Oropharyngeal Cultures of Patients in Protected Environment Units: Evaluation of Semiquantitative Technique During Antibiotic Prophylaxis

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A semiquantitative culture technique was used to monitor the microbial flora of the oropharynx of 30 patients receiving antibiotic prophylaxis in protected environment units. After institution of antibiotic prophylaxis, the median concentration of organisms in the oropharynx fell by 2 logs but gradually increased by 1 log and then remained stable. Neisseria spp., Micrococcus sp., and Strep-tococci were generally eradicated by the antibiotics but were replaced by Lactobacilli and yeast. Four of nine enteric organisms persisted despite in vitro sensitivity to the antibiotic regimens. Yeast were cultured from the initial specimens of only 17% of the 30 patients, but they were cultured subsequently from specimens of 80% of the 20 patients who remained in protected environment units for at least 8 weeks.

Protected environment-prophylactic antibiotic programs currently are being evaluated for patients undergoing cancer chemotherapy who have a substantial risk of developing infection (2, 4, 9, 2a). Microbiological monitoring of the patient and his environment is an important aspect of these programs (1, 3, 5, 8). In some patients, the oropharynx becomes a harbinger of pathogenic organisms which may cause serious infection (5). Recently, we developed a technique for quantitating the microbial flora of the oropharynx (7). This technique has been used to monitor more accurately the oropharyngeal flora of patients in protected environment units. This report describes the results of prophylactic antibiotic regimens on the oropharyngeal flora of 30 patients treated in protected environment units.

MATERIALS AND METHODS

Semiquantitative oropharyngeal culture specimens were collected from 30 patients who received cancer chemotherapy in protected environment units between March 1969 and May 1971. Twenty-three patients had acute leukemia, two had lymphoma, four had metastatic sarcoma, and one had aplastic anemia. The patients ranged in age from 14 to 50 years. Eighteen were males and 12 were females.

The protected environment units used were two laminar air flow rooms and two Life Island units. The Life Island consists of a bed enclosed in a plastic tent, which is provided with filtered air. One entire wall of each laminar air flow room consists of air filters. The filtered air flows in a horizontal direction with a laminar distribution. Detailed descriptions of the units have been published elsewhere (1, 3, 4).

Oropharyngeal culture specimens were obtained from the patients before they entered the protected environment units and weekly thereafter until they were discharged. All specimens were collected at approximately 10 AM, which was 4 hr after a dose of prophylactic antibiotics. The patients gargled 20 ml of isotonic saline for 10 sec and expectorated the solution into a sterile container. Dilutions of 1:10, 1:50, 1:100, 1:200 and 1:400 were made with isotonic saline. A 0.1-ml sample from each dilution was inoculated onto sheep blood agar and was incubated aerobically at 37 C for 24 to 48 hr. A 1.0-ml sample of the original specimen also was inoculated into thiglycollate broth. Representative colonies of each morphological type of organism cultured were identified by the methods of Cowan and Steel (6). Colony counts of each morphological type of organism were determined from the blood agar plate and expressed as the total number of organisms recovered per sample.

The semiquantitative oropharyngeal culture specimens were collected with culture specimens of other body sites which were used to monitor the microbial flora of patients during antibiotic prophylaxis. After the initial cultures were obtained, the patients re-
received one of the prophylactic antibiotic regimens shown in Table 1. The patients swirled the antibiotic solution in their mouths several times before swallowing it. The aerosol solution was sprayed into the nose and mouth, and the antibiotic ointment was applied to the anterior nares and gums four times daily. The antibiotic regimens were similar in their effect on the oropharyngeal flora and were used interchangeably. Detailed descriptions of the antibiotic regimens and their effects on the microbial flora of other body sites have been published elsewhere (2, 5).

The effect of antibiotic prophylaxis on the oropharyngeal flora was evaluated as follows. Organisms which failed to grow from culture specimens during at least the last 3 consecutive weeks of study were considered to be eliminated by the antibiotics. Persistent organisms were those which grew constantly or intermittently from culture specimens during antibiotic prophylaxis. Organisms were considered to be contaminants if they were cultured from only one specimen during antibiotic prophylaxis, and they were not included in the analysis.

RESULTS

The median total log concentration of organisms in oropharyngeal culture specimens was 6.73 before the initiation of antibiotic prophylaxis (Table 2). Immediately after institution of the antibiotic regimens, the median total concentration was reduced by more than 2 logs. During the subsequent month it gradually increased by 1 log, but remained stable thereafter.

Diphtheroids, *Neisseria* spp., *Streptococci*, and *Micrococcus* sp. were the organisms cultured most frequently from the initial oropharyngeal specimens (Table 3). Seven gram-negative bacilli and seven yeasts were cultured from pretreatment specimens. Often *Lactobacillus* and yeast first colonized the oropharynx during antibiotic administration. The antibiotic regimens were especially effective in eliminating *Neisseria* spp., *Micrococcus* sp., and *Streptococci* from the oropharynx. The majority of diphtheroids, *Lactobacillus* sp., yeasts, *Escherichia coli*, and *Pseudomonas aeruginosa* persisted during antibiotic prophylaxis.

The effect of antibiotic prophylaxis on the median log concentration of representative organisms is illustrated in Fig. 1. *Neisseria* spp., *Micrococcus* sp., and *Streptococci* were eliminated promptly by the antibiotics and did not recur. The median log concentration of diphtheroids and *Lactobacillus* fell substantially after initiation of antibiotic prophylaxis, but 1 month later it returned to the pretreatment level where it remained thereafter. Yeasts were seldom cultured from pretreatment specimens, but they colonized the oropharynx promptly after the antibiotic regimens were begun. The median log concentration of yeasts rapidly rose to 10^4 and remained stable thereafter.

Only 17% of the patients had yeasts cultured from their oropharynx prior to antibiotic prophylaxis (Fig. 2). One week after the beginning of the antibiotic regimens, 47% of the patients had yeasts cultured from their oropharynx, and this percentage remained between 45 and 55% during subsequent weeks. The gradual decline in the percentage of patients with yeast during the second month of the study was due to the discharge of some patients from the protected environment units. Of the 19 yeasts cultured, 16 were *Candida albicans*, 2 were *Candida*

### Table 1. Prophylactic antibiotic regimens*

| Antibiotics | Dosage |
|-------------|--------|
| Oral antibacterial agents | Every 4 hr |
| Regimen A | | |
| Paramonocycin sulfate | 500 mg |
| Polymyxin B sulfate | 70 mg |
| Vancomycin hydrochloride | 250 mg |
| or Regimen B | | |
| Gentamicin sulfate | 200 mg |
| Vancomycin hydrochloride | 250 mg |
| Oral antifungal agents | Every 4 hr |
| Nystatin | 3.6 million units |
| or Candicidin | 100 mg |
| Aerosol spray | 4 times daily |
| Neomycin sulfate | 100 mg/ml |
| Vancomycin hydrochloride | 10 mg/ml |
| Polymyxin B sulfate | 5 mg/ml |
| Antibiotic ointment | 4 times daily |
| Neomycin sulfate | 50 mg/g |
| Nystatin | 25,000 units/g |
| Vancomycin hydrochloride | 5 mg/g |
| Polymyxin B sulfate | 2.5 mg/g |

*Antibacterial agents were given as a flavored solution. Candicidin was given as capsules. Nystatin was given as 6 tablets and 6 ml of suspension per dose.

### Table 2. Median log concentration of organisms in oropharynx

| Determination | Pretreatment | Week of stay in protected environment unit |
|---------------|--------------|------------------------------------------|
|               | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| No. of patients | 30 | 30 | 30 | 28 | 27 | 27 | 24 | 23 | 20 | 18 | 13 | 9 |
| Median log conc | 6.73 | 4.04 | 4.34 | 4.55 | 4.77 | 5.00 | 5.20 | 5.07 | 5.38 | 5.14 | 5.25 | 5.17 |
| Range | 3.74-8.90 | Neg.* | Neg. | Neg. | Neg. | 7.17 | 6.30 | Neg. | Broth* | 3.25 | Neg. | Broth |

* Neg. = No growth in any media.

* Broth = Growth only in thioglycollate broth.
Table 3. Effect of antibiotic regimens on oropharyngeal flora

| Organism           | Total isolates | Isolated before therapy | First isolated during therapy | During therapy | Completely suppressed | Persistent |
|--------------------|----------------|-------------------------|-------------------------------|----------------|----------------------|------------|
| Bacillus           | 2              | 1                       | 1                             | 2              | 0                    |            |
| Diphtheroid        | 20             | 17                      | 3                             | 6              | 14                   |            |
| Lactobacillus      | 17             | 5                       | 12                            | 2              | 15                   |            |
| Micrococcus        | 13             | 8                       | 5                             | 13             | 0                    |            |
| Neisseria          | 17             | 17                      | 0                             | 17             | 0                    |            |
| Streptococcus      | 42             | 39                      | 3                             | 37             | 5                    |            |
| Pneumococcus       | 1              | 1                       | 0                             | 1              | 0                    |            |
| Staphylococcus aureus | 2        | 1                       | 1                             | 1              | 1                    |            |
| Enterobacter       | 1              | 1                       | 0                             | 1              | 0                    |            |
| Escherichia coli   | 3              | 2                       | 1                             | 1              | 2                    |            |
| Klebsiella         | 3              | 2                       | 1                             | 3              | 0                    |            |
| Pseudomonas        | 2              | 2                       | 0                             | 0              | 2                    |            |
| Yeast              | 19             | 7                       | 12                            | 19             |                      |            |

spp., and 1 was Torulopsis glabrata. Yeasts were cultured from the oropharynx of 16 of the 20 patients (80%) who remained in the protected environment units for at least 8 weeks.

Figure 3 illustrates the effect of the antibiotic regimen on a 44-year-old woman who received chemotherapy for acute leukemia. After initiation of antibiotic prophylaxis, most of her initial flora was eliminated. Enterococcus was isolated intermittently thereafter from thioglycollate broth cultures. C. albicans colonized her oropharynx and persisted at concentrations of $10^4$ to $10^5$ despite the administration of nystatin.

The antibiotic regimens were unable to prevent serious oropharyngeal infection in a 45-year-old man who underwent chemotherapy for acute leukemia (Fig. 4). The patient had persistent pancytopenia until he achieved a remission of his leukemia during the eighth week of study. After initiation of antibiotic prophylaxis, Klebsiella sp., P. aeruginosa, and C. albicans were cultured from his oropharynx. During the second week he developed thrush, and later he developed acute pharyngitis and left peritonsillar abscess with hemorrhage and necrosis due to P. aeruginosa. His infection finally responded to antibiotic therapy when his peripheral blood became normal. Topical antibiotic regimens failed to eliminate these three organisms, but Klebsiella sp. and C. albicans were eradicated with systemic therapy. P. aeruginosa persisted in high concentrations in oropharyngeal culture specimens despite intensive topical and systemic antibiotic therapy.

DISCUSSION

Protected environment-prophylactic anti-
FIG. 2. Persistence of yeasts in oropharynx.

FIG. 3. Failure of nystatin to eliminate yeast from oropharynx.
Biotic programs are effective adjunctive measures in cancer chemotherapy (2a). The incidence of infection is lower for patients treated in protected environment units than for comparable patients treated in the regular hospital environment. However, some patients develop serious infections despite intensive antibiotic prophylaxis. Usually these infections are due to the inability of current antibiotic regimens to eliminate potential pathogens from the patients' endogenous microbial flora. Organisms often persist in the oropharynx despite antibiotic prophylaxis, probably because there is only brief contact between the antibiotics and the mucosa. Consequently, the drugs fail to penetrate into many areas of the oropharynx.

Previous studies of the semiquantitative oropharyngeal culture technique demonstrated that it is reliable and superior to other culture methods, provided that samples are collected at the same time of day (7). It is a simple and rapid method which can be used as a regular monitoring procedure. We have used this technique for the past 2 years to evaluate the effect of antibiotic prophylaxis on the oropharyngeal flora of patients in protected environment units.

Although the prophylactic antibiotic regimens altered the oropharyngeal flora, the total concentration of organisms was not greatly reduced. Most organisms which are considered to comprise the normal flora of the oropharynx were eradicated by the antibiotics, but were replaced by *Lactobacilli* and yeasts. Four of nine enteric organisms persisted in the oropharynx during antibiotic prophylaxis even though they remained sensitive to these agents in vitro. Occasionally these persistent organisms caused serious infection in either the oropharynx or lung.

It has been suggested that *α* streptococci inhibit the growth of other organisms in the oropharynx (10). Our study supports this observation, since *α* streptococci were eliminated rapidly during antibiotic therapy and during this time yeasts flourished at high concentration. However, *Lactobacilli* appeared to have no inhibitory effect on the growth of yeasts in the oropharynx. These organisms have been used extensively to prevent overgrowth of yeasts and resistant bacteria in the bowel after antibiotic therapy.

The semiquantitative culture technique is useful for monitoring the microbial flora of the
oropharynx during antibiotic prophylaxis, although it may not be sufficiently sensitive to detect organisms present in small numbers. This is suggested by the fact that yeasts were cultured from only 17% of patients prior to antibiotic prophylaxis but were cultured from 80% of the patients after antibiotics were instituted. Presumably these yeasts were present initially in very low concentrations but were not detected until they proliferated during antibiotic therapy. However, in a previous study we demonstrated that this culture technique is superior to the swab technique (7). Current antibiotic regimens are only marginally beneficial in eliminating potential pathogens from the oropharynx. They are totally ineffective against yeasts which persist at high concentrations and often first appear during antibiotic prophylaxis.

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