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Bacterial Interference among Strains of *Staphylococcus aureus* in Man

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Factors related to artificial colonization by and persistence of *Staphylococcus aureus* (strain 502A) were characterized. Carriers of *S. aureus* were treated with antibiotics and antibacterial soaps and challenged with strain 502A. All subjects who lost their original *S. aureus* after treatment with antibiotics were colonized successfully with either 1.5×10^8 or 2×10^9 colony-forming units of strain 502A; these bacteria persisted in 67%–80% of subjects for 23 weeks. Eighty percent of subjects became colonized by strain 502A with persistence in 17% when the original strain(s) of *S. aureus* were reduced but not eliminated. Subjects not treated with antibiotics and challenged with strain 502A were temporarily colonized, and the extent of colonization was related to dose. A challenge inoculum of 10^9 *S. aureus* 502A caused colonization in 100%, while 50% were colonized with 10^7 and 10^5 colony-forming units. Of the 12 subjects in this group, persistence at 10 weeks was noted in only a single individual who received an inoculum of 2.1 $\times 10^9$. Colorization and persistence in subjects who initially resisted colonization by strain 502A and were treated later with antibiotics were similar to those in treated subjects. The data suggest that (*1*) eradication of the original strain of *S. aureus* is an important factor for optimal colonization and persistence of strain 502A; (*2*) it is possible to overwhelm the normal mucosa of persons who carry *S. aureus* with large numbers of strain 502A; and (*3*) carriers who initially rejected artificial colonization can become susceptible to colonization by and persistence of *S. aureus* strain 502A after treatment with antibiotics.

It has been established that interference between strains of *Staphylococcus aureus* is a useful tool in curtailing epidemics of staphylococcal disease in nurseries and interrupting cycles of recurrent staphylococcal furunculosis in individuals [1–5]. However, the factors responsible for establishing colonization and persistence after artificial colonization with marker strains of *S. aureus* have not been defined clearly. The purpose of the present investigation was to uncover some of the factors. The question of use of larger numbers of strain 502A to improve colonization and persistence was investigated. Other questions studied were (*1*) the role of normal flora in influencing colonization by strain 502A after administration of antibiotics; (*2*) the persistence of strain 502A in subjects who lost their resident strain(s) of *S. aureus*, either completely or partially, after treatment with antibiotics; and (*3*) whether carriers of *S. aureus* who initially resisted colonization by strain 502A before administration of antibiotics became susceptible after treatment.

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Materials and Methods

The nasal mucosa of 200 institutionalized male volunteers were cultured for four consecutive weeks. Subjects from whom *S. aureus* was recovered in three of the four cultures were designated as carriers. The staphylococcal carriers whose nasal resident *S. aureus* were sensitive to dicloxacillin, resistant to 2–10 units of penicillin/ml, and sensitive to 5 μg of tetracycline/ml were selected. These criteria facilitated separation of strain 502A from resident strains of *S. aureus*. *S. aureus* strain 502A is sensitive to 0.05 units of penicillin/ml and resistant to 25 μg of tetracycline/ml.

Collection of nasal flora. The nasal flora was collected by using eight calcium alginate swabs [6]. Tenfold dilutions (to 10^4) were made in nutrient broth. These dilutions were adjusted according to the number of nasal flora at different phases of

the study. Sheep's blood agar was used to estimate total counts. Trypticase soy agar (TSA) containing Tween 80 was used for lipophilic diphtheroids, since these bacteria grew poorly on blood agar plates. MacConkey agar and Sabouraud's glucose agar were incorporated for detection of enteric bacteria and yeasts. Tetracycline (25 μg/ml) or penicillin (2 or 10 units/ml) were incorporated into blood agar for detection of strain 502A (which is resistant to tetracycline) and resident strains of *S. aureus* (which are resistant to penicillin). Preliminary data were obtained to determine whether the concentration of penicillin used in culture medium reduced the number of *S. aureus* recovered from volunteers. No reduction in numbers was noted when nasal swabs were cultured on medium containing penicillin and on penicillin free medium. Nasal cultures were obtained from all participants just before treatment with antibiotics, 48 hr after treatment, and at various intervals for 23 weeks following cessation of colonization by strain 502A.

Antibiotic treatment. Five hundred milligrams of sodium dicloxacillin were given orally thrice daily for eight days. At the same time, neosporin ointment was applied locally to the nasal mucosa three times a day. Soaps containing 0.75% hexachlorophene and 0.75% trichlorocarbanilide were used for routine bathing and washing during treatment.

Implantation of S. aureus strain 502A. Forty-eight hours after cessation of antibiotics, various inocula of strain 502A were used to colonize by implantation different groups of individuals. Both sides of the nostrils, chest, axillae, groin, and rectal areas were used for implantation. A 1-ml syringe (without needle) was used to deliver 0.1 ml of bacterial suspension at each specific site. The size of inoculum was determined for each experiment. Implantation was performed daily for seven days. During this week, nongermicidal soaps were used for washing. Strain 502A is coagulase- and mannitol-positive. It is a group 3 *S. aureus* with a bacteriophage type of 6/7/42D/42A/53/54/75/81.

Results

Persistent carriers whose antibiograms and phage types for *S. aureus* were different from strain 502A were divided into three groups. Of these, 17 were treated with antibiotics and divided into two groups (groups 1 and 2) for further study. The remaining 12 were used as controls (group 3) and were not treated with antibiotics.

The total aerobic nasal flora before antibiotic treatment was determined for groups 1 and 2. Nasal bacterial counts for each group of organisms from these 17 individuals were averaged. Coagulase-positive gram-positive cocci (1.7×10^5), coagulase-negative gram-positive cocci (1.1×10^5). lipophilic diphtheroids (0.5×10^5), and nonlipophilic diphtheroids (0.7×10^5) were preponderant. Gram-negative bacilli and cocci were cultured from only two subjects.

The total number of microorganisms decreased in all subjects after treatment with antibiotics. Coagulase-positive gram-positive cocci were not detected in 11 of 17 subjects (65%). Although the number of coagulase-negative gram-positive cocci decreased, this organism was not completely eliminated from any treated subject. Lipophilic and nonlipophilic diptheroids were most sensitive to antibiotic treatment and were not detected in 14 of 17 subjects (82%).

Nine subjects (group 1) were selected for implantation with 1.5×10^8 cfu of strain 502A at each specified site, and were followed up for 23 weeks. Only when strain 502A was not detected on the nasal mucosa were the other sites such as groin, axilla, and rectal area swabbed. Only two of eight subjects had strain 502A in the rectal areas. The other sites were negative. Two of three subjects who retained resident *S. aureus* in reduced numbers after antibiotic treatment became colonized. Strain 502A did not persist in these subjects after eight weeks. However, colonization was observed in the remaining six subjects whose original *S. aureus* was not detected after antibiotic treatment on day 7 after implantation; at the end of 23 weeks, only four of six subjects were colonized.

The original *S. aureus* strain, strain 502A, and/or acquisition of a new strain of *S. aureus* were found simultaneously in the anterior nares of some subjects. In many individuals, when the original *S. aureus* strain and strain 502A were present in equal numbers in the anterior nares, strain 502A was eventually eliminated.

A larger inoculum of strain 502A (2.0×10^9 cfu) was used for colonization of eight subjects. As with the first group, when the resident *S. aureus* was eliminated from the anterior nares by

treatment with antibiotics, 100% of the subjects were colonized. At the end of 23 weeks, this figure was 80% (four of five subjects), not significantly different from that for subjects colonized with 1.5×10^5 cfu of *S. aureus*. Of the three subjects who retained their original *S. aureus* strain, two became colonized, and persistence was seen in only one.

Persistence of strain 502A was compared in the two groups who lost their original *S. aureus* strain, either completely or partially, after treatment with antibiotics (table 1). Persistence of strain 502A depended on successful elimination of resident *S. aureus*. When the resident *S. aureus* was not detected on the nasal mucosa, the persistence rate in 17 subjects was 100% for the first week, 88% (15 of 17) at the 13th week, and 73% (eight of 11) at the 23rd week. Subjects who retained their resident strain of *S. aureus* on the nasal mucosa demonstrated 73% (eight of 11) persistence the first week, 20% (two of 10) at the 13th week, and 17% (one of six) at the 23rd week.

Persistence of strain 502A in relation to the presence or absence of lipophilic and nonlipophilic diphtheroids after antibiotic treatment was compared. No difference in the persistence of 502A was noted when the subjects who lost their nasal diphtheroids, either completely or partially, were compared.

Twelve staphylococcal carriers not receiving sodium dicloxacillin were implanted with strain 502A (figure 1). Strain 502A in three dilutions (2.1×10^9, 2.1×10^7, and 1.1×10^5 cfu) was used to colonize three groups, each comprised of four subjects. Subjects implanted with 10^9 cells of strain 502A were more successfully colonized than those inoculated with fewer bacteria (10^7 and 10^5). One-hundred percent colonization was seen in subjects receiving 10^9 cfu of strain 502A after three days; 50% colonization was seen in groups receiving 10^7 and 10^5 cfu. The colonizing bacteria were eliminated on day 10 in the latter groups. With the exception of subject no. 3 (receiving 2.1×10^9 cfu), none of the participants in this experiment had strain 502A 10 weeks after implantation.

An experiment was designed to determine the effect of antibiotic on the microflora of the same subjects (controls) who rejected colonization by strain 502A when antibiotic was not administered before implantation. The inoculum for implantation was 1.0×10^9 cfu of strain 502A. One-hundred percent colonization was noted for up to 10 days, and strain 502A persisted in five of six subjects (83%) 13 weeks after implantation.

Discussion

Although *S. aureus* strain 502A has been used successfully to combat *S. aureus* infection in neonates [1, 2, 5] and in patients with recurrent furunculosis [3, 4], relapses of furunculosis have been noted when strain 502A is lost and is replaced by the original strain of *S. aureus* [4]. Previously, a 36% persistence rate at the end of 19 weeks was achieved when an inoculum of 1.0×10^6 cfu (three times for four days) was used [7]. One of our goals was to determine whether a larger inoculum (10^8–10^9) of strain 502A than used previously would result in a higher rate of persistence. The results showed that the rate of persistence could be significantly increased by our methods.

Elimination of the original *S. aureus* strain(s) in certain subjects rendered the nasal mucosa more susceptible to artificial implantation with

Table 1. Comparison of persistence of strain 502A in groups of volunteers losing or retaining *Staphylococcus aureus* after treatment with sodium dicloxacillin and neosporin.

Subjects	Persistence of strain 502A		
	7 days	13 weeks	23 weeks
Resident strain(s) of *S. aureus* eliminated by treatment	17/17* (100%)	15/17 (88%)	8/11 (73%)
Resident strain(s) of *S. aureus* not eliminated but reduced in number by treatment	8/11 (73%) ($P = 0.09$)	2/10 (20%) ($P = 0.004$)	1/6 (17%) ($P = 0.027$)

* Eleven subjects used as controls and treated later with antibiotics were also included for comparison after seven days and 13 weeks.

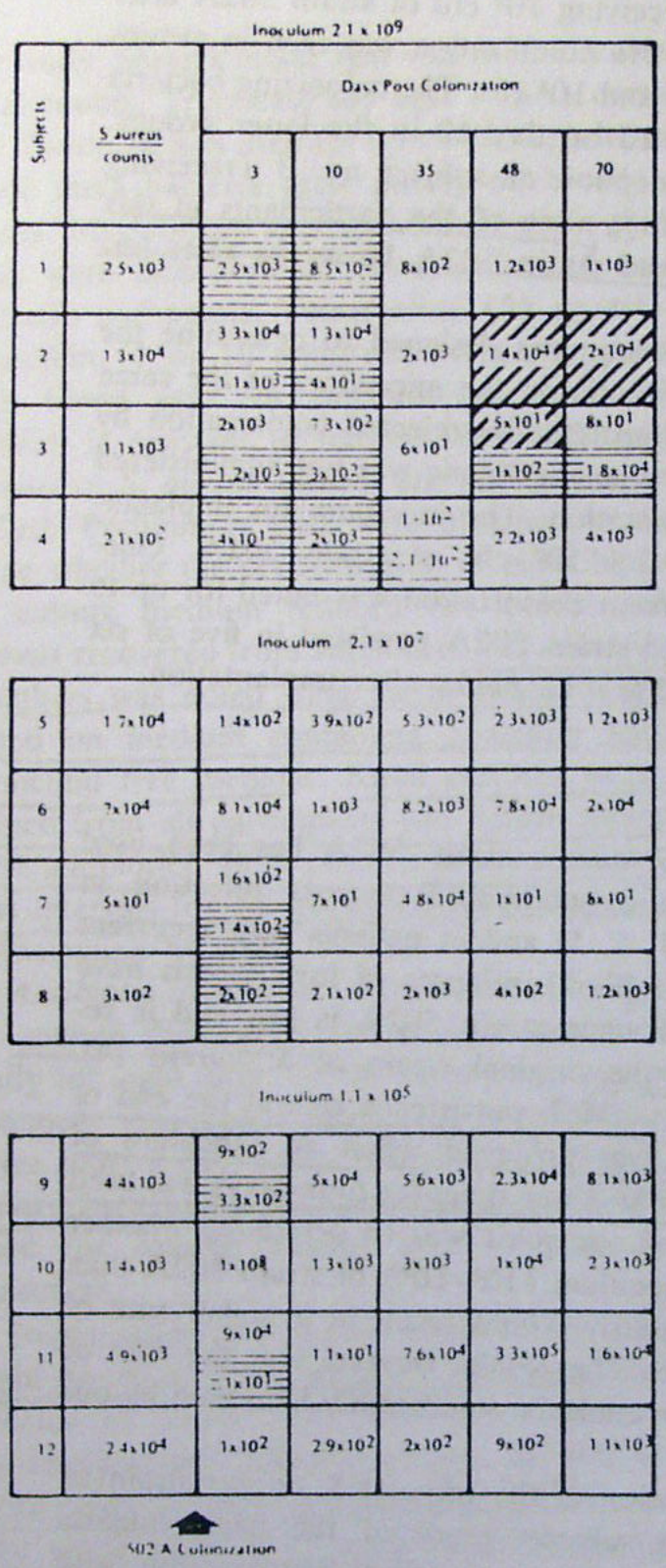

Figure 1. Colonization by and persistence of *Staphylococcus aureus* strain 502A in subjects not treated with antibiotics and implanted with 2.1×10^9, 2.1×10^7, and 1.1×10^5 cfu of strain 502A. ▤ = strain 502A; □ = resident strain of *S. aureus*; ▤ = resident strain *S. aureus* plus strain 502A; ▨ = new strain of *S. aureus*.

strain 502A (67%–80% persistence rate). This relationship correlates with previous reports in recurrent furunculosis of 80% curtailment of the disease in patients implanted with strain 502A. On the other hand, subjects retaining even small numbers of their resident *S. aureus* after treatment were less susceptible to artificial implantation and persistence (17%), thus supporting the hypothesis that removal of *S. aureus* rendered nasal mucosa much more susceptible to 502A colonization and persistence. However, other unknown host factors suppressed by antimicrobial therapy may influence colonization and persistence of implanted *S. aureus*.

A quantitative evaluation of this study indicated that the original *S. aureus* strain and strain 502A can coexist in the anterior nares. In this study, when both organisms were present in equal numbers, the artificially introduced strain 502A was eliminated in most cases. Two subjects transiently acquired new strains of *S. aureus*, which persisted in one. The extent of colonization in subjects who were not treated with antibiotics was dependent on size of inoculum. Half of the subjects inoculated with 10^7 or 10^5 cfu of strain 502A were colonized on day 3, and none was colonized on day 70. All subjects receiving 10^9 cfu of strain 502A were colonized, and persistence was seen in one of four subjects. Thus it was demonstrated that it is possible to overwhelm the host temporarily with larger inocula.

It is possible that rejection of artificial colonization was due to host factor(s) that differed from those of the subjects treated with antibiotics. This hypothesis was studied by treatment and colonization of the subjects (used as controls) who initially rejected colonization by 502A. In this group, high rates of colonization (100%) and persistence (83%) were shown. Administration of antibiotic modified the host defense to favor colonization by strain 502A.

An inverse relationship between diphtheroids and gram-positive cocci has been suggested by reports that these diphtheroids may be responsible for controlling bacterial ecology [6, 8, 9]. Our data permit only limited speculation regarding the interrelationship of these organisms. There were no differences in the persistence rate of strain 502A in those subjects from whose nose the diphtheroids (lipophilic and nonlipophilic) were eliminated, either partially or completely. However, we realize that antibiotics reduced significantly the number of diphtheroids; with reduced numbers of diphtheroids the interfering capacity may have been reduced also.

The present study shed no light on the mechanism of bacterial interference between strains of *S. aureus*. The mechanism of bacterial interference

has been studied in chick embryos [10–13], in broth culture [14], and in experimental burns [15, 16]. Some of these studies have revealed mechanisms for the specific model under study. To extrapolate from either the in vitro or the in vivo results to humans is difficult. Despite these limitations, it has been documented that this phenomenon can be used in specific clinical situations. The data do emphasize the importance of eliminating the original *S. aureus* strain for achieving optimal artificial colonization with strain 502A.

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