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# Reactivity of Coagulase-Negative Staphylococci Isolated from Cow and Goat Milk with Monoclonal Antibodies to *Staphylococcus aureus* Capsular Polysaccharide Types 5 and 8

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**Monoclonal antibodies to *Staphylococcus aureus* capsular polysaccharide types 5 and 8 were used in an enzyme-linked immunosorbent assay to serotype 74 and 42 coagulase-negative isolates from cow and goat milk, respectively. Eighteen (15.5%) isolates were typable: 13 *Staphylococcus haemolyticus*, 1 *S. hyicus*, 1 *S. simulans*, and 1 *S. warneri* from bovine origin and 2 *S. lentus* from caprine origin. Type 5 was predominant, accounting for about 89% of typable isolates. Reactivity with monoclonal antibodies varied considerably according to isolates. The significance and the potential importance of these findings are discussed.**

Studies on encapsulation of coagulase-negative (CN) staphylococci are not numerous. To our knowledge, only six strains have been reported to produce a capsule: one strain of *Staphylococcus simulans* isolated from a clinical case of bovine mastitis (1), one strain of *S. hyicus* isolated from a domestic animal (24), and four strains of *S. epidermidis* from human clinical specimens (22, 25). When examined by electron microscopy, all these strains exhibit a well-defined thick capsule covering the cell wall, as classically defined (21). However, *S. aureus* strains commonly produce polysaccharide microcapsules not demonstrable by light microscopy. Serological distinction of 11 capsular polysaccharide (CP) types has been proposed (11, 18). Using polyclonal antibodies (2, 18) or monoclonal antibodies (MAb) (8), it was shown that 70 to 80% of human *S. aureus* isolates express two predominant capsular serotypes, 5 and 8. Similar data were reported for *S. aureus* isolates from cow, goat, and ewe milk (16).

Recently, four human CN staphylococcal isolates belonging to *S. haemolyticus* and *S. hominis* species have been found to react with MAb to *S. aureus* type 5 and 8 CP (J. M. Fournier, unpublished data). In the study reported here, we examined by enzyme-linked immunosorbent assay (ELISA) the reactivity of CN staphylococcal strains isolated from bovine and caprine milk with MAb against these two CP types.

A total of 116 CN staphylococcal strains belonging to 10 different species were used (Table 1). Strains were collected from 1967 to 1987 from dairy herds of different regions of France. All strains were isolated in pure culture from milk aseptically collected from infected mammary glands of cows or goats. 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 CN staphylococcal strains was established by the method of Devriese et al. (5). Strains Reynolds and Becker are considered as the prototype strains for CP types 5 and 8, respectively (6, 7, 9).

The purity of each CN staphylococcal strain was checked after growth in Columbia medium broth (Difco Laboratories, Detroit, Mich.) and isolation on sheep blood agar plates by comparison of its characteristics with those of the initial isolate. After culture overnight at 37°C on Columbia agar medium, bacterial cells collected on each plate were suspended in 2 ml of phosphate-buffered saline (pH 7) and autoclaved at 120°C for 30 min. After centrifugation, the volume of the supernatant and the wet weight of the bacterial pellet were measured. The supernatant was stored at −20°C until used. Extracts were prepared from the prototype *S. aureus* strains by the same procedure.

Strains were serotyped by a two-step inhibition ELISA performed on the supernatant extracts as previously detailed (16), except that an anti-mouse alkaline phosphatase-conjugated immunoglobulin G (H and L chain specific; Sigma Chemical Co., Saint-Louis, Mo.) was used. After the addition of the enzyme substrate (*p*-nitrophenyl phosphate disodium, 1 mg/ml; Sigma) and incubation at 37°C for about 45 min, the optical density at 405 nm was read with a Titertek Multiskan MCC/340 microplate reader (Flow Laboratories SA, Puteaux, France). A positive ELISA result was defined as any optical density corresponding to 50% inhibition or more. For each typable CN staphylococcal strain and for prototype *S. aureus* strains, twofold dilutions of the supernatant of autoclaved bacteria were submitted to ELISA. A standard inhibition curve was established with serial dilutions of purified type 5 or 8 CP prepared from *S. aureus* as described by Fournier et al. (6, 7). Using this standard curve, we determined a CP equivalence value for each typable strain, defined as the amount of purified *S. aureus* type 5 or 8 CP that gave an equivalent ELISA reactivity to that of the CN staphylococcal strain and expressed in nanograms per milligram of wet bacteria. The production of CP by *S. aureus* reference strains (Reynolds and Becker) was quantified by the same procedure. Tests samples, the negative control (phosphate-buffered saline), and the standard curve were assayed in triplicate. The lower limit of detection of the ELISA, defined as the amount of CP that gave an optical density corresponding to 0% inhibition minus twice the standard deviation, was 0.25 to 0.50 ng.

MAb to *S. aureus* CP types 5 and 8 allowed us to type by ELISA 18 (15.5%) of 116 CN staphylococcal isolates from cow and goat milk (Table 1). Except for two *Staphylococcus lentus* isolates from goats, all typable isolates were from

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TABLE 1. Identity, animal origin, and reactivity of CN staphylococci isolated from milk included in this study

| Organism | Cows | | Goats | | Total no. of strains | No. of strains reacting with MAb | |
|---|---|---|---|---|---|---|---|
| | No. of strains | No. of herds | No. of strains | No. of herds | | Type 5 | Type 8 |
| *S. epidermidis* | 7 | 6 | 7 | 5 | 14 | 0 | 0 |
| *S. haemolyticus* | 19 | 6 | 0 | 0 | 19 | 13 | 0 |
| *S. warneri* | 5 | 3 | 1 | 1 | 6 | 0 | 1 |
| *S. simulans* | 6 | 3 | 5 | 3 | 11 | 0 | 1 |
| *S. hyicus* | 6 | 1 | 0 | 0 | 6 | 1 | 0 |
| *S. chromogenes* | 15 | 5 | 3 | 2 | 18 | 0 | 0 |
| *S. xylosus* | 12 | 5 | 4 | 2 | 16 | 0 | 0 |
| *S. caprae* | 0 | 0 | 13 | 5 | 13 | 0 | 0 |
| *S. lentus* | 0 | 0 | 8 | 3 | 8 | 2 | 0 |
| *S. sciuri* | 4 | 1 | 1 | 1 | 5 | 0 | 0 |

cows. Type 5 was predominant, accounting for about 89% of typable isolates. No *S. epidermidis*, *S. chromogenes*, *S. xylosus*, *S. caprae*, or *S. sciuri* isolate was typable, and most of the typable CN staphylococci belonged to the species *S. haemolyticus*. They were isolated from four different herds; those coming from the same herd were isolated from different animals at different years (data not shown).

The ELISA reactivity of typable CN staphylococcal strains considerably varied according to strains. CP equivalence values ranged from 0.25 to 1460.0 ng/mg for isolates reacting with type 5 CP-specific MAb (16 isolates) and from 0.20 to 230.0 ng/mg for those reacting with type 8 CP-specific MAb (2 isolates). In the culture conditions we used, strains Reynolds (prototype 5) and Becker (prototype 8) produced, respectively, 30.0 and 900.0 ng of CP per mg of wet bacteria.

In a previous report, we demonstrated by ELISA that 70 to 80% of *S. aureus* isolates from cow, goat, and ewe milk produce two predominant CP types, 5 and 8 (16). Using the same ELISA in this study, 15.5% of CN staphylococcal isolates from cow and goat milk were found to react with MAb to *S. aureus* type 5 and 8 CP (Table 1) and consequently can be considered as encapsulated. However, some other strains were suspected of being encapsulated because of the mucoid appearance and sticky texture of the colonies. CN staphylococci are known to produce slime, which is defined as a complex extracellular material that is loosely bound to bacteria and easily removable from the cells (21). Three CN staphylococcal isolates, two reacting in the inhibition ELISA with type 5-specific MAb and one reacting with type 8-specific MAb, were tested in a whole cell ELISA. After bacteria were washed with buffer, they were used to coat microplates. MAb against type 5 CP only bound to type 5 CN staphylococcal strains, and MAb to type 8 CP bound only to the type 8 CN staphylococcal strain (data not shown). This result indicates that the component on CN staphylococci that reacted with MAb in inhibition ELISA is a capsular material located on the bacterial surface. Because none or little of this material was removed from bacteria by washing, it is likely that this material is different from slime. Moreover, slime production is mainly associated with *S. epidermidis* (4), and no isolate belonging to this species was typable (Table 1). Most of the typable CN staphylococcal isolates in this study were *S. haemolyticus* (Table 1); strains belonging to this species rarely produce slime (4, 14).

The reactivity of CN staphylococci with MAb to type 5 or 8 CP observed here could be explained by the fact that (i) CN staphylococci produce CP that share a single common epitope with *S. aureus* type 5 or 8 CP or (ii) CP produced by CN staphylococci are identical to those produced by *S. aureus*. The observation that antibodies against types 5 and 8 are very specific for the homologous polymer (20) could be in favor of the identity hypothesis. Nevertheless, only further chemical and structural studies on CN staphylococcal polysaccharides could allow definitive conclusions about this point. If the structural identity is confirmed, it might be possible to use CN staphylococcal strains from animals, which are less pathogenic than *S. aureus* strains from humans, for large-scale production of type 5 and 8 CP. CP equivalence values (Table 1) indeed suggested that some CN staphylococcal strains produce large amounts of CP and, in the case of strains reacting with type 5-specific MAb, much more than the amount produced by type strain Reynolds.

This study is the first that describes CP cross-reactivity between CN staphylococci and *S. aureus*. Nelles et al. (13) found that *S. epidermidis* clinical isolates exhibited no cross-reactivity with MAb against *S. aureus* type 5 and 8 CP. Similar results were reported by Boutonnier et al. (3) with type strains of the 25 recognized *Staphylococcus* species other than *S. aureus* and with 267 blood cultures from which CN staphylococci (for the most part, *S. epidermidis*) were isolated.

Antibodies to *S. aureus* CP were demonstrated to promote opsonization of encapsulated organisms (10). Since these antibodies cross-react with CN staphylococci involved in mammary gland infection, one would predict significant cross-protection against these bacterial species as recently demonstrated in a mouse virulence model (23). Taking into account the prevalence of staphylococcal infections in mammary glands and the consequences for milk quality (12, 15, 17, 19), the findings presented herein might be useful for development of efficient vaccine against most staphylococcal species involved in udder infection.

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