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The quest for the perfect test: Phenotypic versus genotypic identification of coagulase-negative staphylococci associated with bovine mastitis

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ABSTRACT

Coagulase-negative staphylococci (CNS) are a frequent cause of bovine intramammary infection and the objective of this paper is to discuss the utility of phenotypic identification systems used for species identification of CNS relative to use of genotypic identification. Mastitis control programs have been developed for major mastitis pathogens but few are specifically targeted for control of CNS. Few documented differences in treatment outcomes of mastitis caused by different species of CNS have been published, and at least one study has reported no differences in bacterial cures of mastitis caused by CNS based on genotypic identification. A number of commercial identification kits for species identification of CNS have been evaluated by mastitis researchers. Most phenotypic systems are considered to accurately identify >80% of staphylococci but have not been designed to detect all taxa that have been associated with bovine mastitis. Typical results were observed in the evaluation of agreement between 2 systems used for identification of staphylococci ($n = 54$) isolated from cases of mastitis. Satisfactory agreement (Kappa > 0.87) was achieved for API Staph but low agreement at the species level was seen for the BBL Crystal Gram-Positive system (Kappa = 0.25). Results of this small study are typical of similar studies and confirm that differences occur among phenotypic identification systems. In spite of the limited precision of some phenotypic identification systems, their consistent use with an adequate number of isolates in the diagnostic algorithm is probably sufficient for most mastitis control programs, which are currently not based on species level identification. However, genotypic identification will be useful for advancing knowledge of the role of CNS in bovine mastitis.

1. Introduction

In many modern dairy herds, coagulase-negative staphylococci (CNS) are a frequent cause of bovine mastitis and their importance as mastitis pathogens has been recently reviewed (Taponen and Pyörälä, 2007). On farms that have successfully controlled mastitis caused by *Staphylococcus aureus* and *Streptococcus agalactiae*, opportunistic bacteria such as CNS are frequently associated with bovine mastitis. In Wisconsin, it is quite common for CNS to be recovered from about 15 to 20% of bovine milk samples obtained from cows experiencing subclinical and clinical mastitis (Table 1) (Hoe and Ruegg, 2005; Makovec and Ruegg, 2003; Pol and Ruegg, 2007). Likewise, in prevalence studies conducted in other regions CNS are commonly recovered from milk samples obtained from cows affected by mastitis (Myllys et al., 1998; Wilson et al., 1997; Bradley et al., 2007). CNS are the most common pathogens recovered from heifers and a variety of CNS species have been recovered from teat skin, the streak canal and pre-calving udder secretion obtained from heifers (Nickerson et al., 1995; Borm et al., 2006).

The genus *Staphylococcus* contains at least 40 species and 17 subspecies (http://www.bacterio.cict.fr/s/staphylococcus.html; Bannerman, 2003) and it is conceivable that under the right circumstances most of these organisms

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Table 1
Proportion of coagulase-negative staphylococci recovered from bovine milk samples in selected studies in Wisconsin, USA.

Study	Origin of isolate	Type of sample	Number of samples	Number of farms	Recovery of CNS
Makovec and Ruegg (2003)	State diagnostic laboratory submissions	Quarter and composite	83,650 Milk samples	Not specified	11,062 (13.2%)
Hoe and Ruegg (2005)	Cases of mild or moderate clinical mastitis	Duplicate quarter samples	133 Cases	4 Farms	26 (19.5%)
Pol and Ruegg (2007)	Subclinical mastitis	Single quarter milk samples	5,672 Milk samples	40 Farms	850 (15.0%)

Table 2
Quarter prevalence of *Staphylococcus* species by parity and lactation period (adapted from Matthews, 1992).

Species	Prepartum teat orifice swabs		Parturition (duplicate 1/4 milk)		Week 5 postpartum (duplicate 1/4 milk)	
	Lact. 1 (%)	Lact. 2+ (%)	Lact. 1 (%)	Lact. 2+ (%)	Lact. 1 (%)	Lact. 2+ (%)
S. aureus	6.9	2.3	7.6	0.6	3.5	0.3
S. hominus	2.8	7.1	0.7	0.6	1.4	1.0
S. chromogenes	28.5	28.9	12.5	10.4	9.7	6.2
S. warneri	0.7	3.9			0.7	0.7
S. xylosus	0.7	2.6	0.7			
S. epidermidis	2.1	3.2	2.8	1.0		0.6
S. simulans	3.5	1.0	4.2		2.8	2.8
Staph spp. no ID	0.7	3.6	0.7	0.3		

could be capable of causing bovine mastitis. *S. chromogenes, S. hyicus* and *S. simulans* are generally considered to be predominant species associated with bovine mastitis but many species of CNS have been recovered from milk samples (Sears and McCarthy, 2003). One study that characterized CNS recovered from bovine mastitis occurring on 16 commercial farms in Vermont, [0] identified more than 10 *Staphylococcus* species, i.e. *epidermidis*, *hyicus*, *hominis*, *xylosus*, *simulans*, *warneri*, *haemolyticus*, *sciuri*, *capitis*, *saprophyticus* and *lentus* (Hogan et al., 1987). Many species of CNS have been associated with bovine mastitis, but epidemiological differences among various species have not been well defined. While recovery of *S. aureus* has been associated with stage of lactation and parity, similar trends for individual species of CNS have not been consistently documented (Table 2). In spite of the high incidence of intramammary infections associated with CNS, species specific control programs are rare and most diagnostic laboratories do not even utilize phenotypic identification for clinical specimens. The aim of this paper is to discuss the utility of phenotypic identification systems for CNS in light of evolving systems of genotypic identification, which are described in a companion paper (Zadoks and Watts, 2009).

2. Role of species identification in control programs for mastitis caused by CNS

The principles of mastitis control have been understood for decades and are based on reducing new infections and limiting the duration of existing infections (Bramley and Dodd, 1984). Coagulase-negative staphylococci are generally considered to be opportunistic pathogens that result in mildly elevated somatic cell count and occasional bouts of clinical mastitis. Researchers that ribotyped CNS recovered from subclinical cases of mastitis reported that a variety of types were isolated from different quarters of the same cow and suggested that infections were acquired independently, thus reinforcing their opportunistic nature (Aarestrup et al., 1999).

High rates of spontaneous cure and acceptable responses to antimicrobial treatment are generally reported for mastitis caused by CNS and few differences in these outcomes have been identified among the various species (Wilson et al., 1999; Taponen and Pyörälä, 2007). While some early research has suggested that virulence varies among CNS species (Myllys, 1995; Aarestrup and Jensen, 1997), more recent research has reported only minimal or no difference in virulence based on species (Taponen et al., 2006). A recent study that utilized both species identification and amplified fragment length polymorphisms (AFLP) for identification of CNS, found that there was no association between species and production of β-lactamase, severity of the case or response to antimicrobial treatment (Taponen et al., 2006). Likewise, there was no statistical difference in bacterial cure based on AFLP cluster (Taponen et al., 2006).

Some initial research suggested that duration of infection may vary among species of CNS with particular attention directed toward enhanced persistence of *S. simulans* as compared to other species (Aarestrup and Jensen, 1997). However, more recent research did not confirm that hypothesis, as the same CNS species and isolates with similar AFLP patterns were found in persistent and transient cases of mastitis (Taponen et al., 2007). It is likely that CNS are acquired from both the cow's environment and via contagious transmission from infected or colonized cattle and general mastitis control principles are considered effective (Sears and McCarthy, 2003).

There has been some suggestion that prevalence and distribution of various species of CNS could be influenced by the choice of teat dips but these differences did not appear to be clinically relevant because there was no