Characterization of *Staphylococcus aureus* in a Pediatric Burn Unit

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A one-year study on an endemic strain of *Staphylococcus aureus* phage type 84/85 in a children's burn unit is described. The endemic strain rapidly colonized the burns and nares of acute patients after admission but was not isolated from a patient on admission. Nonendemic strains of *S. aureus* found on some new patients were mostly non-phage typable and did not prevail in burns. The endemic strain was rarely isolated from the nares and skin of reconstructive patients or from the nares of hospital personnel. The endemic strain did colonize the oral cavity, normal skin, and intestinal tract of some acute patients. Endemic and nonendemic strains of *S. aureus* from the burned children were compared in their biochemical activities and antibiotic sensitivities to two groups of *S. aureus* from one other local and one Danish burns unit. The latter groups of strains represented different combinations of staphylococcal phage group III strains. Each of the four groups of strains differed in production of hemolysins, Tween 80 hydrolysis, egg yolk reaction, and proteolysis of casein and gelatin. All of the strains were uniformly sensitive to gentamicin, oxacillin, and cephalothin. Only 4 of 162 strains tested were methicillin resistant. The endemic *S. aureus* strains of phage type 84/85 were uniformly resistant to eight other antibiotics including lincomycin and clindamycin. The endemic strain was not the known cause of a clinically documented infection in a group of 82 acute patients studied. The possible role of *S. aureus* strains of phage group III in burn grafting problems is discussed.

Several studies have been reported which show the changing trends in staphylococcal infections among hospitalized patients. Williams (20) recently described hospital staphylococci as being classified in one of three ways: strains which spread among patients causing infection, strains which spread and colonized patients causing little or no infection, and those strains introduced into hospital wards by carriers but neither spreading or causing infection. Strains of *Staphylococcus aureus* phage group I were once prevalent in many hospitals and responsible for some infections, but this group was eventually replaced by phage group III which in some areas involved the spread of *S. aureus* phage type 83A, 84, and 85 in hospitalized patients (3).

Williams (20) noted that over the last 8 to 10 years staphylococci have tended to decrease in their abilities to spread among patients. Recently, however, an epidemic strain of *S. aureus*, phage type 84, was described as methicillin resistant and responsible for extensive clinically documented infections among burned patients (R. B. Lindberg et al., Abstr. Annu. Meet. Amer. Soc. Microbiol., p. 113, 1972). This report (presented in part at the Annual Meeting of the American Society for Microbiology, Philadelphia, Pa., 1972) describes a strain of *S. aureus* phage type 84/85 which was methicillin sensitive and differed in several respects from other strains of *S. aureus* which were compared to this strain or described in the literature.

**MATERIALS AND METHODS**

**Patients.** All patients were under the age of 14 years. Acute patients were admitted for intensive care of second- and third-degree burns. Reconstructive patients were children who had survived burns and were admitted for plastic surgery of old burns. The latter group was generally in good health.

**Specimens.** Cultures were taken on acute patients at the time of admission and several times per week during hospitalization. Blood cultures were obtained
from taped B-D Vacutainer tubes containing supplemented peptone broth. Burn and oral cultures were taken with saline-moistened swabs. Rectal swabs were taken by the method of Smith and Dayton (14). Swabblings from normal skin and the nares were taken by the method of Smith (12). Foley and intravenous catheter tips were placed directly in thioglycolate medium (BBL). Urine specimens were diluted in saline and spread on media for enumeration purposes. Staphyloccoci were isolated from samples and specimens on 5% human blood agar in Columbia agar base (BBL), phenethyl alcohol agar (BBL), mannitol salt agar (BBL), or methyl green deoxyribonuclease agar (Difco). The latter medium was supplemented with 30 μg of lincomycin per ml as a selective medium for endemic strains of staphylococci. The tube coagulase test (Difco), Gram stain, and catalase test were routinely used to identify S. aureus strains. In comparative studies of the nasal microflora of patients and hospital personnel, gram-negative bacilli were isolated on MacConkey agar (BBL) and yeasts were isolated on Littman oxgall medium (BBL). Lipophilic and nonlipophilic diphtheroids were identified by the methods of Smith (13). Sources of strains. S. aureus strains isolated from children in the Galveston Burn Unit were classified into endemic and nonendemic types. These two groups were separated on the basis of their origins, antibiotic sensitivity patterns, and susceptibility to staphylococcal phages. Phage typing was performed through the courtesy of the Texas State Health Dept., Austin. The endemic strains were not isolated from patients on admission to the Institute. Sealy strains were isolated from the burns and nares of adult patients in the burn unit of the John Sealy Hospital, Galveston, Texas. Each of these strains produced a phage pattern of 47/53/54/75/77/84. Danish strains were isolated from a burn unit and were kindly supplied by Mogens Thomsen, Copenhagen, Denmark. Eighteen of the Danish strains were of phage type 84/85/655/592 and three strains were phage type 83A/84/85/655/592.

Biochemical tests. Hydrolysis of deoxyribonucleic acid (DNA) was determined on methyl green deoxyribonuclease agar (Difco). Tributyrin hydrolysis was detected on Spirit blue agar using the accompanying lipid emulsion (Difco). Caseinolysis was detected using the medium of Martley, Jayashankar, and Lawrence (10), and gelatinase and egg yolk factor were detected by the methods of Arbuthnott et al. (2). Hemolysin production was examined by the method of Marandon and Oeding (8) with rabbit, sheep, and horse blood. The hot-cold incubation-refrigeration method was used for beta hemolysin. Hyaluronidase activity was determined by the method of Arbuthnott et al. (2). Brain heart infusion broth was used in place of nutrient broth, and potassium hyaluronidate was used as the substrate (Sigma Chemical Co., St. Louis, Mo.). Boiled broth supernatants were used as controls for each strain. Hydrolysis of Tween 80 and triolein were determined by the methods of Smith (11).

Antibiotic sensitivity. Strains were tested on Mueller-Hinton agar (BBL) by the standardized Kirby-Bauer method.

RESULTS

Generally, the Institute admits approximately 100 acutely burned children for treatment each year, but, since some patients had already been admitted at the start of this study and others with incomplete data were admitted near the end of the study, data on these patients were not included. During the 1-year study period, the occurrence of S. aureus in clinical specimens of 82 acute patients was determined (Table 1). The endemic strains were all of phage type 84/85, and none was isolated from a new patient on admission. The much smaller number of nonendemic strains were isolated from 34 of the 82 acute patients on admission, but these strains rapidly disappeared from the patients. Only 18 of 71 nonendemic strains were phage typeable and none was of type 84/85.

The mean time in which 68 acute patients acquired the endemic strain in burns was approximately 16 days although some patients were colonized with the endemic strain in less than 1 week. Among the 14 patients who were not colonized by the endemic strains after admission, 7 were discharged and 5 patients died before the strain was isolated from them. Colonization rates of the nares of acute patients was nearly identical to those of burns (Table 2). Acquisition rates of other areas of the body of acute patients varied and were less frequent than those of burns and the nares. Swabblings of normal skin of acute patients revealed that 26 of 42 patients had endemic S. aureus at these sites. In a similar study of 30 reconstructive patients, only 2 had the endemic strain present. Since reconstructive patients seldom remained hospitalized for more than 2 weeks, a comparative study of the nasal microflora was conducted on a group of reconstructive patients, acute patients cultured on admission and hospital personnel. The latter group was included because of their continual exposure to the endemic strain (Fig. 1). The nasal microflora of reconstructive patients and hospital personnel were similar; neither group contained yeasts, and the gram-negative bacilli were transients. These two groups also contained nonlipophilic diphtheroids identified as Corynebacterium pseudodiphthericum. Among the hospital personnel, only two employees yielded endemic S. aureus, but the strain was not isolated a second time from the same individuals. The few reconstructive patients from whom the endemic strain was isolated had been previously acute patients in the Institute for approximately 6 months and 1 year, respectively, prior to the start of the study. The acute patients had the
fewest lipophilic diphtheroids present, no non-lipophilic diphtheroids, and the highest incidence of gram-negative bacilli. The acute patients alone contained yeasts in the nares. The nasal flora of acute patients was dynamic and changed rapidly after admission (Fig. 2) as the predominant organism in the nares became the endemic *S. aureus* strain.

During the 1-year study period, 10 patients died from various complications of burns. Despite the predominant colonization of acute patients with the endemic strain, including some positive blood cultures, autopsy examinations did not reveal a significant role of the endemic *S. aureus* strain in the deaths of these patients, five of whom contained the strain in burns.

Studies were therefore conducted to compare

**TABLE 1. Occurrence of Staphylococcus aureus in clinical specimens of acutely burned children**

| Specimen | Total cultured | Total positive* | Positive cultures with *S. aureus* | No. of patients with endemic strains in cultures |
|----------|----------------|-----------------|-----------------------------------|-----------------------------------------------|
|          |                |                 | *Endemic* | *Non-endemic* | |
| Blood    | 342            | 42              | 5        | 3             | 5  |
| Urine    | 905            | 413             | 25       | 5             | 19 |
| Catheters | 376  | 205             | 23       | 5             | 22 |
| Burns    | 2460           | 1084            | 102*     | 68*           |

*Data based on results of culturing 82 acutely burned children admitted from 1 July 1971 to 30 June 1972.*

*Specimen or culture containing any microorganism including staphylococci. Urines containing staphylococci were all <10^10/ml, whereas some urine specimens containing other microorganisms were >10^10/ml.

*A group of 34 patients yielded these strains in burns on admission and for 1 or 2 weeks after admission.

*Mean time of acquisition of endemic *S. aureus* in burns was 16 days.

**TABLE 2. Incidence and acquisition rate of endemic Staphylococcus aureus in nonclinical specimens and unburned body sites of acute patients**

| Body site or specimen | Total patients studied | Patients colonized | Mean time of acquisition (days) |
|-----------------------|------------------------|--------------------|---------------------------------|
| Oral cavity (saliva)  | 20                     | 8 (40%+)           | 22                              |
| Nares                 | 31                     | 27 (87%+)          | 17                              |
| Feces                 | 40                     | 11 (27%+)          | 36                              |
| Skin                  | 42                     | 26 (56%+)          | 16                              |

*Includes forehead, sternum, axilla, neck, groin, feet, and buttock.

**FIG. 1** Comparative nasal microflora of acutely burned children, reconstructive patients, and hospital personnel. *S. aureus*, nonendemic (coag. pos.), endemic *S. aureus* (end.), lipophilic (LD) and nonlipophilic (NLD) diphtheroids, and *S. epidermidis* (coag. neg.)

**FIG. 2** Sequential changes in the nasal microflora of 31 acutely burned children. Week zero represents nasal microflora of patients on admission.
the endemic and nonendemic strains with two other groups of *S. aureus* strains isolated from burns of adults in an adjacent Galveston burn unit (Sealy Hospital) and a Danish burn unit (Table 3). Compared to the SBI nonendemic strains, the endemic strains were nonhemolytic and exhibited little or no action against casein, gelatin, Tween 80, and egg yolk. The Danish strains which were phage type 84/85 and 83A/84/85 differed from the Galveston endemic phage 84/85 strains in the production of delta hemolysin and action on Tween 80 and casein. The Sealy strains differed from both Danish and endemic strains by production of alpha and delta hemolysins. The majority of all 162 strains tested acted on tributyrin and triolein and were deoxyribonuclease and hyaluronidase positive. Hyaluronidase production ranged from 10 to 20 enzyme units in strains that were positive, but no significant differences were found among the groups of strains tested.

The antibiotic sensitivity patterns of these same four groups of *S. aureus* were compared (Table 4). The endemic strains showed a marked degree of uniformity in antibiotic sensitivity which correlated with the monotypic phage pattern of this group. Over the last 18 months, approximately 1,500 strains of *S. aureus* isolated from burned children produced the same sensitivity pattern, and randomly selected strains representing about 1 of every 15 isolates which were typed consistently were of phage type 84/85. Only occasional variations in sensitivity patterns were observed in the endemic group but were related primarily to measurement of zone sizes. This was considered to be caused by minor variations in technique rather than changes in resistance of the strains. The 15 Sealy strains resembled the nonendemic strains in sensitivity, although the Sealy strains were of phage group III whereas the nonendemic strains were not of this group. The Danish strains differed from the endemic strains primarily in their sensitivity to ampicillin.

### DISCUSSION

Staphylococci are well known for their high incidence of isolation from burns and in burn units. Thomsen (19) determined the increased incidence of multi-antibiotic resistant strains of *S. aureus* phage type 84/85/6557/592 in a Danish burn unit. Thomsen found that this strain was isolated with increased frequency in extensive burns, in cases of prolonged infection, and in patients showing poor takes of grafts. The endemic strain described in this study colonized acute patients irrespective of the above factors reported by Thomsen and also colonized normal skin and other body sites of the acute patients. The strain, however, was essentially absent from the nares of hospital personnel and the nares and normal skin of a group of reconstructive patients who were cultured. The airborne microflora in the burn unit

| Antibiotic          | Groups and no. of strains tested |
|---------------------|----------------------------------|
|                     | 55 En-deemic | 71 Non- endemic | 21 Danish | 15 Sealy |
| Ampicillin          | 0*           | 5              | 21        | 4     |
| Cephalothin         | 55           | 71             | 21        | 15    |
| Erythromycin        | 0            | 45             | 0         | 3     |
| Gentamicin          | 55           | 71             | 21        | 15    |
| Methicillin         | 55           | 71             | 21        | 11    |
| Kanamycin           | 0            | 68             | 0         | 2     |
| Clindamycin         | 0            | 71             | 5         | 15    |
| Lincomycin          | 0            | 71             | 5         | 15    |
| Penicillin          | 0            | 6              | 4         | 1     |
| Oxacillin           | 55           | 71             | 21        | 15    |
| Streptomycin        | 0            | 60             | 0         | 3     |
| Sulfadiazine        | 0            | 62             | 0         | 14    |

* Number of strains sensitive—Kirby-Bauer disc method at 37°C.

### Table 3. Comparative biochemical characteristics of various Staphylococcus aureus isolated from burn patients

| Activity tested | Groups and no. of strains tested | 15 Sealy | 21 Danish |
|-----------------|----------------------------------|---------|-----------|
|                 | Shrine Burns Institute           | 55 En-deemic | 71 Non-endemic |
| Hemolysis       | Alpha                            | 0*       | 8         | 15       | 0         |
|                 | Beta                             | 0        | 34        | 3        | 6         |
|                 | Delta                            | 0        | 3         | 13       | 21        |
| Hydrolysis      | DNA                              | 51       | 65        | 15       | 21        |
|                 | Tween 80                         | 6        | 48        | 3        | 21        |
|                 | Triolein                         | 52       | 68        | 15       | 19        |
|                 | Tributyrin                       | 44       | 70        | 15       | 21        |
| Egg yolk reaction |                                 | 3        | 58        | 3        | 0         |
| Hyaluronidase   |                                  | 51       | 60        | 15       | 21        |
| Caseinolysis    |                                  | 2        | 47        | 0        | 21        |
| Gelatinase      |                                  | 2        | 40        | 2        | 8         |

* Number of positive strains of total tested.
has contained as much as 2 ft³ of endemic *S. aureus* per min (15). The affinity of the organism for only acute patients would indicate that shedding from patients was the primary source of the organism in air.

The origin of this strain is obscure but appeared to be first recognized in this burn unit in 1969-1970 when lincomycin became routinely used for sensitivity testing. Bulow (3) described phage group I staphylococci as being skin staphylococci and those of group III as mucous membrane types. Lacey, Alder, and Gillespie (7) found that phage group I staphylococci survived on skin more readily than group III strains. Bulow (3) later found that these differences in survival on skin could be related to Tween 80 hydrolysis and less quantitative hyaluronidase production by group I strains, whereas group III strains had the opposite biochemical characteristics.

Biochemical activities of staphylococci have often been compared to virulence or some other factors relating to host-parasite relationships. Chesbro, Wamola, and Bartley (4) found gelatinase activity and litmus milk reduction to correlate well with virulence of *S. aureus* but not nuclease, fibrinolysin, hemolysins, or coagulase. Thomsen (16) noted that the Danish strains were isolated more frequently in patients with clinical infection. Representative Danish strains tested produced delta hemolysin, were caseinolytic, and hydrolyzed Tween 80. The Galveston endemic strains were essentially negative as a group for these biochemical activities, and the strain was not the cause of a clinically documented infection during the study period which included data on 82 patients. The isolation of the strain from blood, urine, and catheter tips was difficult to ascribe to a definite etiological role for the organism, and the high colonization rate of patients would make this strain the most likely candidate for contamination of clinical specimens. The same strain might also be the most likely cause of infection in the seriously deteriorated patient. In the study by Linberg et al. (Abstr. Annu. Meet. Amer. Soc. Microbiol., p. 113, 1972), *S. aureus* phage type 84 was reported to cause septicemia and was isolated in autopsy specimens of lung tissue. Strains from these investigators were not available for comparative testing, but their strain was methicillin resistant. It may be significant that hospital-acquired infection had been linked to methicillin-resistant staphylococci (6), and only 4 of the 162 strains tested in this study were resistant to methicillin.

Lincomycin-resistant *S. aureus* strains were relatively unknown in the United States (5), and this appears to be one of the first reports on endemic lincomycin-resistant strains in a burn unit. The addition of lincomycin to media aided in the identification of the endemic strain in clinical specimens. Some prevailing endemic bacteria in burn units have been related to resistance of the strains which developed following the topical use of antibiotics in burn treatment (18). In this burn unit, gentamicin is used most extensively to treat septic patients (1) and clindamycin is given to new patients for 1 week to prevent group A streptococcal infections. Penicillin, oxacillin, and methicillin are used less frequently. The use of these drugs and the sensitivity of the endemic strain to only some of the compounds do not provide a clear explanation as to how antibiotic therapy would selectively promote colonization of burns by this organism. Silver sulfadiazine is used as a topical dressing, and patients are tubbed daily in dilute sodium hypochlorite. The factors involved in permitting any organism to colonize burns under the latter conditions of treatment also require study.

Thomsen (17) was not able to fully evaluate the role of his Danish endemic strains in clinical infection but raised the question of the influence of these burn-colonizing bacteria in tissue graft rejection. This would appear to be a significant consideration. If group III staphylococci are basically superficial burn-colonizing organisms which never contribute to deeper septic conditions, we share the concerns of Thomsen regarding the possible role of *S. aureus* in grafting operations which is an important part of the total welfare of the burn patient. Studies have been underway to evaluate our endemic strains in grafting failure because many purulent rejected grafts contain in excess of 10⁴ endemic staphylococci per gram of tissue. The clinical significance of the Sealy strains is unknown at this time. Finally, since *S. aureus* frequently occurs at skin sites in numbers inversely related to gram-negative bacteria (9); possible interference factors may also be occurring in terms of competitive colonization of burns.

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