Bacterial interference in the treatment of recurrent staphylococcal infections in a family

The concept of bacterial interference was utilized in the treatment of a family with recurrent staphylococcal infections. After eradication of the resistant strain of Staphylococcus aureus phage type 80/81, a penicillin-sensitive strain of Staphylococcus aureus designated 502A was implanted. With the exception of a single superficial infection with the original resident strain, the family has remained free of staphylococcal infection for 17 months.

Richard N. Fine, M.D.,* Jean M. Onslow, B.S., Mary L. Erwin, B.S., and Jay O. Cohen, Ph.D.

LOS ANGELES, CALIF., AND ATLANTA, GA.

RECURRENT STAPHYLOCOCCAL infections are common clinical problems. The offending organism in most instances is a penicillin-resistant staphylococcus; however, the "new" penicillins, effective against penicillinase-producing staphylococci, have not proved entirely effective for the prevention of recurrent infections.[1] Prolonged antibacterial therapy, administration of gamma globulin[2] and commercial and autogenous vaccines,[3] hexachlorophene disinfection, and intranasal application of antibiotic ointments have been tried without uniform therapeutic success.

To avert epidemics of staphylococcal infections[4, 5] Shinefield and co-workers[6] implanted a coagulase-positive, penicillin-sensitive staphylococcus of low virulence around the umbilicus and in the anterior nares of newborn infants to "interfere" with colonization and subsequent infection by a pathogenic penicillin-resistant staphylococcus which was harbored in the nursery. The results of their studies indicated that colonization by the resistant staphylococcus was prevented and epidemics were curtailed.[7]

This concept was carried further in an attempt to eradicate the carrier state. Studies performed on human volunteers[8] who carried the staphylococcus in their anterior nares showed that after eradication of the resident strain, implantation with a penicillin-sensitive strain (502A) prevented recolonization with the resident strain. Furthermore, Strauss, Maibach, and Hurst[9] reported the use, in one patient, of 502A in decreasing the carrier rate of a penicillin-resistant staphylococcus which had caused recurrent attacks of furun-

From the Departments of Pediatrics, Childrens Hospital of Los Angeles and the University of Southern California, and the Communicable Disease Center, United States Public Health Service, Department of Health, Education and Welfare, Atlanta.

Supported by the Fluid Research Fund of Childrens Hospital of Los Angeles.

**Address, Department of Pediatrics, Childrens Hospital of Los Angeles, 4614 Sunset Boulevard, Los Angeles, Calif. 90027.*

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culosis, thereby effecting a decrease in further clinical manifestations.

The present report describes the use of bacterial interference in combating recurrent clinical infections with a penicillin-resistant staphylococcus phage type 80/81 in a family.

DEFINITIONS

Resident strain. Original strain of penicillin-resistant, coagulase-positive *Staphylococcus aureus* recovered during the initial culture period. This proved to be phage type 80/81 in all instances.

Implanted strain. A penicillin-sensitive, coagulase-positive strain of *Staphylococcus aureus* designated 502A.

Variant strain. A strain of *Staphylococcus aureus* recovered from an implantation site, which proved to be penicillin sensitive, coagulase positive, but which differed in colonial morphology, serotyping, phage-typing, or antibiogram from the implanted strain but was believed to have originated from strain 502A.

"Wild" strain. A penicillin-sensitive, coagulase-positive strain of *Staphylococcus aureus* which was not judged to be 502A or variant thereof.

CASE REPORT

The family consists of five members: 30-year-old mother (L. L.), 34-year-old father (J. L.), 10-year-old girl (D. L.), 8-year-old girl (V. L.), and 6-year-old girl (C. L.).

Table I illustrates the incidence and type of infection prior to the start of implantation therapy. A penicillin-resistant staphylococcus was the offending agent each time cultures were obtained. Therapy prior to implantation consisted of washing with hexachlorophene, numerous courses of antimicrobial agents which at times were given to all members of the family concurrently, and daily nasal instillation of an antibiotic ointment. None of these measures proved effective.

METHODS AND MATERIALS

Methods. Initially all possible carrier sites—nares, throat, axilla, antecubital and popliteal fossae, hands, feet, groin, perineum,

Table I. Clinical infections

Month	*Patient*	*Infection*
1963		
July	C. L.	Furuncle
August	C. L.	Sty
September	C. L.	Sty
	J. L.	Furuncle
	L. L.	External otitis
October	C. L.	Sty
	L. L.	External otitis
November	C. L.	Sty
1964		
January	L. L.	Pneumonia
	V. L.	Furuncle
February	L. L.	Pneumonia
	V. L.	Furuncle
May	V. L.	Furuncle
July	J. L.	Furuncle
	L. L.	Furuncle
October	V. L.	Furuncle
November	C. L.	Sty
	J. L.	Furuncle
November	Implantation all members of family	
1965		
January-June		No infections
April	Implantation L. L.	
July	L. L.	External otitis
August-December		No infections
January-April		No infections

and sites of previous infections (which included the eyes in C. L., and the external auditory canals in L. L.)—were cultured for staphylococcus. Subsequent cultures were obtained from sites indicated in Table II.

Cultures were obtained by means of a sterile cotton swab. All swabs were incubated in trypticase soy broth* at 37° C. for 2 to 4 hours and then streaked on sheep blood agar plates (Difco blood agar base). The trypticase soy broth cultures were refrigerated for 8 to 12 hours and then, after a second incubation period of approximately 6 hours at 37° C., restreaked on fresh sheep blood agar plates. All blood agar plates were incubated at 37° C. for 24 and 48 hours, at which time the types of colonies were determined on the basis of size, elevation, luster, margin, hemo-

*Baltimore Biological Laboratories, Baltimore, Md.

Table II. Bacteriologic data

	11/7	11/14	11/21	11/30	12/7	12/19	1/2	1/24
L. L.								
Nose		R	R	R*	N	R		R
Throat		R	N	*		N		N
Ear		N						
J. L.								
Nose		R	N*		502A	502A	502A	W
							W	
Throat		R	N*		502A	502A	N	N
						R		
V. L.								
Nose		R	N*		502A	502A	502A	502A
Throat		R	N*		502A	502A	502A	502A
C. L.								
Nose	R	N	*		502A	502A	502A	502A
Throat	N							
Eye	R							
D. L.								
Nose		N	*		502A	502A	502A	502A
Throat		N						

Key: W, wild—coagulase-positive, penicillin-sensitive staphylococcus which is not 502A.
N, no coagulase-positive staphylococcus isolated.
R, resistant strain 80/81.
502A, implanted strain (or variant thereof).
*Implantation.

lytic activity, and pigmentation. Representative colonies of each type were then streaked on fresh blood agar plates. After incubation at 37° C. for 24 hours they were examined for purity. Tube coagulase tests using blood blank plasma diluted 1:2 with broth were then performed. All coagulase-positive colonies were subcultured on Sabouraud's dextrose agar* where differences in colonial morphology not detected on sheep blood agar plates could sometimes be observed. Antibiotic sensitivity patterns were determined by the disc method with disc concentrations as follows: penicillin, 10 units; tetracycline, 5 and 30 mcg.; chloramphenicol, 30 mcg.; erythromycin, 15 mcg.; Prostaphlin, 1 mcg.; Staphcillin, 5 mcg.; kanamycin, 30 mcg.; and ampicillin, 2 mcg.

Coagulase-positive colonies which differed in any characteristic on sheep blood agar plates, on Sabouraud's dextrose agar plates, and on the antibiograms were selected for phage- and sterotyping. Phage-typing was done by the method of Blair and Williams[10] using the International Set of typing phages. Serotyping was done by the method of Oeding as amended by Cohen and Smith[11] using antisera for factors *a, b, cp* (polyvalent), c_1, *h, i, k, m, n,* and 263-1 (of Hofstad).

Organisms. The staphylococcus used for implantation was strain 502A supplied by Dr. Henry Shinefield.

Antibiotic therapy. After appropriate cultures were obtained, systemic and local antibiotic therapy was instituted. Nafcillin* was given orally in doses of 1 to 5 Gm. per day, depending upon body weight, for 10 days and 1 per cent Nafcillin* ointment was applied to the anterior nares 3 times a day for 10 days. L. L. initially required 20 days for eradication of the resident strain,

*Baltimore Biological Laboratories, Baltimore, Md.

*Supplied by Wyeth Laboratories, Philadelphia, Pa.

3/7	4/5	4/14	4/25	5/9	7/20	8/1	9/17	12/1
R	R*	502A	502A	502A		N	502A	502A
N	N	N		N				N
					R	502A		N
W	W			W		W	W	W
W	W							W
502A	W			W		W	W	502A
								W
502A								W
502A	N			502A		W	W	W
								W
502A	W			W		N	N	502A
								502A

and then required a second 10 day course of therapy when the resident strain reappeared.

Method of implantation. The organism was grown in trypticase soy broth. A sterile cotton swab was placed into the trypticase soy broth and the area of involvement was then implanted. This procedure was repeated once daily for 5 days.

The area to be implanted was determined by the presence of the resident strain on the initial cultures (Table II). In the case of C. L., only the nares were implanted even though the left eye harbored the resident strain, and in the case of D. L. the nares were implanted despite the absence of a resident strain.

RESULTS

Initial cultures. Initially, 4 of 5 members of the family were found to harbor a resident strain (Table II). The sites involved were the nose and throat in L. L., J. L., and V. L., the nose and right eye in C. L. No resident strain was isolated from D.L.

Results of implantation. One course of implantation was successful in colonizing J. L., V. L., C. L., and D. L. with the 502A strain. L. L. proved somewhat refractory to implantation and the resident strain reappeared after the first unsuccessful attempt. A second course of antibiotic therapy was given, and after eradication of the resident strain a second attempt at colonization with 502A was successful. The second course of implantation was limited to the nares because it appeared to be the only site involved. Only the nose and throat were cultured prior to the second course of inoculation.

Persistence of the implanted strain 502A was variable (Table II). However, when the implanted strain disappeared from the sites of inoculation, it was generally replaced by a penicillin-sensitive coagulase staphylococcus ("wild"). In a few instances, no coagulase-positive staphylococcus was cultured from the

implanted site; however, subsequent cultures yielded a wild staphylococcus. In no instance was successful implantation with 502A replaced by the resident strain. The reappearance of 502A in the nares of V. L. and D. L. after an absence of at least 5 months was probably the result of cross colonization from L. L.

Bacteriologic identification. Various strains of coagulase-positive staphylococci can be differentiated by means of colonial morphology, serotyping, phage typing, and antibiogram. The resident strain in the present case could easily be differentiated from the implanted and the wild strains by means of the antibiogram alone. The former was resistant to penicillin, ampicillin, erythromycin, while the latter two were sensitive to these antibiotics. However, differentiation between the implanted and wild strain proved more difficult.

Initially it was considered that the implanted strain could be identified by its colonial morphology, especially on Sabouraud's dextrose agar plates and by its resistance to the 5 mcg. tetracycline disc while being sensitive to the 30 mcg. tetracycline disc. Subsequently, it was observed that strains of staphylococci obtained from implanted sites were morphologically identical to the 502A strain, but were resistant to the 30 mcg. tetracycline disc. Furthermore, strains appeared which were morphologically different from 502A, but whose antibiogram was similar to that of the implanted strain. Extensive serotyping and phage-typing showed these strains to be variants of 502A.

502A cultures were phage type 7 plus minor group III reactions and serotype bc_1. Most of the colonial variants were similar to 502A in phage type and either abc_1mn or bc_1n. The anibiogram of most of the 502A cultures and variants was as previously reported by Shinefield and associates.[6]

These observations indicated that the 502A strain may change in phage pattern without concurrent changes in serotype or antibiograms, may yield variants of altered serotype and phage type without change in antibiogram, can change antibiogram without change in serotype, and can change both serotype and antibiogram. The changes in the antibiogram would have been more convincing if the tube dilution method rather then the disc method had been used.

Clinical results. Except for the single episode of otitis externa in L. L., there were no clinical infections with the resident strain after successful implantation had taken place. The otitis externa was treated successfully with local antibiotics with resultant eradication of the resident strain and subsequent cross colonization with the implanted strain.

DISCUSSION

In the family reported here, 4 of 5 members had recurrent staphylococcal infections over a 17 month period despite vigorous systemic and local antibiotic therapy. Previous therapeutic regimens were probably unsuccessful because of the inability to eradicate the resident strain from all members of the family at one time. Partial eradication of the resident strain from one, two, or three members of the family probably created a "staphylococcal vacuum" at the carrier sites. The vacuum was then filled by any staphylococcus in the vicinity. Since the resident strain was still present in at least one member of the family, it probably filled the vacuum, thereby nullifying any therapeutic effect.

After local and systemic antibiotic therapy had eradicated the resident strain from the carrier sites, a coagulase-positive, penicillin-sensitive staphylococcus of low virulence (strain 502A) was implanted in an attempt to fill the "vacuum." This approach appeared beneficial. Although the mother, L. L., proved somewhat refractory to eradication and implantation and continued to carry the resident strain for 6 months after institution of therapy, the other members of the family, when implantation was successful, resisted colonization by the resident strain and have been free of clinical infections for 18 months.

The mechanism of bacterial interference is unknown. However, it is known that living staphylococci are required. Anthony and

Wannamaker[12] demonstrated that heat-killed staphylococci were not protective. The ability of 502A to interfere with colonization by other staphylococci is not unique to this phage type. The present report, as well as the experience of others,[8] has demonstrated that other phage types are also protective against colonization by 80/81 staphylococci. It would appear that the mechanism of bacterial interference is related to priority of colonization.

The problem of identification of the implanted strain on subsequent cultures appears to be of more academic than practical interest, since both the implanted and the "wild" strains appeared to have a protective effect. However, the identification of variants of 502A makes one aware of the fact that serotyping phage typing, antibiogram, and colonial morphology may all be necessary to identify a specific strain of staphylococcus properly. This could be important if clinical infections occurred with strains other than the resident strain after implantation had taken place.

SUMMARY AND CONCLUSION

A family with recurrent staphylococcal infections affecting 4 of 5 members was treated with local and systemic antibiotics to eradicate the resident staphylococcus phage 80/81. Subsequently, a coagulase-positive staphylococcus of low virulence, strain 502A, was implanted at the initial carrier sites. Except for a single episode of otitis externa in one member of the family, which abated with local antibiotic therapy, the family has been free of staphylococcal infection for 17 months, and is presently free of the resident strain.

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