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Typeability of MALDI-TOF assay for identification of non-*aureus* staphylococci associated with bovine intramammary infections and teat apex colonization

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ABSTRACT

Matrix-assisted laser desorption/ionization time of flight (MALDI-TOF), a culture-dependent assay, has recently been implemented for routine identification of non-*aureus* staphylococci (NAS) species from milk, but the assay has never been investigated for NAS from nonmilk or environmental samples. The objective of this study was to evaluate the typeability of the MALDI-TOF assay for the identification and differentiation of bovine-associated NAS species on aseptically collected quarter milk and teat skin samples in dairy herds. In 8 herds, 14 to 20 cows with elevated somatic cell count were randomly selected for teat skin swabs and foremilk samples from right hind and left front quarters. Teat skin swabs and milk samples were collected aseptically for preliminary identification using bacterial culture on chromogenic and calf blood agars. Colonies from milk and teat skin samples with suspicion of having NAS were identified to species-level by MALDI-TOF assay. Out of 511 isolates from 284 quarters (142 cows), 78% (n = 399) were identified by MALDI-TOF. The percentage of correctly identified NAS from milk (91%, 105/115) using MALDI-TOF was higher than the percentage from teat skin (68%, 268/396). Out of the identified isolates, 93% (n = 373) were successfully identified as NAS, whereas the remaining 26 (7%) were shown to be other bacterial species. Out of 26 NAS isolates, 1 originated from milk (*Corynebacterium stationis*), whereas 25 originated from teat skin representing *Aerococcus viridans* (n = 7), *Bacillus pumilus* (n = 13), *Enterococcus saccharolyticus* (n = 1), *Clostridium septicum* (n = 1), *Corynebacterium stationis* (n = 2), and *Corynebacterium casei* (n = 1). The MALDI-TOF identified 85 (98/115) and 62% (245/396) of the isolates in the first test. Isolates that were not identified to species-level at first test were subjected to a second test, and 47 (8/17) and 32% (48/151) from milk and teat skin, respectively, were identified. After 2 rounds of MALDI-TOF, 22% (n = 112) of the isolates were not identified, representing 103 from teat skin and 9 from milk. Eighteen isolates without identification by MALDI-TOF were successfully identified to species-level using sequencing, where 16 were correctly identified as NAS, whereas the other 2 were *Corynebacterium stationis.* In conclusion, MALDI-TOF is a reliable assay for identification and typeability of NAS species from aseptically collected quarter milk samples. The assay may be used for identification of NAS species from teat skin swabs. However, confirmation using nucleic acid-based tools is vital for accurate species identification of some species and strains.

Key words: non-*aureus* staphylococci, bovine mastitis, teat skin colonization, phenotypic identification

INTRODUCTION

Non-*aureus* staphylococci (**NAS**) are a heterogeneous group of bacterial species (Schukken et al., 2009) regarded as a common cause of IMI in dairy herds (Zadoks and Watts, 2009). Moreover, NAS abundantly colonize the teat skin, teat apex, and teat canal and, hence, many studies have shown that teat colonization with NAS could have a significant role in initiation or development of IMI with NAS in dairy cows (Leroy et al., 2015; De Visscher et al., 2016). Recent studies documented that some species are more important than others in relation to udder health (Supré et al., 2011;

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De Visscher et al., 2016). Furthermore, De Visscher et al. (2014) provided evidence that NAS species are composed of environmental, opportunistic, and host-adapted species, which differ in ecology. Additionally, those authors concluded that some extramammary niches, such as the teat apex, might act as infection sources for IMI-causing NAS. Except for *Staphylococcus xylosus*, an association was observed between teat canal colonization and IMI by all NAS species, in which the majority of IMI were preceded by teat canal colonization (Quirk et al., 2012).

Non-*aureus* staphylococci are known to be a common teat apex colonizer (Braem et al., 2013; Falentin et al., 2016) and are among the mastitis-causing bacteria most likely to enter the mammary gland through the teat orifice (Fox and Norell, 1994), resulting in the establishment of IMI (Pyörälä and Taponen, 2009; Piepers et al., 2010). Braem et al. (2012) identified staphylococci among the bacterial genera with the highest percentage (31%) of colonized teat apices, and they were detected with equal prevalence from teat apices of noninfected, subclinically infected, and clinically infected quarters. Therefore, it is crucial to identify and differentiate the NAS species colonizing teat skin or inhabiting milk that cause IMI to understand their epidemiology and to evaluate the clinical relevance and feasibility of species-specific infection control measures (Zadoks and Watts, 2009). Furthermore, routine microbiological testing is important, because rapid and correct identification of mastitis-causing pathogens will influence the choice of antibiotic before the final determination of antibiotic resistance of the isolate (Nagy et al., 2014). For many years, the species identification of NAS relied on phenotypic characteristics, which is difficult, time-consuming, laborious, and often inaccurate (Watts et al., 1991; Vanderhaeghen et al., 2014). Although biochemical assays such as analytical profile index systems are widely used for identification of NAS, the accuracy and speed is not optimal (Taponen et al., 2006; Capurro et al., 2009; Sampimon et al., 2009; Park et al., 2011).

Matrix-assisted laser desorption/ionization time of flight mass spectrometry is a rapid method that is able to identify a great variety of isolated bacteria based on the composition of conserved ribosomal proteins (Kliem and Sauer, 2012). This technique is based on the acquisition of protein (ribosomal proteins) fingerprints directly from intact microorganisms, as such profiles vary considerably among microorganisms (Singhal et al., 2015). The assay provides a new diagnostic platform that overcomes the limitations of traditional diagnostics for NAS, being time-consuming and laborious, or the need of sugar fermentation or test kits (Watts et al., 1991; Capurro et al., 2009; Vanderhaeghen et al., 2014; Taponen et al., 2016). The technique is increasingly used in human medicine and, recently, it has been expanded as a routine diagnostic tool in veterinary medicine (Randall et al., 2015; Pizauro et al., 2017). In recent years, research studies showed that MALDI-TOF is a powerful and reliable diagnostic tool for identification and discrimination of mastitis-causing pathogens, including NAS from bovine mastitis samples (Tomazi et al., 2014; Gonçalves et al., 2014; Cameron et al., 2017, 2018) and spiked milk samples (Barreiro et al., 2017). The MALDI-TOF assay was validated against other routine diagnostics for NAS, such as the Vitek 2 compact system (Elbehiry et al., 2016) and PCR (Pizauro et al., 2017), where it showed a better performance in identification and discrimination of NAS species from bovine mastitis. To the best of our knowledge, no literature is available describing the performance of MALDI-TOF assay for identification of NAS from nonmilk cow samples or environmental samples in dairy herds. The objective of our study was to evaluate the typeability of the MALDI-TOF assay for the identification and differentiation of bovine-associated NAS species on quarter level from aseptically collected milk (IMI) and teat skin (teat apex colonization) habitats in dairy herds.

MATERIALS AND METHODS

Study Population

Eight dairy herds with Danish Holstein cows were selected to participate in a project on *Streptococcus agalactiae* and *Staphylococcus aureus* IMI. To be eligible for inclusion in the present study, herds had to have automatic milking systems with $\geq$3 milking robots and bulk tank milk PCR cycle threshold value $\leq$32 for *Streptococcus agalactiae*. About 30 to 40 lactating dairy cows were selected randomly from each herd on the basis of the criteria of having no clinical mastitis, SCC $\geq$200,000 cells/mL at the preceding milk recording, and not subjected to antibiotic therapy during the 4 wk before sample collection. From each cow with an odd laboratory running number, teat skin swab and aseptic milk samples were taken from right hind and left front quarters (Mahmmod et al., 2018).

Sampling Procedures

Each herd was visited once to collect teat swab samples and aseptically collected quarter foremilk samples for bacterial culture. The farmers were asked to separate the selected cows for sampling. Cows were fixed in head lockers or tied. Teat swab samples were collected according to the modified wet-dry method (Paduch et al., 2013). Briefly, the teat skin was sampled after cleaning with dry tissue paper. The first rayon swab

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