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Short communication: Outbreak of methicillin-resistant *Staphylococcus aureus* (MRSA)-associated mastitis in a closed dairy herd

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ABSTRACT

Cows are probably the main source of contamination of raw milk with *Staphylococcus aureus*. Mammary glands with subclinical mastitis can shed large numbers of *Staph. aureus* in milk. Because of the risk of this pathogen to human health as well as animal health, the aim of this paper was to describe an outbreak of mastitis caused by methicillin-resistant *Staph. aureus* (MRSA), oxacillin-susceptible *mecA*-positive *Staph. aureus* (OS-MRSA), and methicillin-susceptible *Staph. aureus* (MSSA) on a dairy farm. Milk samples were obtained from all quarters, showing an elevated somatic cell count by the California Mastitis Test. The isolates were identified by phenotypic and genotypic methods. *Staphylococcus* spp. were isolated from 53% (61/115) of the milk samples, with 60 isolates identified as *Staph. aureus* (98.4%) and 1 isolate identified as *Staphylococcus epidermidis* (1.6%). The presence of the *mecA* gene was verified in 48.3% of *Staph. aureus* isolates. Of the *Staph. aureus* isolates, 23.3% were MRSA and 25.0% were OS-MRSA. The total of mastitis cases infected with MRSA was 12.2%. The detection of this large percentage of mastitis cases caused by MRSA and OS-MRSA is of great concern for the animals' health, because β-lactams are still the most important antimicrobials used to treat mastitis. In addition, *Staph. aureus* isolates causing bovine mastitis represent a public health risk.

Key words: methicillin-resistant *Staphylococcus aureus* (MRSA), oxacillin-susceptible *mecA*-positive *Staphylococcus aureus* (OS-MRSA), *mecA* gene, bovine milk

Short Communication

Mastitis is a worldwide problem in dairy herds. It is the most frequent infectious disease in dairy cows, accountable for great losses in dairy production. More than 100 microbial species have been isolated from the mammary glands of cows; however, a relatively small number of these organisms cause mastitis (Owens and Watts, 1988; Costa et al., 1999).

The etiological microorganisms of mastitis are generally classified as "contagious" or "environmental" based on their source, reservoir, and mode of transmission (Ruegg, 2012; Langoni, 2013). For contagious pathogens, the infected mammary glands serve as the primary source of infection, and transmission occurs mainly during the milking period when healthy quarters are exposed to droplets of infected milk or when these droplets are left on milking equipment, towels, or the hands of milkers. The term "environmental pathogens," as it relates to mastitis, refers to microorganisms that are found in a cow's surroundings such as bedding, various surfaces, and in the pasture. Excessive mud, moisture, and manure are commonly associated with these pathogens. Successful control of environmental mastitis is based on maintaining a clean and dry living area, whereas successful control of contagious mastitis is based on reducing exposure to teats contaminated with pathogens found in the milk of infected cows (Ruegg, 2012).

Staphylococcus aureus is one of the main contagious mastitis pathogens. Infected cows are probably the main source of contamination of raw milk with *Staph. aureus* (Jayarao et al., 2004). In particular, cows with subclinical *Staph. aureus* mastitis infections can shed a large number of *Staph. aureus* organisms in their milk. *Staphylococcus aureus* is an important pathogen because of a combination of toxin-mediated virulence, invasiveness, and antibiotic resistance (de Freitas Guimarães et al., 2013; Silva et al., 2014).

In addition, this bacterium is a significant cause of human nosocomial infections. The epidemiology of *Staph. aureus* has changed fundamentally in recent years. In particular, methicillin-resistant *Staph. aureus* (**MRSA**), which was originally restricted to hospitals, has emerged as a significant pathogen in the community (community-acquired MRSA, or CA-MRSA), con-

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siderably increasing the importance of this pathogen to public health as well as to animal health (Vandenesch et al., 2003).

Although *Staph. aureus* is a major cause of bovine mastitis, previously published reports have indicated a low prevalence of bovine MRSA, implying that MRSA is not commonly associated with mastitis (Hendriksen et al., 2008).

Methicillin-resistant *Staph. aureus* was first reported in cows in 1972, when Devriese and coworkers (1972) found 5.2% of Belgian cows on 232 dairy farms to be positive for MRSA. In that study, Devriese et al. (1972) recovered 18 methicillin (cloxacillin)-resistant *Staph. aureus* isolates in 17 of 776 cows (2.2%), and these isolates were associated with *Staph. aureus*-positive milk samples. More recently, MRSA has been sporadically detected in cases of bovine mastitis (Lee, 2003).

Staphylococcus aureus is defined as MRSA when either the *mecA* gene is present or the oxacillin MIC is greater than 4 μg/mL. However, *Staph. aureus* isolates that are positive for *mecA* and *PBP2a* but phenotypically susceptible to oxacillin have also been reported (Sakoulas et al., 2001; Saeed et al., 2010). These isolates are referred to as oxacillin-susceptible *mecA*-positive *Staph. aureus* (**OS-MRSA**; Pu et al., 2014).

The purpose of this study was to describe an outbreak of mastitis caused by MRSA and OS-MRSA in a dairy herd. We studied a herd of Brazilian dairy cows that was located in São Paulo State, exhibited high bulk milk SCC (628,000 cells/mL), and tested positive for clinical or subclinical mastitis. The dairy farm produced an average of 530 kg of milk per day. Improper milking hygiene was reported (lack of pre- and postmilking teat dipping; the California Mastitis Test (**CMT**) was performed only biannually, udder towels were used on more than one cow, and employees were not using gloves. A total of 103 Holstein cows were evaluated. The entire herd of lactating cows was screened using a strip cup and the CMT (Schalm and Noorlander, 1957). Mammary quarters positive by the strip cup test or CMT, or both, were aseptically sampled and stored in refrigerated isothermal boxes for transport to the laboratory for bacteriological analysis (NMC, 1999).

For the bacteriological examination, 0.01 mL of milk was plated onto a blood agar medium containing 5% bovine blood and MacConkey agar, and samples were applied using the streaking technique. Plates were then incubated under aerobic conditions at 37°C and observed after 24, 48, and 72 h of incubation. The samples were considered contaminated when more than 2 types of colonies were isolated from a sample. Isolates were identified based on colony morphology, Gram staining, and catalase, coagulase, and biochemical testing (NMC, 1999; de Freitas Guimarães et al., 2013). Biochemical tests performed included the following: sugar fermentation (trehalose, maltose, and mannitol) and acetoin production (Kloos and Schleifer, 1975). To confirm the identification of *Staphylococcus aureus*, we used the primers Staur 4 and Staur 6, which were previously described by Straub et al. (1999).

Staphylococcus DNA was extracted using an Illustra Blood GenomicPrep Mini Spin Kit (GE Healthcare, Uppsala, Sweden). The gene target was amplified with primers *mecA1* (5′-AAAATCGATGGTAAAGGTTGG-3′) and *mecA2* (5′-AGTTCTGCAGTACCGGATTTG-3′), described by Murakami et al. (1991).

Polymerase chain reactions, used to determine resistance genes, were performed with 10 pmol of each primer, 1.0 U of Platinum Taq DNA Polymerase (Invitrogen, Carlsbad, CA), 200 μ*M* dNTP, 1× PCR buffer, 0.75 m*M* $MgCl_2$, and 3 μL of sample. *Staphylococcus aureus mecA*-positive (ATCC 33591) and *Staphylococcus aureus mecA*-negative (ATCC 25923) samples were used as controls. Incubations were carried out in a Mastercycler gradient EP thermocycler (Eppendorf, Hamburg, Germany). The cycling profile was 94°C for 4 min, followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min, with a final extension at 72°C for 5 min. The PCR products were visualized on a 1.5% agarose gel, as described by Murakami et al. (1991).

Oxacillin MIC were determined using E-test strips (bioMérieux, Durham, NC) with a 0.5 McFarland standard inoculum on Mueller-Hinton agar plates (Remel, Lenexa, KA) according to the manufacturer's manual (EAS 003; AB Biodisk, Solna, Sweden). The MIC values were determined based on the actual endpoint, established according to the manufacturer's recommendations or by rounding up to the next highest doubling dilution, which is the approach recommended by the manufacturer for reporting results (CLSI, 2013).

Staphylococcus spp. were isolated from 61 (53%) of the 115 CMT-positive quarter milk samples, with 60 (98.4%) of these isolates identified as *Staphylococcus aureus* and 1 (1.6%) identified as *Staphylococcus epidermidis*. The following other pathogens were isolated: 25 (21.7%) *Corynebacterium bovis*, 5 (4.3%) *Streptococcus uberis*, 3 (2.6%) *Streptococcus dysgalactiae*, 3 (2.6%) *Klebsiella pneumoniae*, and 1 (0.9%) *Escherichia coli*, as shown in Table 1. These isolates could be classified as 74.8% contagious pathogens and 10.4% environmental pathogens.

The *mecA* gene was detected in 29 (48.3%) of the 60 *Staph. aureus* isolates (Table 2). Of the *Staph. aureus* isolates, 14 (23.3%) *mecA*-positive *Staph. aureus* expressed methicillin resistance (MRSA), 15 (25%) of *mecA*-positive isolates were susceptible to oxacillin (OS-MRSA), and 31(51.7%) *mecA*-negative *Staph. aureus*