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1 **Coagulase-negative staphylococci as cause of bovine mastitis – not so different from**

2 ***Staphylococcus aureus*?**

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1 **Abstract**

2

3 In this review of the literature, mastitis-causing coagulase-negative staphylococci (CNS) and
4 *Staphylococcus aureus* are compared. Staphylococci are the bacteria most commonly isolated from
5 bovine mastitis, and CNS are now predominant over *Staphylococcus aureus* in most countries. CNS
6 include various species, but only a few prevail in bovine mastitis. *S. aureus* can cause clinical mastitis,
7 but often causes subclinical mastitis, which remains persistent and increases milk somatic cell count.
8 CNS, traditionally regarded as minor pathogens, seem to lack the ability to cause severe mastitis. CNS
9 can, however, persist in the mammary gland and moderately increase milk somatic cell count.
10 Resistance to various antimicrobials is more common in CNS than in *S. aureus*, but CNS mastitis
11 responds much better to antimicrobial treatment than *S. aureus* mastitis.

12

13 **Key words:** Mastitis; Bovine; *Staphylococcus aureus*; Coagulase-negative staphylococci

14

1 **1. Introduction**

2

3 Staphylococci are the bacteria most commonly isolated from subclinical mastitis (Pitkälä et al.,
4 2004; Roberson et al., 2006; Tenhagen et al., 2006). In mastitis diagnostics, staphylococci are divided
5 into coagulase-positive staphylococci (CPS) and coagulase-negative staphylococci (CNS) based on the
6 ability to coagulate rabbit plasma. The major pathogen, *Staphylococcus aureus*, is generally coagulase-
7 positive although coagulase-negative strains of *S. aureus* do occur (Fox et al., 1996). Some other
8 *Staphylococcus* species, including *Staphylococcus hyicus*, may also be coagulase-positive (Hajek, 1976;
9 Devriese et al., 1978; Devriese et al., 2005). In some herds, coagulase-positive *S. hyicus* may represent a
10 marked proportion of CPS isolates (Roberson et al., 1996). Although misclassification of species can
11 occur when the classification is based on the coagulase test only, the classification of staphylococci into
12 two groups, CPS or *S. aureus* on the one hand and CNS or *Staphylococcus* spp. on the other hand, has
13 been considered sufficient because CNS usually only cause subclinical or mild clinical mastitis.
14 Currently, staphylococci other than *S. aureus* comprise 39 characterized species. More than ten CNS
15 species have been isolated from mastitis, but only a few species predominate. As mastitis-causing
16 agents, CNS are always separated from *S. aureus*, but are they so different?

17

18 **2. Epidemiological characteristics – comparison of CNS and *S. aureus***

19

20 CNS have traditionally been considered normal skin microbiota that can cause mastitis as
21 opportunistic bacteria (Devriese and De Keyser, 1980). The epidemiology of CNS mastitis is still
22 unclear, although a number of studies have been conducted to identify reservoirs of CNS. A wide variety
23 of CNS species have been isolated from cows' teat canals, skin, and other extramammary sites (Devriese
24 and De Keyser, 1980; Boddie et al., 1987; White et al., 1989; Trinidad et al., 1990a; Matos et al., 1991;
25 Matthews et al., 1992). Species identification in most studies is based on phenotypic characteristics of

1 the bacteria. According to recent studies, phenotypic and genotypic identification of CNS do not
2 necessarily agree (Bes et al., 2000; Taponen et al., 2006). Results of species identification should
3 therefore be considered cautiously. The predominant CNS species in cows' beddings and environment
4 were reported to be *Staphylococcus xylosus*, *Staphylococcus sciuri*, and *Staphylococcus saprophyticus*
5 (Matos et al., 1991). The same species are frequently isolated from cows' hair coat, nares, teat skin, and
6 other sites (Devriese and De Keyser, 1980; Boddie et al., 1987; White et al., 1989). Many other species,
7 including *Staphylococcus chromogenes*, *Staphylococcus warneri*, and *Staphylococcus epidermidis*, are
8 also isolated from cows' skin (Devriese and De Keyser, 1980; Boddie et al., 1987; White et al., 1989). *S.*
9 *chromogenes* was isolated from the skin of the teat apex, teat canal and mammary gland of unbred
10 heifers that were only 10 months old (Boddie et al., 1987; De Vlieghe et al., 2003). *S. chromogenes* was
11 also frequently found to colonize other body sites like nares, hair coat, vagina and teat canals of heifers
12 (White et al., 1989). In the study of De Vlieghe et al. (2003), 20% of heifers had at least one teat apex
13 colonized by *S. chromogenes* and prevalence of teat apex colonization with *S. chromogenes* increased
14 with age. This was not associated with intramammary infection by the same agent.

15 Except for *S. chromogenes*, the CNS species frequently isolated from teat skin, teat apex, and
16 teat canal are mainly different from the CNS species isolated from milk. Devriese and De Keyser (1980)
17 found that the distribution of CNS species in milk samples and on teat skin of dairy cows differed: *S.*
18 *xylosus*, *S. sciuri* and *S. haemolyticus* predominated in the samples obtained from teat skin and apex.
19 Three other species, *S. epidermidis*, *S. chromogenes*, and *S. simulans*, and a group of unidentified
20 staphylococci were frequently isolated from milk samples. The same was shown by Trinidad et al.
21 (1990a). In that study, 7 species of staphylococci were isolated from teat canals, but not from mammary
22 gland secretion samples. The CNS species most commonly isolated from bovine intramammary
23 infections, especially from subclinical mastitis in heifers, is *S. chromogenes* (Trinidad et al., 1990a;
24 Matthews et al., 1992; Nickerson et al., 1995; Aarestrup and Jensen, 1997; De Vlieghe et al., 2003;
25 Rajala-Schultz et al., 2004; Taponen et al., 2006). *S. chromogenes* has been isolated from secretion

1 samples of unbred, pregnant, or freshly calved heifers (Boddie et al., 1987; Trinidad et al., 1990a;
2 Matthews et al., 1991; Matthews et al., 1992; Nickerson et al., 1995; Aarestrup and Jensen, 1997; De
3 Vlieghe et al., 2003). Another species that is frequently isolated, especially during lactation, is
4 *Staphylococcus simulans* (Jarp, 1991; Myllys, 1995a; Waage et al., 1999; Taponen et al., 2006). In some
5 studies in which CNS field isolates originated from clinical or subclinical mastitis, *S. simulans* was the
6 predominant CNS species (Jarp, 1991; Waage et al., 1999; Taponen et al., 2006). *S. hyicus* (Jarp, 1991;
7 Honkanen-Buzalski et al., 1994; Myllys, 1995a; Waage et al., 1999; Rajala-Schultz et al., 2004) and *S.*
8 *epidermidis* (Aarestrup and Jensen, 1997; Thorberg et al., 2006) have also been isolated frequently from
9 CNS mastitis. Various other CNS species are less frequently isolated from milk samples (Devriese and
10 De Keyser, 1980; Rather et al., 1986; Jarp, 1991; Davidson et al., 1992; Aarestrup et al., 1995; Myllys,
11 1995a; Chaffer et al., 1999; Waage et al., 1999; Rajala-Schultz et al., 2004; NORM-VET, 2005; Lütjhe
12 and Schwarz, 2006; Taponen et al., 2006).

13 Most studies on the epidemiology of CNS were performed before accurate methods for strain
14 typing were available. As far as we know, CNS strains from mastitis and other sources were compared
15 in only one study: Thorberg et al. (2006) compared *S. epidermidis* isolates from bovine mastitis and
16 milkers' hands using ribotyping and pulsed-field gel electrophoresis (PFGE). The proportion of cows
17 infected or colonized with *S. epidermidis* in the two study herds was high (22% to 31%), as compared
18 with data from many other studies (Jarp, 1991; Waage et al., 1999). The same strains that were isolated
19 in bovine milk were also isolated from milkers' hands and bends of elbows, indicating that the *S.*
20 *epidermidis* strains causing mastitis could have originated from humans.

21 The epidemiology of CNS infections can be compared with the epidemiology of infection by the
22 major mastitis pathogen *S. aureus*. Like many CNS species, *S. aureus* has been isolated from different
23 extramammary sites in dairy herds (Boddie et al., 1989; Trinidad et al., 1990a; Matos et al., 1991;
24 Roberson et al., 1994). *S. aureus* frequently colonizes body sites of calves and heifers (Boddie et al.,
25 1989; Trinidad et al., 1990a; Matos et al., 1991; Roberson et al., 1994). Roberson et al. (1998) compared

1 *S. aureus* isolates from heifer colostrum with isolates from other milk samples, heifer body sites and
2 environmental sites. The isolates were typed based on biochemical tests, antibiotic sensitivity and phage
3 typing. They found that milk from *S. aureus* infected quarters was the main source of the *S. aureus*
4 strains found in heifer colostrum. Heifer body sites were also found to be a common source of the same
5 *S. aureus* strains. The heifers with *S. aureus* colonies on teat skin had three times higher risk of having *S.*
6 *aureus* mastitis at parturition than heifers without *S. aureus* colonies on teat skin (Roberson et al., 1994).
7 Zadoks et al. (2002) concluded that strains responsible for *S. aureus* mastitis are mainly different from
8 those present on bovine skin, and that milking equipment can transmit both mastitis strains and skin
9 strains. In the study of Zadoks et al. (2002), isolates from cows' skin belonged to the same pulsed-field
10 gel electrophoresis (PFGE) types as skin isolates from milkers' hands. In contrast to the findings for *S.*
11 *epidermidis*, *S. aureus* seems to be predominantly host-specific, i.e. strains of human and bovine origin
12 were mainly different (Zadoks et al., 2002; Smith et al., 2005). Using PFGE and multilocus sequence
13 typing (MLST), Smith et al. (2005) demonstrated that isolates from milk predominantly belong to
14 different strains than isolates from teat skin or from humans, indicating host specificity as well as site
15 specificity, although some *S. aureus* strains can be isolated from milk, milkers' hands, and other sources.
16 Haveri et al. (personal communication) reported contradictory results. In their study of herds with a high
17 prevalence of *S. aureus* mastitis, the predominant *S. aureus* strains causing mastitis in the herds also
18 colonized the cows' teat skin and teat canals. They were also isolated from the milking equipment. It
19 seems that further studies are needed for any conclusions to be reached on the epidemiology of *S. aureus*
20 and CNS infections in dairy herds.

21

22 **3. CNS mastitis is milder than *S. aureus* mastitis**

23

24 *S. aureus* can cause clinical mastitis with moderate to serious local and systemic signs. In
25 addition, *S. aureus* often causes subclinical mastitis, which remains persistent (Anderson, 1983). CNS

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

20 *S. aureus* and CNS both typically increase SCC for a long time (de Haas et al., 2004). *S. aureus*
21 usually increases SCC substantially, but SCC typically remains lower in CNS mastitis than in *S. aureus*
22 mastitis. In a meta-analysis of Djabri et al. (2002), the geometric mean SCC was 138 000 cells/ml in
23 CNS infected quarters and 357 000 cells/ml in *S. aureus* infected quarters. In the study of Rainard et al.
24 (1990), SCC was >500 000 cells/ml in 38% of milk samples taken from CNS infected quarters, in 42% it
25 was 200 000 to 500 000 cells/ml, and in 20% <200 000 cells/ml. Nickerson et al. (1995) reported that

1 the mean SCC of quarters infected with *S. chromogenes* or *S. hyicus* during the first 3 months of
2 lactation were 168 000 cells/ml and 193 000 cells/ml, respectively. In a study on persistent CNS
3 infection (Taponen et al., 2007), the geometric mean SCC for quarters was 657 600 cells/ml and the
4 median SCC for these quarters was 355 400 cells/ml. The geometric mean SCC of 12 quarters infected
5 with *S. aureus* in that study was 3 286 000 cells/ml and the median SCC 1 898 700 cells/ml (data not
6 published). Interestingly, Fox et al. (1996) reported that the mean SCC was 283 000 cells/ml for cows
7 with intramammary infections attributable to a coagulase-negative variant of *S. aureus*, and 373 000
8 cells/ml for cows with intramammary infections attributable to coagulase-positive *S. aureus*, indicating
9 that the ability to coagulate plasma may increase virulence. However, only one coagulase-negative strain
10 caused mastitis in that study, and other virulence factors of that strain may have affected the cell count.
11 These results support the general view that *S. aureus* infections considerably increase milk SCC and are
12 thus much more deleterious for bulk milk quality than CNS infections.

13 Histopathologic changes caused by staphylococcal infection in bovine mammary glands were
14 investigated in 3 studies. According to results from these studies, CNS infection causes a similar type of
15 damage in the mammary gland as *S. aureus* infection, but damage is possibly less serious. Boddie et al.
16 (1987) observed a strong leukocyte response to CNS colonization in the teat canal and mammary tissues
17 of 2 heifers. Trinidad et al. (1990b) studied histopathologic changes in 7 mammary glands of unbred
18 heifers experimentally infected with *S. aureus* Newbould 305 (ATCC 27940), 1 quarter naturally
19 infected with *S. aureus*, and 3 quarters naturally infected with CNS. The quarters infected with *S. aureus*
20 and CNS showed less alveolar, epithelial and luminal areas, more interalveolar stroma and greater
21 leucocyte infiltration than the uninfected quarters. In quarters infected with CNS, histopathologic
22 changes were not as marked as in quarters infected with *S. aureus*. Benites et al. (2002) studied
23 histopathology of lactating dairy cows culled due to mastitis. The histopathologic changes of 99 quarters
24 infected with CNS and 14 quarters infected with *S. aureus* mainly showed a chronic inflammatory

1 response or a chronic inflammatory response with repair, and no differences in the histopathologic
2 changes were observed between *S. aureus* and CNS infected quarters.

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

5
6 Several authors have reported that prepartum intramammary infection can persist into the early
7 lactation, although the prevalence of CNS or *S. aureus* infected quarters decreases markedly during early
8 lactation (Oliver and Mitchell, 1983; Boddie et al., 1987; Matthews et al., 1992). Aarestrup and Jensen
9 (1997) followed quarters of heifers from 4 weeks prepartum until 4 weeks postpartum. They showed that
10 prepartum infections caused by *S. chromogenes* disappeared shortly after parturition. In contrast,
11 infections caused by *S. simulans* were found to persist for longer, indicating that differences between
12 CNS species may exist. It is generally assumed that spontaneous cure rate of CNS mastitis is high, and
13 in many countries, mastitis caused by CNS is commonly left untreated. The observed spontaneous cure
14 probability varies from 60-70% (McDougall, 1998; Wilson et al., 1999) to 16-49% (Rainard and Poutrel,
15 1982; Timms and Schultz, 1987, Deluyker et al., 2005; Taponen et al., 2006). However, several studies
16 have shown that CNS mastitis may persist during lactation (Laevens et al., 1997; Chaffer et al., 1999),
17 with 76% (Rainard et al., 1990) to 85% (Timms and Schultz, 1987) persisting until cessation of milking.
18 Taponen et al. (2007) followed 228 udder quarters of 82 cows on one farm monthly throughout lactation
19 and found that about half of the CNS infections persisted, mostly until the end of lactation. Persistence
20 of the same bacterial strain in the quarters was confirmed with amplified fragment length polymorphism
21 analysis (AFLP). In contrast to the findings of Aarestrup and Jensen (1997), both *S. chromogenes* and *S.*
22 *simulans* were found to persist.

24 **5. Virulence factors of staphylococci**

1 Virulence factors of *S. aureus* and CNS have been investigated both by measuring phenotypic
2 expression of products assumed to be associated with virulence and by screening genes encoding these
3 products. Various virulence factors, including production of haemolysins, leucocidins, exfoliative toxins,
4 enterotoxins, toxic-shock syndrome toxin, and ability to form slime and biofilm have been found in *S.*
5 *aureus* strains isolated from bovine mastitis (Cucarella et al., 2004; Zecconi et al., 2006; Haveri et al.,
6 2007). *S. aureus* isolates from natural bovine intramammary infections have been demonstrated to
7 internalize and live in mammary gland alveolar cells and macrophages (Hébert et al., 2000). Only few
8 studies have focused on virulence factors of CNS isolated from mastitis. Adherence and internalization
9 of CNS into mammary epithelial cells was studied in cell cultures (Almeida and Oliver, 2001; Anaya-
10 López et al., 2006; Hyvönen et al., 2007). CNS were shown to be able to adhere to bovine mammary
11 cells. The adhesive capacity of various CNS was almost equal to the adhesive capacity of *S. aureus*, but
12 the invasive capacity of *S. aureus* was greater than that of CNS strains. In cell cultures, CNS and *S.*
13 *aureus* isolates from mastitis showed cytotoxic activity, possibly caused by a metalloprotease (Zhang
14 and Maddox, 2000). The majority of CNS isolated from caprine mastitis produced at least one type of
15 hemolysin, DNase, and elastase (Bedini-Madani et al., 1998; da Silva et al., 2005). Kuroishi et al.
16 (2003) found that a high percentage of both *S. aureus* and CNS from bovine subclinical, chronic or acute
17 mastitis produced staphylococcal enterotoxins and/or toxic shock syndrome toxin-1. Somewhat
18 surprisingly, production of different staphylococcal enterotoxins and toxic shock syndrome toxin-1 were
19 as common in CNS isolates as in *S. aureus* isolates, and as common in isolates from subclinical mastitis
20 as from chronic or acute mastitis.

21 Various virulence factors of *S. aureus* have been compared with clinical characteristics of
22 mastitis, but no specific virulence factor or combination of factors has been strongly associated with the
23 severity of mastitis (Haveri et al., 2007). Baselga et al. (1993) noted that the severity of ruminant
24 mastitis decreased, but the bacterial capacity to colonize the mammary gland increased when the
25 infection was caused by a mucoid (slime producer) rather than a non-mucoid *S. aureus* isolate. In

1 agreement with that observation Cucarella et al. (2004) showed that a biofilm-producing *S. aureus* strain
2 decreased severity of mastitis but increased colonization capacity in the mammary gland. The ability of
3 staphylococci to generate biofilm can be studied by measuring biofilm formation phenotypically and by
4 screening genes associated with its formation. Biofilm associated proteins were found among bovine
5 mastitis isolates, including *S. aureus*, *S. epidermidis*, *S. chromogenes*, *S. hyicus*, and *S. xylosum*
6 (Cucarella et al., 2004). Oliveira et al. (2006) found 6 out of 16 *S. aureus* and 6 out of 16 *S. epidermidis*
7 isolates from subclinical mastitis phenotypically positive for biofilm production. Fox et al. (2005)
8 showed that biofilm formation was more common in *S. aureus* isolated from milk than in *S. aureus*
9 isolated from teat skin and milking unit liners.

10 Biofilm formation by staphylococci from bovine mastitis or human clinical samples is associated
11 with the loci *ica* (intercellular adhesion), *bap* (biofilm-associated protein), *agr* (accessory gene
12 regulator) and *sar* (staphylococcal accessory regulator), but isolates that form biofilm do not necessarily
13 carry all of these genes, and other mechanisms may be involved in biofilm formation (McKenney et al.,
14 1998; Cramton et al., 1999; Cucarella et al., 2001, 2004; Beenken et al., 2003; Vasudevan et al., 2003;
15 Fox et al., 2005; Lasa and Penadés, 2006; Planchon et al., 2006). The *bap* gene has been identified in
16 biofilm producing staphylococci isolated from mastitis, including isolates of *S. epidermidis*, *S.*
17 *chromogenes*, *S. xylosum*, *S. simulans* and *S. hyicus* (Tormo et al., 2005). Both *bap* and *ica* genes have
18 been identified in mucoid staphylococcal isolates involved in biofilm formation (Cucarella et al., 2004).

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

1

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

3

4 CNS tend to be more resistant to antimicrobials than *S. aureus* and easily develop
5 multiresistance. The most common resistance mechanism in staphylococci is β -lactamase production,
6 which results in resistance to penicillin G and aminopenicillins. The reported percentage of penicillin
7 resistance for CNS isolated from mastitis was 36% in Norway (NORM-VET, 2005), 25% in Denmark
8 (DANMAP, 2001), 41% to 61% in the Netherlands (MARAN, 2004; MARAN, 2005), and 32% in
9 Finland (Pitkälä et al., 2004). Penicillin resistance of *S. aureus* is generally lower than that of CNS,
10 except in Finland. In Norway, the reported penicillin resistance of *S. aureus* was 7% (NORM-VET,
11 2005), in Denmark 18% to 30% (DANMAP, 2001; DANMAP, 2002), in the Netherlands 7% to 12%
12 (MARAN, 2004; MARAN, 2005), and in subclinical mastitis in Finland 52% (Pitkälä et al., 2004). In
13 clinical mastitis in Finland, the proportion of β -lactamase positive *S. aureus* was 13% and that of β -
14 lactamase positive CNS 23% (Nevala et al., 2004), i.e. considerably less than for isolates from
15 subclinical mastitis.

16 Methicillin resistance is very rare in *S. aureus* isolated from mastitis, but it can be found in CNS
17 isolates. In the Finnish survey material (Pitkälä et al., 2004), 4% of *S. aureus* and 10% of CNS were
18 resistant (breakpoint for oxacillin >2 $\mu\text{g/ml}$). Some CNS isolates, but none of the *S. aureus* isolates,
19 carried the *mecA* gene (Pitkälä, personal communication). In the Netherlands, oxacillin resistance was
20 not detected in *S. aureus*, but 5% of CNS were resistant and positive for the *mecA* gene (MARAN,
21 2005). In Korea, 2.5% of *S. aureus* and 2.4% of CNS isolates from mastitis were methicillin resistant
22 (Moon et al., 2007). There is evidence that *S. aureus* has acquired the staphylococcal chromosomal
23 cassette *mec* from CNS (Leonard and Markey, 2007).

24 Resistance of CNS to macrolides and lincosamides was recently reported to be 6 to 7% in
25 Germany (Lüthje and Schwarz, 2006) and 4 to 14% in the Netherlands (MARAN, 2004). Resistance of

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

8

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

10

11 Mastitis caused by *S. aureus* generally responds poorly to antimicrobial treatment. The reported
12 chances of cure vary widely, from 15% to 85%, depending on many factors, including lactation number,
13 SCC before treatment and β -lactamase production of the mastitis-causing isolate (Barkema et al., 2006).
14 There are few published reports on treatment of quarters infected by CNS only. Based on those reports,
15 mastitis caused by CNS seems to respond well to antimicrobial treatment. The chances of bacteriological
16 cure were 80-90% for β -lactamase negative CNS (McDougall, 1998; Pyörälä and Pyörälä, 1998;
17 Taponen et al., 2006). For β -lactamase positive CNS, the probability of cure seems somewhat lower
18 (Taponen et al., 2006), a phenomenon also recorded for *S. aureus* (Sol et al., 2000; Taponen et al.,
19 2003). In most studies, the isolates were tested *in vitro* for susceptibility to penicillin, and penicillin G
20 was used in treatment of penicillin-susceptible CNS mastitis. In the study of Rainard et al. (1990), 87%
21 of quarters infected with CNS and treated with cloxacillin were cured. Penicillin-susceptibility was not
22 reported.

23

24 **8. Differences between CNS species and strains?**

25

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

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

7

8 **9. Conclusions**

9

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

19

20 **Conflict of interest**

21

22 None of the authors (S. Taponen, S. Pyörälä) has a financial or personal relationship with other
23 people or organizations that could inappropriately influence or bias the paper entitled “Coagulase-
24 negative staphylococci as cause of bovine mastitis – not so different from *Staphylococcus aureus*?”.
25

25

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