

## Evaluation of Coagulase-Negative Staphylococci for Ability To Produce Toxic Shock Syndrome Toxin 1

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**A large and diverse group of coagulase-negative staphylococci were assayed for the ability to produce toxic shock syndrome toxin 1 (TSST-1) by immunological reactivity, and whole-cell DNAs from 33 of these strains were hybridized with a TSST-1-specific gene probe. None of the strains tested, including isolates that have been reported as TSST-1<sup>+</sup>, produced the exotoxin, and no DNA homology was found with the gene probe.**

Toxic shock syndrome (TSS) is an acute illness characterized by fever, hypotension, a scarlatinaform rash, desquamation primarily of the palms and soles following the acute phase, and a variable multiorgan component (12, 13). *Staphylococcus aureus* is the causative organism in TSS. Over 90% of menstruation-associated cases are caused by strains that produce TSS toxin 1 (TSST-1; 1, 11; M. S. Bergdoll and P. M. Schlievert, Letter, Lancet ii:691, 1984). *S. aureus* associated with nonmenstrual cases expresses either TSST-1, enterotoxin B, or, rarely, enterotoxin C (9).

Bergdoll and associates (1, 3) reported the identification of coagulase-negative staphylococci that produce TSST-1, and Kahler et al. (5) isolated two coagulase-negative staphylococci that tested TSST-1<sup>+</sup> from a patient with TSS. On the basis of these findings, the researchers suggested that coagulase-negative strains from patients with TSS be tested for TSST-1.

Because of the potential significance of these findings, we tested numerous coagulase-negative species, including some strains previously reported as TSST-1<sup>+</sup> (3, 5), for the ability to produce TSST-1. In addition, whole-cell DNAs from a subset of these strains were examined by DNA hybridization for the presence of the TSST-1 gene (*tst*).

The strains shown in Table 1 were assayed for TSST-1 production by Ouchterlony immunodiffusion. Cultures were grown to stationary phase in a dialyzable beef-heart medium under conditions expected to yield large amounts of TSST-1 (11). Subsequently, the culture fluids were concentrated 100-fold for use in Ouchterlony immunodiffusion assays (10). The sensitivity of this assay corresponds approximately to the usual level of TSST-1 produced by *S. aureus*.

For hybridization analysis by the dot blot method (4), whole-cell DNA was isolated from each strain by a modification of the method of Mekalanos (7). The procedure was adapted for *S. aureus* cells by substituting lysostaphin, at a final concentration of 500 µg/ml, for lysozyme. Approximately 1 µg of the DNA preparation was applied to nitrocellulose by using a Schleicher & Schuell Mini-Manifold. The gene probe was a 297-base-pair *Bam*HI-*Hind*II internal *tst*-specific DNA fragment (2). Hybridization was done under conditions previously described (6).

The data in Table 1 reveal that none of the coagulase-negative staphylococci tested produced TSST-1; nor did any of these strains have detectable homology with the *tst* gene

probe (Fig. 1). The two TSST-1<sup>+</sup> isolates (Fig. 1, A5 and A6), which were previously typed as coagulase negative, were strongly coagulase positive in our study, and both did indeed produce TSST-1. The two strains originally evaluated as TSST-1<sup>+</sup> in the study of Kahler et al. (5) were TSST-1<sup>-</sup>, and, as expected, the TSST-1 gene was absent. Independently and using a different TSST-1 assay, Parsonnet et al. also failed to detect TSST-1 production by coagulase-negative strains (8); the two strains evaluated in the study of Kahler et al. were also shown to be TSST-1<sup>-</sup> (personal communication).

On the basis of these collective findings, there is no convincing evidence that a TSST-1-producing coagulase-negative staphylococcus has been identified. To clearly demonstrate expression of TSST-1 by coagulase-negative strains, we suggest that the following three criteria should be met: (i) the strains should be carefully identified as

TABLE 1. Production of TSST-1 by strains of staphylococci

| Strain  | No. tested | No. producing TSST-1 | No. carrying <i>tst</i> |
|---|------------|----------------------|-------------------------|
| Coagulase <sup>-</sup> , non-TSS <sup>a</sup> | 55         | 0                    | 0/5                     |
| Coagulase <sup>-</sup> , non-TSS from tampons | 22         | 0                    | NT <sup>b</sup>         |
| Coagulase <sup>-</sup> , non-TSS <sup>c</sup> | 22         | 0                    | 0                       |
| Coagulase <sup>-</sup> , TSS <sup>d</sup>     | 2          | 0                    | 0                       |
| Coagulase <sup>-</sup> , TSS <sup>e</sup>     | 2          | 0                    | 0                       |
| Coagulase <sup>-</sup> , TSS <sup>f</sup>     | 9          | 0                    | NT                      |
| Coagulase <sup>+</sup> <sup>g</sup>           | 2          | 2                    | 2                       |

<sup>a</sup> Clinical isolates from wounds, blood, cerebrospinal fluid, catheters, and urine, kindly provided by P. Ferrieri, Departments of Laboratory Medicine and Pathology and Pediatrics, University of Minnesota, Minneapolis.

<sup>b</sup> NT, Not tested.

<sup>c</sup> Strains generously provided by W. Kloos, Department of Genetics, North Carolina State University, Raleigh, and G. Archer, Division of Infectious Diseases, Department of Medicine, Medical College of Virginia, Virginia Commonwealth University, Richmond. These included *S. capitis* (1 strain), *S. caprae* (2 strains), *S. carnosus* (1 strain), *S. caseolyticus* (1 strain), *S. cohnii* (2 strains), *S. epidermidis* (3 strains), *S. gallinarum* (1 strain), *S. haemolyticus* (1 strain), *S. hominis* (2 strains), *S. hyicus* (2 strains), *S. kloosii* (1 strain), *S. lentus* (1 strain), *S. sciuri* (1 strain), *S. simulans* (1 strain), and *S. xylosum* (2 strains).

<sup>d</sup> From Kahler et al. (5).

<sup>e</sup> Strains originated from M. S. Bergdoll, Food Research Institute, University of Wisconsin, Madison, who characterized them as coagulase negative and TSST-1<sup>+</sup>. These strains were provided by R. E. Syverson, Kimberly-Clark Corporation, Neenah, Wis.

<sup>f</sup> Submitted from diverse geographic areas to P.M.S. for routine testing for TSST-1.

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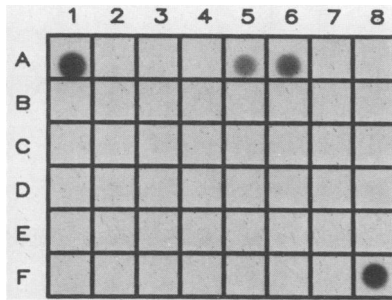


FIG. 1. DNA dot blot hybridization analysis of strains of staphylococci. Approximately 1  $\mu$ g of whole-cell DNA was applied to nitrocellulose and hybridized with a 297-base-pair internal *tst* DNA fragment. Positions contain DNAs from strains as follows: A1 and F8, positive control strain RN4282 (6); A8 and F1, negative control strain MNHO (9); A3, *S. carnosus*; A4, *S. simulans*; A5 and A6, coagulase-positive staphylococci previously characterized as coagulase negative by M. S. Bergdoll; B2 and B6, coagulase-negative staphylococci characterized by M. S. Bergdoll as TSST-1<sup>+</sup>; B3, B4, B5, and F3, representative unidentified coagulase-negative staphylococcus species not associated with TSS; B7, *S. caprae*; C2, *S. hyicus*; C3, *S. cohnii*; C4 and C5, coagulase-negative staphylococci reported by Kahler et al. (5); C6, *S. gallinarum*; C7, *S. hominis*; D2, *S. sciuri*; D3, *S. xylosus*; D4, *S. epidermidis*; D5, *S. cohnii*; D6, *S. epidermidis*; D7, *S. haemolyticus*; D8, *S. epidermidis*; E2, *S. epidermidis*; E3, *S. xylosus*; E4, *S. hominis*; E5, *S. sciuri*; E6, *S. lentus*; E7, *S. capitis*; F4, *S. caseolyticus*; F5, *S. kloosii*; F6, *S. hyicus*.

coagulase-negative staphylococci; (ii) the production of TSST-1 should be rigorously demonstrated; and (iii) the TSST-1 gene should be detected by hybridization.

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