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Bacterial Interference: Effects of Oral Antibiotics on the Normal Throat Flora and Its Ability to Interfere with Group A Streptococci

CHRISTINE C. SANDERS,* W. EUGENE SANDERS, JR., AND DAVID J. HARROWE

Department of Medical Microbiology, Creighton University School of Medicine, Omaha, Nebraska 68178

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The effects of orally administered penicillin and tetracycline on the composition of the normal throat flora and its interference with the growth of group A streptococci were evaluated by throat culture and an agar overlay technique. Tetracycline caused only a slight, transient quantitative decrease in the composition of the flora and interference activity. Penicillin caused significant quantitative and qualitative decreases in both the composition of the flora and interference activity. The diminution in interference activity persisted up to 3 weeks after therapy. The differences observed between the antibiotic regimens correlated with differences in initial susceptibility of the flora to the antibiotic used and emergence of the resistance during therapy. Results indicate that although effects of antibiotics on the composition of the flora are transient, effects on its ability to interfere with group A streptococci may persist long after therapy is discontinued. It is thus possible that penicillin therapy may enhance susceptibility of certain individuals to subsequent infection with group A streptococci.

It has long been recognized that the administration of antibiotics may suppress man's indigenous microflora (6-9). Although this may result in superinfection (12, 13), it is usually without obvious clinical consequence and is soon reversed after cessation of therapy. In a previous prospective study, throat cultures were obtained from children and tested for the presence of organisms that were capable of inhibiting the growth of group A streptococci in vitro (3). During two sequential epidemics of asymptomatic group A streptococcal infections, it was observed that cultures from children who did not become infected more frequently contained inhibitory flora than cultures from children who became infected. Since the presence of inhibitory organisms was shown to be associated with resistance to infection, antibiotic suppression might be expected to diminish any protection afforded by the normal flora. The purpose of the present study was to evaluate the effects of orally administered antibiotics on the composition of the normal throat flora and its ability to inhibit the growth of group A streptococci.

MATERIALS AND METHODS

Throat cultures. Throat cultures were obtained with a dry, sterile cotton-tipped swab pressed firmly around Waldeyer's ring and in a crisscross pattern over the posterior pharyngeal wall. Swabs were immediately placed in 2 ml of brain heart infusion broth (Difco) and shaken vigorously for 3 min. Then 0.01 ml of the broth was placed on the surface of a 5% sheep blood agar plate and streaked for isolation of colonies in the four-quadrant fashion. Cultures were read after incubation for 24 h at 37 C in 10% CO_2 in air. Therefore only aerobic and facultative constituents of the throat flora were considered in this study.

Identification and quantitation of normal throat flora. *Neisseria* sp., *Micrococcus* sp., diphtheroids, and alpha-hemolytic and nonhemolytic streptococci were identified by colonial morphology and type of hemolysis on blood agar, Gram stain, and oxidase and catalase tests. Quantitative estimation of these bacteria was made on a scale of 1+ to 4+ according to their presence in the four quadrants of the sheep blood agar plate. An individual's throat flora was considered to be reduced in numbers if colonies were totally lacking in one or more quadrants that had consistently shown numerous colonies on pretherapy cultures.

Interference assays. An agar overlay technique was used for screening throat flora for inhibitory activity against group A streptococci. The procedure has been described in detail previously (3). Briefly, a throat swab was placed in 2 ml of brain heart infusion broth and vortexed, and 2 ml of a 1:500 saline dilution of the broth was placed on the surface of a brain heart infusion agar plate. During and immediately after the period of drug administration, lower dilutions (1:2 and 1:50) of the broth were processed along with the standard 1:500 dilution. These three dilutions allowed standardization of the inoculum so that consistent numbers of colonies were recovered and were evenly distributed over the entire surface of the agar plates. After overnight incu-

bation, the brain heart infusion agar plate was replica plated onto a sheep blood agar plate and overlayed with brain heart infusion agar-20% sheep blood, and group A streptococci were inoculated onto the overlay surface. The strain used was a clinical isolate (M type 12, T type 12) that has been shown to respond to inhibition by the normal flora in a manner identical to that of other group A streptococci of varying M and T types. After a second overnight incubation, interference was determined by grading the percentage of surface area where growth of the group A streptococcus had been inhibited. Colonies of inhibitory flora present in corresponding areas on the replicate sheep blood agar plate were isolated and identified.

Identification of interfering throat flora. Streptococci were identified by species on the basis of bile solubility, growth on mitis-salivarius agar, growth in 6.5% NaCl broth, fermentation of salicin, mannitol, sorbitol, and raffinose, and release of ammonia from arginine (2). Other organisms were identified by standard procedures (1).

Antibiotic susceptibility testing. Susceptibility of interfering throat flora to penicillin and tetracycline was determined by an agar dilution procedure in which standardized bacterial inocula were deposited on the surfaces of plates with a multiple inoculator device (11). The minimal inhibitory concentration was defined as the lowest concentration of drug preventing all growth.

Selection of volunteers. Thirty healthy young adults between the ages of 19 and 33 were selected for the study based upon three criteria: the presence of interfering organisms in their normal throat flora; no past history of adverse reactions to the drug to be given; and no use of antihistamines, gargles, or other antibiotics for the duration of the study. Informed consent was obtained for each of the volunteers.

Drug compliance. At unannounced intervals during the study (days 4 and 7), urine specimens were collected from all participants. Drug compliance was confirmed by assay for presence of antibiotic activity in the urine with a modified *Sarcina lutea* bioassay (4).

Study protocol. The participants in the study were divided into three treatment groups of ten individuals each. Group I received penicillin V (potassium phenoxymethyl penicillin), 250 mg, four times a day orally for 7 days. Group II received tetracycline HCl, 250 mg, four times a day orally for 7 days. Group III served as an untreated control. These drug regimens were selected because they typify use of bactericidal and bacteriostatic agents by physicians in office practice and not because they are preferred treatment for any single condition. Although participants were aware of the nature of their treatment, laboratory personnel performing all cultures were blinded to the source of cultures by a random numbering system. Throat cultures were obtained to identify and quantify constituents of the normal throat flora and interference assays were performed simultaneously. These procedures were performed on each individual at least twice before therapy, on days 2, 4, and 7 during therapy, and on days 1, 2, 3, 7, 15, and 21 after therapy.

RESULTS

Effect of oral antibiotics on the composition of the normal throat flora. Estimation of quantity of throat flora showed an overall reduction in numbers of colonies on cultures taken from nine of ten individuals in the penicillin group during therapy (Table 1). Cultures taken on one or more occasions from three individuals in this group completely lacked colonies from some constituent of their normal flora. In the tetracycline group, cultures showed reduced numbers of flora during treatment, but no single constituent was eliminated in any of the 10 subjects (Table 1). All of these changes in both groups disappeared within 1 week after completion of therapy. At this time, the composition of all throat cultures appeared identical to pretreatment cultures both qualitatively and quantitatively. No changes were observed in the control group during the study period.

Effect of oral antibiotics on the ability of normal throat flora to interfere with group A streptococci. All cultures from each group taken before therapy demonstrated interference with growth of group A streptococci as assayed by the agar overlay technique (Table 2). During and up to 3 weeks after therapy, the percentage of cultures that showed interference in the penicillin group was significantly less than in the tetracycline or control group (chi-square analysis; $P < 0.0005$). Three weeks after treatment, only five of ten individuals in the penicillin group had cultures that were capable of interfering with growth of the group A streptococci. No significant differences were found between the tetracycline and control groups.

The mean percentage of interference, or percentage of surface area of the overlay plates

TABLE 1. *Number of individuals showing qualitative and quantitative changes in the pharyngeal flora*

Culture period	Group[a]		
	Penicillin	Tetracycline	Control
During therapy			
Numbers reduced[b]	9	10	0
Species absent	3[c]	0	0
3 days after therapy			
Numbers reduced	4	2	0
Species absent	0	0	0
1, 2, and 3 weeks after therapy			
Numbers reduced	0	0	0
Species absent	0	0	0

[a] Ten individuals in each group.
[b] Colonies totally lacking in one or more quadrants that had numerous colonies on pretherapy throat cultures.
[c] Alpha-hemolytic streptococci in one subject and nonhe-

TABLE 2. *Percentage of cultures containing interfering organisms*[a]

Culture period	Group		
	Penicillin	Tetracycline	Control
Before therapy	100	100	100
During therapy	10[b]	80	90
After therapy (days 1, 2, 3, 7, 15, and 21)	35[b]	76	91

[a] Assayed by agar overlay technique.
[b] Significantly different from both control and tetracycline groups (chi-square analysis; $P < 0.0005$). Other apparent differences were not statistically significant.

showing inhibition of growth of the group A streptococci, was compared within each group. In the penicillin group, the mean percentage of interference both during and after therapy was significantly less than its mean value before therapy (t test on small, related samples; $P < 0.02$). Within the tetracycline group, only the mean percentage of interference during therapy was significantly less than the mean value before therapy ($P < 0.02$; Table 3). The control group showed no significant changes throughout the study.

To evaluate any alteration in interpretation of interference assays that may have resulted from the lower number of colonies recovered from treated individuals, assays were also performed using lower dilutions of the swab-inoculated broth. Proportionately larger numbers of colonies grew on brain heart infusion agar plates inoculated with the 1:2 and 1:50 dilutions than those inoculated with the standard 1:500 dilution. However, results of interference assays were identical for plates prepared from each of the three dilutions.

Interfering flora. The constituents of the normal throat flora responsible for interference were also compared within each group. For the penicillin group, the percentage of interfering isolates that were alpha-hemolytic streptococci increased significantly ($P < 0.05$), from 26% before therapy to 60% after therapy (Table 4). Although *Streptococcus salivarius* and other organisms showed a concomitant decrease, this was not statistically significant. No significant changes were observed in the constituents of the normal throat flora that caused interference in cultures from either the tetracycline or control group (Tables 5 and 6).

The large changes in interference observed in the penicillin group, but not in the tetracycline group, suggested a difference in susceptability of interfering isolates to the antibiotic administered to each group. Therefore, the antibiotic susceptibilities of all interfering isolates from tests. The susceptibility of interfering organisms isolated before therapy to the antibiotic to be used was greater for isolates from the penicillin group than those from the tetracycline group (Table 7). The susceptibility of interfer-

TABLE 3. *Mean percentage of interference*[a] *of throat flora from each study group*

Culture period	Group		
	Penicillin	Tetracycline	Control
Before therapy	23 (1–98)[b]	42 (5–100)	20 (5–60)
During therapy	1[c] (0–15)	19[c] (0–70)	15 (0–40)
After therapy	1[c] (0–10)	25 (0–100)	17 (0–50)

[a] Percentage of surface area showing inhibition of growth of group A streptococci in agar overlay technique.
[b] Mean (range).
[c] Significantly different from mean before therapy ($P < 0.02$).

TABLE 4. *Number of interfering organisms*[a] *isolated in cultures from penicillin-treated group*

Interfering organism	Culture period		
	Before therapy	During therapy	After therapy
Streptococcus salivarius	21 (50)[b]	4 (33)	9 (32)
Alpha-hemolytic streptococci	11 (26)	4 (33)	17 (60)[c]
S. mitis	8	4	13
S. sanguis	2	0	2
Streptococcus sp.	1	0	2
Other organisms	10 (24)	4 (33)	2 (8)
Neisseria perflava	5	3	1
Micrococcus sp.	5	0	0
Miscellaneous	0	1	1
Total isolates	42 (100)	12 (100)	28 (100)

[a] Determined by agar overlay technique; each different interfering organism isolated in each culture represents one isolate.
[b] Numbers in parentheses denote percentage of total isolates from group.
[c] Significantly increased over before therapy value ($P < 0.05$).

TABLE 5. *Number of interfering organisms*[a] *isolated in cultures from tetracycline-treated group*

Interfering organism	Culture period		
	Before therapy	During therapy	After therapy
Streptococcus salivarius	18 (36)[b]	20 (40)	34 (38)
Alpha-hemolytic streptococci	16 (32)	17 (34)	34 (38)
S. mitis	16	14	31
S. sanguis	0	1	1
Streptococcus sp.	0	2	2
Other organisms	16 (32)	13 (26)	22 (25)
Neisseria perflava	3	6	14
Micrococcus sp.	12	5	8
Miscellaneous	1	2	0
Total isolates	50 (100)	50 (100)	90 (100)

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...4 (33)	17 (60)
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0	2
4 (33)	2 (8)
3	1
0	0
1	1
12 (100)	28 (100)

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...0 (40)	34 (38)
...7 (34)	34 (38)
...4	31
1	1
2	2
...3 (26)	22 (25)
6	14
5	8
2	0
...0 (100)	90 (100)

TABLE 6. *Number of interfering organisms[a] isolated in cultures from control group*

Interfering organism	Culture period		
	Before therapy	During therapy	After therapy
Streptococcus salivarius	24 (54)[b]	25 (48)	49 (54)
Alpha-hemolytic streptococci	18 (40)	20 (38)	39 (43)
S. mitis	18	20	36
S. sanguis	0	0	0
Streptococcus sp.	0	0	3
Other organisms	3 (6)	7 (14)	3 (3)
Neisseria perflava	2	3	1
Micrococcus sp.	1	3	2
Miscellaneous	0	1	0
Total isolates	45 (100)	52 (100)	91 (100)

[a, b] See Table 4.

TABLE 7. *Susceptibilities of interfering organisms to antibiotic used in therapeutic regimen*

Culture period isolated	Tetracycline MICs[a] (μg/ml)		Penicillin MICs (μg/ml)	
	Tetracycline group	Control group	Penicillin group	Control group
Before therapy	2.5	1.6	0.05	0.04
During therapy	9.0	1.2	0.10	0.05
After therapy (days 1, 2, 3, 7, 15, and 21)	17.7	2.0	0.05	0.05

[a] Geometric mean minimal inhibitory concentrations in (MICs).

ing organisms to tetracycline steadily decreased eightfold over the entire period of the study. No significant changes in susceptibility to penicillin were observed throughout the study (Table 7).

DISCUSSION

The normal bacterial flora of the pharynx appears to be a remarkably stable ecosystem. To date, only antimicrobial agents (6–9), extreme debility (5, 12), and pyogenic infections (12) have been shown conclusively to alter the composition of the flora. In each instance, when the insult has been removed, the composition of the flora has promptly reverted to its original state. More recently, the ability of the normal flora to interfere with growth of the group A streptococci has been associated with resistance to acquisition of these organisms in the throat (3, 10). It thus appeared reasonable to inquire whether transient antibiotic-induced suppression of the throat flora was associated with a concomitant decrease in interfering activity and whether this decrease was equally transient or longer lasting. To answer these questions, three characteristics of the normal flora were assayed: (i) the generic composition of the flora; (ii) the numbers of bacteria recovered; and (iii) the ability of the flora to interfere with growth of the group A streptococci in vitro. These assays were performed on cultures obtained from subjects treated with penicillin V or tetracycline and an equal number of untreated individuals.

As expected, both penicillin and tetracycline resulted in a transient suppression of numbers of bacteria recovered during treatment. The effect of penicillin was quantitatively greater. Organisms from at least one genus appeared to have been eliminated from the flora of three of ten subjects during the administration of penicillin; this effect was not observed in the tetracycline-treated group. All of these changes were only transient. At the end of 1 week after treatment, composition of cultures from drug-treated groups appeared identical both to their respective pretreatment cultures and to cultures from untreated subjects.

Surprisingly, the effect of penicillin treatment on the interfering activity of the flora did not parallel the effect of the drug on the composition of the flora. Interfering activity of the flora was markedly diminished or absent for each of the subjects during penicillin therapy. More importantly, this decrease or absence of interfering activity persisted in eight of the ten subjects for at least 2 weeks after the flora of each had reverted to its respective pretreatment composition. At the end of the study (3 weeks after discontinuation of treatment), interfering activity was absent in cultures from five subjects and profoundly diminished in three. In contrast, tetracycline therapy resulted in only a transient diminution of interfering activity of cultures obtained from each of the subjects during treatment. The disparate effects of penicillin and tetracycline on interfering activity appeared to have resulted from differences in the susceptibility of initial interfering isolates and the rate of emergence of resistance to the two drugs. Interfering isolates from pretreatment cultures were almost uniformly more susceptible to penicillin than to tetracycline. In addition, an increasing resistance of interfering isolates to tetracycline was observed during therapy and for 3 weeks after with tetracycline, whereas no change in susceptibility of isolates to penicillin was noted during the entire period of study. It thus appears likely that flora of penicillin-treated subjects was repopulated by noninhibitory bacteria that appeared identical in all other respects to their pretreatment, more inhibitory counterparts. Whether these noninhibitory strains were de-

rived endogenously or exogenously is unclear; however, the data suggest that they may possess a competitive advantage over the inhibitory stains that they replaced.

In an earlier study, it was observed that interfering activity of the flora of children tended to increase after residence of group A streptococci in the throat. It was then hypothesized that presence of the group A streptococci had exerted a selective pressure that favored inhibitory strains (3). If it is true that interfering activity of one's throat flora is the cumulative result of repeated past exposures to the group A streptococci, it would be reasonable to assume that penicillin-induced suppression of this interfering activity may persist for much longer than 3 weeks—the limited period of post-treatment observation in this study. Clearly, prospective studies to assay the long-term effects of a course of penicillin upon susceptibility to streptococcal infection are indicated. For the present, however, the observed suppression of interfering activity adds another to the growing list of compelling reasons for avoidance of indiscriminate use of penicillin.

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