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Involvement of Coagulase-Negative Staphylococci in Toxic Shock Syndrome

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Coagulase-negative staphylococci that produce toxic shock syndrome toxin 1 (TSST-1) or a staphylococcal enterotoxin or both were isolated from various sources. Coagulase-negative strains that produce TSST-1 alone or with enterotoxin A were the only staphylococci isolated from seven patients with toxic shock syndrome. Two other toxic shock syndrome patients had coagulase-positive staphylococci also, but only the coagulase-negative strains produced TSST-1. Coagulase-positive and coagulase-negative strains that produced TSST-1 were isolated from two other toxic shock syndrome patients. In addition, coagulase-negative staphylococci that produced toxins were isolated from patients with other staphylococcal infections and from food implicated in a case of food poisoning.

During the last 20 years, coagulase-negative staphylococci have been identified as significant pathogens in a variety of clinical situations. *Staphylococcus saprophyticus*, a coagulase-negative *Staphylococcus* species, has been identified as a common cause of primary urinary tract infections, particularly in young women of child-bearing age (8). Coagulase-negative staphylococci are common causes of sepsis in newborns, especially in those needing umbilical vessel catheters, and in cancer patients on long-term vascular catheterization (11). Coagulase-negative staphylococci are associated with all prosthetic devices and have been implicated as the causative organisms in 3.6 to 64% of the cases of septics in patients with prosthetic valves, hip joints, central nervous system shunts, or vascular grafts and in patients undergoing peritoneal dialysis (11).

Vaginal colonization by coagulase-negative staphylococci in healthy women is high; in several studies the incidence ranged from 29.6 to 86% (2, 7, 10, 15); a total of 963 (57.3%) of 1,681 women were vaginally colonized with coagulase-negative staphylococci. The incidence of vaginal colonization by *Staphylococcus aureus* for the same group ranged from 0 to 12%. Toxic shock syndrome (TSS) and its association with *S. aureus* were first described by Todd et al. in 1978 (16). A toxin was subsequently purified from *S. aureus* strains isolated from TSS patients (3, 13) which elicited the signs and symptoms of TSS, except desquamation, in baboons (M. E. Melish, F. S. Chen, and S. M. Murata, Clin. Res. **31**:122A, 1982; F. W. Quimby, M. Olstad, and E. Weiner, Fed. Proc. **43**:378, 1984). This toxic protein is called toxic shock syndrome toxin 1 (TSST-1) (5).

The recovery of *S. aureus* strains that produce TSST-1 or staphylococcal enterotoxins or both from TSS patients was discussed in several papers, but only minimal attention was given to the coagulase-negative staphylococci and their ability to produce these toxins (1–4, 6, 15). In this paper we show that coagulase-negative staphylococci that produce TSST-1, one or more of the enterotoxins, or both, are important in TSS.

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MATERIALS AND METHODS

Patients. All Wisconsin cases were classified as confirmed, probable, unusual, or non-TSS by J. P. Davis, Wisconsin Division of Health. For the four cases from other locations, the physicians submitted hospital discharge summaries that were reviewed and classified according to the case criteria established by the Centers for Disease Control (14).

Cultures. The coagulase-negative staphylococci were received from physicians and public health officers in Wisconsin and four other states. All isolates were grown in brain heart infusion broth for 18 h and analyzed for TSST-1 and the enterotoxins by the membrane-over-agar and optimal sensitivity plate methods; hyperimmune rabbit serum and crude toxin standards were used in the analysis (12).

Coagulase production. The production of coagulase was studied by the tube method. An overnight culture (0.1 ml) in brain heart infusion broth (Difco Laboratories) was added to 0.3 ml of rabbit plasma (coagulase plasma EDTA) and incubated at 37°C. Readings were taken after 30 min and after 2, 4, and 24 h. The results were tabulated by a previously described method (17) as follows: 1+, small unorganized clot; 2+, small organized clot; 3+, large organized clot; 4+, complete clot. Only 3+ and 4+ were considered positive reactions unless the strains that were 2+ were also thermonuclease (TNase) positive.

TNase production. The production of TNase was detected by the plate technique described previously (9).

All strains that were negative for both coagulase and TNase will be referred to as coagulase-negative staphylococci without species designation.

The antibiotic susceptibilities of the cultures were determined by P. J. Wand, State Laboratory of Hygiene, Madison, Wis.

RESULTS

The 19 patients from whom only coagulase-negative staphylococci were isolated were all female and only 1 had nonmenstrual TSS (Table 1). Samples from five patients were cultured after the patients had antibiotic therapy; all of the isolates were toxin negative. Coagulase-negative staphylococci that produced TSST-1, either alone or with staph-

TABLE 1. TSS patients with coagulase-negative staphylococci only

Site	Toxin(s)	Classification[a]
Cultured preantibiotics		
Nose	TSST-1	C-M
Vagina/urine	—/—[b]	P-M
Vagina	TSST-1	P-M
Vagina/tampon	—/—	P-M
Vagina	SEA, TSST-1	C-M
Cervix	—	P-M
Rectum/vagina	SEA, TSST-1/SEA, TSST-1	P-M
Vagina	—	P-M
Cervix/tampon	—/—	C-M
Vagina	—	P-M
Cervix	—	P-M
Vagina	SEA, TSST-1	U-M
Labial lesion/vagina	TSST-1/TSST-1	P-M
Skin lesion	TSST-1	U-N
Cultured postantibiotics		
Eye	—	C-M
Nose	—	C-M
Vagina	—	C-M
Rectum/nose	—/—	C-M
Vagina/rectum	—/—	C-M

[a] C, Confirmed TSS; P, probable TSS; U, unusual, not definable TSS, but TSS cannot be ruled out; M, menstrually related; N, nonmenstrually related.
[b] —, No toxin produced.

ylococcal enterotoxin A (SEA), were isolated from 7 (50%) of the 14 women who had samples cultured before antibiotic therapy was initiated. Coagulase-negative staphylococci were isolated from four patients along with coagulase-positive strains; in two patients, only the coagulase-negative strains produced toxin (Table 2, patients 2 and 4).

Two patients had a recurrence of TSS. In both cases, the staphylococci isolated during the first episode were coagulase-positive, whereas the staphylococci isolated during the second episode were coagulase negative. The coagulase-positive and coagulase-negative isolates from one patient produced the same toxins (SEA and TSST-1), but the strains had different antibiotic susceptibilities (resistant to none versus resistance to ampicillin, penicillin, and tetracycline). This indicated that the coagulase-negative isolate was a different strain and not merely the coagulase-positive strain with an inability to coagulate rabbit plasma. The recurrent episode occurred 9 months after the initial episode for this patient. In the second case, both strains produced TSST-1 but only one strain produced SEA. For this patient, the second episode occurred 28 months after the first episode.

TABLE 2. TSS patients with both coagulase-positive and -negative staphylococci

Patient	Site	Coagulase response[a]	Toxin(s)
1	Tampon	+	Negative
	Tampon	−	TSST-1
	Urine	+	TSST-1
	Urine	−	TSST-1
2	Cervix	+	Negative
	Cervix	−	Negative
	Cervix	−	SEA, TSST-1
3	Vagina	+	TSST-1
	Cervix	+	TSST-1
	Throat	+	TSST-1
	Nose	−	TSST-1
4[b]	Elbow	+	Negative
	Knee	−	Negative
	Knee	−	TSST-1

[a] +, Positive; −, negative.
[b] Four-year-old boy with elbow and knee abrasions.

TABLE 3. Non-TSS patients with toxin-producing coagulase-negative staphylococci

Patient	Site	Toxin(s)
A	Nasopharynx	TSST-1
B	Vagina	SEA
C	Vagina	SEA, TSST-1
D	Tampon	SEC,[a] TSST-1
E	Cervix	SEA
F	Nose	SEC, TSST-1
G	Cervix	SEC

[a] SEC, Enterotoxin C.

Coagulase-negative staphylococci that produced one or more toxins were isolated from five female patients who had illnesses other than TSS, although samples were cultured with TSS in mind (Table 3). The other two women (B and C) were having routine vaginal cultures taken.

Of the *Staphylococcus epidermidis* strains (received from the Centers for Disease Control) that had been isolated from a variety of unused tampons, 5 (18.5%) of 27 produced toxin; TSST-1 was produced by one strain, and staphylococcal enterotoxin C was produced by the other four strains. Six coagulase-negative staphylococcal cultures were isolated from the unused portion of a box of tampons that were being used by a woman who died of menstrually associated TSS. Three of the cultures produced SEA and TSST-1, and the other three were toxin negative. No cultures were available from the woman or from any of the used tampons.

Coagulase-negative staphylococci that produced SEA and TSST-1 were isolated from chicken that was implicated in a recent case of staphylococcal food poisoning. The man who ate it became ill with vomiting and diarrhea 6 h later; the chicken had been smoked in a home cooker.

DISCUSSION

We receive many staphylococcal strains to test for production of the enterotoxins and TSST-1. In addition, we normally test these strains for coagulase and TNase production, especially if they are toxin producers, because these are important characteristics in the classification of a strain as *S. aureus*. The *Staphylococcus* species involved is not of great importance to clinical laboratories and normally they check only for coagulase production. We know of several instances where coagulase-negative staphylococci were discarded because it was felt that they were not important in TSS. We have shown in this paper that coagulase-negative staphylococci can produce toxins that may be important in TSS; they should not be discarded.

Since 1980 our laboratory has received approximately 2,000 staphylococcal cultures that were isolated from possible TSS patients, from humans with other staphylococcal diseases, from colonization studies, from unused catamenial products, from patients with food poisoning, and from animals. About 10% of these cultures were coagulase negative; of these, 33 produced one or more toxins. Admittedly, 33 of over 2,000 cultures tested is not a very large number;

however, of the 19 TSS patients from whom only coagulase-negative staphylococci were isolated and submitted for analysis, 7 had one or more sites with a TSST-1- or SEA- and TSST-1-positive culture (Table 1). A number of serum samples from TSS patients were received for antibody testing without an accompanying staphylococcal culture because either no *S. aureus* was isolated or only coagulase-negative staphylococci were isolated and they were discarded.

No coagulase-negative staphylococci cultured from TSS patients after the administration of antibiotics were toxigenic. It is possible that toxigenic staphylococci were present but were eradicated by the antibiotic, that the proper sites were never cultured, or that there were no toxigenic staphylococci present. We believe that the first hypothesis is the most likely one because the toxin-positive coagulase-negative staphylococci tend to have antibiotic susceptibility patterns that closely resemble that of *S. aureus*, and they would be eradicated by the antibiotic as easily as the coagulase-positive staphylococci. Antibiotic susceptibility testing is usually not done on coagulase-negative staphylococci, but because of our findings, the coagulase-negative staphylococci from TSS patients in Wisconsin are being tested. Of cultures tested from six different patients, three were resistant to ampicillin and penicillin, one was resistant to ampicillin, penicillin, and tetracycline, and two were susceptible to all drugs tested.

Even when coagulase-positive staphylococci are isolated from an appropriate site, that is, tampons or cervix, any accompanying coagulase-negative staphylococci should not be ignored. Both coagulase-positive and coagulase-negative staphylococci were isolated from appropriate sites in two TSS patients, but only the coagulase-negative staphylococci produced toxins. Any staphylococcal culture isolated in a TSS case should be submitted for toxin analysis, regardless of its ability to coagulate rabbit plasma. Coagulase-negative staphylococci that produce one of the enterotoxins or TSST-1 or both have been isolated from many different sources. In addition to further characterization of these strains, additional coagulase-negative staphylococci need to be tested for toxin production. What is most important is that all medical professionals, physicians, and laboratory personnel need to realize that coagulase-negative staphylococci cannot be ignored.

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