JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1984, p. 417–420
0095-1137/84/030417-04$02.00/0

Vol. 19, No. 3

# Epidemiological Investigation of Exfoliative Toxin-Producing *Staphylococcus aureus* Strains in Hospitalized Patients

YVES PIEMONT,[1]* DAFINIE RASOAMANANJARA,[1] JEANNE M. FOUACE,[2] AND TOBIAS BRUCE[3]

*Institut de Bactériologie de la Faculté de Médecine, Université Louis Pasteur, 67000 Strasbourg[1]; Institut Pasteur, Paris[2]; and Service de Néonatologie, Centre Hospitalier Universitaire, Strasbourg,[3] France*

Received 11 August 1983/Accepted 17 November 1983

The incidence of exfoliative toxin-producing strains of *Staphylococcus aureus* was studied. Samples from hospitalized patients of all ages and samples from infants less than 6 weeks old were screened; out of 2,632 coagulase-positive *S. aureus* strains tested, 6.2% synthesized exfoliative toxin. The clinical features could be assessed in 86 patients harboring exfoliative toxin-producing staphylococci. Skin lesions (pustules, blisters, and bullous impetigo) could be observed only when the exfoliative toxin-positive strains were isolated from the skin. Phage nongroup II strains seemed less skin pathogenic than phage group II strains. Outbreaks and sporadic cases were observed.

Epidermal lesions referred to as Ritter von Rittershain syndrome, bullous impetigo, impetigo contagiosa, or more frequently staphylococcal scalded skin syndrome (SSSS) (16) are provoked by an excreted staphylococcal toxin (11, 12) called exfoliative toxin (ET) or exfoliatin. This proteic toxin splits the epidermis of humans and newborn mice between the *stratum granulosum* and the *stratum spinosum* without cell lesions; however, its mechanism of action is as yet unknown (4, 9, 10, 20).

Most of the studies concerning toxigenic staphylococcal strains were performed in patients suffering from SSSS. However, a few authors (2, 5, 6) studied the incidence of ET-producing strains among all strains of *Staphyloccoccus aureus* isolated from hospitalized patients. In this survey, we tried to evaluate the frequency of isolation of exfoliative toxin serotype A (ETA)- and ETB-producing staphylococci from coagulase-positive staphylococci isolated in the 4,000-bed University Hospital at Strasbourg, France. We also studied whether there was an epidemiological association between skin lesions (SSSS) and colonization of the skin or the nares with ET-producing staphylococci. Finally, we wished to determine whether certain ET-producing staphylococcal strains were peculiarly skin pathogenic and whether this ability was related to other biological markers.

## MATERIALS AND METHODS

**Identification of staphylococci.** Staphylococci were identified on the basis of classical tests: Gram staining, colony morphology, yellow pigment production, hemolysis, aerobic acid production from mannitol and sucrose, coagulase production, and phosphatase activity. For coagulase testing, the tube test was performed with reconstituted oxalated rabbit plasma.

**Clinical isolates of staphylococci.** In this survey, 2,632 staphylococcal strains were considered: 2,058 represented all coagulase-positive strains isolated in our laboratory between 1 June 1982 and 15 September 1982 (3.5 months) from patients of all ages hospitalized in the general hospital. The 574 other strains represented all coagulase-positive strains isolated in our laboratory between 1 November 1981 and 30 May 1982 (7 months) from hospitalized infants less than 6 weeks old. This last group of strains was included because the clinicians frequently observed bullae on the skin of these infants; therefore, systematic bacteriological examinations (nose, ear, eyes, skin, throat, and navel) were performed on these infants.

**Detection of ET production.** We prepared rabbit antisera against both ETA and ETB (14), which had been purified in our laboratory (15). ET production was tested by electrosyneresis as described by Piémont and Monteil (14). Cathodic wells were filled with supernatants of staphylococcal cultures grown in TY broth medium (7) under an atmosphere of 10% $CO_2$–90% air; anodic wells were filled with anti-ETA or anti-ETB sera. Both ETA and ETB were thus determined for each strain.

**Phage typing.** The ETA- and ETB-producing strains were phage typed according to the method of Blair and Williams (3). Thus phage group I (types 29, 52, 52A, 79, and 80), phage group II (types 3A, 3C, 55, and 71), and phage group III (types 6, 42E, 47, 53, 54, 75, 77, 83A, 84, and 85) were determined; moreover four nonclassified phages, 81, 94, 95, and 96, were used. For this purpose two concentrations of each phage were used, the critical dilution and a 100-fold-concentrated dilution. The phage type and group are reported on the basis of the more concentrated phage dilution.

**Clinical features.** The clinical features of 86 out of 115 hospitalized carriers of ET-producing strains could be determined on the basis of the written case reports. We could not obtain valuable information for some patients; for others, the case reports were not available. The assessment of the infected patients and the studied cases by age groups is contained in Table 1.

**Comparisons between qualitative parameters.** The statistical analyses were performed by using the chi-square test.

## RESULTS

**Isolation rate of ET-producing staphylococcal strains among the total number of cultured samples.** Among 48,200 clinical samples or swabs tested within a 3.5-month period for the presence of staphylococci and other bacteria, 2,058 *S. aureus* strains (4.3%) were isolated. Of these strains, 113 were ET positive (5.5%); 57 were among 412 (13.8%) strains isolated from infants less than 6 weeks old, and 56 were among 1,646 (3.4%) strains from older patients. During the other isolation period (7 months) involving only infants less than 6 weeks old, 50 ET-positive staphylococci were isolated

* Corresponding author.

TABLE 1. Distribution of ET-infected patients and of patients studied by age group

| Age of Patient | No. of patients harboring ET-positive staphylococci | No. of case reports available | % Cases studied[a] |
|---|---|---|---|
| 0 to 6 weeks | 62 | 43 | 69 |
| >6 weeks | 53 | 43 | 81 |

[a] Average percentage of cases studied, 75%.

out of 574 coagulase-positive strains (8.7%). Among the total 2,632 *S. aureus* strains belonging to both groups of patients and screened for exfoliatin production (163 strains), we observed that ET was most often ETA alone (144 strains; 88%). ETB alone (6 strains; 4%) or strains which were both ETA and ETB (13 strains; 8%) were less frequently encountered.

**Clinical features of patients infected with ET-producing staphylococci.** We could determine the clinical features of only 86 patients, who harbored 129 strains of ET-producing staphylococci. We designated as "skin pathogenic strains" (SPS) the ET-positive staphylococcal strains isolated from the skin or elsewhere and associated only with blisters, pustules, or bullous impetigo. The ET-positive staphylococcal strains leading to a superinfection of skin (itch lesions) or wounds or those found in other locations and not associated with pustules, blisters, or bullous impetigo were called "skin nonpathogenic strains" (SNPS).

**(i) Infants less than 6 weeks of age.** Among 43 infants studied, 13 had pustules or blisters; skin or umbilical swabs or both revealed the presence of ET-positive staphylococci in all of these patients except one, who harbored an ET-producing strain in his nose and not on his skin. Other swabs taken in the framework of systematic bacteriological examinations were also positive from 6 of these 13 patients (especially nasal swabs). No skin lesions related to ET were observed in 30 other infants who were hospitalized for various perinatal disorders. These 30 infants harbored ET-positive staphylococci on the skin only (9 patients) or in other locations (21 patients). Of the 30 patients, 1 had an ET-producing *S. aureus* strain which was recovered from throat, nose, stool, and gastric aspiration without any skin lesion.

**(ii) Patients more than 6 weeks of age.** Among 43 patients studied, 10 had skin lesions caused by SPS recovered from the skin: 7 bullous impetigo, 2 blisters, 1 pustule. Eight patients had other cutaneous injuries (wounds, intertrigo, itch, dermoepidermitis, eczema, or varicose ulcer) from which ET-producing staphylococci were isolated; these strains were therefore called SNPS. For the 25 other patients, toxigenic *S. aureus* strains were isolated from various swabs (nose, ear, eyes, and throat) or samples (sputum, blood, stool, and urine), and the patients concerned had no cutaneous injuries. Of six patients whose urine contained ET-producing staphylococci, three were women in obstetrical units; however their infants had no skin lesions during the first week of life.

The statistical analysis of the data obtained for infants less than 6 weeks old and the total data obtained for adults, children, and infants led to the same results. Blisters, pustules, and bullous impetigo occurred only when ET-producing staphylococcal strains were isolated from the skin or umbilical wound. When ET-producing strains were isolated from the skin, these clinical symptoms appeared in 12 cases of 21 in infants less than 6 weeks old and in 10 cases of 18 in children more than 6 weeks old and adults, i.e., in 22 cases of 39 in the total patient population studied. Moreover, the absence of ET-producing *S. aureus* in skin or umbilical swabs and its presence in other swabs (nose and throat) never resulted in blisters, pustules, or bullous impetigo, except in one case ($P < 0.001$).

**Relationship between phage groups and skin pathogenicity of ET-producing *S. aureus* strains.** Among 163 ET-producing strains, 131 belonged to phage group II (80%) and among 86 patients harboring ET-producing strains, 60 had phage group II strains (70%). When ET-producing strains of *S. aureus* were isolated from various sites in one patient, the phage types were always identical. The most often encountered phage types were those of phage group II, especially the phage type 3A, whether the strain was SPS or not. The phage types belonging to phage groups I and III and to the nonclassified group as well as the nontypable strains were more frequently SNPS than SPS. The distribution of the patients harboring SPS and SNPS according to the phage group of ET-producing staphylococci is reported in Table 2. The data suggested that the strains belonging to phage groups other than phage group II were less pathogenic for the skin ($P = 0.020$ for the 86 patients studied). However, only 22 phage group II ET-producing staphylococci of 60 (37%) were pathogenic for the skin. No correlation could be observed between the phage type or group and the serotype of the ET owing to the low number of ETB-producing strains (6 strains) and of both ETA- and ETB-producing strains (13 strains) which were available. For example, among 114 lysogroup II strains, 97 synthesized ETA, 12 ETA and ETB, and 5 ETB.

**Epidemiology.** The lysotypes of the strains and the serotypes of the toxin (A, B, or both) could be used as markers for epidemiological studies. Two outbreaks were observed during the 3.5-month period. The first involved 14 infants less than 6 months old from an obstetrical service and from an infant care unit, and the causative agent was phage type 55/71, ETA producing. The second outbreak occurred during the same period in another care unit, involving six infants less than 6 months old, and the causative agent was phage type 3A/3C, ETA producing. The durations of the outbreaks were 2 and 1 weeks, respectively. Ten cases were observed jointly with these outbreaks in the same care units and were considered to be sporadic since they had different lysotypic patterns; these last strains synthesized ETA or ETB or both. In this work, the majority of the observed cases were considered to be sporadic.

## DISCUSSION

The frequency of isolation of ET-producing staphylococci among the coagulase-positive staphylococci isolated in a

TABLE 2. Distribution of patients harboring SPS and SNPS according to phage group of ET-producing staphylococci

| Phage group | No. of patients with SPS | No. of patients with SNPS |
|---|---|---|
| II[a] | 22 | 38 |
| I[b] | 0 | 2 |
| III[b] | 0 | 4 |
| I and III | 1 | 2 |
| Nonclassified | 0 | 7 |
| Nontypable | 2 | 8 |

[a] Phage group II alone or associated with other phage groups.
[b] Phage group alone.

general hospital was 5.5%. Kapral, in a previous study (6), also found a frequency of 5%. Arbuthnott and Billcliffe (2) tested 98 *S. aureus* strains for ET production, including some likely producers of ET and also a number of strains selected randomly from an ongoing survey of staphylococci isolated from hospital infections; they found that 11% were ET-producing. Owing to the presence of strains very likely to produce ET in this last study, the percentage of ET-producing *S. aureus* strains should have been lower than 11% in a sample of *S. aureus* strains selected at random. The higher percentage noticed in infants less than 6 weeks old (8.7 and 13.8%, with an average of 10.9%), as compared to the older age group (3.4%), could be due in part to the bias constituted by cultures taken on infants when there was no evidence of skin infection (multiple sampling procedure performed by pediatricians). This discrepancy between both percentages was also partially related to the occurrence of outbreaks (particularly during the 3.5-month period) in infant care units.

The preponderance of ETA-producing strains (88%) over those producing ETB or both ETA and ETB could be related to possible outbreaks involving ETA-producing strains. However, most of the ETA-producing strains represented sporadic clinical cases since the 144 ETA-producing strains isolated were of 37 phage type patterns.

Since SSSS is associated with the presence of ET-producing staphylococcal strains on the skin, the mechanism of pathogenicity of these exfoliatin-producing strains could be explained by local action of the toxin and not by dissemination of ET produced by microorganisms in primary foci of the upper respiratory tract, eyes, or ears as presumed by Kapral (5). This assumption is reinforced by the fact that four patients (three adults and one infant) had septicemia with ET-producing staphylococci without any skin injury. We think that ET or the causative staphylococcal strain crosses the cutaneous barrier by means of latent excoriations or abrasions of the epidermis, since in adult patients skin lesions (bullous impetigo) occurred only on previously damaged skin and since we observed, as previously described by Nishioka et al. (13), that the presence of purified ET on the outer side of newborn mouse skin did not cause either macroscopic or histological changes in the epidermis. However, it must be remembered that *S. aureus* is capable of producing a large array of biologically active products, and the role of undefined staphylococcal substances which help ET to penetrate the skin cannot be excluded. When ET or staphylococci penetrated the cutaneous barrier, skin lesions did not necessarily appear, since antibodies which could neutralize the toxin were present in adults and children. Antibodies against ETA have been found in 20 to 75% of the sera and those against ETB in 4% of the sera (17, 18). However, exfoliatin antibody titers in adults and children were similar (17, 18), and therefore, the humoral immunity did not account for the apparent susceptibility of children to SSSS. Furthermore, we do not know whether the production (or the level of production) of ET in vivo by staphylococcal strains depends on the location of the staphylococcal strains (nose, ear, urine, blood, or skin) as hypothesized by Kapral (6). The presence or absence of ET production in vivo could be demonstrated by using biological fluids (urine, blood, sputum, etc.) in which ET-producing strains were isolated; however, the determination of toxin in these fluids should be performed by techniques more sensitive than electrosynerésis and newborn mice tests.

As expected, phage group II strains were more often associated with SSSS; however, type 3A rather than type 71 was most common. This was a different epidemiological pattern than previously found in the United States and elsewhere. No correlation between the lysotypic pattern of the strains and the type of toxin produced (ETA, ETB, or both) could be established as was also pointed out by Kondo et al. (8).

Frequent outbreaks occurred in groups of infants as described above and elsewhere (1, 19). Sporadic cases of cutaneous injuries were often observed particularly in children, but nasal carriage of such strains was noticed in all patients and may be an important reservoir of pathogenic strains for future outbreaks. Since the epidemiological status of ET-producing staphylococci could vary according to country (in Japan, for example, 19 SSSS cases of 43 were due to phage group III staphylococci [8]), it would be interesting to know whether these results are applicable to other countries.

## LITERATURE CITED


1. **Anthony, B. F., D. M. Giuliano, and W. Oh.** 1972. Nursery outbreak of staphylococcal scalded skin syndrome. Am. J. Dis. Child. **124**:41–44.
2. **Arbuthnott, J. P., and B. Billcliffe.** 1976. Qualitative and quantitative methods for detecting staphylococcal epidermolytic toxin. J. Med. Microbiol. **9**:191–201.
3. **Blair, J. E., and R. E. O. Williams.** 1961. Phage typing of staphylococci. Bull. W.H.O. **24**:771–784.
4. **Elias, P. M., P. Fritsch, M. V. Dahl, and K. Wolff.** 1975. Staphylococcal toxic epidermal necrolysis: pathogenesis and studies on the subcellular site of action of exfoliatin. J. Invest. Dermatol. **65**:501–512.
5. **Kapral, F. A.** 1974. *Staphylococcus aureus*: some host-parasite interactions. Ann. N.Y. Acad. Sci. **236**:267–276.
6. **Kapral, F. A.** 1975. *Staphylococcus aureus* exfoliatin, p. 263–266. *In* D. Schlessinger (ed.), Microbiology—1975. American Society for Microbiology, Washington, D.C.
7. **Kapral, F. A., and M. M. Miller.** 1971. Product of *Staphylococcus aureus* responsible for the scalded skin syndrome. Infect. Immun. **4**:541–545.
8. **Kondo, I., S. Sakurai, Y. Sarai, and S. Futaki.** 1975. Two serotypes of exfoliatin and their distribution in staphylococcal strains isolated from patients with scalded skin syndrome. J. Clin. Microbiol. **1**:397–400.
9. **Lillibridge, C. B., M. E. Melish, and L. A. Glasgow.** 1972. Site of action of exfoliative toxin in the staphylococcal scaled-skin syndrome. Pediatrics **50**:728–738.
10. **Mc Lay, A. L. C., J. P. Arbuthnott, and A. Lyell.** 1975. Action of staphylococcal epidermolytic toxin on mouse skin: an electron microscopic study. J. Invest. Dermatol. **65**:423–428.
11. **Melish, M. E., and L. A. Glasgow.** 1970. The staphylococcal scalded skin syndrome: development of an experimental model. N. Engl. J. Med. **282**:1114–1119.
12. **Melish, M. E., L. A. Glasgow, and M. D. Turner.** 1972. The staphylococcal scalded skin syndrome: isolation and partial characterization of the exfoliative toxin. J. Infect. Dis. **125**:129–140.
13. **Nishioka, K., I. Katayama, and S. Sano.** 1981. Possible binding of epidermolytic toxin to a subcellular fraction of the epidermis. J. Dermatol. **8**:7–12.
14. **Piémont, Y., and H. Monteil.** 1983. Mise en évidence par électrosynérèse des deux sérotypes d'exfoliatine produits par *Staphylococcus aureus*. Ann. Inst. Pasteur **134A**:169–175.
15. **Piémont, Y., and H. Monteil.** 1983. New approach in the separation of two exfoliative toxins from *Staphylococcus aureus*. FEMS Microbiol. Lett. **17**:191–195.
16. **Rogolsky, M.** 1979. Nonenteric toxins of *Staphylococcus aureus*. Microbiol. Rev. **43**:320–360.
17. **Sarai, Y., H. Nakahara, T. Ishikawa, I. Kondo, S. Futaki, and K. Hirayama.** 1977. Detection of anti-exfoliatin antibodies in healthy adults and children by the passive hemagglutination

test. Infect. Immun. **16**:1024–1026.
18. **Wiley, B. B., L. A. Glasgow, and M. Rogolsky.** 1976. Staphylococcal scalded-skin syndrome: development of a primary binding assay for human antibody to the exfoliative toxin. Infect. Immun. **13**:513–520.
19. **Willard, D., H. Monteil, Y. Piémont, R. Assi, J. Messer, J. Lavillaureix, R. Minck, and R. Gandar.** 1982. L'exfoliatine dans les staphylococcies néonatales. Nouv. Presse Med. **11**:3769–3771.
20. **Wuepper, K. D., R. L. Dimond, and D. D. Knutson.** 1975. Studies of the mechanism of epidermal injury by a staphylococcal epidermolytic toxin. J. Invest. Dermatol. **65**:191–200.