Detection of β -lactam antimicrobial residues in the milk of cows treated for mastitis by use of the BetaStar[®] Plus Assay

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Introduction

Rapid assays for on-farm detection of antimicrobial residues in milk collected from individual cows would be advantageous to reduce risks of violative residues in marketed milk. The objective of this project was to evaluate the accuracy of a rapid immunomigration assay (BetaStar[®] Plus, Neogen Corporation, Lansing, MI) for detecting β -lactam antibiotic residues in milk collected from individual dairy cows treated for mastitis. This assay is currently FDA-approved for detecting β -lactam antibiotic residues in commingled milk.

Materials and Methods

From 5 dairy farms, 70 dairy cows with mild to moderate clinical mastitis were enrolled in the study. Cows were treated with β -lactam antimicrobials in accordance with the manufacturers' label directions. Antimicrobials used included ceftiofur hydrochloride (Spectramast[®] LC, Zoetis, Madsion, NJ), cephapirin sodium (Cefa-Lak® or ToDAY®, Boehringer Ingelheim Vetmedica, St. Joseph, MO) or amoxicillin (Amoxi-Mast[®], Merck Animal Health, Summit, NJ). Composite milk samples were collected before first antimicrobial treatment, before the last antimicrobial treatment, at the last milking of the product-labeled milk withhold period, at the first milking after the product-labeled milk withhold period had passed, and at 72 hours after the productlabeled milk withhold had passed. All samples were tested with the BetaStar® Plus assay within 48 hours of collection. Parallel samples were submitted to the Iowa State University Cyclone Analyte Detection Service (CYCADS) for liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. The sensitivity and specificity of the BetaStar® Plus assay was calculated using the LC-MS/MS results as the "gold standard".

Results

A total of 350 individual milk samples were tested with both the BetaStar $^{\tiny (\! B\!)}$ Plus assay and LC-MS/MS and

FDA residue tolerance levels. Of the 70 cows treated. 38 were treated with ceftiofur, 4 were treated with amoxicillin, 21 were treated with cephapirin, 2 were treated with a combination of ceftiofur and systemic ampicillin, and 5 were treated with a combination of ceftiofur and cephapirin. There were 2.1%, 44.3%, 23.6%, 10.0%, and 3.6% BetaStar[®] Plus assay-positive samples at each of the respective time points, regardless of the antimicrobial used. In cows treated with ceftiofur, the most common antimicrobial used in this study, there were 2.6%, 57.9%, 42.1%, 15.8%, and 0.0% BetaStar[®] Plus assav-positive samples at each of the respective time points. The BetaStar[®] Plus assay had a sensitivity of 95.8% (95% confidence interval, 87.8% to 100.0%) and specificity of 77.0% (95% confidence interval, 72.4% to 81.6%), when compared with LC-MS/MS analysis and using FDA published residue tolerance levels for each respective antimicrobial as a threshold. For ceftiofur alone, the assay had a sensitivity and specificity of 93.3% and 78.3%, respectively. For all samples tested, there was only one sample for which the LC-MS/MS analysis detected an antimicrobial residue and the BetaStar[®] Plus did not.

Significance

The BetaStar[®] Plus assay is a useful tool for detection of β -lactam antimicrobial residues in milk obtained from individual cows treated for mastitis before being sold for human consumption. The sensitivity and specificity of the BetaStar[®] Plus assay was similar to those reported for other tests used to detect antibiotic residues in milk from individual cows. The use of the high sensitivity of the BetaStar[®] Plus assay for detection of β -lactam antibiotic residues would significantly reduce the risk of marketing milk with violative residues.