Bovine Mastitis in Finland 2001—Prevalence, Distribution of Bacteria, and Antimicrobial Resistance

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ABSTRACT

A nationwide survey was conducted in Finland to estimate prevalence of bovine mastitis, distribution of mastitis pathogens, and in vitro antimicrobial susceptibility of different mastitis pathogens. In total, 12,661 quarter milk samples were collected from 3282 dairy cows at 216 farms. These were randomly selected from a database covering all Finnish dairy farms. Quarter milk samples collected by the dairy advisors were submitted for somatic cell counting, bacteriological examination, and testing for antimicrobial susceptibility. If the milk SCC of a cow or of a quarter exceeded 300,000/mL, the cow was defined as having mastitis. The results were compared with those of a previous survey done in 1995. The prevalence of mastitis continued to decrease from 38% in 1995 to 31% in 2001. Compared with the study from 1995, the number of quarters with bacterial growth in 2001 increased significantly from 21.0 to 33.5%. This mainly resulted from increased prevalence of *Corynebacterium bovis*. Coagulase-negative staphylococci remained the most common bacterial group, comprising almost one-half of the pathogens isolated, whereas the relative number of *Staphylococcus aureus* isolations decreased from the time of the previous study. According to in vitro antimicrobial susceptibility testing, the enterococci demonstrated the highest level of resistance. Compared with the other Nordic countries, penicillin resistance among the staphylococci was still at a relatively high level in Finland (52.1 and 32.0% for *Staphylococcus aureus* and coagulase-negative staphylococci, respectively). Streptococci isolated from mastitis were very susceptible to β -lactam antibiotics, as also found in the previous survey in 1995.

(**Key words:** mastitis, prevalence, bacteria, antimicrobial resistance)

Abbreviation key: CNS = coagulase-negative staphylococci, MIC₅₀ and MIC₉₀ = minimum inhibitory con-

centrations at which 50 or 90% of isolates, respectively, are at or below, NCCLS = National Committee for Clinical Laboratory Standards.

INTRODUCTION

The first official campaign against mastitis in Finland started in the late 1940s with the goals of eradicating the contagious mastitis pathogen *Streptococcus agalactiae* and improving raw milk quality. Both aims were successful. Bulk milk SCC has been regularly recorded in Finland since the 1980s. During recent years, the SCC has remained at a stable level in the country, and the geometric mean in 2002 was 132,000 cells/mL (Statistics of Finnish Association for Milk Hygiene, 2003). The prevalence of mastitis and its pathogens has been investigated in Finland in several surveys carried out since the late 1970s. In the late 1980s, a national effort was made to decrease mastitis prevalence, and it decreased from 48% in 1988 to 38% in 1995 (Myllys et al., 1998).

In many countries, including Finland, the decreased prevalence of subclinical mastitis has been accompanied by a change in the relative and absolute importance of the main mastitis pathogens, probably because of marked changes in the dairy industry. The prevalence of the classical contagious bacteria *Strep. agalactiae* and then *Staphylococcus aureus* has decreased. Coagulase-negative staphylococci (CNS) and *Corynebacterium bovis*, which are traditionally considered to be minor mastitis pathogens, have become more common (Schepers et al., 1997; Myllys et al., 1998; Huxley et al., 2002). In Finland, CNS has become the most common bacterial group isolated from quarter milk samples (Myllys et al., 1998). Together with this change, the share of resistant staphylococci has increased. From 1988 to 1995, the proportion of strains resistant to at least one antimicrobial has increased both among *Staph. aureus* (from 37 to 64%) and among CNS (from 27 to 50%); most of this change is due to the higher number of penicillin resistant (β -lactamase producing) isolates (Myllys et al., 1998).

Received December 31, 2003.

Accepted April 20, 2004.

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This study describes the results from a national mastitis survey carried out in 2001 in Finland to continue the regular monitoring of mastitis prevalence and the pathogens involved. The antimicrobial resistance of the bacteria isolated was also determined.

MATERIALS AND METHODS

Survey Design

A total of 12,661 quarter milk samples were obtained from 3282 cows at 216 farms. At the end of 2000, a total of 270 dairy farms were selected randomly from a database that included all dairy farms in Finland. Dairy advisors collected quarter milk samples from all milk-producing cows at the farms. The mean size of the herds included in the study was 17.3 cows, the average parity of the cows was 2.5, the mean annual milk production was 7505 kg, and the average geometric mean for SCC was 116,000 cells/mL. At this time, the mean herd size in Finland was 17.9 cows, and the mean annual milk production was 6932 kg. The breeds of the cows were Finnish Ayrshire, 42.1%; Holstein-Friesian, 14.0%; other breeds, 1.0%; and mixed breeds, 42.9%. A great majority of the farms (89.8%) were stanchion barns, and the remainder had loose housing (6.9% warm and 3.2% cold systems). The production and SCC records were available from the national herd records.

Sampling

Quarter foremilk samples were collected aseptically for bacteriological assay as described by Honkanen-Buzalski (1995). Before sampling, the first streams of milk were discarded, and teat ends were disinfected with cotton swabs soaked in 70% alcohol and allowed to dry. The milk samples for SCC were preserved with sodium azide. The milk samples were transported on ice to the laboratory of the Veterinary and Food Research Institute for bacteriology and to Valio Ltd. laboratories in Kouvola, Lapinlahti and Seinäjoki for SCC.

Analysis of Milk Samples

Bacteriology. From each sample, 0.01 mL of milk was cultured on blood-esculin agar and incubated for 48 h at 37°C; the plates were examined after 24 and 48 h of incubation. Bacterial species were identified using accredited methodology based on National Mastitis Council (1999) standards and procedures described by Honkanen-Buzalski and Seuna (1995). A quarter was considered bacteriologically positive when growth of ≥ 500 cfu/mL was detected from a sample. Samples yielding >2 bacterial species were considered to be contaminated (IDF, 1981; NMC, 1999). The diagnosis was

confirmed according to the main pathogen, when 2 different species were present. In such cases, the diagnosis was prioritized by pathogen as follows: *Staph. aureus* $>$ *Streptococcus uberis*/Streptococcus sp. $>$ CNS $>$ Enterococcus sp. $>$ *Aerococcus viridans* $>$ *Lactococcus* spp. $>$ *C. bovis*. The isolates were maintained frozen at -70°C in brain heart infusion broth containing 15% glycerol.

Milk SCC was determined with an electric counter (Fossomatic Milk Analysis, Foss Electric, Hillerød, Denmark) within 1 d of collection. A quarter was determined to have mastitis if SCC exceeded 300,000/mL, and a cow was diagnosed to have mastitis if SCC in at least one quarter exceeded 300,000/mL (Klastrup and Schmidt Madsen, 1974).

Susceptibility testing. The in vitro susceptibility of the isolates to antimicrobials was determined by a commercially available microdilution system (VetMIC; SVA, Uppsala, Sweden) according to the manufacturer's instructions and by following the standards of the National Committee for Clinical Laboratory Standards (NCCLS, 2002a). Prior to the testing, the isolates were subcultured onto blood agar and incubated for 24 h at 37°C. Mueller Hinton Broth (Difco, le Port de Claix, France) was supplemented with 7% defibrinated horse serum as described in the manufacturer's instructions to test the susceptibility of streptococcal isolates. Minimum inhibitory concentration (MIC) was recorded as the lowest concentration of the antimicrobial agent that inhibited bacterial growth. The distributions of the MIC were calculated, and the results were classified using the breakpoints recommended by the NCCLS (2002a) for bacteria isolated from animals. For clindamycin, the NCCLS breakpoint for canine was used, and for enrofloxacin, the NCCLS breakpoint for bovine respiratory disease was used, as they have been used with mastitis pathogens also by other investigators (Gentilini et al., 2000, 2002; Giannechini et al., 2002). No recommendations are currently available from the NCCLS for spiramycin, streptomycin, and neomycin; therefore, the breakpoints used in SVARM (2002) were used. *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were used as quality control strains. The production of β -lactamase was tested immediately after isolation using nitrocefin discs (AB Biodisk, Solna, Sweden). *Staphylococcus aureus* ATCC 29213 was used as a positive control.

Staphylococcus aureus isolates with MIC of oxacillin ≥ 2 $\mu\text{g}/\text{ml}$ were retested using an oxacillin-salt agar screening test described in NCCLS (2002a). The methicillin-resistant *Staph. aureus* strain ATCC 43300 and the methicillin-susceptible *Staph. aureus* strain ATCC 29213 were used as control strains. Quality of the each control strain was tested each day of testing.

Figure 1. Development of mastitis prevalence in Finland in 1980 to 2001. The prevalence of mastitis is given as bars, and the annual change of geometric mean of bulk milk SCC delivered to dairies between 1990 and 2001 is presented as a line.

The differences in bacterial diagnoses and resistance patterns between 1995 and 2001 were compared using the 2-sample proportion test in the statistical software package Statistix (Analytical Software, Tallahassee, FL).

RESULTS

The herd prevalence of bovine mastitis (prevalence of mastitis within herds as a mean of all herds) was 30.6% (95% confidence interval, 28.4 to 33.1) (Figure 1). The prevalence of mastitis increased until the third

calving and then remained at 30%. The proportion of quarters producing high SCC milk decreased, but the proportion of bacteriologically positive quarters increased since the previous survey. The results of bacteriological findings are summarized in Table 1. A total of 33.5% of the samples (4237 out of 12,661) were considered bacteriologically positive. Among the quarters with bacterial growth, CNS was the most commonly isolated bacterial group ($n = 2103$; 49.6% of the positive findings), followed by *C. bovis* ($n = 1458$; 34.4%) and *Staph. aureus* ($n = 431$; 10.2%). *Streptococcus agalactiae* was isolated from 3 samples (0.1% of the positive

Table 1. Bacterial isolates found during the surveys in 1995 and 2001. Figures in the first column each year are percentages of quarter milk samples. In the second column, proportions of different pathogens are presented.

	1995		2001	
	n = 10,337	n = 2172	n = 12,661	n = 4237
CNS ¹	11.24	53.50	16.61	49.63
<i>Staphylococcus aureus</i>	3.50	16.67	3.40	10.17
<i>Streptococcus agalactiae</i>	0.12	0.55	0.02	0.07
<i>Streptococcus dysgalactiae</i>	0.08	0.37	0.05	0.14
<i>Streptococcus uberis</i>	0.72	3.41	0.65	1.94
Other streptococci	1.44	6.86	0.07	0.21
<i>Enterococcus</i> spp. ²			0.40	1.20
<i>Aerococcus viridans</i> ²			0.24	0.71
<i>Lactococcus</i> spp. ²			0.24	0.73
<i>Corynebacterium bovis</i>	3.49	16.62	11.52	34.41
Coliforms	0.28	1.38	0.14	0.42
Other bacteria	0.14	0.64	0.12	0.37
Contaminated samples	4.21		4.15	
No bacteria	74.78		62.38	
Total	100.00	100.00	100.00	100.00

¹CNS = Coagulase-negative staphylococci.

²In 1995, included as other streptococci.

Table 2. In vitro susceptibility of 196 strains of *Staphylococcus aureus*.

Antimicrobial	Breakpoint	% Resistant	MIC ₅₀ ¹ (µg/mL)	MIC ₉₀ ¹ (µg/mL)	Range (µg/mL)
Cephalothin	>16	0	0.25	0.5	0.12 to 0.5
Clindamycin	>2	0.5	1	1	1 to 4
Enrofloxacin	>1	0	0.25	0.5	0.125 to 1
Erythromycin	>4	1.5	0.5	0.5	0.5 to 8
Gentamicin	>8	0	0.5	1	0.25 to 2
Neomycin	>32	0	1	2	1 to 32
Oxacillin	>2	4.1 ²	1	2	0.5 to 8
Oxytetracycline	>8	5.1	1	1	0.5 to 128
Penicillin ³		52.1			
Spiramycin	>32	0	8	16	2 to 16
Streptomycin	>32	4.1	4	16	1 to 256
Trimethoprim/ sulfamethoxazole ⁴	>2	1.5	0.25	0.25	0.25 to 16

¹MIC₅₀ and MIC₉₀ = Minimum inhibitory concentrations at which 50 and 90%, respectively, of isolates are at or below.

²Primary result, oxacillin susceptible according to confirmatory tests.

³β-Lactamase production.

⁴Concentration of trimethoprim is given, tested at concentration ratio 1:19.

samples). The cows infected with *Staph. aureus* had an average of 1.28 *Staph. aureus* positive quarters (in 1995, 1.24 quarters per cow). Also CNS and *C. bovis* were often found from several quarters of a cow. From the bacteriologically positive samples, 3.7% (n = 155) supported growth of 2 bacterial species. In those cases, CNS was involved in 71.6% of the quarters, and *Staph. aureus* was involved in 22.6% of the quarters. Most often the combination was CNS and *C. bovis* (in 36.8% of the quarters), followed by *Staph. aureus* and CNS (in 9.7% of the quarters), CNS and *Enterococcus* spp. (in 9.7% of the quarters), and CNS and *A. viridans* (in 9.0% of the quarters).

The proportion of cows shedding *Staph. aureus* was slightly lower in first and second parity cows than in older cows. The proportion of cows shedding CNS exceeded 30% over all parities. *Corynebacterium bovis* was present at 80% of the farms. From the cows at the first lactation, 15% were positive for *C. bovis*, and this percentage doubled in the older cows. In quarters positive for *Staph. aureus*, milk SCC was >300,000/mL, only in about one-half of the quarters. For CNS and *C. bovis*, the corresponding figures were even lower, 18 and 11%, respectively.

The in vitro activities of each of the antimicrobial agents tested are shown in Table 2 for *Staph. aureus*, in Table 3 for CNS, in Table 4 for *Strep. uberis*, and in Table 5 for Enterococci. The numbers of other streptococci, *Strep. dysgalactiae* (n = 5), *Strep. agalactiae* (n = 2), and *Strep. bovis* (n = 5), were low. *Streptococcus dysgalactiae* was less susceptible to tetracyclines than other streptococci. This finding applied also to *Streptococcus bovis*.

The MIC₅₀ (MIC at which 50% of isolates are at or below) for *Staph. aureus* to penicillin was 0.06 µg/mL, and the MIC₉₀ (MIC at which 90% of isolates are at or below) was 16 µg/mL (0.06 to 16 µg/mL). The MIC₅₀ for CNS to penicillin was 0.06 µg/mL, and MIC₉₀ was 2 µg/mL (0.06 to 16 µg/mL).

Of *Staph. aureus* isolates, 2.0% and of CNS isolates, 3.3% were resistant to ≥3 antimicrobials. Multiresistance was found in 25.4% of Enterococci, including high level resistance (MIC > 1024 µg/mL) to aminoglycosides.

The MIC₉₀ for *A. viridans* (n = 25) was 0.5 µg/mL to penicillin, 2 µg/mL to cephalothin, 1 µg/mL to clindamycin, 1 µg/mL to erythromycin, 4 µg/mL to gentamicin, 8 µg/mL to neomycin, 8 µg/mL to oxytetracycline, 4 µg/mL to spiramycin, 128 µg/mL to streptomycin, and 0.25 µg/mL to trimethoprim-sulfamethoxazole.

DISCUSSION

The prevalence of bovine mastitis in Finland continued to decrease, declining from 38% in 1995 (Myllys et al., 1998) to 31% in 2001. The positive trend in mastitis reduction in Finland noted in recent decades may indicate that the mastitis control method used has been effective. During the same period, consumption of antibiotics for mastitis treatment significantly decreased (Annual Statistics of the National Agency for Medicines, 2003).

The used threshold value of 300,000 cells/mL to indicate mastitis, as recommended by Klastrup and Schmidt Madsen (1974) and as used in the previous study, may be high. The SCC of a cow that is not infected with mastitis pathogens is usually <200,000 cells/mL,

Table 3. In vitro susceptibility of 335 strains of coagulase-negative staphylococci.

Antimicrobial	Breakpoint	% Resistant	MIC ₅₀ ¹ (µg/mL)	MIC ₉₀ ¹ (µg/mL)	Range (µg/mL)
Cephalothin	>16	0	0.25	0.5	0.12 to 0.2
Clindamycin	>2	0.3	1	1	1 to 4
Enrofloxacin	>1	0	0.25	0.5	0.125 to 1
Erythromycin	>4	5.4	0.5	0.5	0.5 to 8
Gentamicin	>8	0	0.25	0.25	0.25 to 1
Neomycin	>32	0	1	1	1 to 32
Oxacillin	>2	9.6	1	2	0.5 to 8
Oxytetracycline	>8	8.7	1	4	0.5 to 128
Penicillin ²		32			
Spiramycin	>32	2.1	8	16	4 to 64
Streptomycin	>32	6.9	2	16	2 to 256
Trimethoprim/ sulfamethoxazole ³	>2	1.8	0.25	0.5	0.25 to 16

¹MIC₅₀ and MIC₉₀ = Minimum inhibitory concentrations at which 50 and 90%, respectively, of isolates are at or below.

²β-lactamase production.

³Concentration of trimethoprim is given, tested in concentration ratio 1:19.

Table 4. In vitro susceptibility of 89 strains of *Streptococcus uberis*.

Antimicrobial	Breakpoint	% Resistant	MIC ₅₀ ¹ (µg/mL)	MIC ₉₀ ¹ (µg/mL)	Range (µg/mL)
Cephalothin	>16	0	0.25	0.25	0.12 to 1
Clindamycin	>2	0	1	1	1 to 1
Erythromycin	>0.5	0	0.5	0.5	0.5 to 0.5
Gentamicin	NR ²		16	32	2 to 64
Neomycin	NR		64	32	8 to 256
Oxytetracycline	>4	1.1	1	1	0.5 to 128
Penicillin G	>2	0	0.064	0.064	0.06 to 0.12
Spiramycin	>16	2.2	4	4	4 to 64
Streptomycin	NR		64	128	16 to 512
Trimethoprim/ sulfamethoxazole ³	>2	0	0.25	0.5	0.25 to 1.0

¹MIC₅₀ and MIC₉₀ = Minimum inhibitory concentrations at which 50 and 90%, respectively, of isolates are at or below.

²NR = Not relevant.

³Concentration of trimethoprim given, tested at concentration ratio 1:19.

Table 5. In vitro susceptibility of 63 strains of Enterococci.

Antimicrobial	Breakpoint	% Resistant	MIC ₅₀ ¹ (µg/mL)	MIC ₉₀ ¹ (µg/mL)	Range (µg/mL)
Ampicillin	>8	0	1	2	0.25 to 8
Erythromycin	>4	19	2	8	0.5 to 8
Gentamicin	NR ^a		8	16	4 to 32
Neomycin	NR		64	2048	16 to 2048
Oxytetracycline	>8	73	64	64	0.5 to 128
Penicillin G	>8	0	4	4	1 to 4
Spiramycin	>32	17.5	4	64	4 to 64
Streptomycin	NR		2048	2048	64 to 2048

¹MIC₅₀ and MIC₉₀ = Minimum inhibitory concentrations at which 50 and 90%, respectively, of isolates are at or below.

²NR = Not relevant.

and, at quarter level, a threshold value of <100,000 cells/mL has been proposed for a truly healthy quarter (Ruegg and Reinemann, 2002; Schukken et al., 2003). According to Schepers et al. (1997), infections caused by *Staph. aureus*, *Strep. uberis*, or *Strep. dysgalactiae* increased SCC more than CNS or *C. bovis* infections. In a meta-analysis on SCC in quarters with IMI (Djarbi et al., 2002), mean SCC was >300,000 cells/mL for *Staph. aureus* only; for CNS and *C. bovis*, the mean values were 138,000 and 105,000 cells/mL, i.e., considerably lower than the limit used here. The sensitivity and specificity of a threshold value depends on which definition of IMI has been used and also on whether the parameters are calculated at the cow level or at the quarter level (Schepers et al., 1997). It is questionable whether the classical survey method based on a threshold level of SCC as high as used here is sensitive enough (Beaudeau et al., 2002; Zecconi and Piccinini, 2002). With any threshold value, however, one part of the results will always be misclassified.

The amount and proportions of positive findings for mastitis pathogens have changed over time. Bacterial growth was detected in 33.5% of the samples, which is significantly more than in 1995 (21.0%; $P < 0.01$). The rise in the number of bacteriologically positive quarters was mainly due to the high frequency of coryneform infections, which increased from 16.6 to 34.4%. For *Staph. aureus*, there was no difference in the findings at cow or quarter levels between the surveys in 1995 and in 2001: the prevalence of cows positive for *Staph. aureus* was 10.1% in 2001 and 11.1% in 1995. The proportion of CNS greatly increased from 1988 to 1995 and has since remained at the same level (Myllys et al., 1998). The herd-level prevalence was not compared between studies because it is influenced by differences in the management and other differences in risk factors among herds.

It is difficult to compare results of surveillance studies because of differences in sample selection and cultivation techniques. Furthermore, the criteria used when diagnosing a sample as bacteriologically positive vary among studies. A wide-ranging mastitis survey was carried out in Norway in 2000. The prevalence of mastitis was found to be comparable with our results. The proportion of quarters with bacterial growth, 22.9%, was clearly lower than in our study, but the criteria for establishing positive bacteriological diagnosis differ from our study (Sølverød and Østerås, 2001). Distribution of udder pathogens in subclinical mastitis has been studied in some countries and regions (Wilson et al., 1997; Schällibaum, 1999; Poelarends et al., 2001). In The Netherlands, *Staph. aureus* was the most common cause of subclinical mastitis when sampling quarters with SCC >250,000 cells/mL (Poelarends et al., 2001).

In Switzerland, subclinical mastitis was followed-up in dairy herds from 1987 to 1996 (Schällibaum, 1999). The most common pathogen found was *Staph. aureus* followed by "other streptococci" (*Strep. uberis*, *Strep. dysgalactiae*, Enterococci) and CNS. In contrast to our findings, the proportions of *Staph. aureus* and CNS did not change during that period; more recent data were not available. In a US study, the proportion of infected cows was as high as 48.5%, and the proportion of *Staph. aureus* was 2 times as high as seen here (Wilson et al., 1997). In that study, composite samples were used, which makes comparison difficult.

The increasing frequency of coryneform infections has been noted elsewhere (Schepers et al., 1997; Hillerton, 2000). In our study, *C. bovis* was isolated at every calving, considerably more often in 2001 than in 1995 ($P < 0.01$), and *C. bovis* was present at 80% of the farms. The reason for this phenomenon is hard to explain, but it is probably related to changes in herd management and consequent bacteriological ecology in the herd environment. In Finland, dairy farms use teat dipping less than in most other countries: 24% of the participating farms in 1995 and 32% of the farms in 2001 used teat dipping.

The age distribution of the cows was the same in 1995 and 2001, but the prevalence of mastitis increased in 2001 until third calving and then stabilized at 30%. In the 1995 survey, the prevalence increased at each subsequent calving and was 57% in cows that had calved >5 times. The occurrence of CNS was considerably higher in 2001 than in 1995 for first and fourth parity cows ($P < 0.01$) and was slightly higher in cows that had calved for the second, third, and >5 times.

The number of cows with *Staph. aureus* growth in quarter milk samples was only slightly lower in first and second parity cows than in older cows and was at the same level as 1995 ($P > 0.05$). In general, prevalence of quarters with *Staph. aureus* growth in our study was low compared with that in other studies (Wilson et al., 1997; Schällibaum, 1999). Older cows are generally believed to be more often infected with *Staph. aureus* than younger cows. We did not note any difference between different parities. In a recent study (Zadoks et al., 2001), age was not found to be a risk factor associated with *Staph. aureus* IMI.

The diversity of Gram-positive, catalase-negative cocci isolated in this study is characteristic of subclinical mammary infection (Devriese et al., 1999). *Aerococcus viridans*, a saprophytic microorganism, which has been frequently misidentified as esculin-positive streptococci, is generally present in air and dust. Aerococci have been associated with human infections (Buu-Hoï et al., 1989). They are infrequently isolated as a cause of bovine mastitis, but are often present in mixed cul-

ture and in contaminated samples (Owens et al., 1990; Devriese et al., 1999).

Antimicrobial susceptibility of most bacteria was quite similar to that in the published data from other countries (Salmon et al., 1998; Gentilini et al., 2000; DANMAP, 2001; NORM-VET, 2001; SVARM, 2001, 2002; Werkenthin et al., 2001; DANMAP, 2002; Gentilini et al., 2002; Giannechini et al., 2002; Rossitto et al., 2002; Guérin-Faubleé et al., 2003). The only internationally recognized reference method for testing mastitis pathogens is NCCLS document M31 (NCCLS, 2002a), but it mentions mastitis-specific interpretive criteria only for penicillin-novobiocin and pirlimycin. Most interpretive criteria used to categorize veterinary pathogens as susceptible or resistant to antimicrobials in vitro are based on data obtained from human pathogens and pharmacokinetics of drugs in humans. The validity of these criteria when used in animal pathogens has been questioned (Watts and Yancey, 1994). Differences in the methodology and breakpoints used make comparison of the results from different countries particularly difficult (Werkenthin et al., 2001; Guérin-Faubleé et al., 2002). Earlier studies of antimicrobial susceptibility of udder pathogens in Finland were performed using the agar disc diffusion method (Myllys et al., 1998).

More than one-half of the *Staph. aureus* isolates from Finland are resistant to penicillin G (52.1%), which is significantly more than reported from other Nordic countries. In Norway, 6.5% of *Staph. aureus* strains isolated from clinical and subclinical mastitis samples were resistant to penicillin (i.e., β -lactamase-positive) (NORM-VET, 2001). In Sweden, 7% of *Staph. aureus* isolated from acute clinical mastitis were resistant to penicillin (i.e., β -lactamase-positive), and the proportion of resistant isolates from subclinical and chronic mastitis was 18% (SVARM, 2002). In Denmark, 25% of *Staph. aureus* isolates in 2001 and 30% in 2002 were reported to be penicillin resistant based on MIC distribution (DANMAP, 2001, 2002). In Finland, wide use of intramammary containing combinations and broad-spectrum antibiotics has probably caused the emergence of penicillin resistance. At the time of the study, the following combinations for intramammary use were available: ampicillin-cloxacillin, cephalexin-dihydrostreptomycin, penicillin-neomycin, and penicillin-streptomycin. Until 1997, an intramammary preparation containing oxytetracycline, neomycin, and oleandomycin was available and widely used. In addition, penicillin G, trimethoprim-sulfonamides, oxytetracycline, spiramycin, and enrofloxacin could be used for parental treatment.

The overall resistance pattern of *Staph. aureus* in this study varied from 0 to 5.1% (0 to 11.7% in 1995),

and the resistance pattern of CNS varied from 0 to 9.6% (0 to 11.5% in 1995), except for penicillin. The difference between β -lactamase-producing *Staph. aureus* and CNS strains in 2001 (52.1 and 32.0%) and in 1995 (50.5 and 34.1%) is not significant ($P > 0.05$).

The interpretive criteria for classification of the staphylococcal strains as susceptible or resistant to penicillin should agree with β -lactamase production of the strains (Devriese et al., 2002). When testing staphylococcal sensitivity to penicillin, strains with borderline MIC results (0.06 to 0.25 $\mu\text{g/mL}$) should be screened for β -lactamase production (NCCLS, 2002a). Even strains with a MIC value of 0.06 $\mu\text{g/mL}$ can have the *blaZ* gene, which mediates resistance to penicillin (Suominen et al., 2002).

In this study, the MIC₉₀ of penicillin G was 0.06 $\mu\text{g/mL}$ for streptococci; thus, this antibiotic is very active against streptococci. However, there are reports from other countries of moderately susceptible or resistant strains of *Strep. uberis* (Watts et al., 1995; Salmon et al., 1998; Erskine et al., 2002; Guérin-Faubleé et al., 2002).

Oxacillin is used to detect methicillin resistance in staphylococci. Methicillin-resistant staphylococci are rare in veterinary medicine (Werkenthin et al., 2001; Guérin-Faubleé et al., 2003). In this study, none of the 196 tested *Staph. aureus* isolates was methicillin-resistant. Coagulase-negative staphylococci with a MIC > 0.5 $\mu\text{g/mL}$ of oxacillin should be tested for possible carriage of the *meaA* gene (NCCLS, 2002b).

In Finland, the difference in proportions of resistant *Staph. aureus* isolates to oxytetracyclines between this (5.1%) and the earlier studies (11.7%) (Myllys et al., 1998) indicates that, despite different methods in susceptibility testing, the decrease of resistance to tetracyclines is actual ($P < 0.05$). The result correlates well with the decreased use of tetracyclines in mastitis therapy in Finland (FINRES, 1999). The tetracycline resistance found in Enterococci was high, 73% with the MIC₉₀ at 64 $\mu\text{g/mL}$.

Macrolide and lincosamide antibiotics have common targets in the bacterial ribosome, and organisms that are resistant to one class can be resistant to the other class (Berger-Bächi, 2002). The overall resistance was low to all macrolides in this study. Enrofloxacin was included in this study for epidemiological purposes as representative for the fluoroquinolones; they are not recommended for mastitis treatment in Finland. Based on the bovine respiratory disease interpretive criteria, all of the staphylococcal strains were susceptible to enrofloxacin.

According to NCCLS breakpoints (2002a), the Enterococci were susceptible to penicillin and ampicillin in our study. The MIC₉₀ for penicillin was 4 $\mu\text{g/mL}$ and for ampicillin was 2 $\mu\text{g/mL}$. The penicillin resistance of

Enterococci can either result from production of penicillin-binding proteins or, more seldom, from production of β -lactamase. In general, Enterococci were the most resistant of all organisms tested here, and their resistance to tetracyclines and macrolides and high level resistance to aminoglycosides is probably acquired rather than intrinsic. The results indicate the importance of identification of mastitis pathogens to species level, as reported by Rossitto et al. (2002) and Zadoks et al. (2003). Since 2001, Enterococci were diagnosed as environmental streptococci in the mastitis diagnosing laboratories in Finland. Even if there are no established MIC breakpoints for *A. viridans*, it was more resistant than streptococci, but less resistant than Enterococci.

The change of resistance factors is greater in an environment with a greater microbial load, such as the gut or barn environments. The environment may harbor a stock of less resistant bacteria, which otherwise would not develop resistance in the practically sterile udder environment. It was noticeable in this study that when 2 microbial species were present in the sample, relatively resistant Enterococci and *A. viridans* were not uncommon.

The frequencies of mastitis and intramammary infections in Finnish herds have been regularly surveyed. This should be continued as it provides important knowledge about mastitis at the national level and helps in evaluating the efficiency of mastitis control strategies. Antimicrobial resistance of udder pathogens is also simultaneously surveyed using standardized techniques and with defined breakpoints and quantitative MIC analysis. In Finland, this was the first survey to monitor MIC of mastitis pathogens on such a large scale. The survey thus stands as a basic study on which further prospective studies can be based.

ACKNOWLEDGMENTS

The authors thank the staff of the Department of Bacteriology at the National Veterinary and Food Research Institute, EELA for their skilful technical assistance. The support of Valio Ltd. was greatly appreciated. The Walter Ehrström Foundation and Ministry of Agriculture and Forestry in Finland supported this study.

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