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SESSION III—ORAL & PERIODONTAL INFECTIONS

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ORAL & PERIODONTAL INFECTIONS

A SYSTEMATIC APPROACH TO EXAMINE THE GENOMIC VARIATIONS AND PATHOGENIC MECHANISMS OF *AGGREGATIBACTER (ACTINOBACILLUS) ACTINOMYCETEMCOMITANS*

Chen, C.;*[1] Kittichotirat, W.;[2] Ngo, V.;[2] Adachi, A.;[1] Chen, W.;[1] Cheng, Y.;[1] Si, Y.;[1] Bumgarner, R.[2]
[1]University of Southern California, Division of Primary Oral Health Care, Los Angeles, CA USA
[2]Department of Microbiology, School of Medicine, University of Washington, Seattle, WA USA

Bacteria often exhibit significant strain-to-strain variations in genomes. Such variations were largely due to acquisition of DNA via horizontal gene transfer (HGT). Genomic islands (GEIs) are large DNA blocks acquired via HGT and may confer upon bacteria additional virulence factors.

Aggregatibacter (Actinobacillus) actinomycetemcomitans, a member of the Pasteurellaceae family, is a recognized pathogen in periodontitis and in extra-oral infections. Some *Aa* strains appeared to be more pathogenic than others. We postulated that *Aa* exhibits significant strain-to-strain genome variations that account for the differences in their virulence potentials. To test this hypothesis, we compared the genome contents of *Aa* clinical strains to identify and evaluate the functions of strain-specific GEIs.

The genome sequences of 2 *Aa* clinical strains (strains D11S and D7S) were determined by a massively parallel sequencing-by-synthesis approach (454 Life Science). The results were annotated and subject to insertion/deletion analysis. The genome structures were compared with a genome alignment tool MAUVE. Selected strain-specific GEIs were deleted to test their contributions to the fitness of *Aa* strains.

The genome of D11S (2,105,764 nucleotides) is similar in size to the known sequenced *Aa* strain HK1651. ~5% of the genome of D11S were strain-specific DNA blocks of >500 bp. This strain harbored a bacteriophage and two plasmids. Structurally the genome of D11S is largely colinear with HK1651 but contains several rearrangements relative to HK1651. The genome sequencing of D7S was near completion with 27 large contigs remained to be assembled. The genome (~2.3 Mb) exhibited significant insertions, deletions and genomic rearrangements relative to HK1651. Approximately 15% of the genome of D7S were >500 bp strain-specific DNA blocks. Selected GEIs of HK1651 and D7S were deleted with a markerless gene deletion protocol. The deletions did not affect the viability or the growth characteristics of the mutants under the laboratory growth conditions.

Acknowledgements. This work was funded by NIDCR grant R01 DE012212

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ORAL & PERIODONTAL INFECTIONS

STUDIES ON *FUSOBACTERIUM NUCLEATUM*

Kinder Haake, S.*
UCLA School of Dentistry, Los Angeles, CA USA

The Gram-negative anaerobe *Fusobacterium nucleatum* plays a key role in oral biofilm ecology. In addition, *F. nucleatum* is an opportunistic pathogen implicated in a wide range of oral and non-oral abscesses and infections. Considerable phenotypic and genotypic diversity is evident within the species, and several subspecies have been defined. Genomic analyses reveal that 25% of the genes identified in *F. nucleatum* ssp. *polymorphum* were not found in the other sequenced *F. nucleatum* strains, and many of these unique genes occur in islands of 5 genes or more. This level of genomic diversity and clustering of unique genes suggests that horizontal gene transfer may be important in the evolution of this species. Several native *F. nucleatum* plasmids, including pFN1, encode putative relaxase (mobilization) genes. Studies on the mobilization properties of pFN1 indicate that it confers a high level of mobilization in *E. coli* to *E. coli* matings, and that the relaxase gene is required for the mobilization properties. These findings are consistent with the hypothesis that native plasmids of *F. nucleatum* were acquired by conjugative mechanisms, and that this mechanism of gene transfer may have contributed to the xenologous origins of *F. nucleatum* genes. Native plasmids were used in the development of gene transfer to facilitate molecular analyses in *F. nucleatum*, enabling assessment of the contribution of specific genes and their corresponding proteins in the native host cell background. Transcriptional analysis of the *aim*1 outer membrane protein mutant demonstrated disruption of expression, and phenotypic analyses indicated a 40% decrease in the ability of the mutant to induce apoptosis in Jurkat cells as compared to the parental strain. Systematic inactivation of a class of large outer membrane protein genes has led to identification of additional genes contributing to interbacterial adherence and apoptosis, properties of importance in colonization and pathogenesis.

Supported in part by NIH/NIDCR PHS Grant No. DE15348 to SKH.

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ORAL & PERIODONTAL INFECTIONS

BACTERIAL DIVERSITY IN PERIODONTAL HEALTH AND DISEASE—A MOLECULAR VIEW

Kumar, P.S.*
The Ohio State University, Columbus, OH USA

Periodontitis is a polymicrobial infection that leads to destruction of the structures that anchor the tooth to the jawbone and results in eventual tooth loss. Epidemiological studies estimate that over four million Americans over 45 years of age suffer from this disease. Both bacterial plaque and the host's response to the bacterial challenge contribute to the disease process.

Bacteria begin to colonize a tooth soon after it erupts into the oral cavity and sequential bacterial colonization in the subgingival crevice (the gap that normally exists between teeth and gums) results in the formation of an organized, complex microbial community called the plaque biofilm. The bacterial etiology of chronic periodontitis has been studied for a number of years, and our understanding of disease pathogenesis has undergone many changes.

Traditionally, cultivation based methods or targeted molecular approaches have been used to identify and enumerate bacteria associated with periodontal health and disease. Current paradigms that implicate certain gram-negative bacteria in the etiology of periodontal disease were based on these methods. However, culturing is very selective, precluding the identification of fastidious organisms and species whose growth requirements are not known. Neither cultivation nor microscopy provides accurate bacterial identification, since they employ phenotypic characteristics for bacterial identification. Targeted molecular approaches examine the presence of selected bacterial species. Since these methods require prior knowledge of the bacterial species found in a health or disease associated community, they are not designed to provide a complete picture of a complex microbial community with several unknown species.

Open-ended approaches such as 16S rDNA cloning and sequencing make no assumptions on the composition of a microbial community and can therefore identify previously unknown and unsuspected species in a sample. Recently, the microbial constituents of many naturally occurring biosystems have been studied using this approach. This approach circumvents the selective bias introduced by culturing and so can identify all bacteria in a sample, including previously uncultivated ones. The 16S sequence information offers an accurate method of bacterial identification. Recent studies using this approach have shown that the subgingival microbial community is extremely diverse with over 300 species, many of which are uncultivated suggesting that traditional, cultivation-based approaches have provided an incomplete picture of the bacterial profile in periodontal health and disease.

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A POSSIBLE ROLE OF INTERSPECIES HYDROGEN-TRANSFER IN PERIODONTITIS ASSOCIATED BIOFILM

Horz, H.P.;[1] Vianna, M.E.;[1,2] Holtgraewe, S;[1] Conrads, G.*[1]
[1]Division of Oral Microbiology and Immunology, Department of Operative and Preventive Dentistry & Periodontology, and Department of Medical Microbiology, RWTH University Hospital Aachen, Germany
[2]Endodontic Area, Department of Restorative Dentistry, Piracicaba Dental School, State University of Campinas, Piracicaba, SP Brazil

Periodontal disease is a polymicrobial anaerobic infection characterized by a complex and dynamic subgingival bacterial biofilm (plaque) that may produce hydrogen (H_2) as one key intermediate during fermentative degradation of organic matter.

Purpose: In order to assess whether H_2-consumption could be a driving force of periodontitis, we investigated the prevalence, distribution, and proportion of three hydrogenotrophic groups (methanogenic archaea, dissimilatory sulfate-reducers, and acetogenic bacteria) in plaque samples obtained from 102 patients and 65 healthy control individuals.

Results: Based on genes encoding for key enzymes involved in H_2- metabolism all hydrogenotrophic groups were found in different constellations in 95% of patient samples, but only one single group, acetogens, also in 64.6% of healthy individuals. Within the subset of periodontitis patients, in 40% of cases only one and in 60% combinations of two or even all three hydrogenotrophic groups were detected. Furthermore, methanogens and sulfate-reducers were present in significantly higher proportions in samples from severe cases compared to moderate cases. In contrast, the proportion of acetogens was significantly lower in the severe cases. The relative population size of each hydrogenotrophic group was significantly elevated when one of the other two functional microbial guilds was not present.

Conclusions: The data indicate negative interactions among hydrogenotrophic groups presumably competing for H_2 as their common substrate with the result of complete niche exclusion in roughly half of the cases. The association of methanogens and sulfate-reducers (of which *Methanobrevibacter oralis* and a *Desulfovibrio fairfieldensis*-like phylotype were identified as predominant organisms) with progression of periodontal disease shows that both groups may serve as alternative syntrophic partners to support growth and activity of secondary fermenting periodontal pathogens.

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IDENTIFICATION OF MICROORGANISMS ASSOCIATED WITH POST-TREATMENT DISEASE

Delboni, M.G.;* Zaia, A.A.; Ferraz, C.C.R.; Souza-Filho, F.J.; Almeida, J.F.A.; Gomes, B.P.F.A.
Department of Restorative Dentistry, Endodontic Division, Dental School of Piracicaba, State University of Campinas, UNICAMP, Piracicaba, SP Brazil

The aim of this study was to detect the presence of *Enterococcus* spp., *Staphylococcus* spp., *Candida* spp. and enterobacteria in saliva and to identify the microorganisms around the coronal restoration and in the canals of 30 root filled teeth with periradicular pathosis. Transport media, specific culture media and adequate gaseous requirements were used to isolate as many strict and facultative anaerobes as possible. Selected media were used to isolate *Enterococcus* spp., enterobacteria and yeasts. From the 114 species of microorganisms isolated from the root canals, 18.5% were strict anaerobes, 81.5% were facultatives, 86% were Gram-positive and 14% Gram-negative bacteria. The most frequently recovered bacterial genera from the root canals were: *Staphylococcus* (15/50%), *Streptococcus* (11/36.7%), *Actinomyces* (12/40%), *Enterococcus*, *Gemella* (8/26.7%), *Propionibacterium*, *Clostridium* (4/13.3%), *Peptostreptococcus*, *Bifidobacterium*, *Eubacterium* (3/10%), *Lactobacillus*, *Prevotella* (2/6.7%) and *Candida* (1/3.3%). *Enterococcus* spp., *Staphylococcus* spp., *Candida* spp. and enterobacteria were isolated in 26.7%, 50%, 3.3% and 16.7% of the canals, in 20%, 40%, 6.7% & 13.3% of the crown and in 43.3%, 56.7%, 50% and 26.7% of saliva, respectively. In conclusion, strict anaerobes are present in small percentage of root-filled canals with periapical lesions when traditional techniques are used. *Enterococcus* spp., *Staphylococcus* spp., *Candida* spp. and enterobacteria could be detected in the saliva, crowns and the root canals of the same patients.

This work was supported by the Brazilian agencies FAPESP (04/11863-0, 05/51653-8) and CNPq (305437/2006-2, 470820/2006-3).

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MICROBIOLOGICAL STATUS OF PERIODONTAL AND PULPAL DISEASES IN LAGOS, NIGERIA

Egwari, L.O.;*[1] Obisesan, B.;[2] Nwokoye, N.N.[1]
[1]Department of Botany and Microbiology, University of Lagos, Akoka, Lagos, Nigeria
[2]Department of Oral and Maxillofacial Surgery, Lagos University Teaching Hospital, Idi-Araba, Lagos, Nigeria

Gingivitis and periodontitis account for between 21-25% of odontogenic diseases among Nigerians. Pulpitis, dentoalveolar abscess and pericoronitis each accounts for less than 5%. The dynamic nature of odontogenic infections made it imperative to study the epidemiology of odontogenic diseases and determine the microbial relationship existing between the different entities. The clinical and microbiological features of 217 patients with odontogenic diseases were analyzed. The advantage of routine antibiotic susceptibility testing for odontogenic pathogens in patient's management was investigated. The incidence of odontogenic diseases were more in female than male with a ratio of 0.60, though the difference was not significant. Age was a determinant factor in susceptibility to different odontogenic conditions with gingivitis occurring more in young adults (20-39 years), while periodontitis and pulpitis were prevalent in older adults (≥40 years). Polybacterial etiology characterized the three clinical conditions; aerobes were the predominant flora in gingivitis with preponderance of *Streptococcus* spp., while anaerobes predominate in periodontitis and pulpitis with species of *Porphyromonas, Prevotella, Fusobacterium* and *Actinobacillus*. The proportion and type of bacteria in periodontitis and pulpitis of patients with both conditions co-existing suggest that both entities derived their microflora independently. Significant reduction in duration of treatment was obtained when patients were treated based on susceptibility results as opposed to empirical knowledge ($p < 0.05$). The diversity of microbial etiology of odontogenic infections may put much demand on routine laboratory investigation for patient management, but the benefit of such practice should be considered in line with widespread of antibiotic resistant bacteria.

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PERIODONTAL CONDITIONS AND PERIODONTAL MICROBIOTA IN NATIVE BRAZILIANS

Gaetti-Jardim, Jr., E.;*[1] Vieira, E.M.M.;[1] Avila-Campos, M.J.[2]
[1]Department of Pathology, Araçatuba School of Dentistry, São Paulo State University, São Paulo, SP Brazil
[2]Department of Microbiology, Institute of Biomedical Science, University of São Paulo, São Paulo, SP Brazil

The aim of this study was to evaluate the periodontal conditions and the occurrence of periodontopathogens in Indians from six ethnic groups from the central region of Brazil. Subgingival samples of 100 native Brazilians were collected and DNA was obtained. Qualitative and quantitative detection of periodontopathogens were performed by using conventional PCR and RT-PCR. Patients evidenced high scores of plaque index, gingival index, and bleeding on probing. Loss of attachment was generalized, but the advanced periodontitis was confined to 12% of the individuals. In 40 patients with periodontitis, *Campylobacter rectus* (72.5%), *Eikenella corrodens* (85%), *Fusobacterium nucleatum* (77.5%), *Porphyromonas endodontalis* (52.5%), *P. gingivalis* (47.5%), *Prevotella intermedia* (72.5%) and *Tannerella forsythia* (52.5%) were the most found. In patients with gingivitis, *E. corrodens*, *F. nucleatum* and *P. intermedia* were more detected. *Porphyromonas gulae* was found in 27% individuals who use monkey meat in diet. *Aggregatibacter actinomycetemcomitans*, *Dialister pneumosintes*, *Prevotella nigrescens*, and *Treponema denticola* were rarely detected. Our results show a peculiar microbiota, particularly with high levels of *F. nucleatum* and black-pigmented anaerobes.

Grants FAPESP 07/51016-3.

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ASSESSMENT OF CULTIVABLE MICROBIOTA IN PRIMARY ENDODONTIC INFECTION AND THEIR DRUG SUSCEPTIBILITY

Martinho, F.C.;* Zaia, A.A.; Ferraz, C.C.R.; Souza-Filho, F.J.; Almeida, J.F.A.; Gomes, B.P.F.A.
Department of Restorative Dentistry, Endodontic Division, Piracicaba Dental School, State University of Campinas – UNICAMP, Piracicaba, SP Brazil

The purpose of the present study was to assess the cultivable microbiota in primary endodontic infection, correlating their presence with clinical signs and symptoms and to determine, *in vitro*, the antimicrobial susceptibility rates of the anaerobic species most frequently recovered from the root canals. Thirty root canals were sampled and microbiologically analyzed. Anaerobic culture techniques were used to isolate and identify microorganisms. Amoxicillin, amoxicillin + clavulanate, azythromycin, benzylpenicillin, clindamycin, cefaclor, erythromycin, and metronidazole were tested against the strains of *Parvimonas micra, Anaerococcus prevotii, Prevotella intermedia, Fusobacterium nucleatum,* and *Porphyromonas gingivalis* by E-test. A total of 182 cultivable isolates were recovered from the root canals. Obligate or microphilic anaerobes accounted for 65.38% of the total species isolated. "Black pigmented bacteria" (BPB) were recovered in 63.3% of the root canals. *Parvimonas micra* (63.3%) and *P. intermedia* (50%) were the two species most frequently isolated. Suggested relationships were found between: *Eggerthella lenta* and tenderness to percussion / pain on palpation, *G. haemolysans* and pain on palpation, *P. micra* and sinus, *Bifidobacterium* and purulent exudates. Amoxicillin, the first line antibiotic for endodontic infection, provided coverage against 87.5% of all strains tested. *P. micra* and *P. intermedia* were susceptible in 91.66% and 80% respectively to amoxicillin. Amoxicillin + clavulanate provided coverage against 100% of all strains tested, even against amoxicillin-resistant strains. In conclusion, a potential complex interaction among cultivable bacteria takes place in the establishment of the root canal microbiota in primary endodontic infections, resulting in characteristic clinical pictures. Even though amoxicillin still exhibits high antimicrobial activity against most of the oral anaerobic species involved in root canal infections, resistance among these species, particularly *P. intermedia*, has increased over the last years. Therefore the need of monitoring its susceptibility patterns must be considered, in order to change, if necessary, the first line antimicrobial agent. In this study, amoxicillin + clavulanate was a potent antimicrobial agent against endodontic pathogens.

This work was supported by the Brazilian agencies FAPESP (04/12436-9, 05/53729-1, 05/53729-1) & CNPq (304282/03-0, 305437/2006-2).

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PREVALENCE OF *HELICOBACTER PYLORI* AND ITS ASSOCIATION WITH ORAL HYGIENE, IRAN, 2007

Nasrolahei, M.; Sharif, M.; Daryani, A.
Mazandaran University of Medical Sciences, Sari, Iran

Background. The role of *Helicobacter pylori* in gastrointestinal disease such as peptic ulcer and gastric cancer is well known. Dental plaque has been considered as a potential reservoir of gastric infection and reinfection. The aim of this study was to determine the prevalence of *Helicobacter pylori* in dental plaque and gastric mucosa in Sari township population and to investigate its association with oral hygiene.

Methods. Two hundred and sixty individuals of rural and urban population of Sari township who had a positive *H. pylori* serology or positive rapid urease test or histologic evidence for the presence of *H. pylori* in their gastric mucosa, were enrolled in the study. These patients were divided into 3 groups determined by Oral Hygiene Index (OHI), scores of good, fair, and poor. Dental plaque was analyzed by a PCR for a specific internal urease A gene. Data were analyzed by using SPSS 11.5 and descriptive and analytical tests.

Results. *H. pylori* was detected in stomach of 57.8% of tested individuals. There was a high prevalence of *H. pylori* (46%) in the dental plaque of individuals with gastric *H. pylori* infection. *H. pylori* positivity in dental plaque was correlated with OHI scores; the positivity was 10.8%, 25% and 64.1% in individuals with good, fair, and poor OHI scores respectively.

Conclusion. The demonstration of the organism in the mouths of a substantial proportion of gastric patients has major implications for the spread of *H. pylori*. However, if the oral cavity is a reservoir for gastric infection, this should be sufficient to warrant a preventive approach that encompasses consideration of the oral reservoir.

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MICROBIOLOGY OF CHRONIC OSTEOMYELITIS IN A BRAZILIAN POPULATION WITH POOR ORAL HEALTH

Okamoto, A.C.;*[1] Avila-Campos, M.J.;[2] Gaetti-Jardim, Jr., E.*[1]
[1]Department of Pathology, Araçatuba School of Dentistry, São Paulo State University, São Paulo, SP Brazil
[2]Department of Microbiology, Institute of Biomedical Science, University of São Paulo, São Paulo, SP Brazil

This study evaluated the microbiota associated to 18 cases of osteomyelitis of the jaws in a Brazilian population with history of poor oral health. After clinical interview, clinical and radiographic examinations, samples of secretions, bone sequestration, and biopsied tissue were collected, cultivated, and submitted to detection of the main oral pathogens by PCR and by RT-PCR. Microbial isolation was performed on fastidious anaerobe agar (FAA) supplemented with hemin, menadione, yeast extract, and horse blood, and on tryptic soy agar (TSA) supplemented with horse blood. The plates containing FAA were incubated at 37ºC in anaerobiosis (90% N_2 + 10% CO_2) during 3, 7 and 21 days, whereas those containing TSA were incubated at 37ºC in aerobiosis during 48 hours. The isolates were identified by commercial kits. DNA extraction was performed using QIAamp DNA Mini Kit and QIAamp Tissue kit. Septic content was cultured in 55.55% of the clinical samples, while PCR and RT-PCR were able to detect bacteria in 77.78% and 83.33% of the samples, respectively. Most clinical samples presented at least 3 bacterial species (61.11%). Regardless of the employed detection method, most microbial genera observed in the patients were originated from dental biofilm, mainly *Actinomyces, Campylobacter, Fusobacterium, Peptostreptococcus, Porphyromonas* and *Streptococcus*.

Grants FAPESP 07/51016-3

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PERIODONTAL MICROBIOTA IN PATIENTS RECEIVING RADIOTHERAPY

Souza, F.R.N.;*[1] Schweitzer, C.M.;[2] Avila-Campos, M.J.;[3] Gaetti-Jardim, Jr., E.;[1]
[1]Department of Pathology, Araçatuba School of Dentistry, São Paulo State University, São Paulo, SP Brazil
[2]Cognition, Computing and Mathematics Center, Federal University of ABC
[3]Department of Microbiology, Institute of Biomedical Science, University of São Paulo, São Paulo, SP Brazil

This study evaluated the occurrence of periodontopathogens in 44 patients, receiving radiotherapy for treatment of malignant head and neck tumors. Oral and periodontal evaluations were performed immediately before radiotherapy, during treatment, and three months after the end of radiotherapy. Supragingival, subgingival and saliva samples were collected and DNA was obtained. Qualitative and quantitative detection of periodontopathogens were performed by using conventional PCR and RT-PCR. Patients evidenced several degrees of mucositis, xerostomia, and periodontal involvement. *Campylobacter rectus* (38.64%), *Eikenella corrodens* (72.72%), *Fusobacterium nucleatum* (65.91%), *P. gingivalis* (47.73%), and *Tannerella forsythia* (57.5%) were the most found. In patients with severe mucositis and periodontitis, *P. gingivalis* was the most frequently detected anaerobe. *Aggregatibacter actinomycetemcomitans* and *Treponema denticola* were rarely detected. The results suggest a relationship between the development of a peculiar microbiota and the severity of oral mucositis and periodontal disease.

Grants FAPESP 07/54851-0.

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CLONAL ANALYSIS OF THE MICROFLORA OF INFECTED ROOT CANALS ASSOCIATED WITH ENDODONTIC ABSCESSES

Jacinto, R.C.;*[1] Shah, H.N.;[2] Gomes, B.P.F.A.[1]
[1]Department of Restorative Dentistry, Endodontic Division, Dental School of Piracicaba, State University of Campinas, UNICAMP, Piracicaba, SP Brazil
[2]NCTC – Molecular Identification Service Unit, Health Protection Agency, Centre for Infections, London, UK

The aim of this study was to examine the diversity of bacterial species in infected root canals of teeth associated with endodontic abscesses by cloning and sequencing techniques. Samples collected from five infected root canals were subjected to PCR with universal 16S rDNA primers. Products of these PCRs were cloned and sequenced. All samples were positive for the presence of bacteria and a range of 7-13 different bacteria were found per root canal sample. A total of 48 different oral clones were detected among the five root canal samples. *Olsenella profusa* was the only species present in all samples. *Porphyromonas gingivalis, Dialister pneumosintes, Dialister invisus, Lachnospiraceae* oral clone, *Staphylococcus aureus, Pseudoramibacter alactolyticus, Parvimonas micra* and *Enterococcus faecalis* were found in 2 of 5 samples. Majority of the taxa were present in only one sample, for example *Tannerella forsythia, Shuttleworthia satelles* and *Filifactor alocis*. Some facultative anaerobes that are frequently isolated from endodontic infections such as *E. faecalis, S. anginosus* and *Lactobacillus* spp. were also found in this study. In conclusion, clonal analysis of the microflora associated with endodontic infections revealed a wide diversity of oral species.

This work was supported by the Brazilian Agencies FAPESP (05/55695-7, 06/60500-3), CNPq (304282/03-0, 305437/2006-2) and CAPES.