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THE BACTERIAL FLORA OF ODONTOGENIC INFECTIONS AND ITS SENSITIVITY TO ANTIBIOTICS

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INTRODUCTION

With the possibility of combatting infection with antibiotics the sensitivity of bacteria becomes a subject of topical interest. Although it is usual in practice to choose the therapy with the guidance of the clinical picture, it is only after performing a bacteriological diagnosis and determining the resistance to antibiotics that the most suitable remedy can be selected. If treatment already begun does not yield a satisfactory result, such an examination may suggest more adequate measures.

Odontogenic infections present special problems. Since the infection is generally exogenous, the bacterial flora of the mouth is reflected in infection foci that frequently contain two or more types of bacteria having different chemoresistance. Moreover, the infections are not unfrequently encapsulated, which makes it difficult to obtain a bacteriostatic concentration in the infection focus. The bacteria may be in the stationary phase and hence not easily accessible for chemotherapy. Other problems are the avoidance of contamination when sampling at the infection focus, and the evolution of methods of cultivating the bacteria and determining their resistance to antibiotics.

THE AIMS OF THE INVESTIGATION

Initially, a comparison was drawn between the types of bacteria recognized in specimens from the mouth and from infection

foci in the same patient, the subjects having different odontogenic infections.

The purpose of the investigation was then to study the resistance of bacteria isolated from the foci to penicillin, streptomycin, sulfa (Elkosin), aureomycin, chloromycetin and terramycin, and on the basis of the results to discuss the application of these antibiotics in the treatment of odontogenic infections.

PREVIOUS INVESTIGATIONS

The information provided by the literature on the constitution of the oral flora varies widely, and any attempt to obtain a clear picture is complicated by frequent inconsistencies in the nomenclature. *Bibby* (1938) has stated that the oral flora varies even from one part of the mouth to another. *Appleton* (1950) finds that there are variations not only from one person to another but also between different ages. He maintains also that the prevalent flora in a mouth maintained in good condition consist of facultative aerobes while anaerobic bacteria are more numerous in less hygienic mouths.

Topley & Wilson (1946) and *Dubos* (1952) state that it is normal to find wide variations in the flora. Common microorganisms include α- and β-streptococci, staphylococci, spirochetes, lactobacilli, fusiform rods and fungi.

The most prevalent of the odontogenic infections of the jaw is periapical osteitis. On account of its various clinical forms it is convenient to group the material of an investigation with respect to the pathogenesis. *Bulleid* (1928, 1931) has recognized this fact and has found that the bacteria of pathogenic importance in apical granulomas consist of various species of streptococci, occasionally in symbiosis with staphylococci. In chronic periapical osteitis, with diffuse bone destruction evident in the radiographs as well as in acute alveolar abscesses, Bulleid always found various species of streptococci. In jaw cysts he reports streptococci and staphylococci. Streptococci thus seem always to be present in the various forms of periapical osteitis. Bulleid performed both aerobic and anaerobic cultivation, paying particular attention to the risk of contamination when taking his samples.

Appleton's summary (1950) throws light on the present-day approach to the bacteriology of apical osteitis: "Streptococci are encountered most frequently, particularly alpha-streptococci. Beta-streptococci are believed by some authors to occur more frequently in acute conditions. Mixed cultures are common. The presence of staphylococci may characterize cases in which pus production is conspicuous".

Reports of sensitivity studies in odontogenic infections are rare. *Ågren* (1951) has examined 30 such cases, mainly with regard to the resistance to penicillin, although sulfa, aureomycin and streptomycin have also been used. In general, the bacteria recognized were sensitive to 1/16—1/4 IU/cc. A small number of strains were shown to be less sensitive or even resistant to penicillin.

Diding & Wallmark (1951) determined the sensitivity to five antibiotics of about 2,500 bacterial strains isolated from clinical specimens. A large number of these strains are common in oral flora. Most bacterial genera have a very variable sensitivity and the necessity of determining this property for suitable therapy was pointed out.

Ever since the introduction of antibiotics various methods have been worked out for determining the sensitivity of the micro-organisms responsible for disease. A rapid and simple method is of great value. Such a procedure has been described by *Jensen & Kiær* (1948) and since modified by *Lund* and collaborators (1951). The sensitivity studies were performed with tablets containing known concentrations of various antibiotics which, placed on blood agar, gave rise to zones of inhibition of varying diameter when the bacteria were spread over the plate. The size of this zone is an expression of the sensitivity of the bacteria studied.

The methods have since been modified by *H. Ericsson* (1953) who, instead of tablets, used circles of blotting paper, 5 mm in diameter, saturated with the antibiotic of suitable concentration. After 15—18 hours in the thermostat the results were read by measuring the diameter of any zone of inhibition observed. The diameter in millimetres may be transformed to degrees of sensitivity by means of tables.

ORIGINAL INVESTIGATION

MATERIAL

The material was selected from patients who came to the Royal School of Dentistry in Stockholm during 1952—53 for treatment of infections of odontogenic origin. In addition there are included one case of naso-alveolar cyst and one of adamantinoma. The diagnosis was in a majority of cases made on the basis of the case history and the radiologic status. 53 persons were examined. Their ages ranged from 19 to 69 years, most of the patients being of middle age. Specimens being taken from infection foci in the jaws and from the mouth. Before taking the specimens the patients had generally not received treatment and in no case had chemotherapy been applied. As far as possible subjects with natural teeth were selected. Oral hygiene had generally been poor. The distribution of the diagnoses is given in Table I

Table I

Distribution of diagnoses

	No. of cases
Chronic rarefying osteitis with granulation tissue	9
Chronic rarefying osteitis with suppuration	2
Acute dento-alveolar abscesses	24
Radicular cysts	14
Follicular cysts	5
Incisive canal cyst	1
Dry sockets	3
Adamantinoma	1
Naso-alveolar cyst	1
Total	60

METHOD

Specimens from the infection foci

On account of the difficulty of avoiding contamination from the mucosa when taking the specimens, various methods were used, depending on the type of case, the position of the focus and the diagnosis.

When removing the contents of the jaw cysts or the pus in

alveolar abscesses the following technique was in some cases applied.

The area from which the specimen was to be taken was isolated with sterile compresses, and the site of the puncture or incision rinsed with Bensalkonium chloride or medicinal grade zinc peroxide powder in 25 per cent water suspension. This treatment was in some cases followed by removal of the mucosa over a small area by thermocautery. A wide cannula was introduced to which was coupled a syringe; on aspiration the exudate was transferred directly from the syringe to Loeffler's medium. Where this technique was not followed the focus was opened by incision, after which a sterile ear-specula was introduced into the incision. A sterile cotton swab was placed in the focus and immediately transferred to Loeffler's medium.

When pieces of tissue such as root tips, small cysts or granulation tissue in chronic resorptive osteitis were examined bacteriologically the method adopted was the following. After carefully reflecting the gum and periosteum, specimens were taken from the bone in the vicinity of the infection focus; the bone was then removed and infected parts taken from the focus and placed in Loeffler's medium. If the bone specimen proved sterile while the specimen from the mucosa and tissue parts revealed growths the latter were considered as uncontaminated. In but one case could bacteria be demonstrated in the bone sample. Growth of bacteria in the bone specimen could be shown only in one of these cases where a destruction in a little area of the jaw bone was found after reflecting gum and periosteum. This had caused a direct communication between the focus of infection and the vestibular corticalis of the jaw bone.

Specimens from the oral mucosa

The specimens were taken with swabs from the gingiva on the left and right sides of the lower jaw. The swab was immediately placed in Loeffler's medium. The tube was then despatched as soon as possible for bacteriological examination. The interval between sampling and bacteriological cultivation was generally not more than eight hours. When in exceptional cases the specimen could not be forwarded immediately it was stored in a refrigerator.

Bacteriological procedure

Since the purpose of the investigation was to determine the chemoresistance no direct microscopic examination of the specimens was performed. In bacteriological examination the expected composition of the flora should determine the choice of medium. As earlier investigations have shown, the oral flora in odontogenic infections invariably contains gram-positive cocci and to some extent gram-negative cocci, bacteria of the coli group, grampositive rods of the genus *Lactobacillus,* and fungi. Spirochetes and fusobacteria occur in the oral flora but there is still no method of cultivating them.

Cultures were made with the following media:

Liquid media

(1) Serum dextrose broth (1 per cent dextrose)
(2) NaCl broth (10 per cent NaCl)
(3) Ascitic broth

Solid media

Aerobic cultivation

(4) Horse blood agar (10 per cent horse blood)
(5) „ „ „ + gentian violet
(6) Phenol-mannitol agar (indicator — phenol red)
(7) Drigalski agar (indicator — bromo-thymol blue)
(8) Chocolate agar + ascitic fluid
(9) „ „ „ „ + penicillin
(10) „ „ „ „ + potassium tellurite
(11) Tomato agar (sodium azide)
(12) Sabouraud's agar

Anaerobic cultivation

(13) Horse blood agar

A majority of the resistance determinations were performed by *H. Ericsson's* paper disc technique (1953). At the time this investigation was begun this method was not yet available, and some ten determinations were made by the tablet method of *Lund* and collaborators.

In the resistance determination the following grading was introduced:

sensitive (S), *moderately sensitive* (MS), *resistant* (R).

These arbitrary degrees of sensitivity correspond to ranges of antibiotic concentration for which complete inhibition of bacterial growth was obtained (Table II).

Table II

Ranges of antibiotic concentration corresponding to the degrees of sensitivity

	Penicillin IU/cc	Streptomycin mcg/cc	Sulfa (Elkosin) mg/%	Aureomycin mcg/cc	Chloromycetin mcg/cc	Terramycin mcg/cc
Sensitive.....	≦ 0.1	≦ 4	≦ 2.5	≦ 1	≦ 4	≦ 1
Moderately sensitive	0.2—2	5—16	4—7.5	2—4	5—16	2—4
Resistant	≧ 3	≧ 25	≧ 10	≧ 5	≧ 20	≧ 5

RESULTS

A majority of cases in this material present various forms of periapical osteitis. For this part of the material a collocation has been made of the clinical and bacteriological data on the basis of the findings of the individual examinations.

CHRONIC RAREFYING OSTEITIS WITH GRANULATION TISSUE

(9 cases. Examination of granulation tissue and root tip)

In all cases the specimens were taken during resection of the root tip. The clinical picture was characterized by an absence of, or very insignificant, symptoms, the radiographs revealing various degrees of bone destruction.

In one case the specimen was sterile. The other cases presented streptococci, either as pure cultures or mixed with other oral bacteria, such as staphylococci, *Neisseria* and lactobacilli. In 6 cases there were *Str. viridans (α)* and in 2 cases non-hemolytic streptococci. When taking the specimens it was found that the bacteria were restricted to the infection foci, and were to be found neither in the connective tissue nor in the bone near the foci as long as there is no communication between the focus of infection and the vestibular corticalis of the jaw bone.

ACUTE DENTO-ALVEOLAR ABSCESSES

(27 cases. Examination of pus)

In all cases the abscesses had arisen through acute flare-up in association with one or more non-vital teeth with clinical or radiographic indications of previous chronic periapical osteitis.

The incision and puncture were made intra-orally. In 8 cases the cultures presented no growth. In all these cases specimens were taken at a late stage in the flare-up (fourth to tenth day). Streptococci were present in all cases but one, where fungi were found. In all cases there were *Str. viridans* (α) in pure cultures, in one case *Str. hemolyticus* (β), in one case *Str. non-hemolyticus anaerob.* In 5 cases the cultivation gave streptococci in mixed cultures with staphylococci, *Neisseria* or fungi. In this form of periapical osteitis, too, streptococci dominated the bacteriological picture.

RADICULAR CYSTS

(14 cases. Examination of pus, fluid or wall + fluid)

13 of the radicular cysts were associated with infected non-vital teeth or roots, one case was a residual cyst from a previously extracted lateral incisor. 6 of these cases presented no clinical symptoms. In the 8 others there were manifest symptoms such as swelling, deviation of teeth, pain, etc. Two of the cysts were purulent and on cultivation showed no growth.

In 8 cases the cultivation gave pure cultures that in 5 cases consisted of *Staph. albus* or *Staph. aureus* and in 3 cases of *Str. viridans* (α). In 4 there were mixed cultures in which streptococci, staphylococci or lactobacilli were present. In the cystic form of periapical osteitis streptococci no longer dominate but were found in as many cases as staphylococci.

The results of the bacteriological cultivation are presented in Tables III & IV.

14 types of micro-organism were isolated, 13 of the 55 oral specimens and 9 of the 60 specimens from the foci of infection. In thirteen cases the latter were sterile (22 per cent).

As expected, *Str. viridans* (α) is dominant both in the oral and the foci specimens, although more marked in the latter. With one exception this species always occurs in the mouth when it is found in the foci specimens. In the small number of cases

Table III

Frequency of bacteria found in the specimens

Micro-organisms	Cultures from foci of infection		Cultures from oral specimens	
	No. strains (76)	Percent. of total specimens (60)	No. strains	Percent. of total specimens (55)
Str. viridans (α)	33	55	50	91
Str. hemolyticus (β)	4	7	4	7
Str. non-hemolyt.	2	3	6	11
Str. non-hem. anaer.	5	8		
Staph. aureus	6	10	10	18
Staph. albus	14	25	21	38
Neisseria	6	10	30	55
Lactobac.	3	2	4	7
Corynebact. pseudodipth.			1	2
E. coli			1	2
Mucoid coli			1	2
Alcaligenes			1	2
H. influenzae			2	4
Fungi	3	5	3	6

with *Str. hemolyticus (β)* there was no marked agreement between the oral and foci flora. Anaerobic non-haemolytic streptococci were found only in specimens from infection foci. In 6 cases staphylococci were found in foci specimens when they could not be demonstrated in oral specimens from the same patient — one of these cases being an infected adamantinoma. *Neisseria,* which was fairly sparsely represented in the foci specimens, occurred in more than one half the oral specimens, when these bacteria could be found in infection foci specimens they were always in the oral samples of the same patient. There was no marked similarity between foci and oral samples in respect of the fungi. Certain bacteria, such as *Corynebacterium pseudo-diphtheriae, Escherichia coli, Alcaligenes* and *Hemophilus influenzae* occurred but sparsely in oral and not at all in foci specimens.

This bacteriological investigation of the oral flora is not claimed to be at all complete and is representative only of the region of the oral cavity where the sample was taken.

To summarize, micro-organisms shown to be present in specimens taken from infection foci were often found in oral specimens from the same patient. This was found to be so in 77 per cent of the cases.

Table IV

Frequency of sterility, pure and mixed cultures

No. of types isolated	Specimens from foci of infection	Specimens from oral cavity
0	13	0
1	28	9
2	11	17
3	6	25
4	2	4

Two or three types of micro-organism occur rather often in the oral specimens, while the specimens from the infection foci generally contain one or two types. In 13 cases the specimens from foci of infection presented no growth.

DISCUSSION

Previous investigations show that odontogenic infections are to a large extent mixed infections in which are included streptococci, mainly *Str. viridans* (α) and staphylococci. The present investigation, performed on untreated cases with particular attention to sterility in sampling, has confirmed this observation and has revealed, moreover, a relatively close agreement between the oral bacteria and the micro-organisms found in the infection foci, although the number of different types is frequently greater in the oral than the focal specimens.

The prevalent odontogenic infection is periapical osteitis. In this material about 80 per cent of the cases consisted of periapical osteitis in various stages. To elucidate the bacteriology of the disease a grouping of the material with respect to the clinical appearance of the appropriate tooth was clearly indicated and the radiographs were examined to determine whether the bone destruction was diffuse or well-defined. The limited material did not permit a more detailed grouping than was performed. The main object was to find which micro-organisms were generally to be expected in various types of odontogenic infection. An interesting finding was the rather frequent occur-

Table V

Results of resistance determinations

	Number of strains	Penicillin			Streptomycin			Sulfa			Aureomycin			Chloromycetin			Terramycin		
		S*	MS*	R*	S	MS	R	S	MS	R	S	MS	R	S	MS	R	S	MS	R
Strept. viridans (α)	33	21	9	3	11	11	11	11	5	17	20	9	4	26	4	3	29	3	1
Strept. hemolyticus (β)	4	3	1		3		1	3		1	3	1		4			3	1	
Strept. non-hemolyticus	2			2			2			2	1	1		1		1	1	1	
Strept. non-hemolyticus anaerob	5	5			2	2	1	1		4	5			5			5		
Staph. aureus	6	3	1	2	1	2	3	1	1	4	1	4	1	3	3		3	2	1
Staph. albus	14	9	2	3	11	2	1	4	6	4	6	5	3	12	2		10	3	1
Neisseria	6	2	3	1	5	1		1	3	2	6			6			5		1
Lactobacillus	3	3			3					3	3			3			3		
Total	73	46	16	11	36	18	19	21	15	37	45	20	8	60	9	4	59	10	4
Percentage		63	22	15	49	25	26	29	21	50	62	27	11	82	12	6	81	14	5

* S = sensitive, MS = moderately sensitive, R = resistant.

The number of sensitive strains is greatest for chloromycetin and terramycin, followed by penicillin and aureomycin. About one half of the tested strains were sensitive to streptomycin, and one third to sulfa. The number of strains resistant to sulfa is about one half of those tested.

Of particular interest is the sensitivity and resistance to penicillin, the antibiotic which is most widely used to-day. Of the 73 tested strains 11 were resistant (R) while 62 were sensitive (S) or moderately sensitive (MS).

rence of sterile specimens from pus in alveolar abscesses and infected cysts.

The resistance determinations show that the bacteria in the infection foci are rather resistant to sulfa and streptomycin. Penicillin and aureomycin assume an intermediate place, while chloromycetin and terramycin exhibit a pronounced activity against the examined bacterial strains. (Table V.)

The usual dosage of the antibiotics mentioned provided a sufficiently high blood concentration and probably a sufficiently high tissue concentration for bacteriostasis against the micro-organisms that have been recorded as sensitive (S) in the resistance determinations. If the question of antibiotic therapy is considered only from the point of view of effectiveness chloromycetin and terramycin should thus be given first place in odontogenic infections. These substances have certain disadvantages, however; they are rather expensive, and after some days use they sometimes cause indisposition, diarrhœa and occasionally pruritus. From the practical point of view it is therefore desirable to use the relatively cheap penicillin, with which the complications over short periods of use are less pronounced. The present investigation indicates that with penicillin with prolonged action a bacteriostatic effect may be obtained against 60 to 85 per cent. of the micro-organisms causing odontogenic infections. Penicillin may thus still be used initially and if this does not lead to a result in 24 to 36 hours chloromycetin or terramycin should be substituted.

SUMMARY

60 bacteria specimens were taken from 53 persons with odontogenic infections, and sensitivity determinations were performed on the strains isolated.

With few exceptions, samples were taken at the same time from the oral mucosa and the infection foci. *Str. viridans* (α) and *Staph. albus* were the most common bacteria in specimens from the foci.

Streptococci and *Neisseria* were commonest in the oral flora.

Resistance determinations in respect to penicillin, streptomycin, sulfa (Elkosin), aureomycin, chloromycetin and terramycin show that the two latter antibiotics had the greatest effect, with

penicillin and aureomycin next. Streptomycin and sulfa appeared to be the least suitable.

For practical reasons penicillin is suggested as the antibiotic of choice in odontogenic infections, with chloromycetin or terramycin only if therapeutic results with penicillin are not obtained within a resonable period.

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RÉSUMÉ

LA FLORE BACTÈRIENNE DANS L'INFECTION ODONTOGÈNE ET SA SENSIBILITÉ POUR LES ANTIBIOTIQES

Sur 53 personnes on a procédé à des déterminations de culture et de résistance de 60 échantillons d'infections odontalgiques. A de rares exceptions on a en même temps prélevé pour culture des échantillons de la flore buccale. Le streptocoque viridans (α) et le staphylocoque albus sont les plus fréquents dans les échantillons d'infection foci, les streptocoques et les neisserias sont habituels dans la flore buccale. La détermination de résistance aux effets de la pénicilline, de la stréptomycine, les sulfas (elkosin), de l'aureomycine, de la chloromycetine et de la terramycine montre que les deux derniers antibiotiques ont le degré d'efficacité le plus grand, la pénicilline et l'aureomycine venant ensuite. La stréptomycine et les sulfas paraissent moins opportuns.

Pour des raisons pratiques nous proposons donc la pénicilline comme premier antibiotique dans les infections odontalgiques, et la chloromycetine ou là terramycine dans les cas où les résultats thérapeutiques de l'application de la pénicilline ne seraient pas atteints en temps raisonnable.

ZUSAMMENFASSUNG

DIE BAKTERIENFLORA BEI ODONTOGENEN INFEKTIONEN UND IHRE EMPFINDLICHKEIT GEGEN ANTIBIOTICA

An 53 Personen hat man Züchtung und Resistenzbestimmung von 60 Proben von odontogenen Infektionen ausgeführt. Mit wenigen Ausnahmen sind gleichzeitig Proben für Züchtung von

der Mundflora genommen. Streptococcus viridans (α) und Staphylococcus albus sind am häufigsten in den Proben von Infektionsfocus. Die erst genannten und Neisseria sind am gewöhnlichsten in der Mundflora.

Resistenzbestimmung mit Penicillin, Streptomycin, Sulfa (Elkosin), Aureomycin, Chloromycetin und Terramycin zeigt dass die zwei späteren Antibiotica den grössten Wirkungsgrad haben, Penicillin und Aureomycin kommen in 2. Stelle. Streptomycin und Sulfa sind nicht so geeignet. Aus praktischen Gründen wird Penicillin als erstes Antibioticum bei odontogenen Infektionen vorgeschlagen und Chloromycetin oder Terramycin, falls terapeutisches Resultat in angemessener Zeit nicht erzielt wird.

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