Effects of a Systemic Antibiotic on Nasal Bacterial Ecology in Man

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Received for publication 22 April 1970

The nasal flora of coagulase-positive staphylococcus carriers and noncarriers was studied in aerobic conditions in 17 individuals. Five hundred milligrams of cephalaxin was given orally four times daily for 12 days, and its effects on the nasal bacteria were determined quantitatively before, during, and after treatment. The total count obtained before the drug treatment was $5.4 \times 10^6$ in carriers and $3.9 \times 10^6$ in noncarriers. The lowest total count observed was 3 days after the cessation of the drug. The increase in gram-negative rods was seen 9 days after antibiotic therapy, not during the greatest reduction of gram-positive bacteria. Coagulase-positive cocci and diphtheroids were most sensitive to drug treatment. After 36 days, the total count was restored to pretreatment level. Diphtheroids did not return to the original number and were replaced by a corresponding increase of resistant coagulase-negative cocci. An inverse relationship between coagulase-negative cocci and lipophilic diphtheroids was seen in the anterior nares of many individuals. No gross difference in nasal ecology to differentiate carriers from noncarriers was seen.

Since the early work of Hallman (3) on the frequency of nasal pathogenic staphylococci, considerable literature has been published on such carriage (2, 13-15). The nose is the primary focus of multiplication and dissemination of organisms onto the skin and into the air, and by depressing nasal staphylococci a rapid reduction of both skin staphylococci and aerial staphylococci is seen (14). While Staphylococcus aureus deserves this attention, the role of other organisms has not been emphasized. The close relationship of these organisms might be a major influence in determining the bacterial ecology of the nose. To gain insight into these factors, we experimentally manipulated this flora by administering an antibiotic (cephalexin) and recording its effects on the composition and density of the aerobic bacterial flora of the nose. Bacteria were sampled before, during, and after antibiotic treatment.

MATERIALS AND METHODS

Subjects. Sixty male volunteers at the California Medical Facility, Vacaville, Calif., were screened by using mannitol salt-agar and citrated rabbit plasma to obtain two groups of subjects: coagulase-positive staphylococcus carriers and noncarriers. Subjects from whom a strain of S. aureus was recovered in at least three out of four consecutive weekly cultures were arbitrarily designated as carriers, and those from whom no S. aureus was recovered were termed noncarriers. Initially, each group was composed of 12 individuals. All subjects were healthy, as judged by the clinical history and review of their institution medical record.

Collection of nasal flora. To obtain a representative sample, each of 16 sterile calcium alginate swabs, individually moistened with nutrient broth, was rotated eight times in the outer half-inch of the right nostril. The 16 swabs were pooled into sets of four, i.e., 1-4, 5-8, 9-12, and 13-16. Our previous experience of utilizing 16 successive swabs demonstrated that these yielded more quantitative and reproducible data than obtained with a single swab (unpublished data). Each set was placed in a separate sterile test tube containing 2 ml of broth plus 1.5% sodium citrate. Sodium citrate was used to dissolve calcium alginate fibers. The swabs were agitated to dissolve partially the calcium alginate and to free bacteria from the fibers. After 10 min, the swabs were removed and compressed against the side of the test tube to remove excess citrate broth. The broth cultures were pooled together and 10-fold dilutions set up in nutrient broth to 10^6. One-tenth milliliter of each serial dilution was plated immediately, by utilizing several media. After two trials, dilutions of $10^{-3}$ and $10^{-4}$ were selected for plating. These dilutions were adjusted by the number of nasal flora at different phases of the study.

Colonies were counted with a Quebec colony counter. Sheep blood-agar plates were used to estimate the total count. Trypticase Soy Agar (TSA) with Tween 80 was used for lipophilic diphtheroid counts, as these bacteria grow poorly on blood-agar plates but luxuriantly in the presence of Tween 80. The count for lipophilic diphtheroids on TSA-Tween 80 did correspond to the count on the blood plates. Lipophilic diphtheroids were differentiated from
nonlipophilic diphtheroids by using the methods of Smith (10).

Colony types were used for picking representative strains of different groups of bacteria. Gram stain was done on each selected colony. By using appropriate physiological and biochemical tests (12), the organisms were classified into five major groups. After taking total counts, percentages for each group were determined.

Averages of total counts and subcounts (for major groups) for 12 subjects were converted to logarithm and plotted in the graphs. Yeasts and gram-negative cocci were seen rarely and thus not considered further. The data are expressed as the total number of organisms per 10 ml swabs. This latter statement is not repeated henceforth except in the figures.

Media used. (i) Blood-agar plate with 5% defibrinated sheep blood added to TSA (Difco) for total count, isolation of strains, and detection of hemolysis; (ii) TSA (Difco) with 0.5% polysorbate 80 (Tween 80) to enhance the growth of lipophilic diphtheroids, as these bacteria grow poorly in the absence of Tween 80 or TSA alone; (iii) TSA plus 10 to 20 μg of cephalixin per ml to study the antibiotic sensitivity of the organisms; (iv) Sabouraud Dextrose Agar (Difco) with antibiotics (penicillin, 20 units/ml, and streptomycin, 40 μg/ml); after inoculations, the medium was overlaid with sterile olive oil; this enhances the growth of Pityrosporum; (v) Eosine Methylene Blue Agar (EMB; Difco) to select enterobacteria; (vi) Mannitol Salt Agar (Difco) to isolate and detect coagulase-positive S. aureus.

All plates were incubated aerobically at 37 °C for 48 hr, except the yeast plates which were incubated for a longer period (6 to 10 days).

Treatment. Five hundred milligrams of cephalixin was given orally four times daily for 12 days. Cephalixin is 7 (β-α-amino-α-phénylacétdamido)-3 methyl-3-carboxylic acid and is distinguished from all other cephalosporins by the methyl group at the 3 position. Its in vitro effectiveness includes Staphylococcus, Streptococcus, Pneumococcus, diphtheroids, Proteus (indole-positive), Escherichia coli, Klebsiella- Aerobacter (nonmotile), Salmonella, and Shigella species.

After the basic screening, the bacterial flora was sampled once weekly for 3 weeks. During the 12 days of antibiotic administration, sampling was performed on the third and seventh days. In the post-treatment phase, sampling was performed on the 3rd, 6th, 9th, 15th, 22nd, and 36th day after stopping the antibiotic.

RESULTS

Composition of the flora. Organisms found in the anterior nares were arranged into the following groups: (i) coagulase-positive cocci (S. aureus), mostly pigmented cocci; (ii) coagulase-negative-gram-positive cocci, pigmented or non-pigmented cocci (Staphylococcus, Micrococcus, Pneumococcus, Streptococcus, and Sarcinia species); (ii) diphtheroids, both lipophilic and nonlipophilic pleomorphic gram-positive rods; and (iv) enterobacteria. The species of enterobacteria recovered were mainly K. pneumonieae, Enterobacter aerogenes, P. mirabilis, and occasionally E. coli. Rarely were yeasts (Pityrosporum) and gram-negative cocci encountered.

Bacteria before drug treatment. For carriers, the average total number of bacteria in three weekly trials before drug treatment was 5.4 × 10^8 (log 6.73). The percentage of organisms in each of the major groups is shown in Table 1. The most common organisms were coagulase-positive cocci and coagulase-negative cocci. These two constituted 74% of the total aerobic flora. Lipophilic diphtheroids (13%) and nonlipophilic diphtheroids (12%) were present in lower densities. Gram-negative rods were noted in three of seven subjects at a maximum of 2% of the total flora.

In noncarriers, the average total number of bacteria was 3.9 × 10^8 (log 6.59). The percent-

| TABLE 1. Per cent nasal flora in coagulase-positive carriers before antibiotic treatment of seven subjects |
|---------------------------------------------------------------|
| Microorganisms                      | Subjects | Avag  |
|-------------------------------------|----------|-------|
| Coagulase-negative gram-positive cocci | 56       | 35.6 ± 27.4* |
| Coagulase-positive gram-positive cocci | 10.5    | 38.3 ± 36.5  |
| Lipophilic diphtheroids              | 18.8     | 13.4 ± 19.1  |
| Nonlipophilic diphtheroids           | 14.6     | 12.5 ± 13.1  |
| Gram-negative rods                   | 0        | 0.5 ± 0.8    |

* Standard deviation.
TABLE 2. Per cent nasal flora in coagulase-negative carriers before antibiotic treatment of 10 subjects

| Microorganisms                      | Subjects | Avg    |
|-------------------------------------|----------|--------|
|                                     |          |        |
| Coagulase-negative gram-positive cocci | 88       | 60 ± 26.8a |
| Lipophilic diphtheroids              | 4        | 23 ± 16.1 |
| Nonlipophilic diphtheroids           | 8        | 17 ± 13.5 |
| Gram-negative rods                  | 0        | 0.2 ± 0.32 |

a Standard deviation.

FIG. 1. Total nasal flora during pretreatment, treatment, and post-treatment of antibiotic.

FIG. 2. Distribution of nasal flora during pretreatment, treatment, and post-treatment of antibiotic in coagulase-positive staphylococcus carriers.

ages of organisms in each of the major groups is shown in Table 2. Coagulase-negative gram-positive cocci were the commonest and in the highest percentage (60%). Lipophilic diphtheroids were the second highest (23%). These two groups of bacteria, when combined (83%), are similar (85%) to that reported by Marples et al. (5). Enterobacteria were seen in 2 of 10 subjects, at a maximum of 1% of the total flora. Except for the higher number of coagulase-negative cocci (60%), no other statistically significant differences were found when the floras of the carrier and non-carriers were compared.

A great range in the distribution of these bacteria was observed from person to person (Tables 1, 2) and is responsible for the large standard deviations.

Change in the nasal flora during antibiotic treatment. A reduction in total bacterial numbers was observed within 3 days. This diminution continued throughout the treatment (Fig. 1). The lowest count in this period (2.9 × 10^3 in carriers and 7.8 × 10^4 in noncarriers) was on the sixth day. Coagulase-negative cocci dominated the aerobic flora (63% in noncarriers and 76% in carriers). In both groups, gram-negative rods became the second dominating (36% in noncarriers and 11% in carriers) organisms. Lipophilic diphtheroids and nonlipophilic diphtheroids constituted less than 2% of the total flora in carrier and noncarrier. The most significant change was seen in coagulase-positive cocci, which were less than 0.6% on the sixth day of treatment.

Change in the nasal flora after administration of antibiotic. The greatest reduction in bacterial count was on the third day (Fig. 1) after stopping the antibiotics. The somewhat lower count in the carrier group was due to greater susceptibility of S. aureus to the drug. The resistance to antibiotics increased to 72% in coagulase-negative cocci on the sixth day. During the period of reduction of gram-positive organisms, an increase in gram-negative rods was observed (Fig. 2). This was maximal (43%) on the ninth day, representing a 10-fold increase. Except for one individual, the increase in gram-negatives was seen only in those subjects who had already carried this before antibiotic therapy. Lipophilic diphtheroids...
were 4% in the carrier group and less than 1% in noncarriers.

Although all organisms were reduced significantly in the carrier, the diminution of coagulase-positive cocci was most striking (38% to 0.6%), disappearing completely in two individuals. These two subjects did not reacquire coagulase-positive cocci, although one of them had carried 94% coagulase-positive cocci. These two subjects, when re-examined after 60 days, had still not reacquired coagulase-positive cocci. Most previous studies on the effect of antibiotic on S. aureus carriage have suggested that the antibiotic eradicated this carriage and intimated that the subsequent reacquisition was from an occult reservoir. In our studies, subject (16 successive swabs) showed the rule was extensive inhibition and not complete eradication.

In the noncarrier, the greatest reduction observed was in lipophilic and nonlipophilic diphtheroids (Fig. 3). None of the subjects lost their microflora completely. Again, a slight increase in gram-negative rods was seen during the reduction period of gram-positive organisms. A significant increase of gram-negative rods (100-fold) was observed in one subject whose gram-positive cocci disappeared completely after the drug treatment.

Although after 36 days the total count returned to the pretreatment level, lipophilic diphtheroids, nonlipophilic diphtheroids, and coagulase-positive cocci did not return to the original number. These organisms were replaced by a corresponding increase in coagulase-negative cocci, which may be due to the resistance of these bacteria to the drug.

Our study on drug sensitivity indicated that before treatment all the microflora (except gram-negative rods in one subject) were sensitive to 10 to 20 μg of cephalixin per ml. In the second week of treatment, the proportion of bacteria resistant to cephalixin increased. By the ninth day after treatment, 53% of the subjects had resistant coagulase-negative cocci and gram-negative rods.

Fungi were studied on Sabouraud plates. Although colony counts with fungi are notoriously inaccurate, we examined the plates at 24 and 48 hr to obtain an estimate. An increase in the number and types of molds after antibiotic treatment was observed.

**DISCUSSION**

There are several reports in the literature indicating that organisms exert important effects upon one another and that it is not uncommon for the growth of one organism to suppress or interfere with another (5, 8, 9, 11). This phenomenon has been studied both in vivo and in vitro recently by many workers (1, 6, 7).

It is shown clearly in this study that there is a marked reduction, but not eradication, in the nasal bacterial count of normal subjects when treated with 500 mg of cephalixin four times daily for 12 days. This treatment not only reduced the count, but also changed the composition of microflora. The failure to eradicate the bacterial flora may be one of the reasons for reacquiring the same original flora when the effects of a drug on the microflora are removed.

Perhaps due to differences in the original flora, all the subjects did not react similarly. Individuals carrying more than 50% diphtheroids showed a marked decrease in their count when the cephalixin was administered. Although both coagulase-negative cocci and diphtheroids were equally sensitive to 10 μg of cephalixin per ml in the pretreatment period, a greater fall in diphtheroids than in coagulase-negative cocci was seen during antibiotic treatment. Also, in the carriers, diphtheroids did not return to the pretreatment level 36 days after cessation of antibiotic. This phenomenon was not seen in the noncarriers. The quick return of coagulase-negative cocci might be due to the increased resistance to the antibiotic, as evidenced by the growth of bacteria in media containing antibiotic. All coagulase-positive cocci were sensitive to 10 μg of cephalixin per ml when tested in the first week after the treatment. A drastic fall, $6.6 \times 10^4$ to $10^1$, was observed in this group.

It was observed that with the possible exception of subject four (Table 1) and subject six (Table 2) an inverse relationship between coagulase-negative cocci and lipophilic diphtheroids.
was seen. A similar situation where heavy carriers of coagulase-positive cocci had depressing effect on diphtheroids was also seen. A study to understand the factors involved in controlling the interrelationship of these organisms is in progress. The interpretation of Marples (5) in other situations was that lipophilic diphtheroids suppressed the growth of cocci and were responsible for controlling the composition of bacteria.

Gram-negative clinical infection in man continues to be a serious matter in spite of the availability of antibiotics for these infections (4, 16). The test population in this study was relatively small because of the extensive quantitative studies needed, yet several healthy subjects carried gram-negative rods in the pretreatment period. One additional subject became heavily colonized after treatment, and the count remained higher after cessation of antibiotic therapy. The practical implications are not clear but are deserving of careful study. No disease due to increase in gram-negatives was seen in these subjects.

Our goal in this experiment was to seek clues as to why part of the population are carriers and others not. Although the answer was not found, certain facts are worthy of comment. We found no difference in the total number of bacteria between the two groups. In the composition of flora (besides the obvious difference of the presence or absence of S. aureus) the noncarriers had a higher percentage of coagulase-negative cocci (60%) than the carrier group (36%). The difference in the number of lipophilic and nonlipophilic diphtheroids between the two groups was not consistent. The response to the antibiotic was quantitatively and qualitatively similar in both groups except for the diminution of the S. aureus in the carriers, thus giving a lower count when compared with other groups of bacteria. None of these aspects of nasal ecology appears to explain why some are carriers and others are not.

ACKNOWLEDGMENTS

This investigation was supported by research grant NGR-05-025-008 from the National Aeronautics and Space Administration.

The valuable technical assistance of Joann Charron is gratefully acknowledged. Adrian Mandel made many valuable suggestions. Lester Pope and Eugene Prout of the California State Department of Corrections cooperated greatly in this investigation.

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