EuroSciCon

12th Euro-Global Summit on

Veterinary and Animal Sciences

October 11-12, 2018
Edinburgh, Scotland

David J Wilson et al., J Vet Med Surg 2018, Volume 2
DOI: 10.4172/2574-2868-C1-002

MICROBIAL IDENTIFICATION OF BOVINE MILK ISOLATES COMPARED BETWEEN CONVENTIONAL CULTURE, MALDI–TOF AND 16S RRNA

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The objective of this study was to compare conventional microbial culture, MALDI–TOF (Matrix Assisted Laser Desorption/Ionization– Time of Flight), and 16S rRNA partial genomic sequencing methods for microbial identification in quarter milk samples from dairy cattle. The same microbial colonies were tested using each method. There is no agreed upon "gold standard" for "true positive" microbial identification. Therefore, this was a study of test agreement, not sensitivity or specificity; the latter calculations require "true" disease status. All 181 bacterial isolates were tested by culture and MALDI–TOF, while 179 were tested by 16S rRNA because two isolates were lost during storage before the latter test was performed. For *Staphylococcus aureus* and *Escherichia coli,* agreement was to the species level in accordance with conventional culture. For all other microbes, agreement was defined as to the genus level or to within the group defined as streptococcal-like organisms, in keeping with culture and accepted industry practices. All samples were mycoplasma-negative. Overall agreement in identification of microbes between all three diagnostic methods was 94% (169/179). Agreement between MALDI–TOF and 16S rRNA was 98% (176/179); culture agreement with each of the other two methods was 95%. Specific microbes were identified with agreement among all three methods ranging from 97% to 100%, all classified "very good" by the Kappa test. Many members of the dairy industry are used to either bacteriological culture or MALDI–TOF for routine mastitis pathogen diagnosis, and there is interest in the agreement between the methods. These results suggest that either method is of practical value. At present 16S rRNA testing is primarily a research tool, but it showed high agreement with the other methods. For purposes of milk quality and udder health monitoring or study any of the three methods are valuable tools for the dairy industry.

Recent Publications

1. **Barreiro J R, J L Gonçalves, P A Braga, A G Dibbern, M N Eberlin, et al. (2017) Non-culture-based identification of mastitis-causing bacteria by MALDI-TOF mass spectrometry. Journal of Dairy Science 100:2928–2934.**
2. **El-Sayed A, W Awad, N Abdou and H Castañeda Vázquez (2017) Molecular biological tools applied for identification of mastitis causing pathogens. International Journal of Veterinary Science and Medicine 5:89–97.**
3. **Tomazi T, J L Goncalves, J R Barreiro, P A de Campos Braga, L F Prada e Silva, et al. (2014) Identification of coagulase-negative *staphylococci* from bovine intramammary infection by matrix-assisted laser desorption ionization time of flight mass spectrometry. Journal of Clinical Microbiology 52:1658–1663.**
4. **Dethlefsen L, S Huse, M L Sogin and D A Relman (2008) The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. PLoS Biology 6(11):e280**

Biography

David J Wilson was born and grew up in Wisconsin. Graduated from veterinary school at Ohio State University in 1982. Practiced in mixed practice, mainly dairy, until 1987. Three-year residency and post-DVM MS specializing in mastitis and udder health completed in 1990 from Michigan State University. Worked in mastitis and udder health at Cornell University for 15 years, also earning a PhD in Epidemiology and Immunology in the Employee Degree Program. Came to Utah State University in 2006 as dairy extension veterinarian and epidemiologist for the Utah Veterinary Diagnostic Laboratory. Worked mainly on mastitis, milk quality, and stray voltage on dairy farms, high throughput disease testing using milk samples, bovine immunology and Johne's disease. Other work has included Bovine Viral Diarrhea and epidemiology of disease in many species tested through the Utah Veterinary Diagnostic Laboratory.

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