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Staphylococcal and other Bacterial Species Associated with Intramammary Infections in Danish Dairy Herds

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Aarestrup F. M., H. C. Wegener, V. T. Rosdahl and N. E. Jensen: Staphylococcal and other bacterial species associated with intramammary infections in Danish dairy herds. Acta vet. scand. 1995, 36, 475-487. – Four thousand six hundred forty-five quarter milk samples from 1179 cows from 20 commercial dairy herds were examined in order to determine the prevalence of bacterial species. A total of 859 isolates from 839 (18.1%) culture positive samples could be assigned to 34 different species and subspecies. Diagnostics of staphylococcal species was based on conventional procedures able to differentiate between all 36 species and subspecies presently acknowledged. *Staphylococcus aureus* was found in 10.2% of the samples and was the most common species isolated. *Streptococcus dysgalactiae* (1.6%) and *Streptococcus uberis* (1.4%) were the second and third most common species isolated. Seventeen different coagulase negative staphylococcal species (CNS) were found in 4.1% of the samples. The most frequently isolated CNS were *S. epidermidis* (1.3%), *S. chromogenes* (1.0%) and *S. simulans* (0.7%). Isolates of *S. aureus* were phage typed, and isolates of *S. epidermidis* were investigated by phage typing, antibiogram typing, and biotyping. A total of 378 (79.9%) isolates of *S. aureus* could be typed by phages, assigning them to 18 different phage types. However, 6 phage types accounted for 92.1% of the typable isolates. One to 2 phage types predominated within each herd. Eleven (18%) isolates of *S. epidermidis* could be typed by phages, assigning the isolates to 3 different types. Biotyping of *S. epidermidis* produced a total of 8 different types, the most common accounting for 29.5% of the isolates. A total of 6 different antibiogram types were observed among all isolates of *S. epidermidis.* Resistance towards penicillin (36.1%), tetracycline (9.8%) and streptomycin (9.8%), were recorded in the isolates of *S. epidermidis.* However, 35 (57.4%) of the isolates were susceptible to all 12 antibiotics tested.

Mastitis; coagulase negative staphylococci; S. aureus; phage types.

Introduction

Staphylococcus aureus has in most countries with intensive dairy production during the last 50 years gradually replaced *Streptococcus agalactiae* as the most common mastitis pathogen. The percentage of animals infected with *Streptococcus uberis* and *Streptococcus dysgalactiae* has remained rather constant throughout the period *(Anon.* 1985, *King* 1981). Especially the role of the coagulase negative staphylococci (CNS) in bovine mastitis has come under increased scrutiny in recent years *(Watts & Owens* 1989, *Todhunter et al.* 1993). Early investigations indicated that infection with CNS might be protective against infections by other pathogens *(Edwards & Jones* 1966, *Bramley* 1978). However, since then it has become increasingly clear that CNS are in fact important mastitis pathogens and the cause of substantial economic losses *(Timms & Schultz* 1987,

Hogan et al. 1987, *Davidson et al.* 1992). Numerous studies have emphasized the importance of identification of CNS from the bovine mammary gland *(Devriese* 1979, *Langlois et al.* 1983, *Watts et al.* 1984, *Watts & Washburn* 1991). The prevalence and distribution of the individual staphylococcal species in bovine intramammary infections has been studied in several countries *(Devriese & De Keyser* 1980, *Watts & Owens* 1989, *Jarp* 1991). In order to investigate the importance and dynamics of infections with CNS, others have followed single herds over time *(Smith & Hagstad* 1986, *Davidson et al.* 1992). Likewise, characteristics of CNS with inflammation and clinical findings have been studied *(Birgersson et al.* 1992, *Todhunter et al.* 1993). A number of studies have investigated the prevalence and distribution of different phage types of *S. aureus* in dairy herds *(Price et al.* 1954, *Parisi & Baldwin* 1963, *Mackie et al.* 1987).

No investigations have been performed on the occurrence of different CNS species and types and different types of *S. aureus* in Danish dairy herds. The purpose of this study was to determine the prevalence of staphylococcal and other bacterial species isolated from intramammary infection in 20 Danish dairy herds and to update the necessary diagnostic procedures. Furthermore, the discriminatory power of traditional typing methods for differentiating *S. aureus* and *S. epidermidis* isolates from intramammary infections was investigated.

Materials and methods

Animals

During a 3 month period (Dec. 1992 – Feb. 1993) 4645 quarter milk samples were obtained from 1179 different cows of a total of 1237 cows present in 20 commercial dairy herds. The herds represented common Danish dairy production systems and management practices. The herds were selected from a number of herds included in a larger study on the basis of diversity in production systems and the owners willingness in cooperating. Milk samples were collected from all cows in lactation and from cows in the dry period if possible.

Sampling and bacteriological identification

Quarter foremilk was sampled according to the recommendations of the International Dairy Federation *(Anon.* 1987). All quarters from all cows in 1 herd were sampled on the same day. The somatic cell count was estimated indirectly by California Mastitis Test (CMT), and 100 µl of each milk sample were streaked on bovine blood agar (Columbia agar, CM 331, supplemented with 5% sterile bovine blood), supplemented with aesculin and incubated aerobically at 37°C overnight. Primary cultures were evaluated by visual examination of the morphology of the bacterial colonies. Plates with pin-point colonies or culturally negative samples of milk with elevated CMT (>3) were further incubated for 24 h in 5-10% CO_2. Plates with aerobic growth of a pathogen were not incubated further. From apparent pure cultures, a single colony was picked for further identification. From cultures showing growth of 2 or 3 colony types, a representative of each colony type was subcultivated on bovine blood agar. Obviously contaminated samples (>3 colony types) were discarded. Genus identification was based on colony morphology and verified with Gram-staining, and test for catalase and oxidase activity.

Diagnostic tests

Coagulase test was performed as a tube test using 1 ml of citrate stabilized horse plasma. One colony from an overnight culture was transferred to the plasma containing tube, which was incubated at 37°C and observed for clot formation after 2, 4 and 24 h. Test for oxidase was

performed as described by *Faller & Schleifer* (1981), and production of hyaluronidase was determined using a strain of *Pasteurella multocida* on bovine blood agar as described by *Devriese et al.* (1985).

Test for the production of urease was performed as described by *Stuart et al.* (1945), for production of β-galactosidase as described by *Maniatis et al.* (1982), and for production of thermostable nuclease by the method of *Lachica et al.* (1971) using toluidine blue DNA agar (Difco).

The production of acid from carbohydrates was tested in Hugh and Leifson's O/F media *(Hugh & Leifson* 1953). The following carbohydrates were used in a concentration of 0.5%: xylose, arabinose, raffinose, sucrose, maltose, mannitol, mannose, lactose, turanose, trehalose, salicin, sorbitol, and ribose.

Identification of staphylococci and micrococci

The original schemes of Kloos & Schleifer *(Kloos & Schleifer* 1975, *Schleifer & Kloos* 1975) and identification schemes of others *(Schleifer* 1986, *Kloos & Lambe* 1991, *Hajek et al.* 1992, *Tanasupawat et al.* 1992, *Chesneau et al.* 1993, *Webster et al.* 1994) formed the basis for the simplified schemes constructed for identification. Initially all isolates were identified to genus level on the basis of colony morphology, Gram-staining and catalase production. Furthermore, production of haemolysis and oxidase was determined. All catalase positive, gram positive cocci appearing in clusters in microscopy, were tested for susceptibility to furazolidone, bacitracin, novobiocin and production of coagulase. Depending on the results obtained, they were subjected to one of 4 panels of second level tests, according to the identification procedures of others *(Devriese* 1986, *Gahrn-Hansen et al.* 1987). Coagulase-negative, bacitracin-resistant, furazolidone, and novobiocin susceptible isolates were tested for aerobic acid production from xylose, sucrose, maltose, mannitol, mannose, and trehalose, and for production of alkaline phosphatase, urease, thermostable nuclease, hyaluronidase, and β-galactosidase. Coagulase-negative, bacitracin-resistant, furazolidone susceptible, and novobiocin resistant isolates were tested for aerobic acid production from xylose, arabinose, raffinose, sucrose, mannose, trehalose, lactose, turanose, and ribose, and for production of urease and the capability to reduction of nitrates. Coagulase-positive, bacitracin resistant, furazolidone, and novobiocin susceptible isolates were tested for aerobic acid production from xylose, sucrose, maltose, mannitol, mannose, and trehalose, and production of hyaluronidase. Coagulase-negative, bacitracin susceptible, and furazolidone resistant isolates were classified as *Micrococcus* species. Isolates that could not be identified by the simplified scheme was identified using additional tests according to the identification scheme of *Kloos & Lambe* (1991). The biochemical identification was verified by colony morphology and production of haemolysins according to the original descriptions.

Identification of Streptococci

Basis for the identification procedures were the schemes by *Barrow & Feltham* (1993). B-haemolytic, CAMP positive, aesculin negative isolates were identified as *S. agalactiae.* A-haemolytic, CAMP negative, aesculin negative isolates were precipitated in capillary tubes using rabbit antisera (C) as described by *Jelinkova* (1977) and tested for production of acid from salicin, trehalose, and sorbitol. Isolates, which precipitated with group C antisera and produced acid from trehalose and not from salicin, were identified as *S. dysgalactiae.* A-haemolytic, CAMP negative, aesculin positive isolates were tested for hydrolysis of hippurate, hydrolysis of arginine, and production of acid from mannitol and inulin. Isolates showing pos-

itive reactions in all these tests were identified as *S. uberis*. Isolates showing deviant results were identified by API-20-Strep (bioMerieux sa, France).

Identification of coliforms, Actinomyces pyogenes and Proteus

Basis for the identification procedure for coliforms were the schemes of *Barrow & Feltham* (1993). Motile isolates, that produced indole and acid from lactose and did not produce acetoin or utilised citrate and that were methyl red positive, were identified as *Escherichia coli*. All other coliforms were identified using API-20E (bioMerieux sa, France).

Small pin-point colonies showing increased growth after incubation in 5-10% CO_2 which appeared as gram positive rods of irregular polymorph shape in microscopy were identified as *A. pyogenes*.

Bacteria showing swarming on blood agar and appearing as gram negative rods in microscopy were identified as *Proteus*.

Phage typing

Phage typing of *S. aureus* and *S. epidermidis* was performed according to the method of *Blair & Williams* (1961). *S. aureus* was typed using the international set of typing phages for human isolates *(Parker* 1983), and *S. epidermidis* was typed as described by *Rosdahl et al.* (1990).

Biotyping

Biotyping of *S. epidermidis* was performed as described by *Jarløv et al.* (1994).

Antimicrobial susceptibility tests

Sensitivity to diagnostic antibiotics and antibiotics used for typing *S. epidermidis* was measured by a tablet diffusion test on Muller-Hinton II agar (Muller-Hinton II, Becton Dickinson Microbiology System, USA, supplemented with 5% sterile bovine blood), using the following antibiotics: bacitracin (0.4 U), furazolidone (50 µg), novobiocin (5 µg), chloramphenicol (60 µg), tetracycline (80 µg), gentamicin (40 µg), netilmicin (40 µg), ampicillin (33 µg), cephalosporin (66 µg), cefuroxime (60 µg), fucidin (400 µg), cefotaxime (30 µg), streptomycin (100 µg), methicillin (29 µg), vancomycin (70 µg), teicoplanin (60 µg) and penicillin (5 µg), according to the manufacturers guidelines (Rosco Diagnostics, Tåstrup, Denmark) *(Casals & Pringler* 1991).

Results

The prevalence of infected quarters varied from 6% to 35% per herd, and of infected cows from 21% to 70% (Table 1).

A total of 859 isolates were recovered from 839 culture positive samples in the 20 dairy herds investigated. Thirty four different species and subspecies were identified. Nine isolates of CNS could not be identified by the scheme used. Four were identified after using additional tests, and 4 were identified as *S. muscae* after restriction fragment length polymorphism of the gene encoding ribosomal RNA (ribotyping) (data not shown). One isolate could not be assigned to a defined species. *S. aureus* was recovered from 473 (10.2%) of the samples. *S. dysgalactiae* (1.6%) and *S. uberis* (1.4%) were the second and third most common species isolated. CNS was isolated from 4.1% of the samples. The most frequently isolated CNS was *S. epidermidis* (1.3%), *S. chromogenes* (1.0%) and *S. simulans* (0.7%) (Table 2).

From 4 to 21 different species were isolated per herd. *S. aureus* predominated in all but 3 herds, and this species accounted for more than 60% of the isolated bacteria in 8 herds. In 3 herds *S. uberis* was the most frequently isolated species. *S. dysgalactiae* was commonly found in herds 6, 8 and 20 (>3% of quarters). CNS was iso-

Table 1. Number of cows examined and perent infected quarters and percent infected cows in 20 Danish herds.

	Herd number																				Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
Number of cows	59	53	53	57	68	67	37	98	59	53	35	37	34	50	50	56	54	46	159	54	1179
Percent infected quarters	19%	6%	14%	10%	16%	24%	15%	16%	21%	16%	15%	18%	11%	28%	15%	11%	11%	19%	35%	15%	18%
Percent infected cows	49%	21%	42%	35%	50%	60%	43%	50%	51%	42%	55%	46%	32%	62%	44%	41%	33%	27%	70%	46%	47%

lated in high numbers in herds 1, 5, 8, 14, 19 and 20 (>5% of quarters). *S. epidermidis* was found in high numbers (>3% of quarters) in herds 1, 5 and 14. Nine isolates of *S. chromogenes* and 7 isolates of *S. haemolyticus* were found in herd 5, where 4 isolates of *S. muscae* were found too. *S. xylosus* was isolated from 5 quarters in herd 12. *S. canis* was isolated relatively frequently in herds 10 and 15 (Table 2).

The relation between bacterial species and the CMT level of the corresponding quarter is shown in Table 3. Infection with *S. aureus, S. xylosus, S. uberis* and *S. dysgalactiae* is associated with high cell counts as measured by CMT. All other bacterial species were associated with low cell counts or were isolated in so low numbers that no distinct pattern could be seen.

The results of phage typing of *S. aureus* are shown in Table 4. A total of 378 (79.9%) of the isolates could be typed by phages, assigning the isolates to 18 different phage types. The most common phage type (47/75/84/85/89) accounted for 22.8% of all the typable isolates. From 1 to 7 different phage types were found within each herd. However, 1 to 2 phage types were found to predominate in each herd. Different phage types were isolated from different quarters of the same cow on 29 out of 114 (25.4%) occasions where more than one quarter of a cow were infected with *S. aureus*.

S. epidermidis was isolated from 10 different herds. The results of phage typing, antibiogram and biotyping of *S. epidermidis* are shown in Table 5. Only 11 (18.0%) of the isolates were typable by phages producing 3 different phage types. Resistance towards penicillin (36.1%), tetracycline (9.8%), and streptomycin (9.8%) was found. However, the majority of the isolates (57.4%) was susceptible to all of the antibiotics investigated. A total of 6 different antibiogram types were observed. Eight different biotypes were found among the 61 isolates of *S.*

Table 2. Distribution of bacteria isolated from bovine intramammary infections in 20 Danish dairy herds.

	Herd number																				All herds
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
S. aureus	21	2	16	4	9	42	12	28	36	20	14	13	12	48	9	4	15	7	157	4	473
S. epidermidis	10	–	–	–	9	–	–	7	1	2	2	–	–	8	1	–	–	–	17	5	61
S. chromogenes	3	2	3	–	9	5	1	5	1	–	–	2	–	1	1	2	2	3	2	2	44
S. simulans	1	–	3	2	1	1	–	3	1	–	–	–	–	1	2	4	2	–	6	7	33
S. xylosus	2	–	–	–	–	–	–	3	–	–	–	5	–	1	1	1	–	–	2	–	15
S. haemolyticus	–	–	–	–	7	–	–	–	–	2	–	–	–	–	–	–	–	–	2	–	11
S. warneri	–	1	–	1	1	1	–	1	–	–	–	–	–	–	–	–	–	–	4	–	9
S. muscae	–	–	–	–	4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	4
S. sciuru	–	1	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	2
S. auricularis	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	1
S. capitis	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	1
S. capitis subsp. *ureolyticum*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	1
S. cohnii	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	1
S. cohnii subsp. *ureolyticus*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	1
S. hominis	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	1
S. lentus	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
S. saprophyticus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	1
Staphylococcus species	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	1
Micrococcus species	–	–	2	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	5
S. dysgalactiae	4	–	–	4	–	10	3	10	11	2	3	4	2	–	3	2	3	3	4	7	75
S. uberis	2	6	6	9	2	3	3	1	2	–	–	2	2	2	3	4	1	2	9	8	67
Enterococcus faecalis	–	–	–	–	1	2	2	2	–	–	–	2	–	–	1	1	–	1	–	1	13
Enterococcus faecium	–	–	–	1	–	–	–	–	–	–	1	–	1	–	–	2	–	–	1	–	6
S. canis	–	–	–	–	–	–	–	–	–	6	–	–	–	–	4	–	–	–	–	–	10
S. lactis	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	1	–	–	–	–	2
S. salivarius	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–	2
E. avium	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	1
E. durans	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	1
Aerococcus viridans	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	1	–	1
Aerococcus hydro	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	1
E. coli	–	–	–	–	–	–	–	1	–	–	–	1	–	–	–	–	–	–	2	1	5
Klebsiella pneumonia	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	1
Proteus	–	1	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	2
A. pyogenes	–	–	–	1	–	2	–	–	1	–	–	–	–	–	1	–	–	–	–	–	5
All species	43	13	30	23	42	67	22	63	53	34	20	29	17	62	29	23	24	16	217	33	859

Table 3. Levels of cell count as estimated by California Mastitis test (CMT) and distribution of microorganisms isolated from intramammary infections in 20 Danish dairy herds.

Bacteria	CMT					Dry cows or quarters without CMT-reactions	Total
	1	2	3	4	5		
S. aureus	73	32	40	113	201	14	473
S. epidermidis	15	12	10	12	3	9	61
S. chromogenes	16	15	3	4	2	4	44
S. simulans	10	8	4	3	3	5	33
S. xylosus	1	–	3	5	5	1	15
Other Staphylococci	16	2	2	6	5	4	35
S. uberis	6	11	7	11	25	6	67
S. dysgalactiae	3	4	11	13	40	4	75

epidermidis. The 3 most common biotypes accounted for 29.5%, 26.2% and 24.6% of the biotypes respectively. The combination of all 3 typing methods produced 22 different types.

Several types of *S. epidermidis* could be found within each herd, but 1 to 2 types predominated within a particular herd. Most types of *S. epidermidis* were unique to a single or a few herds (Table 5).

Discussion

Identification of mastitis pathogens to species level is important for a number of reasons. Firstly, because control and eradication procedures most often will depend on the kind of infection prevalent in a herd. Secondly, because antimicrobial susceptibility patterns should be produced from the relevant causative agens, and thirdly, the validity of epidemiological investigations aiming at determining transmission patterns or the impact of environmental and management factors to a large extent depends on exact bacteriological diagnostics. In this study we identified 34 bacterial species, 18 (53%) of which belonged to the genus *Staphylococcus,* 17 of which were CNS. Conventional procedures for identification of CNS require numerous media and are labour intensive, but several simplified identification schemes have been suggested *(Devriese* 1986, *Gahrn-Hansen et al.* 1987). Commercial identification systems are available, but most of these are not designed for identifying important veterinary pathogens *(Watts & Yancey* 1994). Regarding mastitis pathogens *S. chromogenes* is misidentified as *S. hyicus, S. epidermidis* or *S. simulans (Langlois et al.* 1983, *Watts et al.* 1984, *Matthews et al.* 1990, *Kloos & George* 1991).

The identification scheme used in this investigation was a modification of conventional systems, and it identified all but 9 (1.4%) staphylococci, 8 of which could be speciated using appropriate additional testing.

As expected the infection level varied markedly among herds. From 21% to 70% of all cows and 6% to 35% of all quarters were infected. The herds were not randomly selected, but the most common Danish dairy production systems and management practices were represented. Thus, even though the distribution of species found in the study may have been influenced by the farms selected for investigation, the trends are valid and the diversity of species found in intramammary infections are evident.

The distribution of bacterial species from bovine milk has been investigated in different countries in recent years. It is generally agreed

Table 4. Phage types of isolates of *Staphylococcus aureus* from bovine intramammary infections in 20 Danish dairy herds.

Phage type	Phage pattern	Herd number																				Total
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
47/75/84/85/89	III	3	1	–	–	1	1	–	12	16	9	7	11	–	16	–	–	9	–	–	–	86
96	V	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–1	–	–	76	–	79
29/52	I	5	–	4	3	3	–	–	12	11	5	1	1	7	5	3	3	–	6	4	–	73
3A/3C	II	2	–	9	–	–	38	–	–	–	–	–	–	–	–	–	–	–	–	–	–	49
29/52/80/84	Mix	3	–	2	1	2	3	11	1	1	–	–	1	1	2	–	–	–	–	7	2	37
6/47/75/84/85	III	–	–	–	–	–	–	–	–	2	–	–	–	–	22	–	–	–	–	–	–	24
29/52/84/85	Mix	1	–	–	–	–	–	–	3	–	–	–	–	1	–	–	–	–	–	–	2	7
6/54/77/81/83A/84	III	–	–	–	–	1	–	–	–	–	5	–	–	–	–	–	–	–	–	–	–	6
81		–	–	–	–	–	–	–	–	–	–	–	–	–	–	3	–	–	–	–	–	3
29/52/52A/80/42E/ 53/54/77/83A/84/85/ 95/89	Mix	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	2	–	3
6/42E/47/75/77/81/ 83A/95/93	III	–	–	–	–	1	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	3
29/52/52A/80/42E/ 83A/84/93	Mix	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	1	–	2
29/80/6/42E/47/54/ 75/81/84/85/95/89	Mix	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	1
29/52/42E/95	Mix	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	1
29/52/52A/79/80/ 42E/83A	Mix	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
29/52/52A/79/80/ 55/71/85/93/89	Mix	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
42E/53/77/83A/84/ 85/95/93/89	III	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	1
53	III	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	1
nt		4	1	–	–	1	–	–	–	5	–	6	–	2	2	1	–	6	–	67	–	95

nt = not typeable.

Table 5. Phage type, Antibiogram and biotype discrimination of isolates of *Staphylococcus epidermidis* from bovine intramammary infections in 10 Danish dairy herds.

Biotype	Antibiotical resistance	Phage type	Herd number										Total
			1	5	8	9	10	11	14	15	19	20	
a	pen^r	nt	6	–	–	–	2	–	–	–	–	3	11
a	pen^r tetra^r	nt	–	–	–	–	–	1	–	–	–	–	1
a	tetra^r	nt	–	–	–	–	–	–	–	–	1	–	1
a	strep^r tetra^r	155/157A/275/275A/A6C/B1	–	–	–	–	–	–	–	–	–	1	1
a	None	37/157A/275A	1	–	–	–	–	–	–	–	–	–	1
a	None	nt	2	–	–	–	–	–	1	–	–	–	3
b	pen^r	nt	1	–	1	–	–	–	–	–	–	–	2
b	pen^r strep^r	nt	–	–	4	–	–	–	–	–	–	–	4
b	pen^r tetra^r	nt	–	–	–	–	–	1	–	–	–	–	1
b	None	37/157A/275A	–	–	–	–	–	–	1	–	–	–	1
b	None	nt	–	2	–	–	–	–	6	–	–	–	8
c	pen^r strep^r	nt	–	–	–	1	–	–	–	–	–	–	1
c	tetra^r	nt	–	–	–	–	–	–	–	–	2	–	2
c	None	155/157A/275/275A/A6C/B1	–	–	–	–	–	–	–	–	7	–	7
c	None	nt	–	–	–	–	–	–	–	–	5	–	5
d	pen^r	nt	–	–	1	–	–	–	–	–	–	–	1
d	None	A9C	–	1	–	–	–	–	–	–	–	–	1
d	None	nt	–	5	–	–	–	–	–	–	–	–	5
e	None	nt	–	–	–	–	–	–	–	–	2	–	2
f	pen^r	nt	–	–	1	–	–	–	–	–	–	–	1
g	None	nt	–	–	–	–	–	–	–	1	–	–	1
h	None	nt	–	–	–	–	–	–	–	–	–	1	1

pen^r: resistance towards penicillin; tetra^r: resistance towards tetracycline; strep^r: resistance towards streptomycin. nt = non-typable.

that *S. aureus,* as also seen in this investigation, is the most commonly found mastitis pathogen followed by *S. uberis* or *S. dysgalactiae (Anon.* 1985, *King* 1981).

In contrast the distribution of species of CNS from bovine milk has been reported differently among investigations *(Devriese & De Keyser* 1980, *Jarp* 1991, *Birgersson et al.* 1992, *Todhunter et al.* 1993). Despite variations between herds and countries, *S. chromogenes, S. simulans* and perhaps *S. epidermidis* and *S. xylosus* in general appear to be the most frequently isolated CNS from bovine milk worldwide. Variations between different investigations may in part be due to the diagnostic method applied. In the present Danish study *S. epidermidis* (32.4%), *S. chromogenes* (23.5%) and *S. simulans* (17.6%) were the most commonly isolated species of CNS.

The observed differences in CMT reactions for different bacterial specie supports the findings of others that *S. aureus, S. uberis* and *S. dysgalactiae* cause a severe inflammation in the udder, whereas the CNS generally causes a less severe inflammatory reaction *(Bramley & Dodd* 1984). In this investigation *S. xylosus* was also associated with high CMT.

Previous investigations have shown that strains of *S. aureus* causing bovine mastitis can be referred to many different phage types, but that some of these types predominate both within herds and countries *(Price et al.* 1954, *Mackie et al.* 1987). In this investigation phage typing proved to be a convenient method to differentiate strains of *S. aureus* from bovine mastitis. In all herds investigated, except for the isolates in herd 19, the typability of *S. aureus* was high. Some phage types were found in several herds, whereas others were restricted to one or a few herds. A few types were found to predominate within each herd. This might indicate that only one or a few major sources of infection exist for a particular herd.

Different phage types were relatively frequently isolated from different quarters of the same cow. This is in agreement with other investigations *(Parisi & Baldwin* 1963) and supports the hypothesis that *S. aureus* is primarily transferred from cow to cow during milking and not from an external source specific to a particular cow *(Bramley & Dodd* 1984).

Typing of *S. epidermidis* has not earlier been used in epidemiological investigations of bovine mastitis. Neither phage typing nor antibiogram typing produced satisfactory discrimination in this investigation, whereas biotyping gave good discrimination of the strains. There was a relatively large diversity in types between herds, whereas 1 to 2 types seemed to predominate within a single herd, pointing to a few sources of infection by *S. epidermidis* for each herd.

Conclusion

This study documents the diversity of coagulase negative staphylococci (CNS) and other bacterial species associated with intramammary infections of milking cows in Denmark. *S. aureus* was the most common species found followed by *S. dysgalactiae* and *S. uberis.* The most frequently isolated CNS was *S. epidermidis* followed by *S. chromogenes* and *S. simulans.* This study indicates that relatively few of the species found are important, but also that at least some of these important mastitis pathogens make up a large number of different types.

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Sammendrag

Stafylokokker og andre bakteriearter associeret med intramammære infektioner i danske malkekvægsbesætninger

For at bestemme den relative fordeling af forskellige bakteriearter fra bovin mastitis blev 4645 kirtelprøver fra 20 danske malkekvægsbesætninger undersøgt. Fra 839 af kirtlerne blev der isoleret 859 forskellige bakterieisolater, som kunne inddeles i 34 forskellige bakteriearter og underarter. Stafylokokdiagnostikken var baseret på konventionelle procedurer som var i stand til at skelne mellem alle 36 anerkendte arter og underarter. *Staphylococcus aureus* blev isoleret fra 10,2% af kirtlerne og var den oftest isolerede patogen. Den næst mest almindelige patogen var *Streptococcus dysgalactiae* (1,6%) og trejde *Streptococcus uberis* (1,4%). Der blev isoleret 17 forskellige arter og underarter af koagulase negative stafylokokker. De mest almindelige var *S. epidermidis* (1,3%), *S. chromogenes* (1,0%) og *S. simu-*

lans (0,7%). Isolaterne af *S. aureus* blev fagtypet og isolater af *S. epidermidis* blev undersøgt for fagtype, antibiogramtype og biotype. Ialt kunne 378 (79,9%) af *S. aureus* isolaterne types med fager, som inddelte dem i 18 forskellige typer. Seks fagtyper udgjorde dog 92,1% af alle de typebare isolater. En til 2 forskellige fagtyper dominerede indenfor hver besætning. Elleve (18%) af *S. epidermidis* isolaterne kunne types med fager, som inddelte dem i 3 forskellige typer. Biotypning af *S. epidermidis* gav 8 forskellige typer, hvor den mest almindelige type udgjorde 29,5%. Seks forskellige antibiogramtyper forekom blandt alle isolater af *S. epidermidis*. Resistens mod penicillin (36,1%), tetracyclin (9,8%) og streptomycin (9,8%) blev observeret blandt *S. epidermidis* isolaterne. Femogtredive (57,4%) af isolaterne var dog følsomme over for alle 12 antibiotika, som der blev testet for.

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