BACTERIAL INTERFERENCE: PROTECTION AGAINST STAPHYLOCOCCAL DISEASE*

MARVIN BORIS

Assistant Attending Pediatrician
Stanley J. Lagin Pediatric Research Laboratory
North Shore Hospital
Manhasset, N. Y.

New York Hospital-Cornell Medical Center
New York, N. Y.

NATURALLY occurring or artificially induced staphylococcal colonization of the nasal mucosa of adults and infants has been shown to prevent acquisition of other strains of *Staphylococcus aureus* at this site. This phenomenon, termed bacterial interference, has been investigated and utilized to colonize newborn children during epidemics in nurseries and to alter staphylococcal colonization in individuals carrying a pathogenic strain of *Staph. aureus*. The purpose of this communication is to review briefly the basic concept of bacterial interference and its subsequent therapeutic application in infants and adults.

The initial data were obtained through two basic epidemiological observations in the nursery for newborn children. In a study nursery, routine staphylococcal cultures were being acquired from all newborns and hospital personnel. A nurse carrier of *Staph. aureus*, phage type 80/81, had contact with 68 infants (Table I). Among the 37 infants with whom she had had contact during their first day of life eight, or 22 per cent acquired the 80/81 strain. However, when the 31 infants who were older than one day were handled by this nurse carrier, none became colonized with this strain. Analysis of the available cultures indicated that 26, or 84 per cent, of the infants older than 24 hours were nasally colonized with coagulase positive staphylococci other than type 80/81 prior to contact with the nurse carrier, while there were no staphylococcal carriers in the infants less than one day of age. Several possibilities then existed to explain these observations: 1) the resistance to infection increases with age, or 2) the presence of one strain of

*Presented as part of a *Symposium on Nonchemotherapeutic Approaches to Control of Staphylococcal Infection* held by the Section on Micro- and Molecular Biology at The New York Academy of Medicine, February 8, 1968.

This investigation was supported by Public Health Service Research Grant AI-0709 from the National Institute of Allergy and Infectious Diseases, Bethesda, Md.

TABLE I.—COLONIZATION OF INFANTS WITH STAPHYLOCOCCI 80/81

Time of contact with 80/81 carrier	*No. infants at risk*	*No. infants colonized*	*Percentage*
1st Day of Life	37	8	22
After 1st Day of Life	31	0	0

Staph. aureus interferes with the acquisition of another strain.

A second epidemiological observation and subsequent experiment helped elucidate the fact that it was the presence of *Staph. aureus* on the nasal mucosa which interfered with colonization by other strains of coagulase positive staphylococci.

In one hospital, 56 per cent of infants discharged from Nursery A were colonized with an 80/81 strain. In Nursery B of this hospital, only 11 per cent carried the 80/81 strain on the discharge nasal culture. An epidemiological experiment was arranged. One half of the infants designated to be admitted to Nursery A were initially admitted to Nursery B for 16 hours. The other half of the infants were admitted directly to Nursery A. The discharge colonization rate with type 80/81 among the group transferred from Nursery B to Nursery A was found to be similar (about 15 per cent) to the rate of colonization in newborn infants admitted directly to Nursery B. The infants who were admitted directly to Nursery A still maintained a 50 per cent colonization rate with the 80/81 strain. Therefore the initial 16-hour admission to Nursery B conferred upon the newborn protection against colonization from the 80/81 strain when these children were exposed to the latter organism. It was noted that 80 per cent of the babies prior to their transfer from Nursery B to Nursery A were already colonized with coagulase positive staphylococci other than *Staph. aureus* 80/81. This observation further demonstrates that staphylococci already implanted in the nose inhibit subsequent colonization at this site by other strains of coagulase positive staphylococci.

It was therefore considered worthwhile to attempt to utilize the phenomenon of bacterial interference to prevent colonization of newborn children by epidemic strains during nursery outbreaks where usual methods of control had failed. However, the blocking or interfering strain must have certain characteristics: 1) it must be benign; 2) it should colonize easily; 3) it should persist for a period longer than the

TABLE II.—SITE AND NUMBER OF TAKES IN INFANTS INOCULATED WITH STRAIN 502A

Site	*Number inoculated*	*Take*	*Percentage*
Nose	123	110	89
Umbilicus	133	100	75

sojourn in the nursery; 4) it should be easily identifiable in the laboratory; 5) it should be susceptible to penicillin and many other antibiotics.

Observations on a penicillin-sensitive Group 3 *Staph. aureus* which we designated 502A indicated that this organism would meet these requirements.[1, 2] Initially the strain had been recovered from a nurse. Subsequently it was followed on the nasal mucosa of many infants discharged from the New York Hospital nurseries over a two-and-one-half-year period. During this time, clinical and epidemiological observation revealed a lack of staphylococcal disease among these infants or their family contacts.

The protective effect of artificial colonization with the 502A strain of *Staph. aureus* was then studied in four separate nursery epidemics,[3-6] in four different regions of the United States. In each of these nursery epidemics there was a high rate of staphylococcal colonization and disease, both in the infants and their families. Previous attempts to control the epidemics by antibiotics, hexachlorophene baths, rooming-in, closing the nurseries, ultra-violet light, and sterile techniques had proved unsuccessful. The strains causing disease in these outbreaks were of the 52/52A/80/81 type or a related variant.

During each epidemic, approximately one half the infants were artificially colonized with the 502A strain. No changes were made in nursery procedures. Personnel carriers of epidemic strains were permitted to work and were not informed of their colonization status. Infants were artificially colonized with the 502A strain of staphylococcus by placing approximately 5,000 organisms on each side of the nasal mucosa and on the umbilicus.

Table II demonstrates the number of successful inoculations in infants who were artificially colonized with the 502A organisms. A successful inoculation or "take" is designated as detection of the 502A organisms by culture 24 hours after inoculation. Among the 123 infants

TABLE III.—NASAL COLONIZATION WITH COAGULASE-POSITIVE STAPHYLOCOCCI OTHER THAN 502A IN SUCCESSFULLY INOCULATED AND CONTROL INFANTS DURING HOSPITAL STAY (H) AND AT INITIAL FOLLOW-UP (F)

Category	*Infants colonized with strain other than 502A Number (%)*	
	H	F
Inoculated Takes	5/108 (4.6)	7/93 (7.5)
Control	56/143 (39.1)	68/129 (52.7)

TABLE IV.—RELATION BETWEEN STRAIN OF STAPHYLOCOCCUS IN INFANT'S NOSE AND DEVELOPMENT OF DISEASE

	Lesion	*80/81*	*502A*
Infants	Conjunctivitis	4 (4)	4 (3)*
	Impetigo	16 (10)	1 (1)*
	Abscess	1 (1)	2 (2)
	Omphalitis	1 (1)	
Maternal	Abscess	4 (4)	2 (2)
	Mastitis	1 (0)	1 (0)
	Total	27/53 51%	10/132 8%

Figures in parentheses indicate diagnoses confirmed by culture.
*Strain 502A isolated.

who received the latter organism on their nares, there were 110 successful inoculations, or 89 per cent.

Nasal colonization with coagulase positive staphylococci other than the inoculated 502A strain in successfully inoculated and control infants differed significantly (Table III). In the successfully inoculated group, there were five of 108, or 4.6 per cent, who during the hospital stay acquired a strain other than 502A. At the initial follow-up, usually one week after discharge from the hospital, seven of 93, or 7.5 per cent, were colonized with another strain of *Staph. aureus*. In contrast, in the control group of 143 individuals, 56 or 39.1 per cent, became colonized with a type 80/81 or coagulase positive staphylococcus during their hospital stay and on the initial follow-up, 52.7 per cent of the control

harbored a coagulase positive staphylococcal strain, primarily 80/81.

The principal criteria of the efficacy of colonization would be demonstrated by correlation of lesion rates in infants colonized with the 502A strain compared to infants colonized with the 80/81 strain. Table IV illustrates the relation of the strain of staphylococcus in the infant's nose to subsequent development of disease in both the infant and its mother. In the control group 53 index case infants were followed. Twenty-seven lesions appeared either in the infant or the mother, a rate of 51 per cent. In the group inoculated with 502A there were 10 lesions in the 132 infants followed. However, the 502A strain was isolated from only four lesions in the 502A inoculated babies. These data support the fact that the 502A strain is relatively benign. Further, seven of the babies colonized with *Staph. aureus* 502A had major operations, including cardiac operations and repair of a tracheoesophegeal fistula. No staphylococcal complication occurred in any of these infants. Six additional infants colonized with *Staph. aureus* 502A were hospitalized for bronchiolitis. In no case was the respiratory disease related to the staphylococcus, and in none of the infants did the illness progress to any staphylococcal complication or pneumonia.

Light and Sutherland,[6] during an 80/81 epidemic in a nursery for premature infants in Cincinnati, used artificial colonization with the 502A strain to halt the outbreak. Previous use of neomycin ointment and hexachlorphene baths had failed to halt 80/81 colonization and disease. In a group of several hundred premature infants, no lesions were associated with 502A colonization, and the 80/81 epidemic was terminated rapidly.

The success of artificial colonization to terminate epidemics in nurseries led to several investigations in adults. First, it was determined if it was possible to replace an existing staphylococcal flora with the 502A strain in persistent adult carriers and thereafter protect the individual against a direct challenge with the 80/81 strain. The population utilized were volunteers at a federal penitentiary.[7] Screening of 1,728 inmates revealed 71 persistent staphylococcal carriers.

To summarize this controlled study briefly, one group of persistent carriers were treated with oxacillin, 6 gm. daily systemically, and a local oxacillin ointment inserted three times daily into each nostril. Following this therapy, the 502A strain was inoculated into the nares successfully in 27 of the 29 individuals. Subsequent direct challenge of 50,000 or-

TABLE V.—SUMMARY OF COLONIZATION STATUS IN CONTROL AND 502A INOCULATED FAMILIES DURING FIRST YEAR AFTER TREATMENT†

	Families	*Individuals*	*Individuals treated*	*Recurrence of Original Strains*		*Individuals with lesions*
				Number	*Per cent*	
Control*	12	51	42	31	74	15
502A** Inoculated	16	82	66	18	27	4

*Antibiotic therapy and saline.
**Antibiotic therapy and 502A.
†Reproduced by permission from: Boris, M. *et al.* Bacterial interence: Protection from intrafamilial staphylococcal disease. *Amer. J. Dis. Child. 115*:521-29, 1968.

ganisms of 52/52A/80/81 strain to these persons showed complete rejection in all the 502A carriers. However, one individual became colonized with the challenge 52/52A/80/81 strain; he had failed to become colonized with 502A when inoculated.

In a control group of persistent carriers, 502A was inoculated after four weeks without prior antibiotic therapy. Two of 16 members initially were colonized transiently with the 502A strain, which could no longer be isolated within one week. These individuals were subsequently treated with oxacillin and challenged with the 52/52A/80/81 pathogenic strain; all members of this group became colonized with the latter organism, in contrast to none of the 502A carriers. The challenge strain persisted in 10 of the 15 individuals for at least four weeks. The data demonstrates that colonization of the nasal mucosa with the 502A strain of *S. aureus* is possible after adequate antibiotic therapy. This artificial colonization would subsequently protect individuals from direct challenge with pathogenic strains of staphylococci.

Since it was possible to change the colonization status of persistent carriers, the next study was to demonstrate that artificial colonization could interrupt chronic staphylococcal disease in families.[7]

Families in which more than one member had had recurrent staphylococcal lesions for one year constituted the volunteer group. All had received varied treatments in the past, including toxoids, antibiotics, and antiseptic washes, without much success. All members of the family who harbored the pathogenic, henceforth called the resident strain, were treated with systemic and local antibiotics until two successive sets of nasal cultures, taken at least three days apart after 10 days of treat-

TABLE VI.—STAPHYLOCOCCAL LESIONS IN CONTROL AND 502A-INOCULATED INDIVIDUALS

	Control group		*502A group*		
Type of lesion	*Number lesions*	*Number cultured and original strain isolated*	*Number lesions*	*Number cultured with 502A isolated*	*Number 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

*Twenty-seven lesions in 15 control individuals.

ment, were negative for this resident strain. The families were divided randomly into two study groups. Within 24 hours after cessation of antibiotic therapy, either 502A or sterile saline was applied on four successive mornings to each side of the nostrils of the participants.

Twenty-eight families with 133 members were entered in this study (Table V). In the group inoculated with 502A, all the 66 members who were treated with antibiotics and nasally inoculated with the 502A strain were successfully colonized. Of these 66 individuals, the original resident strain recurred during the subsequent 12-month interval in only 18, or 27 per cent. During the same period, the original resident strain recurred in 31 of 42 control individuals, or 74 per cent. The difference is highly significant statistically.

Among the 15 control individuals, there were 27 distinct pyogenic episodes, compared to only 4 isolated lesions in the 502A inoculated (Table VI). Among these four lesions there were two pustules and one stye from which the 502A strain was isolated. The other pustule was caused by an 80/81 organism, the individual's original resident strain.

The 502A strain of *Staph. aureus* persisted well. After one month 61 of 66, or 92 per cent of the inoculated members still carried the 502A organism (Figure 1). At six months, 71 per cent harbored the

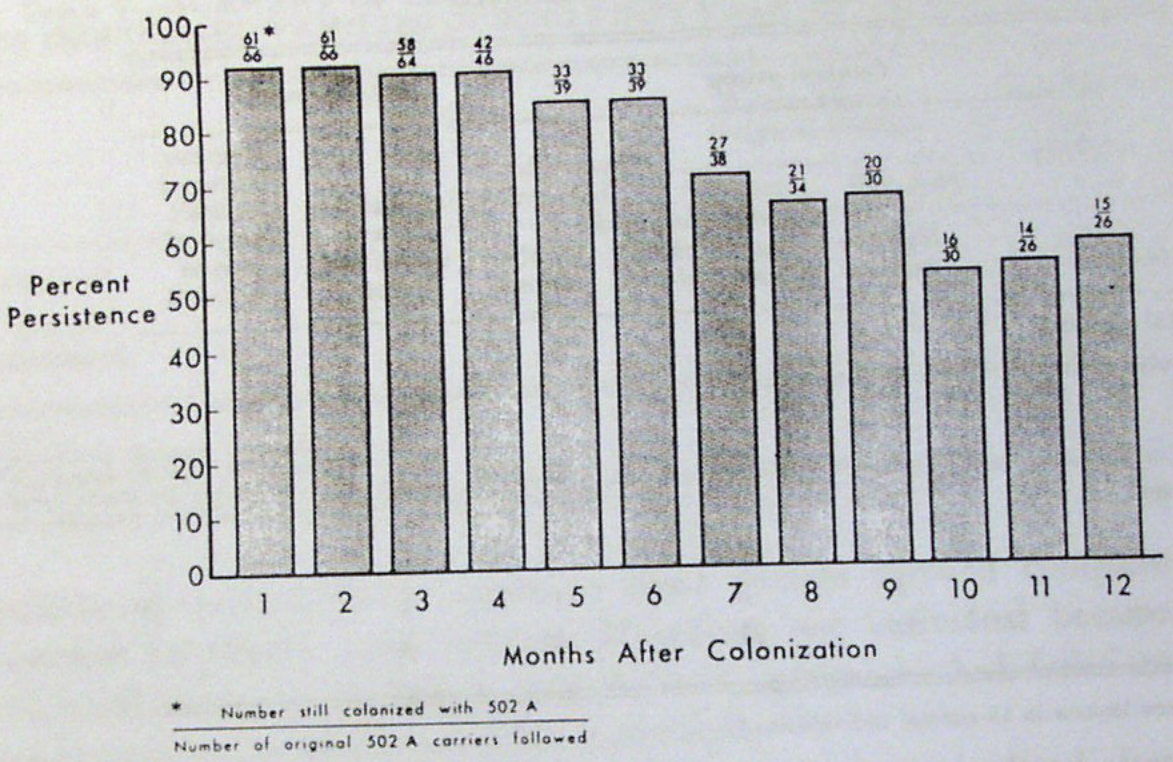

Fig. 1. Persistence *Staphylococcus aureus* 502A in colonized individuals. Reproduced by permission from: Boris, M. *et al.* Bacterial interference: Protection from intrafamilial staphylococcal disease. *Amer. J. Dis. Child. 115*:521-29, 1968.

TABLE VII.—LESIONS AMONG 160 INDIVIDUALS COLONIZED WITH 502A

Lesion	*Nasal colonization status* *502A (128)*	*Relapse with resident strain (32)*
Stye	3*	2**
Pustule	2*	2 (1*) (1**)
Abscess	1**	10 (1*) (9**)
Impetigo	1*	4**

*S. *aureus* 502A isolated.
**Resident *S. aureus* isolated.

strain and, after 12 months, 15 of 26, or 58 per cent, were colonized by the 502A strain.

Since the initial observations 160 additional individuals have been treated with antibiotics and subsequently colonized with the 502A strain and followed for a period of up to three and a half years. Thirty-two of these individuals, or 20 per cent, have had recurrence of their original strain. A total of eight minor lesions due to *Staph. aureus* 502A

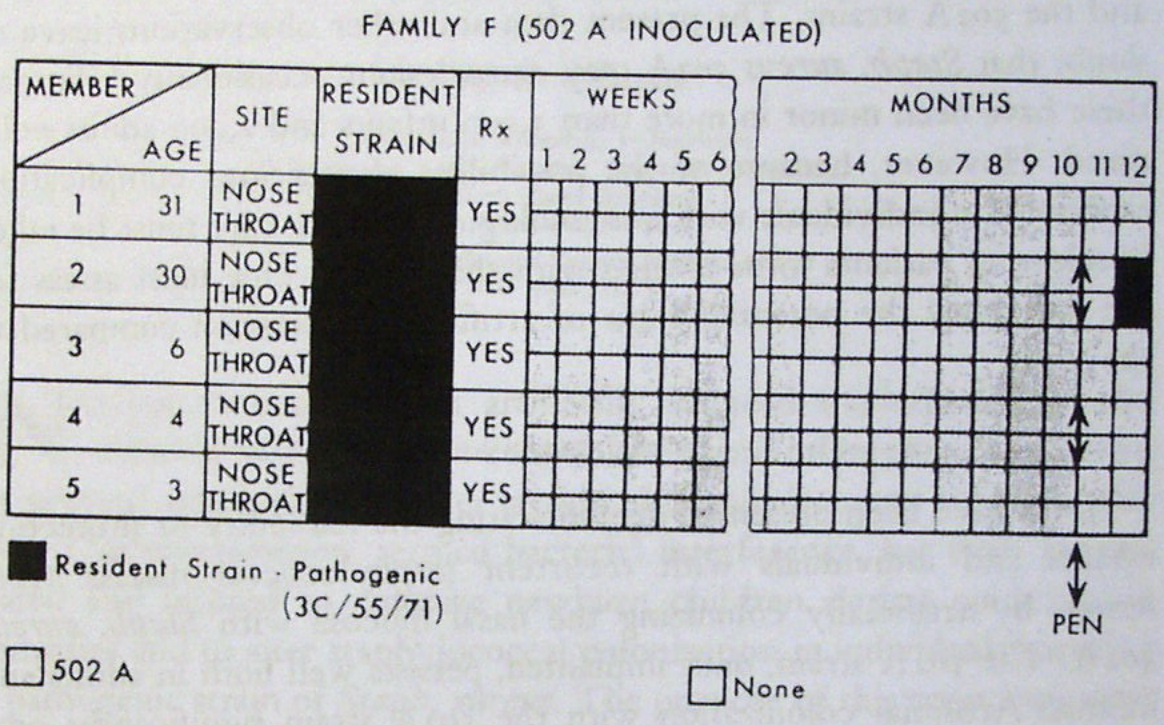

Fig. 2. Summary of *Staphylococcus aureus* cultures before and after treatment.

was observed in these members. All were single pustules except for two styes (Table VII).

Several noteworthy observations were made.

Figure 2 demonstrates *Staph. aureus* cultures before and after treatment in a family colonized with the 502A strain. The family was successfully colonized with 502A, and had no other strains isolated from the nasal mucosa for 10 months. At this time penicillin was administered to two members of the family. Member two lost the 502A strain, and one month later had a recurrence following antibiotic therapy of the resident strain many months after successful colonization with 502A. In addition, there were nine individuals who, between five and 18 months after successful colonization by 502A, lost the 502A strain and, within one month, showed reappearance of their original pathogenic strain. The reason for this is not clear. It may have been due to the persistence of a small number of resident strain organisms on the nasal mucosa, insufficient to be detected by our cultural techniques, or the resident strain may have persisted as an altered variant, such as an L form.

The epidemiological evidence gathered in this study and in previous epidemics that occurred in nurseries suggests that the prime factor responsible for lesion rates in the study and control groups was

the difference in virulence between the resident *Staph. aureus* strains and the 502A strains. The present data and other observations leave no doubt that *Staph. aureus* 502A may cause lesions occasionally.[8] To date these have been minor in more than 3,000 infants and 2,000 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 regime. One must assess for the individual the potential value of artificial colonization compared to the risks.

Summary

Data have been presented demonstrating the feasibility of protecting infants and individuals with recurrent staphylococcal disease from lesions by artificially colonizing the nasal mucosa with *Staph. aureus* 502A. The 502A strain, once implanted, persists well both in adults and infants. Artificial colonization with the 502A strain significantly protected infants and adults from appearance of epidemic strains and lesions. Because minor lesions may be associated with *Staph. aureus* strain 502A, the potential value of therapy must be weighed against the risk in individuals who are to be colonized or recolonized.

REFERENCES

1. Shinefield, H., Ribble, J., Boris, M. and Eichenwald, H. F. Bacterial Interference: Its effect on nursery acquired infection with *staphylococcus aureus*. *Amer. J. Dis. Child. 105*:645-54, 1963.
2. Cohen, J. O., Smith, P. B., Shotts, E. B., Boris, M. and Updike, E. L. VI. Detection of *Staphylococcus aureus* strain 502A by serologic and phage typing. *Amer. J. Dis. Child. 105*:689-91, 1963.
3. Shinefield, H. R., Sutherland, J. M., Ribble, J. C. and Eichenwald, H. R. The Ohio epidemic. *Amer. J. Dis. Child. 105*: 655-62, 1963.
4. Shinefield, H., Boris, M., Ribble, J., Cale, E. and Eichenwald, H. The Georgia epidemic. *Amer. J. Dis. Child. 105*: 663-73, 1963.
5. Boris, M., Shinefield, H., Ribble, J., Eichenwald, H., Hauser, G. and Caraway, C. The Louisiana epidemic. *Amer. J. Dis. Child. 105*:674-82, 1963.
6. Light, I. J., Sutherland, J. M. and Schott, J. E. Control of a staphylococcal outbreak in a nursery, use of bacterial interference. *J.A.M.A. 193*:699-705, 1965.
7. Boris, M., Shinefield, H. R., Romano, P., McCarthy, A. B. and Florman, A. L. Bacterial interference: Protection from intrafamilial staphylococcal disease. *Amer. J. Dis. Child. 115*:521-29, 1968.
8. Drutz, D. J., Van Way, M. H., Schaffner, W. and Koenig, M. G. Bacterial interference in the therapy of recurrent staphylococcal infections. *New Eng. J. Med. 275*:1161-65, 1966.