

Bacterial Interference

Protection of Adults Against Nasal Staphylococcus Aureus Infection After Colonization With a Heterologous S Aureus Strain

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Nasal carriers of pathogenic strains of *Staphylococcus aureus* are a potential hazard to their environment, their contacts, and themselves. At present, there is no known method which permanently eradicates "virulent" strains of *S aureus* from the nose of all persistent carriers. These investigations were undertaken to evaluate a new approach to a solution of the problem presented by adult carriers of virulent staphylococci.

Shinefield and his associates have demonstrated that colonization of the nasal mucosa of newborns with one strain of coagulase-positive staphylococcus interferes with subsequent acquisition of a second strain of *S aureus*,¹ and that, in fact, artificial colonization of newborns immediately after birth with a staphylococcus of low virulence can be

employed to protect infants from infection by virulent "epidemic" strains.²⁻⁵ Epidemiologic data which would support the hypothesis of biologic competition between different strains of staphylococci have been reported by other groups of investigators.⁶⁻⁸

The present communication presents additional and more direct evidence to demonstrate that interference between strains of coagulase-positive staphylococci occurs not only in newborn infants but also in adults, and may thus represent a general phenomenon.

Definitions

For purposes of clarity, the following terms were arbitrarily defined:

Persistent Nasal Carrier: An individual from whose nose a strain of coagulase-positive staphylococcus was recovered each week for four consecutive weeks. Henceforth referred to as a carrier.

Persistent Nasal Noncarrier: An individual from whose nose no coagulase-positive staphylococcus was recovered on weekly cultures for four consecutive weeks. Henceforth referred to as a noncarrier.

Blocking Strain of *S aureus*: A penicillin-sensitive coagulase-positive strain of staphylococcus of low virulence previously designated strain 502A. The organism is lysed by phages 6/7/42E/44D/53/54/75/81 and has serologic type (b) c₁. Immunologic, biologic, and epidemiologic characteristics of this strain have been more completely described elsewhere.^{1,9} After 48 hours of growth on trypticase soy agar, differentiation of this strain from the challenge strain was

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facilitated because of the marked difference in pigmentation between the two strains.

Challenge Strain of *S aureus*: A coagulase-positive, penicillin-resistant strain of staphylococcus which was initially isolated from both a lesion and the nasal mucosa of an adult male. The organism is lysed by phages 52/52A/80/81, has the serologic type a b c h k m. By disc antibiotogram, it is resistant to penicillin, 10 units, tetracycline, 30 μ g, chloramphenicol, 30 μ g, novobiocin, 30 μ g, erythromycin, 10 μ g, and streptomycin, 10 μ g. By the tube dilution method, this organism is susceptible to 3.12 μ g/ml sodium methicillin, 0.78 μ g/ml sodium oxacillin, and less than 0.39 μ g/ml vancomycin hydrochloride.

Criteria of Successful Inoculation or "Take": Recovery of the inoculated strain from the nose by culture 24 to 48 hours after administration.

Material and Methods

1. *Experimental Design.*—The basic design of the experiment consisted of identifying groups of persistent nasal carriers and noncarriers of staphylococci. Artificial colonization was attempted with either the blocking or challenge strain of staphylococcus in some of the groups by placing measured numbers of these bacteria on their nasal mucosa, and using other volunteers to determine whether artificial colonization of the nasal mucosa is possible in individuals whose staphylococcal flora have previously been suppressed with antimicrobial therapy. The inclusion of suitable control groups would clearly indicate whether interference between strains of staphylococci does or does not occur.

The subjects for these observations were drawn from among 1,724 male, adult volunteers, members of the Federal Penitentiary in Atlanta. Nasal

cultures were obtained at weekly intervals for four weeks and on the basis of these results, a pool of 122 persistent nasal carriers of staphylococci and another of 212 persistent noncarriers were defined. These two pools were further subdivided into a total of six groups (Table 1).

Group 1 consisted of 16 carriers of coagulase-positive staphylococci who were simply followed with weekly nasal cultures without any type of treatment or inoculation. Group 2 consisted of ten carriers of coagulase-positive staphylococci who were treated with sodium oxacillin for seven days but were not subjected to artificial colonization with any strain of *S aureus*. Group 3 consisted of 16 carriers of coagulase-positive staphylococci not treated with sodium oxacillin prior to nasal inoculation with strain 502A. Fifteen men in this group were subsequently treated with sodium oxacillin for seven days and then rechallenged with staphylococcus phage type 52/52A/80/81. Group 4 consisted of 29 carriers of coagulase-positive staphylococci who were treated with sodium oxacillin for seven days and then nasally inoculated with *S aureus* strain 502A. Group 5 consisted of 29 noncarriers who were not treated with any antibiotics but in whom colonization with strain 502A was attempted. The individuals of groups 4 and 5 in whom successful colonization with strain 502A was achieved were further divided at random into two subgroups. One group of 20 volunteers was challenged with *S aureus* strain 52/52A/80/81 and another group of 25 subjects was observed without nasal challenge. Group 6 consisted of 20 persistent nasal noncarriers who were not treated with any antibiotics but who were inoculated with strain 52/52A/80/81.

2. *Antibiotic Administration.*—Sodium oxacillin* was administered locally and orally to subjects in groups 2, 3, and 4. They received 2 gm three times daily three hours after meals for a period of seven days. This antibiotic also was applied to both nares three times daily with a cotton

*Sodium oxacillin (Prostaphlin) supplied by Dr. R. T. Towson of Bristol Laboratories, Syracuse, NY.

TABLE 1.—*Experimental Design Staphylococcal Colonization Groups*

Group	No. of Subjects	Persistent Nasal Carrier	Sodium Oxacillin Therapy	Inoculation 502A	Subgroups	No. of Subjects	Challenge 52/52A/80/81
1	16	+	—	—			
2	10	+	+	—			
3	16	+	—	+ →	Sodium oxacillin therapy } → A	15	+
4	29	+	+	+ →	502A } → B	20	+
5	29	—	—	+ →	Carriers } → C	25	—
6	20	—	—	—	————→ D	20	+

swab saturated with sodium oxacillin ointment in a grease base which contained 10 mg of oxacillin per gram. An attempt was made to cover the mucosa to a depth of 3 cm from the nostrils.

3. *Organisms Used for Inoculations.*—Blocking Strain: Blocking strain 502A was inoculated onto the lateral aspect of the nasal mucosa of subjects in test groups 3, 4, and 5 (Table 1) three times over a four day period (day 1, 2, and 4). Each dose of 10^6 organisms was delivered in a 0.001 cc droplet measured with a microburette.

Challenge Strain: Challenge strain 52/52A/80/81 was placed on four different quadrants on the nasal mucosa of each nostril of 20 subjects selected at random from groups 4 and 5 who were found to be colonized with strain 502A. Each area was inoculated with a 0.001 cc. droplet which contained 10^4 organisms. The total dose of 4×10^4 organisms of the challenge strain 52/52A/80/81 was administered in a similar fashion to volunteers in group 6 as well as to subjects in group 3. The latter group was treated with sodium oxacillin before inoculation.

4. *Bacteriological Methods.*—Nasal swabs were obtained weekly from all individuals. In addition, swabs were taken after a course of antibiotic therapy was completed and before each inoculation of *S aureus*. A dry, sterile swab was inserted and rotated gently three times in both nostrils of subjects, and placed into a tube which contained 0.5 cc of trypticase soy broth. Swabs left at room temperature for approximately two hours were then streaked on trypticase soy agar plates and incubated at 37°C for 48 hours at which time they were examined for colonial morphology and pigmentation. For each type of colonial morphology and color combination present, a minimum of three colonies per plate was selected for phage typing. An Accu-drop apparatus which dispensed 24 standard phages was used at the routine test dilution; when indicated, strains were tested at 1,000 times the routine test dilution.

All colonies were tested for coagulase production by a plate method.¹⁰ Antibiotic sensitivity patterns were performed by the disc method on all staphy-

lococcal strains with disc concentrations as follows: penicillin, 2 units and 10 units, tetracycline, 5 and 30 µg, chloramphenicol, 5 µg, erythromycin, 5 µg, streptomycin, 1 µg, novobiocin, 30 µg, bacitracin, 10 units. Serologic typing of selected strains were performed by Dr. Jay O. Cohen, Laboratory Branch, Communicable Disease Center, by the slide agglutination method with the use of ten antigens: a, b, c, polyvalent, c₁, e, h, i, k, m, n. All strains not inhibited by the 10 unit benzathine penicillin G disc were considered to be penicillin resistant.

Results

Strains of all phage groups were recovered from the volunteers who were persistent carriers of *S aureus* (Table 2). Twenty-one of the 71 strains (30.3%) isolated were penicillin resistant. Volunteers who carried penicillin-resistant *S aureus* were distributed at random among the study groups.

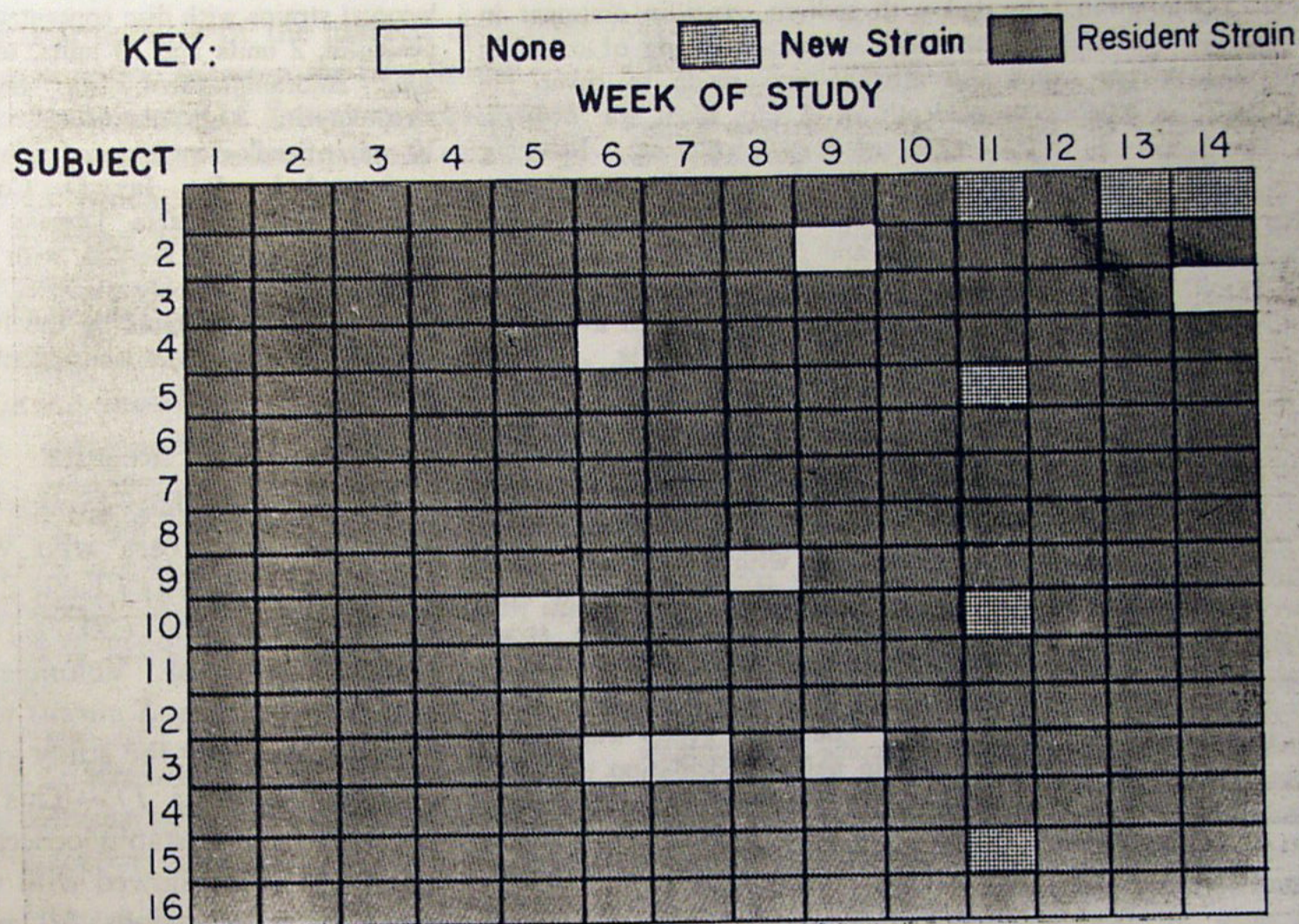
Group 1 (Fig 1).—This group of 16 persistent nasal staphylococcal carriers was untreated and followed with weekly cultures for a 14-week period. All members of this group retained their resident strain throughout the study period except for subject 1 who became colonized with a new strain of coagulase-positive staphylococcus. Transient colonization with a second strain of *S aureus* was observed for one week in three others.

Group 2 (Fig 2).—No coagulase-positive staphylococci could be recovered for one week after local and systemic administration of sodium oxacillin in 9 of the 10 individuals who were carriers of *S aureus*. However, in the subsequent ten-week surveillance period, the original strain of *S aureus* was recovered from the nasal mucosa of six volunteers (60.0%). A new strain appeared either transiently or consistently in four (40.0%) of the subjects. None of the ten subjects remained uncolonized throughout the ten-week period following therapy.

Group 3 (Fig 3).—Sixteen carriers were challenged with *S aureus* 502A inoculated on three occasions over a period of four days. Each dose of 10^6 organisms was placed on the mucosa of both nostrils. The only evidence of a take was the transient appearance of 502A in subjects 27 and 38. After nine weeks of surveillance, 15 volunteers were

TABLE 2.—Characterization of Coagulase-Positive Staphylococci Isolated From Volunteers Prior to Treatment

Phage Group	No. Volunteers in Study With Nasal Colonization of Coagulase-Positive <i>S aureus</i>	
	Penicillin Resistant	Penicillin Sensitive
I	4	7
II	1	5
III	7	10
Miscellaneous	1	3
Nontypable	8	25
Total	21	50

Fig 1.—Nasal colonization with *S aureus*, group 1.

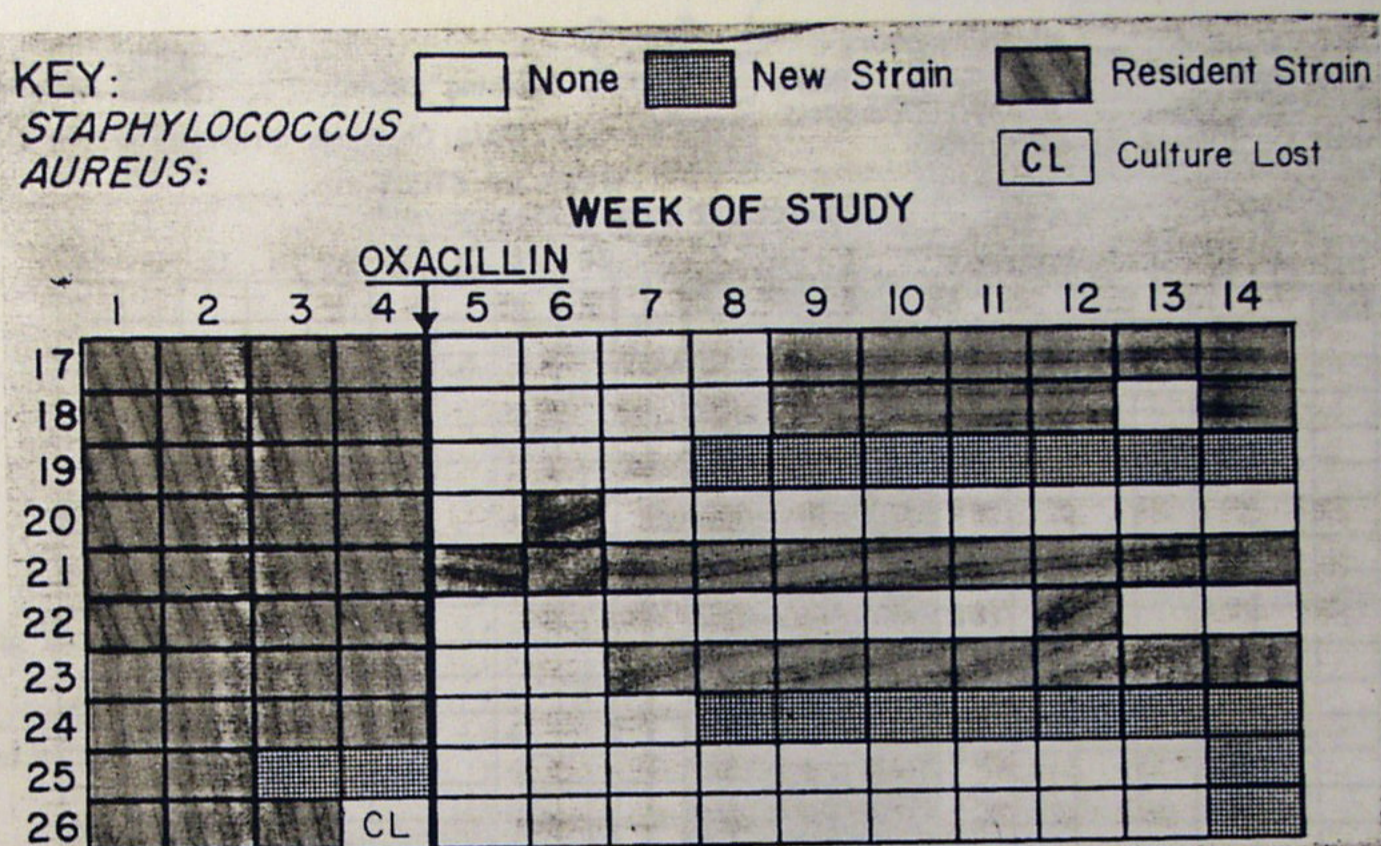
treated with local and systemic sodium oxacillin for seven days and then challenged with 4×10^4 *S aureus* strain 52/52A/80/81. After inoculation, this challenge strain was recovered from all members in the study group and persisted in 10 of the 15 individuals (66.6%) for four weeks.

Group 4 (Fig 4).—The 29 carriers in this group received systemic and local sodium oxacillin therapy for seven days. After therapy, the resident strain of *S aureus* was recovered from only one individual (*S aureus* could be recovered from the nasal mucosa in only one other person in this group). All 29 volunteers were then inoculated with *S aureus* 502A and a take was noted in 27 of the 29 subjects (93.1%). Twelve of the 27 members in the 502A take group selected at random were challenged with an inoculum of 4×10^4 staphylococci strain 52/52A/80/81. The only subject successfully colonized with the 52/52A/80/81 challenge strain of *S aureus* was subject 58, a volunteer who did not have a take with the blocking 502A strain, after sodium oxacillin therapy. At nine weeks after artificial colonization, 25 of

27 volunteers (92.6%) who had an initial take retained the inoculated 502A strain (Fig 6).

Group 5 (Fig 5).—Only 18 of the 29 (62.1%) persistent nasal noncarriers in this group were successfully colonized after artificial inoculation with *S aureus* 502A (subject 83, who was colonized two weeks after inoculation, was not considered a successful take). One week later, 8 of 18 members in this 502A take group were challenged with *S aureus* 52/52A/80/81. Colonization with the challenge strain could not be detected in a single member who was colonized with the blocking strain. Rapid disappearance of the strain was noted in subjects with a successful take. Only 4 of the 18 (22.2%) volunteers retained the marker strain three weeks following inoculation (Fig 6). At nine weeks, only two persons were still carriers.

Group 6 (Fig 7).—This group of 20 persistent nasal noncarriers was challenged with 4×10^4 organisms of *S aureus* phage type 52/52A/80/81. Cultures taken 24 hours after artificial inoculation demonstrated that

Fig 2.—Nasal colonization with *S aureus*, group 2.

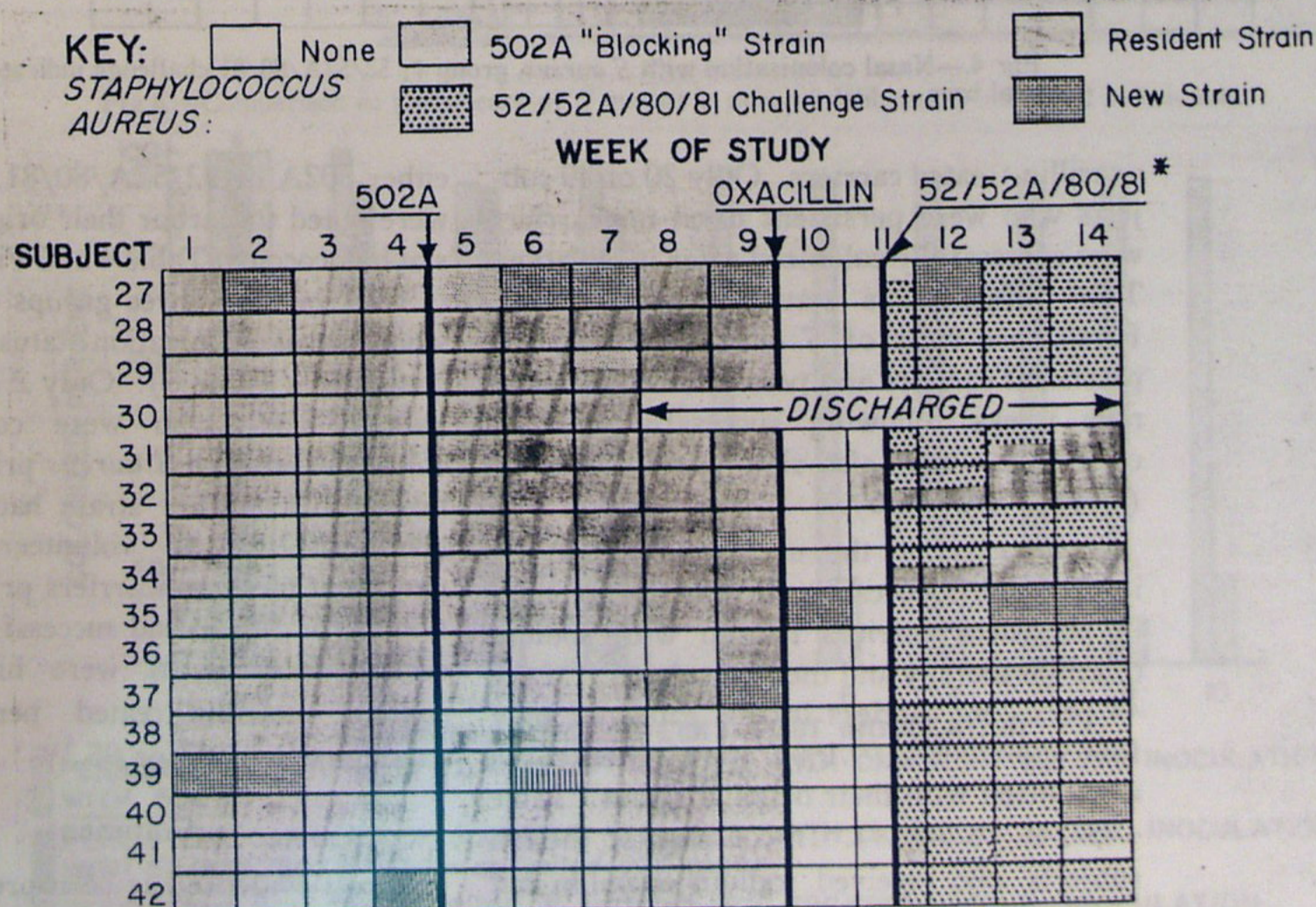
2 of 20 subjects (10%) had a take and carried the challenge strain (Fig 6).

Take and persistence rates after challenge with marker *S aureus* strain † were compared

† Either 502A or 52/52A/80/81.

in subjects who were: (a) persistent nasal *S aureus* 502A carriers treated with sodium oxacillin and (b) persistent nasal non-carriers. Inoculation of marker strains resulted in takes in 42 of 44 sodium

Fig 3.—Nasal colonization with *S aureus*, group 3; 52/52A/80/81 challenge indicated by heavy vertical bars.



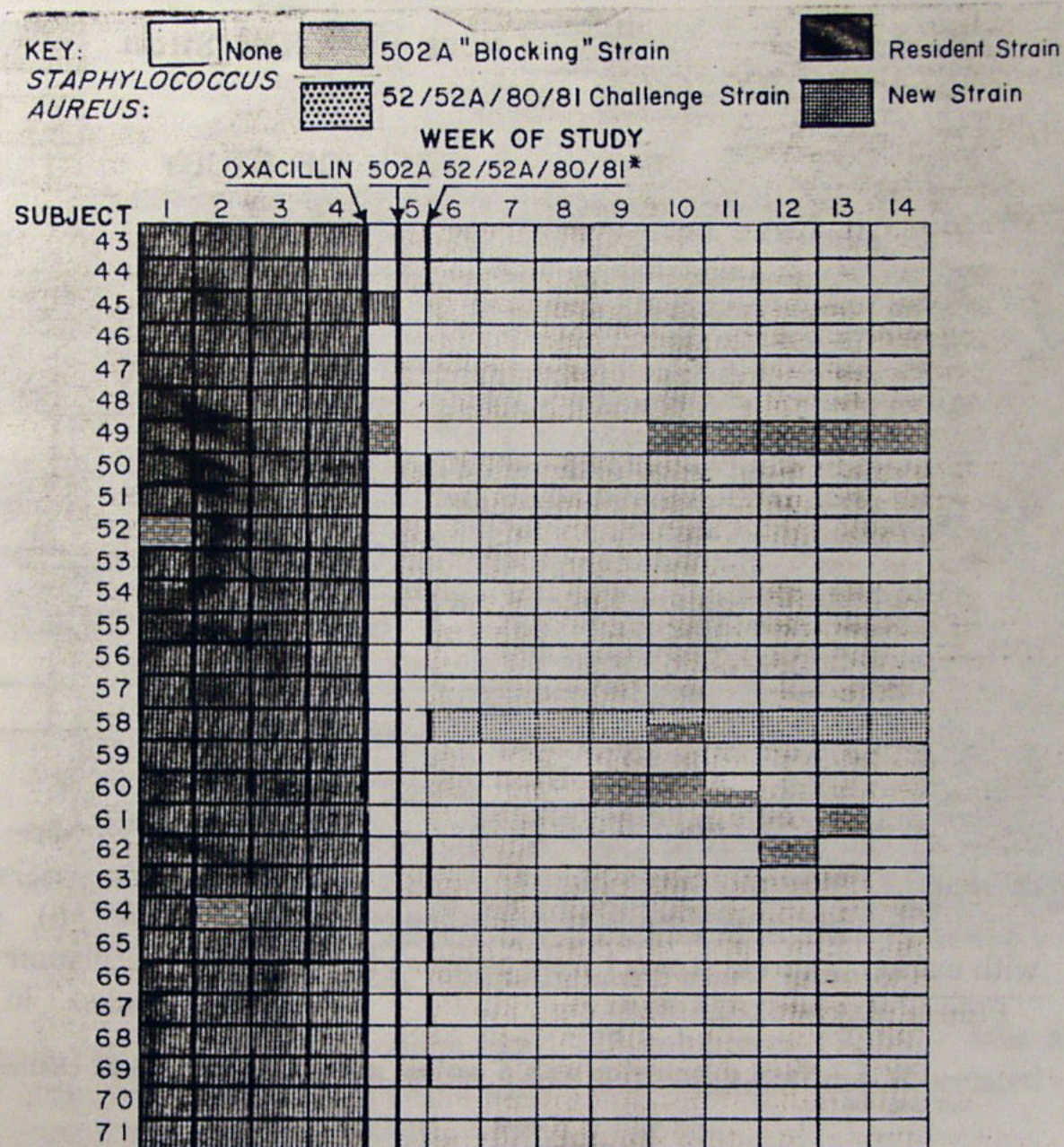


Fig 4.—Nasal colonization with *S aureus*, group 4; 52/52A/80/81 challenge indicated by heavy vertical bars.

oxacillin-treated carriers. Only 20 of 49 subjects who were persistent nasal noncarriers were successfully colonized after inoculation. This difference is statistically significant. Persistence rates of *S aureus* between the persistent carriers and persistent noncarriers nine weeks following successful artificial colonization were also significantly different (Table 3 and Fig 6).

Recurrence on the nasal mucosa of the initial staphylococcal strain was not common in persistent carriers treated with sodium oxacillin therapy and then inoculated. Of the 44 persistent carriers in this category, only four individuals (9.1%) were noted to be recolonized with their original strain (Table 4 and Fig 3 and 4). In contrast, of the ten subjects who received sodium oxacillin but who were not deliberately colonized with

either 502A or 52/52A/80/81, six (60.0%) were noted to harbor their original strain of staphylococcus (Table 4 and Fig 2).

Take rates in three groups of individuals whose nasal colonization status differed were compared (Table 5). Only 2 of 36 individuals (5.5%) who were colonized with coagulase-positive *S aureus* prior to inoculation with a marker strain had a successful take. Of the 48 volunteers who were persistent nasal noncarriers prior to inoculation, 20 or 34.1% had successful takes. Successful inoculations were highest in the sodium oxacillin-treated persistent nasal carriers (41 of 43 or 95.3%).

Comment

Direct evidence has been presented in this communication to support the hypothesis that

fig. 6

Fig 5.—Nasal colonization with *S aureus*, group 5; 52/52A/80/81 challenge indicated by heavy vertical bars.

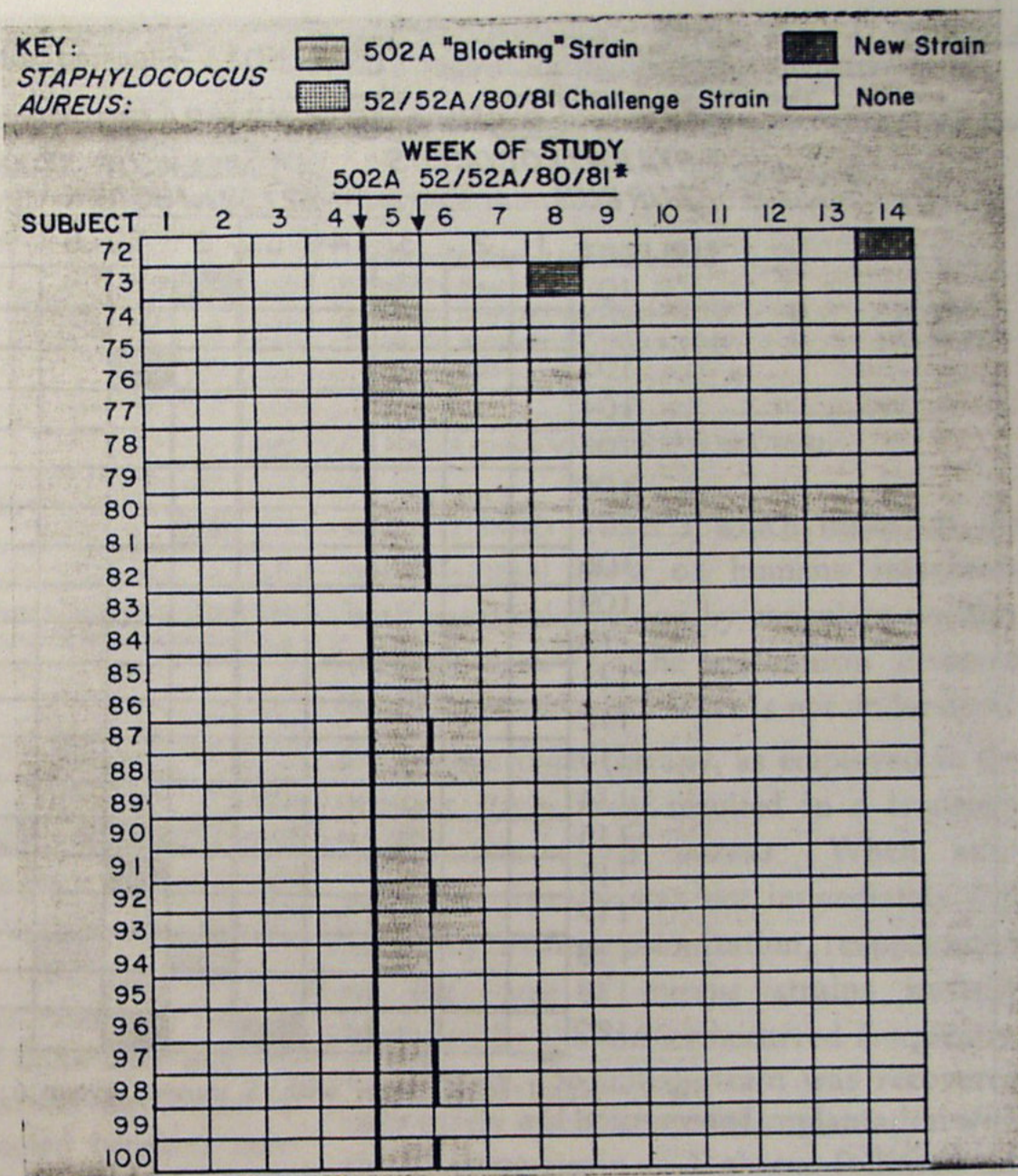
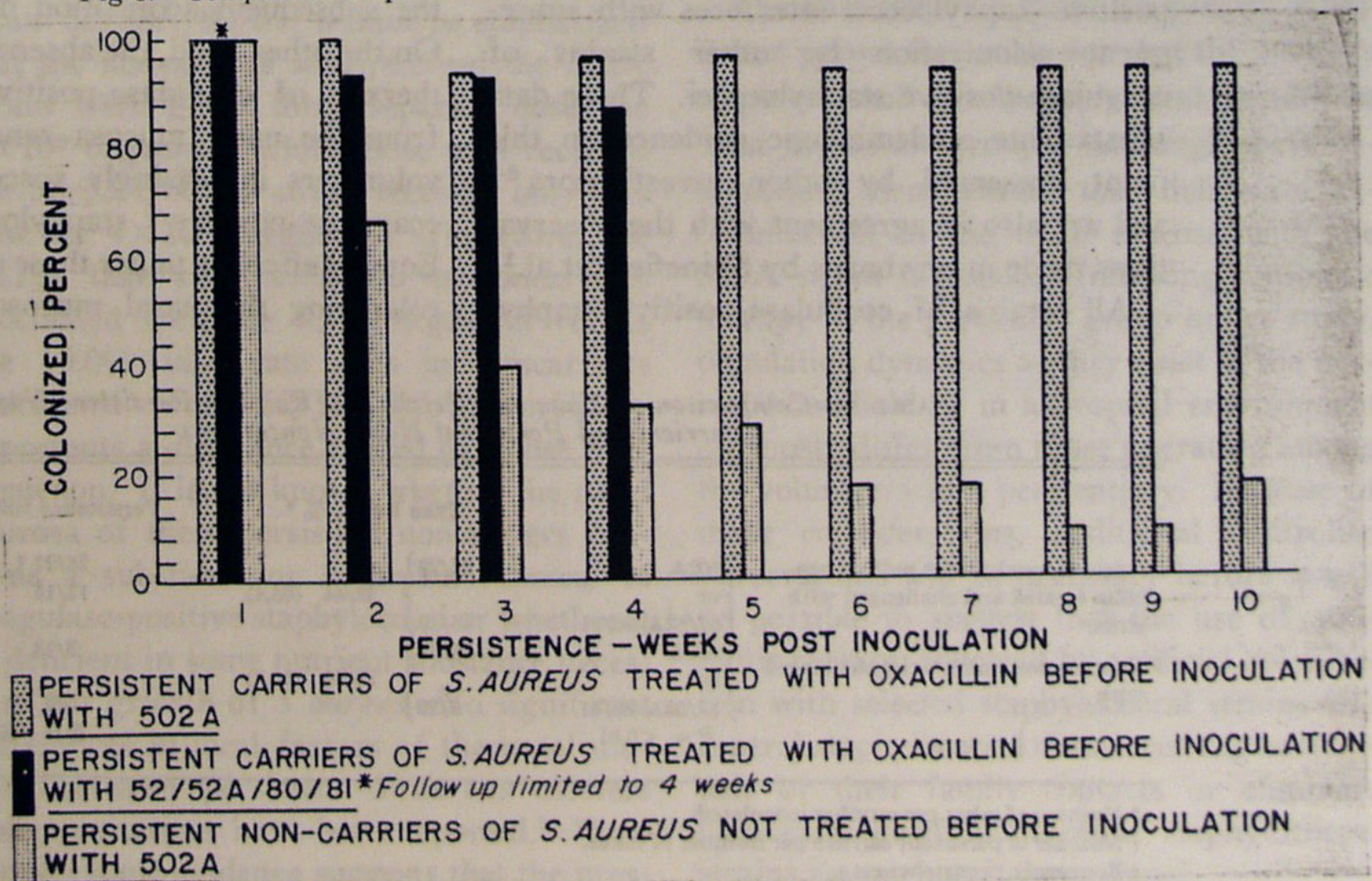


Fig 6.—Comparison of persistence of *S aureus* in nose of deliberately colonized individuals.



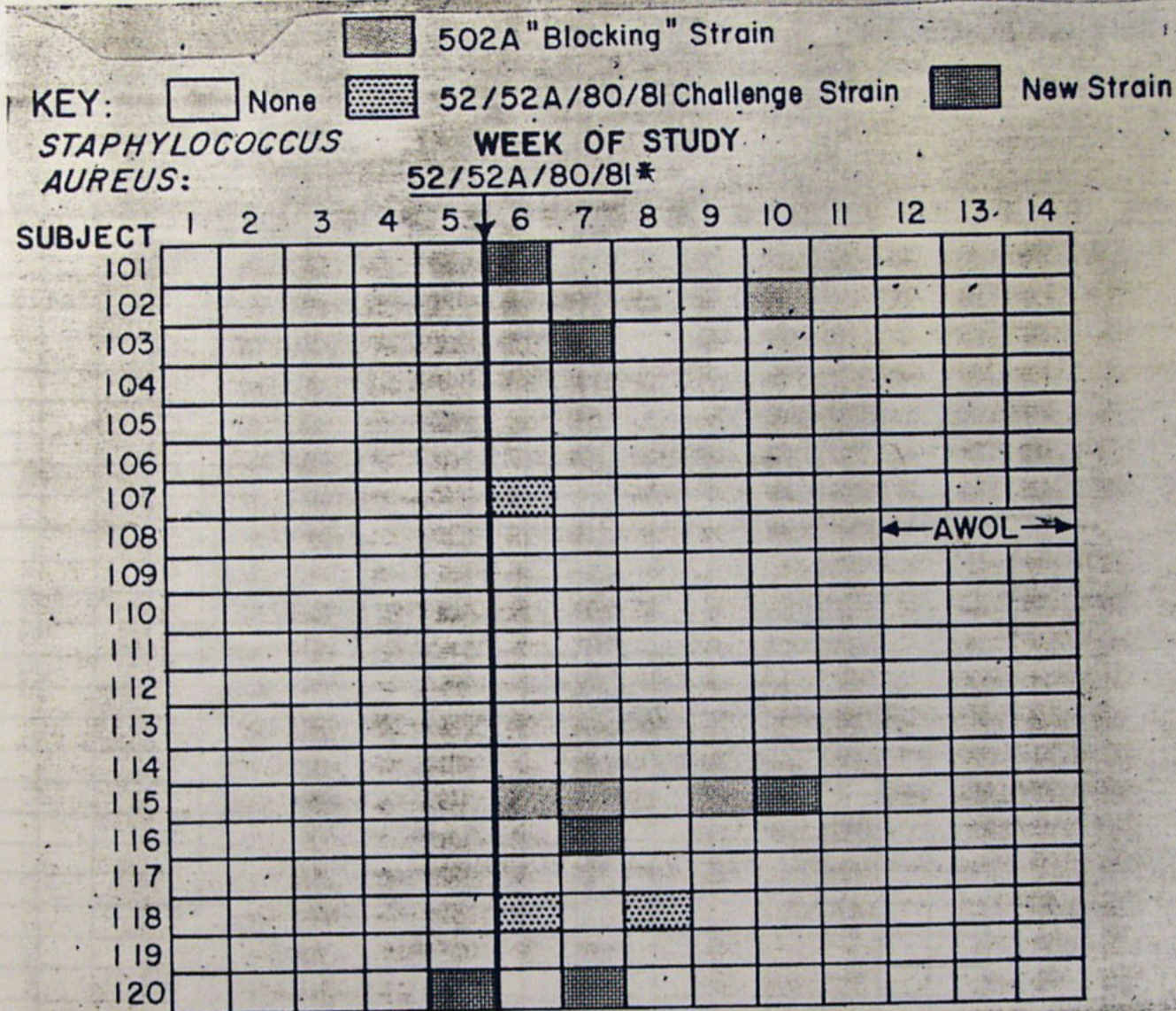


Fig 7.—Nasal colonization with *S. aureus*, group 6; 52/52A/80/81 challenge indicated by heavy vertical bar.

in adults, nasal colonization with coagulase-positive staphylococci interferes with subsequent colonization by other strains of coagulase-positive staphylococci. These data substantiate epidemiologic evidence on this point presented by other investigators⁶⁻⁸ and are also in agreement with the observations made in newborns by Shinefield et al.¹

All strains of coagulase-positive staphy-

lococci proved equally effective in blocking the subsequent acquisition of other strains. On the other hand, the absence or removal by therapy of coagulase-positive staphylococci from the nasal mucosa rendered the adult volunteers increasingly susceptible to other coagulase-positive staphylococcal strains. Equally effective under these circumstances in colonizing the nasal mucosa of volunteers

TABLE 3.—Comparison of Take and Persistence Rates in Persistent Nasal Carriers and Persistent Nasal Noncarriers

		Take Rates, % *		Persistence Rates, % † at 9 Weeks	
Persistent nasal carrier sodium oxacillin treated and challenged with either	502A	27/29	42/44 (95.4)	25/27 †	28/42 (66.7)
	or 52/52A/80/81	15/15		13/15	
Persistent nasal noncarrier challenged with	502A	18/29	20/49 (40.8)	3/18	3/20 (15.0)
	or 52/52A/80/81	2/20		0/2	
		$P = <0.01$		$P = <0.01$	

* Number of takes per number inoculated.

† Number of persistent carriers per number of takes.

‡ Four week persistence rate.

TABLE 4.—Types of Staphylococcal Strains*

Subject	No. With Recovery of Original Strain, % †	No. With Recovery of Strain Other Than Original or Inoculated Strain, % †
Not inoculated with either 502A or 52/52A/80/81 following antibiotics	6/10 (60.0)	4/10 (40.0)
Inoculated with either 502A or 52/52A/80/81 following antibiotics	4/44 (9.1)	9/44 (20.5)

$P = <0.01$

* Types of staphylococcal strains recovered from the nasal mucosa of subjects treated with sodium oxacillin and then either inoculated with marker *S aureus* strains or uninoculated.

† No. positive per total No. in group.

was the penicillin-sensitive 502A strain and the penicillin-resistant 52/52A/80/81 strain.

Of considerable interest is the fact that it proved more difficult to implant staphylococci in persistent noncarriers, and that, in general, deliberately implanted coagulase-positive staphylococci disappeared relatively rapidly from the nasal mucosa of these same individuals. In addition, a marked difference in colonization rates was noted between noncarriers who were inoculated with strain 502A and those who were inoculated with strain 52/52A/80/81; it must be emphasized that the noncarriers who received the 502A strain were given three separate doses of 1×10^6 organisms while those who received the 52/52A/80/81 strain received only one dose of 4×10^4 organisms. Therefore, the 62.1% take rate colonized in noncarriers inoculated with the 502A organism versus the 10.0% take rate seen in noncarriers inoculated with 52/52A/80/81 probably represents a difference related to a dose phenomenon. It is not known whether the nasal mucosa of these persistent noncarriers contains a substance or organism inimical to coagulase-positive staphylococci or whether it is deficient in some nutrient substance necessary for growth of *S aureus*. No significant differences in local factors of the nasal mucosa between persistent *S aureus* carriers and noncarriers have been reported,^{11,12} although some evidence suggests that the pres-

TABLE 5.—Comparison of Take Rates of Inoculated Strains* Related to Nasal Colonization in Three Study Groups

Nasal Colonization Status	No. Subjects Inoculated	No. of Takes, %
Persistent carrier of coagulase-positive <i>S aureus</i>	36	2 (5.5)
Persistent nasal noncarrier	49	20 (40.8)
Persistent nasal carrier treated with sodium oxacillin	44	42 (95.4)

* Marker strains were 502A or 52/52A/80/81.

ence of other bacteria which make up the normal nasal biota of humans interferes with nasal colonization by coagulase-positive staphylococci.^{1,13} The mechanism involved in this type of interference is not understood.

Antimicrobial therapy, as employed in the present study, only resulted in a transient suppression of *S aureus*. When antimicrobial therapy was not immediately followed by artificial colonization, reappearance of the same *S aureus* strains initially harbored by the carrier occurred frequently. Conversely, the initial strain was recovered only rarely when successful implantation with the blocking strain of *S aureus* followed the antibiotic regimen.

The data obtained from the present study do not permit the conclusion that the methods employed will produce identical results in open population groups. Although these observations demonstrate that deliberate recolonization of the nasal mucosa with the 502A strain is feasible following antibiotic therapy in the particular group under study, population dynamics as they exist in the normal household or in a hospital environment obviously differ from those operating among the volunteers in a penitentiary. Because of these considerations, additional controlled observations will be necessary before it will be possible to suggest that the use of antibiotic therapy followed by artificial colonization with selected staphylococcal strains will control staphylococcal disease among individuals or their family contacts or eliminate nasal carriage of "pathogenic" staphylococcal strains among hospital personnel.

Summary

Direct evidence has been presented to support the hypothesis that in adults, nasal colonization with coagulase-positive staphylococci interferes with subsequent colonization by other strains of coagulase-positive staphylococci. All strains of coagulase-positive staphylococci proved equally effective in blocking the subsequent acquisition of another strain. On the other hand, the absence or removal by therapy of coagulase-positive staphylococci from the nasal mucosa made this area more susceptible to colonization with other coagulase-positive staphylococcal strains. No difference in colonizing potential was noted between the penicillin-sensitive 502A strain and the penicillin-resistant 52/52A/80/81 strain.

The ability to colonize persistent non-carriers was significantly less than the ability to colonize carriers pretreated successfully with antibiotics. Persistence rates in these two groups were also strikingly different.

Antimicrobial therapy as employed in the present study only resulted in a transient suppression of *Staphylococcus aureus*. When antimicrobial therapy was not immediately followed by artificial colonization, the original *S. aureus* strain reappeared on the nasal mucosa in a majority of the subjects. Conversely, the resident strain was recovered only rarely when successful implantation with the blocking strain of *S. aureus* followed the antibiotic regimen. The application of this technique to other clinical situations awaits further controlled studies.

Dr. Alexander D. Langmuir, Chief, Epidemic Intelligence Service, Communicable Disease Center, Dr. Philip S. Brachman, Chief Investigation Section, Epidemiology Branch, Communicable Disease Center, and Dr. Carl A. Pirkle, Medical Director, Atlanta Federal Penitentiary, cooperated in the project; laboratory assistance was furnished by John R. Boring, PhD, and Anita High Smith, BS. Warden D. M. Heritage and Mr. Kenneth Holton of the Federal Penitentiary organized and handled the volunteers; and Virginia D. Hines, RN, Orelia C. Dixon, RN, gave nursing assistance.

Generic and Trade Names of Drugs

Tetracycline—Achromycin, Panmycin KM, Polycycline, Tetracycline.
Chloramphenicol—Chloromycetin.
Erythromycin—Erythromycin, Ilotycin.
Sodium methycillin—Dimocillin-RT, Staphcillin.
Sodium oxacillin—Prostaphlin, Resistopen.
Vancomycin hydrochloride—Vancocin.
Bacitracin—Baciguent, Bacitracin.
Benzathine penicillin G—Bicillin.

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