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THE BACTERIAL FLORA OF SUBMUCOUS ABSCESSES ORIGINATING FROM CHRONIC EXACERBATING OSTEITIS

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INTRODUCTION

The composition of the bacterial flora of abscesses originating from chronic periapical osteitis is of great interest from a clinical-therapeutic point of view as well as bacteriologically. The object of the present investigation is to identify the microorganisms which constitute the bacterial flora of abscesses emanating from chronic periapical osteitis. In this paper a study of the bacterial flora of patients with the diagnosis *"Ostitis periapicalis chronica cum abscessu"* is presented. This term refers to a chronic periapical osteitis with exacerbation manifesting itself as an abscess (*Hertz,* 1961). The abscesses were submucous in all cases examined.

Previous investigations

Investigations of the bacterial flora in abscesses emanating from exacerbating chronic periapical osteitis are remarkably few in spite of the fact that osteitis may be considered the most frequent form of jawbone inflammation and that the abscess issuing from it may be regarded as a common complication.

Gilmer and *Moody* (1914) studied the bacterial flora in 16 cases

of acute alveolar abscess and found mainly streptococci of various types — e.g., *viridans* and *haemolyticus* — in aerobic and anaerobic cultures. The mucous membrane over the abscess was cleansed with 50 per cent alcohol; then an incision was made and a sterile pipette was introduced into the wound to draw up the pus. It was not quite clear from the report whether or not all of the abscesses resulted from exacerbating chronic osteitis.

Bulleid (1931) has reported 16 cases of acute alveolar abscesses, 14 of which emanated from nonvital teeth with previous chronic periapical osteitis. Samples from these abscesses were obtained by means of puncture and aspiration, after careful cleansing of the affected area with alcohol and the application of iodine. In 11 of the 14 cases presented in tabular form, *Streptococcus viridans* and *Streptococcus nonhaemolyticus* played a dominant role. Pure cultures of these occurred in 4 cases. There were haemolytic streptococci in 3 cases; one in pure culture. *Staphylococcus albus* occurred in 7 cases, *Staphylococcus aureus* appeared in 2 cases, and *Micrococcus catarrhalis* in 1 case. In addition to the aerobic cultures mentioned above, gram-negative rods were found in 3 cases in anaerobic cultures.

Alin and *Ågren* (1954) have presented a study dealing with 27 cases of acute dento-alveolar abscesses resulting from exacerbations of chronic periapical osteitis. Specimens were taken either by means of aspiration with a syringe or with the aid of a cotton swab pushed through a sterile ear speculum into the incision. The area from which a specimen was to be taken had previously been delimited with sterile compresses, rinsed with an antiseptic solution, and washed with sterile physiological saline. In addition, in some cases thermocautery of the mucous membrane had been performed in the affected area. The specimens were cultured aerobically and anaerobically on a great variety of substrates.

In 8 cases no bacteria could be demonstrated. In all of these cases the samples had been taken at a late stage of the exacerbation. In one case yeasts were found and in all of the remaining cases streptococci, chiefly *Streptococcus viridans,* but also *Streptococcus nonhaemolyticus* and *Streptococcus haemolyticus* were observed in some instances. Streptococci were found in pure cultures, and in other cases in mixed cultures with staphylococci, *Neisseria* or yeasts. The bacterial flora in chronic nonexacer-

bating periapical osteitis has been studied by a number of authors such as *Coriell* (1918), *Fraser* (1923), *Haden* (1926), *Bulleid* (1931), *Burket* (1938), *Hedman* (1951), *Alin* and *Ågren* (1964), *Eklöf* (1955), *Grossman* (1959) and others. The sampling methods used by these workers have varied considerably (see *Grossman,* 1932, 1959; *Engström* and *Frostell,* 1957) and the results have shown great differences. Most of the investigations indicate, in those cases where growth was observed, an apparent dominance of streptococci, mainly of the viridans type.

MATERIAL

About 750 patients treated during 1958—1963 for submucous abscesses were examined as to the periapical and marginal status of the teeth in the abscess area and the condition of the root-canals. The latter have been radiographically classified as follows:

1) no root-filling.
2) root-filling with voids, reaching apex.
3) root-filling with voids, not reaching apex.
4) root-filling with voids, and in excess.
5) root-filling, without voids, reaching apex.
6) root-filling, without voids, not reaching apex.
7) root-filling, without voids, and in excess.

Only cases which satisfied the following conditions were included in the investigations:

There must be no connection between the abscess and the margin; there must be no fistulas or spontaneous perforations which might introduce contamination from the salivary flora; the current condition in question must not have been treated previously in a systemic manner (e.g., with antibiotics) or locally (e.g., via the root-canal); and the current exacerbation must also not have been caused by a recent root treatment.

Cases where for some reason there had been no radiograph were also excluded. Age and sex of the patients were routinely recorded.

Owing to these strict criteria, only 73 (i.e., about 10 %) of the 750 submucous abscess cases available were taken into consideration. In most cases it was possible, by means of clinical and

radiographical examination, to determine definitely from which tooth the abscess had derived. However, in a number of cases these techniques could not show that only one tooth was involved in the inflammatory process. The number of teeth affected in each case and the root status appear in Tables I and II.

Table I.

Distribution of tooth material

Number of teeth affected	Number of cases
1 Tooth	53
2 Teeth	19
3 Teeth	1

In the 53 cases where only one tooth was affected the following distribution of types of teeth occurred:

12 molars (7 upper molars, 5 lower molars)
16 premolars (11 upper premolars, 5 lower premolars)
10 canines (7 upper canines, 3 lower canines)
15 incisors (13 upper incisors, 2 lower incisors)

Table II.

Root Status of Tooth Material
(Cases with only 1 tooth affected)

40 Teeth with no root-filling: (10 molars, 12 premolars, 8 canines, 10 incisors)	
13 Root-filled teeth:	
8 single-canaled teeth (2 premolars, 6 incisors)	1
root-filling with voids, not reaching apex	6
root-filling without voids, not reaching apex	2
5 multi-canaled teeth: (2 molars, 2 premolars, 1 incisor)	1
with a total of 13 root canals	1
no root-filling visible	2
root-filling with voids, not reaching apex	9
root-filling with voids, in excess (not more than 2 mm)	1
root-filling without voids, in excess (not more than 2 mm)	1

METHODS

After anesthetizing the mucous membrane, the site of the incision was delimited by sterile compresses in order to avoid salivary contamination, and rinsed with a solution of a quaternary ammonium compound (benzalkonium chloride 0.1 %). It was then dried with sterile compresses and finally painted with a 5 per cent solution of iodine in alcohol. When the iodine coating had dried the mucous membrane was thermocauterized in the area to be cut and the incision was made. Bacterial samples were taken by collecting the first drop of pus on a cotton swab. The swabs were put in tubes of serumdextrose broth (10 % inactivated horse serum) and in tubes of thioglycollate broth for immediate transport to the bacteriological laboratory in the same building. Besides the samples inoculated into fluid media, samples were also taken in all cases for direct microscopy (Gram staining). To elucidate the degree of risk of contamination in the course of sampling, the following investigation was made: in 20 people selected at random a certain section of the mucosa of the maxillary front was isolated by sterile compresses as previously described when incisions were to be made. The mucous membrane was dried with sterile compresses, washed with a solution of a quaternary ammonium compound (benzalkonium chloride 0.1 %) and dried again. The isolated area was coated with 5 per cent iodine in alcohol, and left to dry. Then samples were taken from the mucous membrane and transferred on a dry cotton swab and also on a cotton swab dampened with Ringer solution to thioglycollate broth tubes. Incubation was at 37° C for 8 days. The result showed that the risk of contamination when using the sampling method just described may be considered very small since only 1 out of a total of 40 specimens thus obtained gave growth from the iodine-treated membrane. (The growth in the only positive specimen proved to be *Staphylococcus epidermidis*). A great number of substrates, selective as well as enriched, were used for cultivation of the samples, in view of the many different types of microorganisms normally existing in the oral flora (*Appleton,* 1950; *Morris,* 1952; *Berger,* 1964; *Richardson* and *Jones,* 1958; *Burnett* and *Scherp,* 1962). In the laboratory transfers were made immediately from the tubes mentioned above into the following media:

Fluid media

1. *Serum dextrose broth.*
2. *Thioglycollate broth.* The fluid media were prepared by the Central Bacteriological Laboratory of Stockholm City.

Solid media for aerobic incubation

1. *Horse-blood agar.* The agar consists of Nutrient broth, with 2 per cent agar-agar and 10 per cent defibrinated horse blood.
2. *Horse-blood agar with gentian-violet.* The agar is as above with the addition of gentian-violet (0.00014 %).
3. *Endoagar* [Difco's Bacto Endo Agar].
4. *Sabouraud agar* [Difco's Bacto Sabouraud Maltose Agar].
5. *Difco Staphylococcus Medium No. 110* [Bacto].

Solid media for anaerobic incubation

1. *Horse-blood agar* (as above).
2. *Rogosa's SL agar* (*Rogosa et al.*, 1951).
3. *"Fusobacterium-agar"* (*Omata* and *Disraely*, 1956).
4. *"Veillonella-agar"* with streptomycin (*Rogosa*, 1956).
5. *"Veillonella-agar"* with vancomycin (*Rogosa et al.*, 1958).
6. *"Actinomyces-agar"* (Horse-blood agar, as above, enriched with 1 % dextrose, poured to form 1 cm thick plates).

The media were all incubated for at least 4 days and some of them for more than 14 days, e.g., the "actinomyces-agar". The anaerobic cultures were incubated in hydrogen with 5 per cent CO_2 in an anaerobic jar with a palladium-asbestos-catalyst (*Frostell*, 1957). In order to obtain pure cultures a great number of subcultures were made from the fluid and solid media to solid selective and enriched media of the types previously mentioned. When the cultures of bacteria on solid media were examined, the terms "rich", "moderate" and "sparse" were used to denote the subjectively estimated amount of growth of the various colonies of bacteria.

Differentiation of the types of the isolated species was per-

formed with the guidance of Bergey's Manual of Determinative Bacteriology (*Breed et al.,* 1957). Certain modifications of the basis for these determinations were made in accordance with the methods used at the Department of Oral Microbiology, Royal Dental School in Stockholm (*Frostell,* 1957; *Engström* and *Frostell,* 1960). The laboratory tests were performed in conformity with standard methods described in the *Manual of Microbiological Methods* (Society of American Bacteriologists, 1957), Topley and Wilson's *Principles of Bacteriology and Immunity* (*Wilson* and *Miles,* 1955) and The Identification of the Genera of Bacteria (*Skerman,* 1959), Bakteriologie und Serologie (*Hallman,* 1961) and Mackie and McCartney's *Handbook of Bacteriology* (*Cruickshank,* 1960).

RESULTS

In 9 of the 73 specimens there was no growth. From the remaining 64 specimens, 123 different strains of microorganisms were isolated. Pure cultures were found in 26 cases, viz. *Streptococcus viridans* (17), anaerobic streptococci (3), *Streptococcus faecalis* (1), diphtheroids (2), *Staphylococcus aureus* (1) and coliform organisms (2). Some rods, which could not be identified with certainty in accordance with the criteria, were placed in the groups "atypical rods".

Streptococci of various species dominated. Thus, in 57 of the 64 (about 90 %) specimens with growth, there were found different varieties of streptococci. Next to the streptococci the diphtheroids occurred most frequently. It should be noticed, however, that the diphtheroids in most cases occurred in samples with sparse growth on the solid media (Table III).

The distribution of the bacterial flora of positive specimens appears in Table III. The flora generally was composed of a pure culture or of a mixed culture with only two strains (Table IV).

Mixed cultures of 3—6 organisms were found in about 20 per cent of the cases. The distribution of the various strains among pure and mixed cultures is given in Table V.

Mixed cultures were observed in 20 different combinations. No clearly dominating combination could be found. There existed a considerable discrepancy between the results from cultures and

those from direct microscopic examination. Thus, complete concordance between the results of the cultures and the results of direct microscopy occurred only in 18 cases.

In general, a considerably greater number of species of microorganisms was found by cultivation than by direct microscopic investigation, but in a number of cases (12) it was quite the contrary (Table VI). The existence of numerous gram-positive rods in the case of direct microscopy indicates that either it was not possible to cultivate those on the media utilized or that the microorganisms in question were dead. In 4 cases where no growth was observed in culture, cells were observed in the direct smears (Table VI). It should, perhaps, be pointed out that nei-

Table III.

Isolated strains and the frequency and density of their growth on solid substrates

Organisms	64 Specimens +++	++	+	Total
Streptococcus viridans-lactis	14	5	27	46
Anaerobic streptococci	4	3	5	12
Streptococcus faecalis	1	—	3	4
Staphylococcus albus*	—	—	2	2
Staphylococcus aureus	—	—	2	2
Neisseria	3	1	3	7
Veillonella	—	3	4	7
Diphtheroids	1	3	10	14
Lactobacilli	—	2	4	6
Leptotrichia	—	2	1	3
Atypical gram-positive rods	1	—	4	5
Atypical gram-variable rods	—	—	3	3
Fusobacteria	—	—	4	4
Bacteroides	—	1	—	1
Coliforms	—	—	4	4
Atypical gram-negative rods	1	—	—	1
Candida albicans	1	—	1	2

+++ rich growth

++ moderate growth

\+ sparse growth or growth by enrichment

* *Staphylococcus epidermidis* according to Bergey's Manual

Table IV.

The distribution of pure cultures and mixed cultures in the specimens

Cultures	Number of Specimens
Pure Cultures	26
Mixed Cultures	
2 Organisms	24
3 Organisms	10
4 Organisms	2
5 Organisms	1
6 Organisms	1
No Growth	9
Total	73

Table V.

The distribution of strains in pure and in mixed cultures

	64 specimens					
	Pure cultures	Mixed cultures				
		2 org.	3 org.	4 org.	5 org.	6 org.
Streptococussus viridans-lactis	17	19	6	2	1	1
Anaerobic streptococci	3	4	4	1	—	—
Streptococussus faecalis	1	2	—	1	—	—
Staphylococcus albus*	—	—	1	—	1	—
Staphylococussus aureus	1	—	1	—	—	—
Neisseria	—	1	2	2	1	1
Veillonella	—	2	4	—	—	1
Diphtheroids	2	7	3	—	1	1
Lactobacilli	—	3	1	1	—	1
Leptotrichia	—	1	2	—	—	—
Atypical gram-positive rods	—	3	1	1	—	—
Atypical gram-variable rods	—	2	1	—	—	—
Fusobacteria	—	2	1	—	—	1
Bacteroides	—	—	1	—	—	—
Coliforms	2	1	1	—	—	—
Atypical gram-negative rods	—	—	—	—	1	—
Candida albicans	—	1	1	—	—	—

* *Staphylococcus epidermidis* according to Bergey's Manual

Table VI.

Cases in which more organisms were found in the direct smears than in the cultures

No.	Strains found in direct smears	Strains found in cultures	Strains found in direct smears but not in cultures
1	Gram-positive cocci intracellular	0	Gram-positive cocci intracellular in leucocytes
2	Gram-positive cocci intracellular in leucocytes	0	Gram-positive cocci intracellular in leucocytes
3	Gram-positive cocci, gram-positive pleomorphic rods	0	Gram-positive cocci, gram-positive pleomorphic rods
4	Gram-positive cocci	0	Gram-positive cocci
5	Gram-positive cocci, gram-negative dipplococci, fusiforms	Streptococcus viridans-lactis	Gram-negative dipplococci, gram-negative fusiforms
6	Gram-positive cocci, gram-positive pleomorphic rods	Streptococcus viridans-lactis	Gram-positive pleomorphic rods
7	Gram-positive cocci, gram-negative dipplococci	Anaerobic streptococci, diphtheroids	Gram-negative dipplococci
8	Gram-positive cocci, gram-variable spiral microorganisms	Streptococcus faecalis, coliforms	Gram-variable spiral microorganisms
9	Gram-positive cocci, gram-negative rods, gram-positive pleomorphic rods	Anaerobic streptococci Veillonella, Bacteroides	Gram-positive pleomorphic rods
10	Gram-positive cocci, gram-positive pleomorphic rods, gram-positive cocci, gram-positive rods similar to Leptotrichia, gram-negative fusiform rods, gram-variable curved rods, gram-variable spiral microorganisms	Streptococcus viridans-lactis, diphtheroids, Neisseria, Staphylococcus albus, gram-negative atypical rods	Gram-positive rods similar to Leptotrichia, gram-negative fusiforms, gram-variable curved rods, gram-variable spiral microorganisms
11	Gram-positive cocci, gram-positive rods	Streptococcus viridans-lactis	Gram-positive rods
12	Gram-positive cocci, gram-positive filaments	Streptococcus viridans-lactis	Gram-positive filaments

ther β-haemolytic pyogenic streptococci nor pneumococci were found in any of the 750 oral abscess cases originally examined, although the possibility of their existence was taken into account.

DISCUSSION

In certain cases in the previous literature, it has been difficult to determine the signification of the clinical diagnosis given. Thus, for instance, *Gilmer* and *Moody* (1914) speak of "acute alveolar abscesses", *Head* and *Ross* (1919) of "apical abscess", *Fraser* (1923) of "periapical abscess", *Haden* (1926) of "chronic periapical dental infection", *Bulleid* (1931) of "acute alveolar abscesses" and *Alin* and *Ågren* (1954) of "acute dento-alveolar abscesses". It is not always clear whether cases with fistula or cases with marginal processes were included in the reported material, a circumstance which is indubitably of importance in relation to the composition of the microbial flora. All possible efforts have been made in the present study to include only representative cases with the exact diagnosis "Ostitis periapicalis chronica cum abscessu".

Streptococci of different species definitely predominate in the flora in our investigation, which is in accordance with studies previously made (*Gilmer* and *Moody,* 1914; *Bulleid,* 1931; and *Alin* and *Ågren,* 1959). The fact that streptococci of the viridans-lactis group were isolated in not fewer than 17 of the 26 cases with pure cultures clearly indicates their dominating role in the development of the disease of apical osteitis and of the abscesses emanating therefrom. It is noteworthy that, contrary to these previous investigations, no "β-haemolytic streptococci" belonging to the pyogenic group were found. In this connection we would point out that none of the 750 abscess cases from which we obtained our material showed any "β-haemolytic streptococci". Also, in cases of infected root-canals, "β-haemolytic streptococci" either have been observed in very small numbers or have been quite absent (*Grossman* and *Christian,* 1952; *Richardson* and *Jones,* 1958; *Winkler* and *van Amerongen,* 1959; *Shovelton* and *Sidaway,* 1960; and *Engström* and *Frostell,* 1964). Anaerobic microorganisms, i.e., anaerobic streptococci, *Veillonella, Fusobacteria* and *Leptotrichia,* on the other hand have occurred with greater

frequency than in previous investigations, due possibly to the progress recently made in anaerobic technique by cultivation in an atmosphere of hydrogen (*Frostell*, 1957) and by utilizing a great number of selective and enriched media.

Previous investigations also have indicated the presence of staphylococci in about 20 per cent of the cases (*Bulleid*, 1931; *Alin* and *Ågren*, 1954 a.o.), a finding which could not be verified in our investigation. The low incidence of *Staphylococcus aureus* observed by us (less than 3 per cent) perhaps may be explained by the fact that this microorganism, like the "β-haemolytic streptococci", seems to be of no particular importance in the common exacerbations of periapical osteitis. If contamination from the surrounding tissues occurs, *Staphylococcus albus* is often found; and the fact that it appeared very infrequently in our investigation might be an indication of the elimination of external microorganisms.*

The proportion of diphtheroids in this material corresponds fairly well to the composition of the oral flora reported by *Berger* and *Hummel* (1964) and *Richardson* and *Jones* (1958) and also to the results of the studies on infected root-canals published by *Shovelton* and *Sidaway* (1960).

Actinomycetes and vibrios have not been demonstrated in the cultures of our material, although other investigators as well as ourselves have often been able to isolate these organisms from samples of plaque material, root-canals and subcutaneous abscesses. In Table VI it can be seen that the microorganisms which were found in direct specimens but which did not appear in cultures very often were gram-positive rods. In a number of cases these rods resembled diphtheroids and might possibly belong to the family *Corynebacteriaceae* or *Actinomycetaceae*. In some cases these apparent rods might be the streptococci found in the cultures since it is well known that under certain conditions streptococci often become elongated and may be mistaken for rods. Since the results of the direct microscopic examination bore little resemblance to that of the cultures, direct specimens alone

* The occurrence of *Staphylococci* and micrococci in human saliva is shown by Jordan, Fitzgerald and Faber (1956): 109 strains of *Staphylococci* and micrococci were isolated from salivas of 46 of 59 persons. Seventeen of the strains were coagulose and mannitol positive.

cannot be used for the bacteriological diagnosis. In addition to the cultures the direct specimens must, however, be considered important (see Table VI). In 9 cases no microorganisms could be found in the cultures. This must not be considered as evidence of absence of microorganisms in these specimens. The reason may simply have been that the substrates were insufficient or that the microorganisms were no longer viable when the incision was made. These hypotheses are supported by the four direct smears which were positive but failed to grow in culture (Table VI).

It should be noted that most of the exacerbating processes arose from teeth which apparently were not previously root-treated (Table II). Only 2 root-canals of the 13 root-filled teeth in the material shown in Table II have tight root-fillings. One of them was filled in excess, the other one with an apical lumen. None of the root-filling in the material shown in the table was without voids and exactly reached the apex. This fact agrees with the results of *Strindberg* (1956), *Grahnén* and *Hansson* (1961), and *Engström et al.* (1964) which show the importance of adequately performed endodontic procedures.

SUMMARY

From 73 patients with *osteitis periapicalis chronica cum abscessu,* which were selected from about 750 cases with odontogenic exacerbations, bacterial specimens for culture were taken by means of incision and were also subjected to direct microscopic examination. Cultures were made on a variety of selective and enriched media under both aerobic and anaerobic conditions. From 64 of the 73 samples strains of different species were isolated. They were generally present in pure cultures or in mixed cultures composed of only two strains. In 4 cases microorganisms were found in smears but could not be demonstrated in cultures. In the remaining 5 cases no microorganisms could be found either in cultures or in smears. As a rule, more microorganisms could be found by cultivation than by direct microscopy. In a number of cases, however, certain types of bacteria, which could not be cultured, were observed by direct microscopic examination. The flora was dominated by streptococci of the viridans-lactis group, the anaerobic groups and diphtheroids.

Streptococci of the pyogenic group were completely lacking and pyogenic staphylococci occurred only in 2 cases. Anaerobic micro-organisms were observed very often. These results are somewhat different from previous investigations; the reasons for this have been discussed.

RÉSUMÉ

LA FLORE BACTÉRIENNE DANS DES ABCÈS SUB-MUQUEUX EMANANT DE OSTÉITES CHRONIQUES EXACERBANTS

Parmi environ 750 patients con exacerbations odontogéniques furent choisis 73 cas con ostitis périapicalis chronica cum abscessu où l'on a effectué, en connexion avec l'incision, des essais bactériologiques pour la culture de bactéries et pour la microscopie directe. La culture se faisait sur un grand nombre d'objets choisis aussi bien dans un milieu aérobic que dans un milieu anaérobic.

De 64 des 73 spécimens pris on a isolé des souches appartenant types différents. Elles se rencontraient généralement dans des cultures pures ou dans des cultures mixtes ne comportant que deux souches. Dans 4 cas on a trouvé des micro-organismes quand on s'est servie de la microscopie mais non en cas de culture. Quand il's agit des 5 cas restants on n'a pu constater aucuns micro-organismes, ni par culture, ni par microscopie directe.

En général il était possible de constater la presence de plus de micro-organismes en cas de culture qu'en cas de microscopie directe. Cependant, dans un nombre de cas on a observé, pour ce qui est de la microscopie directe, certains types de bactéries qu'on n'a pas trouvés dans la culture.

La flore était dominée par des streptocoques appartenant au groupe viridans-lactis, aux groupes anaérobiques et aux diphtéroides.

Il manquait entièrement de streptocoques ("β-hemolytiques") et en ce qui concerne les staphylocoques pyognes on a trouvé seulement 2 cas. Des microorganismes anaérobiques appartenant à la flore orale ont été observés très fréquemment. Cela implique une certaine contradiction en comparaison avec les investigations antérieures, ce dont on a parlé plus en détail dans la discussion.

ZUSAMMENFASSUNG

DIE BAKTERIENFLORE DER SUBMUCÖSEN ABSCESSE AUSGEHEND VON CHRONISCHEN EXACERBIERENDEN OSTITEN

Von 73 Patienten mit Ostitis Periapicalis Chronica cum Abscessu, unter etwa 750 Fällen mit odontogenen Exacerbationen ausgewahlt, wurden im Zusammenhang mit dem Einschnitt Bakterieproben genommen, für sowohl Kultur als für direktes Mikroskopieren abgesehen. Die Kultur umfasste eine grosse Anzahl von ausgewahlten Substraten in sowohl aerobem als in anaerobem Milieu.

Von 64 der 73 genommenen Proben wurden Stämme von verschiedenen Typen isoliert. Sie kamen gewöhnlich in Reinkulturen vor oder in Mischkulturen aus nur zwei Stämmen bestehend. In 4 Fällen konnten Mikroorganismen durch Mikroskopieren aber nicht durch Kultur nachgewiesen werden. In den 5 rückständigen Fällen konnten weder durch Kultur noch durch direktes Mikroskopieren einige Mikroorganismen nachgewiesen werden. Im allgemeinen konnten mehr Mikroorganismen durch Kultur als durch direktes Mikroskopieren nachgewiesen werden. In einigen Fällen wurden doch bei direktem Mikroskopieren gewisse Typen von Bakterien beobachtet, welche bei der Kultur nicht angetroffen wurden.

Dominierend in der Flora waren Streptokokken, den Gruppen viridans-lactis, den anaeroben Gruppen und den Diphteroiden angehörend.

"β-hemolysierende" Streptokokken (Streptococcus pyogenes) fehlten ganz und gar, und pyogene Stafylokokken kamen nur in 2 Fällen vor. Anaerobe Microorganismen der Mundflora angehörend wurden auffallend oft beobachtet. Dieses bildet einen gewissen Gegensatz zu den Ergebnissen von früheren Untersuchungen, was auch in der Diskussion näher besprochen worden ist.

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