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Genetic and Serologic Evaluation of Capsule Production by Bovine Mammary Isolates of *Staphylococcus aureus* and Other *Staphylococcus* spp. from Europe and the United States

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Bovine mastitis caused by *Staphylococcus aureus* is responsible for major economic losses to the dairy industry, and more-effective therapeutic or preventive approaches are sorely needed. The predominance of staphylococcal capsular polysaccharide types 5 and 8 among human isolates from many sources is well documented, but there seems to be a greater variation in the distribution of capsular serotypes among isolates from cows. A total of 636 isolates of *S. aureus* from cases of bovine mastitis in Sweden, Denmark, Finland, Iceland, Ireland, and the United States were investigated for production of capsular polysaccharide types 5 and 8. Approximately half of all the European isolates tested were of serotype 8, although variation among countries and among isolates of clinical and subclinical origin was observed. Sweden had the highest frequency (87%) of serotypeable isolates, and Finland had the lowest (48%). Capsule types 5 and 8 accounted for only 42% of the U.S. isolates tested. A few isolates showed weak reactivity with CP5 antiserum in a colony blot assay, and an enzyme-linked immunosorbent assay inhibition method confirmed that the levels of capsule produced by these strains were <10% of those produced by control strains. Fifty isolates that failed to react with capsular antisera all possessed the genes for production of capsular polysaccharide type 5 or 8. These results underscore the variability in capsule production by bovine isolates of *S. aureus* from different geographic regions. This information is important for the rational design of a capsule-based vaccine to prevent *S. aureus* bovine mastitis.

Mastitis is the most significant cause of economic loss to the dairy industry. Although several bacterial pathogens can cause mastitis, *Staphylococcus aureus* is a prime etiologic agent in most parts of the world. *S. aureus* strains produce capsular polysaccharide (CP) in vivo or under defined culture conditions (20, 36). Encapsulated *S. aureus* strains are more resistant to phagocytic uptake than are nonencapsulated strains, and antibodies to CP opsonize encapsulated strains for phagocytic killing (15, 38). In rodent models of staphylococcal infection (8, 19), capsular antibodies protected animals against death, bacteremia, endocarditis, and metastasis to the spleen, liver, and kidneys.

Eleven CP serotypes have been proposed on the basis of agglutinating reactivity with adsorbed rabbit antiserum and precipitation in double immunodiffusion (16, 34). Whereas there is general agreement that CP5 and CP8 are the predominant serotypes in human *S. aureus* infections, evaluation of capsule production among *S. aureus* strains from ruminants shows varying results. In France, 69% of 212 isolates recovered from cows' milk were of serotype 5 (51%) or 8 (18%) (31). In contrast, only 17% of 18 isolates from bovine mastitis in Israel produced either the serotype 5 or the serotype 8 capsule (34). A recent report (35) indicated that only 14% of 195 bovine isolates from Argentina reacted with antibodies to CP5 or CP8. Guidry et al. (10, 11) evaluated the prevalence of serotype 5 and 8 capsules among *S. aureus* strains from mastitic cows in the United States and in four European countries. They showed that 41 and 70% of the U.S. and European isolates, respectively, were typeable with antibodies to CP5 or CP8.

Strains of *S. aureus* that do not react with antibodies to CP5 or CP8 are referred to as nontypeable (NT). These NT strains also fail to react with specific antibodies to serotype 1 or 2 CP (14, 18). Whether NT strains lack a capsule or produce a heterologous capsule type is unknown, since specific antibodies to proposed serotypes 3, 6, 7, 9, 10, and 11 are not available. Antibodies to serotype 5 react with the only serotype 4 strain that has been identified (14). Moreover, only CPs from serotypes 1, 2, 5, and 8 have been purified and chemically characterized (9, 22, 26, 27). The question of whether NT strains carry genes involved in capsule expression has only recently been addressed (35).

The genes involved in serotype 5 and 8 capsule biosynthesis are chromosomal and allelic (33). Each gene cluster contains 16 open reading frames (ORFs), designated *cap5A* through *cap5P* for type 5 CP and *cap8A* through *cap8P* for type 8 CP. The predicted amino acid sequences of 12 of the 16 ORFs of the *cap5* and *cap8* gene clusters are almost identical. However, four ORFs located in the central region (*cap5H* through *cap5K* or *cap8H* through *cap8K*) bear little homology to each other and are type specific.

The aim of this study was to evaluate the prevalence of serotypes 5 and 8 among bovine mammary isolates of *S. aureus* from four Nordic countries, Ireland, and the United States. Staphylococcal CPs may play a role in the pathogenesis of bovine mastitis because of their virulence-enhancing, antiphagocytic properties. Information concerning the geographical distribution of capsular serotypes is important for the rational design and use of vaccines against *S. aureus* mastitis based on capsular antigens. We also sought to investigate the genotype of NT *S. aureus* strains by hybridization studies and thus to determine whether they carry genes similar to those

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that encode the enzymes for the synthesis and polymerization of CP5 and CP8.

MATERIALS AND METHODS

Bacteria. Six hundred thirty-six isolates of *S. aureus* were obtained from the milk of cows with clinical or subclinical mastitis. The milk samples were collected from dairy herds in the United States, Sweden, Denmark, Finland, Iceland, and Ireland. Most of the isolates were from separate dairy herds within each region. The *S. aureus* isolates were initially selected on the basis of colony appearance and a positive tube coagulase test and their identity was verified by API-Staph (bioMérieux Vitek, Inc., Hazelwood, Mo.) or Rapidec-Staph (bioMérieux, Marcy l'Etoile, France) or by amplification of a 108-bp *S. aureus*-specific DNA fragment by PCR (24). Coagulase-negative staphylococci isolated from clinical cases of bovine mastitis were identified to the species level by standard biochemical methods, API- and Rapidec-Staph, and by their failure to yield an *S. aureus*-specific amplicon by PCR. The bacteria were stored at −70°C in 10% skim milk or heart infusion broth (Difco Laboratories, Detroit, Mich.) with 15% glycerol.

On the basis of clinical data, the isolates from Denmark, Finland, Iceland, Ireland, and Sweden were designated as originating from clinical or subclinical mastitis as defined by the National Mastitis Council (3). In brief, subclinical mastitis was defined as a form of the disease with no detectable change in the udder and no grossly observable abnormalities in the milk. Acute or clinical mastitis was defined as a condition of sudden onset with grossly abnormal milk and redness, swelling, and pain in the udder, with or without systemic symptoms.

Antibodies. Polyclonal antisera were raised in rabbits to heat- or formalin-killed suspensions of prototype *S. aureus* strains Reynolds (serotype 5), Becker (serotype 8), and PS80 (serotype 8). Sera were adsorbed with *S. aureus* Wood 46 and trypsinized cells of acapsular mutants JL243 (2) and JL252 (5) to remove antibodies to noncapsular cell wall determinants. CP5-specific monoclonal antibody S831 and CP8-specific monoclonal antibody S828 were kindly provided by H. K. Hochkeppel.

Capsule serotyping. Our serotyping experiments were performed only with antibodies to CP5 and CP8, since strains expressing CP1 and CP2 possess mucoid colony morphology and are extremely rare (4, 34). Colony immunoblots were performed as described previously (18) with CP5- or CP8-specific antibodies. Reactivity of the bovine isolates was evaluated by comparison with that of the control strains (types 1, 2, 5, and 8 and NT) included on each filter. Each isolate was tested at least twice. Reactions scored as 2+ to 4+ were recorded as positive, and isolates that reacted weakly (0 to 1+) were recorded as NT with the given antisera. Isolates consistently giving weak reactions with antibodies to CP5 or CP8 were further evaluated by immunodiffusion and/or enzyme-linked immunosorbent assay (ELISA) inhibition.

For preparation of crude capsular extracts, *S. aureus* was cultivated for 24 h at 37°C on either tryptic soy agar (Difco Laboratories) or Columbia agar (Difco Laboratories) supplemented with 2% NaCl. The colonies from one 9-cm-diameter plate were harvested in 1 ml of 10 mM phosphate-buffered saline (0.15 M NaCl, pH 7.2), and the bacterial suspension was autoclaved for 1 h at 121°C. The bacteria were sedimented by centrifugation, and the supernatant was passed through a filter (pore size, 0.45 μm) and stored at −20°C. The quality of the capsular extract was verified by its reactivity with teichoic acid antiserum. Reactivity of the capsular extracts with antisera to CP5 and CP8 was evaluated by double immunodiffusion.

An ELISA inhibition method was used to quantitate CP5 produced by 16 bovine *S. aureus* isolates from four countries. This assay, described previously (2, 20), is based on the ability of purified CP5 or whole bacteria (trypsinized to remove protein A) to adsorb capsular antibodies from immune sera.

DNA hybridization experiments. To determine whether NT *S. aureus* isolates carry the genes involved in CP5 or CP8 expression, we performed DNA hybridization studies. Genomic DNA was extracted from 50 NT bovine isolates of *S. aureus* and 14 isolates of coagulase-negative *Staphylococcus* spp. from cases of bovine mastitis. Strains Newman (serotype 5) and Becker (serotype 8), in addition to bovine isolates of serotypes 5 and 8, were included in the hybridization studies as positive controls. DNA from each isolate was digested with *Hin*dIII (Life Technologies, Gaithersburg, Md.) and electrophoresed in a 0.8% agarose gel. The DNA was transferred to a nylon membrane (Gene Screen; NEN Research Products, Boston, Mass.) and probed sequentially with cloned DNA fragments (*cap5ABCD*, *cap5IJK*, *cap8HIJK*, or *cap5MNOP*) that were enzyme labeled with AlkPhos Direct (Amersham Life Science, Inc., Arlington Heights, Ill.). Membrane hybridization and washing steps were performed as directed by the manufacturer at 60°C. Membrane stripping and autoradiography were carried out in accordance with the manufacturer's recommendations.

RESULTS

The results of capsular serotyping of 274 bovine mastitis isolates of *S. aureus* from Europe are shown in Table 1. Marked differences in the distribution of isolates expressing CP5 or CP8 among the various countries were observed. When European isolates from all cases of mastitis (clinical and subclinical) were considered, the majority of isolates from Denmark, Sweden, and Ireland were of serotype 8. Isolates from Iceland showed an equal distribution of serotype 5, serotype 8, and NT isolates, whereas in Finland half of the isolates tested were NT. The typeable Finnish isolates were equally distributed between serotypes 5 and 8. Among the Danish, Swedish, and Finnish *S. aureus* isolates, there was no significant difference between the serotype distributions of clinical and subclinical staphylococcal isolates (Table 1). For Icelandic and Irish isolates, a higher proportion of clinical than of subclinical isolates was of serotype 8 ($P < 0.05$ by chi-square analysis).

Serotyping of the U.S. isolates revealed that only 42% of 362 isolates from seven different states were typeable with the available antisera (Table 2). Serotype 8 strains were recovered

TABLE 1. Results of capsular serotyping of isolates of *S. aureus* from cases of bovine mastitis in Europe

Country and mastitis cases	No. of strains	No. (%) with capsule type:		No. (%) NT[a]
		5	8	
Denmark				
All	39	2 (5)	23 (59)	14 (36)
Clinical	20	1 (5)	12 (60)	7 (35)
Subclinical	19	1 (5)	11 (58)	7 (37)
Sweden				
All	38	4 (11)	29 (76)	5 (13)
Clinical	11	1 (9)	8 (73)	2 (18)
Subclinical	27	3 (11)	21 (78)	3 (11)
Finland				
All	62	17 (27)	13 (21)	32 (52)
Clinical	19	9 (47)	2 (11)	8 (42)
Subclinical	43	8 (19)	11 (25)	24 (56)
Iceland				
All	34	10 (29)	13 (38)	11 (33)
Clinical	11	2 (18)	8 (73)	1 (9)
Subclinical	23	8 (35)	5 (22)	10 (43)
Ireland				
All	101	1 (1)	62 (61)	38 (38)
Clinical	13	0 (0)	12 (92)	1 (8)
Subclinical	88	1 (1)	50 (57)	37 (42)
Total	274	33 (12)	140 (51)	101 (37)

[a] NT, isolates that do not react with antibodies to CP5 or CP8.

TABLE 2. Results of capsular serotyping of isolates of *S. aureus* from cases of bovine mastitis in the United States

State	No. of strains	No. (%) with capsule type:		No. (%) NT
		5	8	
California	32	4 (12.5)	4 (12.5)	24 (75)
Pennsylvania	11	2 (18)	3 (27)	6 (55)
Washington	25	7 (28)	5 (20)	13 (52)
Vermont	20	5 (25)	6 (30)	11 (45)
Kentucky	20	7 (35)	3 (15)	10 (50)
Louisiana	21	6 (29)	2 (9)	13 (62)
New York	233	26 (11)	72 (31)	135 (58)
Total	362	54 (15)	98 (27)	210 (58)

TABLE 3. Correlation between reactivity in the immunoblot and quantitative CP5 expression measured by ELISA inhibition

Expt no. and *S. aureus* strain	Source	Immunoblot reactivity[a]	Amt (μg) of CP5/10^{10} CFU	% of control
1				
Reynolds (control)	Human	4+	518	100
PS15rr (U.S.)	Bovine	4+	518	100
AA405 (U.S.)	Bovine	4+	411	79
Myco 15 (U.S.)	Bovine	4+	278	54
2				
Reynolds (control)	Human	4+	2,400	100
PS Milk (U.S.)	Bovine	2–3+	209	8.7
Gutz 5 (U.S.)	Bovine	2+	91	3.8
3263 (U.S.)	Bovine	2+	42	1.8
KK 13 (U.S.)	Bovine	1+	52	2.2
3				
Reynolds (control)	Human	4+	1,984	100
AA26 (U.S.)	Bovine	2–3+	120	6.0
WSU 1 (U.S.)	Bovine	2+	64	3.2
Myco 11 (U.S.)	Bovine	2+	13	0.7
Hook 14 (U.S.)	Bovine	1–2+	13	0.7
4				
7125-2 (control) (Sweden)	Bovine	4+	1,177	100
6698-1 (Sweden)	Bovine	±	4	0.3
55-46 (Finland)	Bovine	±	4	0.3
78-9 (Finland)	Bovine	±	16	1.4
2076 (Iceland)	Bovine	±	6	0.5

[a] 1+, very weak; 2+, medium; 3+, strong; 4+, very strong; ±, little to none.

almost twice as often as serotype 5 strains, but the majority (58%) of U.S. isolates were NT. As shown in Table 2, there was little difference in serotype distribution among strains from cows in different states. Data on the clinical status of infection were not obtained for isolates from the United States.

Quantitation of CP5 production. We identified a subset of *S. aureus* isolates that consistently reacted weakly with CP5 antiserum in the colony immunoblot assay. Production of cell surface-associated CP5 was quantitated for 12 of these weakly reactive isolates and for five control strains (one of human origin, four of bovine origin) that reacted strongly (4+ reactions) in the colony immunoblot assay with CP5-specific antiserum. A positive control strain was included in each experiment, since there is day-to-day variability in capsule expression by control strain Reynolds. As shown in Table 3, a positive correlation existed between the strength of the reaction in the immunoblot and the quantitative ELISA results. Three *S. aureus* isolates that reacted strongly in the colony immunoblot assays showed >50% of the capsule expression of strain Reynolds, whereas the weakly reactive isolates showed <10% of the cell surface-associated CP5 expression of the positive control strain.

Hybridization of genomic DNA from NT *S. aureus* with the *cap5* and *cap8* genes. To determine whether NT *S. aureus* strains carry the genes involved in CP5 or CP8 expression, DNA hybridization studies were performed. Southern blots of genomic DNA digests from each isolate were probed consecutively with cloned DNA fragments representing either the regions of the capsule gene clusters that are shared by type 5 and 8 strains or the regions that are unique to the *cap5* or *cap8* gene cluster. The results, shown in Table 4, indicate that 48 of the 50 NT isolates tested carry the *cap5* genes, although they did not express detectable levels of CP5. Only two of the NT isolates, one each from Sweden and Iceland, carried the *cap8* genes. The size of the hybridizing bands of the NT strains did not differ from those of the control serotype 5 and 8 strains (not shown), a result suggesting that each NT isolate carries an intact capsule gene cluster. As expected, none of the DNA samples hybridized to both *cap5*- and *cap8*-specific fragments, since the *cap5* and *cap8* loci are allelic.

Hybridization of genomic DNAs from *Staphylococcus* spp. with the *cap5* and *cap8* genes. Poutrel et al. (32) reported that 16 of 74 coagulase-negative staphylococci of bovine origin reacted with monoclonal antibodies to *S. aureus* CP5 or CP8. Thirteen of the 16 reactive strains were *S. haemolyticus* isolates. To examine this phenomenon further, 14 isolates of staphylococcal species other than *S. aureus* were tested for capsule production by serologic methods and for the presence of the *cap5* or *cap8* genes by Southern blot hybridization. The isolates tested, all of which were recovered from bovine milk, included seven isolates of *S. haemolyticus*, three of *S. intermedius*, two of *S. chromogenes*, and one each of *S. xylosus* and *S. simulans*. Except for the *S. haemolyticus* isolates, none of the isolates reacted with the capsular antibodies or carried genes that hybridized with the capsule gene probes (Table 5). In contrast, all seven *S. haemolyticus* isolates reacted with both polyclonal and monoclonal antibodies specific for CP5. Under our standard conditions for hybridization (high stringency, 60°C), genomic DNA prepared from the *S. haemolyticus* isolates did not hybridize to either a *cap5* or a *cap8* gene probe. However, under low-stringency (50°C) conditions, a mixture of *cap5* probes hybridized weakly to chromosomal DNAs from the *S. haemolyticus* isolates.

TABLE 4. Results of hybridization experiments[a]

Source (strain)	No. of strains	Serotype	*cap5ABCD*	*cap5IJK*	*cap8HIJK*	*cap5LMNO*
Human, U.S. (Becker)	1	8	+	−	+	+
Human, U.S. (Newman)	1	5	+	+	−	+
Bovine, Iceland (2317)	1	8	+	−	+	+
Bovine, Sweden (6622-4)	1	5	+	+	−	+
Bovine, U.S. (NY19)	1	5	+	+	−	+
Bovine, U.S.	41	NT	+	+	−	+
Bovine, Sweden	4	NT	+	+	−	+
Bovine, Sweden	1	NT	+	−	+	+
Bovine, Iceland	3	NT	+	+	−	+
Bovine, Iceland	1	NT	+	−	+	+

[a] Genomic DNAs from *S. aureus* isolates were digested with *Hin*dIII, and Southern blots were probed with cloned DNA fragments common to the *cap5* and *cap8* gene clusters (*cap5ABCD* and *cap5LMNOP*), a DNA fragment specific to the *cap5* gene cluster (*cap5IJK*), or a DNA fragment specific to the *cap8* gene cluster (*cap8HIJK*).

TABLE 5. Results of hybridization experiments[a]

Species	Capsule serotype	No. of strains	*cap5ABCD*	*cap5IJK*	*cap8HIJK*	*cap5LMNOP*
S. aureus	5	3	+	+	−	+
S. aureus	8	2	+	−	+	+
S. intermedius	NT	3	−	−	−	−
S. xylosus	NT	1	−	−	−	−
S. chromogenes	NT	2	−	−	−	−
S. simulans	NT	1	−	−	−	−
S. haemolyticus[b]	5	7	−	−	−	−

[a] Genomic DNAs from bovine mastitis isolates of *Staphylococcus* spp. were digested with *Hin*dIII, and Southern blots were probed with cloned DNA fragments common to the *cap5* and *cap8* gene clusters (*cap5ABCD* and *cap5LMNOP*), a DNA fragment specific to the *cap5* gene cluster (*cap5IJK*), or a DNA fragment specific to the cap8 gene cluster (*cap8HIJK*).

[b] Isolates of this species showed weak hybridization to the *cap5* probes under low-stringency conditions.

DISCUSSION

The results of this study underscore the considerable variability that exists in the prevalence of serotype 5 and 8 capsules among bovine mammary isolates of *S. aureus* from different countries. CP8 expression predominated among staphylococcal isolates from Denmark, Sweden, and Ireland. In contrast, Poutrel et al. reported that serotype 5 strains accounted for half of 212 isolates from France while only 18% of the strains were of serotype 8 (31). Our data indicate that 63% of 274 bovine mammary isolates of *S. aureus* tested from five European countries (Denmark, Sweden, Finland, Iceland, and Ireland) expressed either serotype 5 or 8 CPs. At the other extreme are bovine mammary isolates from Argentina, of which 86% are NT (35). In the United Kingdom, Germany, and The Netherlands, there appears to be an equal distribution of serotype 5, serotype 8, and NT isolates (11). We found that only 42% of 369 bovine isolates from the United States expressed CP5 or CP8, consistent with results from a previous study (10).

Our data also suggest a correlation between capsule production and the clinical severity of mastitis in Iceland and Ireland, where >90% of isolates from cows with clinical mastitis were encapsulated. The subclinical isolates from Iceland and Ireland were only 57 and 58% positive, respectively, for capsule production. No such correlation was found for isolates from three other countries (Denmark, Sweden, and Finland), however. The clustering of typeable isolates among the clinical mastitis strains may be influenced by the occurrence of local clones of *S. aureus* that become widely disseminated within a single region. With the use of ribotyping and phage typing, Aarestrup et al. found regional clones of *S. aureus* isolates from bovine mastitis distributed within the Nordic countries (1). Unlike bovine isolates, *S. aureus* isolates cultured from humans residing in diverse geographic regions show only subtle differences in the prevalence of capsule expression. Approximately 75% of all human *S. aureus* isolates (both commensal and disease isolates) are positive for either CP5 or CP8 (4, 13, 34). This difference in capsule production between bovine and human isolates probably reflects limited diversity among the bovine isolates within a particular geographic region.

All 50 of the NT *S. aureus* isolates tested in this study possessed the *cap* genes required for the synthesis of either CP5 or CP8. The fact that 48 of the 50 NT isolates possessed *cap5* genes is noteworthy and merits further investigation. It is possible that these NT isolates have point mutations in one or more of the 16 genes involved in capsule expression, as has been shown for the NT strain *S. aureus* NCTC 8325-4 (6, 21, 39). This strain carries an intact copy of the *cap5* gene cluster, but a mutation in *cap5E* renders it capsule negative. Alternatively, these NT isolates may produce undetectable levels of CP due to poor promoter activity or regulatory effects. Although capsule production is known to be influenced by a number of environmental factors, such as energy availability and source, oxygen supply, and the amino acid content of the growth medium (12, 20, 36, 37), the genetic mechanisms regulating CP expression in *S. aureus* are largely unknown.

We detected a subset of bovine *S. aureus* isolates that expressed <10% of the cell surface-associated CP5 produced by prototype strain Reynolds. These strains were only weakly reactive by the colony immunoblot method. The expression of scant amounts of CP5 by this subset may reflect a defect in the capsule gene cluster or regulatory control mechanisms. Alternatively, production of CP by an individual *S. aureus* isolate may vary under different laboratory growth conditions or during growth in the mammary gland. The clinical significance of *S. aureus* CP production in the pathogenesis of mastitis is unknown, and the biological significance of scant CP production in this disease has not been evaluated. Strain Reynolds was significantly more virulent in a mouse bacteremia model than was either an acapsular mutant (JL243) or a mutant that expressed 9% of the wild-type level of CP5 (JL236). While both mutants were opsonized for phagocytic killing by nonimmune serum with complement activity, strain Reynolds resisted phagocytosis and required capsule-specific antibodies and complement for opsonization (38). In a murine model, septic arthritis was of intermediate frequency and severity in mice inoculated with the CP-deficient mutant JL236, as opposed to the parental strain or the acapsular mutant (29).

Of particular interest in our study was the observation that all seven of the *S. haemolyticus* strains tested showed serologic evidence of CP5 expression, but that genomic DNA prepared from these seven isolates failed to hybridize to the *cap5* genes under high-stringency conditions. Hybridization was observed under low-stringency conditions to genomic, but not plasmid, DNA from these strains, a result suggesting that *S. haemolyticus* does carry *cap* genes with limited homology to those from *S. aureus*. Poutrel et al. showed that 13 of 19 *S. haemolyticus* isolates of bovine origin reacted with monoclonal antibodies to CP5 (32). Nelles et al. (28) showed that *S. epidermidis* clinical isolates did not react with CP5- or CP8-specific monoclonal antibodies. Similar findings were reported by Boutonnier et al. (7), who observed no reactions between capsule-specific monoclonal antibodies and members of 25 *Staphylococcus* species other than *S. aureus*, including one strain of *S. haemolyticus* (ATCC 29970^T). Moreover, of 678 blood culture isolates of coagulase-negative staphylococcal species (primarily *S. epidermidis*), none reacted by ELISA with capsular antibodies.

It is plausible that NT bovine isolates of *S. aureus* make a capsule unrelated to CP5 or CP8 that is not detected by our capsular antibodies or by hybridization with the *cap* gene region-specific DNA probes. Several authors have described the

existence of other, less-defined capsular or surface antigens among bovine mastitis isolates of *S. aureus* (23, 30, 40). Guidry et al. (11) reported that a new antigen called 336 was present on bovine isolates of *S. aureus* that did not produce CP5 or CP8. However, the authors did not distinguish this antigen from teichoic acid (17). A recent study revealed that 29 (35%) of 82 NT *S. aureus* isolates from cases of bovine mastitis in the United States were reactive with antibodies to a newly described staphylococcal surface polysaccharide composed of poly-*N*-succinylglucosamine (PNSG) (25). The relationship of PNSG and CP to previously described bovine staphylococcal surface antigens is largely unknown. PNSG expression was independent of CP5 or CP8 expression; i.e., it was produced by NT strains, as well as by strains positive for CP5 or CP8.

The results of capsule serotyping studies of isolates from different countries are important for the rational design of mastitis vaccines containing staphylococcal capsular antigens. If improved vaccines against bovine mastitis are to be generated, more studies must elucidate the role of these polysaccharides in the pathogenesis of bovine mastitis. The creation and use of isogenic, capsule-negative *S. aureus* mutants of bovine origin in infection models of mastitis would add to the current knowledge. Ongoing studies will determine whether immunization with CP5 or CP8 will be protective against experimental bovine mastitis.

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