# BACTERIOLOGICAL STUDY ON THE SALIVA OF HEALTHY ADULTS

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## Abstract

Quantitative and qualitative studies of the microflora of saliva of 40 healthy individuals aged 20 to 50 years were carried out. Of the cultivable salivary flora anaerobes were found to predominate as compared to aerobes. Streptococci were the predominant aerobes. The group mean for the various categories may be expressed as high, intermediate and low. Variation in microbial counts occurred in different individuals whereas in the same individual each subject had a characteristic zone about which the counts fluctuated. Total anaerobes, total aerobes, streptococci and veillonella were categorised in which small fluctuations occurred on different days. No statistical difference was found between males and females for any of the categories of the various micro-organisms isolated.

## Introduction

Studies conducted on the microbial flora of saliva have indicated a variety of bacterial species (Richardson and Jones, 1958). Development of many of the diseases of the oral cavity depends upon the conditions that favour manifestation of pathogenic potentialities of usually harmless resident micro-organisms (Burnett and Schrep, 1968). In normal mouth a counterbalance exists between Lactobacilli and Candida with the Candida providing nutritional stimulation for the lactobacilli and the latter producing lactic acid which prevents excessive development of yeasts (Young et al., 1956). Alteration of the balance by antibiotics may be a factor in the increased incidence of candidiasis in healthy mouths (Knight and Fletcher, 1971). Streptococci constitute the most numerous group of organisms in the oral cavity and are considered as potential etiological agents of dental caries (Zinsser 1972).

The present investigation was undertaken to determine the normal microbial flora of saliva in healthy adults with stress on the quantitative studies of these organisms. As stability of the adult salivary flora is an important factor in the natural resistance of the host, culture studies on the normal flora of saliva and on serial specimens from three healthy adults were also conducted to confirm whether salivary flora varies from day to day in the same individual and whether it differs among individuals.

## Material and Methods

Specimens of saliva were collected from 40 apparently healthy individuals between 20 to 50 years of age. Subjects were institutional staff, personnel and graduate students. None of them were taking antibiotic or any other drug therapy for the last two weeks.

Ten fold serial dilutions of saliva samples in 9.0 ml sterile saline were prepared within one hour after collection of the specimen. One tenth milliliter of undiluted saliva specimens and the required dilutions were streaked onto the surface of the 4" plates. Table I lists the media employed in the study along with period and temperature of incubation in each case.

Table I: Selective Media and Methods of Incubations

| *Organisms Selected* | *Medium* | *Incubation* | *Saliva Dilution (—log 10)* |
|---|---|---|---|
| Total Anaerobes | Trypticase Soy Agar+5% sheep blood | 37°C—24 hours Anaerobic | 7, 8, 9, 10. |
| Total Aerobes | m Plate count medium | 37°C—24 hours Aerobic. | 6, 7, 8, 9. |
| Streptococci | Blood Agar | 37°C—24 hours Aerobic. | 5, 6, 7, 8. |
| Neisseria | Muller Hinton Agar | 37°C—48 hours Anaerobic. | 3, 4, 5. |
| Veillonella | Veillonella medium (Rogosa 1956) | 37°C—48 hours Anaerobic. | 2, 3, 4. |
| Lactobacilli | Rogosa S.L. Agar (Rogosa et al., 1951) | 37°C—72 hours Aerobic. | 2, 3, 4, 5. |
| Coliforms | McConkey Agar | 37°C—24 hours Aerobic. | 2, 3, 4, 5. |
| Staphylococci | Staphylococci medium 110 | 37°C—48 hours Aerobic. | 2, 3, 4, 5. |
| Yeasts | Sabouraud's dextrose Agar | 28°C—72 hours Aerobic. | 2, 3, 4. |

After incubation total colonies were counted on each plate and representative colonies were picked up from different media and inoculated on slants. Identification of various micro-organisms was based on colony characteristic, microscopic morphology, staining properties and biochemical reactions. Several broad groups of micro-organisms were identified on the above grounds. These were total Anaerobes, total Aerobes, Streptococci, Lactobacilli, Coliforms, Neisseria, Veillonella, Staphylococci and Yeasts.

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## Results

Table II shows the distribution of bacteria in saliva with total mean count and standard deviation for each group. Predominance of anaerobes in saliva was observed as compared to aerobes, while the other organisms were present in lower numbers.

Fig. 2 represents the mean count of various group of organisms per ml of saliva with their standard deviation mentioned. The standard deviations for most of the groups were relatively small as compared to their mean total counts indicating relatively constant population of these organisms in saliva.

Table II: Distribution of Bacteria in Samples of Fresh Saliva

| *Organisms* | *Viable Count per ml* | | | | | | | | | | | | *Mean Viable Count (log 10)* | *S.D. (log 10)* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 10 | 2 10 | 3 10 | 4 10 | 5 10 | 6 10 | 7 10 | 8 10 | 9 10 | 10 10 | 11 10 | 12 10 | |
| Total Anaerobes (40) | — | — | — | — | — | — | — | — | — | 6 | 10 | 18 | 6 | 10.8 | 0.9 |
| Total Aerobes (40) | — | — | — | — | — | — | — | — | — | 10 | 11 | 13 | 6 | 9.0 | 0.5 |
| Streptococci (16) | — | — | — | — | — | — | — | 1 | 10 | 4 | 1 | — | — | 8.7 | 0.6 |
| Neisseria (40) | 20 | — | — | 1 | 1 | 12 | 4 | 2 | — | — | | | | 5.7 | 0.8 |
| Veillonella (20) | 13 | — | — | — | 1 | 5 | 1 | | — | — | — | — | — | 5.5 | 0.8 |
| Lactobacilli (16) | 12 | — | — | — | 3 | 1 | — | — | — | — | — | — | — | 4.8 | 0.5 |
| Coliforms (40) | 36 | — | — | — | 3 | 1 | — | — | — | — | — | — | — | 4.7 | 0.6 |
| Staphylococci (40) | 3 | — | — | 11 | 17 | 7 | 2 | — | — | — | — | — | — | 4.5 | 0.7 |
| Yeast (40) | 26 | 1 | — | 5 | 5 | 2 | 1 | — | — | — | — | — | — | 4.4 | 0.6 |

Figures in parenthesis indicate total number of saliva samples tested.

Fig. 1 illustrates the microbial counts of saliva in healthy adults. The length of the bars represents the range of counts in 95% confidence limit and the vertical lines represent the mean counts.

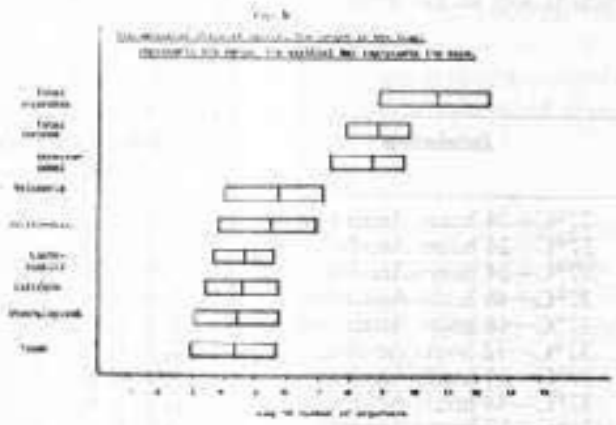

Fig. 1: The bacterial flora of saliva length of block represents the range, the vertical bar represents the mean.

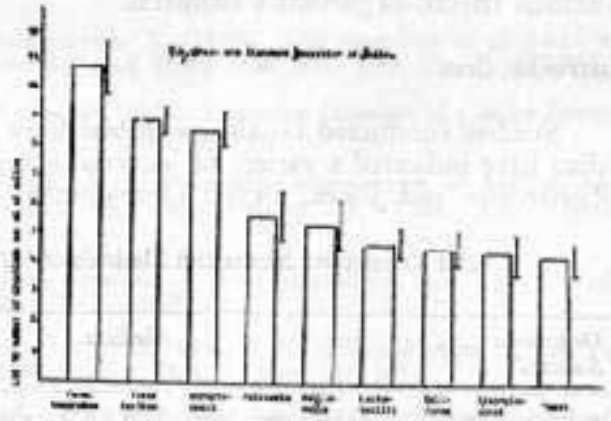

Fig. 2: Mean and standard deviation of saliva

Table III: Microbial Count Categories in Saliva

| *Categories* | *Organisms* | *Mean Count (log 10)* |
|---|---|---|
| High Count group (n x $10^9$/ml) | Total Anaerobes | 60.0 |
| | Total Aerobes | 1.0 |
| | Streptococci | 0.5 |
| Intermediate Count group (n x $10^5$/ml) | Neisseria | 5.0 |
| | Veillonella | 3.0 |
| | Staphylococci | 0.3 |
| Low Count Group (n x $10^4$/ml) | Coliform | 5.0 |
| | Yeast | 2.5 |
| | Lactobacilli | 6.0 |

Arrangement of different groups of organisms in various categories is shown in table III. According to mean total count these organisms have been placed into high, intermediate and low count categories.

A series of saliva specimens collected on six different days over a period of 14 days from three healthy adults were subjected to thorough cultural examination (Fig. 3, 4 and 5). The mean of three subjects is shown in Fig 6. None of the subjects changed diet or received any antimicrobial drug therapy during the period of study. The counts for different groups of organisms are shown on log scale. The major components of the flora on different days are connected by lines and the counts for other organisms present in lower number are drawn as vertical bars on days when they were recovered.

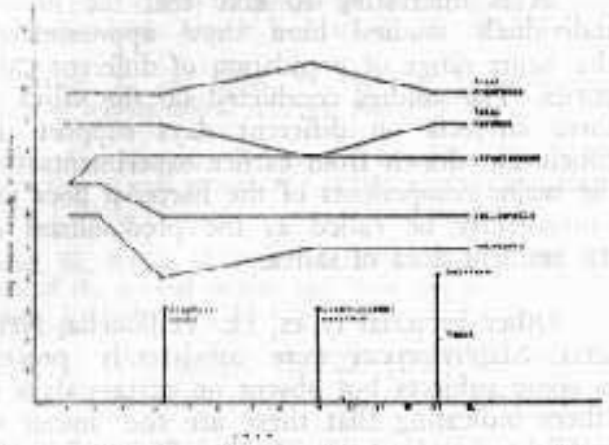

Fig. 3: Microflora of saliva of subject one on different days.

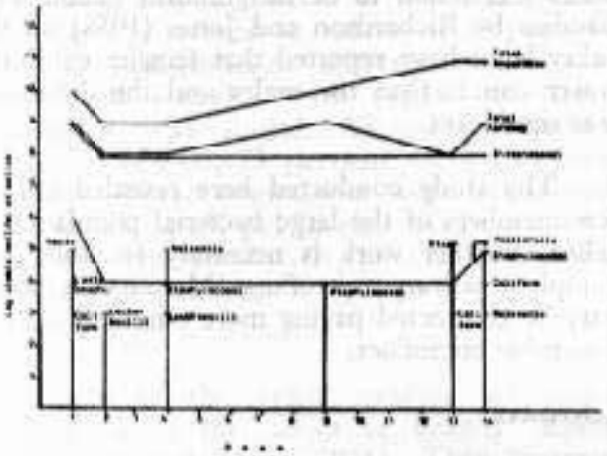

Fig. 4: Microflora of subject two on different days.

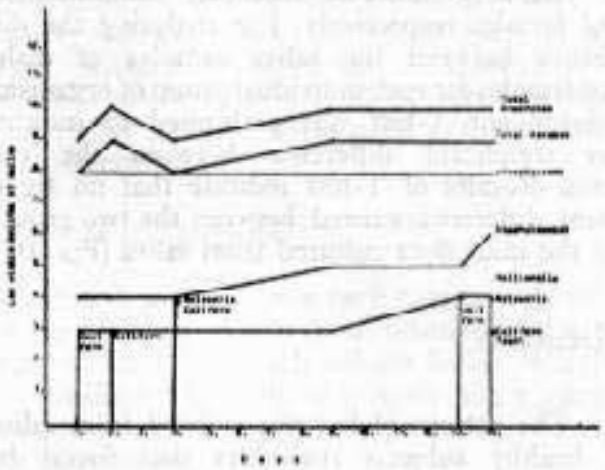

Fig. 5: Microflra of subject three on different days.

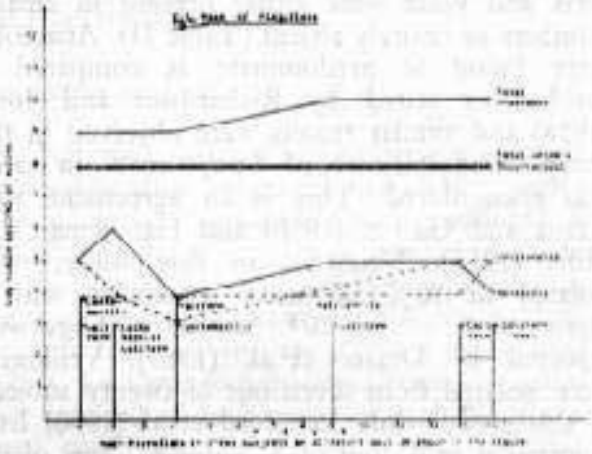

Fig. 6: Mean of microflora.

Table IV: Difference in Microbial Counts between Males and Females (T-Test) for Saliva

| *Groups* | *Sex* | *No. of Cases* | *Mean* | *Standard Deviation* | *Significance* |
|---|---|---|---|---|---|
| Total Anaerobes | M | 19 | 10.87 | 1.00 | P> 0.05 |
| | F | 21 | 10.83 | 0.97 | |
| Total Aerobes | M | 19 | 9.10 | 0.76 | P> 0.05 |
| | F | 21 | 9.13 | 0.69 | |
| Streptococci | M | 18 | 8.62 | 0.66 | P> 0.05 |
| | F | 18 | 8.89 | 0.64 | |
| Neisseria | M | 7 | 5.47 | 0.86 | P> 0.05 |
| | F | 13 | 5.94 | 0.88 | |
| Veillonella | M | 3 | 5.43 | 0.09 | — |
| | F | 4 | 5.48 | 1.19 | |
| Lactobacilli | M | 2 | 4.45 | 0.21 | — |
| | F | 2 | 5.18 | 0.45 | |
| Coliform | M | 3 | 4.77 | 0.84 | — |
| | F | 1 | — | — | |
| Staphylococci | M | 18 | 4.59 | 0.66 | P> 0.05 |
| | F | 19 | 4.46 | 0.80 | |
| Yeast | M | 8 | 4.30 | 0.61 | P> 0.05 |
| | F | 6 | 4.61 | 0.81 | |

Table IV shows the microbial count in males and females respectively. For analysing the difference between the saliva samples of males and females for each individual group of organisms independent T-test was performed to indicate any significant difference between the two sexes. Results of T-test indicate that no significant difference existed between the two groups for the microflora cultured from saliva ($P > .05$).

## Discussion

The pattern of bacteria isolated from saliva of healthy subjects resembles that found by Richardson and Jones (1958). Certain groups of bacteria were recovered from saliva of all normal persons. These included total anaerobes, total aerobes and Streptococci, the other groups e.g. Coliforms and Veillonella were not consistently present while Lactobacilli, Staphylococci, Neisseria and yeast were either present in smaller numbers or entirely absent (Table II). Anaerobes were found to predominate as compared to aerobes as stated by Richardson and Jones (1958) and similar results were observed in this study. Predominance of Streptococci in saliva was encountered. This is in agreement with Kraus and Gaston (1956) and Handleman and Mills (1965). Neisseria, in this study, were isolated in 50% of saliva specimens with a mean value of log $10^3$. Similar findings were reported by Draser et al. (1969). Veillonella were isolated from seven out of twenty subjects in this study while Langford et al. (1950) have reported it in 45 out of 51 subjects. Few of the subjects gave a positive lactobacilli count, but in low numbers (Fig. 2). Similar findings were put forward by Handleman and Mills (1965), while Orland (1959) found it in increased numbers in mouths with active caries. Staphylococci were isolated in 37 out of 40 subjects and in intermediate numbers as also reported by Knighton (1965). Bartels and Blechman (1964) reported on the frequency and distribution of yeast like organism in the oral cavity. They found 43 3% individuals positive for yeasts in oral cavity. In this study 40% of the saliva samples were positive for yeast. All yeast recovered in this study were found to be members of the genus candida. Young et al. (1951) indicated a direct relationship between acidity of saliva and percentage of yeast positive cultures.

Arrangement of different groups of organisms in various categories (Table III) showed that the predominant organisms in saliva were total anaerobes, total aerobes and Streptococci. Similar were the findings of Richardson and Jones (1958) who also divided the different groups of organisms into high, intermediate and low count group.

It is interesting to note that the normal individuals studied here show approximately the same range of population of different categories. The studies conducted on the saliva of three subjects on different days support the conclusion drawn from earlier experiments that the major components of the bacterial flora can conveniently be called as the predominant or the resident flora of saliva.

Other bacterial types, i.e. Veillonella, Neisseria, Staphylococci were consistently present in some subjects but absent on certain days in others indicating that these are the minor or transient components of the flora of saliva (Fig. 3, 4, 5).

To find out the difference between the males and females for each group of organisms studied, independent T-test were performed for the saliva. The difference between the two sexes was found to be insignificant (Table IV). Studies by Richardson and Jones (1958) on the other hand have reported that females exhibited lower counts than the males and the difference was significant.

The study conducted here revealed only a few members of the large bacterial population of saliva. Further work is necessary to study the complete salivary microflora. Moreover a study may be conducted paying more emphasis on the anaerobic microflora.

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