Semiquantitative Oropharyngeal Culture Technique

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Received for publication 30 March 1970

A semiquantitative method for determining the concentration of organisms constituting the normal oropharyngeal flora has been developed. Eleven species of organisms were isolated from the 18 subjects studied. The concentration of organisms in multiple samples, taken at 5-min intervals, was quite similar. The concentration of organisms increased slightly at the end of the day. Obtaining specimens on different days of the week did not appreciably affect the concentration or kinds of organisms. Eating had only a minimal effect on the oropharyngeal flora, but brushing teeth reduced the concentration of organisms substantially. When specimens were obtained 6 months after the initial specimens, the concentration of organisms remained the same but the species of organisms isolated varied considerably. The gargle method was compared to a swab method and proved to be superior. This method of obtaining oropharyngeal culture specimens is reliable and useful as a means of monitoring the normal oropharyngeal flora.

Patients with acute leukemia are highly susceptible to infectious complications. Recently, methods of preventing infections in these patients have been under investigation. These methods include the use of protected environment units (Life Island, laminar air flow rooms) plus prophylactic antibiotic regimens (3, 4). An essential part of this program has been the development of quantitative methods for monitoring microbial contamination of both the patient and his environment (1, 2, 11). Methods have been established for quantitating microbial contamination of the stool and skin of these patients (5, 6). This report describes a method of quantitating the microbial flora of the oropharynx.

MATERIALS AND METHODS

Eighteen normal volunteers participated in this study over a period of approximately 12 months. Quantitative oropharyngeal cultures were obtained by using isotonic saline as the collecting liquid. The subject gargled 20 ml of saline for 10 sec and expectorated it into a sterile container. Saline was added to the specimens to make dilutions of 1:10, 1:50, 1:100, 1:200, and 1:400. A 0.1-ml sample from each dilution was inoculated on a sheep blood-agar plate, and duplicate plates were made of each dilution. The culture specimens were incubated aerobically at 37 C for 24 to 48 hr. Colony counts were determined from the sheep blood-agar plates and expressed as the number of organisms per area. All organisms were identified by accepted methods of identification of oropharyngeal organisms.

Swab specimens were also collected simultaneously for comparison. After swabbing the oropharynx, the applicator was swirled in 3 ml of saline for 1 min and the excess saline was expressed. Dilutions of the specimen were made as above, and 0.1-ml samples were inoculated on sheep blood-agar plates. The same method was used for determining the concentration of organisms as was used with the gargle.

RESULTS

A total of 117 gargle specimens were obtained from the 18 subjects who participated in this study. The mean concentration of organisms from these 117 specimens was 1.6 \times 10^5 per area. There were 11 species of organisms recovered and the most common organisms were Neisseria sp., diphtheroids, \( \alpha \)-hemolytic Streptococcus, Enterococcus, and Micrococcus sp. (Table 1).

The amount of saline used in obtaining the quantitative oropharyngeal cultures had no effect on the total count. This was demonstrated with eight subjects by using 10 and 20 ml of saline. The specimens were collected on the same day at 11:30 AM and at 4:00 PM. Since the concentration of organisms increased slightly in the afternoon, each volume was tested alternately in the morning and in the afternoon. Volumes in excess of 20 ml were not used since they were difficult to gargle. When the smaller volume was used in the morning, the mean log concentration of organisms was 7.11 for a volume of 10 ml compared to 7.50 for a volume of 20 ml. When the larger volume was used in the morning, the mean log concentration
of organisms was 7.34 for a volume of 10 ml compared to 7.27 for a volume of 20 ml. Although concentration of organisms in the latter instance was higher when 10 ml of saline was used, this was because the concentration of organisms increased during the day. Hence, a volume of 10 ml was considered less satisfactory for adequate sampling.

Four subjects were studied to determine the results of repeated gargling. An initial specimen was collected, and subsequent cultures were taken at 5-min intervals for a period of 25 min (Table 2). In all four subjects, there was no substantial reduction in the concentration of organisms. The first specimen was slightly higher than the second, but in subsequent specimens the concentration remained stable.

The effect of time of day on the concentration of organisms in the oropharynx was determined in five subjects. The gargle specimens were collected at 8:30 AM, 11:30 AM, and 4:00 PM. The 11:30 AM specimen was collected before the subject had eaten lunch. The appearance of the culture plates in these specimens is seen in Fig. 1. The mean log concentration of organisms was 7.00 at 8:30 AM (range 6.64 to 7.38), 7.15 at 11:30 AM (range 6.64 to 7.34), and 7.20 at 4:00 PM (range 6.83 to 7.25; Table 3). In most cases, the concentration of each organism also increased during the day. The concentration of Enterococcus and Micrococcus sp. increased more than the other organisms.

The variation in concentration of organisms in the oropharynx on different days was studied in five subjects. The specimens were collected on Monday, Wednesday, and Friday of the same week at 11:30 AM. The mean log concentration was 6.76 on Monday (range 6.60 to 6.90), 6.94 on Wednesday (6.08 to 7.38), and 6.85 on Friday (6.17 to 7.04). In one subject, there was a log variation of 0.78 between Monday and Wednesday, but in the other subjects the total concentration of organisms varied by less than 0.50 log. In most instances, the concentration of each organism remained stable in the three culture specimens (Fig. 2). Enterococcus and diphtheroids were absent in the third culture of two subjects each. Micrococcus appeared in two subjects at the end of the week. Despite these differences, the

TABLE 1. Organisms recovered from total specimens

| Organism            | Per cent of patients | Per cent of cultures | Mean log concn* |
|---------------------|----------------------|----------------------|-----------------|
| Candida             | 6                    | 2                    | 5.71            |
| Diphtheroids        | 87                   | 70                   | 6.09            |
| Enterococcus        | 100                  | 96                   | 6.66            |
| Lactobacillus       | 37                   | 16                   | 6.57            |
| Micrococcus         | 75                   | 31                   | 6.06            |
| Neisseria           | 81                   | 70                   | 6.06            |
| Pneumococcus        | 25                   | 8                    | 6.44            |
| Staphylococcus epidermidis | 6             | 1                    | 5.25            |
| α Streptococcus     | 93                   | 75                   | 6.62            |
| β Streptococcus     | 6                    | 1                    | 4.11            |
| γ Streptococcus     | 18                   | 6                    | 6.25            |

* Mean concentration based on number of positive cultures.

TABLE 2. Variation in log concentration of organisms after repeated sampling

| Subject | Log concn |
|---------|-----------|
|         | Initial | 5 min | 10 min | 15 min | 20 min | 25 min |
| D. J.   | 7.00    | 6.88  | 7.04   | 7.04   | 6.90   | 7.04   |
| D. S.   | 7.34    | 7.04  | 7.17   | 7.04   | 7.14   | 7.07   |
| V. K.   | 6.67    | 6.43  | 6.43   | 6.53   | 6.14   | 6.14   |
| B. G.   | 7.14    | 6.84  | 6.53   | 6.66   | 6.99   | 6.95   |
| Mean    | 7.11    | 6.85  | 6.90   | 6.87   | 6.91   | 6.92   |
total concentrations of each day showed little variation.

Seven subjects were cultured immediately before and after eating lunch to determine the effect of eating on the oropharyngeal flora. The mean log concentration was 6.99 before eating (range 6.38 to 7.34) and 6.98 after eating (6.68 to 7.30). Both increases and decreases in total concentration of organisms, as high as 36% of a log, were observed in individual subjects. The concentration

### Table 3. Log concentration of individual organisms at different times of day

| Organism          | No. of subjects | Mean log conc.\(^a\) |
|-------------------|-----------------|-----------------------|
|                   | 8:30 AM | 11:30 AM | 4:00 PM | 8:30 AM | 11:30 AM | 4:00 PM |
| Diphtheroids      | 4       | 4        | 4       | 6.46    | 6.14     | 6.61    |
| Enterococcus      | 4       | 4        | 5       | 6.17    | 6.60     | 6.59    |
| Micrococcus       | 1       | 1        | 1       | 5.62    | 6.50     | 6.07    |
| Neisseria         | 4       | 4        | 4       | 6.36    | 6.47     | 6.30    |
| \(\alpha\) Streptococcus | 5      | 5        | 5       | 6.64    | 6.83     | 6.69    |
| Total conc.       | 5       | 5        | 5       | 7.00    | 7.15     | 7.20    |

\(^a\) Mean concentration based on number of positive cultures.

**Fig. 2.** Changes in oropharyngeal cultures during a 1-week period. These cultures were collected at the same time on each day.
of different organisms in these cultures varied only minimally. However, in one subject, diphtheroids first appeared after eating and in another subject Neisseria sp. was cultured before eating but disappeared after eating.

The effect of brushing teeth on the oropharyngeal flora was studied in six subjects. Gargle specimens were obtained before and after the first brushing in the morning. All subjects used the same dentifrice (Colgate with fluoride). The total concentration of organisms in each subject was highest before brushing (Table 4). The mean concentration was reduced nearly 1 log after brushing. One subject showed a 2-log decrease in organisms after brushing. The concentration of individual organisms also decreased significantly in cultures taken after brushing. Most organisms were decreased by at least 0.5 log and some, such as Enterococcus, decreased by 1 log. In one subject, diphtheroids were not recovered from the specimen obtained after brushing.

In five subjects, oropharyngeal specimens were obtained 6 months after the initial gargles had been collected (Fig. 3). The culture specimens were collected on the same day of the week and

**TABLE 4. Log concentration of individual organisms before and after brushing teeth**

| Organisms          | No. of subjects | Mean log concn* |
|--------------------|-----------------|-----------------|
|                    | Before brushing | After brushing  | Before brushing | After brushing |
| Diphtheroids       | 5               | 4               | 6.07            | 5.20           |
| Enterococcus       | 4               | 4               | 7.38            | 6.43           |
| Micrococcus        | 1               | 1               | 6.54            | 5.71           |
| Neisseria          | 6               | 5               | 6.04            | 5.72           |
| Pneumococcus       | 1               | 1               | 6.23            | 5.53           |
| \( \alpha \) Streptococcus | 5       | 5               | 7.00            | 6.25           |
| \( \gamma \) Streptococcus | 2       | 2               | 5.89            | 5.77           |
| Total concn.       | 6               | 6               | 7.44            | 6.69           |

* Mean concentration based on number of positive cultures.

**TABLE 5. Log concentration of organisms in swab and gargle cultures**

| Organisms          | No. of sample | Log concn |
|--------------------|---------------|-----------|
|                    | Gargle | Swab | Gargle | Swab |
| Candida            | 1      | 0    | 5.60  | 0    |
| Diphtheroids       | 4      | 4    | 5.53  | 4.55 |
| Enterococcus       | 7      | 7    | 6.46  | 6.63 |
| Lactobacillus      | 3      | 2    | 6.53  | 5.07 |
| Neisseria          | 4      | 3    | 5.59  | 5.41 |
| Pneumococcus       | 1      | 1    | 4.77  | 3.77 |
| \( \alpha \) Streptococcus | 5       | 5    | 6.74  | 5.93 |
| \( \beta \) Streptococcus | 0       | 1    | 0     | 4.11 |

**FIG. 3. Changes in oropharyngeal flora in a 6-month period. The latter cultures were obtained on the same day of the week and at the same time of day as the initial culture.**
at the same time of day. In the majority of subjects, the total concentration varied only slightly. However, in one subject, B. T., the initial culture was 1 log lower than the culture obtained 6 months later. There was considerable variation in the concentration of individual organisms. Subject V. K. acquired an *Enterococcus*, D. S. acquired an α-hemolytic *Streptococcus*, and B. G. acquired a *Pneumococcus*. The concentration and types of organisms varied in V. K. and D. S. but remained stable in D. J.

The gargle method of obtaining quantitative oropharyngeal cultures was compared to a swab method in 19 subjects. The swab specimen was always obtained before the gargle specimen. In 17 instances, the concentration of organisms was higher in the gargle specimens. The mean concentration of organisms obtained by the swab technique was 6.43 ± 0.58 and by the gargle technique was 7.23 ± 0.38. This difference is highly significant (*P* < 0.001). The concentration of individual organisms was evaluated in 7 of the 19 subjects and was consistently lower in the swab culture (Table 5). A β-hemolytic *Streptococcus* was the only organism recovered from a swab specimen but not from the corresponding gargle specimen. There were three organisms (*Neisseria* sp., *Lactobacillus* sp., *Candida* sp.) recovered from gargle specimens but not from the corresponding swab specimen.

**DISCUSSION**

Several methods have been described for obtaining quantitative oropharyngeal cultures. These include a swab method (7), platinum loop (10), gelatin syringe method (9), and mouth wash (8, 12, 13). The swab, loop, and syringe methods have the major disadvantage of not sampling the entire oropharyngeal surface. Organisms located in only tonsillar crypts or other inaccessible areas would not be included.

The mouth wash technique, mentioned above, is similar to the one used in the present study. However, the investigators used marker organisms and did not investigate this method for determining normal flora. Their mouth wash samples were filtered and the filter was cultured. Normal throat flora would be difficult to identify and enumerate on a filter. This method also requires too much equipment and time to be used routinely.

This study demonstrates that a gargle method of obtaining quantitative oropharyngeal cultures is superior to the swab method. Since the gargle specimens are obtained by using an exact amount of saline and having the subject gargle for a specified period of time, there is minimal variation in sampling. There was a greater difference in the mean concentration of organisms recovered from swab specimens collected by different technologists than recovered from gargle specimens. On three occasions, organisms were recovered from gargle specimens and not from the corresponding swab specimen. The opposite situation occurred only once. The mean concentration of organisms was significantly higher in the gargle specimens despite the fact that the swab specimens always were obtained first.

It is important that the gargle specimens be collected at the same time during the day since the concentration of organisms changes during the day. The concentration or number of species of organisms does not vary from day to day over a short period of time, but there can be substantial differences over a period of months. Not only does the concentration change, but some organisms appear or disappear from the flora. Gargle specimens should not be collected immediately after the subject has brushed his teeth since brushing reduces the concentration of organisms. Eating did not substantially affect the concentration of total organisms. However, in two subjects, organisms were acquired or lost after eating.

The gargle method of obtaining quantitative oropharyngeal cultures is very reliable. The concentration of organisms is reproducible after multiple samplings. Because it requires a minimal amount of time, this method can be used as a routine technique. Further investigation is being carried out to determine its usefulness as a monitoring device for patients in protected environments.

**ACKNOWLEDGMENT**

This investigation was supported by Public Health Service grant CA 10042-04 from the National Cancer Institute.

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