Evaluation of four commercial test for detection of ceftiofur in waste milk tank samples

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Introduction

Antimicrobial resistance is a great concern for human and animal health. Over the years increasing concern has been established over drug resistance to 3rd generation cephalosporins, including ceftiofur. Drug use in lactating animals commonly results in drug residues in the milk that in the U.S. is frequently used as a feed source for pre-weaned calves. This milk is commonly called waste milk, and also includes milk from cows with high somatic cell counts (SCC) and fresh cows. Feeding waste milk to preweaned calves has been shown to potentially result in a higher selection of cephalosporin resistant bacteria. The objective on this study was to evaluate factors affecting the specificity, specificity and predictive values of 4 commercially available tests (labeled for detecting beta-lactam drug residues in comingled milk) when testing waste milk (WM) tank samples.

Materials and Methods

Twelve WM samples were collected from 12 different commercial farms in California, and were initially tested using high performance liquid chromatography (HPLC) to assure they were negative for drug residues present above the FDA established tolerance/safe levels. These milk samples were also tested for fat, protein, lactose, solids non-fat (SNF), somatic cell count (SCC), coliform count, and standard plate count (SPC). For this study, each of these WM samples were divided in two aliquot, one labeled as negative for drug residues (WMN) and one as positive for ceftiofur residues (WMPos). The WMPos samples were WMN that were samples spiked with ceftiofur to assure they had a final concentration of 100 ppb of ceftiofur, which is above the FDA tolerance for ceftiofur in saleable milk. The 24 WM samples (12 WMN and 12 WMPos) were tested to evaluate the performance of 4 commercially available tests: Penzyme ® Milk Test, SNAP® β–lactam, Betastar ® Plus and Delvo ®-SP. These test were selected because they function in differently. Negative and positive controls for ceftifour residues were tested using pasteurized whole milk as a media. Four assays in triplicates for the WMN and WMPos were conducted for each WM sample. All samples were vortexed for 30 seconds prior to testing. Testing using the 4 commercial assays followed manufacturer's recommendations. The accuracy of each of the residue detection assays was expressed in terms of specificity, and positive predictive value. Samples were considered true positive only after being spiked with ceftiofur (WMPos). Statistical analysis were conducted using Excel, JMP and SAS.

Results

All WMPos samples were identified as positive by all four tests, rendering 100% sensitivity. The specificities for SNAP β –lactam test, Delvo-SP, Betastar Plus, and the Penzyme Test were 25.8, 33.3, 41.7, 44.4%, respectively, for detection identifying WMN samples as being negative for ceftiofur drug residues. Positive predictive values for the SNAP β –lactam test, Delvo-SP, Betastar Plus, and the Penzyme Test were 58.9, 60, 63.2, and 64.3% respectively. Lower lactose significantly increased false positive (FP) result for the SNAP β-lactam test while a higher value resulted in increased FPs with Betastar Plus. Higher SCC significantly increased FPs for the SNAP β-lactam test and Betastar Plus. Delvo –SP was the only test affected by protein, where lower values resulted in significantly increased FPs. None of the milk characteristics evaluated significantly increased FP results for Penzyme.

Significance

The differences in the number of FPs observed for each test associated with milk components and SCC may be explained by the different mechanisms each test has for detecting residues for each assay. Overall, based on our results, Penzyme resulted in the lowest number of FPs for screening waste milk samples containing ceftiofur concentrations at tolerance levels set by the USDA