UNIVERSIDADE DE TAUBATÉ
Camila Borges Fernandes

O IMPACTO DA PRESENÇA/AUSÊNCIA DENTAL NA COLONIZAÇÃO DE PERIODONTOPATÓGENOS NA CAVIDADE BUCAL

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Tese apresentada para obtenção do Título de Doutor pelo Programa de Pós-Graduação em Odontologia da Universidade de Taubaté.
Área de Concentração: Periodontia
Orientador: Prof. Dr. José Roberto Cortelli

Taubaté-SP
2009

Ficha catalográfica elaborada pelo
SIBi – Sistema Integrado de Bibliotecas / UNITAU

F363i Fernandes, Camila Borges
O impacto da presença/ausência dental na colonização de periodontopatógenos na cavidade bucal / Camila Borges Fernandes. - 2009.
84f.: il.

Tese (doutorado) - Universidade de Taubaté, Programa de Pós-graduação em Odontologia, 2009.
Orientação: Prof. Dr. José Roberto Cortelli, Departamento de Odontologia.

1. Prevalência. 2. Bactérias. 3. Grupos etários. I. Título.

CAMILA BORGES FERNANDES

Data: ___/___/_____

Resultado:_____

BANCA EXAMINADORA

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Dedico este símbolo de vitória aos meus pais, Ivan e Dalva, sempre presentes, fiéis torcedores e incentivadores.

AGRADECIMENTOS

Agradeço a Deus por me permitir alcançar não só este maior sonho, mas também todos os meus objetivos.

Ao meu brilhante Orientador Prof. Dr. José Roberto Cortelli, pela competência do seu trabalho, pela capacidade de passar conhecimento, por sua impecável orientação, e também pela paciência a mim dispensada.

Aos meus pais, Ivan e Dalva, pelo amor e doação incondicionais.

Às minhas irmãs, Karine e Kyra, pelo companheirismo, cumplicidade e união em todos os momentos.

Ao colega Davi Romeiro Aquino por me ensinar Estatística, por ser um maravilhoso amigo, prestativo, com quem pude contar em todos os momentos.

Ao Prof. Dr. Gilson Cesar Nobre Franco pela atenção, boa vontade e colaboração a mim prestadas.

À Juliana Guimarães Santos por sua dedicação e interesse em ensinar-me os segredos do Laboratório de Biologia Molecular.

À equipe de professores da Pós-Graduação da Universidade de Taubaté por seu preparo e competência.

À secretária da Pós-Graduação, Adriana Peloggia, por ter estado sempre disposta a ajudar.

Aos colegas de turma pelos momentos de alegria e descontração.

À Fundação de Apoio à Pesquisa do Estado de São Paulo (FAPESP) pelo suporte financeiro de parte deste projeto de pesquisa.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPQ) pelo fomento ao desenvolvimento do projeto de pesquisa na modalidade produtividade em pesquisa.

Ao programa de Pós-Graduação em Odontologia da Universidade de Taubaté pela aquisição de materiais de pesquisa para o desenvolviemnto dos procedimentos laboratoriais.

“O futuro tem muitos nomes: para o fraco; é o inalcançável; para o medroso, o desconhecido; para o valente, a oportunidade."

Víctor Hugo
(1802-1885)

Fernandes CB. O impacto da presença/ausência dental na colonização de periodontopatógenos na cavidade bucal. [Tese de doutorado]. Taubaté: Universidade de Taubaté, Faculdade de Odontologia. 2009. 81p.

RESUMO

Objetivo: Avaliar o impacto da presença/ausência dental em relação a colonização de periodontopatógenos em recém-nascidos, crianças e adultos/idosos. **Método:** Estudo do tipo transversal, no qual foram incluídos 43 recém-nascidos (2,84±0,16 meses), quarenta crianças com dentição mista (9,33±1,99 anos) trinta adultos/idosos dentados (61,7±7,05 anos) e 31 adultos/idosos desdentados (60,06±8.67 anos). Avaliou-se por reação em cadeia da polimerase a presença de *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, Campylobacter rectus, Eikenella corrodens, Treponema denticola* e *Micromonas micros* dos sítios extra-sulculares (dorso da língua e mucosa bucal) e intra-sulculares (sulco / bolsa periodontal). As frequências bacterianas foram analisadas através do teste Qui-quadrado, e o risco da presença bacteriana em função do dente foi calculado com o auxílio do teste *Odds Ratio*. **Resultados:** *C. rectus* foi a bactéria de maior prevalência em todos os grupos avaliados. *P. gingivalis* não foi detectada em recém-nascidos ou em crianças, enquanto *P. intermedia* apenas não foi encontrada nos recém-nascidos. Os adultos/idosos desdentados, contrariamente aos recém-nascidos, apresentaram *P. gingivalis* em sítios extra-sulculares. Comparando as amostras extra-sulculares conjutamente (dorso de lingual e mucosa bucal), o grupo de crianças apresentou maiores prevalências para todos os patógenos avaliados, em relação aos recém-nascidos. Já nos grupos de adultos/idosos estes apresentaram todas as bactérias examinadas, com diferença estatística para *P. gingivalis, P. intermedia, T. forsythia, T. denticola* e *M. micros* no grupo dentado ($P<0,05$). **Conclusão:** De forma geral, periodontopatógenos foram detectados tanto em indivíduos dentados quanto nos desdentados independentemente da idade. Apesar dos grupos dentados apresentarem maiores prevalências bacterianas, a presença dental não representou risco aumentado para a ocorrência bacteriana. Os resultados sugerem ainda que uma maior atenção profissional deve ser dispensada em relação aos indivíduos desdentados tanto na colonização bacteriana inicial da dentição decídua/mista quanto na colonização tardia relacionada ao planejamento dos implantes dentários.

Palavras-chaves: Prevalência; Bactérias; Grupos etários.

Fernandes CB. The impact of the presence/absence of the teeth in the oral cavity colonization by periodontal pathogens. [Tese de doutorado]. Taubaté: Universidade de Taubaté, Faculdade de Odontologia. 2009 81p.

ABSTRACT

Aim: The aim of this study was evaluate the impact of teeth's presence/absence according to the presence/absence of periodontal pathogens in newborns, children and adults/elderly. **Methods:** This cross-sectional estudy included 43 newborns (2.84 ±1.60 months), forty children with mixed dentition (9.33 ±1.99 years) thirty dentate adults/elderly (61.7±7.05 years) and 31 edentulous adults/elderly (60.06±8.67 years). The presence of *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, Campylobacter rectus, Eikenella corrodens, Treponema denticola* and *Micromonas micros* of extra-sulcus samples (dorsum of the tongue and cheek mucosa) and intra-sulcus samples (gingival sulcus/ periodontal pocket) were evaluated using polymerase chain reaction. Bacterial frequences' were analized by Chi-square test, and the Odds Ratio for the presence of periodontal pathogens colonization according to the presence of the teeth in dentate population, was also calculated. **Results:** *C. rectus* was the more prevalent bacteria in all studied age-groups. *P. gingivalis* was not find in newborns and children, while *P. intermedia* was detected except in newborns. Edentulous adults/elderly, differently from newborns, showed *P. gingivalis* in extra-sulcus samples. When the extra-sulcus samples (dorsum of the tongue and cheek mucosa) were analized together, children's group showed higher prevalences than newborns, for all studied pathogens. Both adults/elderly groups presented all bacteria, with statistical difference for *P. gingivalis, P. intermedia, T. forsythia, T. denticola* and *M. micros* in dentate group ($P<0.05$). **Conclusions:** In general, periodontal pathogens were detected in dentate and edentulous subjects, irrespective of the age. Despite dentate groups showed higher bacterial prevalences, the presence/absence of the teeth did not represent a risk for pathogens' occurrence. The results also sugested that more professional attention must be taken to edentulous subjects for initial colonization of primary and mixed dentition and colonization ralated to subsequent oral implants.

***Keywords*:** Prevalence; Bacteria; Age-groups.

SUMÁRIO

1 INTRODUÇÃO

A colonização bacteriana na cavidade bucal representa uma das mais complexas organizações de desenvolvimento do biofilme em toda a natureza (Socransky & Haffajee, 2002), sendo a idade responsável por mudanças na composição da microbiota normal. Atualmente, estima-se que cerca de setecentas espécies bacterianas diferentes colonizem os tecidos bucais, embora grande parte delas vivam em harmonia com o hospedeiro (Paster et al., 2006).

Ao nascimento, a cavidade bucal é composta apenas pelos tecidos moles dos lábios, bochechas, língua, assoalho e palato, que se mantêm úmidos pela secreção das glândulas salivares. Durante os primeiros dias de vida, inicia-se a colonização das superfícies do hospedeiro por várias bactérias e, gradualmente, algumas formam a microbiota indígena, que constitui um componente integral da função de cada sítio do corpo. A erupção dos dentes permite a colonização por *Streptococcus*, que compõem de 60 a 90% da microbiota inicial. As bactérias remanescentes são primeiramente bastonetes Gram-positivos como *Actinomyces* e *Veillonellae*. Outras cepas de *Streptococcus* aderem-se fortemente à gengiva e bochechas, mas não aos dentes (Nyvad e Kilian, 1987; Könönen, 2005; Todar, 2008).

A formação do sulco gengival promove um habitat favorável à colonização por várias espécies anaeróbicas. Por exemplo, Kimura et al. (2002) observaram que a colonização de vários microrganismos periodontopatogênicos putativos podem existir precocemente na infância sem a presença de sinais clínicos de doença periodontal. Posteriormente, Ooshima et al. (2003) observaram que *Aggregatibacter*

actinomycetemcomitans, Capnocytophaga ochracea, Capnocytophaga sputigena, Prevotella nigrescens, Campylobacter rectus, e Eikenella corrodens são membros comuns da microbiota bucal de crianças saudáveis, enquanto *Porphyromonas gingivalis, Prevotella intermedia* e *Treponema denticola* mostraram-se como microrganismos transitórios.

Segundo Könönen et al. (2007), a complexidade da microbiota bucal continua a crescer com o tempo, embora dependente das espécies distintas, carrega perfil que varia com a idade, nível educacional, tabagismo, e condição periodontal do hospedeiro. Könönen et al. (1991) também relataram que a completa perda do dente pode eliminar algumas espécies patogênicas devido a um ambiente desfavorável.

Em um estudo abordando a colonização precoce de periodontopatógenos putativos em implantes dentários descrito por van Winkelhoff et al. (2000), estes autores concluíram que um controle da infecção periodontal anteriormente à instalação de implantes dentários em pacientes parcialmente desdentados pode precocemente prevenir complicações bacterianas. Quirynen et al. (2006) confirmaram que a colonização inicial de bolsas de periimplantite por periodontopatógenos ocorre dentro de duas semanas. Füsrt et al. (2007) verificaram que a colonização bacteriana ocorre dentro de trinta minutos após a instalação do implante. Estes autores observaram que o padrão de colonização inicial difere entre superfícies dentárias e de implante.

Segundo Gibbons (1989), bactérias bucais demonstram tropismo específico para diferentes superfícies biológicas na cavidade bucal como dente e mucosas ou até por uma outra bactéria. Assim, de acordo com Mager et al. (2003) estas bactérias podem colonizar diferentes superfícies dentro da cavidade bucal como resultado de uma

aderência específica que envolve interações biomecânicas entre componentes da superfície bacteriana (adesinas) e de receptores moleculares de células do hospedeiro.

Estudos longitudinais realizados em diferentes países têm associado a presença de algumas espécies bacterianas em indivíduos periodontalmente saudáveis, portadores de gengivites ou periodontites (Ali et al, 1997; Papapanou et al, 1997; Cortelli et al., 2002). Alguns estudos descreveram ainda que, apesar da incerteza referente às combinações de microrganismos requeridas para induzir a doença periodontal, certas espécies têm sido reconhecidas como patógenos periodontais verdadeiros devido a sua frequente presença em sítios com doença (Genco et al, 1986; Tanner, 1991).

A descrição que se segue tem como objetivo caracterizar os microrganismos aqui estudados sob diferentes aspectos, como por exemplo, morfológicos, bioquímicos e de ocorrência.

Uma destas bactérias facilmente encontrada em sítios doentes é *A. actinomycetemcomitans*, um bastonete Gram-negativo, imóvel, capnofílico, catalase positiva, principalmente isolado de periodontites, podendo ser encontrado em lesões de endocardite e diferentes infecções focais (Slots, 1997). Okada et al. (2000) avaliaram a presença de *A. actinomycetemcomitans* em amostras de biofilme subgengival de crianças periodontalmente saudáveis entre dois e 12 anos de idade. Os resultados indicaram que esse microrganismo foi pouco encontrado. *A. actinomycetemcomitans* é considerado o mais relevante agente etiológico microbiano relacionado a periodontite agressiva, e pode estar associado ao início de perda de inserção periodontal em crianças e adolescentes (Haubek et al., 2008). Cortelli et al. (2008) encontraram

prevalência de *A. actinomycetemcomitans* inferior a 20% em diversas faixas etárias, desde recém-nascidos até idosos.

P. gingivalis é um bastonete pleomórfico curto Gram-negativo, anaeróbio, não-esporulado, não-fermentador de carboidratos. *P. gingivalis* é isolado frequentemente de amostras bacterianas subgengivais de indivíduos com diversas formas de doença periodontal. Heuer et al. (2007) analizaram a formação inicial de biofilme em implantes, porém *P. gingivalis* não foi detectada em amostras do fluido crevicular dos *abutments*.

Em estudos transversais, *A. actinomycetemcomitans* e *P. gingivalis* têm sido detectados em mais de 1/3 de crianças aparentemente saudáveis (Lamell et al., 2000). Fernandes et al. (2007), em um estudo transversal envolvendo crianças, adolescentes e adultos jovens encontraram prevalência de 2% de *P. gingivalis* na população estudada.

Por sua vez, *P. intermedia* é um bastonete Gram-negativo, anaeróbio, não esporulado, fermentador de glicose, lactose, maltose e sacarose. Slots et al. (1986) observaram *P. intermedia* em 58,6% dos sítios com atividade de doença periodontal e, em 36,2% dos sítios não-ativos, tendo correlacionado ainda esse microrganismo com periodontite e gengivite. Em estudo realizado por Ashimoto et al. (1996), a prevalência de *P. intermedia* observada foi de 58% em indivíduos com periodontite avançada, 12% em adultos com gengivite e 18% em crianças diagnosticadas com gengivite. Foram observadas associações positivas entre *P. intermedia*, *P. gingivalis*, *C. rectus* e *Tannerella forsythia*, indicando a ocorrência de uma relação simbiótica entre esses microrganismos no interior de bolsas periodontais. Em um estudo envolvendo crianças de três a 17 anos, foi avaliada a presença de *P. intermedia* em biofilme dental, língua e mucosa jugal em associação com parâmetros clínicos de índice de placa e índice

gengival, este periodontopatógeno foi encontrado nos três sítios avaliados (Tanaka et al., 2006).

T. forsythia é um microrganismo fusiforme, Gram-negativo, anaeróbio estrito, não formador de esporos, imóvel, não fermentador de carboidratos. Este microrganismo tem sido associado às periodontites crônica e agressiva (Sakamoto et al., 2002). *T. forsythia* não foi detectada por Kulekci et al. (2008) em saliva de crianças periodontalmente saudáveis. No entanto, em um estudo prévio, Hayashi et al. (2006) encontraram prevalência moderada de *T. forsythia* em crianças periodontalmente saudáveis, sendo variável a proporção de sítios positivos por indivíduo. Em um estudo utilizando PCR, as frequências encontradas para esta bactéria foram de 74% em indivíduos com periodontite avançada, 52% para gengivite em adultos e 78% para gengivite em crianças (Ashimoto et al., 1996).

C. rectus é um microrganismo anaeróbio, Gram-negativo, com mobilidade, cujas células apresentam-se com extremidades arredondadas ou achatadas, possuindo morfologias curvas, retas ou helicoidais. *C. rectus* ocorre em números elevados em indivíduos com periodontite crônica e, associado com *F. nucleatum* foram os microrganismos mais prevalentes em sítios com doença periodontal quando comparados a controles (Tempro et al., 1997). *C. rectus* é comumente encontrado em crianças, independentemente da sua condição periodontal, distribuído largamente pela cavidade bucal relacionado a estágios precoces da doença (Hayashi et al., 2006). Este patógeno periodontal também foi detectado em frequências elevadas e similares em crianças com dentição decídua, mista e permanente (Umeda et al., 2004). Ávila-Campos et al. (2002) estudaram também através da técnica de PCR, a prevalência de periodontopatógenos em pacientes adultos com doença periodontal e indivíduos sadios

de São Paulo, SP, Brasil. Para os indivíduos saudáveis, a frequência de *C. rectus* encontrada foi de 48%, e para os indivíduos com doença periodontal foi de 80%. Cortelli et al. (2008) em um estudo epidemiológico encontraram que enquanto a prevalência de determinadas espécies bacterianas reduziu com a perda do elemento dentário, *C. rectus* permaneceu elevada em indivíduos edêntulos.

*E. corrodens s*ão cocobacilos pequenos, regulares, Gram-negativos, microaerófilos, assacarolíticos, urease positivos, resistentes a clindamicina e ao metronidazol. Em meios de cultura não seletivos causam corrosão da superfície do ágar sendo o termo *corrodens* decorrente deste fato (Koneman et al., 1997). Predominantemente encontrados no biofilme subgengival podem também causar infecções dos canais radiculares e, infecções extrabucais. Monoinfecções por *E. Corrodens* induzidas em ratos *germ free* acarretam reabsorção óssea severa. *E. corrodens* induz a liberação de mediadores da inflamação como IL-6, IL-8 e PGE_2 pelas células epiteliais que possivelmente exercem papel no início da resposta inflamatória aguda (Yumoto et al., 2001). Ashimoto et al. (1996) observaram as seguintes prevalências deste patógeno: 70% para casos de periodontite avançada, 10% para gengivite em adultos e 14% para gengivite em crianças.

As espiroquetas bucais pertencem ao gênero *Treponema* cujas principais espécies são *T. denticola, Treponema vincentii, Treponema pectinovorum* e *Treponema socranskii.* Pelas dificuldades técnicas muitas espécies de espiroquetas reconhecidas nunca foram cultivadas.

T. denticola são bastonetes helicoidais móveis, com espiras regulares ou irregulares, e com flagelo periplasmático característico. É um microrganismo anaeróbio

estrito, podendo o crescimento ao primeiro isolamento, requerer semanas. Sua principal fonte de energia se dá pela metabolização de aminoácidos podendo também a glicose ser empregada como fonte adicional (Koneman et al., 1997). Inicialmente as espiroquetas foram associadas exclusivamente com a patogênese das doenças periodontais necrosantes. Posteriormente, foi também estabelecida sua relação com outras formas mais prevalentes de doença periodontal inclusive periodontite crônica. A capacidade de invasão tecidual é um fator importante que contribui para a patogênese da doença associada a *T. denticola.* A morfologia e a mobilidade do microrganismo podem ser identificadas em microscopia de campo escuro (Haffajee & Socransky, 1994). Umeda et al. (2004) pesquisaram a distribuição de bactérias periodontopatogênicas em crianças e seus pais, utilizando PCR, e encontraram as prevalências de 41,8% e 88,1%, respectivamente. Em um estudo longitudinal com duração de um ano, Sakai et al. (2007) pesquisaram a prevalência de bactérias periodontopatogênicas em saliva de crianças brasileiras com dentição mista, *T. denticola* apresentou prevalências de 71,9% e 50% na primeira e segunda coletas, respectivamente.

*Micromonas micros s*ão colonizadores bucais associados com infecções anaeróbias inclusive gengivite e periodontite. Esta espécie anaeróbia estrita pode ser isolada com freqüência do ambiente subgengival e morfologicamente se apresenta como células esféricas, Gram-positivas, em geral agrupadas em cadeias. São bactérias assacarolíticas detectadas com maior frequência e em maiores proporções em sítios com destruição periodontal quando comparados a sítios saudáveis ou com gengivite. Lee et al. (2003) pesquisaram a distribuição de periodontopatógenos em uma

população com periodontite agressiva com idade entre vinte e 35 anos, utilizando a técnica de PCR e encontraram 85,9% de prevalência da bactéria *M. micros*.

Ao se avaliar a presença de periodontopatógenos em relação às doenças periodontais, observamos que a prevalência varia de acordo com alguns fatores como por exemplo: idade da população alvo, características sócio-econômicas, raciais, de hábitos, entre outros. Além disso, os dados prevalentes estão também na dependência da sensibilidade do teste microbiano empregado, alguns necessitando da bactéria viável, outros detectados por fragmentos de DNA. Assim, nas comparações entre diferentes estudos devemos observar a semelhança do delineamento do estudo. Neste contexto, buscamos inserir dados prevalentes respeitando as metodologias empregadas, porém, em algumas situações sem a pretensão de estabelecer comparações entre eles.

Gajardo et al. (2005) avaliaram a presença de diferentes patógenos em indivíduos chilenos diagnosticados com periodontite agressiva localizada (idade entre 17-26 anos) e generalizada (idade entre 21-32 anos). Os autores observaram que *C.rectus, P. gingivalis, E. corrodens, M. micros, Capnocytophaga sp*., foram as bactérias mais prevalentes na população estudada.

Cortelli et al. (2005) avaliaram a presença de *A. actinomycetemcomitans* na saliva e compararam sua presença com amostras coletadas de sítios intra e extra sulculares. Foram incluídos 66 indivíduos com idade média de 38,01 anos (±9,28 anos), diagnosticados com periodontite crônica e os resultados mostraram que este patógeno foi isolado em 63,63% dos sítios intra sulculares, 56,06% da saliva e 45,45% da mucosa. Os autores concluíram que em pacientes com periodontite,

A.actinomycetemcomitans detectado na saliva é representativo dos sítios intra e extra-sulculares.

Ávila-Campos et al. (2002) identificaram *A. actinomycetemcomitans, T. forsythia, P. gingivalis, C. rectus, E. corrodens, P. intermedia, F. nucleatum,* e *T. denticola* em cinquenta indivíduos adultos com doença periodontal e em cinquenta indivíduos periodontalmente saudáveis utilizando a técnica de PCR. *A. actinomycetemcomitans, T. forsythia, C. rectus, E. corrodens, P. intermedia,* e *T. denticola* foram estatisticamente significantes em indivíduos com doença periodontal.

A cavidade bucal apresenta numerosas superfícies para colonização bacteriana, e estas superfícies são colonizadas por biofilme de diferentes complexidades microbianas, únicas de cada indivíduo. Como citados anteriormente, vários estudos descrevem o biofilme em pacientes dentados, mas raros são aqueles que abordam os indivíduos desdentados e sua microbiota. Um exemplo deste tipo de estudo foi o realizado por Sachdeo et al. (2008) investigando a presença de biofilme em indivíduos edêntulos com idade média de 59,6 anos (± 11,3). Os autores detectaram a presença de *A. actinomycetemcomitans* e *P. gingivalis* ao contrário do que estudos prévios relatavam ao definirem que estas espécies bacterianas desapareceriam após a remoção total dos dentes naturais. Neste contexto, Cortelli et al. (2008) em um estudo epidemiológico transversal, analisaram a colonização inicial de cinco diferentes periodontopatógenos na cavidade bucal de recém-nascidos, crianças, adolescentes, adultos idosos dentados e desdentados. Estes autores concluíram que embora permaneça incerto exatamente quando e como os patógenos colonizem indivíduos saudáveis, a avaliação da presença microbiana em diferentes faixas etárias permitiu

verificar que a prevalência tende a aumentar com a idade mesmo nos indivíduos saudáveis, todavia, a colonização ocorre desde o nascimento, perdurando até a senilidade, independentemente da presença dental.

Estudando-se métodos para detecção de periodontopatógenos, atualmente a comunidade científica dispõe de uma variedade de técnicas microbiológicas para detecção, identificação e quantificação de bactérias colonizadoras da cavidade bucal. Dentre as técnicas podemos destacar a cultura bacteriana, *checkerboard* DNA-DNA *hibridization* e a reação da polimerase em cadeia.

A técnica de cultura bacteriana elucida a maior parte dos microrganismos presentes, sendo a única capaz de fornecer informações a respeito da susceptibilidade dos microrganismos aos antibióticos. Todavia, esta técnica é dispendiosa e utiliza muito tempo, necessitando de pessoal especializado. Fatores limitantes associados a esta técnica incluem a dificuldade no crescimento de espiroquetas e alguns bacilos móveis em meio de cultura, a perda da viabilidade dos microrganismos durante o transporte e o cultivo e identificação apenas de espécies (Cortelli et al., 2000).

A técnica de *checkerboard* DNA-DNA *hibridization*, utiliza de sondas que contém sequências específicas de genes e são usadas para detectar a presença de um gene particular ou uma sequência de DNA. A visualização da sonda é possível pela marcação desta com elementos radioativos ou enzimáticos. Nas análises que utilizam sondas, se o DNA obtido a partir da amostra de biofilme contiver o microrganismo pesquisado, seu DNA se hibridizará com a sonda e esta reação pode ser identificada através da impressão de películas radiográficas pelos isótopos radioativos. A intensidade da reação aumenta proporcionalmente ao número de microrganismos, permitindo assim sua quantificação na amostra (Cortelli et al., 2000).

Reação da Polimerase em Cadeia (PCR) tem sido utilizada para identificar *A. actinomycetemcomitans* e outros patógenos periodontais em amostras de biofilme dental por ser considerada uma técnica altamente sensível, específica e eficiente, demonstrando-se superior às técnicas de cultura. Por isto, PCR é uma metodologia adequada para a detecção de microrganismos específicos em estudos longitudinais de larga escala (Yuan et al., 2001). PCR também é apropriado para identificação de periodontopatógenos, especialmente em casos de biofilme subgengival em crianças onde há limitado número de patógenos presente (Okada et al., 2004).

Levantamentos epidemiológicos são importantes para o conhecimento da prevalência e tipos de doenças bucais, podendo-se a partir dos dados coletados, planejar, executar e avaliar ações de saúde. É necessário que haja uma metodologia adequada que garanta reprodutibilidade, validade e confiabilidade permitindo assim comparações entre os estudos.

Todavia, poucos autores até o momento se dedicaram a planejar e conduzir estudos transversais ou longitudinais em recém-nascidos, crianças e adolescentes enfocando a associação entre microrganismos periodontopatogênicos e a erupção dentária como também o impacto da perda dental em relação à frequência destes microrganismos, envolvendo indivíduos adultos e idosos. Em função disso, o presente estudo foi conduzido no sentido de avaliar o impacto da presença/ausência dental na colonização de *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythia*, *Campylobacter rectus*, *Eikenella corrodens*, *Treponema denticola* e *Micromonas micros* em uma população de recém-nascidos, crianças, adultos e idosos.

Os capítulos que seguem compõem aos artigos escritos levando em consideração a proposta anterior.

O CAPÍTULO 1 refere-se a um artigo já publicado na revista internacional *Journal of Periodontology* 2008; 79:1962-1965.

O CAPÍTULO 2 refere-se ao artigo submetido à revista internacional *Journal of Medical Microbiology* (ANEXO A).

Por fim, o CAPÍTULO 3 refere-se ao artigo submetido à revista internacional *Clinical Oral Implants Research* (ANEXO B).

2 CAPÍTULOS

2.1 DETECTION OF PERIODONTAL PATHOGENS IN ORAL MUCOUS MEMBRANES OF EDENTULOUS INDIVIDUALS

Background: The purpose of this study was to investigate the colonization of *Campylobacter rectus*, *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans* (previously *Actinobacillus actinomycetemcomitans*), *Prevotella intermedia*, and *Tannerella forsythia* (previously *T. forsythensis*) in the tongue and cheek of newborns and elderly individuals with no teeth.
Methods: Seventy-four edentulous subjects were included in this cross-sectional study. Microbiologic samples were taken from the dorsum of the tongue and cheek mucosa of all individuals and analyzed using a bacterial DNA-specific polymerase chain reaction.
Results: *C. rectus* was the most prevalent species in both groups (20.9% in the cheek of newborns, and 77.4% in the tongue of elderly subjects). *P. gingivalis* and *P. intermedia* were not detected in any of the 43 newborns; however, *P. gingivalis* was recovered from the tongue and cheek (3.2%) of elderly individuals, whereas *P. intermedia* was detected in the tongue (9.6%) and cheek (3.2%) of elderly individuals. *T. forsythia* was detected in newborns as well as elderly individuals, although the highest prevalence was observed in the tongue of newborns (6.9%) and elderly (9.6%) individuals. *A. actinomycetemcomitans* was not found in the tongue of newborns, but we observed *A. actinomycetemcomitans* in the cheek (2.3%) of newborns and in the tongue (12.9%) and cheek (6.4%) of elderly patients.
Conclusions: Although we did not detect *P. gingivalis* and *P. intermedia* in newborns, periodontal pathogens could be detected from the oral mucous membranes of edentulous individuals. Our results suggest that major attention should be paid to edentulous individuals as an important measure in the prevention of the initial colonization of natural teeth and dental implants by periodontal pathogens.
J Periodontol 2008; 79:1962-1965.

KEY WORDS: Bacteria; Cross-sectional studies; Elderly; Epidemiology; Newborn.

The mouth represents a variety of different ecologic situations, with age corresponding to the changes in the composition of the normal microbiota. During the first days of life, the colonization of the host surfaces by various bacteria starts, and gradually, a subset of these forms the indigenous microbiota, which constitute an integral component of the function of each body site. *Streptococcus salivarius* is dominant and may make up 98% of the total oral microbiota until the appearance of the teeth. The eruption of the teeth during the first year of life leads to colonization by *Streptococcus mutans* and *Streptococcus sanguis*. The remaining bacteria are primarily Gram-positive rods, whereas other *streptococci* adhere to the gums and cheek but not to the teeth.[1]

During childhood, the formation of the gingival sulcus will provide a favorable habitat for anaerobic species. Besides other bacterial species, *Aggregatibacter actinomycetemcomitans* (previously *Actinobacillus actinomycetemcomitans*) and *Campylobacter rectus* were found as common members of the oral microbiota of healthy children, whereas *Porphyromonas gingivalis* and *Prevotella intermedia* appear to be transient organisms.[2]

The complexity of the oral microbiota continues to increase with time, althoughd dependent on other variables.[3] In addition, the complete loss of teeth may eliminate some pathogenic bacteria due to an unsuitable microenvironment.[4] Finally, in partially edentulous patients, it was suggested that proper periodontal infection control before installment of dental implants may prevent bacterial complications[5] related to the early colonization previously reported.[6]

Although bacteria can colonize different surfaces within the oral cavity,[7] only a few studies, as reviewed by Sachdeo et al.,[8] considered the presence of

Capítulo 1 _____

periodontopathogens in edentulous individuals. Therefore, the present cross-sectional study evaluated the presence of *C. rectus*, *P. gingivalis*, *A. actinomycetemcomitans*, *P. intermedia*, and *Tannerella forsythia* (previously *T. forsythensis*) in the cheek and dorsum of the tongue of newborns and elderly patients with no teeth.

MATERIALS AND METHODS

Subjects and Exclusion Criteria

The eligible population was recruited from 239 children who were born at the Taubaté University Hospital, Vale do Paraíba, SP, Brazil, and 193 elderly subjects who were seeking dental implant treatment at the Dental School of the University of Taubaté from 2004 to 2006. After screening for the exclusion criteria described below, 74 subjects representing various ethnic groups were divided into two groups: newborns (0 to 4 months) with no teeth and elderly subjects (58 to 79 years of age) with no teeth (edentulous $\geq$12 months and $\leq$36 months).

Medical and dental histories were obtained by individual self-declarations or by parents on a medical questionnaire. Either the subjects themselves or their legal guardians signed an informed consent that was approved by the University of Taubaté's committee on research involving humans (protocol 362/03).

One trained and calibrated examiner conducted all clinical visual examinations and collected the microbial samples. Subjects presenting any of the following conditions were excluded from the study: 1) antibiotics for medical or dental treatment; 2) uncontrolled systemic diseases; 3) immunologic compromise; 4) antibiotic treatment within six months prior to the clinical and microbial examination (elderly group); 5) past or current smokers (elderly group); and 6) individuals with no teeth >36 months prior to the study (elderly group).

Sampling of Microorganisms

Microbial samples were taken from areas ~ 1cm^2 using a cotton swab with reduced Ringer's solution (Oxoid, Basingstoke, Hampshire, UK) rotated six times on the left side of the cheek mucosa and tongue dorsum. Each swab was transferred into a microtube containing reduced Ringer's solution (1ml).

Bacteria-Specific Polymerase Chain Reaction (PCR)

The bacterial cells in the microtube were dispersed using an electricmixer at maximal setting for one minute and then maintained at -80ºC until processing. The presence of *C. rectus*, *P. gingivalis*, *A. actinomycetemcomitans*, *P. intermedia*, and *T.*

forsythia was determined by PCR using specific primer (5'-3') sequences designed based on 16s ribosomal RNA (Table 1). PCR mediated DNA amplification and amplicate analysis were performed as previously published.[9]

Statistical Analysis

The frequencies of bacterial species in the tongue dorsum and cheek mucosa were analyzed using the X^2 test. The presence of all bacteria in each group was evaluated by the Kruskal-Wallis test. All tests were performed using statistical software (BioEstat 4.0, Belém, Pará, Brazil). Results were determined to be statistically significant at $P < 0.05$.

RESULTS

A total of 43 newborns (25 males and 18 females; mean age: 2.84 ± 1.60 months) and 31 elderly subjects (14 male and 17 female; mean age: 60.06 ± 8.67 years) were examined. Elderly patients were toothless ≥12 months but ≤ 36 months (23.74 ± 7.06 months). Advanced chronic periodontitis (n = 17) was the major reason for extractions, followed by caries (n = 8), esthetics/ implants (n = 4), and others (n = 2).

The prevalence of bacteria in the tongue dorsum and cheek between newborns and elderly groups did not show any significant difference (P <0.05). Among the bacteria tested, *C. rectus* showed the highest prevalence in both groups; and a higher bacteria presence of *C. rectus*, *P. gingivalis*, *A. actinomycetemcomitans*, *P. intermedia*, and *T. forsythia* was observed in the elderly group than in the newborn group (Figure 1).

Intragroup analysis revealed that *C. rectus* was more prevalent in the cheek mucosa of newborns (20.9%) and in the dorsum of the tongue in the elderly population (77.4%). However, *P. gingivalis* and *P. intermedia* were not detected in the cheek mucosa and dorsum of the tongue of newborns. *T. forsythia* was detected in newborns and elderly subjects, although the highest prevalence was observed in the dorsum of the tongue in newborns and elderly individuals (6.9% and 9.6%, respectively). *A. actinomycetemcomitans* was found only in the cheek (2.3%) of newborns and at both the tongue dorsum (12.9%) and the cheek (6.4%) in elderly patients.

DISCUSSION

The human mouth provides many sites for oral biofilm colonization,[10] and the tongue and the cheek support their own microbial populations that differ from those found on the teeth. Although soft tissue harbors periodontal pathogens in patients who are partially or totally dentate,[7] little attention has been paid to totally edentate individuals.[8] This is most likely a result of many microorganisms, especially anaerobic pathogens, disappearing from the oral cavity after teeth extraction.[4,11,12]

Because no study, to our knowledge, has addressed the possible presence of periodontal pathogens in both edentate newborns and elderly subjects, considering these pathogens and their associated risk factors with respect to initial colonization when the teeth emerge or dental implants are placed, the present study examined the prevalence of five periodontal pathogens in the oral cavity of these two population groups.

An early colonization of *C. rectus* and *T. forsythia* was demonstrated in both sites of newborns, whereas *A. actinomycetemcomitans* was detected at a low prevalence only in the cheek (2.3%). However, in elderly individuals, all bacterial species were noted in both the tongue and cheek. Our results are in agreement with the study of Sachdeo et al.,[8] which described microbial ecologic relationships in the oral cavity of 61 edentulous subjects and evaluated the microbiota on hard surfaces (palatal surfaces of dentures and denture teeth), eight soft tissue surfaces, and saliva samples and found that the periodontopathogens *A. actinomycetemcomitans* and *P. gingivalis* were present in significant numbers in these subjects. Conversely, Danser et al.[13] did not detect *A. actinomycetemcomitans* and *P. gingivalis* in edentulous subjects. Moreover, our results confirmed that, in the mouth, periodontopathogens could adhere to soft tissues, many of them due to the presence of binding factors such as fimbriae[14] and S-layer.[15]

In general, there was a higher numeric tendency for the elderly subjects to have more bacteria compared to the newborns. However, this difference was statistically confirmed only for *C. rectus* ($P = 0.000$). We can only suppose that the reasons for teeth extraction, lower epithelial desquamation, and lower salivary flow may have favored a higher bacteria presence among elderly subjects (Figure 1).

The presence of pathogens in the tongue dorsum and cheek observed in the present study, except for *P. gingivalis* and *P. intermedia* in newborns, indicates that it is fundamentally important for dentists to plan therapeutic strategies to reduce these microorganisms before either the eruption of the deciduous teeth or installment of dental implants. The early colonization of dental implants by periodontopathogens in partially edentulous patients and the transmission of periodontal disease–associated bacteria from teeth to osseointegrated implants are possible.[5,6,16] We speculated that the presence of periodontal pathogens among elderly subjects could contribute to the microbial colonization of future implants. This theory is also supported by Oringer et al.[17] After comparison of the presence and levels of forty bacterial species between implants and teeth from partially or fully edentulous patients, these authors did not find differences for 38 species, reinforcing the concept of teeth being an important reservoir for bacterial colonization around implants.

Table 1- Bacteria-Specific Primers and PCR-Product Sizes (bp)

Bacteria	Primer	Base Pairs
A. actinomycetemcomitans	5′AAACCCATCTCTGAGTTCTTCTTC3′ 5′ATGCCAACTTGACGTTAAAT3′	550
P. intermedia	5′TTTGTTGGGGAGTAAAGCGGG3′ 5′TCAACATCTCTGTATCCTGCGT3′	575
P. gingivalis	5′AGGCAGCTTGCCATACTGCGG3′ 5′ACTGTTAGCAACTACCGATGT3′	404
T. forsythia	5′GCGTATGTAACCTGCCCGCA3′ 5′TGCTTCAGTGTCAGTTATACCT3′	641
C. rectus	5′TTTCGGAGCGTAAACTCCTTTTC3′ 5′TTTCTGCAAGCAGACACTCTT3′	598

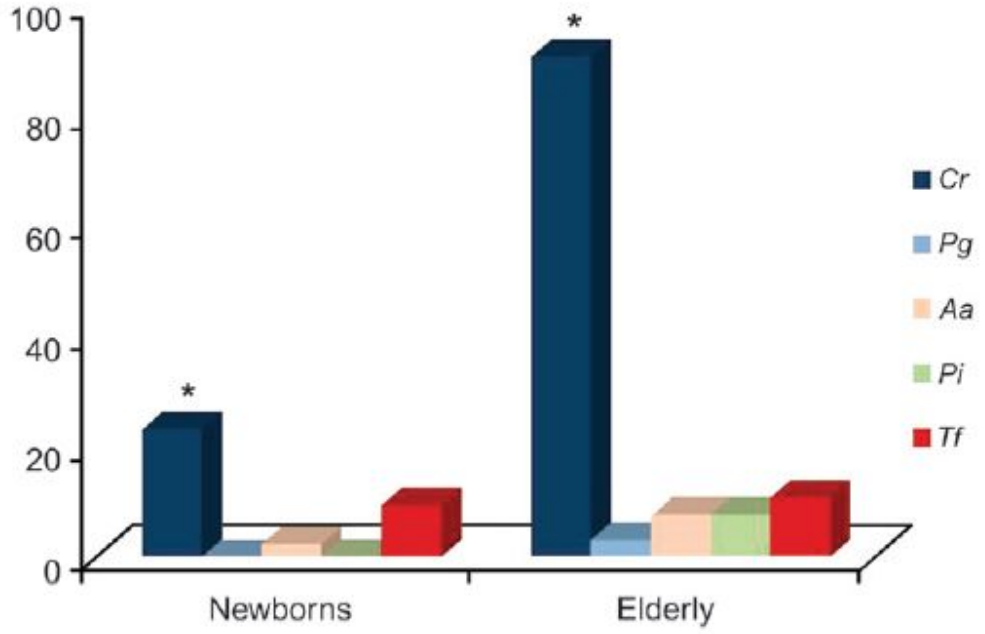

Figure 1- Total prevalence of all periodontal pathogens in newborns and elderly patients. *Statistically significant difference (*P* <0.05; Kruskal-Wallis test)

CONCLUSIONS

Except for *P. gingivalis* and *P. intermedia*, we observed the presence of other pathogens in the tongue and cheek of edentulous newborns. The prevalence of *C. rectus, P. gingivalis, A. actinomycetemcomitans, P. intermedia*, and *T. forsythia* demonstrated differences in their colonization in both types of tissues and age groups. *C. rectus* showed the higher prevalence in the tongue and cheek mucosa in both groups.

ACKNOWLEDGMENTS

The authors are grateful to the São Paulo Foundation for Research, São Paulo, SP, Brazil (grant 04/00256-6), the National Council for Scientific and Technological Development, Brasília, DF, Brazil, and Jonas Carvalho Filho, technician, Department of Molecular Biology, University of Taubaté . The authors report no conflicts of interest related to this study.

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Submitted February 14, 2008; accepted for publication April 8, 2008.

2.2 CHANGES OF PREVALENCE OF PERIODONTAL PATHOGENS IN NEWBORNS AND CHILDREN

SUMMARY

Oral colonization by several bacteria begins early in the life and can represent a risk for disease development in the future. So, the purpose of this cross-sectional study was to investigate the age-specific prevalences of *Campylobacter rectus*, *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia*, *Tannerella forsythia*, *Eikenella corrodens*, *Treponema denticola* and *Micromonas micros* in edentulous newborns and children with mixed dentition. Forty edentulous newborns (zero-four months) and forty children with mixed dentition (six-13 years of age) were included in this study. Microbial samples of the cheek mucosa and the dorsum of the tongue were obtained from all subjects. In addition, subgingival sample was collected from each child from the first molars (n=4 molars/subject) and incisors (n=2 incisors/subject). All samples were analyzed using bacterial DNA-specific polymerase chain reaction. *P. gingivalis* was not detected in any of the groups, and *P. intermedia* was not found in newborns. In the extra-sulcus samples combined, all studied bacteria presented higher prevalences in the group six-13 years of age, with statistically significant differences for *C. rectus* (*P*=0.001), *P. intermedia* (*P*=0.001) and *T. forsythia* (*P*=0.001). *C. rectus*, *P. intermedia*, and *E. corrodens* were more frequently found in the dorsa of the tongues of children with teeth (90.00%, 57.50%, and 64.10%, respectively). Statistically significant differences were also found in cheek mucosa samples for *C. rectus* and *P. intermedia* for the group six-13 years of age (*P*=0.001 and *P*=0.002, respectively). The presence of teeth was associated with a higher prevalence of bacteria. However, periodontal pathogens can be detected in edentulous newborns. In addition, the results underscored the importance of identifying the presence of periodontal pathogens in newborns as well as in children.

Key-words: Bacteria; Children; Newborn; Prevalence.

INTRODUCTION

Periodontopathic bacteria are transmissible among family members, and children seem to acquire periodontal pathogens predominantly from their parents (Sirinian *et al*., 2002). Early childhood years are the critical period for the acquisition of certain bacteria, and close household contacts may be the source of acquisition for infants and children (Okada *et al*. 2004).

Development of the indigenous microbiota begins on the surfaces of the human body after birth, when infants are exposed to continuous person-to-person and environmental contacts with microbes. During the first days of life, the colonization of host surfaces by various bacteria starts, and gradually part of them form the indigenous microbiota, which constitutes an integral component of the function of each body site. However, some clones may contain characteristics that are potentially detrimental to the health of an individual (Könönen, 2005).

The oral cavity is a microbiologic ecosystem consisting of many niches for bacteria to colonize. The intersection between host and bacteria can be very complex. It has been observed that bacteria can thrive in hard and soft tissues in the oral cavity and might even travel from site to site (Leung *et al*., 2006).

In the oral cavity, the age-related pattern of bacterial colonization is at least partly related to the development of the primary dentition: the first teeth erupt around six months of age, and complete dentition is reached around the age of three years. After teeth emerge, more attachment sites and potential niches are available for anaerobic

bacterial colonization (Könönen, 2005). It is important to investigate the presence of periodontal pathogens as the permanent teeth start to erupt (Gafan *et al*., 2004).

In controlling periodontal disease, it may be important to identify children who need more effective oral health programs and to eradicate the organisms before they cause a breakdown in oral health or before they spread to other individuals (Sirinian *et al*., 2002; Gafan *et al*., 2004). Knowing the age at which these pathogens colonize the oral cavity will aid in our understanding of disease development and of how to devise interventions (Tanner *et al*., 2002).

Additionally, the colonization of many putative periodontopathic microorganisms can occur quite early in childhood without clinical signs of periodontal disease. Although their colonization does not necessarily induce an infection that causes destruction of the periodontium, the acquisition of putative pathogens is a prerequisite for developing periodontal disease. Thus, it is important to elucidate the prevalence and distribution of putative periodontopathic bacteria in periodontally healthy children (Kimura *et al*., 2002). Particularly in children with mixed dentition, it is possible that these pathogens remain in the area surrounding exfoliated primary teeth and continue to survive in the gingival sulcus around the permanent teeth in the mixed dentition stage (Okada *et al*., 2000; Hayashi *et al*., 2006).

Thus, the aim of this cross-sectional study was to investigate on the age-specific prevalences of eight periodontal pathogens in edentulous newborns and children with mixed dentition.

METHODS

Subjects and exclusion criteria for recruitment

The present cross-sectional study included newborns up to four months of age recruited from the Hospital of University of Taubaté (São Paulo, Brazil) and children six to 13 years of age, recruited from the Department of Dentistry in the University of Taubaté. Data and personal information related to medical and dental histories of the subjects were obtained from responses by individuals or by their parents to our questionnaire. The legal guardians of subjects signed an Informed Consent form, which was previously approved by the Institutional Committee on Research Involving Human Subjects of the University of Taubaté (protocol 109/08).

In general, we excluded the following subjects: (1) subjects with uncontrolled systemic diseases; (2) subjects with immunological compromise; (3) newborns that had taken antibiotics; (4) subjects who were preterm birth or low birth weight. Specifically, among children six-13 years of age, we also excluded (5) subjects who had taken antibiotics within six months prior to the clinical and microbial examination, (6) subjects who use orthodontic devices; (7) subjects undergoing periodontal treatment 12 months before the beginning of the study; (8) subjects who needed antibiotics prophylaxis for dental treatment; and (9) subjects with no permanent first molars and central incisors.

Clinical measurements

For newborns, the examination was only by visual inspection. For children, the clinical examination comprised the Visible Plaque Index (VPI) and the Gingival Bleeding Index (GBI) according to Ainamo & Bay (1975). One trained and calibrated examiner conducted all clinical measurements and collected the microbial samples.

Sampling of microorganisms

Microbial samples of the left side of the cheek mucosa and the dorsum of the tongue were obtained from all subjects. These samples were taken from areas of approximately 1cm^2 using a swab with a reduced Ringer's solution (Oxoid Ltd., Basingstoke, Hampshire, UK), rotated six times. Each swab was transferred into a microtube containing reduced Ringer's solution (1ml). In addition, a pooled subgingival sample was collected from each child six-13 years of age from the mesio-buccal aspect of first molars (n=4 molars/subject) and incisors (n=2 incisors/subject), using sterile paper points inserted into the depth of the sulcus after the removal of supragingival plaque using sterile curettes. The paper points were removed sixty seconds after being placed into the sulcus and immediately placed in a microtube containing reduced Ringer's solution.

Bacterium-specific PCR

The bacterial cells in the microtube were dispersed using a vortex mixer at maximal setting for one minute and then maintained at -80°C until laboratory processing. The presence of *A. actinomycetemcomitans, P. gingivalis, P. intermedia, T. forsythia, C. rectus, E. corrodens, T. denticola* and *M. micros* was determined by polymerase chain reaction (PCR), as described below.

The bacterial suspensions were thawed and centrifuged at 12,000 x r.p.m. for 3 min, and the DNA was extracted from the bacterial pellet using a DNA isolation kit, following the manufacturer's instructions (InstaGene purification matrix; BioRad Laboratories, Hercules, CA). PCR reaction was carried out using 10µl of the sample and 15µl of reaction mixture containing 2.5µl of 10 X PCR buffer (Promega, Madison, WI), 1.25 units of *Taq* DNA polymerase (Promega), and 0.2mM of each of the deoxyribonucleotides (Pharmacia LKB, Piscataway, NJ). PCR amplification was performed using a thermalcycler (Perkin-Elmer, Wellesley, MA). The bacterium-specific primer (5'-3') sequences used in this study are shown in Table 1. The primers for *P. gingivalis*, *T. forsythia*, *C. rectus*, *E. corrodens* and *T. denticola* have been described previously by Slots *et al*. (1995). The primers for *A. actinomycetemcomitans* and *P. intermedia* have been described by Ashimoto *et al*. (1996), and the primer for *M. micros* was designed for this study.

Table 1- Bacteria-specific primers and PCR-product sizes (base pairs)

Bacteria	Primers	Base Pairs
A. actinomycetemcomitans	5'AAACCCATCTCTGAGTTCTTCTTC3' 5'ATGCCAACTTGACGTTAAAT3'	550
P. gingivalis	5'-AGGCAGCTTGCCATACTGCGG-3' 5'-ACTGTTAGCAACTACCGATGT-3'	404
P. intermedia	5'-TTTGTTGGGGAGTAAAGCGGG-3' 5'-TCAACATCTCTGTATCTGCGT-3'	575
T. forsythia	5'-GCGTATGTAACCTGCCCGCA-3' 5'-TGCTTCAGTGTCAGTTATACCT-3'	641
C. rectus	5'-TTTCGGAGCGTAAACTCCTTTT-3' 5'-TTTCTGCAAGCAGACACTCTT-3'	598
E. corrodens	5'- CTACTAAGCAATCAAGTTGCCC-3' 5'-CTAATACCGCATACGTCCTAAG-3'	688
T. denticola	5'-TAATACCGAATGTGCTCATTTACAT-3' 5'-TCAAAGAAGCATTCCCTCTTCTTCTTA-3'	316
M. micros	5'-AGTGGGATAGCCGTTGGAAA-3' 5'-GACGCGAGCCCTTCTTACAC-3'	328

PCR products were separated in a 1.5% agarose gel (Sigma, Dorset, United Kingdom) by an electrophoresis performed at 5V/cm in Tris-acetate-EDTA buffer (Promega). The DNA bands on the gel were stained with 0.5% µg/ml ethidium bromide

(Amersham, Arlington Heights, IL) and photographed under 300-nm UV light illumination.

Statistical Analysis

The frequencies of bacterial species in the dorsum of the tongue and cheek mucosa were analyzed using the Chi-square test. We also estimated the odds ratios for the presence of periodontal pathogens colonization according to the presence of teeth in children with six-13 years of age. All tests were performed using the statistical software BioEstat 5.0. Results were statistically significant at $P<0.05$.

RESULTS AND DISCUSSION

A total of eighty individuals, forty newborns (24 male and 16 female) and forty children (twenty male and twenty female) were included in this study (Table 2).

Table 2- Distribution of age and gender of the study population

	Newborns	Children	Total
Male	24	20	44
Female	16	20	36
Total (mean±sd)	40 (2.20±1.30) Months of age	40 (9.33±1.99) Years of age	80

sd= standard deviation

Children six-13 years of age exhibited a healthy periodontal profile, based on the mean values of VPI (0.44 ± 0.19) and GBI (0.03 ± 0.05).

P. gingivalis was not detected in any of the samples and *P. intermedia* was not found in samples from newborns.

When the extra-sulcus samples from the dorsum of the tongue and cheek mucosa were analyzed together, the frequencies were significantly higher for *C. rectus*, *P. intermedia* and *E. corrodens* in children with mixed dentition than newborns (Figure 1).

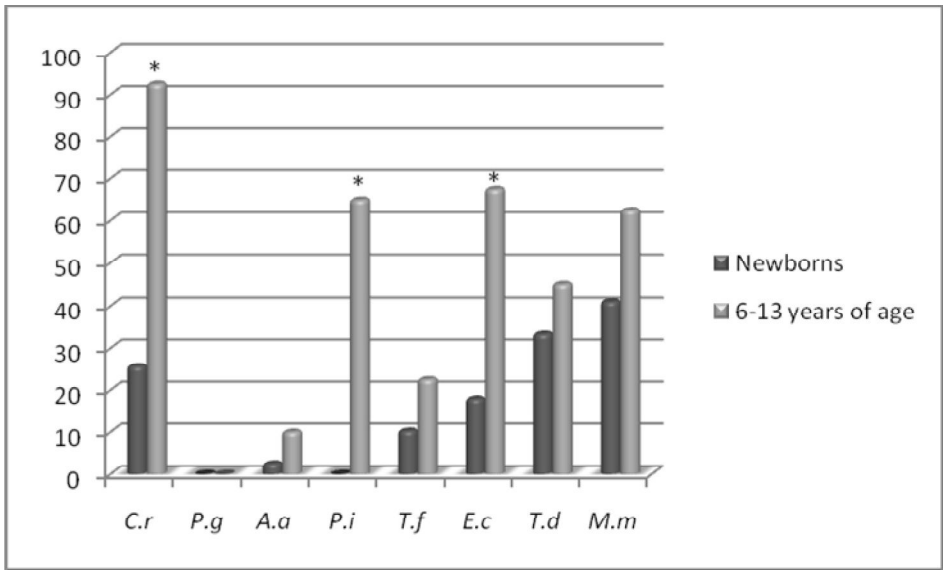

Figure 1.-Comparison of extra-sulcus samples among groups. *Statistically significant difference (*P*<0.05; Chi-Square test)

The prevalences of all bacteria examined in the dorsa of the tongues as well as on the cheek mucosa, according to age-groups, are shown in Tables 3 and 4.

Table 3- Prevalences from the dorsa of the tongues, by age-groups

	C.r	*P.g*	*A.a*	*P.i*	*T.f*	*E.c*	*T.d*	*M.m*
Newborns	20.51	0.00	0.00	0.00	7.69	12.82	20.51	25.64
6-13 y	90.00	0.00	10.00	57.50	15.00	64.10	38.46	53.85
P value	0.001*	-	0.130	0.001*	0.504	0.001*	0.096	0.014*

*Statistically significant difference. Y – years of age. Chi Square test

Table 4- Prevalences from the cheek mucosa, by age-groups

	C.r	*P.g*	*A.a*	*P.i*	*T.f*	*E.c*	*T.d*	*M.m*
Newborns	23.08	0.00	2.56	0.00	2.56	12.82	23.08	23.08
6-13 y	57.50	0.00	2.50	25.00	10.00	30.00	27.50	17.50
*P*value	0.001*	-	0.485	0.002*	0.370	0.063	0.651	0.537

*Statistically significant difference. Y – years of age. Chi Square test

When only the sulcus samples of the group six-13 years of age were analyzed, we found the following prevalences: *C. rectus*, 95.00%, *P. gingivalis*, 0.00%, *A. actinomycetemcomitans*, 10.00%, *P. intermedia*, 75.00%, *T. forsythia*, 52.50%, *E. corrodens*, 82.50%, *T. denticola*, 42.50% and *M. micros*, 67.50%.

Finally, we attempted to establish the odds of the presence of each studied pathogen according to the presence of teeth, among the group with mixed dentition. There were no statistically significant odds ratios for any of the pathogens (Table 5).

Table 5- Risk of presence in teeth for each periodontal pathogen

	C.r	*P.g*	*A.a*	*P.i*	*T.f*	*E.c*	*T.d*	*M.m*
OR	0.010	_	0.230	0.008	0.100	0.040	0.670	0.330
P value	0.001	_	0.370	0.001	0.001	0.001	0.543	0.032
CI <	0.003	_	0.025	0.001	0.030	0.014	0.271	0.133
CI >	0.089	_	2.220	0.072	0.345	0.147	1.688	0.839

OR= Odds ratio, CI= confidence interval

The detection of periodontal pathogens is an important aspect of the identification, diagnosis and future treatment of periodontal diseases. Teeth eruption causes alterations in the oral environment and promotes the installation of microbiota. More specifically, the oral cavity provides a variety of surfaces for bacterial attachment and possible colonization. These surfaces include the saliva, epithelium of the cheeks, gums, tongue and teeth. As the formation of the gingival sulcus area provides a favorable habitat for the colonization of several anaerobic species. Thus, we also speculated in this study whether the presence of teeth is associated with greater colonization of target periodontal pathogens.

Previously, in a prevalence study of four periodontopathic bacteria in Brazilian children with mixed dentition, Sakai *et al*. (2007) found that a high percentage of children harbored at least one of the putative periodontal pathogens in saliva. So they concluded that it is of particular importance to investigate the early colonization of periodontal pathogens, which can have important implications for the prevention and treatment of periodontal disease.

In our investigation, when we compared the presence of periodontal pathogens in the extra-sulcus samples by age-groups, newborns showed lower prevalences of the target pathogens (Figure 1). This confirmed the role of time, the presence of teeth and the subgingival area on bacterial colonization in the different sites of the oral cavity. *C. rectus*, *P. intermedia* and *E. corrodens* were statistically significantly more frequent in children with mixed dentition (Figure 1), however, based on the odds ratios values, the presence of teeth was not associated with risk for these colonization (Table 5).

C. rectus was the most prevalent bacterium in the group with mixed dentition (92.50%), but this was not the case among newborns (25.64%). The presence of teeth

in the oral cavity seems to be a permissive factor for colonization by this and other bacteria in children's oral cavity. Kimura *et al*. (2002) found *C. rectus* prevalence about 50% in sulcus samples from children, while we detected a frequency of 95.00% in the same site. This disagreement in the results may have occurred due to differences in age of the study population. In our study, the mean age of children with mixed dentition were higher than those in Kimura's *et al*. (2002) investigation, thus, our participants' teeth and subgingival sulci had more time to provide the opportunity for colonization by *C. retus*, as well as the others bacteria.

Kamma *et al*. (2000) found prevalences of 15-25% for *T. forsythia* and 51-52% for *E. corrodens* in the sulcus samples of children. They used a culture for bacterial isolation that was anaerobic and was in 10% CO_2 plus air using selective and nonselective media. In the present study, we found higher prevalences for the same bacteria (52.50% and 82.50%, respectively) in sulcus samples of children with mixed dentition. Differences in the sensitivity of techniques might explain the different prevalences.

Fine *et al*. (2007) studied the relationship of *A. actinomycetemcomitans* on the incidence of disease in healthy adolescents and concluded that the detection of this bacterium in periodontally healthy children can serve as a risk marker for the initiation of localized aggressive periodontitis. This species has been extensively studied as a possible casual factor for aggressive periodontitis in adolescents because of its increased prevalence in individuals with the disease, detectable specific antibody responses in patients and its possession of certain disease-relevant virulence factors (Haubek *et al*., 2008). There have been several studies investigating *A. actinomycetemcomitans* prevalences in young populations showing much variation in the frequencies (Alaluusua & Asikainen, 1988; Okada *et al*., 2000; Lamell *et al*., 2000;

Yuan *et al*., 2001). In our survey, the prevalence of *A. actinomycetemcomitans* was low for all age-groups (2.56% for newborns and 10% for children). But, even in low levels, we found this pathogen in a young population.

Kimura *et al.* (2002) observed that *P. intermedia* was relatively infrequently detected in healthy children. In the present study, this bacterium was not present in newborns, but was found in 65.00% of the extra-sulcus samples and in 75.00% of the sulcus samples of children six-13 years old. Könönen (2005) also did not find this pathogen in a study of children ranging in age from two months to two years of age. Additionally, Kamma *et al.* (2000) found prevalences of this bacterium of 35-42.50% in children with mixed dentition. This significant difference among newborns and dentate children can be explained by teeth eruption and by *P. intermedia* being an anaerobic species.

Tanner *et al*. (2002) found higher frequencies in young children who were six-18 months of age than in newborns of periodontal pathogens from samples of the dorsum of the tongue. They also reported the following prevalences: *A. actinomycetemcomitans*, 30%, *P. intermedia,* 29%, *P. gingivalis*, 23%, *T. forsythia*, 11%, and *T. denticola*, 36%. However, we did not detected *A. actinomycetemcomitans*, *P. intermedia* and *P. gingivalis* in newborns' samples of the dorsum of the tongue, and we found prevalences of 7.69% for *T. forsythia* and 20.51% for *T. denticola* (Table 3). The higher frequencies of periodontal pathogens in the study by Tanner *et al.* (2002) may have resulted from their children being older than those included in our study.

Comparing tongue samples in different age-groups, children with mixed dentition showed significantly higher frequencies of *C. rectus*, *P. intermedia*, *E. corrodens* and *M. micros* (Table 3). Thus, these data confirm that the presence of the teeth can influence

the colonization of other sites in the oral cavity, which is consistent with the results found by Tanaka *et al.* (2006).

P. gingivalis is considered to be a major pathogen of adult periodontitis and is occasionally detected in samples from the oral cavities of children (Lamell *et al*., 2000). We did not find this bacterium in any of the samples from any of the sites across age-groups, and this was consistent with the reports by Frisken *et al*. (1990) and Kimura *et al*. (2002). But many other previous studies reported the occurrence of *P. gingivalis* in children (Tanner *et al*., 2002; Tanaka *et al*., 2006; Kulekci *et al*., 2008). This may be explained by differences in factors related to chosen microbiological methods, levels of sensitivity of species detection, dentition phase, ethnic background and even by contact with diseased periodontal parents.

M. micros is not frequently investigated in studies with young children. In the present study, there was a slightly higher frequency in the cheek mucosa in newborns than in children aged six-13 years (Table 4), but this was not statistically different. However, in samples from dorsum of the tongue, this pathogen was more prevalent in children with mixed dentition. Kamma *et al*. (2000) reported frequencies of *M. micros* of about 12.5 – 15% in sulcus samples of children aged seven-eight years, but for the same site, we found a prevalence of 67.50% in children aged six-13 years.

Other species that have also been related to the disease is *T. denticola*, which was not detected in children in Kimura's *et al.* (2002) study. However, in our survey, this pathogen showed prevalences of 33.33% for newborns and 52.50% for children, and was present on all three sites, even among children with healthy periodontal status. Thus, even in periodontally healthy children, it is important to identify the presence of *T.*

denticola, as well as the others periodontopathic bacteria, which are risk factors for disease in the future.

In conclusion this study indicated that a wide range of periodontal pathogens can be detected in edentulous newborns and periodontally health children with mixed dentition. In addition, this investigation confirmed the role of teeth and the subgingival area in facilitating the colonization of several bacteria after teeth eruption, as evidenced by the bacterial frequencies that increased with age for all periodontal pathogens under study. Therefore, the best approach to managing periodontitis is prevention, with early detection of periodontal pathogens.

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2.3 DO ELDERLY EDENTULOUS PATIENTS HARBOR PERIODONTAL PATHOGENS?

ABSTRACT

Aim: The purpose of the present investigation was to compare the presence of *Campylobacter rectus*, *Prophyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia*, *Tannerella forsythia*, *Treponema denticola*, *Eikenella corrodens* and *Micromonas micros* in dentate and edentulous elderly subjects.
Methods: Thirty subjects (mean age 61.7±7.05) with teeth and thirty subjects (mean age 65.8±8.69 years) without teeth were included in this cross-sectional study. Microbiologic samples of cheek mucosa and the dorsum of the tongue were taken from all subjects. In addition, sulcus samples were taken from the dentate group. All samples were analyzed using a bacterial DNA-specific polymerase chain reaction.
Results: All studied pathogens were present in both groups. In the extra-sulcus samples combined, only *C. rectus*, *A. actinomycetemcomitans* and *E. corrodens* were present in the same amounts, irrespective of the presence of teeth, while the other species were more prevalent in the dentate group ($P<0.05$). *P. intermedia* and *T. denticola* were present in higher frequencies in the cheek mucosa samples in the group with teeth (26.67% and 66.67%, respectively). *P. gingivalis* and *T. forsythia* were more prevalent, in the dorsum of the tongue, in dentate subjects (26.67% and 56.67%, respectively).
Conclusions: In conclusion, the absence of teeth reduced, but did not eliminate the periodontal pathogens. Thus, care must be taken to maintain oral health given this finding that periodontal pathogens colonize mouth sites irrespective of the presence of teeth and may also colonize subsequent oral implants.

Key-words: Bacteria; Edentulous; Ederly; Prevalence.

INTRODUCTION

Over seven hundred species of bacteria have been identified to reside in the human oral cavity. Of these, over four hundred species were found in the periodontal pocket, while the remaining three hundred species were localized to other sites within the oral cavity such as the tongue, oral mucous membranes, carious lesions, and endodontic infections (Paster et al., 2006).

Periodontitis differs from many other types of infections, as it is not caused by a single bacterium, but rather by a group of bacteria (Nonnenmacher et al., 2004). Most bacteria colonizing higher-order organisms grow into populations by adhering to solid surfaces and ultimately forming mixed culture biofilms. The early colonizers of dental plaque are of great importance in the successive stages of biofilm formation and its overall effect on the oral health of the host (Li et al., 2004).

Full mouth extraction is the most predictable form of therapy for severe periodontitis and eliminates potential variables that could interfere with the pursuit of the question (Danser et al., 1994). The complete loss of teeth presents a dramatic ecological change for oral bacteria and may lead to the elimination of some oral pathogens due primarily to a lack of suitable habitats, such as tooth surfaces and subgingival sites (Socransky & Manganiello, 1971).

Use of osseointegrated oral implants has been shown to be an excellent method for the replacement of missing teeth (Heuer et al., 2007). Occasionally, failures occur and inflamed tissues and formation of pockets around the implant may be seen. These failures have been ascribed to several factors, which include surgical trauma, bacterial

infection, and (premature) fixture overload. Periodontitis and peri-implantitis are linked to the presence of several key pathogens (Quirynen et al., 2006), since implants revealing signs of peri-implantitis contain subgingival microbiota similar to those of natural teeth with periodontitis (Heuer et al., 2007).

It has been suggested that the presence of periodontal bacteria in the subgingival plaque of the remaining dentition may promote colonization of newly incorporated implants and influence their fate (Danser et al., 1997). Further, mucosal surfaces may function as a source of reinfection for the periodontal tissues after treatment (Danser et al., 1996).

To date, there has been limited research regarding biofilms in the oral cavities of edentulous patients. As we wish to evaluate whether elderly edentulous subjects show different profiles of colonization of periodontal pathogens as compared with elderly dentate subjects, the purpose of this study was to compare the prevalence of *Campylobacter rectus*, *Prophyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia*, *Tannerella forsythia*, *Treponema denticola*, *Eikenella corrodens* and *Micromonas micros* in the studied populations.

MATERIALS AND METHODS

Human subjects

In this cross-sectional study, we recruited thirty elderly subjects (mean age 61.7±7.05) with teeth and thirty elderly subjects (mean age 65.8±8.69) with no teeth, from the Department of Dentistry in the University of Taubaté (São Paulo, Brazil).

Data and personal information related to medical and dental histories of the subjects were obtained from responses by individuals to our questionnaire. All subjects signed an Informed Consent form, which was previously approved by the Institutional Committee on Research Involving Human Subjects of the University of Taubaté (protocol 109/08).

Exclusion criteria

Subjects presenting with uncontrolled systemic diseases, subjects who were immunocompromised, subjects who had taken antibiotics within six months prior to the clinical and microbial examination, and subjects who were wearing prosthetics were not included in the study.

For the dentate group, subjects with a need for antibiotic prophylaxis for dental treatment, subjects who had been undergoing periodontal treatment 12 months prior to the beginning of the study, and subjects who had no first or second molars and incisors or who had fewer than three molars or only one incisor remaining were also excluded.

Clinical measurements

For elderly patients without teeth, the oral examination was performed by visual inspection. To determine periodontal status of the elderly patients with teeth, the clinical examination was comprised of the Visible Plaque Index (VPI) and Gingival Bleeding Index (GBI), according to Ainamo & Bay (1975); in addition, we measured Periodontal Probing Depth (PPD) and Clinical Attachment Level (CAL). One trained and calibrated examiner conducted all clinical measurements and collected the microbial samples. The calibration protocol for the optimization of intra-examiner difference followed methods similar to those published by Araujo et al. (2003). Baseline data analysis was performed to determine if the intra-examiner reliability was calibrated. Using kappa statistics (*K*) for the categorical clinical measurement variables, such as PPD and CAL, the standard error of these measurements was monitored. The examiner's clinical measurement technique was considered calibrated if the standard error for the measurements was ≤0.8 and had a *K* value that ranged from between 0.8 and 0.95.

Sampling of microorganisms

Microbial samples of the left side of the cheek mucosa and the dorsum of the tongue were obtained from all subjects. These samples were taken from areas of

approximately 1cm^2 using a swab with a reduced Ringer's solution (Oxoid Ltd., Basingstoke, Hampshire, UK), rotated six times. Each swab was transferred into a microtube containing reduced Ringer's solution (1ml). In addition, a pooled subgingival sample was collected from each subject with teeth from the mesio-buccal aspect of the first molars (n=4 molars/subject) and the mesial incisors (n=2 incisors/subject) using sterile paper points inserted to the depth of the sulcus after the removal of supragingival plaque using sterile curettes. For subjects without the aforementioned teeth (i.e., first molars and mesial incisors), microbial samples were obtained from second molars and/or lateral incisors. The paper points were removed sixty seconds after being placed into the sulcus and immediately placed in a microtube containing reduced Ringer's solution.

Bacterium-specific PCR

The bacterial cells in the microtube were dispersed using a vortex mixer at maximal setting for one minute and then maintained at -80^{O}C until laboratory processing. The presence of *A. actinomycetemcomitans, P. gingivalis, P. intermedia, T. forsythia, C. rectus, E. corrodens, T. denticola* and *M. micros* was determined by polymerase chain reaction (PCR), as described below.

The bacterial suspensions were thawed and centrifuged at 12,000 x R.P.M. for 3 min, and the DNA was extracted from the bacterial pellet using a DNA isolation kit, following the manufacturer's instructions (InstaGene purification matrix; BioRad

Laboratories, Hercules, CA). PCR reaction was carried out using 10µl of the sample and 15µl of reaction mixture containing 2.5µl of 10 X PCR buffer (Promega, Madison, WI), 1.25 units of *Taq* DNA polymerase (Promega), and 0.2mM of each of the deoxyribonucleotides (Pharmacia LKB, Piscataway, NJ). PCR amplification was performed using a thermal cycler (Perkin-Elmer, Wellesley, MA). The bacterium-specific primer (5'-3') sequences used in this study are shown in Table 1.

Table 1- Bacteria-specific primers and PCR-product sizes (base pairs)

Bacteria	Primers	Base Pairs
A. actinomycetemcomitans	5'AAACCCATCTCTGAGTTCTTCTTC3' 5'ATGCCAACTTGACGTTAAAT3' Ashimoto et al. (1996)	550
P. gingivalis	5'-AGGCAGCTTGCCATACTGCGG-3' 5'-ACTGTTAGCAACTACCGATGT-3' Slots et al. (1995)	404
P. intermedia	5'-TTTGTTGGGGAGTAAAGCGGG-3' 5'-TCAACATCTCTGTATCTGCGT-3' Ashimoto et al. (1996)	575
T. forsythia	5'-GCGTATGTAACCTGCCCGCA-3' 5'-TGCTTCAGTGTCAGTTATACCT-3' Slots et al. (1995)	641
C. rectus	5'-TTTCGGAGCGTAAACTCCTTTT-3' 5'-TTTCTGCAAGCAGACACTCTT-3' Slots et al. (1995)	598
E. corrodens	5'- CTACTAAGCAATCAAGTTGCCC-3' 5'-CTAATACCGCATACGTCCTAAG-3' Slots et al. (1995)	688
T. denticola	5'-TAATACCGAATGTGCTCATTTACAT-3' 5'-TCAAAGAAGCATTCCCTCTTCTTCTTA-3' Slots et al. (1995)	316
M. micros	5'-AGTGGGATAGCCGTTGGAAA-3' 5'-GACGCGAGCCCTTCTTACAC-3' Access # U60326.1	328

PCR products were separated in a 1.5% agarose gel (Sigma, Dorset, United Kingdom) by electrophoresis performed at 5V/cm in Tris-acetate-EDTA buffer

(Promega). The DNA bands on the gel were stained with 0.5% µg/ml ethidium bromide (Amersham, Arlington Heights, IL) and photographed under 300-nm UV light illumination.

Statistical Analysis

The frequencies of bacterial species on the dorsum of the tongue and cheek mucosa were analyzed using the Chi-Square test (X^2). In addition, we estimated the odds ratios for the presence of periodontal pathogen colonization according to the presence of teeth for the dentate group. All tests were performed using statistical software (BioEstat 5.0, Belém, Pará, Brazil). Results were statistically significant at *P* values <0.05.

RESULTS

Both groups had evidence of colonization by all of the studied bacteria. The characteristics of the included population by age and gender are shown in Table 2. In general, the periodontal profile of the studied dentate population did not include active periodontal disease (Table 3).

Table-2. Distribution of population according to age and gender

	With teeth	Without teeth	Total
Male	11	14	25
Female	19	16	35
Total (ma±sd)	30 (61.7±7.05)	30 (65.8±8.69)	60 (63.8±8.05)

ma= mean age; sd= standard deviation

Table-3. Clinical parameters of dentate group

	VPI	GBI	PPD	CAL
Mean value	0.43	0.39	2.79	3.26
Standard deviation	± 0.38	± 0.28	± 0.66	± 1.59

VPI= visible plaque índex; GBI= gingival bleeding índex; PPD= periodontal probing deph; CAL= clinical attachment level

When the extra-sulcus samples (i.e., samples from the cheek mucosa and dorsum of the tongue) were analyzed together, most of the frequencies were significantly higher for the dentate group (Figure 1).

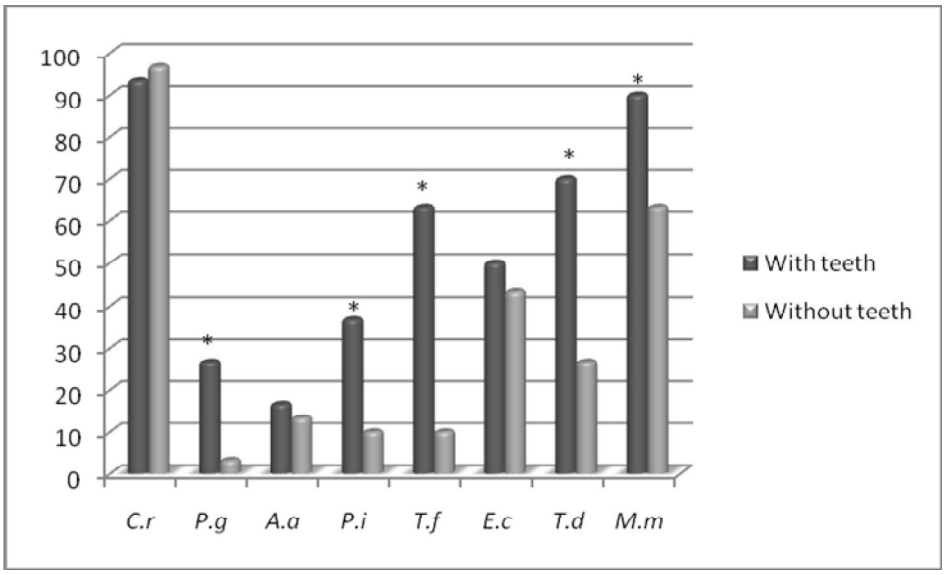

Figure 1- Comparison of extra-sulcus samples (cheek mucosa + dorsum of the tongue) among groups. *Statistically significant difference (*P*<0.05; Chi Square test)

When we compared cheek mucosa samples between both groups, *P. intermedia* (*P*=0.030) and *T. denticola* (*P*=0.0001)were more prevalent, for the group with teeth. When samples from the dorsum of the tongue between both groups were analyzed, *P. gingivalis* and *T. forsythia* were present in higher frequencies in the group with teeth (Tables 4 and 5, respectively).

Table 4- Comparison of the prevalence of various bacteria in cheek mucosa among groups

	C.r	*P.g*	*A.a*	*P.i*	*T.f*	*E.c*	*T.d*	*M.m*
With teeth	66.67	10.00	0.00	26.67	20.00	23.33	66.67	36.67
Without teeth	60.00	3.33	6.67	3.33	3.33	23.33	10.00	33.33
P-value	0.592	0.604	0.472	0.030*	0.107	-	0.001*	0.786

*Statistical difference. Chi Square test

Table 5- Comparison of prevalence of various bacteria on dorsum of the tongue among groups

	C.r	*P.g*	*A.a*	*P.i*	*T.f*	*E.c*	*T.d*	*M.m*
With teeth	80.00	26.67	16.67	30.00	56.67	39.29	53.57	57.14
Without teeth	83.33	3.33	13.33	10.00	10.00	40.00	26.67	40.00
P-value	0.738	0.030*	1.00	0.052	0.001*	0.790	0.063	0.300

*Statistical difference, Chi Square test

When we analyzed the frequencies of the eight periodontal pathogens in sulcus samples from the dentate group, the prevalences were as follows: *C. rectus* 90.00%, *P. gingivalis* 46.67%, *A. actinomycetemcomitans* 16.67%, *P. intermedia* 36.67%, *T. forsythia* 73.33%, *E. corrodens* 51.72%, *T. denticola* 86.21% and *M. micros* 62.07%.

Finally, we attempted to establish the odds of the presence of each studied pathogen according to the presence of teeth among the dentate group. There were no statistically significant odds ratios for any of the pathogens (Table 6).

Table 6- Risk of having periodontal pathogen according to the presence of teeth

	C.r	*P.g*	*A.a*	*P.i*	*T.f*	*E.c*	*T.d*	*M.m*
OR	0.310	25.370	1.300	5.210	24.750	1.300	13.750	0.860
P value	0.604	0.001	1.000	0.032	0.001	0.795	0.001	1.000
CI <	0.030	3.050	0.312	1.278	5.855	0.473	3.917	0.306
CI >	3.167	211.112	5.404	21.237	104.608	3.614	48.266	2.460

OR= Odds ratio, CI= confidence interval. Chi-square test

DISCUSSION

The oral cavity has many ecologically distinct sites for bacterial colonization, including the teeth, with its sulcus areas that provide a favorable environment for anaerobic species. However, the complete loss of teeth represents a breakdown in this microbial habitat, resulting in changes in the periodontal bacterial flora. However, such loss of teeth does not completely eliminate the occurrence of bacterial colonization. Considering future treatments of edentulous patients with implant therapy, we attempted to study the presence of several periodontal pathogens among the edentulous in order to establish the potential risk of bacteria colonizing the surfaces of the implants on installation.

Sachdeo et al. (2008) analyzed biofilms in the edentulous oral cavity. One of the major findings in their study was their detection of periodontal pathogens in significant numbers within that population, a surprising result as these species were thought to disappear after removal of all natural teeth. Further, they reported that samples from the dorsal surfaces of the tongue exhibited the highest bacterial counts, while the lowest mean counts were found in samples from the buccal mucosa. A larger number of bacteria would likely adhere to the dorsum of the tongue due to papillae providing an increased surface area and possibly a more consistently moist environment. A similar finding was previously reported by Lee et al. (1999) who detected higher levels of different species from the dorsum of the tongue samples.

In our study, all of the putative periodontal pathogens were present in both groups, even in the absence of teeth. Five of the eight investigated bacteria were

statistically more prevalent in the dentate group (Figure 1). Thus, periodontal pathogens can colonize cheek mucosa and/or the dorsum of the tongue irrespective of the presence of teeth, which may be a potential source for species colonizing future new implants.

Based on calculated odds ratios, the presence of teeth was not associated with significantly higher risk for periodontal pathogen colonization, as expressed in Table 6.

In our research, the frequencies of pathogens present on the dorsum of the tongue from edentulous subjects for *C. rectus*, *P. gingivalis*, *E. corrodens, T. forsythia* and *T. denticola* were, respectively, 83.33%, 3.33%, 40.00%, 10.00% and 26.67% (Table 5). For the same species, Lee et al. (1999) studied pre- and post-implantation microbiota of the tongue, teeth, and newly-placed implants, using whole genomic DNA probes in a checkerboard assay. In samples from the tongue, they found prevalences for the same bacteria of approximately 50%, 25%, 63%, 25% and 13%, respectively. Variations in percentages between our study and Lee's study may have occurred due to differences in techniques. Those authors also concluded that species levels from tongue samples were higher than those from teeth and implants, although species prevalence was similar, suggesting that larger samples were obtained from the dorsum of the tongue.

Griffen et al. (1998) analyzed the prevalence of *P. ginginvalis* in sulcus samples of a periodontally healthy population and found a prevalence of 25% using a PCR method. They concluded that this species may not be a normal inhabitant of periodontally healthy dentition. In our study, we found a higher prevalence of this pathogen (46.67%), but our population was not exclusively healthy, despite presenting a generally healthy profile. On the other hand, van Winkelhoff et al. (2000) investigated the occurrence of *P.*

gingivalis in subjects with mean age 52.8 (±8.4) and found sulcus prevalence of this pathogen to be 15%, using anaerobic culture, a less sensitive technique than PCR.

We found the prevalence of *A. actinomycetemcomitans* to be approximately 13.33% in edentulous subjects not wearing dentures, exactly the same value related by Devides et al. (2006) for a population without teeth. Könönen et al. (1991) and Danser et al. (1994, 1995) did not find it in any of the samples collected from edentulous subjects using a culture technique and thus concluded that this bacterium may not be a part of the normal flora of edentulous subjects wearing dentures. When samples from the sulcus were analyzed in a dentate population, we found a prevalence of 16.67% of that bacterium, which is a result not dissimilar to that of van Winkelhoff et al. (2000), who detected a prevalence of 10% in subjects of similar age.

In our study, we observed that the frequency of *P. intermedia* in edentulous patients was 10%. In a study by Danser et al. (1994), the authors investigated the short-term effect of full-mouth extraction on periodontal pathogens colonizing the oral mucous membranes. They detected *P. intermedia* in all subjects that had been colonized with this pathogen prior to full-mouth extraction and concluded that this species can colonize the oral mucous membranes of edentulous subjects, irrespective of the presence of a subgingival microflora. A possible reason for the disagreement between our study results and those in Danser's study is that Danser et al. performed microbiological examinations at one and three months after tooth extractions, while our population had been edentulous for at least 12 months. Devides et al. (2006) reported *P. intermedia* prevalence of 46.7% in a completely edentulous population using PCR, however, their

samples were collected by rubbing the mucosa with three standardized sterile paper points.

Studying the prevalence of detectable putative periodontal pathogens in subgingival samples of elderly patients, van Winkelhoff et al. (2000) found *M. micros* and *T. forsythia* in frequencies of 50 and 45%, respectively. In our study, we found, from the same site, prevalences of 62.07% and 73.33%, for the same bacteria. For extra-sulcus samples in a similar population, they detected 20% of *M. micros* and 25% of *T. forsythia*, prevalences lower than the frequencies of 90 and 63.33%, respectively, that we obtained in our study. Those authors detected the presence and numbers of the putative periodontal pathogens on anaerobic blood Agar plates, while we used PCR.

C. rectus showed the highest prevalences in all sites for both studied groups, according to Cortelli et al. (2008). The author concluded that this bacterium remained in high levels irrespective of the presence of teeth.

In our research, we found *A. actinomycetemcomitans* and *P. ginginvalis* in edentulous subjects, which contrasts with prior reports in the literature that suggested that these species disappeared from the oral cavity after extraction of all teeth and did not reappear even when hard surfaces were provided (Danser et al., 1994-1997). In both of these studies, the authors used culture as the microbiologic identification method, as this the bacteria must be alive and viable, which is different from PCR. Devides et al., (2006), did not find *P. gingivalis* in edentulous subjects, although, after implant placement, the authors found that bacterium was detected with a frequency of 46.7%. Devides et al., (2006) also detected *P. intermedia* before extraction but could not find it shortly after full mouth extraction. Upon placement of the dental implants, however, *P. intermedia* was detected again in low percentages, suggesting that the absence of this bacterium shortly

after extraction may reflect only a temporary event (Danser et al., 1995). These data support that oral membranes can be a source for species colonization of new implants and individuals colonized with these pathogens should be identified by microbiologic methods in order to establish the risk of developing peri-implantitis. The primary source of colonization for dental implants in edentulous patients is the oral mucous membranes (Danser et al., 1997), which is of great importance given our finding of periodontal pathogens in the edentulous oral cavity. This has major implications regarding future dental treatment and the general health of individuals (Sachdeo et al., 2008).

In conclusion, the absence of teeth reduced, but did not eliminate the periodontal pathogens. Thus, care must be taken to maintain oral health given this finding that periodontal pathogens colonize mouth sites irrespective of the presence of teeth and may also colonize subsequent oral implants.

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3 CONCLUSÕES

Os dados aqui apresentados permitem concluir que uma considerável variedade de bactérias periodontopatogênicas podem ser encontradas nas diversas faixas etárias, incluindo desde recém-nascidos desdentados, crianças, adultos/idosos e até idosos desdentados. A presença do elemento dental esteve associada a uma maior prevalência bacteriana, porém, apesar do sulco/bolsa periodontal representar o habitat preferido de colonização, patógenos periodontais foram também observados nas mucosas lingual e jugal da população estudada.

A partir destes achados podemos destacar que além dos cuidados com crianças e adultos/idosos dentados, uma atenção também deve ser dispensada para os indivíduos desdentados. Particularmente aos recém-nascidos, pois estes em um curto espaço de tempo estarão recebendo os primeiros dentes decíduos, e aos idosos desdentados, pois estes já contam com a possibilidade de uma melhor qualidade de vida principalmente pela restauração da função mastigatória obtida pela colocação dos implantes dentais. Percebendo-se que tanto nos recém-nascidos, a partir da erupção dental, quanto nos idosos pela instalação dos implantes, os microrganismos periodontais encontrarão seu habitat preferido. Logo, os profissionais de saúde bucal devem contemplar medidas preventivas eficazes nestas populações minimizando os possíveis riscos futuros periodontais ou perimplantares.

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APÊNDICES

APÊNDICE A - FICHA DE ANAMNESE E AVALIAÇÃO PERIODONTAL

UNIVERSIDADE DE TAUBATÉ
DEPARTAMENTO DE ODONTOLOGIA

Nome do paciente _____Número:_____

Nascimento___/___/___Idade_____ Gênero _____

Endereço_____

Cidade_____ Cep _____ Fone: ( ) _____

Profissão_____Estado Civil _____

R.G.: _____ C.I.C.: _____

Data da coleta ___/___/ ___

Há quanto tempo foi a sua última consulta médica?_____

Qual o motivo?_____Médico: _____

No momento está fazendo algum tratamento médico?_____

Está tomando algum medicamento? Sim ( ) Não ( )

NOME	DOSAGEM	TEMPO DE USO

Tem sensibilidade a algum anestésico ou alergia a algum medicamento:_____

Pressão arterial:_____ Fuma?_____ Quantidade. cigarros/dia:_____

Ex.- fumante ?_____ Tempo que fumou:_____ Há quanto tempo parou?

_____ Está grávida? _____Quantos meses?_____

Usa fio dental? Sim ( ) Não ( )

Quantas vezes você escova os dentes por dia? _____Você já passou por um tratamento periodontal? _____ Há quanto tempo?_____

OBSERVAÇÕES

_____

_____

_____

_____

Ficha pava avaliação periodontal

		18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
V	PS																
	PIC																
	PLACA																
	SANGRAMENTO																
L	PS																
	PIC																
	PLACA																
	SANGRAMENTO																
		48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38
V	PS																
	PIC																
	PLACA																
	SANGRAMENTO																
L	PS																
	PIC																
	PLACA																
	SANGRAMENTO																

APÊNDICE B - TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Título: O IMPACTO DA PRESENÇA DENTAL NA COLONIZAÇÃO DE PERIODONTOPATÓGENOS NA CAVIDADE BUCAL

Responsável: Prof. Dr. José Roberto Cortelli

JUSTIFICATIVA

A doença de gengiva é uma infecção causada por bactérias (germes) encontradas na boca. Estas bactérias fazem com que a gengiva fique inchada, vermelha, podendo sangrar. A gengiva pode também retrair (afastar) e então aparece a raiz do dente. Estas bactérias causam mau hálito, tártaro e podem deixar alguns dentes com mobilidade. A doença de gengiva acontece mais em adultos, mas pode também acontecer em crianças ou jovens. Geralmente, mais de uma pessoa na família tem problema de gengiva. Assim, é importante saber quem tem a doença de gengiva e que bactéria a pessoa tem na boca para evitar esses problemas ou tratá-los o mais rápido possível. Se o dentista sabe qual bactéria a pessoa tem, ele também pode passar um remédio (antibiótico) que ajuda no tratamento.

Este estudo tem por objetivo examinar recém-nascidos, crianças, adolescentes, adultos e idosos para saber se estas pessoas apresentam doença de gengiva e se elas têm na boca bactérias chamadas *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, Campylobacter rectus, Eikenella corrodens, Treponema denticola* e *Micomonas micros*. Todos os dentes serão examinados e será coletado um pouco de líquido da bochecha e língua com um

cotonete de algodão ou da gengiva com um cone de papel. O exame leva aproximadamente dez minutos, não dói e não causa qualquer problema. Se a pessoa não quiser participar deste estudo será feito apenas um exame mais rápido para ver que tipo de tratamento dentário ela precisa. Se a pessoa quiser participar, mas durante o exame não gostar de alguma coisa é só falar com o dentista que ele irá parar na mesma hora. O tratamento dentário será realizado de qualquer maneira mesmo que não queira participar do trabalho.

Observação: O Termo de Consentimento Livre e Esclarecido nos casos das crianças e menores de 18 anos deverá obrigatoriamente ser assinado por um dos pais ou pelo seu responsável legal.

CUSTO E PAGAMENTO

Não haverá nenhum custo para participar deste trabalho.

SIGILO

Caso você aceite participar deste estudo será feito um cadastro numa ficha que pertence ao professor responsável pela pesquisa. Será mantido segredo do seu nome e não será divulgado o seu nome em trabalhos apresentados na faculdade, congressos etc.

INDENIZAÇÃO E DANOS

Você deve saber que a coleta do líquido da bochecha e língua com um cotonete de algodão ou da gengiva com um cone de papel não vai causar nenhum dano a você, assim, não haverá qualquer tipo de pagamento pelo exame.

CONSENTIMENTO VOLUNTÁRIO

Você deve ter entendido tudo o que leu. Uma cópia deste Termo será entregue para você e outra ficará arquivada com o professor responsável. A assinatura abaixo significa que você concorda em participar deste estudo.

DECLARAÇÃO

Eu,.....

Nacionalidade....., Nascido (a) em / /

Na cidade de....., estado de.....

Portador da cédula de identidade número.....

Residente a .....

.....

Declaro ter sido inteiramente esclarecido sobre o estudo e ter lido e entendido o termo que estou assinando abaixo.

_____ _____

Assinatura do paciente Assinatura do responsável

Taubaté, ____de _____ 200___.

ANEXOS

ANEXO A - COMPROVANTE DE SUBMISSÃO DE ARTIGO

Dear Dr Camila Borges Fernandes

JMM paper no. JMM/2009/011361: CHANGES OF PREVALENCE OF PERIODONTAL PATHOGENS IN NEWBORNS AND CHILDREN.

Thank you for submitting your paper to JMM. It has been assigned the reference number shown above. Please quote this number in any correspondence.Your paper is being considered for publication by Professor David Beighton ("David Beighton" <david.beighton@kcl.ac.uk>), who will inform you of the decision in due course. If you have any queries about the progress of your paper prior to receiving the decision please contact Professor Beighton. If you are asked to revise the paper, please submit your revised manuscript via the Bench>Press system.Your paper will be reviewed on the understanding that all the authors have agreed to the submission and to the order of their names on the title page. They must also have agreed that you, as corresponding author, have their agreement to act on their behalf throughout the editorial review and publication process. You are responsible for obtaining such agreement.If your paper is accepted for publication, it is a condition of acceptance that you assign copyright to the Society for General Microbiology (the URL for the copyright assignment form will be sent to you at the appropriate stage).We welcome the submission of striking pictures, preferably in colour, for possible use on the front cover of Journal of Medical Microbiology. Pictures need not be linked with a paper in the journal. We will pay GBP75 towards expenses for any pictures used.

Yours sincerely

Dr Melanie Scourfield

ANEXO B - COMPROVANTE DE SUBMISSÃO DE ARTIGO

31-Mar-2009

Dear Dr. Fernandes:

Your manuscript entitled "DO ELDERLY EDENTULOUS PATIENTS HARBOR PERIODONTAL PATHOGENS?" has been successfully submitted online and is presently being given full consideration for publication in Clinical Oral Implants Research.

Your manuscript ID is COIR-Mar-09-OR-1090.

Please mention the above manuscript ID in all future correspondence or when calling the office for questions. If there are any changes in your street address or e-mail address, please log in to Manuscript Central at http://mc.manuscriptcentral.com/coir and edit your user information as appropriate.

You can also view the status of your manuscript at any time by checking your Author Center after logging in to http://mc.manuscriptcentral.com/coir .

Thank you for submitting your manuscript to Clinical Oral Implants Research.

Sincerely,
Clinical Oral Implants Research Editorial Office

Camila Borges Fernandes

Taubaté, abril de 2009.

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