The Veterinary Journal 203 (2015) 44–51

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Review

Identification, typing, ecology and epidemiology of coagulase negative staphylococci associated with ruminants

Wannes Vanderhaeghen [a,*,1], Sofie Piepers [a], Frédéric Leroy [b], Els Van Coillie [c], Freddy Haesebrouck [d], Sarne De Vliegher [a]

[a] M-team and Mastitis and Milk Quality Research Unit, Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium
[b] Research Group of Industrial Microbiology and Food Biotechnology, Faculty of Sciences and Bio-engineering Sciences, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussels, Belgium
[c] Technology and Food Science Unit, Institute for Agricultural and Fisheries Research (ILVO), Brusselsesteenweg 370, 9090 Melle, Belgium
[d] Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

ARTICLE INFO

Article history:
Accepted 3 November 2014

Keywords:
Ruminants
Dairy cattle
Subclinical mastitis
Intramammary infections
Coagulase negative *Staphylococcus* spp

ABSTRACT

Since phenotypic methods to identify coagulase negative staphylococci (CNS) from the milk of ruminants often yield unreliable results, methods for molecular identification based on gene sequencing or fingerprinting techniques have been developed. In addition to culture-based detection of isolates, culture-independent methods may be of interest. On the basis of molecular studies, the five CNS species commonly causing intramammary infections (IMI) are *Staphylococcus chromogenes*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus simulans* and *Staphylococcus xylosus*. Current knowledge suggests that *S. chromogenes* is a bovine-adapted species, with most cases of IMI due to this bacterium being opportunistic. *S. haemolyticus* also appears to be an opportunistic pathogen, but this bacterium occupies a variety of habitats, the importance of which as a source of IMI remains to be elucidated. *S. xylosus* appears to be a versatile species, but little is known of its epidemiology. *S. epidermidis* is considered to be a human-adapted species and most cases of IMI appear to arise from human sources, but the organism is capable of residing in other habitats. *S. simulans* typically causes contagious IMI, but opportunistic cases also occur and the ecology of this bacterium requires further study. Further studies of the ecology and epidemiology of CNS as a cause of IMI in cattle are required, along with careful attention to classification of these bacteria and the diseases they cause.

Introduction

Mastitis is the most common and costly disease in the dairy sector worldwide (Halasa et al., 2007; Huijps et al., 2008). It can occur in clinical or subclinical forms, the latter indicated by an increase in somatic cell count. Mastitis typically results from bacterial intramammary infection (IMI), the most common causative agents being staphylococci, streptococci and coliforms (Tenhagen et al., 2006; Bradley et al., 2007). These infectious agents can be grouped on the basis of their impact on udder health, with major pathogens, such as *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus agalactiae* and *Streptococcus uberis*, being distinguished from minor pathogens, such as *Corynebacterium* spp. and coagulase negative staphylococci (CNS).

Mastitis pathogens are also differentiated by their main reservoirs and principal mode of transmission within a herd into contagious, environmental and (teat skin-associated) opportunistic pathogens (Pyörälä and Taponen, 2009; Zadoks et al., 2011). The principal source of contagious pathogens is assumed to be the cow or the infected udder, with spread among cows primarily through vectors, such as the milking machine, human hands or flies. Environmental pathogens are assumed to originate from the cow's environment, contaminating teats mainly between milkings, especially under suboptimal housing conditions. Skin-associated opportunists are pathogens normally residing on the skin that only cause disease under conditions favouring colonisation of the udder.

The category of skin-associated opportunists was established principally to classify CNS found in milk, even though sound data on their habitats and main sources was lacking (Pyörälä and Taponen, 2009). Despite their current classification into 48 species and 23 subspecies,[1] CNS have long been regarded by mastitis researchers

* Corresponding author. Tel.: +32 92 647545.
E-mail address: wannes.vanderhaeghen@amcra.be (W. Vanderhaeghen).
[1] Present address: Centre of Expertise on Antimicrobial Consumption and Resistance in Animals (AMCRA vzw), Salisburylaan 133, 9820 Merelbeke, Belgium.

[1] See: http://www.bacterio.net/s/staphylococcus.html (accessed 28 October 2014).

http://dx.doi.org/10.1016/j.tvjl.2014.11.001
1090-0233/

as a homogeneous group of little interest. However, CNS are the leading cause of subclinical mastitis on well-managed dairy farms that have controlled contagious pathogens (Bradley, 2002; Pitkälä et al., 2004; Tenhagen et al., 2006; Bradley et al., 2007; Piepers et al., 2007; Schukken et al., 2009).

The economic importance of subclinical mastitis is well established (Seegers et al., 2003; Halasa et al., 2007; Huijps et al., 2008) and recent research has focused on the ecology and epidemiology of CNS associated with mastitis. The aim of this review is to assess the progress that has been made in the identification and typing of ruminant-associated CNS and to outline how this has improved our understanding of the prevalence, ecology and epidemiology of CNS species associated with mastitis in dairy cows. In this review, CNS are regarded as pathogens causing IMI and leading to mastitis. However, the pathogenic role of CNS is complex and some CNS species/strains might have a beneficial effect on udder health (Vanderhaeghen et al., 2014).

Identification and typing of coagulase negative staphylococci from ruminants

Phenotypic identification

CNS constitute the majority of the *Staphylococcus* genus; only six Staphylococcus (sub)species (*S. aureus, Staphylococcus delphini, Staphylococcus intermedius, Staphylococcus lutrae, Staphylococcus pseudintermedius and Staphylococcus schleiferi subsp. coagulans*) are typically coagulase positive and two (*Staphylococcus hyicus and Staphylococcus agnetis*) are coagulase variable. Except for *S. aureus* and *S. hyicus/S. agnetis*, coagulase positive staphylococci are rarely isolated from cases of ruminant mastitis. Reliable phenotypic identification within the CNS group is laborious, since it requires a large number of biochemical tests (Kloos and Schleifer, 1975; Devriese et al., 1985; Watts et al., 1991; Thorberg and Brändström, 2000).

CNS species-specific data were traditionally obtained using commercial identification kits, such as API Staph ID (bioMérieux) and Staph-Zym (Rosco), although these tests were principally developed for human clinical settings. API Staph ID has been recommended by the American National Mastitis Council for differentiation of CNS (Hogan et al., 1999). However, the typeability (i.e. the ability to obtain a species name) and accuracy (i.e. the ability to obtain a correct species name compared with a gold standard) of these systems for identification of CNS isolates from cows (Thorberg and Brändström, 2000; Taponen et al., 2006, 2008; Capurro et al., 2009; Sampimon et al., 2009b; Park et al., 2011) or small ruminants (Onni et al., 2010, 2012; Koop et al., 2012a) is limited.

Using API Staph ID or Staph-Zym, 11–59% unidentified isolates have been reported from milk samples from healthy cows or cows with subclinical or clinical mastitis (Thorberg and Brändström, 2000; Capurro et al., 2009; Sampimon et al., 2009b) and from goat milk (Koop et al., 2012a). The accuracy of API Staph ID was 77%, 76% and 41% compared with conventional biochemical methods (Thorberg and Brändström, 2000), 16S rRNA sequencing (Park et al., 2011) and *rpoB* sequencing (Sampimon et al., 2009b), respectively. The accuracy of Staph-Zym was 94%, 61% and 31% compared with conventional biochemical methods (Thorberg and Brändström, 2000), *tuf* sequencing (Capurro et al., 2009) and *rpoB* sequencing (Sampimon et al., 2009b), respectively. In particular, problems have been encountered in the identification of *Staphylococcus chromogenes*, *Staphylococcus haemolyticus* and *Staphylococcus simulans*, which are common species causing IMI, along with *Staphylococcus cohnii*, *Staphylococcus equorum* and *Staphylococcus warneri*, which less frequently cause IMI (Thorberg and Brändström, 2000; Sampimon et al., 2009b; Onni et al., 2010; Park et al., 2011; Koop et al., 2012a). Hence, these phenotypic systems should be abandoned for scientific and routine use in bovine CNS species identification.

Molecular identification

Sequencing of (housekeeping) genes is considered to be the most reliable method for species identification of bacteria (CLSI 2007; Zadoks and Watts, 2009). The 16S rRNA gene is most universally targeted and several protocols have been established for sequencing the staphylococcal 16S rRNA gene (Boerlin et al., 2003; Becker et al., 2004; Heikens et al., 2005). However, staphylococcal 16S rRNA sequences have a relatively high sequence similarity (mean similarity 97.4%) (Kwok et al., 1999; Shah et al., 2007), making the gene unsuitable for distinguishing closely related species and subspecies (Becker et al., 2004; Taponen et al., 2006). Therefore, sequencing of other genes is preferable and protocols have been validated for *hsp60*/*cpn60*/*groEL* (heat shock protein 60) (Goh et al., 1996; Kwok et al., 1999; Kwok and Chow, 2003), *rpoB* (β subunit of RNA polymerase) (Drancourt and Raoult, 2002), *sodA* (superoxide dismutase A) (Poyart et al., 2001), *gap* (glyceraldehyde-3-phosphate dehydrogenase) (Ghebremedhin et al., 2008; Bergeron et al., 2011), *dnaJ* (chaperone DnaJ) (Shah et al., 2007) and *tuf* (elongation factor Tu) (Capurro et al., 2009; Bergeron et al., 2011).

Mean interspecific sequence similarities are 77.6% for *dnaJ*, 81.5% for *sodA*, 82% for *hsp60*, 86% for *rpoB* and 86–97% for *tuf* (Kwok et al., 1999; Poyart et al., 2001; Drancourt and Raoult, 2002; Kwok and Chow, 2003; Mellmann et al., 2006; Bergeron et al., 2011). *rpoB* has been shown to be useful for discrimination of staphylococcal subspecies (Mellmann et al., 2006; Bergeron et al., 2011).

Genotypic methods used to generate bacterial 'fingerprints' that have been optimised for identification of CNS isolates from bovine mastitis include transfer-RNA intergenic spacer PCR (tDNA-PCR) combined with capillary gel electrophoresis (Supré et al., 2009; Koop et al., 2012a), 16S-23S rDNA gene internal transcribed spacer PCR (ITS-PCR) (Bes et al., 2000) and $(GTG)_5$-PCR fingerprinting, a repetitive DNA sequence-based PCR (rep-PCR) technique (Braem et al., 2011). It is important to include isolates originating from both the environment and host in reference libraries generated for these techniques. $(GTG)_5$-PCR has a typeability of 94.7% and an accuracy of 94.3% for identification of CNS (Braem et al., 2011). tDNA-PCR has a typeability of 91% and an accuracy of 99.2% (Supré et al., 2009); an advantage of this technique is the availability of a free software programme (BaseHopper),[2] which allows rapid and convenient species identification from a pattern, but reference patterns are specific to the particular equipment and consumables used within a laboratory (Koop et al., 2012a).

PCR-restriction fragment length polymorphism (RFLP) applied to the *groEL*, *rrs* or *gap* genes has been used to identify CNS from bovine (Santos et al., 2008; Park et al., 2011), ovine (Onni et al., 2010) and caprine milk (Onni et al., 2012). PCR-RFLP of the 16S rRNA gene is not sufficiently discriminative, in particular for the closely related species *S. epidermidis*, *Staphylococcus caprae* and *Staphylococcus capitis* (Onni et al., 2010). Performance of RFLP-PCR might depend on the restriction enzyme used (Park et al., 2011). Amplified fragment length polymorphism (AFLP) has relatively high discriminatory power and can be used to distinguish most closely related CNS species, with a typeability of 98.4% and accuracy of 99.2%, but is labour intensive and expensive (Taponen et al., 2006, 2007; Piessens et al., 2010). Ribotyping performs well in distinguishing bovine CNS species (Bes et al., 2000; Taponen et al., 2008).

[2] See: www.basehopper.be (accessed 28 October 2014).