Evaluation of the API® 20 Strep system for identification of *Lactococcus lactis* subspecies *lactis* isolated from bovine milk samples

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Introduction

Environmental streptococci and streptococci-like bacteria, including *Lactococcus lactis* ssp *lactis,* account for approximately 40% of clinical mastitis cases in cows. Recently, *Lactococcus lactis* ssp *lactis* was associated with intramammary infections on several New York farms. Although *Lactococcus lactis* ssp *lactis* has been isolated from the bovine udder, its importance as a mastitis pathogen is unclear because of misidentification by use of traditional bacterial identification techniques. Molecular identification has improved correct identification of bacterial pathogens, but it is expensive for the clinical practitioner. In this study, the sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV), and kappa (k) of the API 20 Strep system for identification of *Lactococcus lactis* ssp *lactis* were assessed by the use of molecular identification results as the standard.

Materials and Methods

Samples submitted to the Keseca Veterinary Clinic Milk Laboratory identified as pure cultures of nonhemolytic, esculin-positive, *Streptococcus* species were inoculated onto API 20 Strep test strips. Both 4-hour and 24-hour results were obtained for each isolate in accordance with manufacturer instructions (bioMérieux), regardless of the quality of the 4-hour result. Isolates were then sent to Quality Milk Production Services (Ithaca, NY) for molecular identification by use of PCR assay. Isolates with inconclusive molecular identification or API identifications qualified as "Unacceptable", "Doubtful", or "Not Valid", were excluded from analyses. Three different cutoffs for quality of API identification were used for test evaluation. For each cutoff analyzed (Low Discrimination, Acceptable, and Good), API identifications of *Lactococcus lactis* ssp *lactis* with quality scores equal to or better than the cutoff were considered positive, and those with lower quality than the cutoff were considered negative. Two-by-two tables were evaluated with WinEpiscope 2.0 to calculate the Se, Sp, PPV, NPV, and κ for each cutoff by use of molecular identification (ie, PCR assay) results as the standard.

Results

Out of 79 total isolates, 6 were excluded because of inconclusive molecular identifications. Of the remaining 73 isolates, 24 (32.9%) were identified as *Lactococcus lactis* ssp *lactis*; 11 isolates were excluded from the 4-hour analysis; and 13 were excluded from the 24-hour analysis because of poor quality of API identification. At 4 hours, each of the Good, Acceptable, and Low Discrimination cutoffs had unacceptable Se (< 4.3%), high Sp (100%), and little agreement with molecular identification (k < 0.05). At 24 hours, the Good and Acceptable cutoffs had similar results (Se = 9.5% [95% confidence interval {CI}, 0% to 22.1%]; Sp = 100% [95% CI, 100% to 100%]; PPV = 100% [95% CI, 100% to 100%]; NPV = 67.2% [95% CI, 55.2% to 79.3%]; and κ = 0.12). However, the 24-hour Low Discrimination cutoff had markedly increased Se (76.2%; 95% CI, 58.0% to 94.4%), while Sp (100%; 95% CI, 100% to 100%) remained high. This increased NPV and yielded the highest observed agreement between API and molecular identification results for all cutoffs and time periods when evaluating to the subspecies level (PPV = 100% [95% CI, 100% to 100%]; NPV=88.6% [95% CI, 79.3% to 98%]; κ = 0.81]). At the species level, test agreement was even higher at the 24- hour Low Discrimination cutoff (Se = 81% [95% CI, 64.2% to 97.8%]; Sp = 100% [95% CI, 100% to 100%]; PPV = 100% [95% CI, 100% to 100%]; NPV = 90.7% [95% CI, 82.0% to 99.4%]; κ = 0.85).

Significance

With a 24-hour turnaround time and less required overhead, the API 20 Strep system is an accurate and less expensive alternative to molecular identification for identifying *Lactococcus lactis* ssp *lactis*, but only when results are evaluated at 24 hours and the Low Discrimination cutoff is used to establish a positive identification. If identification to the species is sufficient, the test has even higher accuracy at this cutoff. Despite these promising results, the Se at this cutoff requires a practitioner to consider the consequences of lower NPV in populations with higher disease prevalence before choosing it as a tool for identifying and managing *Lactococcus lactis* ssp *lactis* as a mastitis pathogen.