Original Articles

ERRATUM

In the original article by Boris et al, "Bacterial Interference," which appeared in the May 1968 issue of the ARCHIVES (115:521-529), the following corrections should be noted:

Doctor Florman is associated with the North Shore Hospital, Manhasset, NY, and not with the New York Hospital and Cornell Medical Center, New York.

The second sentence in the first paragraph under results (page 522) should have read, "This consisted of 12 families of 51 members in the control group and 16 families of 82 members in the 502A-inoculated group."

The second and third sentences of the summary (page 528) should have read, "In a control group of 42 individuals treated only with antibiotics, the original pathogenic strain recurred in 31 or 74% of the group, and 15 individuals had 27 staphylococcal lesions. In the group treated with antibiotics then colonized with 502A, the original pathogenic strain recurred in 18 of the 66 individuals, or 27%, and there were only four minor staphylococcal lesions in this group."

Amer. J. Diseases of Children.

Bacterial Interference

Protection Against Recurrent Intrafamilial Staphylococcal Disease

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IT HAS been shown that colonization by one strain of *Staphylococcus aureus* of the nasal mucosa of adults [1-4] and the nasal mucosa and umbilicus of infants [5-11] interferes with subsequent colonization at those sites by other strains of coagulase positive staphylococcus. This phenomenon, called bacterial interference, has been successfully utilized to curtail epidemics of *S aureus* in newborn nurseries.

A similar protective effect has been demonstrated in adults. In two separate controlled studies, carriers of *S aureus* who were deliberately colonized following local nasal and systemic antibiotic therapy were protected from recolonization when directly challenged by a second strain of *S aureus*.[1,2] Cessation of recurrent bouts of furunculosis following recolonization was reported recently in one individual [3] and in one family.[4]

The present study demonstrates that artificial colonization with the 502A strain not only protects families against recolonization by the original resident strain of *S aureus* but also significantly interrupts chronic familial staphylococcal disease.

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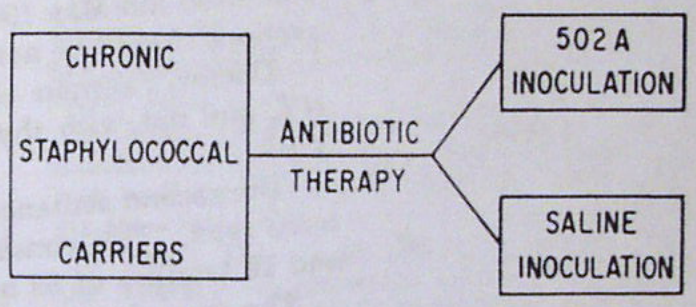


Fig 1.—Experimental design.

Materials and Methods

Families in which more than one member had recurrent staphylococcal lesions for at least one year constituted the volunteer group. All had received varied treatments in the past including toxoids, antibiotics, and antiseptic washes without success. The nares and throat of each member were cultured. If a single strain was found in several of the members, the family qualified for admission into the study. The family was randomly placed in the control or 502A-inoculated group. Cultures were then taken from the nares, throat, ears, axillae, trunk, perineum, and rectum. All members who harbored this strain, henceforth called the resident strain for that family, were treated with systemic and local antibiotics until two successive sets of nasal cultures, taken at least three days apart after ten days of therapy failed to yield the resident strain of *S aureus*. All antibiotics were discontinued the day prior to each culture. Most individuals in the study received antibiotics for two to four weeks.

The local antibiotic therapy consisted of applying, with a saturated cotton swab, oxacillin sodium ointment (10 mg of sodium oxacillin per gram of grease paste) to a depth of 3 cm from the nostrils. A neomycin and bacitracin ointment was substituted and applied in a similar manner in families where there was a history of penicillin allergy.

Systemic antibiotics utilized were either dicloxacillin, oxacillin, or lincomycin. The antibiotics were given in doses of 2 gm three times daily for adults; 1 gm four times daily to children 7 to 11 years of age; and 0.5 gm three times daily to children from 4 months to 2 years of age.

Figure 1 shows the experimental design. Within 24 hours after cessation of antibiotic therapy either 502A staphylococci or sterile saline was applied on four successive mornings to each side of the nose of the participants.

The 502A strain of *Staphylococcus* was inoculated by placing a cotton swab into an 18-hour fresh 502A broth culture and then rotating the swab twice in each nostril of the subject. Approximately $(10)^6$ organisms were inoculated by this process.

Cultures were then taken from the control and study groups weekly for the first two weeks, bi-weekly for a month, and monthly for the remainder of the year. Participants were instructed to report any possible lesion and have it cultured at our laboratory. Cooperation of the patients was excellent.

Bacteriological Methods.—A dry sterile swab was applied to the site to be cultured and rotated three times. It was then placed into a tube containing 2 ml of trypticase soy broth. This was incubated at 37 C for approximately two hours. The swabs were then streaked on a mannitol salt agar plate and a mannitol salt agar plate containing 0.2 units of aqueous penicillin per milliliter of agar (the penicillin medium was utilized to isolate small numbers of resident penicillin-resistant strains if present among many of the 502A organisms). After incubation at 37 C for 48 hours, the plates were examined for colonial morphology and pigmentation. For each type of colonial morphology and color combination a minimum of two colonies were selected for phage typing. Twenty-four standard phages were utilized at routine test dilution. These were 29, 52, 52A, 79, and 80 in group 1; 3A, 3B, 3C, 55, and 71 in group 2; 6, 7, 83A, 42E, 47, 53, 54, 73, 75, 77, and 83 in group 3; and 42D, 187, and 81 in the miscellaneous group. Nontypable colonies were tested for coagulase activity by a plate method.[12] Antibiotic sensitivity patterns were performed by the Kirby disk method,[13] on all strains isolated with disk concentration as follows: penicillin, 2 units; tetracycline, 5 and 30μg; chloramphenicol, 5μg; lincomycin, 5μg; oxacillin, 10 units; erythromycin, 5μg; dicloxacillin, 2 units; novobiocin, 30μg.

The 502A strain utilized for colonization was a penicillin sensitive; non-penicillinase-producing, coagulase positive strain of staphylococcus of low virulence. It phage typed with one or more members of the following group: 6, 7, 54, 53, 75, 77, 42D, 83, 81. The 502A strain has the serological agglutination pattern of $(b)c_1$. A more detailed description may be found in references 1, 2, 5-9, and 14.

When *S aureus* 502A was tested against a 5μg disk of tetracycline, there was no visible inhibition of growth around the disk. With the 30μg disk, there was a 2 to 3 mm zone of inhibition. By tube dilution studies, the 502A was resistant to 25μg and sensitive to 50μg of tetracycline. This inhibition of growth around the tetracycline disks was used as a marker even though the 502A strain cannot be considered sensitive to therapeutic amounts of tetracycline. This is the identical sensitivity of the 502A strain used previously for inoculation of infants and adults.[1,2,5-8]

Results

Twenty-eight families with 133 members were entered in the study. This consisted of 12 families of 51 members in the 502A-inoculated group. In both groups, the proportion of family members with nasal carriage of resident pathogenic *S aureus* was almost identical; in the control group, 42 of 51 (82%), in the inoculated group, 66 of 82 (81%).

Among the 12 control families, 11 of the staphylococcal strains isolated were resistant to a screening concentration of a two unit disk of penicillin G and one strain was sensitive to this penicillin G disk. By similar tests in the 16 families subsequently inoculated with 502A, 14 had a resident strain resistant to penicillin G and two had a penicillin G sensitive strain.

The distribution by phage group of the staphylococcal resident strains isolated in the controls consisted of one strain in phage group 1, one strain in phage group 2, seven strains of the 52/52A/80/81 complex, one miscellaneous, and two nontypable strains. In the 502A group, three were in group 1, two in group 2, nine had the 52/52A/80/81 complex, one was a miscellaneous strain, and one non-typable.

All of the 66 family members who were treated with antibiotics and nasally inocu-

Table 1.—Colonization Status in Control and 502A-Inoculated Families During First Year After Treatment

	No. of Families	No. of Individuals	No. of Individuals Treated	Recurrence of Original Strains		No. of Individuals With Lesions
				Number	%	
Control *	12	51	42	31	74	15
502A † inoculated	16	82	66	18	27	4

* Antibiotic therapy and saline.
† Antibiotic therapy and 502A.

lated with the 502A strain were successfully colonized. Successful colonization is defined as isolation of the inoculated 502A strain one week after implantation. There were three individuals who remained persistent perineal carriers, two individuals who remained axillary carriers, six individuals who remained throat carriers, and one who was an ear carrier, who were colonized in the nares despite failure to eradicate the resident strain from the aforementioned sites.

Staphylococcus aureus 502A was recovered from 61 of 66 (92%) of these individuals after one month. At six months, 33 of 39 (71%) and at twelve months 15 of 26 (58%) still were colonized with 502A strain. (Fig 2).

Of the 66 individuals who received antibiotic and 502A, the original strain recurred during the subsequent twelve month interval in only 18 (27%), (Table 1). During the same period of time the original strain recurred in 31 of 42 individuals (74%) in the control group who had received antibiotic and saline inoculation. This difference is significant at $P<0.0001$.

Among the 15 control individuals with lesions there were 27 distinct pyogenic episodes (Table 2). There were eight large cutaneous abscesses, five cases of impetigo, four small pustules, five styes, three ischial rectal abscesses, one episode of peritonitis, and one case of otitis externa. Twenty-two of these 27 lesions were cultured and in 21 the original resident strain was recovered. In one abscess an unrelated strain of *S aureus* was isolated. In the treated group colonized with *S aureus* 502A, there were four lesions, one in each of four individuals. These were three small pustules and one stye. From two of the pustules and the stye, the 502A strain was isolated. The other pustule was caused by an 80 81 organism, the individual's original resident strain.

Individual observations of the families in both the control and 502A-inoculated groups demonstrated several interesting phenomena.

Family R (Fig 3) represented the fairly typical control family. All four members carried the same pathogenic strain. Following 17 days of intensive antibiotic therapy the resident strain could no longer be isolated from any members. However, within four weeks of cessation of therapy, the original resident 52 52A/80 strain was again recovered from all members. This was accompanied by the recurrence of lesions.

Family AD (Fig 4) was another control family. Of the five members, three (individuals 1, 2, and 4) harbored the same pathogenic strain. Again, within a three-week period following cessation of antibiotic therapy, the resident pathogenic strain reappeared in all three members. It is of interest that members 3 and 5 who were not treated with antibiotics because they did not have the resident pathogenic strain, were carriers of *S aureus*, type 71. Despite multiple lesions in the other members of their family, these two individuals did not acquire any lesions during the seven-month period of followup.

Family F, a 502A study family of five members (Fig 5), was successfully colonized with the 502A strain of *S aureus* after antibiotic therapy. Monthly cultures showed persistence of the 502A strain during the first 11 months. During this period the original penicillin-resistant strain

Table 2.—Staphylococcal Lesions in Control and 502A-Inoculated Individuals

Type of Lesion	Control Group		502A Group		
	No. of Lesions	No. of Cultured and Original Strain Isolated	No. of Lesions	No. Cultured With 502A Isolated	No. Cultured With Original Strain Isolated
Ischial rectal abscess	3	3	...	...	...
Cutaneous abscess	8	6	...	...	...
Impetigo	5	4	...	...	...
Pustule	4	3	3	2	1
Otitis externa	1	1	...	...	...
Peritonitis	1	1	...	...	...
Styes	5	3	1	1	...
Total	27 *	21	4	3	1

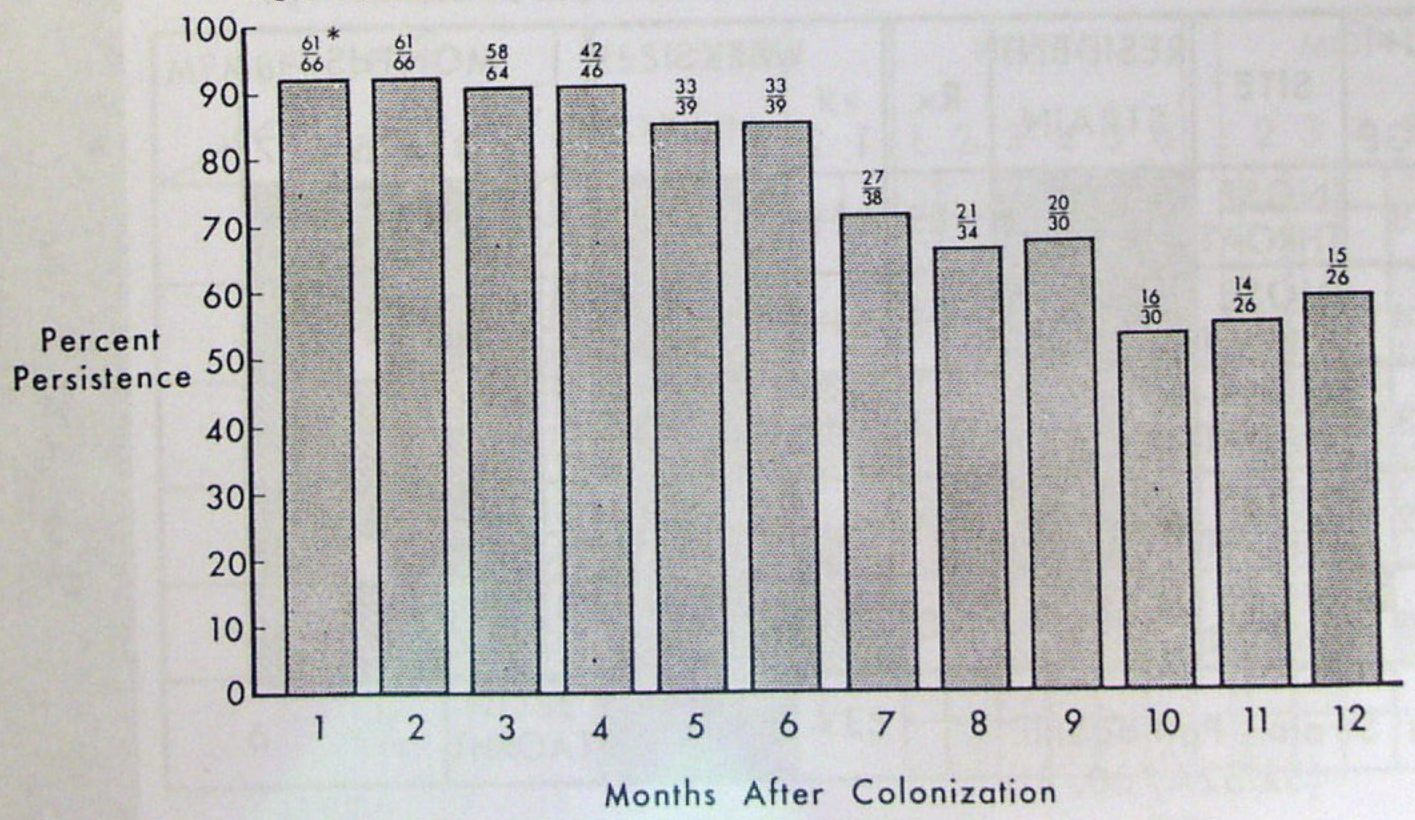
* 27 lesions in 15 control individuals.

could never be detected despite repeated attempts to isolate it using penicillin-containing agar in addition to the standard media. In all, 80 swabs were taken and 342 colonies phage-typed. Between the eleventh and twelfth month penicillin was administered for an intercurrent infection to members 2 and 4. Member 2 did not have the original pathogenic coagulase positive strain isolated during the first eleven months; however, at the twelfth month, the latter 3C/55/71 strain was isolated.

The recurrence of the original resident strain was noted in an additional six of ten individuals after they received penicillin therapy.

Family H was also in the 502A inoculated group, (Fig 6). It consisted of six members. The resident pathogenic strain had a 29/80 phage type. Following antibiotic therapy and 502A inoculation all members became carriers of the 502A strain. After three months, however, member 5 spontaneously lost 502A and had a nasal and throat recurrence of the original 29/80 resident pathogenic strain.

Fig 2.—Persistence of **Staphylococcus aureus** 502A in colonized individuals.



* Number still colonized with 502 A / Number of original 502 A carriers followed

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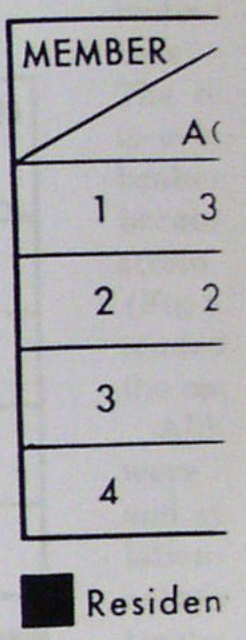

Residen

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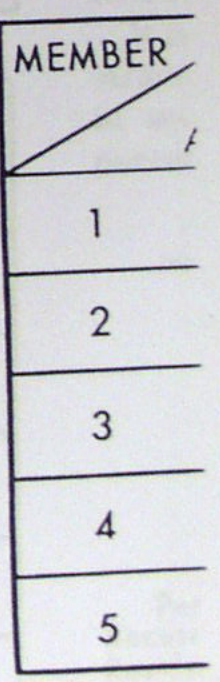

Reside

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Comment

Chronic furunculosis has been defined by different criteria by various investigators. Because of the lack of uniformity in defining this disease and the lack of controls in most studies it is difficult to evaluate various therapeutic regimens proposed in the treatment of this problem.[15] We have selected for the present study only those patients who have had intermittent disease for one year or more.

Data presented demonstrate the feasi-

Fig 3.—**Staphylococcus aureus** cultures before and after treatment (family R [control]).

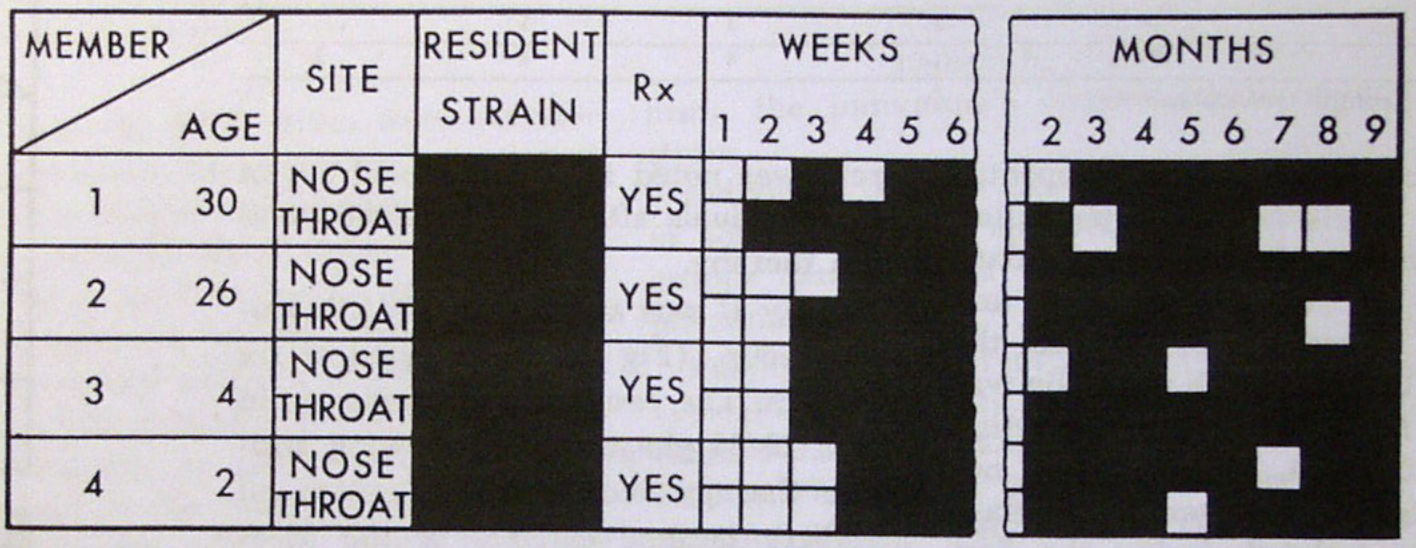
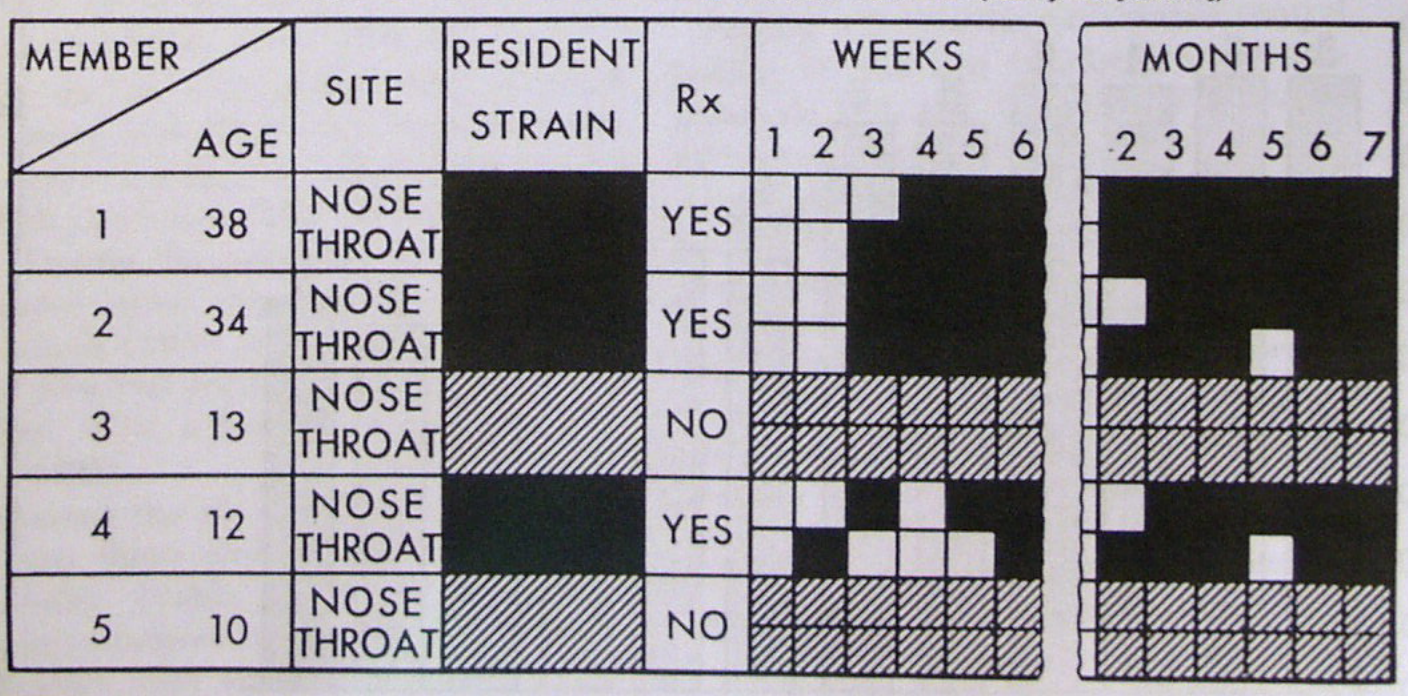


Fig 4.—**Staphylococcus aureus** cultures before and after treatment (family AD [control]).



bility and beneficial effects of altering the nasal colonization status of persistent staphylococcal carriers who have recurrent pyogenic lesions. This was accomplished by nasal implantation of the 502A strain of *S aureus* after systemic and local nasal antibiotic treatment removed the original resident strain.

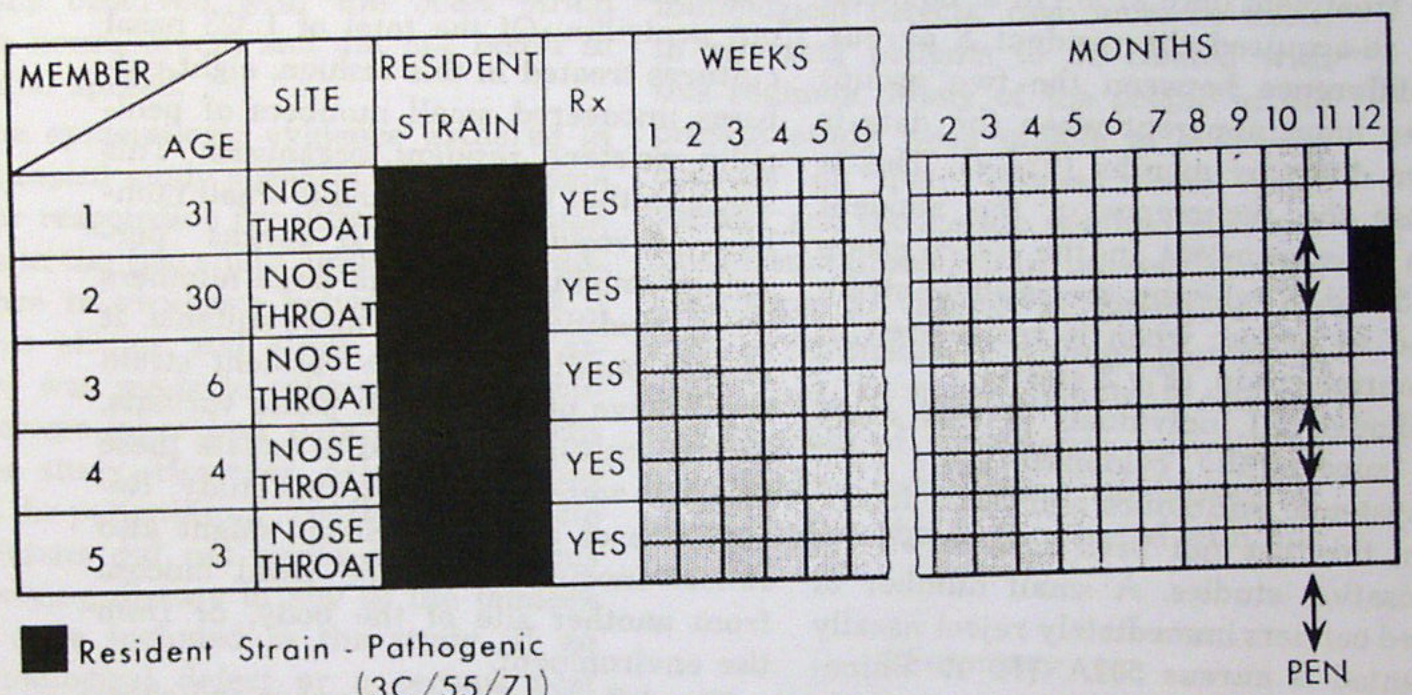
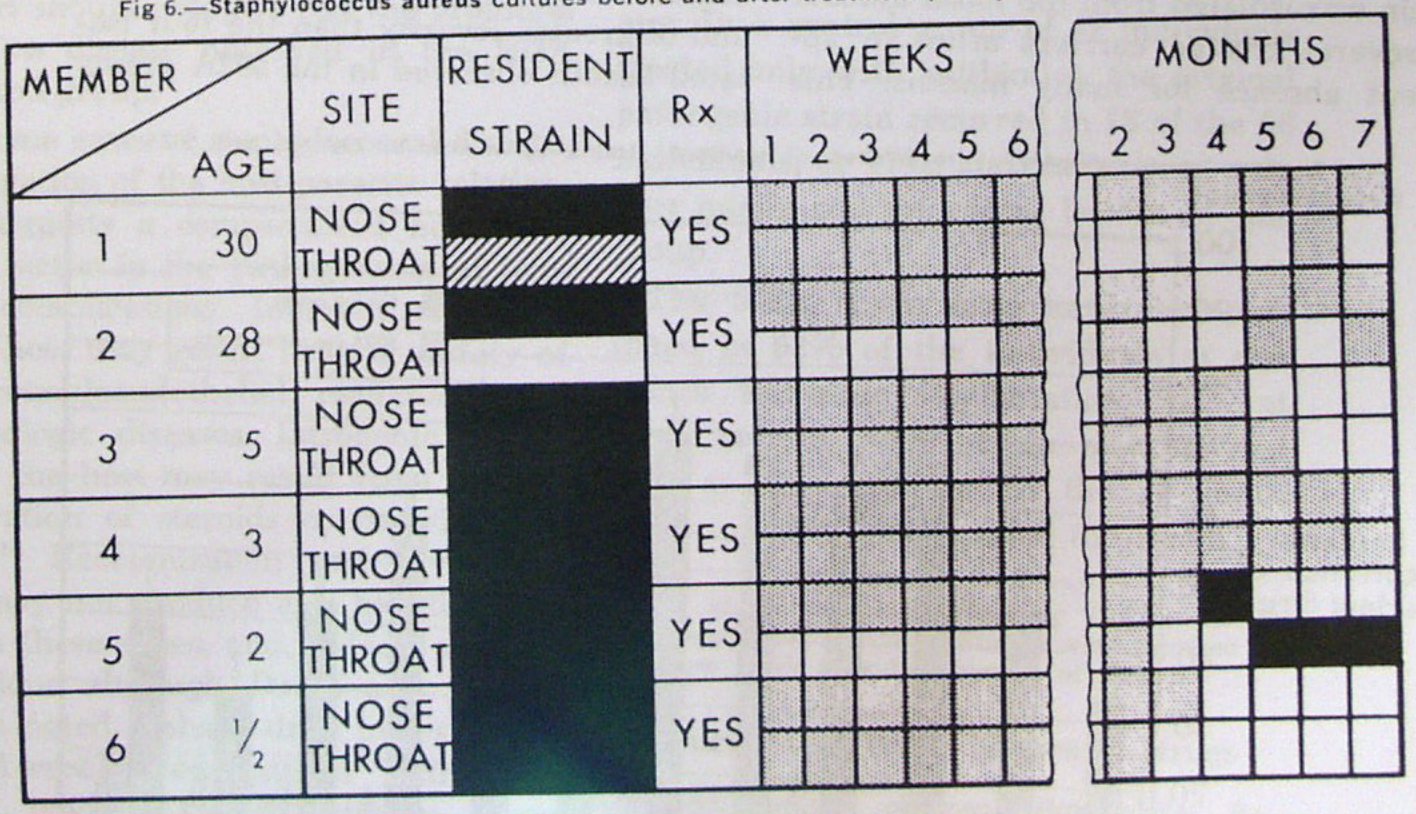


Fig 5.—Staphylococcus aureus cultures before and after treatment (family F [502A inoculated]).

Fig 6.—Staphylococcus aureus cultures before and after treatment (family H [502A inoculated]).



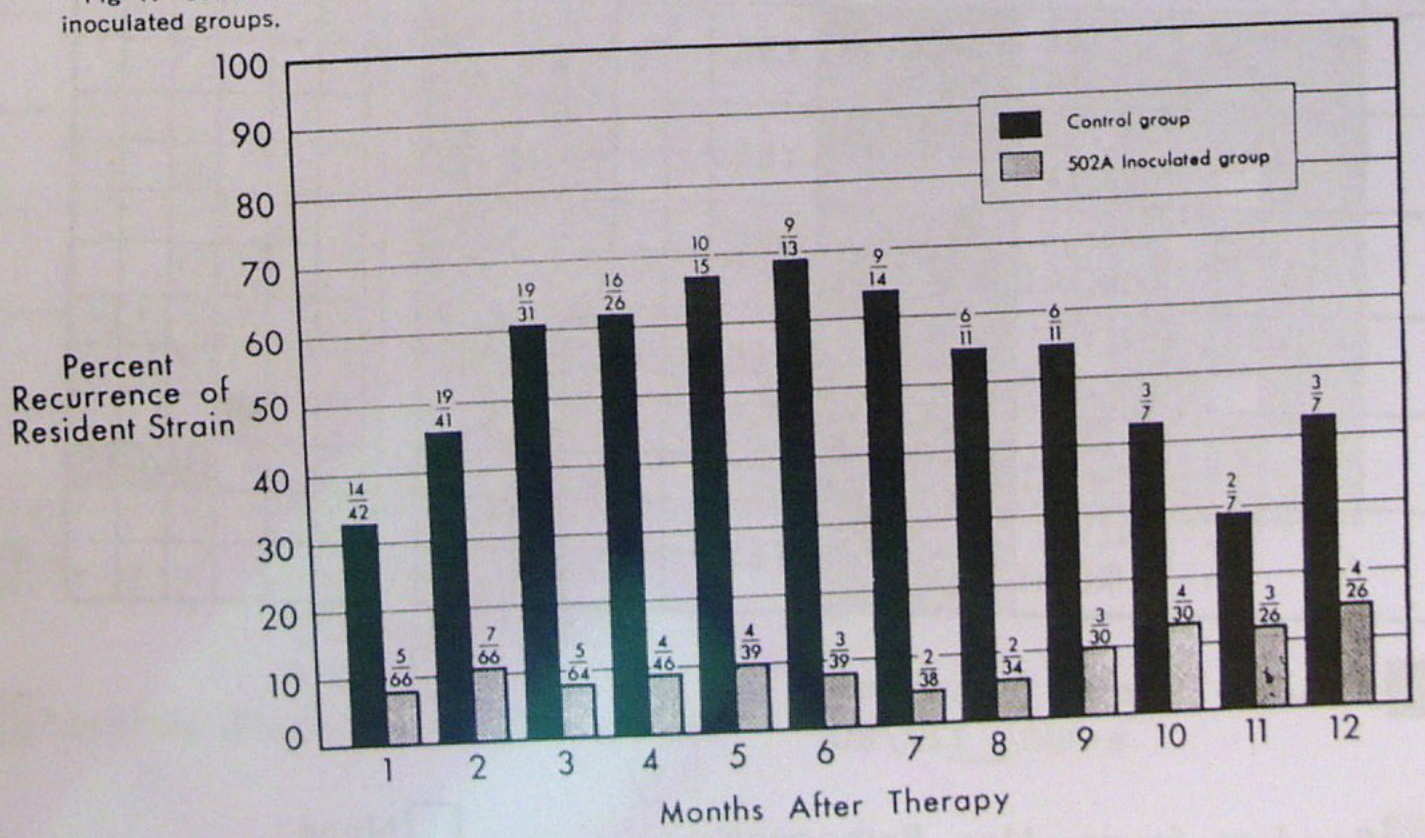
In the control group who received antibiotics and saline, the recurrence rate on the nasal mucosa of the original resident strain was 74% (31 of 42 members). In the group inoculated with 502A after antibiotic treatment only 27% (18 of 66 members) re-acquired the resident *S aureus*. The difference between the two groups is even more apparent when the data is broken down by months (Fig 7). This is because the recurrence of the resident strain was transient in the 502A group (Fig 5 and 6) whereas the resident strain tended to persist when it reappeared in the control group (Fig 3 and 4).

Although all individuals in this study were successfully colonized after local and systemic antibiotics and 502A inoculation, this has not been true in all recolonization studies. A small number of treated carriers immediately reject nasally implanted *S aureus* 502A (H. R. Shinefield and M. Boris, unpublished data). Host factors responsible for this phenomenon remain to be elucidated.

It is not clear why the original resident strain was isolated from the nasal mucosa in several treated carriers after an apparent absence for many months. This might have been because a small number of resident strain organisms persisted on the nasal mucosa but were not detected by our cultural techniques. To determine if this was a factor, swabs were plated in duplicate on agar plates with and without penicillin. Of the total of 1,323 nasal cultures treated in this fashion, eight cultures uncovered small numbers of penicillin-resistant resident organisms. This suggests that in some patients small numbers of resident strain *S aureus* "persisters" might reappear in large numbers when conditions become more suitable. It is also possible that the resident strain might have persisted as L phase variants. Special cultures required to isolate these variants were not used in this study. Recurrence of a resident strain might also reflect inoculation of the nasal mucosa from another site of the body, or from the environment.

The difference in the number and character of lesions in the control and 502A-inoculated groups was marked. The 27 staphylococcal lesions observed in the 15 control individuals were more extensive and of greater severity than the four isolated lesions observed in the 502A group.

Fig 7.—Cumulative summary of recurrence of resident strains by month in control and 502A-inoculated groups.



In the latter group there were three single pustules and one stye which responded well to local therapy. One of the three pustules was due to the resident pathogenic strain. From the stye and the other two pustules only 502A was isolated. The lesions observed with the 502A strain were nonrecurrent and did not result in familial spread.

The epidemilogic evidence gathered in the present study suggests that the prime factor responsible for difference in lesion rates in the two study groups was the difference in virulence between resident *S aureus* strains and strain 502A. No attempt was made to collect complete immunologic laboratory data on individuals in the study. However, detailed histories from the patients and reports from private physicians did not uncover any serious underlying disease in any of the families who were included in the study. If an immunological defect or a serious host factor were responsible for the recurrent staphylococcal disease in the patients studied, a change in the strain of *S aureus* carried by the host from resident strain to 502A should not have had the profound effect on disease observed in the 502A colonized group.

In some cases of staphylococcal disease, investigation of the host-parasite relationship suggests a compromised host as a major factor in the pathogenesis of pyogenic complications. Inherent alteration of the host may result from a variety of skin, reticuloendothelial, malignant, and immunologic diseases. Iatrogenic alteration of the host may result from the administration of steroids or certain antibiotics.[16] Recolonization with *S aureus* 502A may not produce any beneficial effects in these cases, and, indeed, may be deleterious although Drutz and his associates noted a sharp drop in lesions in a 502A-recolonized patient with skin disease who was inadvertently given steroids.[17]

The present data and other observations leave no doubt that *S aureus* 502A may occasionally cause lesions.[9-11,18] To date these have not been of a serious nature in the more than 3,000 infants and 1,000 adults colonized. However, because of the possibility of a serious complication, especially in individuals with immunological defects, care must be taken in selecting patients to be treated with this regimen. Many of the people in the present study were unable to hold jobs because of their illness, some were pariahs, and the disease resulted in severe psychiatric problems in others. All had undergone a variety of therapies for chronic furunculosis without any beneficial effect. In these individuals the potential value of colonization outweighed the potential risk. Future candidates for recolonization should be selected with this in mind.

Summary

It is feasible to protect individuals from recurrent staphylococcal disease by artificially colonizing the nasal mucosa with *S aureus* 502A following antibiotic therapy. In a control group of 42 individuals treated only with antibiotics, the original pathogenic strain recurred in 18 of the 66 individuals, or 27%, and there were only four minor staphylococcal lesions in this group.

The 502A strain once implanted persisted in 92% of the individuals at one month following implantation, 91% at three months, 85% at six months, and 67% at nine months. At the 12-month follow-up, 58% still carried the strain.

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Generic and Trade Names of Drugs

Oxacillin sodium ointment—*Prostaphlin, Resistopen.*

Neomycin and bacitracin ointment—*Bacimycin HC ointment.*

Lincomycin—*Lincocin.*

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THE PHILANTHROPIC FOUNDATION

Foundation giving belongs to the best democratic tradition: ruggedly individualistic, proud of its great wealth—and guilty enough about possessing it to consider investing it in unselfish ways.—The Foundation as Pioneers, *Time*, Jan 19, 1968, p 17.