J. Dairy Sci. 97:2959–2964
http://dx.doi.org/10.3168/jds.2013-7523

Short communication: Prevalence of methicillin resistance in coagulase-negative staphylococci and *Staphylococcus aureus* isolated from bulk milk on organic and conventional dairy farms in the United States

K. M. Cicconi-Hogan,*[1,2] N. Belomestnykh,* M. Gamroth,† P. L. Ruegg,‡ L. Tikofsky,*[3] and Y. H. Schukken*
*Department of Population Medicine and Diagnostic Sciences, Cornell Veterinary Medicine, Cornell University, Ithaca, NY 14853
†Department of Animal Sciences, Oregon State University, Corvallis 97331
‡Department of Dairy Science, University of Wisconsin-Madison, Madison 53706

ABSTRACT

The objective of this study was to evaluate the presence of methicillin-resistant *Staphylococcus aureus* and coagulase-negative *Staphylococcus* spp. in bulk tank milk samples from 288 organic and conventional dairy farms located in New York, Wisconsin, and Oregon from March 2009 to May 2011. Due to recent publications reporting the presence *mecC* (a *mecA* homolog not detected by traditional *mecA*-based PCR methods), a combination of genotypic and phenotypic approaches was used to enhance the recovery of methicillin-resistant organisms from bulk tank milk. In total, 13 isolates were identified as methicillin resistant: *Staph. aureus* (n = 1), *Staphylococcus sciuri* (n = 5), *Staphylococcus chromogenes* (n = 2), *Staphylococcus saprophyticus* (n = 3), *Staphylococcus agnetis* (n = 1), and *Macrococcus caseolyticus* (n = 1). The single methicillin-resistant *Staph. aureus* isolate was identified from an organic farm in New York, for an observed 0.3% prevalence at the farm level. The methicillin-resistant coagulase-negative staphylococci prevalence was 2% in the organic population and 5% in the conventional population. We did not identify *mecC* in any of the isolates from our population. Of interest was the relatively high number of methicillin-resistant *Staph. sciuri* recovered, as the number of isolates from our study was considerably higher than those recovered from other recent studies that also assessed milk samples. Our research suggests that the presence of a potential methicillin-resistant *Staphylococcus* reservoir in milk, and likely the dairy farm population in the United States, is independent of the organic or conventional production system.

Key words: dairy, organic, methicillin resistance, *Staphylococcus*

Short Communication

The organic dairy industry has seen rapid growth from 2000 to 2008, and has since been a consistently large contributor to the dairy industry (USDA-ERS, 2013). Organic milk quality, management, and animal health have been assessed in comparison with conventionally managed dairy farms (Zwald et al., 2004; Ruegg, 2009; Cicconi-Hogan et al., 2013a,b; Richert et al., 2013; Stiglbauer et al., 2013). Antimicrobial resistance in agriculture has become a major concern among consumers and scientists alike, as it presents potential health risks to animals and humans. The lack of use of antimicrobials on organic farms and how prevalent antimicrobial-resistant organisms are in relation to conventional farms is of interest, as is the possibility of various antimicrobial-resistant microorganisms in milk.

Staphylococcus aureus is a major mastitis-causing pathogen on dairy farms and has been found more frequently on organic dairy farms than conventional farms in recent studies (Ruegg, 2009; Cicconi-Hogan et al., 2013b). Methicillin-resistant *Staph. aureus* (**MRSA**) is of major concern in the human population, as it is difficult to treat. The *mecA* gene confers methicillin resistance by encoding a penicillin-binding protein, known as PBP-2α. It is located on a mobile element called staphylococcal cassette chromosome, known as SCC*mec*, which allows other species of *Staphylococcus* to pick up the methicillin resistance. Although detection of MRSA in bulk tank milk is often performed with molecular methods searching for a single conserved cassette (Virgin et al., 2009; Haran et al., 2012), recent research has found a variant that is undetectable through these methods. A divergent homolog, $mecA_{LGA251}$ has been described in García-Álvarez et al. (2011) and in

Received September 24, 2013.
Accepted January 7, 2014.
[1]Corresponding author: kmc277@cornell.edu
[2]Current affiliation: United States Department of Agriculture, Agricultural Research Service, Russell Research Center, Poultry Processing and Swine Physiology Research Unit, Athens, GA 30605.
[3]Current affiliation: Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO 64506.

Paterson et al. (2012) that only presents 70% similarity at the DNA level to the traditional *mecA* gene, and is officially identified as *mecC* (Ito et al., 2012).

Methicillin-resistant *Staph. aureus* has been isolated from bulk tank and quarter milk samples in Europe (Spohr et al., 2011; Kreausukon et al., 2012), but the MRSA detection rate in bulk tank milk in the United States is quite low (Virgin et al., 2009; Haran et al., 2012). Although research has been done on MRSA and methicillin-resistant CNS (**MRCNS**) at the cow level in the United States (Tikofsky et al., 2003) and in limited geographical locations (Sato et al., 2004), no research has specifically targeted MRSA or MRCNS in organically produced milk at the herd level in a representative sample of the organic dairy population from across the United States. Due to the mobile nature of SCC*mec*, assessing the prevalence of MRCNS in bulk tank milk is of interest. The objective of this study, which was part of a larger project designed to assess differences between organic and conventional dairy farms, was to assess the presence of MRSA and MRCNS in bulk tank milk samples from New York, Wisconsin, and Oregon.

Data and Sample Collection

Herd inclusion, eligibility, and recruitment criteria are described in Cicconi-Hogan et al. (2013a), Richert et al. (2013), and Stiglbauer et al. (2013). Following recruitment and a matching period, 192 organic and 100 conventional farms in New York, Oregon, and Wisconsin were visited between March 2009 and May 2011. The conventional farms were included in the study based on proximity to the organic farms and were matched based on herd size category (0–99 adult cows, 100–199 adult cows, or ≥200 adult cows). Farms were compensated with bulk tank milk testing and testing of clinical mastitis samples. At the time of the visit, a questionnaire was administered to the person primarily responsible for farm management and animal health. More detailed information on the questionnaire can be found in other published material from the study (Cicconi-Hogan et al., 2013a,b; Richert et al., 2013; Stiglbauer et al., 2013) and at the project website (http://milkquality.wisc.edu/organic-dairies/project-c-o-w/).

Six bulk tank milk samples were collected from each farm at the time of the visit. After the bulk tank had been agitated for a minimum of 5 min, all samples were taken directly from the bulk tank with a sterile sampler, put on ice, and transported to Quality Milk Production Services (Ithaca, NY) for testing. Of the 292 farms visited, 2 farmers requested that their bulk tanks not be sampled or analyzed, and 2 samples were unable to be analyzed, leaving a total of 288 samples. All samples were kept frozen at −20°C until testing.

Genotypic and Phenotypic Testing for MRSA and MRCNS

Two parallel assays were performed to assess the presence of methicillin-resistant organisms in the bulk tank milk. A genotypic approach (Virgin et al., 2009) was initially used to determine if the *nuc* gene, which encodes the thermostable nuclease of *Staph. aureus* (Brakstad et al., 1992), and a 174-bp portion of the *mecA* gene (Martineau et al., 2000), were present in the isolates cultured from the bulk tank milk. Approximately 20 μL of milk was swabbed onto Trypticase soy agar with 5% sheep blood and 0.1% esculin (bioMérieux Inc., Durham, NC), and incubated at 37°C. Plates were assessed for growth at 24 and 48 h. Colonies were initially identified as *Staphylococcus* spp. by appearance, hemolysis, catalase and coagulase production, and UV light assessment, and were then isolated on blood agar plates. Up to 10 colonies per sample were isolated for further testing. DNA templates from all isolates in the study were obtained using a Qiagen DNA Mini Kit (Qiagen Inc., Valencia, CA), according to the procedure for gram-positive bacterial organisms. All PCR reactions in this study were done on an iCycler (Bio-Rad Laboratories Inc., Hercules, CA). The PCR protocol was run as described in Virgin et al. (2009). A negative lysate preparation and a negative for the PCR reaction served as the negative controls for the experiment. The positive control used was strain QMP S1-027 (Virgin et al., 2009), an MRSA isolate from heifer milk that was *mecA* and *nuc* positive, confirmed by PCR (a gift of J. Barlow, University of Vermont, Burlington).

Concomitantly, a phenotypic approach was used to enhance the recovery of MRSA and MRCNS from the milk and to identify methicillin-resistant genotypes other than those that could be identified with the previously described PCR protocol. Milk samples were defrosted overnight at 4°C. To encourage the growth of *Staphylococcus* spp., a 2-step selective enrichment method was used (Haran et al., 2012). In brief, 10 mL of milk was added to 40 mL of Mueller-Hinton broth with 6.5% NaCl, and the samples were incubated for 24 h at 37°C. One milliliter of the initial broth was added to 9 mL of phenol red mannitol broth (with 75 mg of aztreonam/mL and 5 mg of cefoxitin/mL), and was incubated at 37°C for 24 h. Then, 100 μL of the resulting culture was spread onto MRSA*Select* plates (Bio-Rad Laboratories Inc., Redmond, WA) and incubated for 24 h at 37°C. The plates were assessed for pink colonies, indicating MRSA, or for off-white colonies, indicating MRCNS. All resulting colonies were replated on MRSA*Select* plates to confirm growth and on blood agar plates to reevaluate *Staphylococcus*-like colonies for appearance. Isolates were tested for