The use of bacterial interference to prevent infection[1]

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For decades, bacterial strains of low virulence were occasionally used in man to replace or to block colonization by the more virulent organisms and thereby prevent bacterial infection. This paper reviews the topic and presents recent information on the implantation of strain 215 α-hemolytic streptococcus (α-strep) in the nasopharynx of neonates in the intensive care unit. A single inoculation of strain 215 can change abnormal colonization of the pharynx to "normal" (α-strep predominant) in 48–72 h in most neonates. Following implantation, α-strep with strain 215 like characteristics fluctuate among naturally occurring strains of α-strep, sometimes persisting in dominance and sometimes decreasing rapidly as new strains appear. Strain 215 can survive in the pharynx during subsequent antibiotic therapy and can be recalled to dominance by such therapy. It seems remarkably stable *in vivo*. There is no evidence of its nosocomial spread in the nursery. Streptococcus with strain 215 like characteristics occurred naturally in 1–6% of neonates in our intensive care unit. No infection (disease) attributable to strain 215 occurred in implanted infants.

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Depuis bon nombre d'années on utilise à l'occasion chez l'homme des souches bactériennes de faible virulence pour remplacer ou bloquer la colonisation par des organismes plus virulents dans le but de prévenir l'infection bactérienne. Le présent article fait une revue de la question et apporte des données nouvelles sur l'implantation de la souche 215 de streptocoque α-hémolytique (strep-α) dans le nasopharynx de nouveaux-nés placés dans une unité de soins intensifs. Une seule inoculation de la souche 215 peut changer la colonisation anormale du pharynx en une colonisation "normale" (prédominance des strep-α) en 48–72 h. Après son implantation, le strep-α ayant les caractéristiques de la souche 215 entre en compétition avec les souches de strep-α présentes naturellement dans le milieu et parfois il demeure prédominant et parfois il disparaît rapidement pour faire place à une nouvelle souche. Cette souche 215 peut persister dans le pharynx durant une antibiothérapie et elle peut même redevenir prédominante suite à une telle thérapie. Cette souche est exceptionnellement stable *in vivo*. Malgré l'utilisation de cette souche, il n'y a eu aucune dispersion nosocomiale dans la pouponnière. Des streptocoques possédant des propriétés comparables à la souche 215 se retrouvent chez 1–6% des nouveaux-nés de notre unité des soins intensifs. Aucune infection (maladie) attribuable à la souche 215 n'a été enregistrée chez les enfants venus en contact avec cette souche.

[Traduit par la revue]

The use of bacteria to fight bacteria, a concept at least a century old, continues to provide a challenge. Florey's (1946) review *The Use of Microorganisms for Therapeutic Purposes* notes that the first attempted replacement of one organism by another in man was by Cantani in 1885. Cantani claimed success in treating a case of tuberculosis by replacing the tubercle bacillus in the lungs by insufflation with "Bacto. termo," a so-called "harmless organism." Florey (1946) mentions the work of many others, emphasizing that of Metchnikoff in the early 1900s and including that of Nissle, who in 1916 used a strain called "Bact. coli" made commercially available as "mutiflor" for treatment of various intestinal problems including constipation.

In recent times the outstanding example of use of a low virulence bacterial strain to block colonization by a more virulent one was that by Shinefield *et al.* (1963) in the early 1960s of the strain 502A *Staphylococcus aureus* to block neonatal acquisition of *S. aureus* phage type 80/81, a very virulent organism causing high rates of morbidity and mortality in the nurseries at that time. The group implanted 502A, a naturally occurring strain of low virulence, in the anterior nares and on the umbilicus of newborns before they became colonized by the type 80/81 strain. Implantation was achieved with strain 502A, colonization by other strains of staphylococcus

was blocked, and epidemics were interrupted. Use of this strain became widespread. It is still available but has been little used in recent years, partly because of lack of need and partly because of infections that have been reported following its use (Blair and Tull 1969; Drutz *et al.* 1966; Fine *et al.* 1967; Houck *et al.* 1972; Light *et al.* 1967). Blair and Tull (1969), studying passage characteristics of the strains obtained from lesions in infants during an epidemic episode, were disturbed by "the instability and nonhomogeneity of this particular 502A culture." However, they were studying behavior of the strain following passage at low dilutions and noted that large doses of 502A had been used in epidemics known to be associated with lesions. They and others (Houck *et al.* 1972; Light *et al.* 1967) have correlated the incidence of lesions (primarily small periumbilical pustules) with the number of organisms used for implantation. Light *et al.* (1967) demonstrated a 0.4% infection rate with an inoculum of 4000 organisms, 5% with ~25 000 organisms, and 14–24% with ~10^7 organisms. It seems reasonable to conclude from the published information and personal communication with Dr. Shinefield that the risk of serious infection from properly conducted implantation with strain 502A is extremely low and that the benefits of its use as a means of aborting an epidemic with more virulent strains clearly outweigh the small risk incurred.

In recent years there have been several additional short-term uses of bacterial implantation in man for various purposes. Ehrenkranz (1970) implanted diphtheroids and *S. epidermidis* to block *S. aureus* colonization in neonates. Implantation was achieved without harmful effect, but did not accomplish its purpose. Lodinova *et al.* (1967, 1973

TABLE 1. Pharyngeal colonization patterns of intensive care unit
neonates not receiving antibiotics

Flora pattern	June 1978– Sept. 1979	1980	1981
Normal	105(92)*	89(91)	109(91)
Low titer or no growth	0	0	0
High titer abnormal	9(8)	9(9)	10(8)
Total infants	114	98	119

*Percentages are given in parentheses.

reproducibly and successfully implanted a benign type 083 *Escherichia coli* in the gut of neonates. However, the technique was used primarily as a research tool to investigate development of antibodies to gut flora. Raibaud *et al.* (1975) ...cessfully implanted *B. licheniformis* in an immunodeficient infant to limit colonization by *Clostridium perfringens*, bu... ...he effect was brief and high titers of the clostridia recu...red. Implantation of bacterial strains is currently being developed for prevention of dental caries (Kurasz *et al.* 1986) and urinary tract infection (Reid *et al.* 1985).

It is evident that interest in the use of bacteria to "fight" bacteria is a venerable and current concept. There is a resurgence of interest in a field in which we became involved because our interest in preventing infection in high-risk neonates pushed us inexorably in that direction.

Significance of normal flora

Renewed interest has as its base recognition of the bacterial flora that are normal for sites such as the oropharynx, gut, skin, and vagina, and the presumption that the interplay of bacterial species constituting the normal flora provides a host defense mechanism against abnormal colonization and infection (Sprunt and Redman 1968; Rosebury 1962a). *In vitro*, some species enhance and some inhibit the growth of others; some coexist without demonstrated activities (Rosebury 1962b). *In vivo*, the coexisting species provide a dynamic matrix at a site that apparently limits invasion by a foreign bacterium or overgrowth of a minority member of the matrix. The equilibrium can be upset by various events. Suppression of presumed inhibitors by antibiotics, for instance, can result in a prompt overgrowth of previously suppressed strains Sprunt and Redman 1968). Overgrowth is potentially serious because it can be followed by "superinfection," or clinical disease caused by the abnormal colonizing strain.

Superinfection

In 1963, Louria and Brayton (1963) showed that up to 29% of patients receiving large doses of penicillins for respiratory tract infections developed bacterial overgrowth in the oropharynx. In the complete study using different doses and routes of administration of penicillin, 15 of 83 treated patients developed overgrowth and 6 developed clinical superinfection; 4 of the 6 died. In the 71 abnormally colonized patients reviewed by Tillotson and Finland (1969), among 148 individuals given antibiotics for respiratory tract infection, 24 developed superinfection; 16 of the 24 died. The superinfections were caused by the organism overgrowing in the oropharynx. Disturbance of normal flora is obviously potentially serious. Our natural bacterial flora appear to be advantageous. Key constituents of our flora can be used to help preserve or reconstitute "normal" circumstances (Sprunt *et al.* 1971, 1980).

α-hemolytic streptococci, key constituents in normal pharyngeal flora

Our past work *in vivo* and *in vitro* supports the evidence that α-hemolytic streptococci (α-strep) are key constituents in maintaining and promoting a normal bacterial flora pattern in the pharynx (Sprunt and Redman 1968; Sprunt *et al.* 1971). Their suppression or elimination by antibiotics increases the risk of bacterial overgrowth at the site (Sprunt and Redman 1968) and therefore increases the risk of infection. Successful implantation of a selected strain of α-strep should lead to normalization of the pharyngeal colonization pattern and thereby decrease the risk of infection and limit the spread of the potential pathogen.

Development of pharyngeal flora in the intensive care unit for neonates

Further discussion of this concept requires definition of the terms we use (Sprunt *et al.* 1978).

In our experience neonates in the intensive care unit (ICU) develop their pharyngeal flora by 3–5 days of life according to one of three patterns: (i) "normal," in which α-strep predominate in cultures showing at least moderate growth on routine plating; (ii) "high titer abnormal" or bacterial overgrowth, in which any other organism predominates in cultures showing at least moderate growth; and (iii) "low titer colonization" or "no growth" pattern, which develops in the majority of infants receiving antibiotics. Our published evidence (Sprunt *et al.* 1978) showed that significant infections have occurred only in infants with high titer abnormal colonization of the pharynx, i.e., in 21% of the 117 of 223 infants abnormally colonized (53%). (See, however, the two exceptions mentioned below in the section on Exceptions to general rule of risk associated with abnormal colonization.)

Subsequent data for the years 1978–1981 (Table 1) show that ~90% of ICU neonates not receiving antibiotics developed normal flora in the first few days of life and ~10% showed abnormal colonization and were at risk of infection from their abnormal colonizers (Table 1, data subsequent to that in Sprunt *et al.* 1978). As shown in Table 2, up to 15% of infants receiving antibiotics may also develop normal flora (predominantly α-strep) in spite of their antibiotic treatment. Where α-strep persisted, overgrowth did not occur. However, high titer abnormal overgrowth did occur initially in about a third of the infants. The remaining group of infants receiving antibiotics yielded a low titer or no growth pattern. Infants in this group were apparently not at risk of infection while in this state, but when antibiotics were withdrawn, approximately 50% or more of the group subsequently developed abnormal colonization and were at risk of infection. Not all infants in this group could be followed after their treatment. However, for those followed it is evident that infants abnormally colonized initially plus those abnormally colonized after therapy comprise a little more than half the antibiotic-treated infants, i.e., ~50% were at risk of superinfection in comparison to ~10% in the untreated groups.

Abnormal colonization and risk of infection

These abnormally colonized infants were the subgroup at risk of infection. Prospectively collected data obtained before the infection rate change in 1980–1981 show that only 2 of 372 infants (0.5%) with "normal" flora became infected, but 47 of 321 (15%) abnormally colonized infants became infected (p < 0.001) (Sprunt 1985). Infection in the two infants with normal flora was caused by an organism found at an infected

TABLE 2. Pharyngeal colonization pattern of intensive care unit neonates receiving antibiotics

Flora pattern	June 1978– Sept. 1979	1980	1981
Normal	29(15)	29(13)	25(13)
Low titer or no growth	96(49) (N = 35, Abn = 35)*	127(58) (N = 27, Abn = 55)*	132(67) (N = 37, Abn = 61)*
High titer abnormal	69(36)	64(29)	39(29)
Total no. of infants	194	220	196
No. at risk of infection	69 + 35 = 104(54)	64 + 55 = 119(54)	39 + 61 = 100(51)
% infected	15	6	4

NOTE: N, normal; Abn, abnormal. Percentages are given in parentheses.
*After antibiotics discontinued.

TABLE 3. Pharyngeal implantation of strain 215 α-streptococcus in 42 neonates in the intensive care unit

Implantation result	Neonates	
	Number	Percentage
Success[a]	31	74
Failure	11	26
Explicable	7	
Not explicable	4	
Success discounting explicable failures	31/35[b]	89

[a]Success, recovery of implant strain.
[b]Total no. of neonates – explicable failures = 42 – 7 = 35.

peripheral source (heel stick, IV site). Otherwise, infection was caused by an organism with the cultural characteristics of the high titer abnormal colonizing strain. These infections were significant: sepsis, meningitis, pneumonia, and urinary tract infection as opposed to conjunctivitis or pustules.

Exceptions to general rule of risk associated with abnormal colonization

The relationship between abnormal colonization of the pharynx of neonates in the ICU and infection has been remarkably consistent during our approximately 12-year observation. Recently two exceptions have become evident. The abnormal colonization rate was remarkably constant throughout the 1970s, as was the proportion of abnormally colonized infants who became infected. However, in 1980 and 1981 (Table 2), while the abnormal colonization rate was little changed (54–51%), there was a significant drop in the clinical infection rate (15 to 6% and 4%). There were no changes in cultural techniques or observation methods for infection in the course of the study.

Review of the data showed that 238 infants classified as abnormally colonized had an infection rate of only 5% in contrast to the usual 15–20%. Breakdown of the data showed that 189 of the 238 (79%) were abnormally colonized by *S. epidermidis* with an infection rate of 1.8%. The infection rate of the remaining 49 infants was 20%, in the expected range.

The second exception provided reaffirmation of a previously noted association of *S. aureus* with infections in four

infants (Sprunt *et al*. 1978) even though it was not th predominant or abnormally colonizing organism and man or predominant proportions of α-strep were present. Th recent data review included 252 infants with "normal" flo (α-strep predominant). There were no infections in the 2(whose flora did not include *S. aureus* and six infections in th 47 with "normal" flora in which 10% or more of the residu population consisted of *S. aureus*. We cannot explain th exception to the general rule for the protective effect of α strep. However, it appears to be a persistent observation

Development of normal pharyngeal flora by implantation α-strep strain 215

On the assumption that normal flora provides an impo tant host defense mechanism against infection and th "normal" denotes α-strep as the predominant organism the pharynx, we investigated conversion of abnorm oropharyngeal colonization of ICU infants to "normal" t nasopharyngeal implantation of a carefully selected ar naturally occurring strain of α-strep obtained from a norm neonate.

Details of the selection of the α-strep we use as an impla strain (strain 215) have been reported (Sprunt *et al*. 1980 Notably, strain 215 is a good inhibitor *in vitro* of commc overgrowing organisms, it has a relatively low degree (resistance (resistant to 1, susceptible to 3 μg/mL ampicilli to penicillin and ampicillin, such that once established, persists in the uninflamed pharynx during ampicillin therap and it has a series of other genetic markers by which it can t identified in mixed streptococcal populations. The strain ha been given as a single nasopharyngeal dose of about 1(colony-forming units to 42 abnormally colonized infant: From this still limited experience, we know that implantatio can be achieved reliably, that α-strep can be expected t predominate in pharyngeal cultures within 48–72 h, and tha the implant strain can be identified in mixed streptococc; populations. Furthermore, no successfully implanted infar has become infected with the implant strain or with the abno mally colonizing organism.

Figure 1 shows the abrupt shift in flora that occurs in mo: infants following successful implantation (Sprunt *et al*. 1980 The proportions of α-strep increased sharply as the abnorm; colonizing strain decreased. Figure 2 illustrates anothe response pattern in which the proportions of the abnorm; colonizing strain decreased over a period of days. The distinc
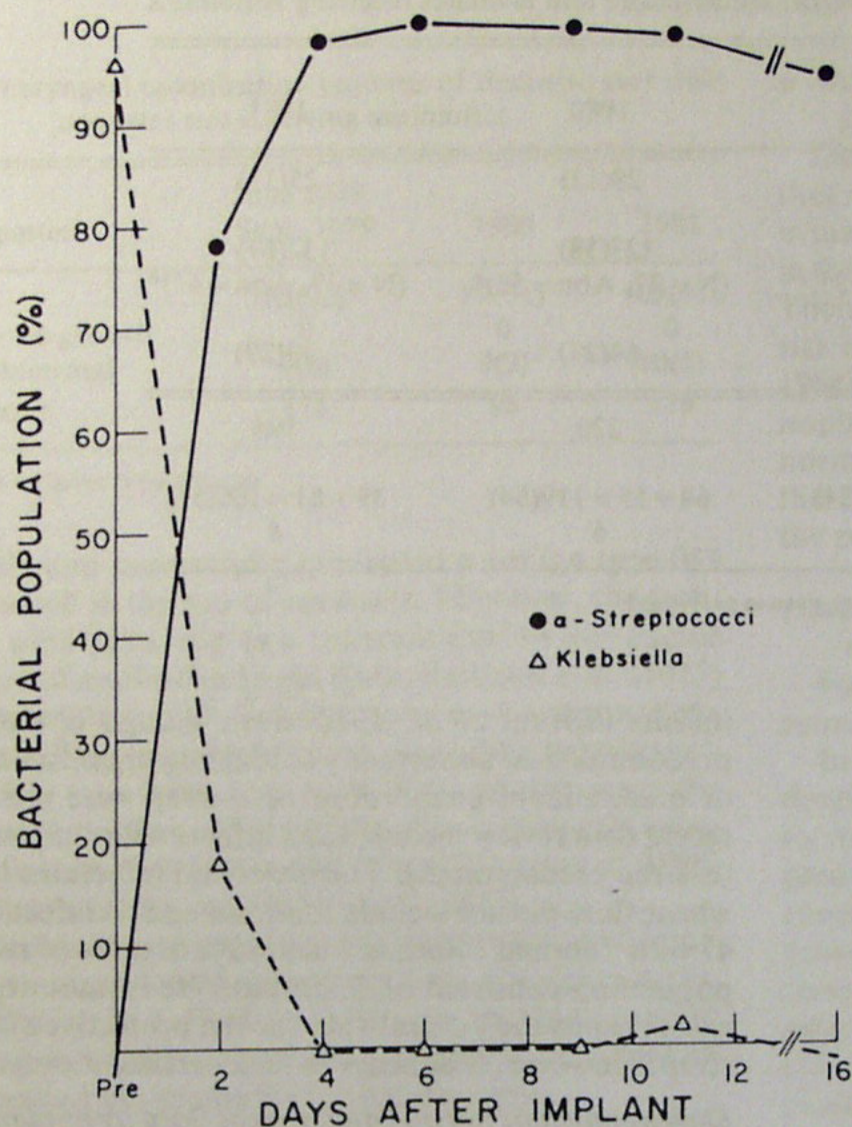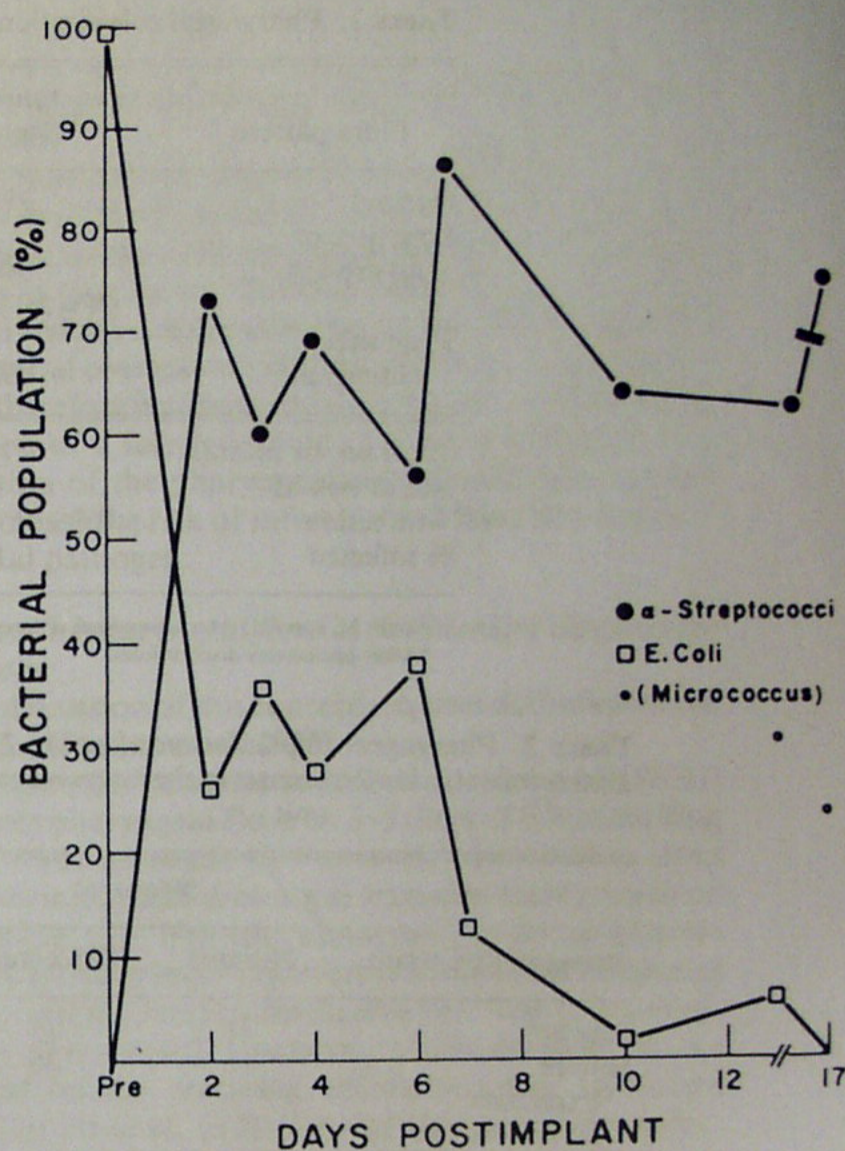
FIG. 1. Percent α-hemolytic streptococci and *E. coli* for 17 days after implantation of strain 215. No α-streptococci were found prior to implantation; only the implant strain was recovered for 13 days. pre, prior to inoculation.

FIG. 2. Percent α-hemolytic streptococci and *E. coli* for 17 da after implantation of strain 215. No α-streptococci were found pri to implantation; only the implant strain was recovered for 4 day

TABLE 4. Marker characteristics of strains of α-hemolytic streptococci different from strain 215 in Fig. 1

		Infant 1			Infant 2	Infant 3	Infant 4		Infant 5		Infant
Marker	Strain 215	1*	2	3	1	1	1	2	1	2	1
Routine											
Group H (Difco)	+	Weak +	+	–	+	–	–	+	+	+	–
Arginine hydrolysis	–	–	+	–	+	–	–	+	–	–	–
Ampicillin											
Resistant (R)	R1		R2		R2			R2			
Susceptible (S)	S3	S2	S5	S2	S5	S2	S2	S5	S2	S2	S2
Sugar utilization											
Raffinose	–	–	–	–	–	–	–	+	–	+	–
Salicin	–	+	–	+	–	–	–	+	+	–	–
Trehalose	–	–	–	+	–	–	–	+	–	–	–
Maltose	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+	+
Mannose	+	–	–	+	–	–	– or slow	+	+	+	– or slow
Other											
Morphology		diff.	diff.		diff.		diff.		diff.		diff.
Turbid growth					+		+	+	+		

NOTE: +, positive reaction; –, negative reaction; diff., morphology different on agar media; R1 and R2, resistant to 1 and 2 μg ampicillin/mL, respectively; S2, S3, and S susceptible to 2, 3, and 5 μg ampicillin/mL, respectively.
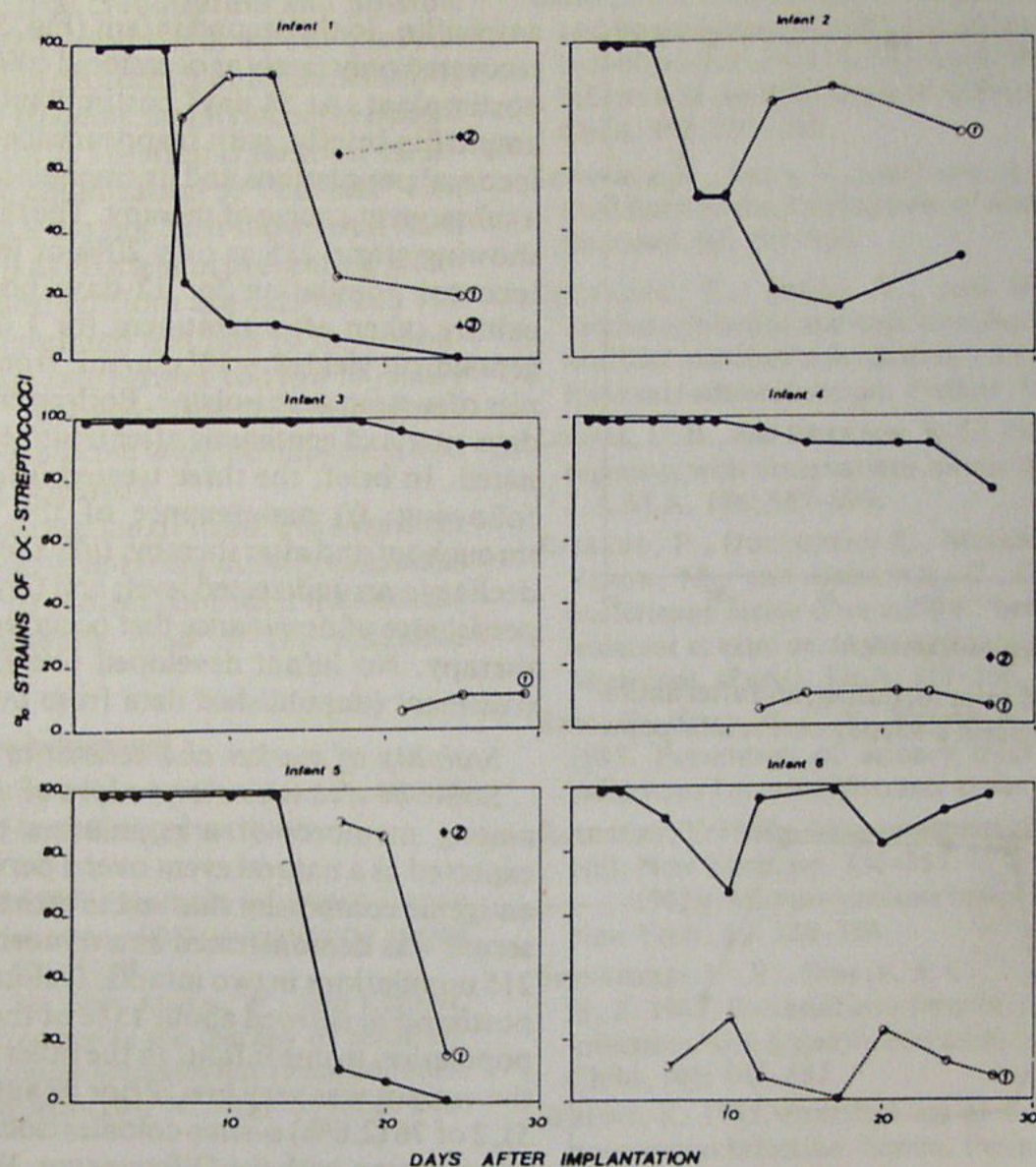*Strain number.

FIG. 3. Competition of strain 215 with other strains of α-streptococci as they arise. ●, strain 215; ○, strain 1; ♦, strain 2; ▲, strain 3.

tion between patterns is not important as far as we know, except perhaps, epidemiologically.

The data in Table 3 show the general success rate in establishing normal flora following a single dose of the implant strain. There are 31 successful implants, i.e., shift in flora and recovery of the implant strain, and 11 failures; 7 of the failures are explicable. One infant, the first implanted, received a very low dose of strain 215. The other six infants were on antibiotics at the time of implantation or were given antibiotics within 24 h of the implant procedure before the strain could become established. If we exclude these failures, 31 of 35 (89%) of the single implant procedures were successful.

It should be pointed out that abnormally colonized babies can develop α-streptococcal predominance naturally in a 24- to 48-h period. However, we are convinced of the role of strain 215, because it has been the only streptococcal strain recovered from 12 infants following implantation, a state that has been observed to persist for from 3 to 4 days to as long as 18 days.

Stability of strain 215 in the pharynx of implanted neonates

Implantation of strain 215 in ICU neonates carries with it concern for the future of that strain *in vivo* once it has accomplished its immediate purpose.

Competitive ability among naturally occurring α-strep

The competitive ability of the strain *in vivo* was evaluated in a group of six closely studied infants from our ICU who had only the implant strain of α-strep after implantation (Fig. 3). For three of the six (infants 3, 4, and 6), strain 215 main-

tained marked dominance for at least 30 days, during which time only one or two other strains were recovered in the cultures. In contrast, the cultures of the other three infants showed a rapid rise to dominance of the first new strain to appear, followed in two of the three (infants 1 and 5) by establishment of dominance of another new strain, all within 30 days postimplantation. Strains arising subsequent to implantation differed from strain 215 in at least two of the routine markers (Table 4).

In brief, the subsequent rise of one or two new strains of α-strep may have no effect on the dominant status of the implant, or may lead to its rapid decline. In other infants in whose initial cultures the implant strain was mixed with other strains of α-strep, 215 may emerge to dominance, continue at a moderate level, or decline. It appears that strain 215 behaves like a usual strain of α-strep, fluctuating in competition with other strains as apparently occurs normally in the neonatal pharynx.

Effect of subsequent antibiotic therapy

A basic marker for the implant strain is its resistance to a low concentration of penicillin (Sprunt *et al*. 1980). This characteristic resistance provided a selective recovery tool for the strain, and in addition, contributed to its ability to persist or to be recalled in the pharynx during and after a course of antibiotic therapy. Three infants have received ampicillin or oxacillin at some time after successful implantation. Strain 215 was still dominant when therapy was started in one infant (Fig. 4). It remained dominant during and after the course of
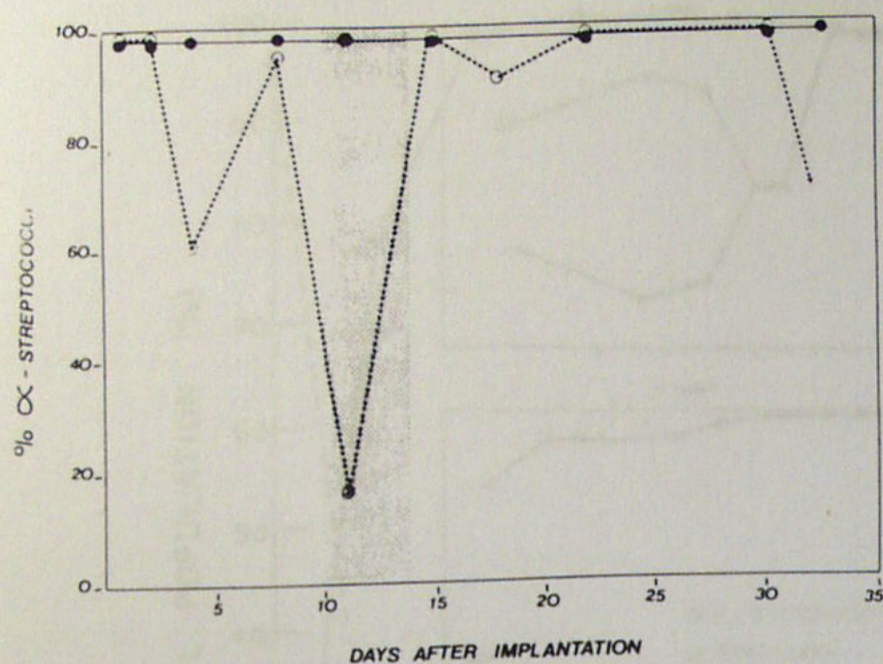
FIG. 4. Dominance of strain 215 prior to, during, and after antibiotic treatment. ●, strain 215; ○, percent a-streptococci in total population; ⊞, antibiotic therapy.

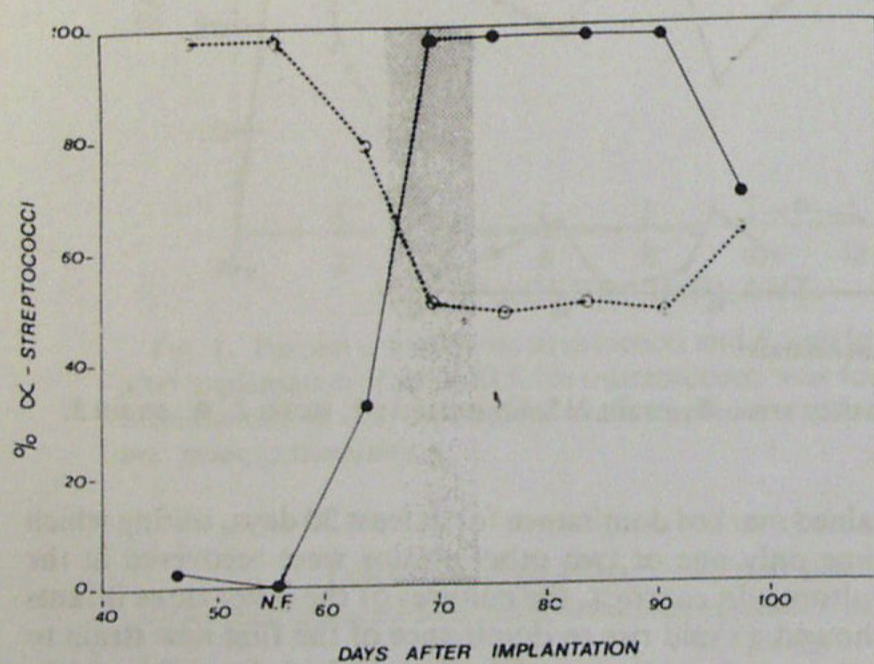

FIG. 5. Decline of strain 215 and its reemergence to dominance after antibiotic treatment. ●, strain 215; ○, percent α-streptococci in total population; ⊞, antibiotic therapy. N.F., not found.
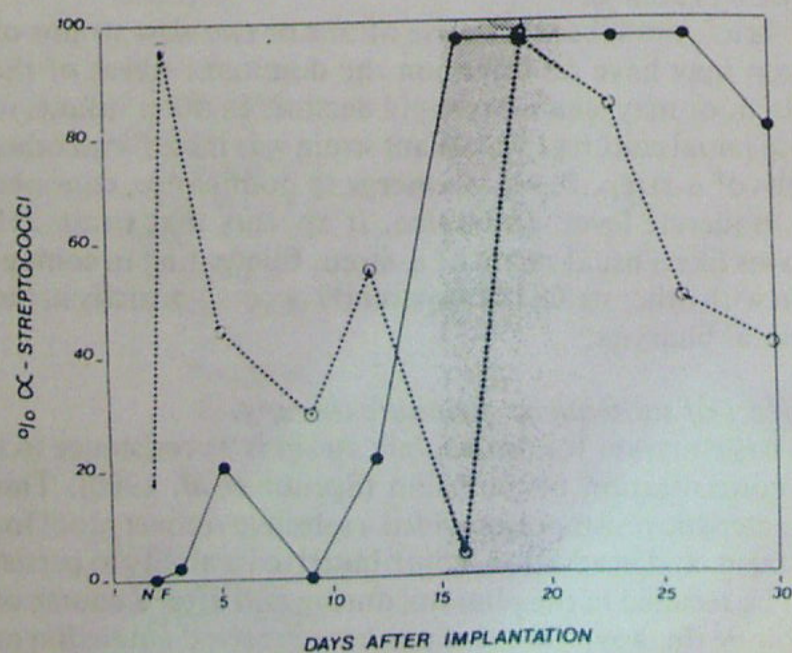

FIG. 6. Dominance of strain 215 established only with antibiotic treatment. ●, strain 215; ○, percentage α-streptococci in total population; ⊞, antibiotic therapy.

ampicillin. In the second infant (Fig. 5), strain 215 had been recovered only rarely as occasional colonies after the 24th day postimplant. At 54 days postimplant this infant was given ampicillin briefly, with reappearance of strain 215 in streptococcal populations and its emergence to dominance during a subsequent course of therapy. The third infant had cultures showing strain 215 as only 20% or less of the mixed streptococcal population for 12 days postimplant (Fig. 6). A culture taken after treatment for 1 day with oxacillin and gentamicin yielded ~ 10^3 cfu/mL from which only two colonies of α-strep were isolated. Both resembled strain 215. Three days later and continuing after treatment, strain 215 predominated. In brief, the three treated infants demonstrated the following: (i) maintenance of the implant's dominance throughout and after therapy; (ii) its recall to dominance after decline to an undetected level; and (iii) the establishment and persistence of dominance that occurred only during and after therapy. No infant developed overgrowth during or after treatment (unpublished data from our laboratory).

Stability of marker characteristics

Some in vivo mutation to loss of a single genetic marker among members of a population of strain 215 may be expected as a natural event over a period of time. Loss of the antigenic component that reacts with the Difco group H antiserum was demonstrated as a minority finding in the strain 215 populations in two infants. Cultures from 19 and 23 days postimplant showed about 15% of the strain 215 like variant population in one infant. In the other infant, carriage rate of the variant was very low. Prior to antibiotic treatment (Fig. 5), 2 of 76 (2.6%) α-strep colonies isolated did not yield a positive reaction with the Difco serum. While the infant was on antibiotics, only four colonies of α-strep were found. They resembled the implant. After treatment only 2 of 52 implant-like colonies were similar to the presumed variant. These presumed mutants, resembling strain 215 in other markers, failed to react with the group H anti-serum even when the antigen was diluted. However, the same antigen preparations yielded a precipitate with rabbit serum produced in our laboratory against strain 215.

There is no evidence of a selective advantage of the presumed mutant relative to the implant strain in one infant before and after antibiotic treatment. To generalize, it seems fair to state that strain 215 appears to be remarkably stable in vivo for extended periods after implantation.

Frequency of α-strep resembling strain 215 in cultures of unimplanted neonates in the ICU

We reviewed our culture data to determine the frequency with which α-strep with the marker characteristics of strain 215 occurred naturally in the ICU. We were also interested in evidence for nosocomial spread of strain 215 once it was introduced, a finding that is possibly desirable. Our data show no evidence for this. From 1978 through 1981 the flora of 151 to 258 infants per year was screened for strain 215 like strains using penicillin resistance marker as a starting point. In brief, only 1–6% of the infants naturally carried strain 215 like strains. They occur normally in the environment but are not frequent. There was one 3-month interval in 1979 when 10 infants carried such strains at a time when successfully implanted infants were present in the nursery, but this concentration of strains has not recurred.

In conclusion, there seems to be a role for artificial implantation of carefully selected strains of bacteria to increase host

resistance to abnormal bacterial colonization and possible subsequent infection. The 502A strain of *S. aureus* has been demonstrated to be effective in protecting individual infants and preventing the spread of epidemic staphylococcal disease in nurseries. The role of strain 215 α-strep is yet to be established. It can alter abnormal colonization to "normal" in a high proportion of trials, but it has not been shown that flora made "normal" artificially is as efficient in preventing infection as naturally acquired normal flora appears to be. The controlled experiment to determine this had to be discontinued when the ICU infection rate became too low to justify proceeding. It can be said, however, that no successfully implanted infant has become infected with the implant strain or the abnormal colonizing organism.

We envision the use of implantation of strain 215 to protect selected high-risk neonates and to interrupt nursery epidemics where it would be expected to protect colonized individuals and decrease the environmental load of the epidemic pathogen.

Acknowledgement

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