates was also determined by multiloccus sequence typing. Fifteen (6.2%) positive *S. aureus* samples were found in this study: 9 from milk and 6 from teat skin samples. Four (2%) MRSA isolates were detected and, using multiloccus sequence typing genotyping, these were designated to clonal complexes CC133 (n = 2; milk samples) and CC5 (n = 2; teat skin). Three (1.25%) coagulase-negative staphylococci isolates from the teat skin also harbored the *mecA* gene. Although, the MRSA isolated from milk samples is not a typical human-associated lineage, the CC5 clone isolated from teat skin is a common and widespread clonal complex associated with humans, suggesting that this extramammary niche could be a relevant reservoir of methicillin-resistant staphylococci. Furthermore, the fact that 75% of MRSA were recovered from 1 farm showing poor hygiene practices strengthens the hypothesis that good hygiene practices could be useful to prevent persistence and spread of MRSA at a farm level.
Key words: livestock-associated methicillin-resistant staphylococci, MLST, dairy goat

Short Communication

Mastitis is the most costly disease in dairy goat production (Persson and Olofsson, 2011; Zhao et al., 2015). Among mastitis pathogens, CNS are the most common cause of IMI in dairy goats, followed by *Staphylococcus aureus* (Bergonier et al., 2003; Zhao et al., 2015). The infected mammary gland is the primary reservoir of staphylococci in ruminants; however, it can be isolated from extramammary sites, such as the teat skin, which may contribute to the spread of staphylococci in dairy goat herds (Bergonier et al., 2003; Mørk et al., 2010).

The importance of staphylococci in dairy goat herds is not only limited to animal production, but is also a relevant to public health. For instance, staphylococci resistance to antimicrobial drugs has been observed in several studies (França et al., 2012; Eriksson et al., 2013; Cortimiglia et al., 2015). Likewise, the importance of antimicrobial resistance of *Staph. aureus* was recently highlighted by the World Health Organization (WHO), as this pathogen is regarded among the priority pathogens that pose the greatest threat to human health in terms of growing global resistance to antimicrobial agents (WHO, 2017). A special public health concern is the potential risk of transmission of methicillin-resistant *Staph. aureus* (**MRSA**) and CNS (**MRCNS**) to humans (van Rijen et al., 2008; Pantosti, 2012; Larsen et al., 2016).

The potential for zoonotic transmission of staphylococci between livestock, companion animals, and humans (van Rijen et al., 2008; Pantosti, 2012; Larsen et al., 2016) has been exemplified by the emergence of MRSA ST398 (Neyra et al., 2014). Thus, accurate and

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rapid detection and typing of *Staph. aureus* is crucial to a better understanding of the *Staph. aureus* epidemiology and control this infectious organism among animal production systems. Conversely, scarce information exists about the occurrence of MRSA and MRCNS and their epidemiology in dairy goat herds. To the best of our knowledge, ours is the first report on the detection of MRSA and MRCNS isolated from milk and teat skin samples from US dairy goat herds. Furthermore, MRSA isolates were characterized by a molecular typing method to better understand the implications of the isolates to public health.

Farms were selected based on the Ohio State University database (Infectious Diseases and Molecular Epidemiology Laboratory Database). Farmers were invited to join the study by email, 5 farms agreed to join the experiment, and visits were scheduled. Teat swabs and milk samplings were collected from a random subset of 120 lactating dairy goats during the milking routine procedures on each farm. All farms were located in a radius of 200 miles from Columbus, Ohio, and had Nubian, Toggenburg, and Saanen breeds as base of their herds. Dairy products were the primary activity in 3 of the farms, and 4 of them participated in fairs and expositions. A description of the number of animals sampled, routine milking practices of each farm, and type of parlor is shown in the Table 1.

Teat surface samplings were performed by rubbing a sterile moistened swab onto each teat and transferring them into a sterile tube containing 5 mL of Müller-Hinton broth (BBL Mueller Hinton Broth, Heidelberg, Germany) with 6.5% NaCl. Afterward, the teat ends were scrubbed with cotton containing 70% ethanol and composite milk samples from both halves were aseptically collected into 10-mL sterile vials after discarding the first 3 milk streams. One hundred twenty animals were sampled and milk samples were kept under refrigeration conditions for transportation until processing at the laboratory.

Teat swab samples were initially incubated at 37°C for 12 h and then streaked onto mannitol salt agar (**MSA**; BD, Heidelberg, Germany) and Oxacillin Resistant Screen Agar (BD) in parallel and incubated at 37°C for 12 h. Homogeneous colonies that were circular, pinhead, convex with entire margins, and light yellow were selected. These colonies were streaked onto Müller-Hinton agar plates (BD Mueller Hinton II Agar, Heidelberg, Germany) and incubated for 24 h at 37°C for further identification. Additionally, these colonies were also streaked onto Oxacillin Resistant Screen Agar (BD) and incubated at 37°C for 24 h. Growth was identified and results were recorded. Catalase-positive colonies were tested by coagulase production by means of a commercial kit (BBL Coagulase Plasma, BD). For DNA extraction, a commercial kit (Qiagen DNeasy Blood and Tissue kit, Qiagen, Valencia, CA) was used according to the manufacturer's protocol (DNeasy Blood & Tissue Handbook; https://www.qiagen.com).

Bacterial isolates were tested by PCR assay targeting the genes *nuc* to identify *Staph. aureus* and *mecA* to detect MRSA and MRCNS. Briefly, 1 μL of DNA template was added to a 24-μL master mix using a commercial kit (Illustra PuReTaq Ready-To-Go PCR beads, GE Healthcare, Little Chalfont, UK) with primer sequences and under PCR conditions listed in Table 2. Electrophoresis of PCR products was performed on 1% agarose gel stained with ethidium bromide. The DNA fragments were visualized in a UV transilluminator and photographed.

Sequencing analysis of the MRSA isolates was performed regarding 7 housekeeping genes (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, *yqiL*), as previously described by Enright et al. (2000). Based on the sequence analysis, multilocus sequence typing (**MLST**) was determined by analyzing the allelic profiles using the MLST database (http://saureus.mlst.net/) and the identification of sequence types.

Out of 120 teat swabs samples, 53 (44%) and 5 (4%) bacterial isolates were recovered by MSA and oxacillin screen agar, respectively. Among the 120 milk samples, 12 (10%) and 2 (2%) isolates grew in MSA and oxacillin screen agar, respectively. All samples with growth on MSA plates were tested for coagulase and catalase. Among those, 9 samples (14%) from milk and 6 samples (94%) from teat skin samples were phenotypically identified as coagulase-positive. Those 15 (64%)

Table 1. Characteristics of farms enrolled in the study, with description of number of animals sampled per farm, type of milking parlor, and milking routine procedures

Farm	Number of animals sampled	Type of milking parlor	Teat dipping
Farm A	48	Parallel milking parlor	Not used
Farm B	22	Manual milking	Pre- and postdipping
Farm C	15	Milking machine	Pre- and postdipping
Farm D	20	Milking machine	Pre- and postdipping
Farm E	15	Manual milking	Postdipping