

Speciation of coagulase-negative staphylococci isolates from bovine mastitis and characterization of their virulence factors

Joo Youn Park¹, Lawrence K. Fox¹, Keun Seok Seo², and Gregory A. Bohach²

¹ College of Veterinary Medicine, Washington State University, Pullman, 99164, USA.

² Department of Microbiology, Molecular Biology and Biochemistry, University of Idaho, Moscow, ID
83844, USA.

Abstract

The overarching hypothesis that will be tested is coagulase negative staphylococci (CNS) that cause mastitis are often misidentified as minor pathogens as many species of this genus possess the ability to produce enterotoxins and have other virulence factors. Thus proper speciation is a critical first step in testing this hypothesis. Currently biochemical assays are the cornerstone of CNS speciation of mastitis isolates. In an effort to test the accuracy of the phenotypic vs. a genotypic speciation test, 68 coagulase-negative staphylococci (CNS) isolates from bovine milk were identified by commercial biochemical assay API STAPH ID 20 (bioMérieux) and PCR-RFLP for *gap* gene encoding glyceraldehyde-3-phosphate dehydrogenase. Only 34 CNS isolates (50%) were identified by API STAPH commercial kit with $\geq 80\%$ of identification accuracy whereas 63 isolates (92.6%) were identified by *gap* gene PCR-RFLP. Based on *gap* gene PCR-RFLP results, *S. chromogenes* (n=43, 63.2%) was the most common species followed by *S. xylosus* (n=10, 14.7%), *S. haemolyticus* (n=6, 8.8%) and *S. sciuri* (n=2, 2.9%). To examine the presence of genes encoding 19 staphylococcal enterotoxins (SEA-SEU, except SEF, SES, and SET; TSST-1) in these CNS isolates, multiplex PCR were performed. Of the 68 CNS isolates tested, 60 isolates (88.2%) were found to harbor one or more SE genes. Of these SE gene harboring CNS isolates, 50 isolates (83.3%) have genes encoding newly identified SEs with or without combination of classical SEs (SEA-SEE). Current results indicate that the prevalent genotypes of SEs in CNS isolates were *selo* (n=34, 50.0%), followed by *seu* (n=18, 26.5%). Taken together, *gap* PCR-RFLP appears to be a more accurate tool than the phenotypic method for CNS identification and the prevalence of CNS with genetic constructs for enterotoxins suggests that CNS might be potential source of classical and newly identified SEs and contribute to the pathogenesis of mastitis typically associated with the *S. aureus* pathogen.