APPLIED MICROBIOLOGY, Sept. 1971, p. 397-400

Vol. 22, No. 3
Printed in U.S.A.

Quantitative Nasal Culture: a Tool in Antibiotic Research

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Received for publication 29 April 1971

The use of the quantitative nasal culture was investigated as a means of evaluation of new antimicrobial drugs in man. Cyclacillin was somewhat more active in vitro than penicillin G against penicillin G-resistant organisms. Cyclacillin was highly effective in suppressing staphylococci susceptible to penicillin G in nasal carriers but did not suppress staphylococci resistant to penicillin G. Although in previous studies by others cyclacillin was effective in treating mice infected with penicillin G-resistant staphylococci, in the present studies cyclacillin was not effective in suppressing nasal penicillin G-resistant staphylococci in man at doses which markedly suppressed penicillin G-sensitive organisms.

There are problems in investigating the effectiveness of a new antistaphylococcal drug in treating clinical infections. Mild infections, such as furuncles, often are self-limiting and the effect of chemotherapy is hard to evaluate. In serious, deep-seated infections the mortality rate is often high even when treated with effective drugs, and the investigator may be hesitant to use an investigative drug by itself. It would be preferable to obtain initial evidence of efficacy in humans through treating a benign condition where there would be no hazard if the drug failed to work.

In previous studies, the antistaphylococcal enzyme lysostaphin was evaluated by following the effects of therapy on the quantitative nasal cultures of staphylococcal carriers (3, 4). In those studies, the in vivo efficacy of lysostaphin was demonstrated while the potential hazards of systemic administration of the drug were avoided.

Preliminary studies had indicated that cyclacillin (Wy-4508) was not highly active in vitro against penicillinase-producing staphylococci. However, it was as effective as nafcillin in protecting mice against lethal infections with penicillinase-producing staphylococci (14). The unusual situation in which there is disagreement between in vitro and in vivo tests made it desirable to evaluate the effectiveness of cyclacillin against penicillinase-producing staphylococci in man.

In the present study, the effect of oral cyclacillin on *Staphylococcus aureus* from the anterior nose of healthy staphylococcal carriers was investigated. Fifteen carriers of penicillin-sensitive *S. aureus* were treated to document that the drug has in vivo activity against these strains and that the methods can detect this activity. In addition, a controlled double-blind study was performed on 30 carriers of penicillinase-producing staphylococci, in which 15 subjects received cyclacillin and 15 subjects received an identical placebo. These studies demonstrate that cyclacillin is active against penicillin G-sensitive staphylococci in the anterior nose, but there is no effect of the drug on the carrier state for penicillin G-resistant staphylococci.

MATERIALS AND METHODS

Quantitative nasal cultures were performed as described previously (10). Nasal carriers of coagulase-positive staphylococci were defined as subjects with *S. aureus* present on at least three consecutive quantitative nasal cultures over a 2-week period. Staphylococcal strains were tested for coagulase production by using human plasma. The staphylococcal isolates were tested for penicillin susceptibility by inoculating one drop of overnight broth cultures on Trypticase soy agar containing twofold dilutions of penicillin G. Strains inhibited by 0.195 μg of penicillin G per ml or less were considered to be penicillin-susceptible.

Subjects were medical students and house officers from the Indiana University Medical Center without clinical disease. The study was explained to the volunteers, and informed consent was obtained from all participants before they were enrolled in the study. Fifteen nasal carriers of penicillin G-susceptible staphylococci were treated with 250 mg of cyclacillin orally four times daily for 1 week. Thirty subjects who were carriers of penicillin-resistant staphylococci were randomly assigned to treatment with either 250 mg of cyclacillin orally four times daily or an identical

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appearing placebo. There were 15 subjects in each group. Coded supplies of cyclacillin and placebos were furnished by the Wyeth Co., and the codes remained unbroken until the end of the study. All subjects were followed by serial quantitative nasal cultures. For each subject, at least three pretreatment cultures were obtained. Two cultures were performed during the week of therapy, post-treatment cultures were obtained 1 day after therapy and at weekly intervals for 4 weeks, and a final culture was obtained 2 months after the treatment was completed. Over 90% of planned cultures were obtained from each subject. Colonies of pretreatment and post-treatment staphylococci were stored on agar slants and saved for in vitro twofold serial tube dilution sensitivity testing, with a final concentration of 1:100 dilution of overnight cultures of staphylococci in broth and a final volume of 1.0 ml of heart infusion broth.

RESULTS

The 30 staphylococcal strains isolated from carriers of penicillin G-resistant organisms before treatment were tested for in vitro susceptibility to penicillin G and cyclacillin by tube dilution methods. These isolates required inhibitory concentrations of penicillin G ranging from 6.25 μg/ml to greater than 50 μg/ml; only 23% of the strains were inhibited by 50 μg/ml (Table 1). Cyclacillin was over twice as active in vitro as penicillin G against these strains. Coagulase-positive staphylococci isolated from carriers of penicillin G-sensitive strains were all inhibited by 0.4 μg/ml or less of penicillin G; 6.25 μg of cyclacillin per ml was required to inhibit all 15 of the same strains (Table 2).

Oral administration of cyclacillin was followed by the expected suppression of *S. aureus* in nasal cultures from carriers of strains susceptible to penicillin G. There was a decrease in the carrier rates from 100% during the control period to 25% at the end of the week of therapy (Fig. 1). Most subjects who reacquired staphylococci in their nose did so during the first week after the drug was stopped, when carrier rates rose to 60%. Five of the 15 subjects (33%) continued to have negative cultures for staphylococci during the 2 months after treatment was finished. In contrast, there was no significant change in the carrier rates for carriers of *S. aureus* resistant to penicillin G whether they were treated with cyclacillin or placebo. Carrier rates for both groups were similar throughout the study, remaining above 85% during and after therapy.

The mean colony counts for coagulase-positive staphylococci were similar for all three groups during the pretreatment control period (Fig. 2). The log of the mean of *S. aureus* colonies from positive cultures did not differ consistently between the three groups during or after therapy. In carriers of penicillin G-susceptible staphylococci treated with cyclacillin, the mean counts of positive cultures from subjects who remained car-

TABLE 1. *Susceptibility of staphylococci isolated from 30 carriers of penicillin G-resistant strains before therapy*

Concn (μg/ml)	Cumulative per cent susceptible	
	Penicillin G	Cyclacillin
3.1	0	0
6.25	7	3
12.5	7	27
25	20	53
50	23	77

TABLE 2. *Susceptibility of staphylococci isolated from 15 carriers of penicillin G-sensitive strains before therapy*

Concn (μg/ml)	Cumulative per cent susceptible	
	Penicillin G	Cyclacillin
0.025	13	0
0.05	60	7
0.1	87	27
0.2	93	33
0.4	100	33
0.8	100	33
1.6	100	73
3.1	100	93
6.25	100	100

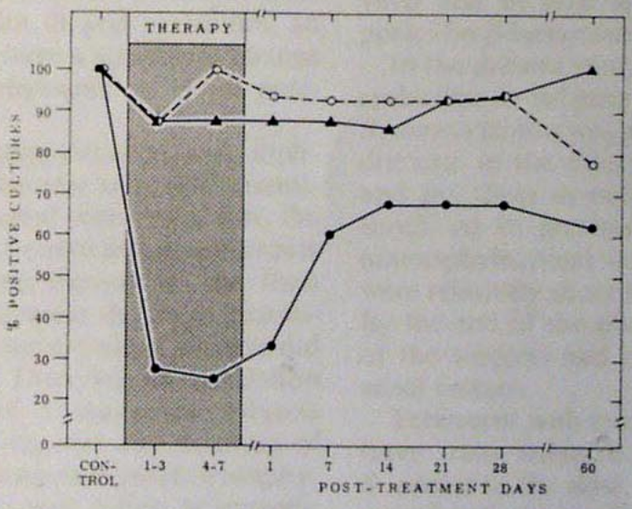

FIG. 1. *Effect of treatment of nasal carriers of staphylococci on carrier rates. Symbols: ●, carriers of penicillin G-sensitive staphylococci treated with cyclacillin; ○, carriers of penicillin G-resistant staphylococci treated with cyclacillin; ▲, carriers of penicillin G-resistant staphylococci treated with placebo.*

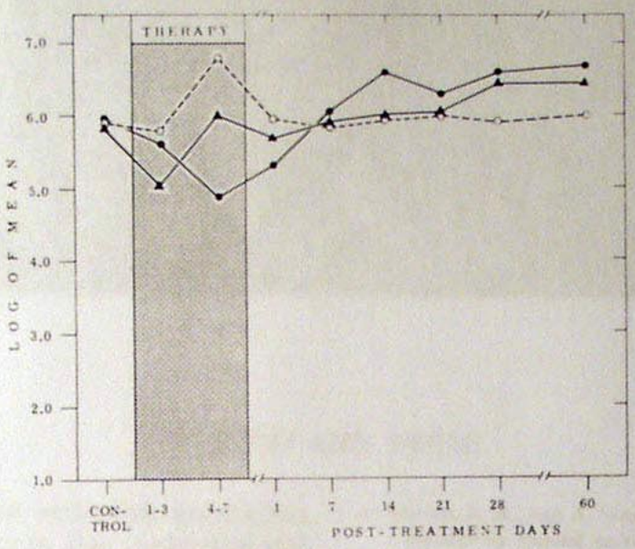

FIG. 2. *Effect of treatment of nasal carriers of staphylococci on the numbers of staphylococci isolated from positive cultures. Symbols:* ●, *carriers of penicillin G-sensitive staphylococci treated with cyclacillin;* ○, *carriers of penicillin G-resistant staphylococci treated with cyclacillin;* ▲, *carriers of penicillin G-resistant staphylococci treated with placebo.*

riers dropped one log from control levels by the end of therapy. Fluctuations of less than one log occurred in the mean values for carriers of penicillin G-resistant staphylococci treated with either cyclacillin or placebo.

All coagulase-positive staphylococci isolated from carriers after treatment were tested for their susceptibility to penicillin G and cyclacillin. In none of the carriers was there a significant change in the resistance of staphylococci to either penicillin G or cyclacillin.

Coagulase-negative staphylococci and diphtheroids isolated from the nose were also quantitated. In all subjects treated with cyclacillin, the quantities of both *S. epidermidis* and diphtheroids isolated decreased during therapy to less than 10% of control levels, whereas the mean quantities of these strains in placebo-treated subjects did not change significantly. There was no correlation between reacquisition of *S. aureus* by subjects whose cultures became negative and numbers of organisms other than coagulase-positive staphylococci cultured from the nose before or immediately after therapy.

DISCUSSION

The anterior nose has been consistently documented to be the most important human reservoir of coagulase-positive staphylococci (8, 12). The majority of subjects who become nasal carriers will harbor the same strain of *Staphylococcus* in the nose for weeks or months (5). Thus, the staphylococcal carrier is a suitable subject for monitoring the in vivo effectiveness of antistaphylococcal drugs.

Prior studies with the quantitative nasal cultures demonstrated that either local or systemic administration of an agent with good in vitro activity will decrease the carrier rates from staphylococci and reduce the numbers of organisms cultured from subjects who continue to carry staphylococci during therapy (3, 4, 6, 9, 11). After the antistaphylococcal agent is stopped, nasal cultures show a return of staphylococci to pretreatment levels in the majority of subjects.

In contrast, administration of an antimicrobial agent to which the *S. aureus* strain from the nose shows in vitro resistance has little effect on the nasal cultures. The numbers of staphylococci cultured may even increase with the use of an antibiotic to which the organisms are resistant (1).

In vitro studies of the activity of cyclacillin against staphylococci indicated that the spectrum of activity was similar to that seen with penicillin G, ampicillin, or other penicillin analogues which can be degraded by staphylococcal penicillin β-lactamase. In spite of the relatively poor activity in vitro against staphylococci which produce β-lactamase, mice could be protected from otherwise lethal infection with a staphylococcal strain producing β-lactamase by the administration of cyclacillin. The protection afforded by cyclacillin was at least as good as comparative protection provided by nafcillin, which is effective both in vitro and in vivo against *S. aureus* producing penicillin β-lactamase (2).

In the present study, the oral administration of cyclacillin to subjects carrying penicillin-sensitive *S. aureus* strains was accompanied by the expected decrease in the rates of staphylococcal isolation and the drop in numbers of staphylococci cultured. As in previous studies with other active antistaphylococcal drugs, the effects of therapy were relatively short term in most of the subjects. By the end of the observation period, two-thirds of the subjects had reacquired staphylococci on nasal culture.

Treatment with cyclacillin did not alter the cultures from subjects with penicillin G-resistant *S. aureus* in the nose. The frequency of staphylococcal isolation and the numbers of organisms grown were similar in subjects treated with cyclacillin or with a placebo.

Treatment of active staphylococcal infections may differ from treatment of the nasal carrier state in several important variables including the number of bacteria present, growth phase of the organisms, the role of phagocytoses and serum factors, and penetration of antibiotics into active infections as compared to the nasal mucosa. How-

ever, previous observations with other antibiotics and the present observations that cyclacillin did suppress the carrier rate of penicillin G-susceptible staphylococci suggest that there is a good correlation between the effect of antibiotics on nasal staphylococci and its effect in systemic infections.

The lack of effect of cyclacillin on nasal penicillin G-resistant staphylococci suggests that this agent would not be effective against the large numbers of these organisms present in clinical infections in man.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service training grant AI-00343 and research grant AI-07902 from the National Institute of Allergy and Infectious Diseases and by a grant from Wyeth Co., Philadelphia, Pa.

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