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Species identification of coagulase-negative staphylococci: Genotyping is superior to phenotyping

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ABSTRACT

Coagulase-negative staphylococci (CNS) are isolated commonly from bovine milk and skin. Their impact on udder health and milk quality is debated. It has been suggested that sources and consequences of infection may differ between CNS species. Species-specific knowledge of the impact and epidemiology of CNS intramammary infections is necessary to evaluate whether species-specific infection control measures are feasible and economically justified. Accurate measurement of impact, sources, and transmission mechanisms requires accurate species level identification of CNS. Several phenotypic and genotypic methods for identification of CNS species are available. Many methods were developed for use in human medicine, and their ability to identify bovine CNS isolates varies. Typeability and accuracy of typing methods are affected by the distribution of CNS species and strains in different host species, and by the ability of test systems to incorporate information on new CNS species into their experimental design and reference database. Generally, typeability and accuracy of bovine CNS identification are higher for genotypic methods than for phenotypic methods. As reviewed in this paper, DNA sequence-based species identification of CNS is currently the most accurate species identification method available because it has the largest reference database, and because a universally meaningful quantitative measure of homology with known species is determined. Once sources, transmission mechanisms, and impact of different CNS species on cow health, productivity and milk quality have been identified through use of epidemiological data and accurate species identification methods, appropriate methods for routine use in research and diagnostic laboratories can be proposed.

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1. Introduction

The role of coagulase-negative staphylococci (CNS) as etiological agents of bovine mastitis has not been fully elucidated and previous studies in this area have yielded contradictory results. For example, some researchers regard CNS as an important cause of bovine mastitis (Pyörälä and Taponen, 2009), while others consider them minor pathogens with limited impact on milk quality and udder health (Schukken et al., 2009). Presence of CNS is associated with clinical mastitis and with somatic cell counts (SCC) that are, on average, higher than those in culture-negative quarters (Schepers et al., 1997; Kudinha and Simango, 2002). Increased SCC is generally associated with decreased milk production (Seegers et al., 2003) but subclinical intramammary infections by CNS have been associated with increased milk production (Wilson et al., 1997). By contrast, clinical CNS mastitis was linked to decreased milk production (Gröhn et al., 2004) and increased risk of culling (Gröhn et al., 2005). Mere

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detection of CNS in a milk sample was not associated with an increased risk of treatment or culling for mastitis (Reksen et al., 2006). In addition to debate about the impact and relevance of CNS infections, there is debate on whether or not specific CNS species are associated with the outcome of infection. A Finnish study showed an association between CNS species and severity of clinical symptoms (Honkanen-Buzalski et al., 1994), but a different study from the same country did not show such an association (Taponen et al., 2006). One CNS species, *Staphylococcus chromogenes*, is thought to protect the udder from intramammary infection (Matthews et al., 1990a; De Vliegher et al., 2003), whereas *Staphylococcus hyicus*, a closely related species, does not have this effect. CNS has been categorized into human and animal-associated species (Watts and Owens, 1989). Human-associated CNS species, specifically *Staphylococcus epidermidis*, are thought to be more likely to invade and infect the udder than animal-associated species (Devriese and De Keyser, 1980; Watts and Owens, 1989). Prevalence of *S. epidermidis* may be associated with herd management factors. Specifically, *S. epidermidis* is more common in herds that use linear dodecyl benzene sulphonic acids for postmilking teat disinfection than in herds that use iodine (Hogan et al., 1987; Watts and Owens, 1989). Whether species-specific transmission routes and control strategies exist for other CNS species is largely unknown. Because little species-specific information on control of CNS mastitis is available, identification to the group level, possibly supplemented with antimicrobial susceptibility testing, is currently sufficient for most therapeutic and management decisions. The defining characteristic of CNS as a group is the lack of coagulase expression, which is a phenotypic trait. To evaluate whether species-specific infection control measures are feasible and economically justified, species-specific knowledge of the impact and epidemiology of CNS infections is necessary. Accurate measurement of the impact, sources, transmission mechanisms and control options for individual CNS species requires accurate species level identification of CNS (Thorberg and Brändström, 2000; Heikens et al., 2005; Sivadon et al., 2005). In this contribution, merits of phenotypic and genotypic methods for CNS species identification are compared with special consideration of identification of CNS isolated from bovine milk.

2. The species concept

Interpretive criteria for the definition of bacterial genera and species are not consistent in the literature and may differ between species, genera and authors (Freney et al., 1999; Lan and Reeves, 2001; CLSI, 2007). In fact, the whole concept of what defines a bacterial species is a matter of debate (Lan and Reeves, 2001). Standards for description of new staphylococcal species were last defined by the Subcommittee on the taxonomy of staphylococci and streptococci of the International Committee on Systematic Bacteriology in 1999 and were based largely on phenotypic criteria (Freney et al., 1999). Nowadays, combinations of phenotypic and genotypic methods are used to define new species, such as biochemical profiling, gas chromatographic analysis of cellular fatty acids, ribotyping, sequencing of the 16S rRNA gene and sequencing of additional housekeeping genes (Becker et al., 2004; Carretto et al., 2005). For many species, there is only a single or a limited number of type strains, and their phenotype and genotype defines the species (Becker et al., 2004; Shah et al., 2007). The aim of this paper is not to discuss the definition of species, but the accurate designation of species names to clinical isolates. Given that species and type strains exist, our task as diagnosticians and scientists is to determine to which species the CNS isolates that we study belong.

3. Phenotypic identification of CNS

Phenotypic identification methods are based on evaluation of the expression of genetically encoded characteristics by bacterial isolates. Phenotypic traits include morphology, growth characteristics, ability to metabolize substrates, antimicrobial resistance, and other features that result from DNA-expression but that are not based on detection of the bacterial DNA itself. Over the years, many phenotypic methods have been developed for the identification of staphylococci in diagnostic laboratories. Methods include commercial test systems such as the API 20 Staph system (bioMérieux), API ID 32 Staph (bioMérieux), Staph-Zym (Rosco), the Vitek system (bioMérieux) and other combinations of biochemical tests, which may not be available in commercial formats (Bannerman et al., 1993; Devriese et al., 1994; Watts and Yancey, 1994; Ieven et al., 1995).

An inherent weakness of phenotypic methods is that there is variability in expression of phenotypic characteristics by isolates belonging to the same species (Bannerman et al., 1993; Ieven et al., 1995; Heikens et al., 2005). Furthermore, the interpretation of phenotypic tests can be subjective (Carretto et al., 2005). Variability in the expression and interpretation of phenotypic characteristics limits the reproducibility of tests, i.e. the ability to generate the same results every time the tests are used. In addition to reproducibility, the typeability and accuracy of phenotypic testing are imperfect. Typeability is the proportion of isolates that are assigned a type by a typing system (Struelens, 1996). An increase in the number of tests that is included in a system generally improves typeability. For example, a study of human CNS isolates with an API-system based on 20 biochemical reactions showed a typeability of only 37% (Carretto et al., 2005) whereas a system that included 32 reactions had a typeability of 85% (Maes et al., 1997).

Accuracy does not have a single standard definition. The concept can be interpreted in two major ways. First, accuracy can denote the level of certainty assigned by a test to its own results. The statement "Isolate 13 was identified to *S. cohnii* by API Staph with 99.1% accuracy" (from Heir et al., 1999) would be an example of this interpretation. Second, accuracy can denote the level of agreement between a method and a reference method, i.e. the correctness of the identification. The statement "Isolate 13, identified to *S. cohnii* by API Staph with 99.1% accuracy was identified as *S. caseolyticus* by 16S rRNA gene sequence

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