Coagulase-negative staphylococci as cause of bovine mastitis—Not so different from *Staphylococcus aureus*?

Suvi Taponen *, Satu Pyöälä

University of Helsinki, Faculty of Veterinary Medicine, Department of Production Animal Medicine, Helsinki, Finland

1. Introduction

Staphylococci are the bacteria most commonly isolated from subclinical mastitis (Pitkälä et al., 2004; Roberson et al., 2006; Tenhagen et al., 2006). In mastitis diagnostics, staphylococci are divided into coagulase-positive staphylococci (CPS) and coagulase-negative staphylococci (CNS) based on the ability to coagulate rabbit plasma. The major pathogen, *Staphylococcus aureus*, is generally coagulase-positive although coagulase-negative strains of *S. aureus* do occur (Fox et al., 1996). Some other *Staphylococcus* species, including *Staphylococcus hyicus*, may also be coagulase-positive (Hajek, 1976; Devriese et al., 1978, 2005). In some herds, coagulase-positive *S. hyicus* may represent a marked proportion of CPS isolates (Roberson et al., 1996). Although misclassification of species can occur when the classification is based on the coagulase test only, the classification of staphylococci into two groups, CPS or *S. aureus* on the one hand and CNS or *Staphylococcus* spp. on the other hand, has been considered sufficient because CNS usually only cause subclinical or mild clinical mastitis. Currently, staphylococci other than *S. aureus* comprise 39 characterized species. More than 10 CNS species have been isolated from mastitis, but only a few species predominate. As mastitis-causing agents, CNS are always separated from *S. aureus*, but are they so different?

2. Epidemiological characteristics—comparison of CNS and *S. aureus*

CNS have traditionally been considered normal skin microbiota that can cause mastitis as opportunistic bacteria (Devriese and De Keyser, 1980). The epidemiology of CNS mastitis is still unclear, although a number of studies have been conducted to identify reservoirs of CNS. A wide variety of CNS species have been isolated from cows’ teat canals, skin, and other extramammary sites (Devriese and De Keyser, 1980; Boddie et al., 1987; White et al., 1989; Trinidad et al., 1990a; Matos et al., 1991; Matthews et al., 1992). Species identification in most studies is based on phenotypic characteristics of the bacteria. According to recent studies, phenotypic and genotypic identification of
CNS do not necessarily agree (Bes et al., 2000; Taponen et al., 2006). Results of species identification should therefore be considered cautiously. The predominant CNS species in cow's beddings and environment were reported to be Staphylococcus xylosus, Staphylococcus sciuri, and Staphylococcus saprophyticus (Matos et al., 1991). The same species are frequently isolated from cow's hair coat, nares, teat skin, and other sites (Devriese and De Keyser, 1980; Boddie et al., 1987; White et al., 1989). Many other species, including Staphylococcus chromogenes, Staphylococcus warneri, and Staphylococcus epidermidis, are also isolated from cow's skin (Devriese and De Keyser, 1980; Boddie et al., 1987; White et al., 1989). S. chromogenes was isolated from the skin of the teat apex, teat canal and mammary gland of unbred heifers that were only 10-months-old (Boddie et al., 1987; De Keyser and De Vliegher, 2003). S. chromogenes was also frequently found to colonize other body sites like nares, hair coat, vagina and teat canals of heifers (White et al., 1989). In the study of De Vliegher et al. (2003), 20% of heifers had at least one teat apex colonized by S. chromogenes and prevalence of teat apex colonization with S. chromogenes increased with age. This was not associated with intramammary infection by the same agent.

Except for S. chromogenes, the CNS species are frequently isolated from teat skin, teat apex, and teat canal, but not from mammary gland secretion samples. The CNS species most commonly isolated from bovine intramammary infections, especially from subclinical mastitis in heifers, is S. chromogenes (Trinidad et al., 1990a; Matthews et al., 1992; Nickerson et al., 1995; Aarestrup and Jensen, 1997; De Vliegher et al., 2003; Rajala-Schulz et al., 2004; Taponen et al., 2006). S. chromogenes has been isolated from secretion samples of unbred, pregnant, or freshly calved heifers (Boddie et al., 1987; Trinidad et al., 1990a; Matthews et al., 1991, 1992; Nickerson et al., 1995; Aarestrup and Jensen, 1997; De Vliegher et al., 2003). Another species that is frequently isolated, especially during lactation, is S. simulans (Jarp, 1991; Myllys, 1995a; Waage et al., 1999; Taponen et al., 2006). In some studies in which CNS field isolates originated from clinical or subclinical mastitis, S. simulans was the predominant CNS species (Jarp, 1991; Waage et al., 1999; Taponen et al., 2006). S. hyicus (Jarp, 1991; Honkanen-Buzalski et al., 1994; Myllys, 1995a; Waage et al., 1999; Rajala-Schulz et al., 2004) and S. epidermidis (Aarestrup and Jensen, 1997; Thorberg et al., 2006) have also been isolated frequently from CNS mastitis. Various other CNS species are less frequently isolated from CNS mastitis. The predominant S. aureus strains causing mastitis in the herds also colonized the cow's teat skin and teat canals. They were also isolated from the milking equipment. It seems that further studies are needed for any conclusions to be reached on the epidemiology of S. aureus and CNS infections in dairy herds.
3. CNS mastitis is milder than S. aureus mastitis

S. aureus can cause clinical mastitis with moderate to serious local and systemic signs. In addition, S. aureus often causes subclinical mastitis, which remains persistent (Anderson, 1983). CNS have been regarded as minor pathogens that mostly infect heifers around calving, do not cause clinical signs, cause only a slight increase in the somatic cell count (SCC), and disappear soon after parturition (Myllys, 1995b). It is generally held that in clinical CNS mastitis, only mild local signs are usually seen, such as slight swelling and changes in the milk appearance, but thorough investigations of clinical characteristics of mastitis caused by CNS are rare (Jarp, 1991; Taponen et al., 2006). In a Norwegian study, Jarp (1991) reported that CNS mastitis can produce severe local and systemic signs, but no details were provided in that article. In the study of Taponen et al. (2006), half of the intramammary infections were clinical, but in the majority of cases, the clinical signs were mild. Elevated body temperature was only detected in 7% of cows with CNS mastitis. In two recent studies, cases of toxic mastitis caused by staphylococci other than S. aureus, including CNS, were reported (Bleul et al., 2006; Bravard and Schmitt van de Leemput, 2006). In a pilot study in which five lactating cows were experimentally infected with S. chromogenes, concentrations of inflammatory mediators in milk were 10–100 times lower than in an experimentally induced Escherichia coli mastitis, and the clinical signs were very mild (Simojoki et al., 2007). In the study of Waage et al. (1999) from Norway, S. aureus was most frequently associated with clinical mastitis in heifers (44% of samples), and in mastitis with systemic signs the proportion of S. aureus was >50%. The proportion of CNS in clinical mastitis was 13%. S. simulans represented 54% of the CNS species, followed by S. chromogenes and S. hyicus (15% each). S. hyicus was more often associated with systemic signs than other CNS species. CNS species identification was based on phenotype.

S. aureus and CNS both typically increase SCC for a long time (de Haas et al., 2004). S. aureus usually increases SCC substantially, but SCC typically remains lower in CNS mastitis than in S. aureus mastitis. In a meta-analysis of Djibri et al. (2002), the geometric mean SCC was 138,000 cells/ml in CNS infected quarters and 357,000 cells/ml in S. aureus infected quarters. In the study of Rainard et al. (1990), SCC was >500,000 cells/ml in 38% of milk samples taken from CNS infected quarters, in 42% it was 200,000–500,000 cells/ml, and in 20% <200,000 cells/ml. Nickerson et al. (1995) reported that the mean SCC of quarters infected with S. chromogenes or S. hyicus during the first 3 months of lactation were 168,000 cells/ml and 193,000 cells/ml, respectively. In a study on persistent CNS infection (Taponen et al., 2007), the geometric mean SCC for quarters was 657,600 cells/ml and the median SCC for these quarters was 355,400 cells/ml. The geometric mean SCC of 12 quarters infected with S. aureus in that study was 3,286,000 cells/ml and the median SCC 1,898,700 cells/ml (data not published). Interestingly, Fox et al. (1996) reported that the mean SCC was 283,000 cells/ml for cows with intramammary infections attributable to a coagulase-negative variant of S. aureus, and 373,000 cells/ml for cows with intramammary infections attributable to coagulase-positive S. aureus, indicating that the ability to coagulate plasma may increase virulence. However, only one coagulase-negative strain caused mastitis in that study, and other virulence factors of that strain may have affected the cell count. These results support the general view that S. aureus infections considerably increase milk SCC and are thus much more deleterious for bulk milk quality than CNS infections.

Histopathologic changes caused by staphylococcal infection in bovine mammary glands were investigated in three studies. According to results from these studies, CNS infection causes a similar type of damage in the mammary gland as S. aureus infection, but damage is possibly less serious. Boddie et al. (1987) observed a strong leukocyte response to CNS colonization in the teat canal and mammary tissues of two heifers. Trinidad et al. (1990b) studied histopathologic changes in seven mammary glands of unbred heifers experimentally infected with S. aureus Newbould 305 (ATCC 27,940), 1 quarter naturally infected with S. aureus, and 3 quarters naturally infected with CNS. The quarters infected with S. aureus and CNS showed less alveolar, epithelial and luminal areas, more interalveolar stroma and greater leukocyte infiltration than the uninfected quarters. In quarters infected with CNS, histopathologic changes were not as marked as in quarters infected with S. aureus. Benites et al. (2002) studied histopathology of lactating dairy cows culled due to mastitis. The histopathologic changes of 99 quarters infected with CNS and 14 quarters infected with S. aureus mainly showed a chronic inflammatory response or a chronic inflammatory response with repair, and no differences in the histopathologic changes were observed between S. aureus and CNS infected quarters.

4. CNS mastitis may persist—like S. aureus mastitis

Several authors have reported that prepartum intramammary infection can persist into the early lactation, although the prevalence of CNS or S. aureus infected quarters decreases markedly during early lactation (Oliver and Mitchell, 1983; Boddie et al., 1987; Matthews et al., 1992). Aarestrup and Jensen (1997) followed quarters of heifers from 4 weeks prepartum until 4 weeks postpartum. They showed that prepartum infections caused by S. chromogenes disappeared shortly after parturition. In contrast, infections caused by S. simulans were found to persist for longer, indicating that differences between CNS species may exist. It is generally assumed that spontaneous cure rate of CNS mastitis is high, and in many countries, mastitis caused by CNS is commonly left untreated. The observed spontaneous cure probability varies from 60–70% (McDougall, 1998; Wilson et al., 1999) to 16–49% (Rainard and Poutrel, 1982; Timms and Schultz, 1987; Deluyker et al., 2005; Taponen et al., 2006). However, several studies have shown that CNS mastitis may persist during lactation (Laevens et al., 1997; Chaffer et al., 1999), with 76% (Rainard et al., 1990) to 85% (Timms and Schultz, 1987) persisting until cessation of milking. Taponen et al. (2007) followed 228 udder quarters of 82 cows on one farm monthly throughout lactation and found that about half of the CNS infections persisted, mostly until the end of
lactation. Persistence of the same bacterial strain in the quarters was confirmed with amplified fragment length polymorphism analysis (AFLP). In contrast to the findings of Aarestrup and Jensen (1997), both S. chromogenes and S. simulans were found to persist.

5. Virulence factors of staphylococci

Virulence factors of S. aureus and CNS have been investigated both by measuring phenotypic expression of products assumed to be associated with virulence and by screening genes encoding these products. Various virulence factors, including production of haemolysins, leukocidins, exfoliative toxins, enterotoxins, toxic-shock syndrome toxin, and ability to form slime and biofilm have been found in S. aureus strains isolated from bovine mastitis (Cucarella et al., 2004; Zeconi et al., 2006; Haveri et al., 2008). S. aureus isolates from natural bovine intramammary infections have been demonstrated to internalize and live in mammary gland alveolar cells and macrophages (Hébert et al., 2000). Only few studies have focused on virulence factors of CNS isolated from mastitis. Adherence and internalization of CNS into mammary epithelial cells was studied in cell cultures (Almeida and Oliver, 2001; Anaya-López et al., 2006; Hyvönen et al., 2007). CNS were shown to be able to adhere to bovine mammary cells. The adhesive capacity of various CNS was almost equal to the adhesive capacity of S. aureus, but the invasive capacity of S. aureus was greater than that of CNS strains. In cell cultures, CNS and S. aureus isolates from mastitis showed cytotoxic activity, possibly caused by a metalloprotease (Zhang and Maddox, 2000). The majority of CNS isolated from caprine mastitis produced at least one type of hemolysin, DNAse, and elastase (Bedini-Madani et al., 1998; da Silva et al., 2005). Kuroishi et al. (2003) found that a high percentage of both S. aureus and CNS from bovine subclinical, chronic or acute mastitis produced staphylococcal enterotoxins and/or toxic-shock syndrome toxin-1. Somewhat surprisingly, production of different staphylococcal enterotoxins and toxic shock syndrome toxin-1 were as common in CNS isolates as in S. aureus isolates, and as common in isolates from subclinical mastitis as from chronic or acute mastitis.

Various virulence factors of S. aureus have been compared with clinical characteristics of mastitis, but no specific virulence factor or combination of factors has been strongly associated with the severity of mastitis (Haveri et al., 2008, Baselga et al. (1993) noted that the severity of ruminant mastitis decreased, but the bacterial capacity to colonize the mammary gland increased when the infection was caused by a mucoid (slime producer) rather than a non-mucoid S. aureus isolate. In agreement with that observation Cucarella et al. (2004) showed that a biofilm-producing S. aureus strain decreased severity of mastitis but increased colonization capacity in the mammary gland. The ability of staphylococci to generate biofilm can be studied by measuring biofilm formation phenotypically and by screening genes associated with its formation. Biofilm associated proteins were found among bovine mastitis isolates, including S. aureus, S. epidermidis, S. chromogenes, S. hycicus, and S. xylosus (Cucarella et al., 2004). Oliveira et al. (2006) found 6 out of 16 S. aureus and 6 out of 16 S. epidermidis isolates from subclinical mastitis phenotypically positive for biofilm production. Fox et al. (2005) showed that biofilm formation was more common in S. aureus isolated from milk than in S. aureus isolated from teat skin and milking unit liners.

Biofilm formation by staphylococci from bovine mastitis or human clinical samples is associated with the loci ica (intercellular adhesion), bap (biofilm-associated protein), agr (accessory gene regulator) and sar (staphylococcal accessory regulator), but isolates that form biofilm do not necessarily carry all of these genes, and other mechanisms may be involved in biofilm formation (McKenney et al., 1998; Cramton et al., 1999; Cucarella et al., 2001, 2004; Beeken et al., 2003; Vasudevan et al., 2003; Fox et al., 2005; Lasa and Penadés, 2006; Planchon et al., 2006). The bap gene has been identified in biofilm-producing staphylococci isolated from mastitis, including isolates of S. epidermidis, S. chromogenes, S. xylosus, S. similans and S. hycicus (Tormo et al., 2005). Both bap and ica genes have been identified in mucoid staphylococcal isolates involved in biofilm formation (Cucarella et al., 2004).

Leitner et al. (2003) studied virulence of S. aureus and CNS using a mouse model. Seven strains of S. aureus and one strain of each of S. chromogenes and S. intermediusisolated from chronic bovine mastitis were studied. Mice were experimentally infected in one limb and thereafter inspected for morbidity (arthritis and gangrene) and mortality. One S. aureus isolate producing α-hemolysin was most virulent, followed by isolates producing α + β-hemolysin and β-hemolysin. The least virulent isolates were the non-hemolytic S. aureus strains, but even they were more virulent than the S. chromogenes and S. intermedius isolates tested. These two CNS isolates did not cause any morbidity or mortality.

6. CNS are more resistant to antimicrobials than S. aureus

CNS tend to be more resistant to antimicrobials than S. aureus and easily develop multiresistance. The most common resistance mechanism in staphylococci is β-lactamase production, which results in resistance to penicillin G and aminopenicillins. The reported percentage of penicillin resistance for CNS isolated from mastitis was 36% in Norway (NORM-VET, 2005), 25% in Denmark (DANMAP, 2001), 41–61% in the Netherlands (MARAN, 2004, MARAN, 2005), and 32% in Finland (Pitkälä et al., 2004). Penicillin resistance of S. aureus is generally lower than that of CNS, except in Finland. In Norway, the reported penicillin resistance of S. aureus was 7% (NORM-VET, 2005), in Denmark 18–30% (DANMAP, 2001; DANMAP, 2002), in the Netherlands 7–12% (MARAN, 2004; MARAN, 2005), and in subclinical mastitis in Finland 52% (Pitkälä et al., 2004). In clinical mastitis in Finland, the proportion of β-lactamase positive S. aureus was 13% and that of β-lactamase positive CNS 23% (Nevala et al., 2004), i.e. considerably less than for isolates from subclinical mastitis.
Methicillin resistance is very rare in *S. aureus* isolated from mastitis, but it can be found in CNS isolates. In the Finnish survey material (Pitkälä et al., 2004), 4% of *S. aureus* and 10% of CNS were resistant (breakpoint for oxacillin >2 µg/ml). Some CNS isolates, but none of the *S. aureus* isolates, carried the mecA gene (Pitkälä, personal communication). In the Netherlands, oxacillin resistance was not detected in *S. aureus*, but 5% of CNS were resistant and positive for the mecA gene (MARAN, 2005). In Korea, 2.5% of *S. aureus* and 2.4% of CNS isolates from mastitis were methicillin resistant (Moon et al., 2007). There is evidence that *S. aureus* has acquired the staphylococcal chromosomal cassette mec from CNS (Leonard and Markey, 2008).

Resistance of CNS to macrolides and lincosamides was recently reported to be 6–7% in Germany (Lüthje and Schwarz, 2006) and 4–14% in the Netherlands (MARAN, 2004). Resistance of CNS isolated from mastitis to oxytetracycline was 9% in Finland (Pitkälä et al., 2004) and 16% in the Netherlands (MARAN, 2004). Fusidic acid is not widely used in mastitis treatment, but resistance has been reported among CNS isolated from mastitis. Recently, fusidic acid resistance has been reported to be mediated by the fusB gene (Yazdankhah et al., 2006). The resistance figures in the annual reports of some countries (DANMAP, MARAN, NORM-VET) vary slightly from year to year, but the tendency is that most *S. aureus* isolates were generally susceptible to all or almost all antimicrobials tested, whereas more than half of the CNS isolates were resistant to one or more antimicrobials tested.

7. Response to treatment differs between CNS and *S. aureus*

Mastitis caused by *S. aureus* generally responds poorly to antimicrobial treatment. The reported chances of cure vary widely, from 15% to 85%, depending on many factors, including lactation number, SCC before treatment and β-lactamase production of the mastitis-causing isolate (Barkema et al., 2006). There are few published reports on treatment of quarters infected by CNS only. Based on those reports, mastitis caused by CNS seems to respond well to antimicrobial treatment. The chances of bacteriological cure were 80–90% for β-lactamase negative CNS (McDougal, 1998; Pyörälä and Pyörälä, 1998; Taponen et al., 2006). For β-lactamase positive CNS, the probability of cure seems somewhat lower (Taponen et al., 2006), a phenomenon also recorded for *S. aureus* (Sol et al., 2000; Taponen et al., 2003). In most studies, the isolates were tested in vitro for susceptibility to penicillin, and penicillin G was used in treatment of penicillin-susceptible CNS mastitis. In the study of Rainard et al. (1990), 87% of quarters infected with CNS and treated with cloxacillin were cured. Penicillin-susceptibility was not reported.

8. Differences between CNS species and strains?

Many of the earlier studies evaluating CNS have not differentiated among species, or the identification has been performed with phenotypic test series which recently have been found to be inaccurate (Bes et al., 2000; Taponen et al., 2006, 2007). Hence, a number of general assumptions have been made about differences between CNS and *S. aureus* that may not hold true after introduction of new identification methods and molecular fingerprinting techniques. Studies of CNS virulence factors are scant, and some CNS species may possess similar virulence factors to *S. aureus* while others may not. Different strains within species may also have different virulence, as known from *S. aureus* (Lipman et al., 1996; Smith et al., 1998). It is commonly assumed that differences between CNS species can exist in clinical characteristics and persistence of the intramammary infection. The fact that *S. chromogenes* is particularly associated with heifers around parturition (Trinidad et al., 1990a; Rajala-Schulz et al., 2004), whereas *S. simulans* predominates in samples originating from mastitis during lactation (Jarp, 1991; Waage et al., 1999; Taponen et al., 2006), supports this assumption. However, to date, only few studies have been able to detect any significant differences in clinical characteristics of mastitis caused by different CNS species, perhaps because of the limited size of materials in field studies. In heifers, a significantly higher proportion of *S. hyicus* was isolated from cases with systemic signs of disease than from those without clinical CNS mastitis (Waage et al., 1999). *S. hyicus* was also the most pathogenic CNS species in a study by Myllys (1995a). It was most often isolated from quarters with clinical CNS mastitis, and the activity of N-acetyl-β-D-glucosaminidase in *S. hyicus* infected quarters was significantly higher than in quarters infected with other CNS species. *S. simulans* was also frequently isolated from clinical mastitis (Myllys, 1995a). In some studies, *S. simulans* tended to be associated with clinical mastitis more often than other CNS species (Jarp, 1991; Taponen et al., 2006), but without statistical significance. Aarestrup and Jensen (1997) found differences in persistence between infections caused by *S. chromogenes*, *S. simulans*, and *S. epidermidis*, but such differences were not found by Taponen et al. (2007). The number of infected quarters was small in both studies.

Before any control strategies for prevention of CNS mastitis can be launched, more knowledge is needed about CNS species associated with mastitis and the nature and virulence of the different species. Currently the identification methods for staphylococci are under reassessment and methods based on molecular genetics are being developed. With identification methods based on bacterial genotype, easily available and at reasonable cost, and consensus about criteria for CNS species identification, substantial advances in knowledge about bovine CNS mastitis can be expected.

9. Conclusions

Mastitis caused by *S. aureus* may be subclinical or clinical with severe systemic signs, but CNS seem to lack the ability to cause severe mastitis. Many putative virulence factors have been identified in CNS and, especially, *S. aureus* isolates from mastitis, but the association of different virulence factors with severity of mastitis remains unexplained. *S. aureus* mastitis easily persists. CNS are also able to persist in the mammary gland and cause moderate increase of milk SCC. CNS mastitis...
responds much better to antimicrobial treatment than *S. aureus* mastitis, but resistance to different antimicrobials is more common in CNS than in *S. aureus*. Among all *Staphylococcus* species, including *S. aureus*, some strains seem to be more virulent than others. It is concluded that CNS are clearly less pathogenic than *S. aureus*. CNS should, however, be considered to be mastitis pathogens and not just teat canal microbiota.

**Conflict of interest**

None.

**References**


Mylly, V., 1995b. Staphylococcal mastitis in heifers and dairy cows. Academic dissertation. Department of Food Hygiene, National Veterinary and Food Research Institute, and Department of Clinical Veterinary Medicine, College of Veterinary Medicine, Helsinki, Finland. Ylioppistopaino, Helsinki, Finland.


