

Conventional culture, MALDI-TOF and 16S rRNA compared for agreement in diagnosis of bovine mastitis pathogens

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Introduction

Tests for bacterial mastitis pathogens include those in use for some time and some that have only become available recently (Barreiro et al, 2017). The objective of this study was blind comparison of culture, matrix assisted laser desorption ionization - time of flight (MALDI-TOF), and 16S rRNA genomic sequencing for test agreement in identification of mastitis pathogens from bovine milk.

Materials and Methods

Ten μ l of milk from all quarter samples submitted to The Dairy Authority (TDA) laboratory on 1 day were streaked onto Columbia blood agar, MacConkey agar, and Modified Hayflick medium. Bacteria isolated within 48 hr were sub-cultured onto CBA and plates were number coded, paraffin sealed, and shipped overnight with cold packs to the University of Missouri (MU). Culture at TDA identified bacteria to genus level except for speciation of *Staphylococcus aureus* and *Escherichia coli*, and grouping of streptococcal-like organisms. Mycoplasma-speciating PCR was available if any colonies were detected on modified Hayflick medium. At MU, a MALDI-TOF mass spectrometer (Bruker Daltonics) tested colonies in duplicate. Comparison with the Biotyper database of known bacteria produced scoring between 1.7 and 1.99 for genus level identification and ≥ 2.0 for species level. 16S rRNA colony lysate PCR products were Sanger sequenced, and sequences were compared to GenBank data using nucleotide-BLAST at MU. All microbiologists were blind to other results. Number coded results were entered into a database program (Microsoft Excel) and all test agreement comparisons were calculated, pairwise for each combination of 2 tests, and for whether all 3 methods agreed. Analysis of strength of test agreement used McNemar's test.

Results

Culture and MALDI-TOF tested 181 isolates; 16S rRNA tested 179 (2 lost during storage). No *Streptococcus agalactiae* or *Mycoplasma* spp were detected, consistent with previous results from the source herd. In accordance with culture, *S. aureus* and *E. coli* agreement was to species level, within streptococcal-like organisms, or all others to genus level. Overall agreement between the 3 diagnostic methods was 94% (169/179); agreement between MALDI-TOF and 16S rRNA was 98% (176/179), both defined as good agreement by McNemar's test. Most bacteria were identified with good agreement among all 3 methods, including 94% (80/85) of isolates defined by culture as *Staph* spp, with 22 isolates defined by culture as *S. aureus*, *Enterobacter* spp, *Klebsiella* spp, *Pasteurella* spp, or *T. pyogenes* showing 100% agreement among all 3 methods. Lowest agreement among all 3 methods was 90% (36/40) for streptococcal-like organisms.

Significance

Many members of the dairy industry are comfortable using either bacterial culture or MALDI-TOF for routine milk bacteria diagnosis, while 16S rRNA is mainly a research tool but becoming more utilized. Newer test method availability has led to some questions within the dairy industry about whether 1 test method is "better" than another, especially regarding the newer methods in comparison to the old. The results suggest that for purposes of milk quality and udder health monitoring or study, any of the 3 methods are valuable tools for the dairy industry.