EVALUATION OF BACTERIAL PATTERNS AND THE PATHOGENICITY LEVEL IN THE ENDO-PERIODONTAL SYNDROME

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Abstract

The present study investigates the content of microbial flora in the infected canals and periodontal pockets of the teeth with the endo-periodontal syndrome, for establishing the frequency of the bacterial associations thus determined. Materials and method. The content of 25 radicular canals and of 20 periodontal sites, with pockets between 3-5 mm, taken over from 20 patients, clinically and radiographically diagnosed with the endo-periodontal syndrome, was evaluated by microbial analysis. Identification of the isolated bacterial species considered: the growing aspect of the colonies (colony morphology) and also the morpho-tinctorial aspect in some isolated colonies. Results and discussion. The 25 endodontic microbial samples contained cultivable microorganisms. The average number of CFU ml⁻¹ was 8x10⁴ per sample, while the number of species per canal varied between 4 and 7 (with a mean value of 5.1). The microorganisms from the periodontal pockets were all positive for the anaerobic flora. Out of their total number, 50 bacterial species were identified, 4 strains remaining unidentified. Conclusions. The results obtained show that the endodontic pathogenic agents do not occur randomly, being present, instead, in specific combinations, which may contribute to the manifestation of some clinical signs and symptoms. The diseases of the marginal periodontium are undoubtedly related to the existence of the Gram-negative microbial species present at subgingival level. The microbiological tests provide important data for a correct selection of the antibiotic treatment recommended by the antibiogram.

Keywords: endo-periodontal syndrome, microbial determinations

INTRODUCTION

As generally known, a strong interdependence exists between endodontium and parodontium, so that, when one of them is affected, a response may be noticed in the other (1-3). Actually, this interrelation occurs because the tooth and the parodontium represent a functional unit (4-6). A good functioning of a tooth depends on the health of the parodontium (7). A disease in this area may result from: extension of the pulp disease to the periodontal tissues (8-12), progression towards the apical area of the gingival inflammation, which may affect the cement, the ligament or the alveolar bone (13,14).

The first stage necessary for determining the microbial species and their interactions involves the study of their prevalence, simultaneously with their being isolated (15-17).

The scope of the study was to investigate the content of the microbial flora in the infected canals and periodontal pockets of the teeth with the endo-periodontal syndrome, and to determine the frequency of the bacterial associations found.

MATERIALS AND METHOD

The content of 25 radicular canals and of 20 periodontal sites, with 3-5 mm pockets, taken over from 20 patients clinically and radiographically diagnosed with endo-periodontal syndrome, was evaluated by microbial analysis. The conditions for their being included in the investigation were as follows:

- clinically healthy patients, having no anti-biotherapy six month prior to the analysis,
- asymptomatic teeth (negative vitality tests),
- no previous antibacterial treatment of the teeth under investigation, the radiographic images evidencing periapical – marginal bone loss.

Working algorithm

Following cleaning and perfect isolation of the tooth, the crown was disinfected and the pulp
room was opened with sterile burrs, then rinsed with reduced transport fluid, the absorption of the liquid being made with sterile cones. Five sterile cones made of absorbing paper were placed consecutively in the canal and left for 10 sec, then immersed into sterile tubes containing 1 ml R.C. broth regenerated through boiling. Study of the flora from the gingival ditch /the periodontal pockets (sampling had been made exclusively from the deepest periodontal pocket ± purulent secretion) made use of cones of sterile paper #20, maintained in the pocket for 10 -15 sec, then poured into tubes containing 0.5 ml medium transport soil. In the laboratory, the samples were incubated at 37ºC, under anaerobiosis, for 48 hr. When this time interval was over, 10 seriated dilutions of the samples were made, and 100 µl from each dilution from each tube were processed on Petri plates with Agar Wilkins-Chalgren with 5% human blood (25 ml/plate Ф 90 mm).

The plates were anaerobically incubated at 37ºC, for 7 days, after which the total number of colony-forming units (CFU) and the various microbial morphotypes were counted with a stereomicroscope (16x magnification).

**Identification of bacteria**

Bacteria identification is based on the study of the biochemical characters, through cultivation on a large number of tubes with special media (kit). The modifications produced in the medium may be directly observed, due to the chemical indices, being written down on a record permitting identification of the isolated bacterium. Identification of the isolated bacterial species considered:

- the growing aspect of the colonies (colony morphology): pigmentation, size, form,
- Morpho-tintorial aspect of the isolated colonies.

**RESULTS**

**A. Assessment of the endodontic flora**

The 25 endodontic microbial samples contained cultivable microorganisms. The average number of CFU ml⁻¹ was of 8x10⁴ per sample. The number of species per canal varied between 4 and 7 (average value: 5.1).

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Number of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fusobacterium spp</td>
<td>18</td>
</tr>
<tr>
<td><em>P. oralis</em></td>
<td>9</td>
</tr>
<tr>
<td><em>P. intermedia</em></td>
<td>19</td>
</tr>
<tr>
<td><em>P. buccae</em></td>
<td>7</td>
</tr>
<tr>
<td><em>P. melaninogenica</em></td>
<td>3</td>
</tr>
<tr>
<td><em>P. prevotii</em></td>
<td>5</td>
</tr>
<tr>
<td>Bacteroides spp</td>
<td>7</td>
</tr>
<tr>
<td>Capnocytophaga SPP</td>
<td>14</td>
</tr>
</tbody>
</table>

**Table I – Gram negative Bacilli species from the infected canals**

Out of the Gram negative bacilli, there were identified Fusobacterium spp, Prevotella spp (*P. oralis, P. intermedia, P. buccae, P. melaninogenica, P. prevotii*), as well as Bacteroides and Capnocytophaga SPP species (Fig 1).

<table>
<thead>
<tr>
<th>Microbial species</th>
<th>Number of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eubacterium spp</td>
<td>15</td>
</tr>
<tr>
<td>Actinomyces SPP</td>
<td>9</td>
</tr>
<tr>
<td>Bifidobacterium SPP</td>
<td>7</td>
</tr>
<tr>
<td>Propionibacterium spp</td>
<td>21</td>
</tr>
</tbody>
</table>
As to the Gram positive bacilli, the following types were isolated: *Eubacterium* spp, *Actinomyces* spp, *Bifidiobacterium* spp, *Propionibacterium* spp (Table II, fig. 2).

### Table III - Cocci - species from infected canals

<table>
<thead>
<tr>
<th>Microbial species</th>
<th>Number of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-Cocci Veillonella spp</td>
<td>7</td>
</tr>
<tr>
<td>G+Cocci Peptostreptococcus micros</td>
<td>25</td>
</tr>
<tr>
<td>Gemella spp</td>
<td>3</td>
</tr>
<tr>
<td>Staphylococcus spp</td>
<td>5</td>
</tr>
</tbody>
</table>

Out of the G- cocci there were isolated Veillonella spp while, out of the G+ cocci - Staphylococcus spp, *Peptostreptococcus micros* and Gemella spp (table III, fig 3).

### Table IV - Bacterial species from the infected canals

<table>
<thead>
<tr>
<th>Microbial species</th>
<th>Number of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-Bacilli</td>
<td>63</td>
</tr>
<tr>
<td>G- Bacilli</td>
<td>52</td>
</tr>
<tr>
<td>G+Cocci</td>
<td>33</td>
</tr>
<tr>
<td>G-Cocci</td>
<td>7</td>
</tr>
</tbody>
</table>

![Diagram](image-url)
As to the ratio of cocci, the highest value was recorded for *Peptostreptococus micros* (62%), followed by *Veillonella* (17%), *Staphylococcus* (13%) and Gemella spp (8%) (fig. 7).

![Figure 7](image-url)  
**Figure 7.** Ratio of Cocci G-/+ microbial species

### B. Identification of microorganisms from the periodontal species

Identification was based on the growing aspect of the colonies and also on their morpho-tintorial aspect. The 20 samples were all positive for the anaerobic flora.

54 bacterial strains were isolated: 2-3 strains/sample, out of which 50 bacterial species were identified, 4 strains remaining unidentified.

The anaerobic species isolated are: Gram-negative bacilli - 33 strains (66%), Gram-positive bacilli - 7 strains (14%), Gram-positive cocci - 6 strains (12%), 4 strains (8%) - non-identified (fig 8).

![Figure 8](image-url)  
**Figure 8.** Ratio of anaerobic species isolated from the periodontal pockets

The types of anaerobic bacterial species isolated are listed in Table VI.

<table>
<thead>
<tr>
<th>Gram+ Cocci</th>
<th>Gram- Bacilli</th>
<th>Gram+ Bacilli</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. asaharolyticus</em></td>
<td><em>P. endodontalis</em></td>
<td><em>Actinomyces spp.</em></td>
</tr>
<tr>
<td><em>Peptostreptococcus</em></td>
<td><em>P. asaharolytica</em></td>
<td><em>Clostridium perfringens</em></td>
</tr>
<tr>
<td><em>spp.</em></td>
<td><em>P. gingivalis</em></td>
<td><em>Clostridium spp.</em></td>
</tr>
<tr>
<td><em>P. magnus</em></td>
<td><em>Fusobacterium spp.</em></td>
<td><em>Eubacterium lentum</em></td>
</tr>
<tr>
<td><em>P. niger</em></td>
<td><em>Capnocytophaga spp.</em></td>
<td><em>Eubacterium lentum</em></td>
</tr>
<tr>
<td><em>Bacteroides spp.</em></td>
<td><em>B. capillosus</em></td>
<td><em>Eubacterium lentum</em></td>
</tr>
<tr>
<td><em>Clostridium spp.</em></td>
<td><em>Bacteroides spp.</em></td>
<td></td>
</tr>
<tr>
<td><em>P. niger</em></td>
<td><em>Porphyromonas spp.</em></td>
<td><em>Eubacterium spp.</em></td>
</tr>
<tr>
<td><em>Fusobacterium spp.</em></td>
<td><em>Fusionbacterium spp.</em></td>
<td></td>
</tr>
<tr>
<td><em>Porphyromonas spp.</em></td>
<td><em>Porphyromonas spp.</em></td>
<td></td>
</tr>
<tr>
<td><em>Prevotella spp.</em></td>
<td><em>Porphyromonas spp.</em></td>
<td></td>
</tr>
<tr>
<td><em>Fusobacterium spp.</em></td>
<td><em>Fusobacterium spp.</em></td>
<td></td>
</tr>
<tr>
<td><em>F. nucleatum</em></td>
<td><em>F. nucleatum</em></td>
<td></td>
</tr>
<tr>
<td><em>F. necrophorum</em></td>
<td><em>F. necrophorum</em></td>
<td></td>
</tr>
</tbody>
</table>

### DISCUSSION

In most cases, the bacterial invasion of the necrotic pulp causes periapical inflammation.

The usual way followed by bacteria in their invasion of the necrotic pulp involves the cariogenic process, even if, out of the large number of species present on the tooth surface and in the gingival sulcus, only a small part will be able to develop in the medium offered by the endodontic space (18). Even if deprived of pathogeneity, when occurring in the oral cavity, in the infected canal, such species play an important role in inducing inflammation and necrosis.

An accessory canal can be found anywhere along the root, thus creating a periodontic-endodontic communication and a possible penetration of the pulp, if the periodontal tissues lose their integrity. In the periodontal disease, development of a periodontal pocket may expose an accessory canal, thus permitting to the microorganisms or to their metabolic products to access the pulp. The presence of accessory canals leads to exchanges of inflammation products, degradation processes between pulp and the periodental tissues, thus influencing the result of endodontic therapy and maintaining a periodontal health condition (19,20).

Microorganisms and other irritating agents may reach the pulp through a progressive loss of periodontal attachment, when the periodontal disease destroys the supporting tissues, permitting...
exposure of the accessory canals or even of the auxiliary foramen (21).

The bacterial plaque, associated with the process of periodontal disease, may cause pulpititis and necrosis of the pulp tissue. Pulp exposure occurs without caries or traumatisms, yet with a considerable penetration of the irritating agents. Nevertheless, complications of this type are not frequent and, in most cases, the pulp remains vital and functional, even in teeth with advanced loss of the marginal periodontium.

Even if a clear-cut cause-effect relation has been established between the endodontic infection and the periapical lesions, penetration of bacteria in opposite direction (towards the pulp) during the periodontal disease is still under debate, as a controversial topic of the current investigation (22).

Theoretically, the lateral patent canals and the apical foramen adjacent to the periodontal pockets should facilitate the access of the oral microorganisms to the canalicular system of the root (23).

Grossman experimented on monkey gingiva the passage of the Serratia marcescens microorganism, subsequently discovered in the pulp tissue; other authors, too, reached the conclusion that the periodontal ligaments provide penetration ways of such microorganisms into the pulp tissue (24-26).

Even if bacteria do not invade the pulp in the usual way, through the canalules of the dentine not affected by the caries, inflammatory pulpar reactions will be nevertheless induced, through the permeabilization created by bacterial products (27).

Transitory bacteriemy was considered to be a possible way for bacteria penetration, through the hematogenic route, in the necrotic pulp. Several studies were carried out to find out whether the bacteria present in the blood might be attracted by the dental pulp during some traumatism or procedures causing inflammation, without inducing pulp exposure. Such attraction is known as anachoresis – classically described by Menkin.

The researches performed by Burke and Knighton, Smith and Tappe demonstrated that tooth traumatism might attract bacteria, in the absence of pulp exposure. Robinson and Dohing, Gier and Mitchell collected organisms injected in the non-exposed pulp of teeth to which pulpitites had been induced during the operative procedures. The injured pulp offers an excellent culture medium for continued microorganisms growth (28).

The hematogenic way of bacterial invasion (anachoresis) involves: a partially or totally injured pulp (necrosis); dissemination, in the sanguine flow, of bacteria capable of leaving behind the circulation to penetrate the injured pulp (29).

The bacteria may install themselves in any part of a necrotic or injured tissue. During bacteriemy (after dental extractions and even after dental brushing), the microorganisms may be attracted and localized in the inflammed or necrozed tissues, as actually demonstrated by Robinson, Bowling and Csernyiei on laboratory animals.

In spite of the presence of the granulation tissue and of the multiple colonies of microorganisms, the blood and the nerves may penetrate the lesion at a normal vascularization flow. The living conditions of the pathogenic microbial flora from the level of the radicular canal depend on 3 factors - the reduction potential, the sanguine flow, of bacteria capable of leaving behind the circulation to penetrate the injured pulp (29).

The occurrence of microorganisms in apical periendonditis, localized in the main canal, may be explained by such weak defense mechanisms, as well as by the presence of nutritive substances. Localization of bacteria in the lateral canals in the vicinity of the apex, at furcation level or in other regions of the root was not studied as a
separate topic; however, bacterial penetration of the lateral canals in apical periodontitis was demonstrated (30,31).

The bacteria from the main radicular canal may spread into the surrounding dentine through invasion of the dentinarian canalicules. Data on the extent and frequency of microbial invasion in the dentinarian canalicules from the apical canal are scarce. Equally poor is the information on the various bacterial species – being nevertheless known that the Gram+ species may invade the dentinarian tubules much more easily than the Gram- species (15,32).

Most of the endodontic microorganisms may be considered as opportunistic pathogenic agents that cause infection, following an environmental modification. After necrosis, the pulp tissue loses its defensive capacity and may be – at least theoretically – colonized by any microorganism (12,33).

Knowing that an important objective of the endodontic treatment is to eliminate bacteria from the canals of the teeth with necrotic pulp, it is equally logical and indispensable to check the treatment efficiency from this perspective (34,35). It goes without saying that determination of the presence, and identification of microorganisms in the infected canals and in the adjacent area may be extremely important, elimination of such microorganisms being essential in endodontic therapy.

Association of some microbial species in infections of the radicular canal is predisposed by the presence of nutrients and also by the reduced surface tension, which causes serious polymicrobial infections.

The manoeuvres in periodontal therapy may increase the inflammatory pulp response. At the same time, the pulp may be susceptible to the substances from the plaque whenever external resorption of the radicular wall occurs (36).

For establishing both the diagnosis and microbial susceptibility, the microbiological samples should be taken and cultivated using technologies permitting culture of anaerobic microbes and, optionally, of anaerobic ones, together with establishing the relative ratio of descendents present. The technology necessary for the cultivation and identification of anaerobic bacteria is new, requiring specialized training.

Development of a culture should lead to conclusive results, illustrating in a most precise way the microbial conditions in the canal.

The flora contaminating the necrotic pulp/the periodontal pocket includes only a few bacterial species, being by far less complex than the bacterial plaque, the anaerobic species prevailing, even if, occasionally, a large number of optionally anaerobic elements may also appear. Analysis of the biological products taken from the patients with endodontic-periodontal syndromes shows, in all cases, the presence of anaerobic flora, in which the Gram-negative bacilli are of majority.

**CONCLUSIONS**

- The results of the study show that the endodontic pathogenic agents do not occur randomly, being instead present in some specific combinations, which may contribute to the manifestation of some specific clinical symptoms and signs.
- The diseases of the marginal periodontium are undoubtedly related to the existence of the Gram-negative microbial species at subgingival level.
- The microbiological tests performed aimed at isolating and identifying the Gram-negative anaerobic bacterial species known as being involved in periodontal diseases.
- Equally, the microbiological tests provide significant data for a correct selection of the antibiotic treatment recommended by the antibiogram, cooperation with the microbiological laboratory being essential in such situations.

**References**


