

Anaerobe 2010

The 10th Biennial Congress of the
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BACTERIAL INTERFERENCE AND PROBIOTICS IN MAINTAINING HEALTH OF THE ORAL CAVITY

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The concept of supplementing the diet with certain microorganisms to promote health is well established for the gastrointestinal tract. This same concept has been applied to oral health by demonstrating that the regular introduction of select species of naturally occurring oral bacteria will promote oral health by reducing the number of pathogenic bacteria responsible for dental caries and periodontal disease. In a healthy periodontal site, certain viridans streptococci, notably *Streptococcus oralis* and *Streptococcus uberis* are commonly found in significant amounts, while the levels of periodontal pathogens including *Tannerella forsythensis*, *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Peptostreptococcus micros*, *Campylobacter rectus*, and *Prevotella melaninogenica* are usually quite low. The opposite situation prevails in disease sites where, in fact, *S. oralis* and *S. uberis* are usually undetectable. Hydrogen peroxide production by *S. oralis* and *S. uberis* is the basis for the dose dependent negative interaction with periodontal pathogens. Similarly, a strong negative bacterial interaction has been shown to occur between a naturally occurring lactic acid-deficient *Streptococcus rattus* strain and *Streptococcus mutans*. Twice daily administration of Probiora3®, a proprietary combination of *S. oralis*, *S. uberis*, and *S. rattus* strains, was shown in a pilot study to be safe and effective in reducing the levels of oral pathogenic bacteria.

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THE ORAL MICROBIAL “CANARY IN THE COAL MINE” OF HUMAN DISEASE

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Based on culture-dependent and culture-independent molecular methods using sequence analysis of 16S rRNA genes, there are approximately 700 predominant oral bacterial species, of which about 35% have not yet been cultivated in vitro. The Human Oral Microbe Identification Microarray, or HOMIM, is a high sample-throughput technology which allows the simultaneous detection of over 300 of these bacterial species, including not-yet-cultivated species. HOMIMs and other molecular methods have been used to identify the potential role of specific bacterial species or bacterial complexes in oral health and oral infectious diseases, such as periodontitis and caries. However, HOMIM has utility beyond determining bacterial associations with oral health status. Oral microbial profiles may also serve as potential biomarkers for systemic diseases, such as pancreatic cancer, Crohn's disease and heart disease. Consequently, specific bacterial profiles may be useful to determine those people at risk for oral and systemic diseases, e.g., a “danger” profile may indicate early signs of disease, hence the proverbial “canary in the coal mine” of human disease.

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ANAEROBIC CULTURE OF SEVERE EARLY CHILDHOOD CARIES

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Severe early childhood caries (S-ECC) is an aggressive dental infection that devastates the primary dentition of pre-school-aged children. While recent studies have characterized S-ECC using non-cultural molecular methods, this study determined the cultivable species associated with dental caries to provide strains for pathogenicity testing.

S-ECC and caries-free (CF) children (2-6 years) were recruited and plaque samples taken from primary molars. Samples were cultured anaerobically on blood (pH 7), and acid (pH 5) agars. Isolates were identified by comparing partial 16S rRNA sequences with taxa (species) in the Forsyth Human Oral Microbiome Database (www.homd.org).

Over 5,000 isolates were sequenced from 42 S-ECC children and 40 CF children. S-ECC children had an average of 12 carious teeth, and significantly higher gingivitis scores than CF children. Isolates were identified to 230 different taxa, many belonging to unnamed, including previously uncultivated, species. The greatest microbial diversity was isolated from blood agar, with higher detection frequencies of *Actinomyces*, *Selenomonas*, *Capnocytophaga*, *Prevotella*, *Campylobacter*, *Fusobacterium* and *Campylobacter* species than from acidic agar. The acid agar selected for mutans streptococci, *Lactobacillus*, *Scardovia* and *Veillonella* species. Species significantly associated with caries from blood agar included *S. mutans* ($p < 0.001$), *Scardovia wiggsiae* (HOT 195) ($p < 0.01$), *S. cristatus* ($p < 0.05$), *A. gerensceriae* ($p < 0.05$), *V. parvula* ($p < 0.05$), and *Porphyromonas catoniae* ($p < 0.05$). These caries-associated species were also detected more frequently in gingivitis, with additional higher detection frequencies for *F. nucleatum*, *P. nigrescens*, *P. denticola* and 2 unnamed *Prevotella* species. Detection frequencies from caries children for *S. mutans*, *S. wiggsiae*, *Parascardovia denticolens*, and *S. sobrinus* were higher from acid compared with blood agar.

We conclude that in young children anaerobic culture on blood agar detected a wide diversity of species, comparable to clonal analyses. The acid-tolerant microbiota, however, selected for putative caries pathogens. Our findings suggest that several species other than *S. mutans* may play a role in this aggressive caries infection.

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RHEUMATOID ARTHRITIS AND PERIODONTAL DISEASE: A LINK BY *PORPHYROMONAS GINGIVALIS*?

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Rheumatoid arthritis (RA) and periodontal disease are chronic inflammatory disorders characterized by dysregulation of the host inflammatory response. Increased secretion of proinflammatory mediators results in soft and hard tissue destruction. The etiology of both diseases is multifactorial, and they have risk factors in common like smoking and aging. In North America and Northern Europe, the incidence of RA is estimated at 20-50 cases per 100,000 population and the prevalence at 0.5-1.1%, whereas severe periodontitis occurs in 10%-15% of the adult population. *Porphyromonas gingivalis* is a strong bacterial marker for destructive periodontal disease in adults. *P. gingivalis* has direct access to the systemic circulation and endothelium by transient bacteremia. Protein citrullination has been identified as a primary focus of the RA-specific autoantibody response. Anti-Citrullinated Protein Antibodies (ACPAs) are specific serological markers for the majority of patients with RA, with a sensitivity of 67% (95% CI, 62% to 72%), and a specificity of 95% (CI, 94% to 97%). Citrullinated proteins are generated posttranslationally when arginine residues are deiminated by peptidylarginine deiminase (PAD). Citrullination is a common process in inflammation and apoptosis of largely unknown significance. Autoantibodies in RA recognize various naturally citrullinated proteins, including fibrin, vimentin, and filaggri. ACPAs are frequently present early in the disease process and often precede the clinical symptoms which strongly suggests that these antibodies are markers of the specific events that initiate autoimmunity in RA.

In recent years, it has become apparent that patients with RA and periodontitis share common pathogenetic characteristics, such as a proinflammatory traits. RA and periodontitis are both associated with the HLA SE alleles. Studies have shown that periodontitis is more common in RA, and that periodontal therapy reduces the severity of RA. Antibody titers to *P. gingivalis* are elevated in patients with RA. So far *P. gingivalis* is the only micro-organism described to express PAD, suggested to be a virulence agent. Sequence similarity and cross-reactivity with immunodominant epitopes of citrullinated proteins and their bacterial variants, and/or molecular mimicry of antibodies may indicate a role for bacterial infection, particularly with *P. gingivalis*, in priming autoimmunity in a subset of patients with RA.

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SMOKING AFFECTS SUBGINGIVAL BACTERIAL ACQUISITION AND COLONIZATION

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Introduction: Smokers have a higher incidence and severity of chronic periodontitis than non-smokers, with 42% of disease attributable to smoking. The role of bacteria in disease etiology is established, however; the effect of smoking on bacterial acquisition and colonization during biofilm formation is not elucidated.

Objective: The purpose of this study was to examine the effect of smoking on supragingival and subgingival bacterial colonization during early plaque formation using 16S cloning and sequencing for bacterial identification and enumeration.

Methods: 15 current and 15 non-smokers over 18 years of age with no history of systemic disease, current or planned pregnancy, recent or prophylactic antibiotic use were recruited. Following baseline evaluation and prophylaxis, stents were fabricated to protect 3 adjacent teeth in 2 quadrants during brushing. Clinical data, gingival crevicular fluid, supragingival and subgingival plaque samples were collected at day 0, 1, 2, 4, 7, 14 and 21. At each visit the patient was scaled, polished and flossed to ensure uninterrupted plaque formation for the next visit. Bacterial DNA was isolated from each sample; 16S rRNA genes were amplified by PCR, cloned into *E.coli*, and sequenced. 50 clones were sequenced from each sample and compared to sequences in GenBank for identification (18,000 total clones, over 27 million base pairs). Crevicular fluid levels were measured using a Periotron and the levels of 27 immune mediators were analyzed using a multiplexed bead-based assay. Cytokine levels were normalized for the amount of fluid collected. Within-subject and between-subject comparisons were made using Wilcoxon signed rank and Kruskal-Wallis tests respectively.

Results: The data was divided into groups based on clinical changes—Health (1 and 2 days), Transition (4 and 7 days), and Disease (14 and 21 days). There were no differences in the microbial profiles within these groups in either smokers or non-smokers. Smokers displayed a greater bacterial diversity than non-smokers at all time points ($p < 0.05$, Kruskal Wallis analysis). The bacterial population of smokers in health was dominated by species belonging to *Campylobacter*, *Hemophilus*, *Corynebacterium*, *Prevotella* and *Fusobacterium* while species belonging to *Veillonella*, *Streptococcus* and *Neisseria* formed 82% of the community in non-smokers. There were no significant differences in the supragingival microbial profiles of smokers and non-smokers at any time point. Smokers also demonstrated a significant increase in the levels of TNF- α , RANTES, IL-7, IL-2 and MCAF-1 on day 4 as compared to non-smokers.

Conclusions: Smoking significantly alters the composition of the subgingival biofilm beginning with early colonization. Starting with initial accumulation, there is an increase in bacterial diversity and levels of disease-associated bacteria, along with early increases in cytokine levels in smokers when compared to non-smokers.

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IN VITRO ANTIMICROBIAL ACTIVITY OF MANUKA HONEYS AGAINST ORAL BACTERIA

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Purpose: Honey has been used since ancient times and more recently, for the healing of wounds and against infectious diseases. The aim of our study was to investigate the effect of manuka honeys showing different potencies of their antibacterial activity, on potentially cariogenic bacteria in their planktonic and biofilm-embedded states.

Methods: Two Manuka honeys were tested, Manuka^① (30+ UMF) and Manuka^② (16+ UMF).

The Unique Manuka Factor (UMF) relates to the potency of the antibacterial activity of the honey. The UMF numbers derive from a standard laboratory test with 'Active' Manuka being compared with a standard antiseptic (phenol) to prove its potency. For example, a honey with a UMF[®] rating of 10+ would be equivalent to the antiseptic potency of 10% solution of phenol. From these tests UMF factors are shown to relate to the strength of an antiseptic solution.

The antimicrobial activity was examined by determining the MIC and MBC using the macro dilution broth technique. The effect on the adherence was tested on growing cells of *Streptococcus mutans* on a glass surface and on a multi-species biofilm model grown on saliva-coated hydroxyapatite discs.

Results: As expected, the antibacterial activity of Manuka^① (with higher potency of antibacterial activity) was the most important. The two tested honeys weakly inhibited the adherence of *S. mutans* cells to a glass surface at sub-MIC concentration.

Manuka^① showed a total inhibition of multi species biofilm at the concentration of 2000 µg/ml. Manuka^② inhibited biofilm formation weakly at the concentration of 2000 µg/ml but firmly at the concentration of 5000 µg/ml.

The results showed that Manuka honeys could reduce the growth and adherence of oral bacteria. These two honeys appear to be able to control dental biofilm deposit.

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FUSOBACTERIUM IDENTIFICATIONS IN PERIODONTAL DISEASE

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The genus *Fusobacterium* seems involved in many human diseases, including *Fusobacterium nucleatum* in oral diseases (endodontic infections and periodontal disease). However, identification of *Fusobacterium* species is difficult.

The aim of our study is to compare biochemical and molecular identification of *Fusobacterium* reference strains and wild strains from periodontal diseases.

We have taken a sample of subgingival plaque from 23 patients with different periodontal diseases: 10 aggressive periodontitis, 5 chronic and 5 in maintenance phase. After harvesting and culture, genus identification was achieved by observation of colony and Gram stain. Biochemical identification was performed according to several criteria: fermentation of carbohydrates, indole production, gas production, esculin hydrolysis, lipase activity, α and β galactosidase activities. This identification has been completed by a study of terminal metabolic acids by capillary electrophoresis.

The molecular identification was performed by extraction, sequencing of 16S rDNA, and construction of a phylogenetic tree. Identifications, biochemical and molecular, have been performed on 12 ATCC *Fusobacterium* reference strains and 37 wild strains from different periodontal disease.

For the reference strains, we showed that biochemical characteristics do not allow relevant identification to the species level, owing to their fickleness. But our results may provide new directions. The results for molecular identification agree with others studies.

Biochemically, among the 37 wild strains we find: 38% of *F. nucleatum*, 19% *F. naviforme*, 11% of *F. gonidiaformans*, 8% of *F. russii* and 5% of *F. periodonticum*; in 19% of biochemical identification, no result is found. We can observe that others *Fusobacterium* species than *F. nucleatum* are found in periodontal disease. Moreover, we note that *F. periodonticum* have the same biochemical characteristics as *F. nucleatum*. With molecular techniques, we describe 84% of *F. nucleatum*, 2,5% of *F. periodonticum*, 11% of uncultured bacterium and in 2,5% we find no identification result. Between the two identification methods, the concord is only 30%. The rDNA sequencing gives subspecies identification level in 19%. For more accurate results, new media, new biochemical characteristics are considered. The sequencing of another part of the genome is also considered. On a clinical level, it doesn't seem to be any correlation between periodontal disease and species identified.

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EFFECT OF CHITOSAN ON ANAEROBIC BACTERIA

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To assess the prospective use of chitosan to prevent and control oral diseases, its bacterial activity against characteristic oral bacteria was investigated. Five oral-related bacteria were used in this study: *Streptococcus mutans*, *Streptococcus mitis*, *Prevotella buccae*, *Aggregatibacter actinomycetemcomitans* and *Tannerella forsythia*. To determinate antibacterial activity the minimal inhibitory concentration (MIC) and minimal bactericide concentration (MBC) for each bacterium were investigated. In general low molecular weight chitosan showed activity at lower concentrations when compared with high molecular weight chitosan. The only exception was *T. forsythia*, which presented a MIC of 0,1% (v/v) and MBC of 0,5% (v/v) for high molecular weight against a MIC of 0,3% (v/v) and MBC of 0,7%(v/v) for low molecular weight. Chitosan of high and low molecular weight showed a significant antibacterial action against the studied bacteria and thus this results support the utilization of chitosan in oral disease prevention and control.

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DISTRIBUTION OF TETRACYCLINE AND MACROLIDE-LINCOSAMIDE-STREPTOGRAMIN RESISTANCE GENES IN ORAL MICROORGANISMS ISOLATED FROM PATIENTS UNDERGOING HEAD AND NECK RADIOTHERAPY

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This study evaluated the distribution of tetracycline and macrolide-lincosamide-streptogramin resistance genes in oral microorganisms isolated from 50 patients undergoing head and neck radiotherapy. In the tests, 250 isolates of *Enterobacteriaceae* and enterococci, as well as 320 isolates of genera *Actinomyces*, *Aggregatibacter*, *Campylobacter*, *Capnocytophaga*, *Eikenella*, *Eubacterium*, *Fusobacterium*, *Parvimonas*, *Porphyromonas*, *Prevotella*, *Propionibacterium* and *Veillonella* were submitted to susceptibility tests to tetracycline and erythromycin by means of an agar dilution method. Strict anaerobes and microaerophiles were tested on Wilkins-Chalgren agar supplemented with horse blood, while facultative microorganisms were tested on Mueller-Hinton agar, incubated in anaerobiosis and aerobiosis, at 37°C, for 48h. The DNA of resistant isolates were extracted and the presence of *tet(A)*, *tet(B)*, *tet(C)*, *tet(D)*, *tet(E)*, *tet(G)*, *tet(K)*, *tet(L)*, *tet(M)*, *tet(O)*, *tet(Q)*, *tet(S)*, and *tet(T)* tetracycline resistance genes, as well as *ermA*, *ermB*, *ermC*, *ermF*, *ermT*, and *ermX* macrolide resistance genes was detected by PCR. A total of 42 strict anaerobes and facultative anaerobes from oral microbiota were resistant to tetracycline and 31 were resistant to erythromycin. In addition, 78 isolates of *Enterobacteriaceae* and enterococci were resistant to tetracycline. The most frequently detected tetracycline resistance genes in enteric bacteria were *tet(A)* gene, observed in 18.7%, *tet(B)*, observed in 16.0%, *tet(K)*, detected in 12.0%, and *tet(M)*, observed in 18.7% of the resistant isolates, whereas in oral microorganisms *tet(M)* or *tet(Q)* were present in 60.% of the resistant isolates. The most common genes coding for proteins that confer resistance to macrolides were *ermB* and *ermF* in oral microorganisms, detected in 58.2% of the erythromycin resistant strains. The distribution of resistance genes was not influenced by radiation dosage nor the frequency or severity of side effects of radiotherapy.

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PREVALENCE OF PERIODONTAL PATHOGENS AND ENTERIC BACTERIA IN HIV-POSITIVE PATIENTS WITH GINGIVITIS OR PERIODONTITIS RECEIVING HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAAT) OR INITIATING TREATMENT FOR HIV

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In this study, the occurrence of pseudomonads, family *Enterobacteriaceae*, *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis* and *Tannerella forsythia* in HIV patients with gingivitis or chronic periodontitis was evaluated. One hundred HIV-positive patients were grouped into four categories: patients with gingivitis or chronic periodontitis; as well as receiving highly active antiretroviral therapy (HAAT) for at least five years or initiating treatment for HIV. The HIV-positive patients who were initiating antiretroviral therapy presented recently diagnosed HIV infections. One hundred HIV-negative patients presenting similar periodontal conditions took part in this study as control groups. After analyses of medical and dental histories, extra and intra oral, periodontal and radiographic examinations were performed. Bacterial isolation was carried out on selective and non-selective agar, incubated in anaerobiosis or aerobiosis at 37°C for 4 to 14 days. Bacterial identification was performed by both biochemical tests and PCR in addition to direct identification of pathogens in the clinical samples using real-time PCR with specific primers and probes. Prevalence and odds ratio analysis were performed using Cochran and Mantel-Haenszel statistics. Quantitative data were analyzed by ANOVA. The HIV-patients with chronic periodontitis displayed higher plaque index than those with gingivitis; periodontal attachment loss was particularly severe in HIV-positive patients initiating antiretroviral therapy. The occurrence and populations of *A. actinomycetemcomitans*, *F. nucleatum*, *P. gingivalis*, and *T. forsythia* were significantly higher in HIV-positive patients with chronic periodontitis in comparison to patients with gingivitis; HIV-positive patients initiating antiretroviral therapy showed a higher prevalence of these periodontal microorganisms and enteric bacteria, particularly *E. cloacae*, *Proteus* spp., and *P. aeruginosa*. In the biofilm of HIV-positive patients with gingivitis and receiving HAAT, the prevalence of these microorganisms was similar to that observed in HIV-negative patients with the same periodontal status.

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MICROBIOLOGY OF MUCOSITIS IN PATIENTS UNDERGOING HEAD AND NECK RADIOTHERAPY FOR CANCER TREATMENT

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The aim of this study was to evaluate the oral microbiota of patients undergoing radiotherapy (RT) for treatment of head and neck cancer. Fifty patients receiving RT were examined before, during RT, immediately after RT, and 30 days after RT. Clinical examinations were carried out in order to evaluate the presence and severity of radiation side effects. Saliva, mucosa, and biofilm samples from 50 patients (38 males, 12 females; aged 18-80, 59.5 ± 11.5 years) were collected and plated on fastidious anaerobe agar supplemented with hemin, menadione and horse blood for anaerobes; and on tryptic soy agar supplemented with yeast extract and horse blood for facultative bacteria and aerobes. Plates were incubated in anaerobiosis and aerobiosis, at 37°C during 14 and 3 days, respectively. The subgingival biofilm and saliva samples were also transferred to 300 µL ultrapure water and maintained at -196°C until DNA extraction. The quantitative detection of *A. actinomycetemcomitans*, *C. rectus*, *F. nucleatum*, *P. micra*, *P. gingivalis*, *P. intermedia*, *P. nigrescens*, *T. forsythia*, and *T. denticola* was carried out using real-time PCR by means of specific primers and probes. Microbial identification was performed by using commercial kits and biochemical tests. Prevalence and odds ratio analysis were performed using Cochran and Mantel-Haenszel statistics and interrelations between clinical and microbiological parameters were assessed using Mann-Whitney's test. Quantitative data were analyzed by ANOVA. After RT, mucositis and xerostomia were detected in 90% and 94% of the patients, respectively; severe mucositis, with necrotic and ulcerated areas in oral mucosa was observed in 58% of the patients. In these patients, the prevalence of family *Enterobacteriaceae* changed from 25% to 71.4% in the subgingival biofilm, the same was also observed in relation to *P. micra* (from 25% to 46.4%), *P. gingivalis* (from 50% to 82.1%), *P. intermedia* (from 46.4% to 71.4%) and *T. forsythia* (from 28.6% to 67.9%). In addition, the populations of these anaerobes represented 4.9% of total microbial load before RT and 19.1% after the conclusion of the treatment. The results reinforce the concept that Gram-negative anaerobes may be involved in the exacerbation of inflammatory reactions in radiation induced oral mucositis.

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MICROBIOTA ASSOCIATED WITH CHRONIC OSTEOMYELITIS OF THE JAWS

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Chronic osteomyelitis of the maxilla and mandible is rare in industrialized countries and its occurrence in developing countries is associated with trauma and surgery, and its microbial etiology has not been studied thoroughly. The aim of this investigation was to evaluate the microbiota associated with osteomyelitis of the mandible or maxilla from 22 Brazilian patients. After clinical and radiographic evaluation, samples of bone sequestra, purulent secretion, and biopsies of granulomatous tissues from twenty-two patients with chronic osteomyelitis of the mandible and maxilla were cultivated and submitted for pathogen detection by using a PCR method. Each patient harbored a single lesion. Bacterial isolation was performed on fastidious anaerobe agar supplemented with hemin, menadione and horse blood for anaerobes; and on tryptic soy agar supplemented with yeast extract and horse blood for facultative bacteria and aerobes. Plates were incubated in anaerobiosis and aerobiosis, at 37°C during 14 and 3 days, respectively. Bacteria were cultivated from twelve patient samples; and genera *Actinomyces*, *Fusobacterium*, *Parvimonas*, and *Staphylococcus* were the most frequent. By PCR, bacterial DNA was detected from sixteen patient samples. The results suggest that cases of chronic osteomyelitis of the jaws are usually mixed anaerobic infections, reinforcing the concept that osteomyelitis of the jaws are mainly related to microorganisms from the oral environment, and periapical and periodontal infections may act as predisposing factors.

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OCCURRENCE OF PERIODONTAL PATHOGENS IN TEN ETHNIC GROUPS FROM INDIAN RESERVATIONS IN WEST CENTRAL BRAZIL

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This study evaluated the occurrence of periodontal pathogens in the subgingival biofilm of 150 native Brazilians of ten ethnic groups living in the borders of Amazon forest. Periodontal examinations were carried out in accordance with the Periodontal Screening and Recording protocols and the visible plaque index was also recorded; 34 natives were periodontally healthy subjects, 58 presented chronic periodontitis and 58 showed gingivitis. Diet, general health, and genetic miscegenation were also evaluated. The subgingival biofilm was collected from the three most diseased periodontal site and transferred to 300 µL ultrapure water and maintained at -196°C until DNA extraction. Bacterial isolation was performed on fastidious anaerobe agar supplemented with hemin, menadione and horse blood for anaerobes; and on tryptic soy agar supplemented with yeast extract and horse blood for facultative bacteria and aerobes. Plates were incubated in anaerobiosis and aerobiosis, at 37°C during 14 and 3 days, respectively. The quantitative detection of a selected group of periodontal pathogens was carried out using real-time PCR by means of specific primers and probes. Prevalence and risk analysis were performed using Cochran and Mantel-Haenszel statistics and interrelations between clinical and microbiological parameters were assessed by Fisher exact test and Mann-Whitney's test. Presence of *E. corrodens*, *C. rectus*, *F. nucleatum* and *P. gingivalis* was associated with poor oral hygiene, while *F. nucleatum*, *T. forsythia*, *P. gingivalis*, *P. endodontalis*, *P. intermedia* and *P. gulae* were associated with gingival bleeding, while periodontal attachment loss was associated with *T. forsythia* and *P. gingivalis*. This study revealed that the microbiota observed in natives without significant genetic miscegenation and living based on hunting and fishing presents characteristics frequently associated with periodontopathies in non-natives. However, peculiarities were noted, including a modest occurrence of *T. denticola* and *A. actinomycetemcomitans* and high frequency of other microorganisms such as *C. rectus*, *T. forsythia*, *P. gingivalis*, *P. intermedia* and *P. nigrescens* in periodontitis subjects, while *P. gulae* was just detected in clinical specimens from natives living a traditional way of life based on hunting and fishing.

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OCCURRENCE OF 10 PERIODONTAL PATHOGENS IN PREGNANTS OF A PUBLIC HEALTH PROGRAM

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This study evaluated the selected periodontal pathogens from pregnant patients with different periodontal conditions. Initially, 86 women (mean age $24 \pm 11,5$ years) with pregnant age between 4th and 7th month. The pregnant women were submitted at pre-natal attendance in Health Basic Unities of Brazilian Health System (SUS), and subjected to periodontal examination and general health evaluation. These patients were grouped into 3 categories: 50 patients were periodontally healthy, 27 with gingivitis and 9 with chronic periodontitis. After clinical interview and clinical examinations, samples of subgingival biofilm were collected by mean of sterilized absorbent paper points and transferred to 300 μ L ultrapure water. DNA extraction was performed using QIAamp DNA Mini Kit. The detection of the periodontal pathogens was carried by PCR. Prevalence and odds ratio analysis were performed using Cochran and Mantel-Haenzel statistics and interrelations between clinical and microbiological parameters were assessed using Mann-Whitney's test. *A. actinomycetemcomitans* was detected in 12% of periodontally healthy women, while its occurrence in patients with gingivitis or periodontitis were 22%, and 33%, respectively. Other microorganisms were found in a higher frequency, such as *P. gingivalis* found in 14%, 22%, 66%; *T. forsythia* in 12%, 29%, 66% *P. intermedia*, 22%, 22%, 77% and *F. nucleatum*, the most prevalent, detected in 42% healthy subjects, 51% gingivitis patients and in 88% women with periodontitis. The prevalence of these anaerobes and microaerophiles was similar in gingivitis and periodontally healthy subjects, but significantly exacerbated in pregnant women with periodontitis.

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EVALUATION OF SUBINHIBITORY CONCENTRATIONS OF PENICILLIN G AND CHLORHEXIDINE ON HYDROPHOBICITY, HEMAGGLUTINATION, ADHERENCE AND ULTRASTRUCTURE OF *PREVOTELLA INTERMEDIA*, *PORPHYROMONAS ENDODONTALIS* AND *PORPHYROMONAS GINGIVALIS*

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Species of *Prevotella* and *Porphyromonas* are Gram-negative, nonmotile, black-pigment producers, strict anaerobes and they make part of the microbiota of the human and the animal oral cavity. These organisms are frequently involved in the etiology on mixed anaerobic infections, particularly periodontitis, endodontic infections and periimplantitis, as well as generalized sepsis in patients with immune impairment. In order to control these pathogens several strategies have been employed, specially chemical and mechanical control of dental biofilm. However, the residues of these antimicrobials may remain in the oral cavity for hours and may affect the expression of microbial virulence factors and ultrastructure. In this study, the effects of subinhibitory of penicillin G and chlorhexidine on hydrophobicity, hemagglutination, adhesion to the oral epithelial cells as well as on microbial ultrastructures were evaluated. In the tests, 33 isolates of *Prevotella intermedia*, 10 *Porphyromonas endodontalis* and 9 *P. gingivalis* isolates obtained from 25 patients with pulpal necrosis and endodontic infections. The hydrophobicity capacity varied significantly among the tested anaerobes and only 13 agglutinated human blood. The adhesion to oral epithelial cells was universal, but fimbriae were not detected using transmission electronic microscopys. Subinhibitory concentrations of penicillin G and chlorhexidine affected the microbial hydrophobicity and hemagglutination capacities, but the effects did not present a defined pattern in all tested strains. In the presence of the antimicrobials used, some isolates evidenced morphologic alterations, with loss of cytoplasmic components, producing spindle shaped cells and ghost cells, what affected the adhesion on human structures and cells. Grants: Fundunesp

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SPIRAMYCIN RESISTANCE IN HUMAN PERIODONTITIS MICROBIOTA

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Purpose: The occurrence of *in vitro* resistance to therapeutic concentrations of spiramycin, amoxicillin, and metronidazole was determined for putative bacterial pathogens isolated from 37 consecutive adults with untreated severe periodontitis in the United States, where spiramycin is not routinely available.

Methods: Subgingival plaque specimens were anaerobically cultured, with isolated putative pathogens identified to species level, and tested *in vitro* for susceptibility to spiramycin at 4 µg/ml, amoxicillin at 8 µg/ml, and metronidazole at 16 µg/ml (*van Winkelhoff et al. 2000*).

Results: Antibiotic-resistant putative pathogens were recovered from 18 (48.7%) subjects with 4 µg/ml of spiramycin, 23 (62.2%) subjects with 8 µg/ml of amoxicillin, and 10 (27.0%) subjects with 16 µg/ml of metronidazole. Spiramycin *in vitro* resistance occurred among *Fusobacterium nucleatum* (44.4% of organism-positive subjects), *Prevotella intermedia/nigrescens* (11.1%), *Parvimonas micra* (10.8%), *Streptococcus constellatus* (10%), *Streptococcus intermedius* (10%), *Porphyromonas gingivalis* (6.7%), and *Tannerella forsythia* (5.3%). Amoxicillin *in vitro* resistance was found in *P. intermedia/nigrescens* (55.5%), *T. forsythia* (15.8%), *S. constellatus* (10%), *F. nucleatum* (5.6%), and *P. micra* (2.7%), with only *S. constellatus* (70%) and *S. intermedius* (40%) exhibiting *in vitro* resistance to metronidazole. When subject-based *in vitro* resistance data for 4 µg/ml of spiramycin and 16 µg/ml of metronidazole were jointly considered, all isolated putative periodontal pathogens were inhibited *in vitro* by one or the other of the antibiotic concentrations, except for one strain each of *S. constellatus* and *S. intermedius* from one study subject. Similarly, either 8 µg/ml of amoxicillin or 16 µg/ml of metronidazole inhibited *in vitro* all recovered putative periodontal pathogens except one *S. constellatus* subject strain.

Conclusions: These findings indicate that *in vitro* spiramycin resistance in putative periodontal bacterial pathogens in the United States occurred in approximately one-half of severe periodontitis patients evaluated, particularly among subgingival *F. nucleatum* species. *In vitro* resistance patterns also suggest that therapeutic concentrations of spiramycin plus metronidazole may have potential antimicrobial efficacy in chronic (non-*Aggregatibacter actinomycetemcomitans*-associated) periodontitis similar to amoxicillin plus metronidazole, which may be beneficial for patients hypersensitive to beta-lactam antibiotics.

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TLR2 SENSING OF *F. NUCLEATUM* AND *S. SANGUINIS* DISTINCTLY TRIGGERED GINGIVAL INNATE RESPONSE

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Gingival tissue faces constant exposure to micro-organisms. It functions as part of the host response, an anti-microbial barrier that recognizes and discriminates between commensal and pathogenic bacteria. This study aimed to evaluate and compare the effects of different periodontal bacteria, commensals *Streptococcus sanguinis* and *Fusobacterium nucleatum* and the pathogen *Porphyromonas gingivalis*, on the innate immune response of gingival keratinocytes and the role of TLR2 in regulating this. The Japanese Cancer Research Resources Bank (JCRB) provided Ca9-22 cells. They were grown in Dulbeccos's modified Eagle's minimum essential medium (DMEM) supplemented with 10% FBS. Ca9-22 cells were transfected with the plasmid pUNO-hTLR2-HA (Invivogen) with jetPEI, following manufacturer's instructions. The medium was changed to medium supplemented with 7,5 µg/mL of blasticidine to select the transfected cells. The colonies were screened by real time quantitative PCR and flow cytometry and the best clone Ca9-22/TLE2 was amplified. These two immortalized keratinocytes cells lines were exposed to bacteria at different multiplicity of infection (50 to 300).

We assayed mRNA levels to determine the expression of human beta-defensins (hβD-2, hβD-3) and interleukin-1α and 8. We studied levels of IL-8 liberated in the medium by ELISA test. Results showed that *Streptococcus sanguinis* has not only any effect on beta-defensins induction but has any effect on IL-8 liberation too: *S. sanguinis* did not upregulate the host response. *F. nucleatum* induced beta-defensins and inflammatory marker mRNA expression at higher levels than *Streptococcus sanguinis*. Our results put in an obvious place a dose-effect for *Fusobacterium nucleatum*. Therefore, TLR2 receptor seems to be implicated in recognition of *Streptococcus sanguinis* and induction of beta-defensins: infection of Ca9-22/TLR2 cells by *Streptococcus sanguinis* induced expression of beta-defensins and we observed an effect-dose. Extinction of this receptor inhibited the upregulation of beta-defensin and cytokine transcripts by *F. nucleatum* but, in contrast, led to a weak induction of hβD-3 after challenge with *S. sanguinis*. Although *F. nucleatum* strongly induces innate immune and inflammatory mediators, *S. sanguinis* limits their expression through TLR2. Together, our data demonstrate that gingival keratinocytes recognize and discriminate between Gram-positive and Gram-negative commensal bacteria, in part through TLR2, to activate different signaling pathways of the innate immune host response. The aim of future studies will be working with native gingival keratinocytes and responses of cells in contact with an artificial biofilm composed of commensal and pathogenic bacteria.