

Importance of Exfoliatin Toxin A Production by *Staphylococcus aureus* Strains Isolated from Clustered Epidemics of Neonatal Pustulosis

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Clustered epidemics of pustulosis due to *Staphylococcus aureus* occurred in two geographically distant newborn nurseries. In nurseries A and B an attack rate of pustulosis of 0.8 and 2.0 cases per 100 live births occurred, respectively. Experimental phage type 1046/1116 belonging to phage group II dominated clustered epidemics in nursery A, while group II phage type 3A/3C/55/71 and 3A/3C/55 occurred in nursery B. Other group II strains also occasionally produced clustered epidemics. These epidemic strains were found to be making heat-stable dermal exfoliatin toxin A (ETA) which had a pI of 6.8 and a molecular weight of 32,000 and 33,000. ETA-bearing strains did not make bacteriocin. Children infected with ETA-producing strains developed extensive bullous pustulosis. Surveillance cultures of personnel revealed an ETA-bearing strain in only one person. This strain was not the same phage type as the epidemic cluster. In contrast, ETA-bearing epidemic strains were found in the inanimate environment of both nurseries. ETA protein acts as an important virulence factor in the production of neonatal pustulosis infection and appears to be linked with the ability of *S. aureus* organisms to stick to the inanimate environment.

In the 1950s *Staphylococcus aureus* 80/81 caused a pandemic of outbreaks of pustulosis and abscess formation in newborns (5, 8, 10, 35, 37, 44). These epidemics have ceased with better means of infection control (2, 12, 13, 30, 45). Recently, however, group II organisms have been reported to produce sporadic nursery outbreaks (1, 3, 4, 14, 22, 34) characterized by bullous impetigo, erythema, and the scalded skin syndrome (28). These dermal disorders are related to the production of an exfoliatin toxin (7, 23, 27, 28) which in the newborn mouse can cause a characteristic separation of cells at the midepidermal junction (7, 23, 27), allowing skin to be readily denuded with gentle pressure (24, 25, 46). By use of the newborn mouse model, a heat-labile protein responsible for exfoliation has been isolated (16, 18, 27, 32) from culture fluid supernatants of *S. aureus* strains associated with scalded skin syndrome. The generation of this protein is plasmid mediated (41, 42). The same plasmid also carries genetic information for bacteriocin production (15, 19, 26, 31, 39, 40, 43). Cultivation at 44°C eliminates this plasmid. A second exfoliatin has been described which is chromosomally mediated and heat stable (20, 36). These two exfoliatins, although having the same molecular weight and isoelectric point, are antigenically distinct. They are now called exfoliatins B (ETB) and A (ETA) (ETB, heat labile and plasmid induced; ETA, heat stable and chromosomally mediated) (43).

Production of toxin is not exclusive to phage group II strains (20), and epidemics of scalded skin syndrome have occurred with organisms other than group II (6, 9, 11). While

toxin ETA and ETB have been shown to be responsible for isolated cases of staphylococcal pustulosis complicated by exfoliation, the full extent of their importance in outbreaks of pustulosis in the newborn has been only briefly investigated (1, 3, 4, 14, 22, 34). In this study we show that ETA-producing strains of different phage types dominated two different nursery outbreaks of pustulosis. These same strains could not be found in personnel caring for these children. In contrast, they were found in the inanimate environment and they are believed to be the source of the continued epidemic. The importance of this observation is discussed.

MATERIALS AND METHODS

In nursery A systematic prospective surveillance for pustular disease of the newborn has been occurring since 1980. In nursery B active surveillance began in September 1982 when an outbreak of pustulosis was noted. These two nurseries are separated geographically by about 50 miles and have never shared patients. All pustules occurring in hospitalized neonates were cultured for the presence of *S. aureus*. Physicians caring for children discharged from the nursery were regularly asked to report children who developed pustulosis after discharge. All readmissions of neonates with staphylococcal infections were evaluated to ascertain whether the child contracted the infection while in the newborn nursery. In instances where private physicians performed a culture of a pustule which grew *S. aureus*, attempts were made to retrieve the organism for phage typing.

Infection index. All charts of children with pustulosis hospitalized in nursery A were reviewed retrospectively to determine the site of pustular involvement. An attempt was made to quantitate the extent of pustulosis. Each area of skin

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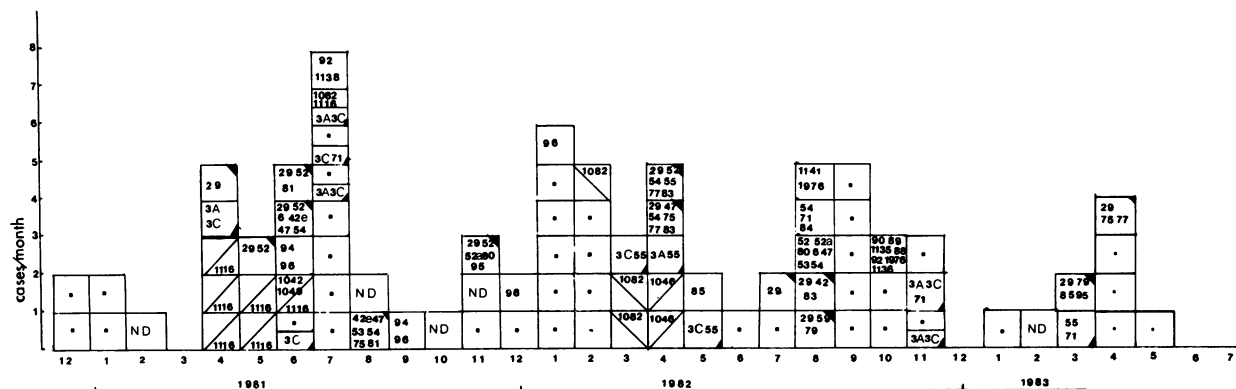


FIG. 1. Each box represents a case of pustulosis in nursery A. The following symbols indicate different phage types: \square , 1046/1116; \square , 1116; \square , 1046/1116/3C; \square , 1046/1116/3A/3C; \square , 1046; \square , 1046/1116/3C/71; \square , 1046/1082/1116/3A/3C; \square , strains not typed. Boxes with black triangles in the right lower corner are phage type 2 organisms. Boxes with black triangles in the right upper corner are phage type 1 organisms. Note that the epidemic strains 1046/1116 and 1116 occur sporadically in clusters superimposed on a background of many different staphylococcal strains.

involved was given a score of one to include the left or right groin, axilla, leg or arm, and the chest, neck, face, back, perineum, or periumbilical area. The total of all areas involved were added up to give the infection index. In nursery B, since most cases of pustulosis occurred after the child was discharged from hospital, indexing was not possible.

Bacteriophage typing. Staphylococcal phage typing was carried out by the New York City Department of Health, using the international phage set and experimental phages.

Identification of *S. aureus*. Cultures of skin or nasopharynx were obtained with cotton swabs and inoculated onto blood agar plates. Colonies characteristic of *S. aureus* were differentiated by testing for coagulase production and other properties characteristic of this organism (17).

Preparation of *S. aureus* culture filtrate. Each organism was grown in a chemically defined medium described by van de Rijn and Kessler (38). Organisms were grown in 100 ml of medium for analysis by isoelectric focusing or for analysis by polyacrylamide gel electrophoresis and in 1,000-ml batches for preparative isoelectric focusing. Organisms were incubated at 37°C for 72 h. After growth, organisms were separated from culture filtrate by centrifugation at $16,300 \times g$. The supernatant was then passed through 0.45- μ m-pore-size Nalgene sterilization filter units (Nalge Co., Rochester, N.Y.) to eliminate bacteria. Crude culture filtrates were concentrated to powder by freeze drying at -20°C and were reconstituted in sterile distilled water to be concentrated $50\times$. This solution was then clarified by centrifugation at $16,300 \times g$ for 30 min. Crude culture filtrate was dialyzed in tubing with a molecular weight cut off of 12,000 (3787-D 42; Arthur H. Thomas, Co. Philadelphia, Pa.) against distilled water and stored at -20°C before use.

Bacterial reference strains. Bacteria which produced ETA or ETB were kindly given to us by M. Rogolsky (University of Missouri, Kansas City, Mo.) and R. L. Warren (University of Ohio, Dayton, Ohio). These strains were RW 1001 and UT0002 (which produced both ETA, ETB, and bacteriocin [BacR1]), UT0007 and UT0001 which produced ETB and BacR1 and UT0100 and UT0003 which failed to produce ETB or BacR1. Purified enterotoxin F (toxic shock antigen) and antiserum to enterotoxin F were generously given to us by M. Bergdoll (University of Wisconsin, Madison, Wis.).

Bacteriocin assay. This was carried out according to the method of Anthony et al. (3). Indicator strain 502A grown overnight at 37°C in Trypticase soy broth was diluted 1:100 in saline solution, and 0.1 ml of inoculum was spread as a lawn onto Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, Md.). This lawn was then stabbed with freshly grown cultures of staphylococci. Bacteriocin activity was considered to be present if the organism in the lawn failed to grow about the stabbed culture after 24 h at 37°C. Reference strains UT0002 and UT0007 were used as positive controls, and UT0003 and UT0100 were used as negative controls. Similar methodology was used to test culture filtrates for bacteriocin, except that wells were cut out to hold 0.1 ml of solution.

Exfoliatin toxin assay. Mice under 3 days of age were injected subcutaneously in the back with 0.1 ml of culture filtrate or proteins purified by isoelectric focusing. Animals were then inspected at 4 to 6 h and again after 24 h for evidence of exfoliation, skin wrinkling, skin hemorrhage, or for death.

Rabbit antisera. Albino rabbits (2 to 3 months old) were immunized with protein antigens described in this paper. Antigen (0.5 ml) mixed with equal volumes of Freund incomplete adjuvant (Difco) was injected subcutaneously. A booster dose with adjuvant was given after 7 to 10 days. Antigens were then administered each week intravenously starting at 0.05, 0.1, 0.2, 0.4, 0.6, and finally 0.8 ml. Animals were bled by cardiac puncture 7 days after the last injection.

Isoelectric focusing. Concentrated culture filtrates were analyzed for their protein constituents by isoelectric focusing using the Ampholine-polyacrylamide gel plates pH 3.5 to 9.5 (LKB, Bromma, Sweden). A 20- μ l amount of each filtrate was placed approximately 20 mm from the cathode. Plates were electrofocused for 1.5 h at 30 W and 1,500 V at 10°C. Gels were fixed in trichloroacetic acid and sulfasalicylic acid and stained in 0.115% Coomassie blue at 60°C. Direct pH measurement of the gels was made with a Corning electrode (Corning Glass Works, Medfield, Mass.).

Preparative isoelectric focusing. Dialyzed culture filtrate (1 liter) was concentrated by lyophilization to approximately 50 to 60 ml. Ampholine solution (5 ml) (pH 3.5 to 9.5) (LKB) was added to this concentrate, and distilled water was added to make a final volume of 95 ml. Ultradex (4 g) (LKB) was

TABLE 1. Nursery A

Group	Phage type	No.	Prolonged hospital stay	Readmission (days)	Infection score	Day of onset	♂	♀
II	1046/1116	34	6	8	2.5	4.6	13	21
	1116	5	0	2	2.4	6.6	2	3
	1046/1116 plus other	5	0	0	1.6	5.2	2	3
	Other group II	7	0	0	1.4	4.2	4	3
	1046	2	1	0	1.3	3.0	1	1
I	Multiple types including 29 or 52	13	0	0	1.7	4.0	11	2
III	85/or multiple	2	0	0	1.0	3.5	1	1
Other	Experimental phage	7	0	0	1.0		5	2
	94/96 or 94	4	0	0	0.8	5.0	2	2
	Not typed	5	0	0	NA"			

" NA. Not applicable.

then added, and the slurry was poured into a gel tray to create an even suspension. Water was evaporated from the slurry to the evaporation limit specified by the manufacturer. Electrofocusing was performed at 4°C and 8 W overnight. A Zymogram paper print was taken of each gel before dividing the gel into 30 sections. Direct pH measurements were obtained for each fraction. Fractions containing proteins of interest were collected and tested for biologic activity.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Molecular weight determinations were made on 13% sodium dodecyl sulfate-Laemmli gels (21). Culture filtrate (40 µl) or purified protein (40 µl) was combined with 20 µl of lysis buffer (0.25% bromphenol blue, 0.5 M Tris hydrochloride [pH 6.8], 10% glycine, 10% sodium dodecyl sulfate, 5% 2-mercaptoethanol) and boiled for 3 min. Electrophoresis was performed in a Protean cell (BioRad Chemical Div., Richmond, Calif.) with Tris-glycine running buffer (pH 8.3) overnight at 30 V, 40 mA at 10°C. Electrophoresis was

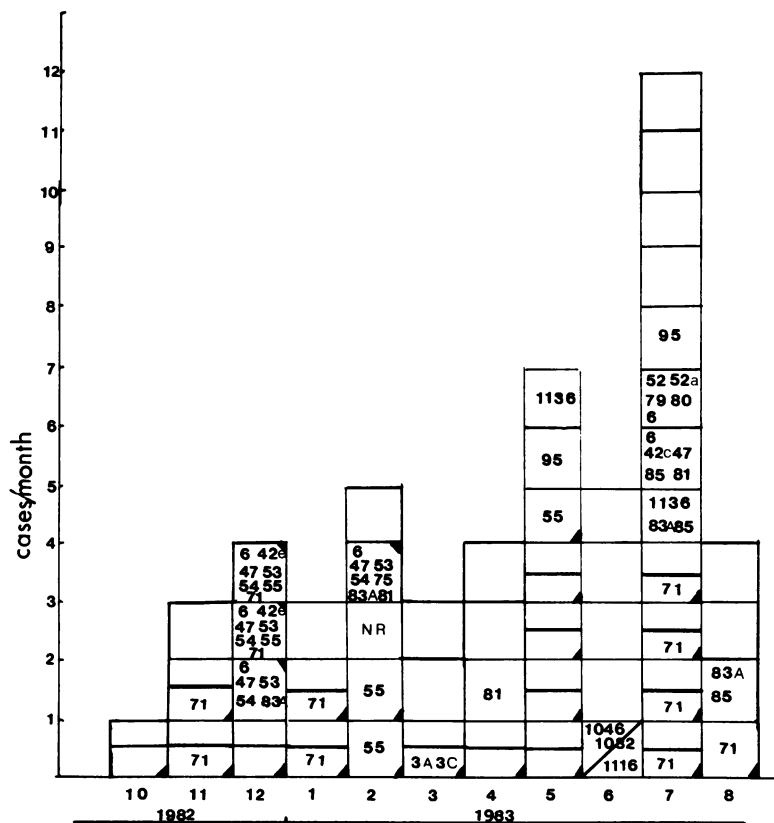






FIG. 2. Each box represents a case of pustulosis in nursery B. The following symbols indicate different phage types: , 3A/3C/55; , 3A/3C/55/71; , strains not phage typed; , strains that did not react with any phage type. Boxes with a black triangle in the right lower corner are phage type 2, and those with a black triangle in the right upper corner are phage type 1.

TABLE 2. Nursery B

Group	Phage type	No.	Prolonged hospital stay	Readmission (days)	Day of onset	♂	♀
II	3A/3C/55 or 3A/3C/55/71	15	0	4	9	8	7
	Other	6	0	1	9	3	3
I	81 and 52/52A/79/80/6	2	0	1	14	2	0
III	Multiple types	5	0	1	7	2	3
	Other	2	0	1	3	2	0
Other	Experimental phage	4	0	0	7	4	0
	Not typed	17	0	0	13	NA ^a	

^a NA, Not applicable.

stopped when the dye reached the end of the gel. Gels were fixed in 50% trichloroacetic acid, stained in 0.5% Coomassie blue, and destained in 30% ethanol-10% acetic acid. High- and low-molecular-weight standards (Pharmacia Fine Chemicals, Piscataway, N.J.) were run with each gel.

RESULTS

Characteristics of the pustular epidemic in nurseries A and B. In nursery A, 84 cases of *S. aureus* pustulosis occurred in 10,442 newborn infants (0.8 cases per 100 live births) over 32 months (Fig. 1). The dominant infecting strain of *S. aureus* failed to type with standard phages but did type with an experimental phage, 1046/1116. The 46 infants infected with organisms that typed with 1046 or 1116 developed extensive infection, with an infection index of 2.5 (Table 1). Of these infants, 10 had to be rehospitalized for a total of 55 days for treatment of their pustulosis shortly after being discharged from the hospital. Seven additional neonates required hos-

pitalizations of greater than 7 days after they were born (average, 11 days). Children infected with these strains demonstrated extensive bullous impetigo, with multiple sites of involvement. Of 46, 2 had typical scalded skin syndrome. In contrast, of 31 children infected with other phage group strains, 1 child was readmitted to hospital for treatment of a positive blood culture which proved to be a procurement contaminant, and only 1 child was treated for extensive pustulosis. The average pustule score was low (Table 1).

In Nursery B, 51 of 2,384 newborn infants developed *S. aureus* pustulosis (2 of 100 live births) over an 11-month period (Fig. 2). The dominant epidemic strains belonged to phage group II and typed as 3A/3C/55/71 (8) or 3A/3C/55 (7). A total of five children infected with the group II strains in nursery B also had to be rehospitalized for treatment of extensive pustular disease (Table 2). The onset of pustulosis due to the major epidemic strains 3A/3C/55/71 and 3A/3C/55 in nursery B was later than that of pustulosis due to epidemic strain 1046/1116 in nursery A (9 days versus 4.6 days).

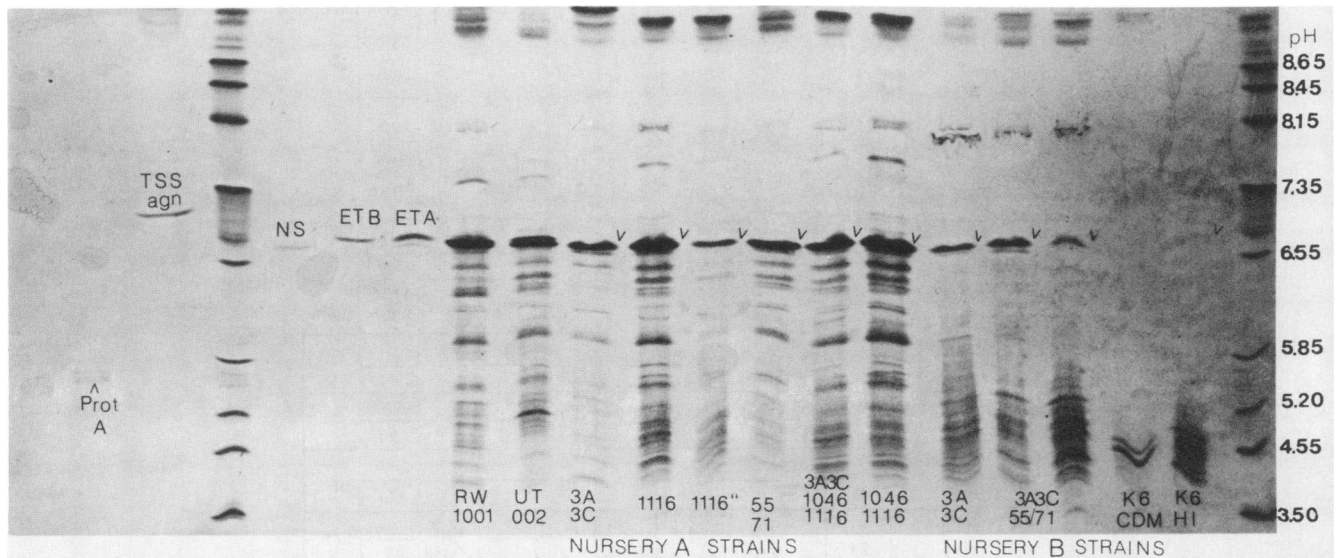


FIG. 3. Protein patterns produced by isoelectric focusing of culture filtrates of pustulosis strains isolated from babies in nurseries A and B. Protein A is purified protein A (Pharmacia). T55 antigen is the toxic shock antigen (kindly given to us by M. Bergdoll). NS is a highly antigenic protein common to most *S. aureus* strains. Note the second band with ETA. This represents NS as described in the text. RW1001 and UT002 are reference strains which make ETA and ETB. Nursery A and nursery B strains are from representative isolates from each nursery which were making ETA as indicated by the arrow. Strain K6 produced only minimal quantities of ETA when grown in chemically defined media but produced detectable amounts when grown in medium containing heart infusion dialysate.

TABLE 3. Analysis of ETA production in representative strains isolated from infants with pustulosis

Group	Nursery A			Nursery B		
	Phage type	ETA (+)	ETA (-)	Phage type	ETA (+)	ETA (-)
II	1046/1116	13	1	3A/3C/55/71	8	0
	1116	3	0	3A/3C	1	0
	1046/1116	1	0	1046/1082/1116	1	0
	3A/3C					
	1046/3A/3C	1	0			
	3A/3C	1	0			
	55/71	1	0			
I	29/multiple and 29	0	2	52/52A/multiple	0	1
Other	94/96,96,95	0	3	95 and 85/95	0	2
III				6/42a/multiple and 81	0	4

Protein patterns of epidemic strains in both nurseries. Evidence supporting the importance of ETA protein in the epidemic. In preliminary studies, culture filtrates of representative strains were analyzed by isoelectric focusing to study the protein patterns of pustular strains. The 1046/1116 strain produced considerable amounts of a protein which we suspected was ETA with an isoelectric point of 6.8 (Fig. 3). On careful inspection of the isoelectric focusing gel, a second protein could be seen which focused at about the same pH. This protein is called new substance (NS). These two proteins were purified by preparative isoelectric focusing as described with a representative 1046/1116 strain. These two proteins were difficult to separate to great purity by this technique. Nonetheless, semipurified material was injected into rabbits, producing an antiserum which demonstrated two bands on immunodiffusion (anti-ETA and anti-NS) when tested against proteins that focused at pH 6.8. To distinguish anti-ETA from anti-NS, we found another group II strain which lacked exfoliatin activity in the newborn mouse model but which had one distinct protein at pH 6.8. Individual proteins in 1,000 ml of culture filtrate of this strain were separated by preparative isoelectric focusing. The material focusing at pH 6.8 reacted with our bivalent antiserum, producing only a single band on immunodiffusion. This protein proved to be NS. It was then used in immunodiffusion plates as a reference protein to enable us to distinguish between precipitin lines due to NS and those due to ETA when we tested unknown culture filtrates or purified proteins for presence of ETA activity using our bivalent antiserum.

Culture filtrates from all available *S. aureus* isolates from infants with pustulosis were prepared as described, and their protein composition was analyzed by isoelectric focusing. Clustered epidemic strains 1046/1116 and all 1116 strains from nursery A, all 3A/3C/55/71 and 3A/3C/55 strains in nursery B, and other group II strains in both nurseries were found to possess a strong band at pH 6.8 (Fig. 3). Culture filtrates from these strains were then tested in immunodiffusion assay for evidence of ETA protein. All but one available 1046/1116 strain and all 1116 in nursery A and all 3A/3C/55/71 and 3A/3C/55 strains in nursery B were found to possess ETA protein (Table 3). Phage type 1046/1116 produced far greater quantities of ETA than did strain 3A/3C/55/71 or 3A/3C/55. Some group I and group III strains had bands at pH 6.8, but on immunodiffusion these proved to be NS.

Biologic activity of ETA. Culture filtrates from representative strains that reacted to ETA antisera uniformly produced

exfoliation in the newborn mouse assay. Purified NS and strains that contained NS only but not ETA did not produce exfoliation in this assay. Purified ETA protein was not inactivated by heating at 60°C when tested either in the

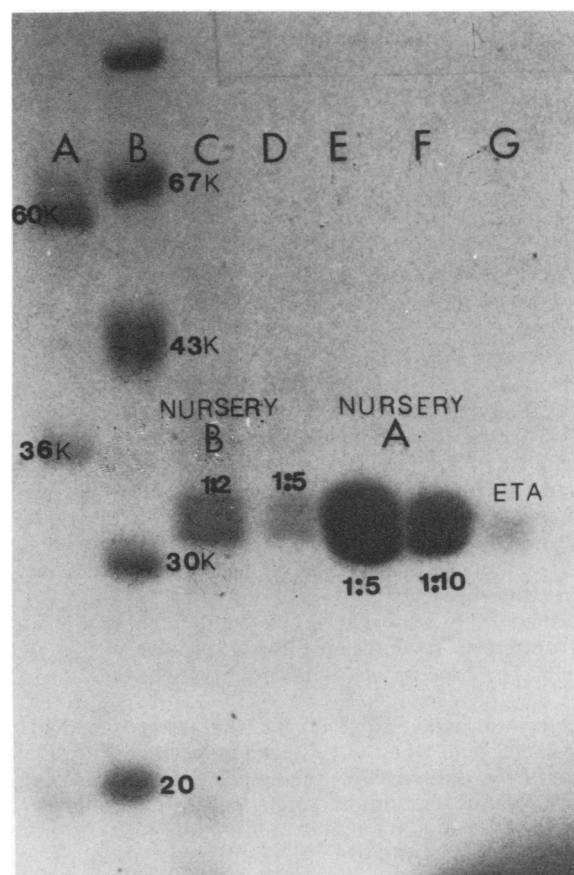


FIG. 4. Laemmli gel of purified ETA from strain 3A/3C/55/71 of nursery B and from strain 1046/1116 of nursery A. The molecular weight is estimated to be 32,000. Bands appear as doublets. Strain 1046/1116 produced considerably more ETA than did strain 3A/3C/55/71 when the two strains were grown in the same volume of chemically defined medium. By analyzing the culture filtrate for ETA by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, strain 1046/1116 produces very intense bands in contrast to strain 3A/3C/55/71 as shown in this representative gel.

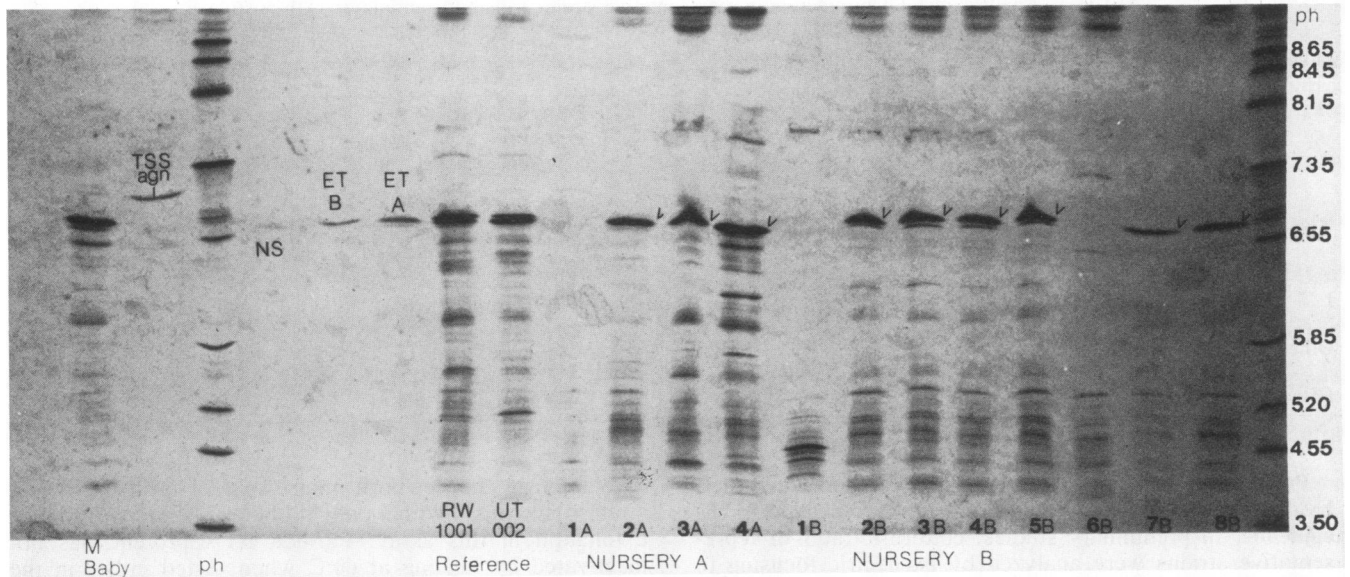


FIG. 5. Protein pattern on isoelectric focusing of culture filtrates of environmental isolates in nurseries A and B. M baby, strain 1046/1082/1116 isolated from a child in nursery A; TSS, toxic shock antigen (M. Bergdoll); RW 1001 and UT0002, strains producing both ETA and ETB; 1A, closet handle (note absence of ETA); 2A, magazine isolate; 3A, stethoscope bell isolate 1046/1116; 4A, ophthalmoscope isolate 1046/1116/3C/71; 1B, isolated from the floor of nursery B (note the absence of ETA); 2B-5B, 7B, and 8B, isolates obtained from different air ducts in nursery B typing with 3A/3C/55/71; and 6B, a strain that typed with 6/53/83A/84/81 (note absence of ETA). Arrows indicate ETA.

newborn mouse assay for exfoliatin activity or in antigen assay against antisera ETA. This suggested that it was ETA, which is known to be a heat-stable exfoliatin (19, 20, 31, 33, 43). To prove this, we obtained representative ETA and ETB strains from R. L. Warren and M. Rogolsky. Our strains showed lines of identity to strains that were known ETA-bearing organisms. We had also isolated a phage group II strain from a 1-year-old child who had acute tonsillitis characterized by extensive exfoliation about the lips, chin, neck, and face. This strain also had a protein that focused at pH 6.8. This protein was similarly semipurified by preparative isoelectric focusing and an antisera was produced. This antisera also contained two precipitin bands. One line proved to be anti-NS. The other band proved to be ETB when tested by immunodiffusion against known ETB strains. This antisera failed to detect any ETB-bearing strains isolated from any of the infected children or colonized personnel in either nursery.

Purified ETA was also subjected to polyacrylamide gel electrophoresis to determine the molecular weight of the protein. The protein has a molecular weight of 32,000 (Fig. 4).

Bacteriocin assay. All ETA-bearing strains failed to produce bacteriocin when tested against reference strain 502a.

Search for the source of the epidemic strains. During the major epidemics in both nurseries, cultures were taken from 458 personnel and 206 noninfected infants to try to find the source of the epidemic strains. An epidemic strain was found in only one person in nursery B and in one infant without pustulosis, despite isolation of numerous *S. aureus* organisms from noninfected personnel and infants (Table 4). A few individuals did however carry nonepidemic group II organisms. Not all of these cultures were available for study. However, when available strains were analyzed by isoelectric focusing and tested for the presence of ETA antigen (Table 5) using our ETA antisera, no ETA-bearing strains were found except in one nurse who had a paro-

nychia. In this nurse, the group II strain was not the same as the epidemic strain as determined by its pattern in isoelectric focusing (Table 5).

In contrast, cultures of the inanimate environment of nursery A and nursery B were found to harbor the epidemic strains. In nursery A in two repeated instances 1046/1116 colonized an ophthalmoscope that was used to examine all newly admitted neonates to one of the nursery rooms. In another instance it was found to be present on a stethoscope bell that was used by many physicians to examine newborns just admitted to the nursery. In a third instance it was found contaminating a common laundry cart. In nursery B the epidemic strains 3A/3C/55/71 and 3A/3C/55 were found colonizing the entire air duct system going into the nursery rooms (Table 6). Culture filtrates of these environmental strains from both nurseries were tested by isoelectric focusing and found to contain a protein with pH 6.8 (Fig. 5), which proved to be ETA by immunodiffusion analysis. The association of ETA strains with the environment is striking in

TABLE 4. Distribution of phage types in colonized infants and personnel

Group or type	Nursery A		Nursery B	
	Personnel	Infants	Personnel	Infants
I	20	5	3	3
III	10	5	3	1
94/96 and 95	11	2	7	0
Experimental and nontypeable	11	5	4	3
II-nonepidemic strains	3	5	1 ^a	3
Not typed			7	
Total	55	22	25	10
Total no. cultured	322	206	136	

^a Isolate from paronychia.

contrast with its lack of association with noninfected infants and personnel. Thus, 11 of 13 environmental strains produced ETA, in contrast to 2 of 42 isolates that were available for study from noninfected personnel and infants (Table 5).

DISCUSSION

The appearance of epidemics of pustulosis in the newborn continues to be a major public health problem. In this study, we describe two separate nurseries which had cluster outbreaks of pustulosis. In nursery A, a strain which typed with an experimental phage, 1046/1116, dominated one epidemic, while in nursery B, two strains, 3A/3C/55 and 3A/3C/55/71, appeared (Fig. 1 and 2). In nursery A, two other outbreaks appeared in small clusters. These clustered outbreaks were superimposed on a background of pustulosis due to many different phage types. Characteristically, the strains for these clustered epidemics produced a heat-stable protein which we have shown to be an exfoliatin toxin. These strains reacted with antisera developed in our laboratory to ETA. These strains failed to make bacteriocin. All these features indicate that these strains were characteristic of ETA-producing *S. aureus*.

Epidemics of pustulosis in newborns due to exfoliatin toxin-bearing strains have begun to appear in recent years. Two outbreaks caused by phage type 71 strain have been described, afflicting 14 infants in one study and 3 infants in another nursery (14). Light et al. (22) described an outbreak of bullous impetigo in 19 children due to a strain that lysed with phage type 3B/3C/55/71. Outbreaks due to 3B/71, affecting 34 infants in one nursery (1) and 11 infants in another nursery (34) have also been described. Recently Curran and Al-Salihi (6) described a massive nursery epidemic of scalded skin syndrome attacking 68 newborns in a short time. The epidemic strain was found to type as 29/79/80/3A/3C/54/75. Another epidemic has been found to be due to 42E/54/75 (11). In these outbreaks, the exfoliatin toxin has never been typed, nor have the proteins produced by these strains been studied in any great detail. In the outbreak described by Anthony et al. (3), their strain produced bacteriocin, which is a property of ETB. Piemont et al. (29) recently described a sporadic epidemic involving 14 infants due to ETA-producing strain 55/71 and another sporadic outbreak involving six infants on an obstetrical unit due to ETA-producing strain 3A/3C. To our knowledge, our epidemics represent the clearest documentation of the role of ETA-producing strains in newborn pustulosis.

Children infected with epidemic strains clearly had more severe infections, with several areas of skin involved with

TABLE 5. Analysis of ETA production in representative and strains isolated from colonized personnel and infants

Group or type	Nursery A		Nursery B	
	ETA (+)	ETA (-)	ETA (+)	ETA (-)
I	0	6	0	5
III	0	2	0	4
94/96, 95 or 96	0	4	0	6
Other	0	3	0	7
II				
10/46	0	1	2 ^a	2
3C/71	0	1		
3A/3C/55/71	0	1		

^a One nurse with paronychia and one colonized infant (3A/3C/55/71).

TABLE 6. Presence of phage group II epidemic strains in the inanimate environment

Organisms in nursery A (no.)
<i>S. aureus</i> (5)
Stethoscope bell, 1046/1116 ^a
Ophthalmoscope handle, 1046/1116 3C/71
Otoscope handle, 1046/1116
Laundry cart, 1046/1116
Magazines nursery F, 71
<i>S. epidermidis</i> (11)
Gram-negative rods (14)
Other (2)
Negative (47)
Organisms in nursery B (no.)
<i>S. aureus</i> (8)
Nursery air, 3A/3C/55/71
Nursery air, 3A/3C/55/71
Airplate 3, room II, 3A/3C/55/71
Airplate 4, 3A/3C/55/71
Airplate 3, 3A/3C/55/71
Airplate 5, 3A/3C/55/71
Outside air, 3A/3C/55/71
Nursery 1, 6/53/75/83A/84/81
Coagulase-negative staphylococci (3)
Gram-negative rods (0)
Other (5)
Negative (46)

^a Phage type.

bullous impetigo. However, in nursery A only a few children actually had the full-blown scalded skin syndrome or erythema so characteristic of the expanded clinical illness produced by exfoliatin-producing strains in the past. This may be due to early detection and treatment of pustulosis which minimized toxin production. The absence of the scalded skin syndrome and the late onset of infection in children from nursery B may well be due to the reduced amount of toxin produced by strain 3A/3C/55/71 in contrast to strain 1046/1116.

In our search for the origin of these strains, a significant number of personnel and infants were found with *S. aureus* in nasopharyngeal and skin cultures, but the epidemic cluster strains could not be found despite over 600 surveillance cultures. Much literature on staphylococcal pustulosis of the newborn stresses the importance of personnel in carrying epidemic strains (5, 12, 13, 30, 45). These studies, most of which were done between 1950 and 1970, involved epidemics of phage group I strains. It was shown that these strains could readily colonize infants although disease was often not seen. These strains were transmitted readily by the hands of personnel from one baby to another. In a recent study, it was suggested that crowding and understaffing were mostly responsible for nursery outbreaks (13). What was so surprising to us was the finding that ETA-producing strains dominated the inanimate environment in both nurseries. Of the 141 environmental surveillance cultures, 13 were found to have *S. aureus*, of which 11 were the epidemic strains and all were ETA producing. The tenacity of these strains to colonize the environment was particularly apparent in nursery B, where the air ducts to this nursery were found to be colonized with these strains. The association of ETA production with the ability to colonize the inanimate environment is a new finding. Our nurseries are not geographically related. Nonetheless, only ETA-producing strains were found (Fig. 5) to

be associated with clustered epidemics of pustular disease. ETA-bearing strains may be more common than suspected as causes of epidemic neonatal pustulosis. Since we have found the inanimate environment to be a source of epidemic strains, we now control new cases of pustulosis differently. First, we phage type all strains of *S. aureus* producing pustulosis. If any bullous impetigo or scalded skin syndrome appears, we immediately increase awareness of hand washing among our personnel. We also close the room in which new cases are appearing. Environmental cultures are taken and we terminally clean the entire room, including all instruments. We have also instituted a policy of alcohol swabbing all common instruments in our nurseries daily, as these instruments may carry organisms to our neonates. We are pleased to note that with the introduction of these measures, we have successfully eliminated epidemic clusters.

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