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Bacterial interference between strains of *Staphylococcus aureus*

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Endogenous bacterial flora protects the host from deleterious bacterial infection. Mechanisms of this interaction are complex and not well understood. We have been interested in the interrelationship between strains of *Staph. aureus*. I would like to summarize data collected over the past ten years concerning this phenomenon. In 1961, epidemiologic observations during a nursery outbreak of staphylococcal disease suggested to us that colonization of the nasal mucosa or umbilical stump of an infant with *Staph aureus* prevented subsequent colonization at the same site by a second strain of *Staph. aureus*.

In order to test this hypothesis, further observations by direct inoculation of *Staph. aureus* were made on a series of infants, medical students, nurses, and prisoner volunteers. For inoculation, a coagulase positive strain of *Staph aureus* of low virulence susceptible to penicillin and incapable of being induced to produce beta-lactamase was used. The organism is lysed by group III staphylococcal phages and is referred to as strain 502A.

Table 1. Types of organisms present on nasal mucosa related to successful takes* in infants over 24 hours old; inoculum 500 or more bacteria

	Total infants	Other than *Staphlococcus*		*Staphylococcus*			
				Coagulase-negative		Coagulase-positive	
		Present	Absent	Present	Absent	Present	Absent
Take	68	38	30	28	40	0	68
No take	10	7	3	8	2	4	6
		$x^2 = 0.31$;	$p = 0.62$	$x^2 = 4.04$;	$p = 0.05$	$x^2 = 13.88$;	$p = 0.001$

* Presence of marker 502A strain detected at 24 hours after inoculation

Table I presents the data on a series of 78 babies deliberately colonized. A striking relationship was noted between prior presence of *Staph. aureus* and the failure to implant 502 A strain. Coagulase negative staphylococci exerted a much effect while under the conditions of the experiment. No interference could be demonstrated by other organisms that colonized the nasal mucosa.

Observations on adults offered an opportunity for additional manipulation and studies. The human volunteer experiments involved persistent carriers and non-carriers of *Staph. aureus*, some of whom were treated locally and systemically with antimicrobials and then challenged with either marker strains of 502A or a second strain phage

type 52/52A/80/81. Cross-challenge experiments were also performed. The patients and volunteers involved were usually observed for ten weeks following challenge. The summary of the data on one group of volunteers is presented in table 2. The data offer direct evidence that in adults as well as infants, nasal colonization with coagulase positive staphylococci interferes with subsequent colonization by other strains of coagulase positive staphylococci. The ability to colonize non-carriers was independent of whether the individuals were treated with antimicrobials prior to challenge. However, persistence of the inoculated strain was significantly higher in non-carriers who received antimicrobials than in subjects who did not receive antibiotics before inoculation.

Table 2. Comparison of 'take' and persistence rates in peristent nasal carriers and persistent nasal noncarriers

Subjects	Take		Persistence			
			3 weeks		13–14 weeks	
	Rate	%	Rate	%	Rate	%
Carriers:						
Treated with sodium oxacillin and challenged with *Staph. aureus* 502A	13/13	100	11/13	85	7/13	54
Treated with placebo and challenged with *Staph. aureus* 502A	9/14	64	3/9	33	1/9	11
Noncarriers:						
Treated with sodium oxacillin and challenged with *Staph. aureus* 502A	15/18	83	11/15	73	7/15	47
Treated with placebo and challenged with *Staph. aureus* 502A	17/18	94	7/17	41	5/17	29

Other observations demonstrated interference between strains of *Staph. aureus* was site-specific (Table 3). It can be seen that a resident strain on the nasal mucosa could

Table 3. Sites of successful takes in 8 carriers treated with oxacillin and inoculated with *Staph. aureus* 502A

	Nose		Throat	
	Resident strain	502A	Resident strain	502A
Before therapy	8	—	8	—
After therapy	0	—	5	—
One month after inoculation with 502A	0	8	6	1

be eliminated by intensive antimicrobial therapy. One month after inoculation with 502A, persistence at that site with strain 502A was noted. However, antimicrobial therapy did not influence carriage of resident staphylococci on the oropharynx. Throat carriage of resident *Staph. aureus* was not influenced by nasal inoculation of 502A.

Another set of observations offered evidence that the ability to interfere was a quantitative phenomenon (Table 4). It can be seen that individuals with large numbers of resident *Staph. aureus* on the nasal mucosa are better able to withstand a challenge inoculation than those individuals with smaller numbers of *Staph. aureus*.

Additional cross-over experiments demonstrated that the ability to interfere with colonization was not the property of a single strain, and the nasal mucosa which had

Table 4. Number of *S. aureus* on the nasal mucosa of carriers related to 502A take and persistence rates

Resident *Staph. aureus* on nasal mucosa	Takes with *Staph. aureus* 502A		Persistence 1 month after colonization	
Number	Rate	%	Rate	%
$> 10^3$	2/12	16	1/12	8
$< 10^2$	5/10	50	3/10	30

been resistant to super-infection with the second strain could easily be colonized by a second strain if the interfering strain was removed with antimicrobial.

In summary, the data from observations on direct colonization with marker strains demonstrate heavy colonization of the nasal mucosa of adults with *Staph. aureus* interfered with subsequent colonization by other *Staph. aureus* strains. The capacity to interfere was not restricted to a single *Staph. aureus* type. In addition, interference between strains of *Staph. aureus* was site-specific. The resistance to colonization by a second *Staph. aureus* strain appears to depend partly on the local presence of large numbers of resident staphylococci, since removal or suppression of this original strain renders the nasal mucosa increasingly susceptible to artificial colonization. The data collected also suggest that factors exist other than the mere physical presence of staphylococci which interferes with attempts to colonize the nasal mucosa. The unknown protective factors can be suppressed with antimicrobial therapy or can be overwhelmed by repeated administration of large doses of *Staph. aureus.*

Application of the phenomenon of bacterial interference utilizing staphylococci in humans in therapy was first described by a Danish physican *Schiøtz* in 1909. He noted that a patient with a staphylococcal throat infection wrongly diagnosed as diphtheria and placed in the diphtheria ward did not become ill with disease. He then deliberately sprayed suspensions of staphylococci into throats of the diphtheria carriers and claimed good results in eliminating the carrier state.

We first used the phenomenon of interference in controlling severe outbreaks of staphylococcal disease in nurseries. Several nurseries which cared for different population groups and engaged in a variety of nursery practices were studied. In common, were high infant colonization and disease rates due to a single strain of staphylococcus. Initially, half of the infants in the nursery were artificially colonized on the nasal mucosa and umbilical stump with strain 502A in the first few hours of life, while the other infants received placebo consisting of saline solution. Hospital personnel who carried epidemic strains were permitted to continue to work, and the infants were followed at home for a period of a year to determine nasal colonizations status and disease rates. It was conclusively demonstrated that nasal colonization with 502A afforded newborn infants virtually complete protection. Over the past seven years, at least eight nursery staphylococcal epidemics in which colonization with *Staph. aureus* 502A was used as a control measure have been reported. In no instance has this technique failed to curtail the epidemic.

More than 4,000 infants have been colonized. Five to fifteen percent of the infants colonized with 502A developed tiny vesicles around the umbilical area in the first few days of life. These spontaneously disappeared and were not a cause of concern. In one nursery, in a group of 50 infants, the rate of periumbilical lesions was reported to be 34%. None of these infants developed any serious disease on careful follow-up during a period of more than one year. Conjunctivitis has also been seen in the newborn

Table 5. Pustular lesions related to inoculum size of *Staph. aureus* 502A

Authors	Number of organisms and method	Number of newborns	Newborns with pustules (%)
Shinefield et al.	2×10^3 to 4×10^3 (microburette)	524	1.0
Light et al.	2×10^3 to 5×10^4 (cotton swab)	584*	<5.0
Light et al.	2×10^3 to 5×10^4 (cotton swab)	687	3.5
Houck et al.	2×10^3 to 5×10^4 (cotton swab)	644	4.7
Light et al.	1×10^8 (cotton swab)	85	14.0
Blair and *Tull*	1×10^8 (cotton swab)	50	34.0
Houck et al.	? (cross infection)	444	0.5

* 470 full term; 114 premature

associated with *Staph. aureus* 502A. There has been a single case of severe infection following colonization of an infant with *Staph. aureus* 502A. This was an immature infant of a diabetic mother who had been colonized at three hours of age. At eight hours of age the infant was noted to be apneic, sluggish, and the infant was hypoglycemic. Through this colonized umbilical site, a polyethylene catheter was inserted into the umbilical vein and infusion begun with 15% glucose. Treatment was delayed until the infant was 68 hours old. He died at 84 hours of age of septicemia and meningitis.

Cultures from the blood and peritoneum grew both *Staph. aureus* 502A and *E. coli*. Post-mortem cultures of the meningis grew *Staph. aureus* 502A. It should be noted that this was a premature hypoglycemic baby who was catheterized through an infected site and profused with a highly irritating solution of 15% glucose.

Experience has demonstrated that the pustular lesions can be minimized since they are related to the inoculum size of *Staph. aureus* 502A (Table 5). It can be seen deliberate colonization with two to four thousand bacteria results in a pustular rate of 1%. When a cotton swab technique is used, which results in application of approximately 2,000 to 50,000 bacteria, the pustular rate is in the range of 5%, while colonization with 1×10^8 bacteria results in a lesion rate of 14–34%. It should be noted that in a large group of infants who were cross-infected the pustular rate was approximately 0.5%. All the data suggest small numbers of inoculated organisms minimize the pustular rate. Since colonization can be accomplished with relatively few bacteria, it would seem wise to colonize with about 5,000 to 10,000 502A during epidemics (Table 6).

The data substantiate the fact that artificial colonization of newborn infants with 502A in nursery situations where there is a high colonization rate and disease rate due to a virulent hospital strain of staphylococcus is an effective and safe method for curtailing epidemics provided reasonable precautions are followed.

Another situation where the concept of bacterial interference has been useful is in the treatment of patients with recurrent furunculosis. Here the technique has been one of recolonization rather than colonization. Prior to the nasal application of strain 502A, individuals with recurrent furunculosis are treated with antibiotics systemically and also with application of an antimicrobial cream to the nasal mucosa. This technique

Table 6. Frequency of 'takes'* in relation to inoculum size of *Staph. aureus* 502A

Authors	Method and number of organisms	Site of inoculation	Number of patients	'Takes'	
				Number	%
Shinefield et al.	Microburette 2000–4000	Nose	42	39	93
		Umbilicus	42	30	72
Boris et al.	Microburette 2000–4000	Nose	25	21	84
		Umbilicus	25	23	92
Light et al.	Cotton swab moistened with broth containing (estimated 2000–5$\times 10^4$)	Nose	584	530	91
		Umbilicus	584	520	89

* 'Take' indicates presence of marker 502A strain detected at 24 hours after inoculation

Table 7

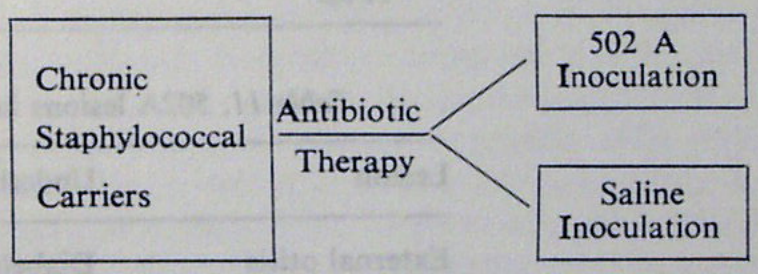

Table 8. Summary of colonization status in control and inoculated families during first year after treatment

	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.

eliminates the staphylococcal carrier strain related to the disease and is necessary to assure effective nasal colonization with 502A.

Tables 7 and 8 outline a controlled study utilizing this technique. It can be seen that about 80% of individuals with recurrent furunculosis were cured. Additional data has been collected on approximately 600 patients over a seven year period of time. In this group of individuals relapse or recurrence of the original staphylococcal strain on the nasal mucosa of treated individuals within twelve months was 21% (Table 9). A

Table 9. Relapses in 587 patients colonized with *Staph. aureus* 502A

Underlying disase	Patients treated	Relapsed
Diabetes	5	4
Eczema	3	3
Acne	11	5
Total relapse	122/587	(21%)

high relapse rate was noted in patients with diabetes, eczema, or acne. Of interest was the fact that relapse rate was 15% of the patients treated with a penicillin derivative, while patients suspected of penicillin allergy and treated with lincomycin exhibited a relapse rate of 45% (Table 10).

Table 10. Relapses related to initial therapy

	Total treated	Relapse	%
Dicloxacillin	470	69	15
Lincomycin	117	53	45
Total	587	122	21

Table 11. 502A lesions in 587 recolonized patients

Lesion	Underlying disease	Patients
External otitis	Diabetes	1
Impetigo	Eczema	2
Pyarthrosis	Diabetes	1
Pustuls (1 or 2)	Diabetes	1
	None	3
Stye	None	3
Total		11

Disease associated with 502A was noted in eleven patients (Table 11). Three patients were diabetics and two had extensive eczema. To date there is only one case of recolonization associated with a 502A lesion classified as more than mild. This was a diabetic with pyarthrosis that responded well to antibiotics. The patient had no further staphylococcal disease despite numerous lesions prior to colonization.

Other workers reported lesions associated with the 502A strain of staphylococcus. An occasional pustule was seen by *Maibach* and his group. *Drutz* and his co-workers reported a patient with primary skin disease developed a 502A abscess while on steroids.

The importance of the nasal colonization status in patients with furunculosis is illustrated in a report of recolonization by *Strauss* and his associates. Three patients with recurrent furunculosis recolonized by the standard technique became free of furunculosis. After a period of six months to a year, they were again noted to be nasal carriers of the original pathogenic strain. At this time episodes of recurrent furunculosis related to the original carrier strain were again noted in all patients.

Recolonization is not helpful in all situations. After recolonization, no decrease in disease rate was noted in an institution with a mild but chronic staphylococcal problem. Therefore, utilization of this technique must be individualized as to patient and environment.

The mechanism responsible for this phenomenon in humans is not understood. The possibilities include the development of an unfavorable growth environment, as a result of initial colonization. This may result from production of inhibitors, creation of unfavorable pH or redox potential, accumulation of toxic metabolic products or the production of an antimicrobial substance which may result in bacterial antagonism. Another possibility is that the colonizing strain depletes the environment of an essential nutrient and thus inhibits growth of a second strain of a similar organism. As a matter

of fact, the precise mechanism of interference between two staphylococcal strains has been determined in some experimental models. *Ribble* found that bacteria-free filtrate prepared from broth cultures of coagulase negative staphylococci was less able to support growth of coagulase positive staphylococci than fresh broth. He offered evidence that the mechanism of action in this in vitro model could be attributed to the production of a non-protein dialyzable heat labile substance. Primary action of the substance was to interfere with the utilization of an essential nutrient of the organism, niacinamide, and thus interfere with staphylococcal growth. It is of interest when allantoic fluid instead of broth is used as a medium for bacterial growth, interference is the result of nutrient exhaustion, in as much as addition of a combination of amino acids results in the restoration of the ability of filtrate to support the growth of coagulase positive staphylococci.

The in vivo models that have been used to study the phenomenon of bacterial interference between two strains of staphylococci include the fertile hens egg and full thickness burns of the skin of rabbits. Many interesting observations have been made in these models, but the exact explanation for interference in these circumstances has not been completely understood.

It is clear that although the phenomenon of bacterial interference between strains of *Staph. aureus* has been subject to intensive investigation, there is no explanation for the well described observations in humans. Despite these limitations, some useful information and therapeutic tools have been developed. In at least two situations, nursery outbreaks of staphylococcal disease and recurrent furunculosis in humans, the host can be protected by deliberate implantation or manipulation of the flora of the upper respiratory tract. It is of interest that attempts are being made to utilize this phenomenon in protecting the host against other organisms as well. New and imaginative investigation may lead to clarification of this interesting phenomenon forthcoming.

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