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Expression of Type 8 Capsular Polysaccharide and Production of Toxic Shock Syndrome Toxin 1 Are Associated among Vaginal Isolates of *Staphylococcus aureus*

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A colony immunoblot method was developed for serotyping the capsular polysaccharides expressed by *Staphylococcus aureus* isolates. The method was rapid and specific and was performed with either polyclonal or monoclonal antibodies specific for each of the capsule types. *S. aureus* isolates were obtained from patients with toxic shock syndrome (TSS) or other staphylococcal infections and from asymptomatic women with vaginal colonization. Among the vaginal isolates of *S. aureus*, expression of the type 8 capsule was significantly ($P < 0.001$) more frequent among strains that produced TSS toxin 1 (TSST-1) than it was among TSST-1-negative strains. In contrast, the frequency of type 8 capsule expression was similar among both TSST-1-positive and -negative strains of *S. aureus* from patients with nonvaginal TSS. When all vaginal and nonvaginal isolates were compared, TSST-1-negative *S. aureus* strains were equally distributed among the type 5 and 8 and nontypeable capsule groups, whereas TSST-1-positive strains were predominantly capsule type 8.

A characteristic feature of toxic shock syndrome (TSS)-associated strains of *Staphylococcus aureus* is production of TSS toxin 1 (TSST-1). This protein is secreted by virtually all strains isolated from menstruation-related cases of TSS, 50 to 60% of strains isolated from non-menstruation-related cases of TSS, and 15 to 20% of *S. aureus* strains isolated from other clinical sources (4, 7, 19, 22, 25). TSS-associated isolates from nonmenstruation-related cases that do not secrete TSST-1 often express one or more of the staphylococcal enterotoxins, which share many of the biological properties of TSST-1 (15, 21). When these purified toxins are administered to experimental animals, they develop many of the clinical signs of a TSS-like illness (5, 16, 26).

Other phenotypic characteristics typical of TSS-associated strains of *S. aureus* include resistance to arsenate, mercury, cadmium, and penicillin; susceptibility to bacteriocin; and increased proteolysis. TSS isolates are less likely to produce α-hemolysin or to carry plasmid DNA (3, 24, 25). None of these traits reliably distinguishes TSS-associated strains from other *S. aureus* isolates.

There have been no reports on the capsular serotypes of *S. aureus* strains isolated from patients with TSS. Examination of strains from routine suppurative infections revealed that over 90% of *S. aureus* isolates are encapsulated (1, 2, 9, 11, 18, 23) and can be assigned to 1 of 11 serologically distinct capsule types (23). Highly encapsulated, mucoid strains usually belong to serotypes 1 or 2 and are rarely isolated from clinical specimens (2, 11, 23). The other serotypes are characterized as "microencapsulated," i.e., they produce nonmucoid colonies and their capsules are not apparent by negative strains like India ink. Type 5 and 8 strains predominate, making up ~22 and ~53%, respectively, of all isolates examined, including strains from diverse geographic areas, human and animal sources, superficial and deep-seated infections, and the skin and mucosal surfaces of normal individuals (1, 2, 9, 11, 18, 23). Only a few subpopulations of *S. aureus* isolates have been associated with a unique capsular serotype. Notably, oxacillin-resistant *S. aureus* strains and bovine milk isolates from France are predominantly capsule type 5 (2, 6, 18). Thus, type 5 and 8 microcapsules are commonly expressed by *S. aureus* isolates, and with the exceptions noted above, the distribution of capsule types from different sources (both pathologic and commensal) is quite similar. The clinical relevance of the *S. aureus* microcapsule has not been established.

In this report, we describe a colony immunoblot method developed to serotype capsules produced by TSS-associated staphylococci, vaginal isolates of *S. aureus* from healthy carriers, and strains isolated from patients with infections other than TSS. Using this technique, we found a significant association between production of TSST-1 by vaginal *S. aureus* isolates and type 8 capsule expression.

MATERIALS AND METHODS

Bacteria. Two hundred three isolates of *S. aureus* were collected between 1982 and 1990 from asymptomatic women and from patients with staphylococcal infections (both TSS and non-TSS). The clinical isolates included strains sent to the Channing Laboratory for TSST-1 testing; their sources are cited in Table 1. *S. aureus* strains from patients with TSS were included in the study only if the diagnostic criteria of certain or probable TSS were met (20). *S. aureus* isolates from patients with non-TSS infections included those isolated from blood, skin, bone, or soft tissue infections. Control *S. aureus* strains included previously described (11) reference cultures for capsule types 1, 2, 5, and 8 (strains M, Smith diffuse, Reynolds, and Becker, respectively) and five type 8, four type 5, and four nontypeable isolates that were previously serotyped by immunoprecipitation (2). Stock suspensions of all strains were maintained at −70°C in 5% skim milk.

Antibodies. Polyclonal antisera were raised in rabbits to heat- or Formalin-killed suspensions of prototype *S. aureus* strains producing capsule types 1, 5, or 8 (10). Monoclonal antibodies specific for type 5 (immunoglobulin M [IgM];

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TABLE 1. Origins of *S. aureus* isolates (n = 203) included in the study

Source	No. of isolates
Normal vaginal isolates from asymptomatic women (n = 77)	
Hospital personnel, Boston, Mass.	47
Nonhospital personnel, Boston, Mass.	17
Vancouver, British Columbia, Canada[a]	8
Centers for Disease Control, Atlanta, Ga.[b]	5
TSS-associated vaginal isolates (n = 31)	
Washington, D.C.[c]	6
Centers for Disease Control, Atlanta, Ga.[b]	5
Minnesota[d]	1
Massachusetts	9
Pennsylvania	1
Laboratory Centre for Disease Control, Ontario, Canada[e]	9
TSS-associated nonvaginal isolates (n = 40)	
Centers for Disease Control, Atlanta, Ga.[b]	22
Massachusetts	16
Connecticut	1
Alabama	1
Non-TSS infections (n = 55)	
Canada[f]	10
Massachusetts	44
Alabama	1

[a] Provided by A. Chow.
[b] Provided by A. Reingold.
[c] Provided by M. Pollock.
[d] Provided by P. Schlievert (strain MN8).
[e] Provided by E. Ewan.
[f] Provided by A. Chow and R. Lannigan.

17-23-1) and 8 (IgG3; 18-2-2) capsules were described previously (13).

Immunoblot. Tryptic soy agar or Mueller-Hinton agar plates supplemented with 4% NaCl–0.8 mM $MgCl_2$–1.4 mM $CaCl_2$ were spot inoculated in a grid pattern with as many as 100 *S. aureus* isolates and incubated overnight at 37°C. The colonies were blotted onto nitrocellulose filter membranes (diameter, 82.5 mm; Schleicher & Schuell, Keene, N.H.) for 5 min at ambient temperature. Adherent colonies were fixed to the membranes by heating them at 60°C for 15 min. After washing them twice in 5 mM sodium phosphate buffer–0.85% sodium chloride (phosphate-buffered saline [PBS]) to remove excess cells, the filters were immersed in a solution of trypsin (1 mg/ml; Sigma Chemical Co., St. Louis, Mo.) for 60 min at 37°C to remove protein A from the bacterial cells. After two washes in PBS, the filters were blocked with 0.05% skim milk for 1 h and washed in PBS containing 0.05% Tween 20 (PBST). Capsule type-specific polyclonal antisera (diluted ≥1:5,000 in PBST) or monoclonal antibodies (diluted 1:1,000 in PBST) were incubated with each filter at 37°C for 2 h. After washing in PBST, horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse immunoglobulin (diluted ≥1:1,000 in PBST; Organon Teknika-Cappel, West Chester, Pa.) was incubated with each filter for 2 h at 37°C. After three washes, substrate (3 mg of 4-chloro-1-naphthol [Sigma] per ml of methanol diluted 1:5 in PBS and containing 0.1% H_2O_2) was added to the filters. A purple color developed within 15 min and was scored visually from 0 to 4+.

The reactivities of the clinical isolates were evaluated by comparison with those of 12 to 17 control *S. aureus* strains (type 1, 5, and 8 and nontypeable isolates) included on each filter membrane. Positive reactions were scored as 2+ or greater. Each clinical isolate was tested at least twice with polyclonal or monoclonal antibodies specific for capsule types 1, 5, and 8. A few strains reacted weakly (1 to 2+) with all of the antibodies tested, and these were recorded as nontypeable isolates.

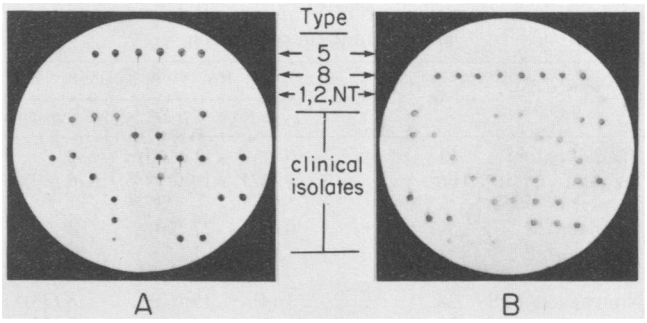

FIG. 1. Colony immunoblot reaction of control *S. aureus* strains (types 5, 8, 1, and 2 and nontypeable [NT]) and clinical isolates. Filter membranes were reacted with rabbit serum (diluted 1:8,000) raised to the type 5 strain Reynolds (A) or rabbit serum (diluted 1:5,000) raised to the type 8 strain Becker (B).

Ten strains that were nontypeable by colony immunoblots were tested by rocket immunoelectrophoresis to determine whether the latter method might be more a sensitive method for detecting capsule production. Rocket immunoelectrophoresis was carred out as described by Hochkeppel et al. (9) by using 1% agarose containing 0.5 to 1% polyclonal rabbit serum raised to either the Reynolds or Becker strain.

Toxin detection. *S. aureus* strains were cultivated by using the membrane-over-agar method (8). TSST-1 production was evaluated by using a competitive enzyme-linked immunosorbent assay (17), and the enterotoxins were detected by immunodiffusion (14).

RESULTS

Capsule typing by colony immunoblot. The immunoblot is a rapid (completed within 7 h) and specific assay for differentiating *S. aureus* strains by capsule type. Polyclonal rabbit sera raised to prototype *S. aureus* strains or capsule-specific monoclonal antibodies reacted only with *S. aureus* strains that produced the homologous capsule type (Fig. 1). Nonencapsulated controls or colonies of coagulase-negative staphylococci showed weak or negative reactions in the assay. None of the strains reacted on filters incubated with conjugate alone, and none of the clinical isolates reacted with capsule type 1 antiserum (data not shown). The latter observation confirms earlier reports by others that type 1 capsules are rare among clinical isolates of *S. aureus* (1, 2, 9, 11, 18, 23). Strains that did not react with antibodies to capsule types 1, 5, or 8 were recorded as nontypeable; these included strains that were nonencapsulated, as well as those that produced microcapsules other than types 1, 5, or 8. Extracts prepared from 10 strains determined to be nontypeable by immunoblotting were also nonreactive when tested by rocket immunoelectrophoresis against type 5 and 8 antisera (data not shown). Because ~75% of staphylococcal strains are typeable with antibodies specific for type 5 and 8 strains (1, 2, 9, 11, 18, 23), no further serologic distinctions were made within the nontypeable group.

Capsule typing of vaginal isolates of *S. aureus*. Using the immunoblot method, we compared the capsule types of *S.*

TABLE 2. Capsule types of *S. aureus* isolates analyzed by source and toxin production

Source of *S. aureus*	No.	TSST-1	No. (%) of isolates		
			Type 5	Type 8	Nontypeable
Vaginal colonization	11	+	0	11 (100)	0[a]
	66	−	21 (32)	19 (29)	26 (39)
Vaginal TSS	30	+	0	27 (90)	3 (10)
	1[b]	−		1	
Nonvaginal TSS	24	+	1 (4)	15 (63)	8 (33)
	16	−	3 (19)	11 (69)	2 (12)
Non-TSS infections	5	+	0	4 (80)	1 (20)
	50	−	21 (42)	14 (28)	15 (30)

[a] $P < 0.001$, as determined by chi-square analysis by comparing the distribution of capsule types of TSST-1-positive and -negative strains from women asymptomatically colonized with *S. aureus*.
[b] This strain made enterotoxin B.

TABLE 3. Correlation between capsular type 8 and TSST-1 production among *S. aureus* isolates

Source of *S. aureus*[a]	No.	TSST-1	No. (%) of isolates		
			Type 5	Type 8	Nontypeable
Vaginal	41	+	0	38 (93)	3 (7)[b]
	67	−	21 (31)	20 (30)	26 (39)
Nonvaginal	29	+	1 (3)	19 (66)	9 (31)[c]
	66	−	24 (36)	25 (38)	17 (26)

[a] Includes both TSS and non-TSS strains of *S. aureus*.
[b] $P < 0.001$, as determined by chi-square analysis by comparing the distribution of capsule types of TSST-1-positive and -negative vaginal isolates of *S. aureus*.
[c] $P = 0.003$, as determined by chi-square analysis by comparing the distribution of capsule types of TSST-1-positive and -negative nonvaginal isolates of *S. aureus*.

aureus strains isolated from the vaginas of asymptomatic, healthy carriers with those isolated from the vaginas of women with TSS. Vaginal TSS cases included menstrually related disease, as well as nonmenstrually related cases associated with postpartum infection or use of a diaphragm or contraceptive sponge. TSST-1-negative, vaginal isolates from asymptomatic women were equally distributed among the type 5 and 8 and nontypeable groups (Table 2). Of 77 of the normal vaginal isolates, 11 (14%) produced TSST-1, and all 11 expressed type 8 capsules. The distribution of capsule types between TSST-1-positive and -negative strains from healthy carriers of vaginal *S. aureus* was significantly different ($P < 0.001$) by chi-square analysis. When we analyzed the normal vaginal isolates by source, there were no differences in the distribution of capsule types between *S. aureus* strains carried by healthy hospital personnel and *S. aureus* strains carried by women in the community (data not shown). Of the TSS-associated vaginal isolates, 30 of 31 were TSST-1 positive. Like the colonizing strains, 27 of 30 (90%) TSST-1-producing organisms elaborated type 8 capsules.

Capsule typing of TSS-associated, nonvaginal isolates of *S. aureus*. Nonvaginal (nonmenstrual) cases of TSS are often caused by *S. aureus* strains that do not produce TSST-1 (7, 19). As shown in Table 2, 60% (24 of 40) of the TSS-associated nonvaginal isolates in our study were TSST-1 positive. These strains were isolated from skin or soft tissue infections, including surgical wounds. Although the majority of toxin-producing, nonvaginal TSS isolates were serotype 8, the distribution of capsule types was not significantly different between TSST-1-positive and -negative strains. Both toxin producers and nonproducers were predominantly serotype 8 strains, in proportions similar to those reported for *S. aureus* from other clinical sources (1, 2, 9, 11, 18, 23).

Capsule typing of *S. aureus* isolates from non-TSS infections. Fifty-five clinical isolates from patients with staphylococcal infections other than TSS were tested for toxin production and capsular serotype. Similar to findings reported earlier by others (1, 2, 9, 11, 18, 23), we found a predominance of capsule types 5 (42%) and 8 (28%) among 50 TSST-1-negative *S. aureus* isolates (Table 2). Only five of the non-TSS strains produced TSST-1, and four of these made type 8 capsules.

When we compared the capsule types of *S. aureus* strains isolated from either vaginal or nonvaginal sources (Table 3), the TSST-1-producing strains from both groups were predominantly capsule type 8. Among the vaginal isolates, strains that produced type 8 capsules accounted for 38 of 41 (93%), whereas 19 of 29 (66%) of the nonvaginal isolates produced type 8 capsules. The distribution of capsule types was very similar for TSST-1-negative *S. aureus* from both vaginal and nonvaginal sources (Table 3).

Twenty *S. aureus* strains in our collection were known to produce staphylococcal enterotoxin B. These strains showed the expected distribution of capsular types (35% type 5, 50% type 8, and 15% nontypeable). Only 11 of our isolates were known enterotoxin A producers; 1 of these was type 5, 5 were type 8, and 5 were nontypeable. Enterotoxins C, D, and E were detected in too few strains for evaluation of their capsule types.

DISCUSSION

In previous studies demonstrating the predominance of capsule types 5 and 8 among *S. aureus* strains, serotyping methods such as immunoprecipitations (2, 11), bacterial microagglutinations (9, 13, 23), or enzyme-linked immunosorbent assay inhibitions (1, 6, 18) were used. These techniques are tedious for screening large numbers of isolates, since trypsinized bacterial suspensions or autoclaved extracts must be prepared for each bacterial strain. We developed a colony immunoblot method to facilitate capsule typing of large numbers of *S. aureus* isolates. This rapid and specific assay was used to compare the capsular serotypes of TSS-associated *S. aureus* strains with strains from other sources.

Our results showed that >90% of vaginal, TSST-1-positive *S. aureus* strains expressed a type 8 capsule. This included toxin-positive, TSS-associated vaginal isolates, as well as toxin-positive, vaginal isolates from healthy carriers. In contrast, only one-third of the TSST-1-negative, normal vaginal isolates produced a type 8 capsule. TSS-associated, nonvaginal strains of *S. aureus* were also predominantly type 8, but there was no significant difference between the distribution of capsule types for TSST-1-positive and -negative strains. Only five *S. aureus* strains from patients with infections other than TSS produced TSST-1, and four of these elaborated type 8 capsules.

Our results demonstrate a striking association between the production of type 8 capsules and TSST-1 by vaginal isolates of *S. aureus*. The role of the type 8 capsule in promoting staphylococcal colonization of the urogenital tract has not been examined. However, it is unlikely that capsules are

important for colonization since TSST-1-negative, vaginal isolates from asymptomatic women were equally distributed among the type 5 and 8 and nontypeable groups. The data do support the premise that TSST-1-producing, vaginal isolates of *S. aureus* are clonally distributed. Genetic relatedness among TSS isolates was recently reported by Musser et al. (12), who analyzed 315 TSST-1-producing *S. aureus* isolates using multilocus enzyme electrophoresis. Their study revealed that 88% of 187 toxin-positive *S. aureus* isolates from the urogenital tract were grouped into one closely related cluster of electrophoretic types. This "clone" also accounted for 53% of 47 nonvaginal, TSST-1-positive strains and 28% of 53 non-TSS vaginal isolates. Their study did not include TSST-1-negative strains isolated from nonvaginal cases of TSS. TSST-1-positive *S. aureus* from vaginal cultures appear to be a more homogeneous group of strains than toxin-positive strains isolated from other sources such as patients with nonmenstrual TSS are. Other reports have also shown that TSS-associated *S. aureus* strains have distinct phenotypes compared with non-TSS strains (3, 24, 25). These traits, like capsule type, have not been associated with the disease process and may reflect characteristics shared by phylogenetically related strains of *S. aureus*.

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