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Multidrug residues and antimicrobial resistance patterns in waste milk from dairy farms in Central California

P. N. Tempini,* S. S. Aly,*† B. M. Karle,‡ and R. V. Pereira*[1]
*Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, Davis 95616
†Veterinary Medicine Teaching and Research Center, School of Veterinary Medicine, University of California, Davis, Tulare 93274
‡Cooperative Extension, Division of Agriculture and Natural Resources, University of California, Orland 95963

ABSTRACT

Waste milk (WM) is a common source of feed for preweaned calves in US dairy farms. However, limited information is available about characteristics of this product, including concentration of drug residues and potential hazards from antibiotic-resistant bacteria present in the milk. The aims of this cross-sectional study were to (1) identify and measure the concentration of antimicrobial residues in raw WM samples on dairy farms in the Central Valley of California, (2) survey farm management practices for factors associated with the occurrence of specific antimicrobial residues in raw WM, (3) characterize the antimicrobial resistance patterns of *E. coli* cultured from raw WM samples, and (4) evaluate the potential association between WM quality parameter and risk of identifying drug residues in milk. A single raw bulk tank WM sample was collected from dairy farms located in California's Central Valley (n = 25). A questionnaire was used to collect information about farm management practices. Waste milk samples were analyzed for a multidrug residue panel using liquid chromatography–tandem mass spectrometry. Bacteria were cultured and antimicrobial resistance was tested using standard techniques; milk quality parameters (fat, protein, lactose, solids-not-fat, somatic cell count, coliform count, and standard plate count) were also measured. Of the 25 samples collected, 15 (60%) contained detectable concentrations of at least 1 antimicrobial. Of the drug residue–positive samples, 44% (11/25) and 16% (4/25) had detectable concentrations of β-lactams and tetracycline, respectively. The most prevalent drug residues were ceftiofur (n = 7, 28%), oxytetracycline (n = 4, 16%), and cephapirin (n = 3, 12%). No significant associations were identified between farm characteristics or management practices and presence of drug residues in WM. In this study, 20% of farms did not pasteurize WM before feeding to calves. Two of the 10 *Escherichia coli* isolated from WM samples were multidrug resistant. *Streptococcus* spp. (n = 21, 84%) was the most common genus cultured from WM samples, followed by *Staphylococcus* spp. (n = 20, 80%) and *E. coli* (n = 10, 40%). *Mycoplasma* spp. was cultured from 2 WM samples (n = 2, 8%). The presence of drug residues in WM at concentrations that increase selection of resistant bacteria indicates the need for additional studies targeting on-farm milk treatments to degrade drug residues before feeding to calves. The presence of multidrug-resistant *E. coli* in WM urges the need for on-farm practices that reduce calf exposure to resistant bacteria, such as pasteurization.

Key words: waste milk, drug residue, ceftiofur, antibiotic resistance

INTRODUCTION

The use of antimicrobials in livestock production and its effect on the development of antimicrobial resistance are a current public health concern. Although prevention approaches can reduce disease and the need for use of antimicrobial drugs to treat animals, drug use in food animals is an important tool to help ensure animal health and welfare. Despite these benefits, there is considerable concern from public health, food safety, and regulatory stakeholders regarding the use of antimicrobials in food animals because of possible selection of antimicrobial-resistant bacteria (Oliver et al., 2011). The selection of resistant bacteria can especially occur when antimicrobials are administered to food animals at regimens outside of labeled dosage and duration (Maron et al., 2013).

In the dairy industry, most antimicrobial drugs used to treat cows result in the milk from these animals being withheld from sale because of the presence of drug residues above the tolerable concentration established by the US Food and Drug Administration (**FDA**). Milk from cows treated with drugs that require a milk with-

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[1]Corresponding author: rvpereira@ucdavis.edu

drawal period and transitional milk (transitioning from colostrum to saleable milk) are considered nonsaleable for human consumption. One-third of dairy farms in the United States use waste milk (**WM**) to feed preweaned dairy calves (USDA, 2008a). A study examining the effects of feeding pasteurized WM to preweaned calves found that calves consuming pasteurized WM had increased weaning weights and decreased mortality rates compared with calves consuming milk replacer (Godden et al., 2005). Additionally, the total savings of feeding pasteurized WM was $0.69/calf per day (Godden et al., 2005). However, milk from cows receiving antimicrobial treatment during lactation and colostrum from cows treated with antimicrobials at dry-off may contain concentrations of drug residues, and such milk could lead to increased fecal shedding of antimicrobial-resistant bacteria when fed to preweaned dairy calves (Pereira et al., 2014a; Maynou et al., 2017a; Ricci et al., 2017). Not all dry cow treatments have zero withdrawal milk days (in addition to the minimum dry period length required). As an example, the commercial drug used for dry cow treatment containing cephapirin benzathine (ToMorrow, Boehringer Ingelheim, Ingelheim am Rhein, Germany) states on its label that milk from treated cows must not be used for food during the first 72 h after calving.

In a recent study conducted in New York State, 82% of WM fed to calves contained β-lactam drug residues, including ceftiofur, penicillin G, and ampicillin (Pereira et al., 2014b). A subsequent study evaluating the effect of ingestion of raw milk containing antimicrobial drugs found an increased prevalence of antimicrobial resistance in *Escherichia coli* isolated from the feces of dairy calves (Pereira et al., 2014a). Based on 16S RNA sequencing of fecal samples, feeding milk containing drug residues also affected the developing enteric microbiota of calves, resulting in a microbiota that discriminated at the genus level in their weekly microbial profile (Pereira et al., 2016). A follow-up evaluation using shotgun sequencing to evaluate the effects of feeding WM on the function of the microbiota revealed differences between treatment and control groups for genes linked to stress response, regulation and cell signaling, and nitrogen metabolism (Pereira et al., 2018). For example, calves in the treatment group had a greater abundance of microbial genes related to zinc-regulated enzymes, allowing this microbiota to adapt better to lower concentrations of zinc in feed. Calves in the control group had a greater abundance of microbial genes related to osmotic stress, which could translate into this microbiota being able to withstand greater osmotic pressure variation, such as that caused by feed with high concentration of sugars or salts. Changes in the microbiota functions could directly affect the resilience of the microbiota to adjust to changes such as variations in nutrient content or TS percentage in milk fed. To the author's knowledge, no studies have evaluated the effects of drug residues at concentrations observed in WM on the gastrointestinal epithelium.

Limited information is available on antimicrobial drug residues and resistant bacteria in WM fed to preweaned dairy calves. As the leading dairy state in the United States, with 1.7 million lactating cows and contributing almost 20% of the country's milk supply in 2015 (Monson et al., 2015), the California dairy industry is particularly poised to address this research need. Public awareness and increasing concerns about the development of antimicrobial resistance associated with the use of antimicrobials in livestock production and the possible spread of resistance to human medicine require field data to provide scientific information for stakeholders.

The aims and hypotheses for this study were as follows. First, we aimed to identify and measure the concentration of antimicrobial residues in raw WM samples on dairy farms in the Central Valley of California; our hypothesis was that more than 50% of dairy farms in California would have drug residues in bulk tank WM and that ceftiofur would be the most common drug residues detected in WM based on previous reports screening drug residues in WM (Pereira et al., 2014a; USDA, 2016). Second, we aimed to survey farm management practices for factors associated with the occurrence of specific antimicrobial residues in raw WM; our hypothesis was that use of specific antimicrobial drugs or management practices that affect the frequency of antimicrobial drug use would result in higher prevalence of specific drugs in the WM bulk tank. Third, we aimed to evaluate the association between WM quality parameters and identification of drug residues in milk; our hypothesis was that milk quality parameters could be associated with prevalence of drug residues. It was also an objective of the study to characterize the antimicrobial resistance patterns of *E. coli* cultured from raw WM samples. To our knowledge, this is the first study to focus on addressing all the aims and objectives on WM from dairy farms in California.

MATERIALS AND METHODS

Study Population and Design

Raw hospital WM samples from 25 dairy farms were collected from herds in the following counties in the Central Valley in California: Tulare (n = 12), Kings (n = 2), Fresno (n = 2), Madera (n = 1), Solano (n = 1), Placer (n = 1), Glenn (n = 5), and Butte (n = 1). Samples were collected from August 2016 to February