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CNS SPECIES AND ANTIMICROBIAL RESISTANCE IN CLINICAL AND SUBCLINICAL BOVINE MASTITIS

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Abstract

Coagulase-negative staphylococci (CNS) are often associated with bovine mastitis. Knowledge about the relative importance of specific CNS species in different types of mastitis, and differences in antimicrobial resistance among CNS species is, however, scarce. Therefore, the aims of this study were to compare prevalence and antimicrobial susceptibility of CNS species in clinical and subclinical mastitis using material from

two national surveys. Overall, *S. chromogenes* and *S. epidermidis* were the most common CNS species found followed by *S. simulans* and *S. haemolyticus. S. epidermidis* was significantly more prevalent in subclinical than in clinical mastitis, and a similar trend was observed for *S. saprophyticus*, while *S. hyicus* was significantly more common in clinical mastitis. The prevalence of β-lactamase producing isolates varied markedly between CNS species, and was significantly higher in *S. epidermidis* and *S. haemolyticus* (~40%), than in *S. simulans* and *S. chromogenes* where none or a few of the isolates produced β-lactamase. Resistance to more than one antimicrobial substance occurred in 9% and 7% of the clinical and subclinical isolates, respectively. In conclusion, the distribution of CNS species differed between clinical and subclinical mastitis indicating inter-species variation of pathogenicity and epidemiology. Overall, the prevalence of antimicrobial resistance was low, but some variation between CNS species was observed.

Keywords: bovine mastitis, CNS species, antimicrobial susceptibility

1. Introduction

Coagulase-negative staphylococci (CNS) are associated with bovine intra-mammary infections (IMI) and may cause both subclinical and clinical mastitis. In most cases, however, the inflammatory reaction is relatively mild. The prevalence of CNS IMI may vary markedly between regions and countries, but is in most cases lower in studies on clinical than in studies on subclinical mastitis (for review see Pyörälä and Taponen, 2009). In national Swedish surveys on clinical (Ericsson Unnerstad et al., 2009) and subclinical (Persson Y., unpublished results) mastitis the CNS prevalence was 6% and 17%, respectively.

The control of CNS mastitis is complicated by the heterogeneity of this bacterial group. Today more than 15 CNS species have been identified in association with bovine IMI. Species identification of CNS can be performed using phenotyping or genotyping, but genotyping is nowadays considered superior to phenotyping (Zadoks and Watts, 2009). The distribution of species varies between studies, but in recent studies using genotyping for CNS speciation *S. chromogenes*, *S. epidermidis*, *S. haemolyticus*, *S. simulans* and *S. xylosus* are commonly found (Taponen et al., 2006; Taponen et al., 2008; Capurro et al., 2009; Sampimon et al., 2009; Perry et al., 2010). Knowledge about the relative prevalence of specific CNS species in different types of mastitis is, however, scarce. In a Finnish study comparing prevalences of CNS species among cases of CNS in clinical and subclinical mastitis, Taponen et al. (2006) found no difference in the prevalences of *S. chromogenes* and *S. simulans*. To our knowledge, however, similar studies have not been performed on national level.

Antimicrobials are an important tool in mastitis control programs. Therefore, surveillance of antimicrobial resistance is important to ensure optimal results of antimicrobial use and minimize the risk for development and spread of antimicrobial resistance. Very few studies have investigated differences in antimicrobial resistance among CNS species identified by genotyping (Sampimon, 2009), and no study has compared antimicrobial resistance in CNS species found in clinical and subclinical mastitis.

The aims of this study were to compare prevalence and antimicrobial susceptibility of CNS species in clinical and subclinical mastitis using material from two national surveys.

2. Material and methods

2.1 CNS isolates and bacteriological analyses

CNS isolates included in the study originated from national surveys on prevalence of udder pathogens in acute clinical mastitis (Ericsson Unnerstad et al., 2009), and subclinical mastitis (Persson Y., unpublished). In Ericsson Unnerstad et al. (2009), milk samples were collected by field veterinarians distributed all over the country. The number of cases per practice was proportional to the number of dairy cows in the county. In each practice, cases were enrolled in order of appearance until the sampling quota was filled. Only cases of acute clinical mastitis given specific inclusion criteria were eligible for sampling. The enrolment of veterinary practices, numbers of cases per practice and selection of cases were similar in the study on subclinical mastitis. In the latter study aseptic quarter milk samples (CMT>2; scale 1-5) were taken from one cow

with new infection and one cow with chronic infection in each herd according to criteria specified in Table 1.

Milk samples were directly cultured (10µl) on 5% bovine blood agar plates (Oxoid Ltd., Cambridge, UK). The agar plates were incubated at 37°C for 16-24 h. The isolates were identified as CNS by phenotypic appearance and negative reaction in the tube coagulase test according to recommendations by the National Mastitis Council (Hogan et al., 1999). Isolates were stored at -20°C in trypticase soy broth (Oxoid Ltd) containing 15% glycerol. Species differentiation of CNS isolates was made by sequencing part of the *tuf* gene as previously described (Capurro et al., 2009).

Antimicrobial susceptibility for the following substances was investigated; penicillin, oxacillin, erythromycin, tetracycline, gentamicin, ciprofloxacin and trimethoprim. Minimum inhibitory concentrations were determined by using a microdilution method (Bengtsson et al., 2009). Examination of β-lactamase production was performed using the "clover-leaf" method (Bryan and Godfrey, 1991). For quality control, the strains *S. aureus* ATCC 29213 and *S. aureus* ATCC 25923 were used. For testing of oxacillin susceptibility 2% NaCl was added to the broth and isolates with a MIC for oxacillin >1 mg/L were examined for presence of the *mecA*-gene by PCR according to Smyth et al. (2001).

Isolates were classified as susceptible or resistant based on species-specific epidemiological cut-off values issued by European Committee on Antimicrobial Susceptibility Testing (EUCAST) (http\\www.eucast.org). For trimethoprim, a cut-off for CNS was not available. Therefore the cut-off for *S. aureus* was used (>2 mg/L).

Classification of staphylococci as resistant to penicillin or oxacillin was based on production of β-lactamase and presence of *mecA* gene, respectively.

2.2 Statistical analyses

Differences in prevalence were investigated using the Chi-square or Fisher's exact tests (Statistica 6.0, StatSoft, Inc., Tulsa, OK, USA). All CNS isolates (62 clinical; 98 subclinical) were included in the data set when comparing prevalence of CNS between clinical and subclinical cases. The isolates originated in 55 and 94 herds, respectively. When evaluating the distribution of CNS species and antimicrobial resistance (positive (β+) or negative (β-) in the β-lactamase test) only the first isolate per CNS species and herd was included in the clinical (n=56) and subclinical (n=94) group. Thus, 4 subclinical cases (1 *S. chromogenes* (β-), 1 *S. epidermidis* (β+), 1 *S. saprophyticus* (β+), 1 *S. simulans* (β-)) were excluded from that evaluation. However, when comparing new and chronic subclinical cases, and in the descriptive statistics given in the tables, all isolates (n=98) were included. Statistical evaluation was not performed when the total number of isolates in the two groups compared was less than 5.

3. Results

CNS was significantly (P<0.001) more prevalent in subclinical than in clinical cases of mastitis (Table 2). Among cases with specific infection (i.e. excluding samples with no growth and contaminated samples), CNS was also significantly (P=0.022) more common in new than in chronic subclinical mastitis (Table 2).

In total, 14 CNS species were identified, 9 of those in clinical and 12 in subclinical cases of mastitis (Table 3). Seven species (*S. chromogenes*, *S. epidermidis*, *S.*

haemolyticus, *S. hyicus*, *S. simulans*, *S. warneri/pasteuri*, *S. xylosus*) were found in both groups, while 2 species (*S. aureus* (coagulase-negative), *S. lentus*) were found only in clinical cases, and 5 species (*S. arlettae*, *S. gallinarum*, *S. pseudintermedius*, *S. saprophyticus*, *S.* spp.) only in subclinical cases. *S. chromogenes*, *S. simulans* and *S. haemolyticus* were the most common findings in clinical mastitis, while *S. epidermidis* followed by *S. chromogenes*, *S. simulans* and *S. haemolyticus* were most common in subclinical mastitis (Table 3). *S. epidermidis* ($P<0.001$) was significantly more prevalent in subclinical than in clinical mastitis, and a similar trend was observed for *S. saprophyticus* ($P=0.057$). The opposite was the case for *S. hyicus* ($P<0.001$), while no significant difference ($P>0.05$) between clinical and subclinical cases was found for *S. chromogenes*, *S. haemolyticus*, *S. simulans*, *S. warnerii/pasteuri* and *S. xylosus*. The distribution of CNS species did not differ significantly ($P>0.05$) between cases of new and chronic subclinical mastitis.

β-lactamase production (β+) was significantly more common ($P=0.003$) in subclinical cases than in clinical cases (Table 3), but new and chronic subclinical mastitis did not differ significantly ($P>0.05$). The proportion of β+ isolates varied markedly between CNS species (Table 3). Overall among species with at least 10 isolates, the prevalence of β+ isolates was significantly higher ($P<0.004$) in *S. epidermidis* and *S. haemolyticus* (just over 40%), compared to *S. simulans* and *S. chromogenes* where none or a few of the isolates were β+. Within these 4 species the prevalence of β+ did not differ significantly between clinical and subclinical cases. Among CNS species found less frequently, the proportion of β+ was very high (86%) in *S. saprophyticus*, and high (40-67%) also in *S. warneri/pasteuri* and *S. xylosus*, while none of the *S. hyicus* isolates was β+.

Resistance to more than one antimicrobial substance occurred in 5 (8.9%) and 7 (6.7%) of the clinical and subclinical isolates, respectively. Among the clinical isolates, 3 (one each of *S. aureus*, *S. haemolyticus* and *S. xylosus*) were resistant to penicillin and tetracycline, gentamicin or erythromycin. One isolate (*S. lentus*) was resistant to erythromycin and tetracycline, and 1 isolate (*S. epidermidis*) to penicillin, oxacillin and tetracycline. The latter isolate was also methicillin resistant (*mec*A gene positive by PCR). Among subclinical isolates, 4 (1 *S. arlettae*, 3 *S. haemolyticus*) were β+ and resistant to trimethoprim, 1 (*S. cohnii*) was β+ and resistant to tetracycline, 1 (*S. epidermidis*) was β+ and resistant to erythromycin, and 1 (*S. epidermidis*) was β+ and resistant to erythromycin and tetracycline. Ten (6 *S. saprophyticus*, 2 *S. gallinarum*, 1 *S. warneri* and 1 *S. haemolyticus*) isolates had a MIC >2 mg/L for oxacillin, but none of the isolates was positive for the *mec*A gene. All 13 *S. simulans* isolates, and 13 of 14 *S. haemolyticus* isolates were resistant to trimethoprim, i.e. MIC >2 mg/L. These 26 isolates constituted 79% of all trimethoprim resistant CNS isolates.

4. Discussion

Overall, CNS was more common in subclinical than in clinical cases of mastitis, which is in line with earlier studies on either clinical or subclinical mastitis (for review see Pyörälä and Taponen, 2009). Very few studies have, however, presented data on both clinical and subclinical mastitis from the same region or country. In a Finnish study, the prevalence of CNS among all cases sampled was similar in clinical (18%) and subclinical (24%) mastitis (Koivula et al., 2007). We also found that CNS was more prevalent in new than in chronic subclinical cases. The reason for this is not clear, but it may indicate that transient CNS infections are relatively common.

Overall, *S. chromogenes* and *S. epidermidis* were the most common CNS species found followed by *S. simulans* and *S. haemolyticus.* These species were also found in varying proportions in previous studies based on genotyping (Taponen et al., 2006; Taponen et al., 2008; Capurro et al., 2009; Sampimon et al., 2009; Perry et al., 2010). The distribution of CNS species differed somewhat between clinical and subclinical mastitis. *S. hyicus* was more common among clinical cases, while *S. epidermidis* and *S. saprophyticus* (tendency) were more common among subclinical cases. In line with Taponen et al. (2006) the prevalences of *S. chromogenes* and *S. simulans* were similar in clinical and subclinical cases. Even though the number of isolates was small, the fact that *S. hyicus* was mainly found in clinical cases may indicate that this species is relatively virulent. This hypothesis is supported by the finding by Perry et al. (2010) that *S. hyicus* was uncommon among subclinical cases of mastitis, but that quarters infected with this species had very high somatic cell count (SCC). Moreover, in a study using phenotypic species differentiation *S. hyicus* was described as the most pathogenic CNS species (Myllys, 1995). That *S. epidermidis* and *S. saprophyticus* were mainly found in subclinical cases may indicate that they are less virulent and/or cause more persistent infections than other CNS. In line with this hypothesis, Thorberg et al. (2009) found that persistent *S. epidermidis* IMI were common. In the same study, *S. saprophyticus* was only found in udder quarters with relatively low SCC indicating a minor effect on udder health.

β-lactamase production is the most common resistance mechanism in staphylococci. Overall, such production was more prevalent among subclinical CNS isolates than in clinical isolates, which is in line with a Norwegian study (Jarp, 1991). Taponen et al.

(2006) found a similar numerical, but not significant, difference. The proportion of β+ subclinical CNS isolates (29%) was similar to those reported from subclinical mastitis or IMI in Finland (32%), Norway (36%) and Netherlands (37%) (Pitkälä et al., 2004; Østerås et al., 2006; Sampimon, 2009). The relatively low proportion of β+ clinical isolates supports the Swedish veterinary policy of benzyl penicillin as the first drug of choice in clinical CNS mastitis.

The marked difference between CNS species in β-lactamase production is an important finding. Among the most frequently isolated species, such production was common in *S. epidermidis* and *S. haemolyticus*, but not in *S. chromogenes* and *S. simulans*. These results are in line with Sampimon (2009) who found that resistance to penicillin was 70%, 33%, 18% and 0% in *S. epidermidis*, *S. haemolyticus*, *S. chromogenes* and *S. simulans*, respectively. Similar proportions of β-lactamase production for *S. epidermidis* (46%) and *S. chromogenes* (18%) was also reported in a US study (Sawant et al., 2009). In that study, however, phenotypic species identification was performed.

The results indicate that the higher proportion of penicillin resistance in subclinical than in clinical isolates was due to the high prevalence of *S. epidermidis* in subclinical mastitis in combination with a high proportion of resistance among less common species such as *S. saprophyticus*, *S. warneri/pasteuri* and *S. xylosus.*

Overall, resistance to other antimicrobials than penicillin was uncommon, and was markedly lower than in other studies for example for erythromycin, oxacillin and tetracycline (Lüthje and Schwarz, 2006; Rajala-Schultz et al., 2009; Sampimon, 2009; Sawant et al., 2009). Moreover, Sampimon (2009) found that 30% of the CNS isolates

expressed resistance to more than one antimicrobial compound. Differences between CNS species in antimicrobial susceptibility have also been observed. Both Sampimon (2009) and Sawant et al. (2009) found that *S. epidermidis* exhibited lower susceptibility to several antimicrobials than other species. Sampimon (2009) found for example that the *mec*A gene, i.e. methicillin resistance, was significantly more common in *S. epidermidis* than in other species.

In line with the results for β-lactamase, trimethoprim resistance differed between CNS species. A majority of the resistant isolates was *S. simulans* or *S. haemolyticus.* The reasons behind differences in antimicrobial susceptibility between species are not known. It may be hypothesized that the resistant isolates belonged to the same clonal group within each species, and that this group also inhabit virulence factors important for spread of infection. Thus, further genotypic analyses of differences within species are needed.

Care should be taken when comparing different studies as study design and methodology, including definitions of mastitis, may vary.

5. Conclusion

In conclusion, the distribution of CNS species differed between clinical and subclinical mastitis indicating inter-species variation of pathogenicity and epidemiology. Overall, the prevalence of antimicrobial resistance was low, but some variation between CNS species, especially in β-lactamase production, was observed.

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References

Bengtsson, B., Ericsson Unnerstad, H., Ekman, T., Artursson, K., Nilsson-Öst, M., Persson Waller, K., 2009. Antimicrobial susceptibility of udder pathogens from cases of acute clinical mastitis in dairy cows. Vet. Microbiol. 136, 142-149.

Bryan, L., Godfrey, A., 1991. Beta-lactamase antibiotics: mode of action and bacterial resistance. In: Lorian, V. (Ed.). Antibiotics in Laboratory Medicine. William and Wilkins, Baltimore, USA, p. 648.

Capurro, A., Artursson, K., Persson Waller, K., Bengtsson, B., Ericsson Unnerstad H., Aspán, A., 2009. Comparison of a commercialized phenotyping system, antimicrobial susceptibility testing, and *tuf* gene sequence-based genotyping for species-level identification of coagulase-negative staphylococci isolated from cases of bovine mastitis. Vet. Microbiol. 134, 327-333.

Ericsson Unnerstad, H., Lindberg, A., Persson Waller, K., Ekman, T., Artursson, K., Nilsson-Öst, M., Bengtsson, B., 2009. Microbial aetiology of acute clinical mastitis and agent-specific risk factors. Vet. Microbiol. 137, 90-97.

Hogan, J.S., González, R.N., Harmon, R.J., Nickerson, S.C., Oliver, S.P., Pankey, J.W., Smith, K.L., 1999. Laboratory Handbook on Bovine Mastitis. National Mastitis Council, Verona, WI.

Jarp., J. 1991. Classification of coagulase-negative staphylococci isolated from bovine clinical and subclinical mastitis. Vet. Microbiol. 27, 151-158.

289 Koivula, M., Pitkälä, A., Pyörälä, S., Mäntysaari, E.A., 2007. Distribution of bacteria
290 and seasonal and regional effects in a new database for mastitis pathogens in Finland.
291 Acta Agricult. Scand. A 57, 89-96.
292 Lüthje, P., Schwartz, S., 2006. Antimicrobial resistance of coagulase-negative
293 staphylococci from bovine subclinical mastitis with particular reference to macrolide-
294 lincosamide resistance phenotypes and genotypes. J. Antimicrob. Chemother. 57, 966-
295 969.
296 Myllys, V., 1995. Staphylococci in heifer mastitis before and after parturition. J. Dairy
297 Res. 62, 51-60.
298 Østerås, O, Sølverød, L., Reksen, O., 2006. Milk culture results in a large Norwegian
299 survey – effects of season, parity, days in milk, resistance, and clustering. J. dairy Sci.
300 89, 1010-1023.
301 Perry, J., Middleton, J.R., Dufour, S., Scholl, D., Calloway, C., Anderson, S., Dohoo. I.,
302 2010. Association of coagulase negative staphylococcal species and milk somatic cell
303 count of cows from the Canadian national cohort of dairy farms. NMC Annual Meeting
304 Procedures, Albuquerque, New Mexico, 204-205.
305 Pitkälä, A., Haveri, M., Pyörälä, S. Myllys, V., Honkanen-Buzalski, T., 2004. Bovine
306 mastitis in Finland 2001 – prevalence, distribution of bacteria, and antimicrobial
307 resistance. J. Dairy Sci. 87, 2433-2441.
308 Pyörälä, S., Taponen, S. 2009. Coagulase-negative staphylococci – Emerging mastitis
309 pathogens. Vet. Microbiol. 134, 3-8.
310 Rajala-Schultz, P.J., Torres, A.H., DeGraves, F.J., Gebreyes, W.A., Patchanee, P., 2009.
311 Antimicrobial resistance and genotypice characterization of coagulase-negative
312 staphylococci over the dry period. Vet. Microbiol. 134, 55-64.

313 Sampimon, O.C., 2009. Coagulase-negative staphylococci mastitis in Dutch dairy herds.
314 Thesis, Dutch Animal Health Serive (GD), Deventer, The Netherlands.
315 Sampimon, O.C., Barkema, H.W., Berends, I.M.G.A., Sol, J., Lam, T.J.G.M., 2009.
316 Prevalence and herd-level risk factors for intramammary infection with coagulase-
317 negative staphylococci in Dutch dairy herds. Vet. Microbiol. 134, 37-44.
318 Sawant, A.A., Gillespie, B.E., Oliver, S.P., 2009. Antimicrobial susceptibility of
319 coagulase-negative *Staphylococcus* species isolated from bovine milk. Vet. Microbiol.
320 134, 73-81.
321 Smyth, RW., Kahlmeter, G., Liljequist, BO., Hoffman, B., 2001. Methods for
322 identifying methicillin resistance in *Staphylococcus aureus*. J. Hosp. Inf. 48, 103-107.
323 Taponen, S., Simojoki, H., Haveri, M., Larsen, H.D., Pyörälä, S., 2006. Clinical
324 characteristics and persistence of bovine mastitis caused by different species of
325 coagulase-negative staphylococci identified with API or AFLP. Vet. Microbiol. 115,
326 199-207.
327 Taponen, S., Björkroth, J., Pyörälä, S., 2008. Coagulase-negative staphylococci isolated
328 from bovine extramammary sites and intramammary infections in a single dairy herd. J.
329 Dairy Res. 75, 422-429.
330 Thorberg, B.-M., Danielsson-Tham, M.-L., Emanuelson, U., Persson Waller, K., 2009.
331 Bovine subclinical mastitis caused by different types of coagulase-negative
332 staphylococci. J. Dairy Sci. 92, 4962-4970.
333 Zadoks, R.N., Watts, J.L., 2009. Species identification of coagulase-negative
334 staphylococci: Genotyping is superior to phenotyping. Vet. Microbiol. 134, 20-28.

Table 1. Composite SCC (cells/ml) inclusion criteria for cows in the survey on subclinical mastitis

Category	Latest monthly test milking	Previous monthly test milking
New infection	≥200 000	<100 000
Chronic infection	>300 000	>300 000

Table 2. Prevalence of CNS in acute clinical (Ericsson Unnerstad et al., 2009) and subclinical (new/chronic infection) mastitis when including all cases or only cases with specific infection i.e. excluding samples with no growth and contaminated samples

Type of mastitis		**All**	**Specific infection**
		% (n/N)	**% (n/N)**
Clinical	Total	6[a] (62/1056)	7[a] (62/896)
Subclinical	Total	17 (98/584)	28 (98/351)
	New	*18 (52/284)*	*34[b] (52/152)*
	Chronic	*15 (46/300)*	*23 (46/199)*

[a] Significantly different (P<0.001) from total subclinical mastitis within column.

[b] Significantly different (P=0.022) from chronic subclinical mastitis within column.

Table 3. Distribution of CNS species among isolates from cases of acute clinical mastitis (CM), and cases of subclinical mastitis (SCM; new and chronic infection), and numbers (%) of isolates producing β-lactamase (β+) within species

Species	CM		SCM						Total	
			New		Chronic		Total SCM			
	n (%)	β+ n (%)	n (%)	β+ n (%)	n (%)	β+ n (%)	n (%)	β+ n (%)	n (%)	β+ n (%)
S. arlettae	0	-	1 (2)	1	1 (2)	1	2 (2)	2	2 (1)	2
S. aureus[d]	2 (4)	1	0	-	0	-	0	-	2 (1)	1
S. chromo-genes	16 (29)	1 (6)	9 (17)	0 (0)	12 (26)	2 (17)	21 (21)	2 (10)	37 (24)	3[c] (8)
S. epidermi-dis	4[a] (7)	2	17 (33)	9 (53)	13 (28)	3 (23)	30 (31)	12 (40)	34 (22)	14 (41)
S. gallinarum	0	-	1 (2)	1	1 (2)	1	2 (2)	2	2 (1)	2
S. haemolyti-cus	8 (14)	2 (25)	8 (15)	4 (50)	6 (13)	3 (50)	14 (14)	7 (50)	22 (14)	9 (41)
S. hyicus	6[a] (11)	0 (0)	0	-	1 (2)	0	1 (1)	0	7 (5)	0 (0)
S. lentus	1 (2)	1	0	-	0	-	0	-	1 (<1)	1
S. pseud-intermedius	0	-	1 (2)	1	0	-	1 (1)	1	1 (<1)	1
S. sapro-phyticus	0[b]	-	4 (8)	3	3 (7)	3	7 (7)	6 (86)	7 (5)	6 (86)
S. simulans	14 (25)	0 (0)	6 (12)	0 (0)	7 (15)	0 (0)	13 (13)	0 (0)	27 (18)	0[c] (0)
S. sp.	0	-	1 (2)	0	0	-	1 (1)	0	1 (<1)	0
S. warneri/ pasteuri	3 (5)	0	2 (4)	2	0	-	2 (2)	2	5 (3)	2 (40)
S. xylosus	2 (3)	1	2 (4)	1	2 (4)	2	4 (4)	3	6 (4)	4 (67)
Total	56 (100)	8[a] (14)	52 (100)	22 (42)	46 (100)	15 (33)	98 (100)	37 (38)	154 (100)	45 (29)

[a] The prevalence was significantly different (P<0.05) from total SCM within row (based on 94 cases of SCM, see Statistical analyses).
[b] The prevalence tended to differ (0.05>P<0.10) from total SCM within row (based on 94 cases of SCM, see Statistical analyses).
[c] The prevalence was significantly different (P<0.05) from that of *S. epidermidis* and *S. haemolyticus* within column (based on 94 cases of SCM, see Statistical analyses).
[d] The strains were coagulase-negative.