Epidemiol. Infect. (2002), **129**, 193–202. © 2002 Cambridge University Press
DOI: 10.1017/S095026880200715X Printed in the United Kingdom

Spread of *Staphylococcus aureus* resistant to penicillin and tetracycline within and between dairy herds

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(*Accepted 7 March 2002*)

SUMMARY

One hundred and seven bovine isolates of penicillin and tetracycline resistant *Staphylococcus aureus*, recovered from 25 different dairy herds in various parts of Norway, were characterized using antimicrobial susceptibility testing, multilocus enzyme electrophoresis, ribotyping, plasmid analysis and serotyping of capsular polysaccharide. Forty-one isolates from one particular herd, 37 isolates from 5 herds that used a common pasture and milking parlour in summer and 21 isolates from 12 herds in 8 different counties belonged to the same strain. The remaining 8 isolates, which originated from herds in 5 different counties, were assigned to 6 different strains. Seven out of these 8 isolates had the same plasmid restriction profile. In conclusion, penicillin and tetracycline resistant *S. aureus* occurring in dairy herds in Norway mainly seems to represent one particular strain that has achieved widespread distribution or belong to one of several different strains carrying identical plasmids.

INTRODUCTION

Mastitis is the disease that causes the greatest economic losses to the dairy industry. Incidence rates of clinical mastitis varying from 0·2 to 0·6 cases per cow-year have been reported from different countries [1–3]. A substantial proportion of the clinical cases of mastitis is caused by *Staphylococcus aureus*; different studies have found *S. aureus* in 27–44% of such cases [3–5]. Even greater losses than those resulting from clinical mastitis are caused by the high prevalence of mammary quarters with a chronic subclinical infection. *S. aureus* is a predominant cause of subclinical bovine mastitis and has been found in 40–60% of infected quarters [6, 7]. Because of its strong ability to evade the udder defence mechanisms [8] and to survive antibacterial treatment [9], intramammary infections caused by *S. aureus* often persist for long periods, thus representing reservoirs for the further transmission of this organism to quarters of other cows.

The antimicrobial susceptibility pattern of bovine isolates of *S. aureus* varies among countries, among

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regions within countries and among herds within the same region [7, 10, 11]. Although the incidence of clinical mastitis is high in dairy cows in Norway [2], and penicillin G has been, and still is, the antibacterial drug most commonly used for the treatment of cases of mastitis, the proportion of bovine *S. aureus* isolates that are resistant to penicillin is relatively low. Susceptibility testing of more than 70000 *S. aureus* isolates obtained over the last 10 years from cases of bovine clinical or subclinical mastitis revealed that almost 90% of the isolates were sensitive to penicillin [7]. Of the penicillin resistant isolates approximately 10% were also resistant to tetracycline. Herds in which penicillin and tetracycline resistant *S. aureus* (PTRSA) was present were located in different parts of the country. However, in some areas, clusters of affected herds were observed. Within some herds, PTRSA was recovered from a great proportion of the infected quarters.

The question arose as to whether the observed PTRSA isolates represented several different strains that had independently acquired resistance to penicillin and tetracycline or whether they belonged to one strain or a few strains that had spread within and between the affected herds. To decide whether bacteria of the same species obtained from different sources are epidemiologically related, precise characterization of isolates is essential. Various methods have been used to differentiate human or bovine isolates of *S. aureus*, including antimicrobial susceptibility testing [12], plasmid analysis [12–14], ribotyping [12, 15, 16], multilocus enzyme electrophoresis (MEE) [12, 17, 18] and serotyping of capsular polysaccharide (CP) [19, 20]. To achieve sufficient discrimination for epidemiological purposes it is recommended that at least two typing methods be used [12].

To study the relatedness of bovine PTRSA, isolates recovered from dairy herds in different parts of Norway were characterized using various typing methods.

MATERIALS AND METHODS

Herds, cows and isolates of *S. aureus*

Based on the results of routine examinations of quarter milk samples (QMS) at mastitis laboratories in Norway, dairy herds were identified in which cases of mastitis caused by PTRSA occurred. In several of these herds, QMS were collected within a 1-year period (1996) for the identification and further characterization of PTRSA isolates.

A dairy herd (EE), located in Østfold County, was included in the study because repeated examinations of QMS during the previous 2 years had revealed a persistently high prevalence of quarters infected with PTRSA. On one occasion, all lactating cows ($n = 37$) in the herd were sampled. Additional QMS were taken from some cows with clinical or subclinical mastitis. The time interval between the first and last collection of samples was 11 months. Of the PTRSA recovered from the herd, 41 isolates from 20 different cows were randomly selected for further examination. One cow supplied 5 isolates, 6 cows 3 isolates, 5 cows 2 isolates and 8 cows 1 isolate. Two isolates were obtained 6 months apart from the same quarter of the same cow; remaining multiple isolates from particular cows were from samples collected simultaneously from different quarters.

Four herds (HH1–HH4) that used the same pasture and milking parlour during summer were enrolled in the study because of a recent detection of PTRSA in two of these herds. A fifth herd (HH5), which had used the same pasture as HH1–HH4 until 2 years prior to this study, was also included. The common pasture, located in Hedmark County, was established in 1981; HH1, HH2 and HH3 had used this pasture since the beginning and HH4 and HH5 since 1990. On one occasion, QMS were collected from all lactating cows in the herds. In three of the herds, additional QMS were collected from cows with clinical or subclinical mastitis. The time interval between the first and last sampling was 12 months. Thirty-seven PTRSA isolates were selected for further characterization; 1 isolate was from a cow in HH1, 6 isolates from 4 different cows in HH2, 21 isolates from 12 different cows in HH3, 4 isolates from 3 different cows in HH4 and 5 isolates from 5 different cows in HH5. Pairs of isolates were obtained from the same quarter of 4 different cows; 3 pairs were from cows in HH3 from which samples were collected 11 months apart and 1 pair was from a cow in HH4 from which samples were collected 1 month apart.

In addition, 29 PTRSA isolates from mastitic quarters of 23 different cows in 19 different herds in 12 counties (Fig. 1) were included. Sampling of all lactating cows was not performed in these herds.

Except for one herd (MR2), all herds included in the study were enrolled in the National Production Recording Scheme, from which information on herd size was obtained. Herd incidence rate of clinical mastitis was based on recordings in the Norwegian Health Card System for Cattle [21]. Information on

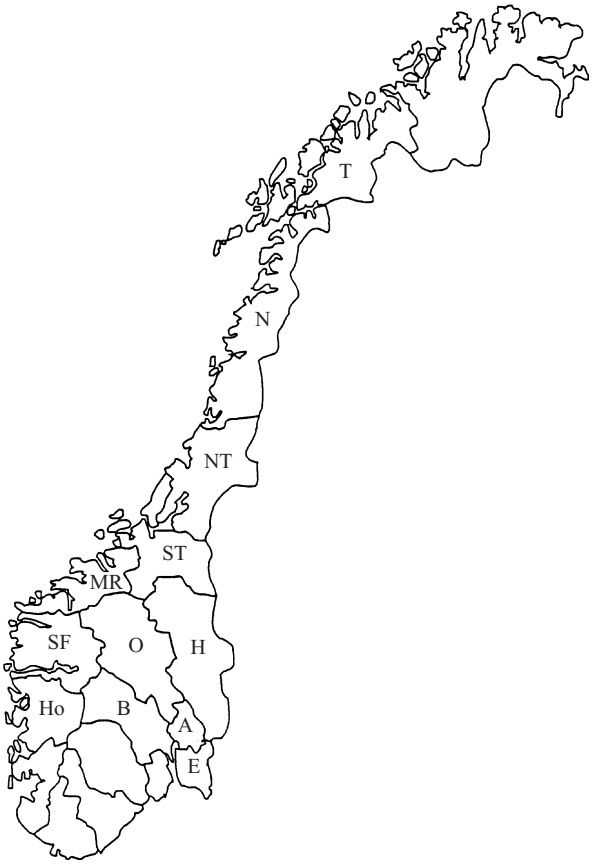

Fig. 1. Map of Norway showing the counties of location for 25 dairy herds from which *Staphylococcus aureus* isolates subjected to typing were recovered. The counties were Akershus (A), Buskerud (B), Østfold (E), Hedmark (H), Hordaland (Ho), Møre og Romsdal (MR), Nord-Trøndelag (NT), Nordland (N), Oppland (O), Sogn og Fjordane (SF), Sør-Trøndelag (ST) and Troms (T).

Table 1. *Herd size and incidence of clinical mastitis for six of the herds included in the study**

Herd	Herd size (cow-years)	Clinical mastitis (cases/100 cow-years)
EE	47·0	27
HH1	6·0	0
HH2	12·0	66
HH3	16·3	61
HH4	26·4	41
HH5	21·2	33

* Data from 1996.

herds EE and HH1–HH5 is given in Table 1. For the remaining 18 herds for which data for 1996 were available, herd size varied from 7·0 to 34·9 cow-years (mean, 14·8 cow-years) and the incidence rate of clinical mastitis varied from 5 to 111 episodes per 100 cow-years (mean, 46·3 episodes per 100 cow-years).

Examination of QMS

QMS were examined at the mastitis laboratories of the National Veterinary Institute. The methods employed by the laboratories [22] were in accordance with recommendations given by the International Dairy Federation [23]. Staphylococci that produced coagulase and acetoin, but not β-galactosidase, were considered to be *S. aureus*. Isolates were stored at -70 °C in heart infusion broth (Difco Laboratories, Detroit, MI) containing 15% (v/v) glycerol.

Antimicrobial susceptibility testing

The susceptibility of *S. aureus* to antimicrobial substances was determined by an agar diffusion method using Neo-Sensitabs® (Rosco Diagnostica, Taastrup, Denmark) [24]. Tablets containing the following antimicrobials were used: penicillin, oxytetracycline, minocycline, bacitracin, trimethoprim, sulfamethizol, streptomycin, cephalothin, neomycin, gentamicin, chloramphenicol, erythromycin, lincomycin, spiramycin, nitrofurantoin, enrofloxacin, fusidic acid and oxacillin. In addition, minimum inhibitory concentration (MIC) of penicillin was determined for all isolates using Etest® (AB Biodisk, Solna, Sweden).

Ribotyping

Ribotyping was performed as described previously [25]. Purified DNA was digested using *Eco*RI. Fragments were separated by electrophoresis in a 0·8% agarose gel and transferred by Southern blotting onto a Hybond-N nylon membrane (Amersham, Arlington Heights, IL). Genes encoding rRNA were detected by hybridization with a digoxigenin labeled rDNA probe. Isolates showing a single band difference were considered different ribotypes.

MEE

Methods of protein-extract preparation, starch-gel electrophoresis and selective enzyme staining were similar to those described by Selander and colleagues [17]. The 13 enzymes assayed were nucleoside phosphorylase, phosphoglucose isomerase, glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, mannitol 1-phosphate dehydrogenase,

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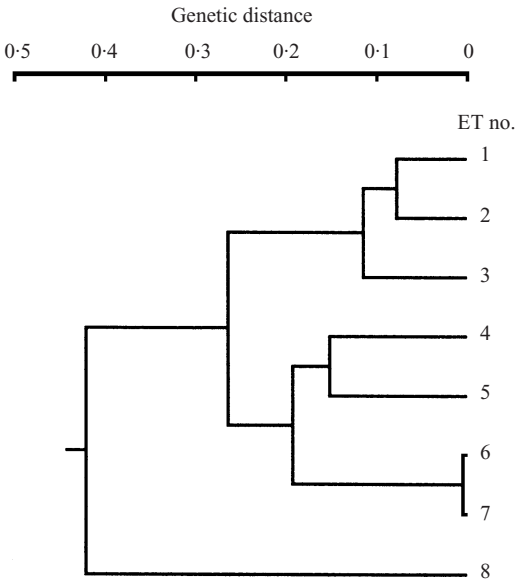

Fig. 2. Results of typing of *Staphylococcus aureus* using multilocus enzyme electrophoresis showing genetic relationships among 8 electrophoretic types (ETs) of 107 isolates resistant to penicillin and tetracycline recovered from cases of bovine mastitis in Norway. The dendrogram was generated by the average-linkage method of clustering from a matrix of coefficients of genetic distance based on 13 enzyme loci. ETs are numbered sequentially from top to bottom.

lactate dehydrogenase, alcohol dehydrogenase, NAPD-linked glutamate dehydrogenase, two peptidases, two adenylate kinases and catalase. Each isolate was characterized by its combination of alleles at the 13 enzyme loci. Distinctive multilocus genotypes were designated as electrophoretic types (ETs). Genetic distance between pairs of ETs was expressed as the proportion of enzyme loci at which dissimilar alleles occurred, and clustering was performed from a matrix of genetic distances by the average-linkage method [26]. The ETs were numbered sequentially according to their position in the dendrogram (Fig. 2).

Plasmid analysis

Plasmid DNA was isolated using the SNAP Miniprep Kit (Invitrogen, Carlsbad, CA), following the procedure recommended by the manufacturer. Lysostaphin (Sigma Chemical Co., St. Louis, MO) was added to the resuspension buffer to a final concentration of 35 μg/ml. Unrestricted plasmid DNA and plasmid fragments produced by using the restriction enzymes *Eco*RI and *Hae*III (Life Technologies, Gaithersburg, MD) were electrophoresed through 1·0% agarose gel. Molecular weight markers used were Supercoiled DNA Ladder (Life Technologies) and plasmids of *Escherichia coli* strain V517 [27] for unrestricted plasmids and 1 kb DNA Ladder (Life Technologies) and a combination of bacteriophage λ DNA digested with *Hind*III (Life Technologies) and ϕX174 RF DNA digested with *Hae*III (Life Technologies) for restricted plasmids. The banding patterns produced by the restriction enzymes were used to differentiate isolates.

Capsular serotyping

Polyclonal antisera against CP of types 5 (CP5) and 8 (CP8) were raised in rabbits to heat- or formalin-killed suspensions of prototype *S. aureus* strains Reynolds (CP serotype 5) and PS80 (CP serotype 8). Sera were adsorbed with trypsinized suspensions of strain Wood-46, acapsular mutant JL243 [28] and acapsular mutant JL252 [29] to remove antibodies to noncapsular cell wall determinants. CP5-specific monoclonal antibody S831 and CP8-specific monoclonal antibody S828 were kindly provided by Dr H. K. Hochkeppel [30].

Serotyping was performed using both colony immunoblots and double immunodiffusion. Colony immunoblots were performed as described previously [31]. Reactivity of the bovine isolates was evaluated by comparison with that of control strains (CP types 1, 2, 5 and 8 and one nontypeable isolate), which were included on each filter membrane. For double immunodiffusion, crude capsular extracts were prepared. Isolates were cultivated for 24 h at 37 °C on Columbia agar (Difco Laboratories) supplemented with 2% NaCl. The colonies from one 9-cm plate were suspended in 1 ml of 10 mM phosphate buffered saline, pH 7·2, and the bacterial suspension was autoclaved for 1 h at 121 °C and subsequently sedimented by centrifugation. Double immunodiffusion was performed in a 1·0% agarose gel.

RESULTS

Herd prevalences of PTRSA

Examination of QMS collected on particular occasions from all lactating cows in herds EE and HH1–HH5 revealed point prevalences of subclinical mastitis at the mammary quarter level between 20% (4 out of 20 quarters in HH1) and 37% (55 out of 148

https://doi.org/10.1017/S095026880200715X Published online by Cambridge University Press

Table 2. *Results of examination of secretion samples collected at particular points of time from mammary quarters of all lactating cows in each of six dairy herds in Norway**

Herd	No. of mammary quarters (cows) examined	No. of mammary quarters (cows)				Percentage of quarters (cows) infected with PTRSA
		Subclinical mastitis†	Infectious subclinical mastitis‡	Presence of *S. aureus*	Presence of PTRSA§	
EE	148 (37)	55 (29)	43 (25)	38 (20)	31 (14)	20·9 (37·8)
HH1	20 (5)	4 (3)	1 (1)	1 (1)	1 (1)	5·0 (20·0)
HH2	47 (12)	17 (10)	11 (7)	6 (5)	4 (3)	8·5 (25·0)
HH3	60 (15)	22 (11)	19 (9)	15 (8)	14 (8)	23·3 (53·3)
HH4	81 (21)	20 (13)	11 (8)	6 (4)	4 (4)	4·9 (19·9)
HH5	75 (20)	17 (13)	13 (10)	12 (9)	4 (3)	5·3 (15·0)
Total	431 (110)	135 (79)	98 (60)	78 (47)	58 (33)	13·5 (30·0)

* Five of the herds (HH1–HH5) used a common pasture.
† Signs of inflammation (increased somatic cell count in quarter secretion) with or without presence of organisms.
‡ Organisms were detected in quarter secretion (*S. aureus* ($n = 78$ quarters), coagulase-negative staphylococci ($n = 14$ quarters), *Streptococcus dysgalactiae* ($n = 4$ quarters), *Streptococcus uberis* ($n = 1$ quarter), *Enterococcus* sp. ($n = 1$ quarter)).
§ PTRSA: penicillin and tetracycline resistant *S. aureus*.

quarters in EE) (Table 2). *S. aureus* was isolated from 78 (80%) of 98 infected quarters, and PTRSA was present in 58 (74%) of those quarters that were infected with *S. aureus* (Table 2). The herd prevalence of cows that had at least one quarter infected with PTRSA varied between 15% (HH5) and 53% (HH3), and the overall proportion in these herds of cows infected with PTRSA was 30%.

Typing of *S. aureus*

In Table 3, the 107 *S. aureus* isolates that were subjected to typing are classified according to the results of the various methods. Based on MEE, isolates were grouped as separate types. Isolates that were identical according to the results of all typing techniques were classified as a separate subtype.

All isolates contained plasmids. One particular plasmid (restriction profile PL1) was present in all isolates from herd EE. This plasmid, approximately 20 kb, was also found in 14 isolates from 7 different herds located in 5 different counties. All isolates from HH1–HH5 and 3 isolates from 3 additional herds carried an approximately 26-kb plasmid (restriction profile PL2). Comparison of the *Hae*III banding patterns indicated that 4 of the 5 bands of PL1 were present in PL2. The largest fragment of PL1 differed in size from the largest fragment of PL2, and PL2 also showed two additional bands. The unrestricted profile and restriction patterns indicated that the isolate with the PL4 restriction profile carried the plasmid showing the PL2 restriction profile together with a second approximately 3·5-kb plasmid. Seven isolates from 6 different herds located in 4 different counties had a separate restriction profile (PL3). This profile and PL5 were related. Isolates of either profile contained two different plasmids; the smallest plasmid of the isolates with PL3 and the largest plasmid of the isolate with PL5 seemed to be of identical size (approximately 4·3 kb). Two additional restriction profiles were observed in isolates from herd ST1; the isolate with PL11 seemed to carry the plasmid showing the PL1 profile together with an approximately 1·7-kb plasmid, and isolates with PL12 seemed to include the two plasmids showing the PL11 profile as well as a third approximately 2·3-kb plasmid. The PL11 isolate and one of the isolates with PL12 were from secretions collected simultaneously from two different quarters of the same cow.

Apart from being resistant to penicillin and tetracycline, 98 (91·6%) of the isolates were resistant to fusidic acid. The degree of resistance to penicillin and tetracycline varied. For isolates with the PL3 plasmid restriction profile, MIC of penicillin was consistently higher than for the remaining isolates, and the diameter of the tetracycline inhibition zone was smaller than for all but one of the remaining isolates. None of the 107 isolates was resistant to any of the other antimicrobials used for susceptibility testing.

Two different ribotypes were identified. One hundred and two isolates (95·3%) belonged to ribotype

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Table 3. *Distribution of 107* Staphylococcus aureus *isolates resistant to penicillin and tetracycline, obtained from 75 cows in 25 dairy herds, grouped as types* and subtypes according to specific combinations of antibiotic susceptibility pattern†, plasmid restriction profile‡, ribotype, electrophoretic (ET) as determined by multilocus enzyme electrophoresis, and capsular polysaccharide (CP) serotype*

Herd§	No. of isolates	Anti-biogram‖	Plasmid profile	Ribotype	ET	CP serotype	*S. aureus* type	*S. aureus* subtype
EE	3	P_2T_2F	PL1	R1	ET6	NT¶	Sa-1	Sa-1-1
EE	34	P_2T_2F	PL1	R1	ET7	NT	Sa-1	Sa-1-2
EE	4	P_2T_2F	PL1	R2	ET7	NT	Sa-1	Sa-1-3
HH1–HH5	37	P_2T_2F	PL2	R1	ET7	NT	Sa-1	Sa-1-4
E1, E2, A1, H2, SF1, MR2	11	P_2T_2F	PL1	R1	ET7	NT	Sa-1	Sa-1-2
MR1	1	P_2T_2F	PL1	R1	ET7	NT	Sa-1	Sa-1-2
MR1	2	P_2T_2	PL1	R1	ET7	NT	Sa-1	Sa-1-5
H1, O2, ST2	3	P_2T_2F	PL2	R1	ET7	NT	Sa-1	Sa-1-4
B1	1	P_2T_2F	PL4	R2	ET7	NT	Sa-1	Sa-1-6
ST1	1	P_2T_2F	PL11	R1	ET7	NT	Sa-1	Sa-1-7
ST1	2	P_2T_2F	PL12	R1	ET7	NT	Sa-1	Sa-1-8
T1	2	P_1T_1	PL3	R1	ET8	NT	Sa-2	Sa-2-1
Ho1	1	P_1T_1	PL3	R1	ET1	NT	Sa-3	Sa-3-1
Ho2	1	P_1T_1	PL3	R1	ET2	NT	Sa-4	Sa-4-1
Ho3	1	P_1T_1F	PL3	R1	ET2	NT	Sa-4	Sa-4-2
NT1	1	P_1T_1	PL3	R1	ET3	NT	Sa-5	Sa-5-1
O1	1	P_1T_1	PL3	R1	ET4	NT	Sa-6	Sa-6-1
N1	1	P_2T_1	PL5	R1	ET5	NT	Sa-7	Sa-7-1

* Isolates of different ETs classified as separate types, except for ET6 and ET7 (genetic distance ≈ 0) which were considered the same type.

† MIC of penicillin measured by Etest®; susceptibility to remaining antibacterials determined by tablet diffusion (using Neo-Sensitabs®).

‡ Using *Eco*RI and *Hae*III.

§ Counties where the herds were located were Akershus (A1), Buskerud (B1), Østfold (EE, E1, E2), Hedmark (HH1–HH5, H1, H2), Hordaland (Ho1–Ho3), Møre og Romsdal (MR1, MR2), Nord-Trøndelag (NT1), Nordland (N1), Oppland (O1, O2), Sogn og Fjordane (SF1), Sør-Trøndelag (ST1, ST2), Troms (T1).

‖ P_1 = penicillin resistant, $1{\cdot}5 \leqslant MIC \leqslant 3{\cdot}0$; P_2 = penicillin resistant, $0{\cdot}50 \leqslant MIC \leqslant 1{\cdot}0$; T_1 = tetracycline resistant, $10 \leqslant$ zone diameter $\leqslant 12$ mm; T_2 = tetracycline resistant, 14 mm $\leqslant$ zone diameter $\leqslant 16$ mm; F = fusidic acid resistant.

¶ NT = nontypeable.

R1. The remaining 5 isolates, of which 4 were from herd EE, were of ribotype R2.

MEE identified 8 different ETs. Ninety-six isolates (89·7%), recovered from 18 different herds, were assigned to ET7. Three isolates were assigned to ET6, which was very closely related to ET7 (genetic distance ≈ 0) (Fig. 2). The remaining ETs were represented by isolates from single herds (ET1, ET3, ET4, ET5 and ET8) or from two different herds (ET2). Except for herd EE, from which isolates of both ET6 and ET7 were recovered, only a single ET was identified within each herd.

Five different plasmid restriction profiles were found in isolates of ET7. PL1 and PL2 were observed only in isolates of ET7 and ET6, while PL3 was detected in isolates of 5 different ETs. With the exception of 2 isolates in 1 herd, the isolates of ET7 or ET6 were resistant to fusidic acid. Of the 8 isolates of the remaining ETs, 7 were sensitive to fusidic acid; the isolate resistant to fusidic acid was of the same ET (ET2) and had the same plasmid restriction pattern (PL3) as a fusidic acid sensitive isolate from another herd in the same county.

CP serotyping revealed that none of the isolates reacted with antisera against CP5 or CP8, and no isolates expressed mucoid colony morphology. Thus, all isolates were classified as nontypeable.

DISCUSSION

Resistance to both penicillin and tetracycline is uncommon in bovine *S. aureus* isolates in Norway.

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Routine examination of a large number of QMS during the past decade has shown that approximately 1% of the *S. aureus* isolates is PTRSA. In this study, 107 PTRSA isolates from cases of bovine mastitis in 25 different herds were characterized. The majority of these isolates was also resistant to fusidic acid, while all isolates were sensitive to the remaining 15 antibacterial agents used for susceptibility testing.

Of the typing methods we used, MEE was considered the most suitable for strain differentiation. Eight different ETs were identified. MEE did not fully distinguish between fusidic acid sensitive and fusidic acid resistant isolates. Although the majority (99 out of 100) of the fusidic acid resistant isolates belonged to ET6 or ET7, which were very closely related (Fig. 2), both fusidic acid sensitive and resistant isolates were found within each of two different ETs. A study comparing different methods for typing of *S. aureus*, using epidemiological data from well-characterized outbreaks as the 'gold standard', demonstrated that MEE was able to differentiate most outbreak-related isolates from other isolates [12]. Other studies using MEE have identified a variety of different ETs among human and bovine isolates of *S. aureus* and have demonstrated that this method is suitable for estimating the degree of genetic relationship among isolates [17, 18, 32]. Based on the results of a recent survey of *S. aureus*, the ETs represented in the current study do not seem to be common in dairy herds in Norway; of 86 isolates recovered from cows in herds in different parts of Norway only 1 isolate (a PTRSA) was assigned to one of the ETs found in our study [33]. Furthermore, 13 *S. aureus* isolates sensitive to penicillin and tetracycline that were recovered from herds EE, HH4 and HH5 were all assigned to ETs different from those for the PTRSA in this study (S. Waage, D. A. Caugant, unpublished data).

Only two different ribotypes were identified. Ribotype R1 included 95% of the 107 isolates, and the 8 different ETs were all represented among isolates of this ribotype. The isolates classified as ribotype R2 belonged to the one ET that included most of the R1 isolates as well. In general, ribotyping is relatively effective in differentiating among bovine *S. aureus* isolates. For example, examination of 96 isolates from cases of clinical mastitis in primiparous cows in Norway identified 32 different ribotypes [16]. However, as compared with MEE, ribotyping seems to be less capable of discriminating between epidemiologically unrelated isolates of *S. aureus* [12].

Plasmids were present in all isolates included in this study. Using the restriction enzymes *Eco*RI and *Hae*III, seven different plasmid restriction profiles were identified. Some studies have concluded that plasmid analysis is a useful method for typing of *S. aureus* for epidemiological purposes [34]. Tenover and colleagues found that, although plasmid analysis was relatively insensitive when used to identify epidemiologically related isolates, the specificity was high; however, the reproducibility of this method was moderate [12]. In our study, neither the sensitivity nor the specificity of plasmid analysis was very high when classification based on MEE was used as the standard. The PL3 plasmid restriction profile was found in *S. aureus* of 5 different ETs, and, moreover, 5 different plasmid restriction profiles were found in isolates of one particular ET (ET7).

The degree of resistance to penicillin and tetracycline varied among isolates. All isolates showing the PL3 plasmid restriction profile had higher MIC for penicillin and a smaller zone of inhibition of tetracycline than the isolates of ET6 or ET7, which had other plasmid restriction profiles. One isolate, which had a separate plasmid restriction profile and was assigned to a separate ET, was in an intermediate position by exhibiting the higher level of resistance to tetracycline and the lower level of resistance to penicillin.

Serotypes 5 and 8 are shown to be the predominant *S. aureus* CP types [20, 35]. A recent study of *S. aureus* from cases of bovine mastitis in Norway found that 95% of the isolates expressed CP8 and only 3 out of 86 isolates were nontypeable [34]. In contrast, all isolates in the present study were nontypeable. Isolates expressing CP of type 1 or 2 are extremely rare and form mucoid colonies [35]. All isolates in our study produced nonmucoid colonies; thus, it is unlikely that CP serotype 1 or 2 was represented among those isolates. Other CP serotypes have been suggested [19]; however, prototype strains of such types or antisera against these were not available and testing for those serotypes was not performed.

Forty-one of the isolates in this study originated from one particular herd (EE). All of these were of ET7 or the very closely related ET6 and carried a single, approximately 20-kb plasmid. Two ribotypes were identified. However, MEE and the other typing methods did not differentiate isolates of the two ribotypes and all PTRSA isolates from EE were considered representatives of a single strain.

PTRSA from seven herds located in various parts of Norway were identical to the predominant subtype

found in EE (Table 3). Furthermore, a few isolates from another herd were very similar to that subtype, the only difference being that the isolates from the latter herd carried 1–2 small plasmids in addition to the approximately 20-kb plasmid present in isolates from EE. Two of these isolates, which carried one and both of the small plasmids, respectively, were from samples collected simultaneously from different quarters of the same cow, thus supporting the previous observation that loss of plasmids or acquisition of additional plasmids seems to occur relatively frequently in staphylococci [12].

The fact that all PTRSA isolates recovered from four herds (HH1–HH4) that used a common pasture during summer belonged to the same strain clearly demonstrates the contagious nature of *S. aureus* in dairy herds. Bacteriological examination of QMS from all lactating cows in these herds and in EE revealed a high prevalence of PTRSA. The proportion of cows with at least one PTRSA infected quarter was 30% in HH1–HH4 and 38% in EE. During the grazing season, the cows from HH1–HH4 were all milked in the same parlour. Transfer by the milking machine, which is considered an important mechanism for the transmission of *S. aureus* between quarters of the same cow and from cow to cow [36], was most likely the main cause for the spread of PTRSA. Other studies also have found that a single strain of *S. aureus* can be widely disseminated within a herd. Using antibiogram, biotyping and phage typing to characterize isolates of *S. aureus* from a dairy herd, Smith and colleagues reported a rather constant prevalence of approximately 20% infected cows over an 18-month period due to one particular strain [37].

Cows from a herd (HH5) that had used the same common pasture as HH1–HH4 until 2 years prior to this study, harboured PTRSA identical to the strain in HH1–HH4. Thus, once introduced, PTRSA can be present for a long time within a herd. Infected quarters may constitute a persisting reservoir, as shown by the detection of identical PTRSA in milk from particular cows at intervals of up to 11 months.

PTRSA identical to the strain in HH1–HH5 was found in three additional herds located in three different counties. The only difference between this subtype and the predominant subtype in EE, as detected by the typing methods used in our study, was that the former carried a larger plasmid, approximately 26 kb in size. Restriction analysis indicated that this plasmid was closely related to that in the isolates from EE. Thus, these PTRSA subtypes were most likely of relatively recent common origin, the plasmid of one of the subtypes probably having been derived from that of the other by the insertion or deletion of approximately 6 kb DNA. One isolate from a herd in another county was closely related to these subtypes. It belonged to the same ET as the isolates from HH1–HH5, was of the same ribotype as some of the isolates from EE and carried a plasmid apparently identical to the plasmid in the isolates from HH1–HH5. In addition, it contained a second, small plasmid.

It is not clear how this PTRSA strain was spread between herds in several different counties. Purchase of infected animals is one possible explanation. Transfer by humans, which was considered a likely explanation for the occurrence of methicillin resistant *S. aureus* in several horses treated at a veterinary hospital [38], is another possibility.

The plasmid restriction profile PL3 was shown by PTRSA isolates assigned to 5 different ETs. These isolates were from 6 different herds located in 4 different counties. Although these isolates belonged to the same ribotype, the dendrogram of genetic relationships among ETs (Fig. 2) indicated that at least some isolates were relatively distantly related. Plasmids may be transferred to *S. aureus* from other gram-positive bacteria [39, 40]. Thus, it is possible that bacteria other than *S. aureus* are a source for the plasmids with the PL3 restriction profile. In some herds, those plasmids might have been transferred into *S. aureus*.

In conclusion, the high prevalence of PTRSA observed in one particular dairy herd and in four herds that used a common pasture and milking parlour in summer was due to the spread of a single strain. This strain also occurred in several other herds in different parts of Norway. The remaining PTRSA, which were recovered from herds in different counties, belonged to six different strains; however, except for one isolate, these isolates carried apparently identical sets of plasmids. While strain classification was based on MEE, the other methods used for characterization showed that the PTRSA were similar in certain respects; all isolates were nontypeable according to CP serotyping, most isolates belonged to one particular ribotype and, with the exception of differences in susceptibility to fusidic acid, the antibiogram was identical for all isolates. The results of plasmid analysis suggest that loss or acquisition of plasmids or plasmid fragments occurred rather frequently in the PTRSA.

ACKNOWLEDGEMENTS

The authors thank the regional mastitis laboratories in Norway, which supplied some of the bacterial isolates included in this study. Technical assistance concerning MEE typing was provided by Torill Alvestad. The study was supported by the Research Council of Norway, grant no. 116112.

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