The use of chromogenic culture media for on-farm identification of milk pathogens associated with mastitis in dairy cows

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Introduction

Clinical mastitis is a highly prevalent and costly disease to the dairy industry. In fact, mastitis is the major cause of antibiotic use in dairy herds and represents a large portion of animal health expenses (Erskine et al., 2003; Pol and Ruegg, 2007). Selective treatment based on on-farm culture of milk pathogens decreased antibiotic usage by 50% without impairing health and production traits (Lago et al., 2011a; Lago et al., 2011b). However, increasing the range of microorganisms to be identified often reduces accuracy for observers without microbiological training (McCarron et al., 2009). Chromogenic culture media (CCM) has been used for pathogen identification in human and animal specimens (Perry and Freydiere, 2007; Kalchayanand et al., 2013). Chromogenic culture media allows for rapid and accurate characterization of a broad spectrum of microorganisms based on media selectivity and color change. Hence, the objectives were to develop an on-farm CCM system for identification of milk pathogens associated with clinical mastitis (Accumast). Accuracy of Accumast was evaluated based on results from Cornell Quality Milk Production Services (QMPS; https://ahdc.vet.cornell.edu/sects/qmps/) and identification of cultured pathogens using 16S rRNA sequencing.

Materials and Methods

Three CCM were selected based on accuracy and variety of detectable microorganisms and combined into a single sectioned plate (Accumast; http://www.feraanimalhealth.com/). Milk from cows with clinical mastitis was sampled in a single commercial dairy herd located in upstate New York, which milked approximately 2,800 cows thrice daily in a double-52 milking parlor. Milk samples (n=904) were collected aseptically by trained farm personnel and plated on Accumast using a sterile cotton swab. The same milk samples were submitted to QMPS for microbiological culture. Plates were incubated at 37°C and read 16 to 24 h later by a single technician blinded to QMPS results. Growth, number, and color of colonies were recorded. Bacteria were identified according to Accumast guidelines. Additionally, isolates from 214 cases of clinical mastitis previously cultured using the Accumast system were subjected to DNA extraction. The 16S rRNA gene was amplified by PCR and sequenced for phylogenetic determination. Accumast sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV), and accuracy (Ac) were calculated based on QMPS results, and PPV was also calculated based on 16S rRNA sequencing results. In addition, the agreements between QMPS culture or 16S rRNA sequencing and Accumast were assessed by simple Cohen's kappa coefficient (κ) using the FREQ procedure of SAS version 9.3 (SAS Institute Inc., Cary, NC).

Results

The overall accuracy of Accumast compared with QMPS results was 88% for both Gram-positive and Gram-negative bacteria. Moreover, Se, Sp, PPV, NPV, Ac, and κ were 78%, 99%, 93%, 96%, 95% and 85% for the identification of Gram-negative bacteria; 100%, 99%, 88%, 100%, 100%, and 93% for the identification of *S. aureus*; 73%, 96%, 60%, 98%, 95%, and 63% for the identification of *Staphylococcus* sp.; and 92%, 89%, 88%, 93%, 91%, and 81%, respectively, for the identification *Streptococcus* sp. All kappa coefficients were associated $P<0.001$. The overall PPV and κ of Accumast compared to 16S rRNA sequencing were 93% and 86% for *Escherichia coli*, 100% and 100% for *Enterococcus* sp., 100% and 95% for *Klebsiella* sp., 88% and 93% for *Staphylococcus* sp., and 95% and 92% for *Streptococcus* sp., respectively.

Significance

The diagnostic system evaluated in the present study is suitable for use under field conditions and presented overall sensitivity and specificity of 83% and 92% compared with standard laboratory diagnosis, which was confirmed by 16S rRNA gene sequencing. Accumast provides a unique approach for on-farm identification of mastitis associated pathogens, mostly through its straightforward color-based classification of bacteria that can be easily interpreted by individuals with limited microbiology training.