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Prevalence of Capsular Polysaccharide Types 5 and 8 among *Staphylococcus aureus* Isolates from Cow, Goat, and Ewe Milk

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Monoclonal antibodies to *Staphylococcus aureus* capsular polysaccharide types 5 and 8 were used to serotype by enzyme-linked immunosorbent assay 212, 54, and 33 isolates from cow, goat, and ewe milk, respectively. Capsular types 5 and 8 accounted for 69.4% of bovine isolates and 71.5 and 78.8% of goat and ewe isolates, respectively. Type 5 was predominant in strains from bovine sources (51.4%), whereas type 8 was prevalent in strains from caprine (68.5%) and ovine (75.8%) sources.

Earlier reports on the existence and the frequency of encapsulation of *Staphylococcus aureus* isolated from bovine milk are confusing and contradictory. Using the formation of diffuse colonies in serum soft agar (SSA) as evidence of encapsulation, several authors concluded that staphylococci involved in bovine mastitis are encapsulated (10–12, 16). According to Yokomizo et al. (16), diffuse-type growths were exhibited by 0.92% of bovine isolates from stock cultures and 6.32% of freshly isolated strains. Norcross and Opdebeeck (10, 12), culturing the bacteria directly from milk in SSA without prior isolation, reported that about 94% of *S. aureus* isolates from lactating cows were encapsulated. They demonstrated that encapsulation was lost upon three or four subcultures or short storage on artificial medium. By comparison of the growth in SSA and India ink staining, Anderson (1) rejected the conclusions of these previous reports, claiming that strains from cases of mastitis are not encapsulated and that growth as diffuse colonies in SSA is not a reliable test of encapsulation. Recently, Rather et al. (14) confirmed both the results of Norcross and Opdebeeck and those of Anderson and concluded that fresh bovine milk isolates of *S. aureus* produce slime, not true capsules. These authors suggested that capsule, as defined as a covering, thick polysaccharide layer outside the cell wall, demonstrable by light microscopy, occurs in very few strains. Instead, strains may more commonly produce polysaccharide microcapsules, not demonstrable by light microscopy.

Capsular polysaccharides (CP) have been identified in human clinical isolates of *S. aureus*, and serological distinction of 11 CP types has been proposed (8, 15). A method for the serological typing of the CP with polyclonal rabbit antisera has been described (7). Surveys of human clinical isolates from various geographic origins, using polyclonal antibodies (2, 15) or monoclonal antibodies (MAbs) (6), have shown that two capsular types, 5 and 8, account for about 70 to 80% of serological types. Recently, Sompolinsky et al. (15) found 1 type 5 and 2 type 8 isolates among 17 isolates from bovine mastitis. Nevertheless, 13 other isolates were considered as encapsulated on the basis of nonagglutinability with anti-teichoic acid serum.

In this study, we used MAbs to examine by enzyme-linked immunosorbent assay (ELISA) the presence of type 5 and 8 CP in a large collection of *S. aureus* strains isolated from bovine milk. In addition we investigated the frequencies of these CP types among isolates from goat and ewe milk.

MATERIALS AND METHODS

Bacterial strains. The 299 strains of *S. aureus* used in this study (Table 1) were collected from 1958 to 1987 from dairy herds of different regions of France; 265 strains were isolated from subclinical or clinical cases of bovine, caprine, or ovine mastitis, and 34 strains were taken from bovine bulk milk. The strains were kept either freeze-dried in the collection of our laboratory (Institut National de la Recherche Agronomique, Nouzilly, France) or frozen in broth medium after isolation on sheep blood agar plates. Identification of *S. aureus* was based on colony morphology, Gram stain, hemolytic pattern, tube coagulase test, and clumping factor.

Preparation of extracts for typing. Each strain was checked for purity by comparison of its characteristics with those of the initial isolate and then cultivated on Columbia medium agar (Difco Laboratories, Detroit, Mich.) overnight at 37°C. Bacterial cells were suspended in 2 ml of phosphate-buffered saline (PBS) (pH 7) and autoclaved at 121°C for 30 min. After centrifugation, the supernatant was retained and stored at −20°C until typed.

Serotyping of *S. aureus* by ELISA. *S. aureus* strains were serotyped by a two-step inhibition ELISA technique using purified type 5 and 8 CP and MAbs to these types. Type 5 and 8 CP were purified as described by Fournier et al. (4). The preparation of MAb to type 5 and 8 CP has been reported by Hochkeppel et al. (6). A flat-bottom microplate (Nunc, Roskilde, Denmark) was coated (100 μl per well) with a 5-μg/ml solution of type 5 or 8 CP. After incubation at 37°C for 1 h, the plate was washed with PBS-Tween 20, blocked with gelatin (0.5% gelatin in PBS; Prolabo, Paris, France) at 37°C for 30 min or overnight at 4°C, and washed with PBS-Tween 20. The wells of a second gelatin-blocked plate received 100-μl volumes of test samples (supernatants of autoclaved bacteria) and 100 μl of MAb at a concentration giving an optical density at 492 nm of 0.2 to 0.5 and determined by preliminary titration. After incubation at 37°C for 1 h and then overnight at 4°C, 100-μl volumes were transferred well to well from the second plate to the first one and incubated at 37°C for 1 h. After washing with PBS-Tween 20, an anti-mouse peroxidase-conjugated immunoglobulin G (H and L chain specific) (Diagnostic Pasteur, Marnes La Coquette, France) was added to the wells and incubated at 37°C for 45 min. Enzyme substrate (*o*-phenyl-

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enediamine, 0.4 mg/ml; Dakopatts, Copenhagen, Denmark) was added in citrate buffer (pH 5.2) with 0.03% hydrogen peroxyde. After 20 min at room temperature, the reaction was stopped by addition of 3 N HCl (50 μl per well). The optical density at 492 nm was read with an MR 610 automated microplate reader (Dynatech Inc., Torrance, Calif.). For each ELISA run, negative control (well not receiving test sample but PBS) and purified CP titration were performed to determine assay sensitivity. A positive ELISA result was defined as any optical density corresponding to 50% inhibition or more.

Statistical methods. Chi-squared analysis was used to examine the significance of differences in the frequency of capsular types in isolates from different sources.

RESULTS

MAbs to CP types 5 and 8 used in ELISA allowed us to type about 73% of all *S. aureus* isolates from cow, goat, and ewe milk tested (Table 2). The distribution of capsular types was different according to the animal carrier. Type 5 was represented by 51.4% of strains of bovine origin, and 18% were type 8. Type 8 was significantly ($P < 0.001$) more frequent among caprine and ovine strains (68.5 and 75.8%, respectively), and type 5 was significantly less frequent, accounting for 13 and 3%, respectively. By contrast, distribution of types 5 and 8 did not differ between caprine and ovine isolates.

DISCUSSION

Previous studies of encapsulation of *S. aureus* strains from bovine origin used growth as diffuse colonies in SSA as the main criterion of encapsulation and led to contradictory conclusions (1, 10–12, 14, 16). That may be due to the lack of chemical or immunological characterization of the capsular material of these strains. By contrast, the presence of CP in *S. aureus* strains from human sources is well established, and clinical isolates have been classified into 11 capsular types (8, 15). Moreover, the type 8 CP has been purified and chemically and immunologically characterized (4), and the type 5 and 8 CP-binding sites on the bacterial surface have been visualized by electron microscopy (6).

Predominantly two CP types, 5 and 8, occur among *S. aureus* human isolates, accounting for about 70 to 80% of all isolates (2, 6, 15). Similar figures were obtained in our survey, in which 73% of *S. aureus* isolates from cow, goat, and ewe milk were typable by ELISA with MAbs to type 5 and 8 CP (Table 2). The distribution of capsular types among bovine milk isolates was different from the distribution reported for human clinical isolates (2, 6, 15). In our study, type 5 was represented by about 51.5% of the isolates and type 8 accounted for about 18%, whereas the figures are, respectively, about 20 and 60% for human isolates. However, a significant correlation between capsular types and phage patterns was found (15), and bovine strains usually are not susceptible to the human set of phages (3). Our data concerning prevalence of types 5 and 8 were not in agreement with previous studies. Opdebeeck and Norcross (12) reported complete or partial suppression of capsule formation in SSA for 72.5% of *S. aureus* isolates from bovine milk in the presence of antiserum to the Smith diffuse strain. It was previously shown that serotype 2 CP is serologically identical to the capsular antigen of the Smith strain (8). Serotype 2 was not investigated in our study, but it is obvious that it was not prevalent among the isolates we typed. Recently, Sompolinsky et al. (15) found that 13 of 16 isolates from bovine mastitis tested had nontypable capsules. Although the distribution of types 5 and 8 was similar in the United States and Europe for human isolates (6), differences according to the geographic origin of isolates cannot be set aside for bovine strains. These discrepancies could also be explained by methods used for characterization of capsules. Antisera to the Smith strain were prepared by Opdebeeck and Norcross (12) by hyperimmunizing rabbits with crude bacterin preparation. Consequently, they were not specific for the type 2 CP, and probably cross-reactivity with other epitopes existed. The lack of serum and milk antibodies to capsular preparations from the Smith strain for cows with spontaneous mammary gland infection with *S. aureus* (9) might mean that the Smith strain is not representative of CP type for most isolates involved in bovine mastitis. Capsular polysaccharides were investigated by Sompolinsky et al. (15) by precipitation and agglutination techniques with 11 monospecific sera. It was recently demonstrated (6) that agglutination is less sensitive than rocket immunoelectrophoresis for CP typing. Although a comparison between agglutination and ELISA has never been studied, it is likely that the ELISA technique with MAb we used in our study was both more specific and more sensitive, which allowed us to type low-CP-containing bacteria.

To our knowledge, this study is the first report concerning the typing of CP for *S. aureus* isolated from goat and ewe milk. About 80% of strains were typable, and, as previously described for human isolates, CP type 8 was found to be predominant (Table 2). However, the collection of isolates from goat and ewe sources we tested here was not large enough to give a definitive conclusion on the distribution of CP types.

In the present investigation, we showed that the presence of CP is a common feature of most *S. aureus* isolates involved in mammary gland infection. Considering adhesion of strains to host tissues (5) and antiphagocytic properties (13), the capsule of *S. aureus* is considered to be a virulence factor and consequently may play a significant role in the establishment of udder infection. The findings presented herein might lead to useful developments in diagnosing mammary infections and in devising vaccination strategies.

TABLE 1. *S. aureus* isolates included in this study

Animal origin	Total no. of strains	No. of strains isolated from milk:		No. of herds
		Bulk	Udder	
Cow	212	34	178	96
Goat	54		54	25
Ewe	33		33	9

TABLE 2. Capsular types of *S. aureus* isolated from cow, goat, and ewe milk

Animal origin	No. (%) of isolates		
	Type 5	Type 8	Nontypable[a]
Cow	109 (51.4)	38 (18.0)	65 (30.6)
Goat	7 (13.0)	37 (68.5)	10 (18.5)
Ewe	1 (3.0)	25 (75.8)	7 (21.2)

[a] Isolates that were neither type 5 nor 8.

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