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MICROBIOLOGICAL STATUS OF DIFFERENT AREAS OF TOOTH

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Summary. *A healthy oral cavity represents a complex microsystem changeable in a number and type of bacteria, fungi, viruses and protosoa. Bacterial flora is different on different areas of tooth. These areas were cllassified as open and closed ecosystems. The surfaces of enamel and exposed dentin and cementum, with dental plaque, could be considered as open tooth ecosystems. The root channel could be seemed as closed dentin ecosystem.*

The aim of this study was to isolate and identify prevalent microorganisms from:

– dental plaque (open ecosystem)

– infected root channel (closed ecosystem)

The investigated group included 30 adult patients with Periodontitis periapicalis. The most common type of periodontitis was Periodontitis periapicalis chronica granulomatosa (63%). Both, paque and channel swabs were taken, at the same time, from the same tooth. Swab were immediately disseminated, in a dentist's office, on the medium for anaerobic, microarephilic and aerobic cultivation.

Mediums for aerobic cultivation were incubated 20 hours in a thermostat at 37°C. Microaerophil and anaerobic condition were achieved by means of GAS-PAC bags (bio Merieux, Torlak).

Members of streptococcus species were identified by the folowing methods: API-STREPTO system and SLIDEX STREPTO-Kit (bio Merieux) and by folowing tests: optohin, bacitracin, CAMP. Yeastes of Candida species were identified after incubation of 7 days, at the temperature of 37°C from Sabourad base.

The polimorphic bacterial flora and Candida were isolated from plaque and channel swabs. The most common species of microbs in dental plaque were Streptococci spp. (nonhemolitics and viridans group). The most common species of microorganisms, in channel swabs, were Prevotella - Porphyromonas group or gram negativ bacills. Porphyromonas gingivalis had been found in 60% of channel swabs and in 20% of plaque swabs (statistical significans of $p < 0.05$).

In a closed ecosystem (root channel) Streptococci decreased whereas Lactobacilli increased.

Key words: *Teet, infection, bacteria, candida*

Introduction

A healthy oral cavity represents a complex micro system, changeable in a number and type of bacteria, fungi, viruses and protozoa, which can be found there as commensales or as a part of flora. In the oral cavity, $4{,}5\times10^7$ bacteria can be found (8). Almost all the types of bacteria found in the mouth have sufficient pathogenic potential to induce inflammatory processes on teeth and soft tissues.

Bacterial flora is different in different areas of teeth. These areas are classified by Edwardsson (10) as open and closed tooth ecosystems.

The exposed dentin surfaces could be considered as open dentin ecosystems. There are many physiological or pathological processes which can expose dentin to the oral cavity. Such processes are abrasion, attrition, erosion, cavity preparation, scaling and even tooth brushing. The exposed surfaces are rapidly colonized by microorganisms and covered by dental plaque (1). In this process, environment factors as saliva, diet, oral hygiene have certain access.

Other dentin areas are more closed. Those are: dentin enamel junction beneath white spot lesion, gaps between cavity walls and restoration, fissures, deep areas of penetrated caries, remaining carious dentin beneath restoration, root channels. Bacterial flora is different and changeable in open and closed tooth ecosystems.

The Aim

The aim of this study was to isolate and identify prevalent microorganisms from dental plaque - as open ecosystem, and from infected root channel - as closed ecosystem.

Material and Methods

The examination was performed at the Stomatological Clinic and Institute of Public Health in Niš; Stomatological and Clinical laboratory department of the Health Center in Aleksinac.

Plaque and channel swabs were taken from thirty adult patients with Periapical periodontitis.The most common type of periodontitis was Periodontitis periapicalis chronica granulomatosa (63%). Both, plaque and channel swabs were taken, at the same time, from the same tooth. Swab were immediately disseminated, in a dentist's office, on the medium for anaerobic, micro aero philic and aerobic cultivation. Solid mediums were disseminated with Miller needle, whereas liquid mediums were disseminated width chrome wire, which was sterilized together with liquid medium.

Table 1. Microorganisms in closed and open ecosystem.

	Occurence in infected root canal	Occurence in tooth plaque
Gram negative anaerobic bacills		
Prevotella/Porphyromonas groups	high 80%	
Porphyromonas gingivalis	high 60%	medium 20.9%
Prevotella intermedia	high 53.3%	low 16.2%
Bacteroides ovatus, fragilis, ruminocola	medium 33.3%	low 4.65%
Nonhemolitic and viridans streptococci		
Streptococcus salivarius, sanguis, cremoris, mutans, mitis	high 77%	high 95%
Anaerobic and microaerobic streptococci		
Peptostreptococcus spp Streptococcus intermedius Streptococcus constelans Streptococcus asaccharolyticus	high 73%	high 76%
Staphylococci		
Staphylococcus aureus, epidermidis, saprophyticus	medium 33.3%	medium 37%
Gram-positive anaerobic bacills		
Lactobacillus acidophylus, fermentum, minutus, odontolyticus	medium 30%	low 16.27%
Acinomycess spp		
Actinomycess viscosus, odontolyticus, naeslundi, israeli	medium 33.3%	low 13.95%
Neiseriae spp		
Neisseria mucosa, sicca, catarrhalis, flavescens, perflava	low 16.6%	
Candida spp	medium 26.6%	medium 25%

Mediums for aerobic cultivation were incubated 20 hours in a thermostat at 37°C. Microaerophil conditions were achieved by means of GAS-PAC bags for microaerophil cultivation (Torlak), and the incubation lasted 48 hours. Mediums for anaerobic cultivation were incubated 48 hours under anaerobic conditions achieved in pots for anaerobic cultivation by means of GAS-PAC bags for anaerobic incubation (bio Merieux). The identification of the isolated bacterial types was performed on the basis of microscopic, cultural, physiological - biochemical and antigenic characteristics (10).

Members of the *Streptococcus* species were identified by the following methods: API-STREPTO system (bioMerieux), SLIDEX STREPTO - Kit (bioMerieux) and by the following tests: optohin, bacitracin, CAMP. According to need, some additional examinations of biochemical and physiological characteristics were used.

Bacteria of the *Neisseria* species were identified on the basis of cultural characteristics, microscopic preparations, biochemical features, and growth capacity on a nutritious agar, growth capacity at the room temperature and oxidase test.

Gram negative bacteria from the *Enterobacteriacea* family were identified on the basis of cultural characteristics, microscopic preparations and physiological properties, the examination of which is performed in a regular laboratory work.

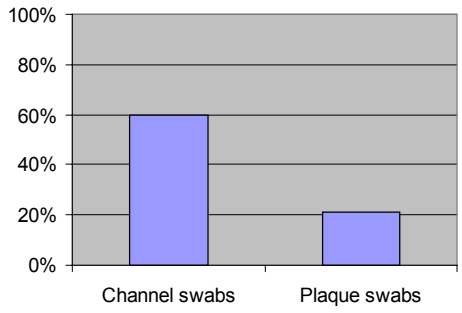
Anaerobic bacteria were identified on the basis of cultural characteristics (for example dark pigmentation of bacterial colonies of *Prevotella* and *Porphyromonas* species), microscopic properties, aerotolerance test, growth capacity on Schaedler agar with the addition of 20% gall, growth capacity on Schaedler agar with vancomycine. In a final identification, API 20 A (bioMerieux) was used.

Yeasts of the *Candida* species were identified after an incubation of 7 days, at the temperature of 37°C primarily from Sabourad base (with the addition of maltose and 0.8% chloramphenicol) on the basis of cultural, biochemical and microscopic characteristics.

Results

The anaerobic, microaerophilic and aerobic flora and Candida, were isolated from plaque and root channels swabs.

Gram negative bacills, *Prevotella-Porphyromonas* spp were the most common isolated bacterial types in channel swabs. They were found in 24 (80%) of samples, out of 30 examinated subjects.



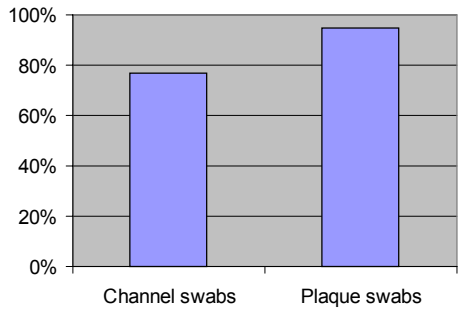
Graph 1. Porphyromonas gingivalis in channel swabs and plaque swabs.

The most common species of microorganism in channel swabs was *Porphyromonas gingivalis*. It had

been found in 60% of channel swabs and in 20% in plaque swabs. (statistical significans of $p<0.05$).

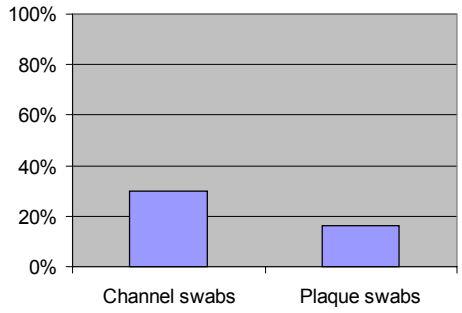
Streptococci spp were isolated from swabs of infected root channel and plaque, very often and seemed, very pathogenic. Gram postiv microaerophilic alpha hemolitic streptococci were found in, almost, identical frequency in both kind of swabs.

Nonhemolitic and viridans species of Streptococci were isolated in 77% from channel swabs and in 95% from plaque.



Graph 2. Nonhemolitic and viridans Streptococci in swabs of channel and plaque.

Lactobacillus spp were found in 30% of channel swabs and in 16.27% of plaque swabs.



Graph 3. Lactobacillus spp in swabs of channel and plaque.

Frequency of isolation of *Candida spp* was, almost, identical in both exominated ecosystem.

Discusion

The previous investigation were proved that the bacterial types observed on dentin and cementum surfaces were on the avarage comparable to that found in dental plaque on smooth enamel surfaces. The main bacterial types, isolated from dental plaque were among gram positive - *Streptococcus* and *Actinomyces*, among gram negative-*Veillonella, Neisseria* and *Bacteroides* (Edwardsson 1987) (10).

We also noted a high frequency of isolation of *Streptococci spp* in plaque (specialy, nonhemolitic and viridans groups).

During the development of dental caries, some groups of microorganisms increase in plaque, whereas the other decrease. During caries progress an increase was found of *Streptococcus mutans, Actinomyces, Lactobacillus* and Yeastis, but decrease of *Streptococcus sanguis* and *Veilonella* (Thylstrup et al, 1986) (9).

A microbiological observation of initial root caries lesions have shown less aciduric flora. *Streptococcus mutans* and *Lactobacilli* were not constantly present. The results obtained that *Actinomyces viscosus* strains play a crucial role in the pathogenesis of root surface caries. *Streptococcus mutans* (serotip d) had a very low affinity for root dentinal surfaces (Guggenheim, 1987) (10).

Microflora in gaps betwen cavity wals and restoration have showen dominance of cocci, in comparison to that of the rods and filaments. Streptococci dominated. *Actinomyces* and *Streptococcus mutans* were in low numbers, *Lactobacilli* were not found (Brannstrom,1987) (3).

The examination of bacterial flora of soft caries dentin and decalcified deep areas of dentin has revealed mainly gram positive cocci and rods, such as *Streptococcus spp* and *Actinomyces spp*. Both, in soft dentin and in deep areas, gram positive bacteria-*Streptococci, Actinomyces* and *Lactobacilli* dominated (Edwardsson, 1987) (10).

Our investigation have showen dominance of gram negative bacills in channels flora, whereas *Streptococci* dominated in plaque. The number of *Lactobacilli* was large in channal flora.

Gram negative anerobic bacilli are the usual inhabitans of oral cavity. Most of them can be found in supra, and subgingival plaque. The significant presence of them in infected root channels could be explained by comunication between oral environtment and root channels.

The common presence of *Candida spp* could be explained as disturbance of immunological system of patients.

Gomes et al (8), analysed channel flora in 70 patients with *Periodontitis periapicalis*. Statistical significant conection was noted between tootache and positive percusion and *Prevotella spp* (gram negativ bacils), $p<0.01$.

According to these findings and our investigation, it is obvious that gram negative bacilli play a very important role in periapical lesions. We could notice that it is difficult to determinate the predominant strain of microoganisms in root channel and plaque. Also, it is difficult to compare the results from other investigators because of using rather different methods and techniques for identification of microbs.

Conclusion

1. The polimorphic anaerobic, microaerophilic and aerobic flora and Candida were isolated from plaque and root channel swabs.
2. The most common species of microbs in supragingival plaque were nonhemolitic and viridans *Streptococci.*
3. The prevalent flora in infected root channels was anaerobic, gram negativ anerobic bacills.

4. The most common species of microorganisms in root channels were *Prevotella-Porphyromonas groups*.
5. In closed ecosystem (root channel) *Streptococci* decrease whereas *Lactobacilli* increase, in comparation with open ecosystem (dental plaque).

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MIKROBIOLOŠKI STATUS RAZLIČITIH POLJA ZUBA

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Kratak sadržaj. *Zdrava usna duplja je složeni mikro sistem, promenljiv u broju i tipu bakterija, gljivica, virusa i protozoa. Na zubnom organu se razlikuju otvoreni i zatvoreni eko sistemi ili polja zuba koje karakteriše različita bakterijska flora.*

Cilj ovog rad bio je utvrđivanje mikrobiologije a) zubnog plaka (otvoreni ekosistem) b) inficiranog kanala korena (zatvoreni ekosistem). Obrađeno je 30 briseva odraslih pacijenata sa dijagnozom periapikalnog parodontita. Najčešći tip parodontita bio je Periodontitis periapicalis chronica granulomatosa (63%). Od svake ispitivane osobe istovremeno su uzimani bris supragingivalnog plaka i kanala korena, sa istog zuba..

Brisevi su zasejavani u stomatološkoj ordinaciji, neposredno po uzimanju, prvo na podloge za anaerobnu a zatim na podloge za mikroaerofilnu i aerobnu kultivaciju. Podloge za aerobno kultivisanje inkubirane su u termostatu na 37°C, 20 časova. Mikro aerofilni i anaerobni uslovi postizani su pomoću GAS-PAC kesica (bioMerieux, Torlak). Pripadnici roda Streptococcus identifikovani su sledećim metodama: APO-STREPTO sitemom i SLIDEX STREPTO-KIT-om (bioMerieux) kao i testovima sa optohin-om, bacitracin-om, CAMP. Kvasnice iz roda Candida identifikovane su posle inkubacije od 7 dana, na temperaturi od 37°C, sa Sabourad podloge.

Iz briseva plaka i briseva kanala korena izolovana je polimorfna bakterijska flora.

Dobijeni rezultati ispitivanja briseva plaka pokazali su dominaciju Streptokoka (nehemolitičkih i viridans grupe) u suprafingivalnom plaku.

Rezultati ispitivanja briseva kanala korena istakli su najveću zastupljenost gram negativnih anaerobnih bacila, Prevotella - Porphyromonas grupe u inficiranom kanalu korena.

Razlika u zastupljenosti Pporphyromonas gingivalisa u brisevima kanala, u odnosu na briseve plaka, bila je statistički značajna za nivo značajnosti $p < 0,05$.

U zatvorenom ekosistemu (kanal korena) Streptokoke su bile u opadanju, dok se broj Laktobacila povećavao.

Ključne reči: *Zubi, infekcija, bakterije, kandida*