

# The Microbiological Status of Patients with Periodontitis in Southern Estonia after Non-surgical Periodontal Therapy

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## SUMMARY

Our objective was to investigate the presence of periodontal pathogens in comparison with the total degree of microorganisms after non-surgical periodontal therapy. The study material consisted of microbiological samples from periodontal pockets originating from 140 consecutive patients with chronic generalized severe periodontitis. The subgingival samples from periodontal pockets were obtained by a sterile curette, placed into 2 ml of the VMGA III medium, homogenized and serially diluted in the Brucella broth. 100 µl aliquots from the dilutions were inoculated onto the Brucella and the TSBV agar. The plates were incubated in an anaerobic chamber and under microaerobic conditions. The isolates were identified according to colonial and cellular morphology, the potency disk pattern, and the biochemical profiles. After instrumentation, no periodontal pathogens were isolated in 46 (33%) patients, while 94 patients (67%) were infected with one to five different periodontal pathogens. However, higher degree of the total microflora was positively correlated with number of isolated pathogens, a putative indicator of their presence. Therefore, due to the occurrence of residual microorganisms after non-surgical mechanical treatment, information about the pattern of these pathogens is needed for application of antimicrobial therapy. The level of microbial load in gingival pockets, including both pathogenic and non-pathogenic species, is one of the determinants of presence of residual pathogens after non-surgical periodontal therapy.

**Key words:** invasive pathogens antimicrobial susceptibility

## INTRODUCTION

Periodontitis is a chronic infectious disease, which leads to the destruction of periodontal ligament fibres and alveolar bone until tooth loss [1]. Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans, Tannerella forsythensis, Fusobacterium nucleatum, Prevotella intermedia/nigrescens, Micromonas micros, and Campylobacter rectus have been suggested as periodontal pathogens [2,3]. Dominating periodontal pathogens may vary geographically and regionally [4]. However, in different populations the presence of the pathogens may reflect rather the microecology of the mouth; on the other hand, the presence of suspected pathogens may not be strictly related to the disease [5,6].

Oral hygiene instruction, scaling and root planing in local anaesthesia should constitute the basis for periodontal therapy [7,8]. Non-surgical periodontal therapy alone may fail to eliminate pathogens because of their location within the gingival tissues or in tooth structures inaccessible to periodontal instruments. Evidence of bacterial specification in periodontitis patients has led to the use of periodontal instrumentation combined with systemic antibiotic treatment for certain patients [9]. However, the choice of antibiotics depends on the spectrum of the main pathogens and their susceptibility patterns [10-12]. Beginning from 2001, patients with periodontitis from Southern Estonia have been investigated microbiologically after the mechanical debridement. Our objective was to investigate the presence of periodontal pathogens in comparison with the total level of microorganisms after non-surgical periodontal therapy.

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## MATERIALS AND METHODS

The study material was collected from January 2001 to April 2003 from 140 adult patients with chronic generalized severe periodontitis referred to the Policlinic of the Tartu University Dental Clinic. The diagnosis was defined by CPITN score 4 in at least three sextants (periodontal pocket

depth 6 mm, clinical attachment loss 5 mm) in at least two teeth and the minimum radiographic marginal alveolar bone loss 1/3 of the root length in at least three quadrants. The patients were systemically healthy and had not received antibiotics within three months prior to entering the investigation. Non-surgical periodontal treatment (scaling and root planing under local anaesthesia at 4 up to 6 appointments during 2-3 weeks) was performed and three weeks after completion of the treatment periodontal status was clinically evaluated by periodontal specialists. From patients with clinical signs of inflammation, such as bleeding and/or suppuration on probing, redness and swelling of gingivae, the subgingival samples from six deepest periodontal pockets were obtained by a sterile curette [3], placed into 2 ml of the VMGA III medium [13] and taken to the laboratory within 4 hours. The samples were homogenized with a Vortex mixer. The bacterial suspension was then serially diluted in 5-fold steps in the Brucella broth (Oxoid, Basingstoke, Hampshire, UK), 100 µl aliquots from the dilutions were inoculated onto the Brucella agar (Oxoid) enriched with 5% horse blood and 1% menadione, and the TSBV (Oxoid) agar. The Brucella Agar plates were incubated in an anaerobic chamber (Sheldon Manufacturing Inc., Cornelius, Oregon, USA; gas mixture: 5% CO<sub>2</sub>, 5% H<sub>2</sub>, 90% N<sub>2</sub>) and TSVB plates under microaerobic (Oxoid, CampyPak) conditions. After incubation at 35°C for 5 to 7 days, the isolates were identified according to colonial and cellular morphology, the potency disk pattern (Vancomycin, Kanamycin, Colistin, Brilliant Green, and Oxgall), catalase, oxidase and spot indole reactions, long-wave UV light fluorescence, and MUG assay [3,14]. All anaerobic microorganisms were tested for absence of growth under microaerobic conditions. The total level of microbial load was calculated as the logarithmic value of colony forming unit per 1mL (log<sub>10</sub> CFU/ml).

Ethics Review Committee (ERC) on Human Research of the University of Tartu approved our study protocol.

Statistical analysis (Descriptive Statistics and Spearman's correlation) was carried out with the

JandelSigmaStat 2.0 program, and the p-values less than 0.05 were considered statistically significant.

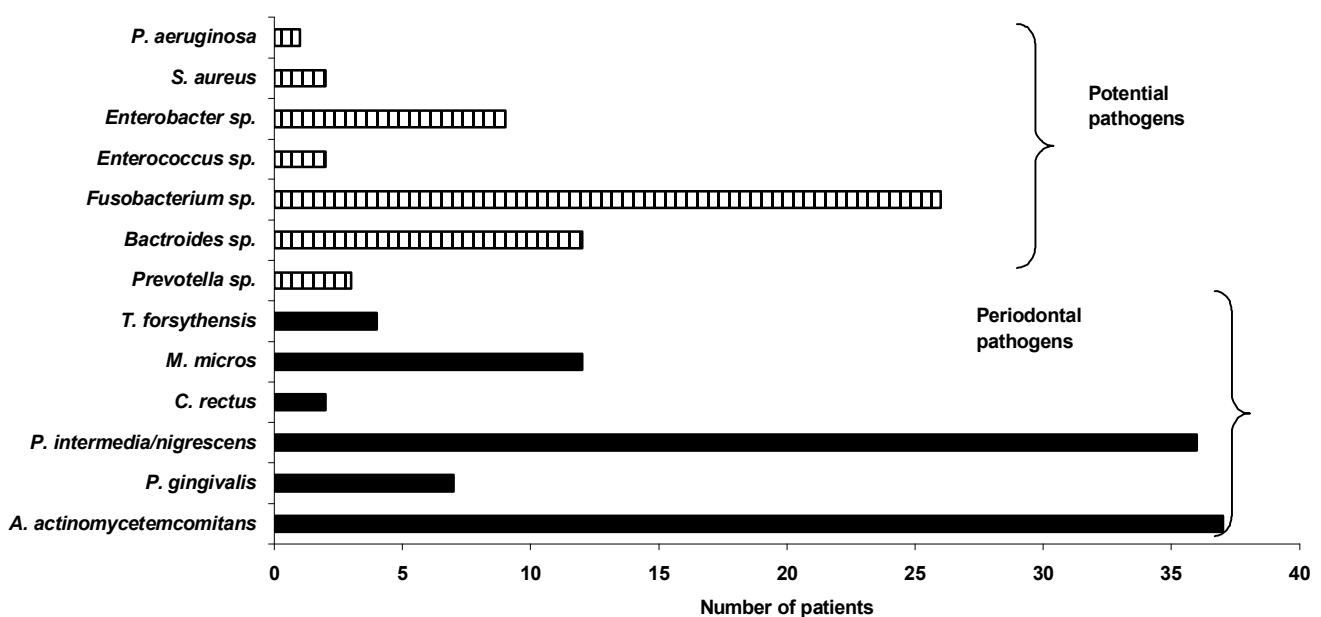
**RESULTS AND DISCUSSION**

After instrumentation, no periodontal pathogens were isolated in 46 (33%) patients, while 94 patients (67%) were infected with one to five different periodontal pathogens (53 patients harboured one, 27 harboured two, 12 harboured three and 2 patients harboured five pathogens).

The pathogens isolated from the periodontal pockets of Estonian patients with chronic generalized severe periodontitis (n=140) are depicted in Figure 1.

One third of the patients remained pathogen-free, in concordance with the results of other authors, where primary therapy reduced the amount of pathogens [7,8]. On the other hand, we found that 67% of the primarily treated patients were equally infected with mostly one pathogen, but also with combinations of different pathogens. However, scaling and root planing alone do not eliminate periodontal pathogens effectively [15]. Therefore, due to the occurrence of residual microorganisms after non-surgical mechanical therapy, information about the pattern of these pathogens is needed for application of antimicrobial therapy on patients who do not respond to mechanical treatment.

Periodontal microbes can be divided into different risk groups according to their association with periodontal disease, where the "red" group includes real pathogens and the "green" group comprises the normal oral microflora [16]. Among the well-known pathogens of periodontitis, some other potentially pathogenic bacteria, eg. *Fusobacterium* sp., *Bacteroides* sp., *Prevotella* sp., enterococci, enterobacteria, and others can play some role in different populations. Among the periodontal pathogens, *Prevotella intermedia/nigrescens* (37 patients) and *Actinobacillus actinomycetemcomitans* (36 patients) were dominating, *Micromonas micros* was detected in 12, *Porphyromonas gingivalis* in 7, *Tannerella forsythensis* in 4, and



**Fig. 1.** Specific and potential periodontal pathogens in Estonian patients with periodontitis (n=140) after non-surgical periodontal therapy

*Campylobacter rectus* in 2 patients. Proportional recovery of *Prevotella intermedia/nigrescens* varied from 2.3 to 63% (median 16.9), of *Actinobacillus actinomycetemcomitans* from 2.4 to 100% (median 23), of *Micromonas micros* from 3 to 44% (median 21.8), of *Porphyromonas gingivalis* from 9 to 65% (median 29), of *Tannerella forsythensis* from 6.3 to 20% (median 9.6), and of *Campylobacter rectus* from 4.5 to 33% (median 18.8). The spectrum of most the important gingival pathogens found in Estonian patients is similar to those reported in literature [2,3]. However, information about pre-treatment microbial ecology is requested for comparison of Estonian data with the findings of other studies.

The total level of microbial load (log<sub>10</sub> CFU/ml) of all isolated microbes varied from zero to 8.4 log, whereas sterile samples occurred rarely, four cases per 140. The median of colonization after treatment was 5.5 log (according to 3x10<sup>5</sup> CFU/ml), indicating the substantial effect of instrumentation in viable counts of microbes. Scaling and root planing alone reduces the numbers of microorganisms in the subgingival area, but due to the limitations of cultivation (detection limit 10<sup>3</sup>) there may occur false negative results. We found that higher microbial load was positively correlated ( $p < 0.001$ ) with number of residual pathogens. In the case of periodontitis with developed deep gingival pockets, the in-

creased amount of the microflora creates good possibilities for overgrowth of anaerobes, indicating also the presence of periodontal pathogens.

## CONCLUSIONS

Therefore, due to the occurrence of residual microorganisms after non-surgical mechanical treatment, information about the pattern of residual pathogens is needed for application of appropriate antimicrobial therapy for patients not responding to non-surgical treatment. The higher level of microbial load in gingival pockets, including both pathogenic and non-pathogenic species, may be one of the determinants of presence of residual pathogens after non-surgical mechanical therapy. As periodontitis is a chronic recurrent infection, successful diagnosis and therapy should be based on the individual microbiological examination for reasons including quality control of mechanical therapy as well as an aid of treatment planning.

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