UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

Rogério Heládio Lopes Motta

Cirurgião-Dentista

Prevalência, resistência e patogenicidade de *Staphylococcus aureus* colhidos no ambiente clínico odontológico.

Tese apresentada à Faculdade de Odontologia de Piracicaba, da Universidade Estadual de Campinas, para obtenção do grau de Doutor em Odontologia, Área de Farmacologia, Anestesiologia e Terapêutica do Programa de Pós-Graduação em Odontologia.

PIRACICABA – SP
2005

UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

Rogério Heládio Lopes Motta

Cirurgião-Dentista

Prevalência, resistência e patogenicidade de *Staphylococcus aureus* colhidos no ambiente clínico odontológico.

Tese apresentada à Faculdade de Odontologia de Piracicaba, da Universidade Estadual de Campinas, para obtenção do grau de Doutor em Odontologia, Área de Farmacologia, Anestesiologia e Terapêutica do Programa de Pós-Graduação em Odontologia.

Orientador:
Prof. Dr. Thales Rocha de Mattos Filho

Co-orientador:
Prof. Dr. Francisco Carlos Groppo

Banca Examinadora:
Prof. Dr. Carlos Eduardo Pulz Araújo
Prof. Dr. Fernando de Sá Del Fiol
Prof. Dr. Márcio de Moraes
Prof. Dr. Thales Rocha de Mattos Filho
Prof. Dr. Vitoldo Antonio Kozlowski Jr.

PIRACICABA – SP
2005

FICHA CATALOGRÁFICA ELABORADA PELA
BIBLIOTECA DA FACULDADE DE ODONTOLOGIA DE PIRACICABA
Bibliotecário: Marilene Girello – CRB-8ª. / 6159

M858p

Motta, Rogério Heládio Lopes.
Prevalência, resistência e patogenicidade de *Staphylococcus aureus* colhidos no ambiente clínico odontológico. / Rogério Heládio Lopes Motta. -- Piracicaba, SP : [s.n.], 2005.

Orientadores: Thales Rocha de Mattos Filho, Francisco Carlos Groppo
Tese (Doutorado) – Universidade Estadual de Campinas, Faculdade de Odontologia de Piracicaba.

1. Prevenção e controle. 2. Isolamento e purificação. 3. Crescimento e Desenvolvimento. 4. Patogenicidade. I. Mattos Filho, Thales Rocha de. II. Groppo, Francisco Carlos. III. Universidade Estadual de Campinas. Faculdade de Odontologia de Piracicaba. IV. Título.

(mg/fop)

Título em inglês: Prevalence, resistance and pathogenicity of *Staphylococcus aureus* isolated from dental clinic environment.
Palavras-chave em inglês (*Keywords*): Prevention and control; Isolation and purification; Growth and development; Pathogenicity
Área de concentração: Farmacologia, Anestesiologia e Terapêutica
Titulação: Doutor em Odontologia
Banca examinadora: Thales Rocha de Mattos Filho; Carlos Eduardo Pulz Araújo; Fernando de Sá Del Fiol; Márcio de Moraes; Vitoldo Antonio Kozlowski Jr.
Data da defesa: 17/02/2005

Dedicatória

A **DEUS PAI**, pela vida...

A meus pais queridos, **CARMELINDA** e **HELÁDIO**, pelo amor, carinho, dedicação, doação e sem os quais essa jornada não teria começado...

A **CRISTIANE**, minha noiva, pelo amor, carinho, compreensão e por você ser tão **VOCÊ...**

A minha irmã **LUZIA,** e meu cunhado **MARCO**, amigos e conselheiros de todas as horas...

A meu irmão **ALESSANDRO (*in memorian*)**, por ser meu guia...

A minha avó **APARECIDA**, por tantos ensinamentos e conselhos...

A **PAULA** e **TINHO**, pela convivência e crescimento juntos...

A meus tios **ROSA, MARIA, CACILDA, ELENICE, BENIGNA (*in memorian*), RENATO, SÉRGIO, IRINEU (*in memorian*)** e ao amigo **NIVALDO (*in memorian*)...** pelo apoio e por tudo o que foram, são e sempre serão para mim...

A **CAMILA**, **MAIARA**, **THAÍSA** e **BENTO**....

A minha **FAMÍLIA...**

Ao **MENINO**...

A família **FARMACOLOGIA** da **FOP/UNICAMP**, pelo carinho e por me ajudar nesse contínuo processo de "lapidação" ...

dedico este trabalho

Agradecimentos Especiais

Ao **Prof. Dr. THALES ROCHA DE MATTOS FILHO**, meu grande amigo e orientador, agradeço pela orientação, pela compreensão, pelo incentivo, pela confiança, pelos conselhos de todas as horas e por tornar-me uma pessoa melhor. Muito obrigado por tudo.

Ao **Prof. Dr. FRANCISCO CARLOS GROPPO**, meu co-orientador: não tive a oportunidade de conhecer meu irmão Alessandro Francisco, mas a vida me presenteou com um irmão chamado Francisco. Muito obrigado por, entre tantas e outras, ensinar-me a "Metodologia de Chico Modificada" para viver a minha vida.

Ao **Prof. Dr. JOSÉ RANALI,** pela amizade, dedicação, confiança, ensinamentos, oportunidades, e por fazer-me crer que muito podemos fazer por aqueles que em nada crêem.

Ao **Prof. Dr. EDUARDO DIAS DE ANDRADE**, pela amizade, dedicação, confiança, ensinamentos, oportunidades e pelas aulas inesquecíveis.

A **Profa. Dra. MARIA CRISTINA VOLPATO,** pela amizade, dedicação, confiança, ensinamentos, risadas e pelas palavras amigas e encorajadoras nos momentos mais difíceis.

Ao **Prof. Dr. PEDRO LUIZ ROSALEN,** pela amizade, dedicação, confiança, ensinamentos, oportunidades, e pelo auxílio sempre presente dentro e fora do laboratório.

A **MARIA ELISA DOS SANTOS, ELIANE MELO FRANCO** e **JOSÉ CARLOS GREGÓRIO,** pela convivência, ajuda, prontidão, e acima de tudo, a amizade.

A minha irmã de dedos **JULIANA,** e ao meu "velho" grande amigo-irmão **SINVALDO**, companheiros de todas as horas: obrigado por me suportarem, pelo auxílio na fase experimental deste trabalho e pelo apoio incondicional....

A **MÔNICA, FRANCISQUINHO E SOFIA,** pela grande amizade e convivência.

Aos meus "amigos-irmãos" farmacológicos: **GIOVANA**, **REGIANE, SIMONE, ROBERTA, MARCELO, GÍLSON, RAFINHA, LUCIANA, ANA PAULA, RODRIGO, HUMBERTO, FILIPE, TAMINATO, ALCIDES, MARCOS, KARINA, MYRELLA, LUCIANA, CAROL, GUGA, MICHELLE, FERNANDO, ALINE, FLÁVIA, PATRÍCIA, VANESSA, FÁBIO, RAMIRO, PAULO, FABIANO, CARINA, JÉSSICA, CARINA, FERNANDA, GUSTAVO, LEANDRO, FELIPE, BRUNO, KARLA** e aos "amigos-irmãos" não-farmacológicos: **ANDRÉ, PATRIQUE, LEANDRO, RONALDO, EDIVANDRO, KÁTIA, LUCIANA, REGIANE, GUTO, MARIA, SEU TONINHO, DONA MÁRCIA, DIGUINHO, ADRIANO, JABÁ, DEISE, MARCELO, ROSANA, FRANZ, MARLA, CLÉBER, CLEVINHO, DANIEL, RICARDO, JOÃO, BRUNO, GUILHERME, HENRIQUE, NILESH, TAQUEO, FRUTOSE, ANDRÉ, WAGNER, HERMETO & JULIANA, RAFAEL, ED, ROGÉRIO, MURILO, NEWTON, FERNANDO, SEU LEANDRO, SEU ANTONIO,** e tantos outros **AMIGOS**, por toda a ajuda, pelos bons momentos e pela amizade.

A **JORGE VALÉRIO** e sua esposa **JULIANA,** pela eterna ajuda em todos os momentos e pela grande amizade, que acredito também será eterna.

Aos amigos do **AMAZONAS**.

Agradecimentos

A Universidade Estadual de Campinas, UNICAMP, na pessoa do Magnífico Reitor, **PROF. DR. CARLOS HENRIQUE DE BRITO CRUZ** e à Faculdade de Odontologia de Piracicaba, FOP, por meio do Diretor **PROF. DR. THALES ROCHA DE MATTOS FILHO**.

Ao **PROF. DR. PEDRO LUIZ ROSALEN**, coordenador dos Cursos de Pós-Graduação da Faculdade de Odontologia de Piracicaba, Universidade Estadual de Campinas.

Ao **PROF. DR. FRANCISCO CARLOS GROPPO**, coordenador do Programa de Pós-Graduação em Odontologia da Faculdade de Odontologia de Piracicaba, Universidade Estadual de Campinas.

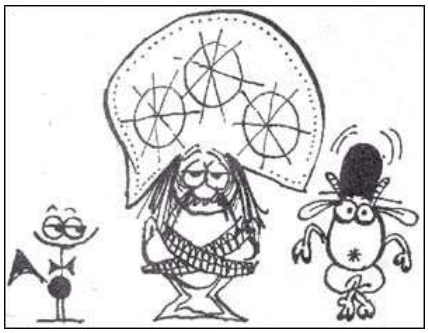
A **CAPES**, Centro de Aperfeiçoamento de Pessoal Especializado, pelo apoio financeiro.

A **ÉRICA ALESSANDRA PINHO**, secretária da Coordenadoria do Programa de Pós-Graduação da FOP-UNICAMP, pela amizade e pelos auxílios nesses anos;

A **HELOÍSA MARIA CECCOTI e MARILENE GIRELLO**, bibliotecárias da Faculdade de Odontologia de Piracicaba, pela amizade e preciosa ajuda;

Aos amigos **MARCOS ANTONIO CAVALLARI JÚNIOR** e **PAULO ROBERTO RIZZO DO AMARAL**, pela ajuda e convivência.

Aos professores, alunos e funcionários da Faculdade de Odontologia de Piracicaba.



"Se não houver frutos, valeu a beleza das flores; se não houver flores, valeu a sombra das folhas; se não houver folhas, valeu a intenção da semente."

Henfil

Sumário

Resumo

Os objetivos destes estudos foram: avaliar a contaminação de superfícies de equipamentos antes, durante e após procedimentos clínicos odontológicos; determinar a quantidade e a susceptibilidade de *Staphylococcus aureus* colhidos em ambiente clínico odontológico e avaliar a patogenicidade de infecções estafilocócicas induzidas por *S. aureus* ATCC 25923 sensível à penicilina (PSSA) e *S. aureus* meticilina resistente (MRSA) isolado em ambiente clínico odontológico e observar a eficácia da amoxicilina e vancomicina contra esses microrganismos em um modelo de infecção em ratos. **Material e Métodos:** Foram colhidas amostras através de swabs esterilizados de superficies de diversos equipamentos: botão de cadeira odontológica, seringa tríplice, cone de raio-X, tecla "Enter" de computadores, maçanetas de porta e alças de refletores, antes, durante e depois de procedimentos clínicos. Essas amostras foram inoculadas em BHI agar, incubadas a 37^{o}C por 24 horas, contadas e classificadas usando técnica de Gram e testes bioquímicos. Discos de papel contendo diversos antimicrobianos foram usados no teste de susceptibilidade antimicrobiana para as cepas isoladas de *S. aureus*. As unidades formadoras de colônias (UFC) foram contadas e analisadas atráves do testes de Kruskal-Wallis e Dunn (*alpha*=5%). Para o modelo de infecção, 4 esponjas de poliuretano foram colocadas no dorso de 180 ratos. Após 14 dias, dois tecidos granulomatosos receberam 0.5ml de 10^{8} ufc/ml de PSSA e MRSA. Dois dias depois, os ratos foram divididos em seis grupos e os antimicrobianos foram administrados. Após 6, 24, e 48 horas da administração das drogas, 10 ratos de cada grupo e período foram sacrificados. Os tecidos granulomatosos infectados foram removidos e transferidos para tubos tipo eppendorf com 1 ml de 0.9% NaCl e pesados. Os tecidos infectados foram então sonicados e inoculados em Salt Mannitol Agar. Após a incubação por 24h a 37^{o}C, as colônias foram contadas manualmente. As contagens de UFC e os pesos dos tecidos foram submetidos ao teste de ANOVA multifatorial (alfa=5%). **Resultados:** Um aumento no número de microrganismos foi observado durante os procedimentos clínicos ($p<0.05$). As maiores taxas de resistência antimicrobiana foram observadas em relação aos beta-lactâmicos. Todas as cepas de *S. aureus* foram sensíveis a vancomicina e 2% das amostras foram resistentes à meticilina.

Não houve diferença estatisticamente significativa entre os pesos dos tecidos não infectados ($p>0.05$). Os grupos com tecidos infectados por MRSA mostraram os maiores valores de peso de tecido e de contagem de microrganismos ($p<0,05$). As cepas (PSSA ou MRSA) não afetaram as concentrações séricas ou nos tecidos não-infectados ($p>0,05$), mas reduziram significativamente a concentração de amoxicilina no tecido infectado ($p<0,05$). **Conclusões:** Os procedimentos clínicos odontológicos podem aumentar a contaminação ambiental e conseqüentemente a proporção de *S. aureus* multirresistentes no ambiente clínico odontológico. A amoxicilina em dose única não foi capaz de interferir na infecção induzida pela cepa de *S. aureus* sensível à penicilina. A cepa de *S. aureus* resistente à meticilina, isolada do ambiente clínico, mostrou uma alta patogenicidade em comparação com a cepa sensível à penicilina e, além disso, a mesma não foi erradicada por uma dose única de vancomicina.

Palavras-Chave: contaminação ambiental, *Staphylococcus aureus*, contaminação cruzada, infecção estafilocócica, modelos *in vivo*.

Abstract

The purposes of these studies were to verify environmental contamination by collecting microorganisms from dental equipment surfaces before, during and after clinical procedures, to determine the number and the susceptibility of *Staphylococcus aureus* collected in a dental clinical environment, to evaluate the pathogenicity of staphylococcal infection induced by a *Staphylococcus aureus* ATCC 25923 strain (S1) and a methicillin-resistant *Staphylococcus aureus* (S2) strain isolated from dental clinic environment and to observe the efficacy of amoxicillin and vancomycin against these microorganisms in an infection model in rats. **Material and Methods:** Cotton sterile swabs were used to collect the samples from dental-chair push buttons, 3-in-1 syringes, X-ray tubes, computer keys, doorknobs, and light handles before, during and after clinical procedures. These samples were spread on BHI agar, incubated at 37°C for 24 hours, counted and classified using the Gram staining and biochemical tests. Commercial paper disks containing widely prescribed antimicrobial agents were used to perform the antimicrobial susceptibility tests for *S. aureus*. The counts among the periods and groups were analyzed by Kruskal-Wallis and Dunn tests (alpha=5%). For infection model, 4 polyurethane sponges were placed in the back of 180 rats. After 14 days, two granulomatous tissues received 0.5 ml of 10^8 cfu/ml (S1) and (S2). Two days later, the rats were divided into six groups and the drugs were administered. After 6, 24, and 48 hours of drug administration, 10 rats of each group and in each period were killed. Each infected granulomatous tissue sample was then removed, placed in eppendorfs with 1 ml of 0.9% NaCl and weighed. Infected tissues were dispersed in a sonic system and were spread on salt mannitol agar. After incubation for 24h at 37°C, the colonies were counted using a manual colony counter. Microorganim counts and wet-weight results were submitted to the Kruskal–Wallis test (alpha=5%). **Results:** An increase in the number of microorganisms was observed during clinical procedures ($p<0.05$). The highest bacterial resistance rates for *S. aureus* were observed for beta-lactam group. All *S. aureus* strains were sensitive to vancomycin and two percent of the samples were resistant to methicillin. No statistically significant difference ($p>0.05$) was observed among the wet weights of non-infected tissues. The groups 2 and 6 showed the higher wet-weight values

and microorganism counts (p<0,05). Both drugs were quantified only at 6-hours after their administration. The strains (PSSA or MRSA) did not affect the serum or the non-infected tissue concentrations (p>0.05) but significantly reduced the amoxicillin concentration in the infected tissue (p<0.05). **Conclusions:** Clinical activities increased the number of microorganisms and the proportion of resistant *S. aureus* dispersed in dental clinical environment. A single dose of amoxicillin did not affect the curse of infection induced by a penicillin-susceptible strain. The MRSA strain isolated from a clinical environment showed high pathogenicity in comparison to a penicillin-susceptible *S. aureus* strain and it was not eradicate by a single dose of vancomycin.

Key words: environmental contamination, *Staphylococcus aureus*, cross-contamination, staphylococcal infection, *in vivo* models.

1. Introdução

Nas últimas décadas, o controle de contaminação cruzada nos consultórios odontológicos tem sido um grande desafio. Durante séculos, os profissionais de Odontologia realizaram seus trabalhos inconscientes dos riscos de contaminação inerentes à sua prática, até que se compreendeu que as infecções poderiam ser transmitidas no ambiente clínico odontológico (SAMARANAYAKE *et al*., 1995; WARREN *et al.*, 2001).

A introdução de instrumentos de alta rotação e ultra-sônicos, na década de 50, contribuiu significativamente para que os consultórios fossem contaminados por aerossóis (COTTONE *et al.*, 1991). Nas clínicas das escolas de Odontologia, onde profissionais e pacientes encontram-se envolvidos com trabalhos clínicos simultaneamente, este risco assume um significado especial (MILLER *et al*., 1990; MATTOS-FILHO *et al*., 2005).

Segundo KEDJARUNE *et al.* (2000), os aerossóis constituem-se de partículas as quais têm massa e energia cinética suficiente para realizarem longas trajetórias no ambiente de clínica odontológica, contaminando objetos e equipamentos mesmo que distantes da cadeira odontológica. As infecções por aerossóis em clínicas odontológicas podem ser causadas por várias fontes, incluindo pacientes, profissionais, visitantes, ventilação e sistema de ar-condicionado (KEDJARUNE & LEGGAT, 2001).

BENTLEY *et al.*, (1994) relatam que a distribuição dos aerossóis contaminados por bactérias é extremamente variada e pode estar influenciada por diferentes fatores, tais como os níveis de microrganismos na boca do paciente (higiene bucal adequada), a posição de trabalho do profissional em relação ao paciente, o tipo de procedimento realizado, a posição do dente que está sendo tratado, a movimentação de pessoas dentro do ambiente de

clínica, entre outros. Segundo KEDJARUNE & LEGGAT, 2001, essa distribuição também pode ser influenciada pela umidade, temperatura e o tamanho das partículas geradas. Além desses fatores, os autores relatam que o período do dia pode influenciar a contaminação dos aerossóis.

CRAWFORD (1983) relata que as partículas produzidas pelo uso de equipamentos rotatórios permanecem viáveis no ambiente (Quadro 1). GUIMARÃES JR. (2001) apresenta dados importantes a respeito da sobrevivência de alguns microrganismos sobre superfícies, mostrando que uma grande variedade deles consegue sobreviver por tempo prolongado em diversos materiais de uso rotineiro em odontologia, como fichas clínicas, peças de mão, papel, gaze, pele e luvas.

QUADRO 1 - Distribuição do microrganismo, fonte/procedência e viabilidade no ambiente.

Microrganismo	Fonte/Procedência	Viabilidade
Staphylococcus aureus	Saliva, pele, exsudato	5 dias
Streptococcus pyogenes	Saliva, secreções	2 dias
Mycobacterium tuberculosis	Escarro	2 Semanas
Vírus Herpes simplex	Saliva, vesícula	Minutos
Vírus Herpes zoster	Saliva, vesícula	Horas
Epstein Barr	Saliva	Horas
Vírus Influenza (gripe)	Saliva, secreções	12 horas
Vírus Hepatite A	Saliva, sangue, urina	Semanas
Vírus Hepatite B	Saliva, sangue	Semanas
Vírus HIV – AIDS	Sangue	Minutos
Grupo mutans – cárie	Saliva	Horas

Fonte – Crawford., 1983

Dentre as várias espécies de microrganismos encontrados na cavidade bucal, os *Staphylococcus aureus* têm sido considerados um dos mais versáteis e perigosos patógenos humanos (HONMA *et al.*,1994). Além da capacidade de sobreviver em superfícies de diferentes ambientes por um período de até 5 dias, esses microrganismos têm demonstrado

um preocupante aumento de resistência aos antimicrobianos, tais como a meticilina e vancomicina (OIE *et al.*,2002; SINGH *et al.*,2002).

Staphylococcus aureus meticilina resistentes (*MRSA)* demonstram taxas significativas de mortalidade em pacientes idosos e em pacientes imuno-comprometidos, sendo menos prevalentes e fatais em pacientes jovens. Não são freqüentemente encontrados em hospedeiros saudáveis, entretanto, quando isolados podem ser um indicador de debilidade clínica do paciente (BRADLEY, 1999). Embora tenham uma alta relevância clínica, não existem relatos na literatura avaliando modelos de infecções *in vivo* induzidas por esses microrganismos quando isolados em ambientes clínicos.

Diversos modelos de infecção em tecidos de ratos têm sido utilizados (DEL FIOL *et al.*,2000). A indução de tecido granulomatoso, por exemplo, tem sido proposta para avaliar a infecção e a concentração tecidual de antimicrobianos em ratos (GROPPO *et al.*,2000). Além disto, este método tem demonstrado reprodutibilidade e eficácia (BAGLIE *et al.*,2000).

A amoxicilina e a vancomicina têm sido usadas em modelos de infecção com *Staphylococcus aureus* para avaliar a farmacocinética desses antimicrobianos em tecidos e suas interferências no desenvolvimento de processos infecciosos (GERBER *et al.*,1993; VANDAUX *et al.*,2002). Dentre diversas metodologias, a cromatografia líquida de alta eficiência (CLAE) tem demonstrado ser confiável e muito eficiente para a quantificação desses antimicrobianos (PIRES DE ABREU *et al.*,2003; SAITO *et al.*,2004).

2. Proposição

Foram objetivos deste trabalho:

- Investigar o grau de contaminação de utensílios, materiais e equipamentos utilizados na clínica de Graduação da Faculdade de Odontologia de Piracicaba – FOP/UNICAMP, determinando os microrganismos mais prevalentes e os períodos e os locais de maior possibilidade de quebra da cadeia asséptica;

- Investigar o grau de contaminação de utensílios, materiais e equipamentos utilizados na clínica de Graduação da Faculdade de Odontologia de Piracicaba – FOP/UNICAMP por *Staphylococcus aureus* e estudar o grau de resistência bacteriana destes microrganismos frente a diferentes antimicrobianos.

- Avaliar a patogenicidade de uma cepa de *Staphylococcus aureus* meticilina resistente isolada no ambiente clínico da FOP-Unicamp e uma cepa de *Staphylococcus aureus* ATCC 25923, observando a eficácia da amoxicilina e vancomicina contra esses microrganismos em um modelo de infecção em ratos.

3. Capítulos

Essa tese está baseada na Informação CCPG/001/98/Unicamp que regulamente o formato alternativo para tese de Doutorado e permite a inserção de artigos científicos de autoria ou co-autoria do candidato.

Desta forma, esta tese é composta de três artigos, os quais foram submetidos ou encontram-se em fase de submissão para publicação em revistas científicas, conforme descrito abaixo:

3.1. – Artigo 1 - "Environmental Contamination Before, During and After Dental Treatment." , artigo aceito para publicação na *American Journal of Dentistry*.

3.2. – Artigo 2 – "Isolation of Methicilin-resistant *Staphylococcus aureus* in a Dental Clinic Environment.", o qual foi submetido para publicação na *Infection Control and Hospital Epidemiology*.

3.3. – Artigo 3 – "The effect of amoxicillin and vancomycin against MRSA infection. *In vivo* study in rats.", o qual foi submetido para publicação na *Antimicrobial Agents and Chemoterapy*".

3.1. Title: **Environmental Contamination Before, During and After Dental Treatment**

Running Title: **Environmental Contamination in Dentistry**

Thales Rocha de Mattos-Filho[a]* , DDS, MSc, PhD, Chairman

Francisco Carlos Groppo[a] , DDS, MSc, PhD, Associate Professor

Juliana Cama Ramacciato[a] , DDS, MSc, PhD

Aline de Barros Nóbrega Dias Pacheco[a] , DDS, MSc, PhD

Rogério Heládio Lopes Motta[a] , DDS, MSc, PhD

[a] *Department of Physiological Sciences, Area of Pharmacology, Dentistry School of Piracicaba, UNICAMP, Brazil*

*Corresponding author:

Rogério Heládio Lopes Motta

Department of Physiologic Sciences

Dental School of Piracicaba, University of Campinas,

Av Limeira # 901, Areião – CEP (zip code)13.414-903

Piracicaba, São Paulo, Brazil.

Phone: 55 1934125310 Fax: 55 1934210144

e-mail:rogeriomotta@fop.unicamp.br

De: godoy@nova.edu
Enviado em: sexta-feira, 2 de abril de 2004 00:16
Para: rogeriomotta@fop.unicamp.br
Assunto: Paper Accepted

Dr. Motta:

I am pleased to inform you that your paper "Environmental contamination before, during and after dental treatment" has been accepted for publication
in the American Journal of Dentistry.

Before publication you will receive page proofs for your approval.

Sincerely,

Prof. Dr. Franklin Garcia-Godoy
Editor, American Journal of Dentistry
Professor and Associate Dean for Research
Director, Bioscience Research Center
Director, Biomaterials Research Center
Director, Clinical Research Center
College of Dental Medicine
Nova Southeastern University
3200 South University Drive
Fort Lauderdale, Florida 33328

Tel: (954) 262-7373
Fax: (954) 888-9137
E-mail: godoy@nsu.nova.edu

Title: Environmental Contamination Before, During and After Dental Treatment

Summary

Purpose: to verify environmental contamination by collecting microorganisms from dental equipment surfaces before, during and after clinical procedures. **Setting:** Undergraduate clinic of the Dental School of Piracicaba, University of Campinas, Brazil. **Materials and Methods:** Sterile swabs were used to collect the samples from dental-chair push buttons, 3-in-1 syringes, X-ray tubes, computer keys, doorknobs, and light handles before (P1), during (P2) and after (P3) clinical procedures. These samples were spread on BHI agar and incubated at 37^{o}C for 24 hours. The resulting microorganisms were counted and classified using the Gram staining and biochemical tests. Microorganism counts among the periods and groups were analyzed by Kruskal-Wallis test (alpha=5%). **Results:** The most prevalent microorganisms were *Viridans* group streptococci*, Sthaphylococcus epidermidis* and *Baccillus subtilis*. Push buttons were the most contaminated (p<0.05). Microorganisms were most prevalent during clinical procedures (p<0.05). Microorganism counts were higher in P2 than in P1 and P3 (p<0.05). **Conclusion:** Clinical activity caused an increase in the number of environmental microorganisms, where *Viridans* group streptococci were the most prevalent contaminant found on equipment surfaces.

Key words: environmental contamination, viridans group streptococci, cross-contamination, dentistry, infection control.

Clinical significance: *Considering the risk of cross-contamination in dental settings caused by microorganisms spread in fluids such as blood and saliva during dental procedures, studies involving clinical environmental contamination are still necessary.*

Introduction

Cross-contamination in dental settings caused by viruses, bacteria, and fungi present in fluids such as blood and saliva has been of great concern to dental professionals during the last decades. [1,2] Microorganisms which cause respiratory and blood diseases are found in dental equipment and can be hazardous for dental patients and staff when infection-control routines are not done effectively. [3] Although it is well known that air, surfaces, dental materials and instruments, and water in dental units could be vehicles for cross contamination in dental settings, more research in microbial contamination involving this environment is needed. [4]

Some studies have been performed in order to verify the bacterial contamination of air samples in dental offices collected during dental procedures. [5,6,7] These studies concluded that infectious aerosols might be generated during dental practice, especially when high-speed handpieces or ultrasonic scalers are used. The presence of potentially pathogenic microorganisms such as *Legionella pneumophila*, and *Pseudomonas aeruginosa* has been related in the water of dental units. [8,9] High bacteriological contamination of water and air as well as a widespread bacterial contamination were often found during a survey. [4]

The implementation of barriers and aseptic conditions usually reduces cross-contamination risks, which are considerably high in dental schools due to the presence of professionals and patients simultaneously involved in the treatment. [10] Barrier systems remarkably reduced the number of streptococci in the air of waiting room in a dental clinic. [11] Organizations such as Center of Disease Control and Prevention, the American Dental Association, schools of dentistry, many other health agencies and professional associations supported the effectiveness of procedures to control infection and universal precautions in the dental settings to prevent microbial pollution and cross-contamination. Since the end of

the 1980s many surveys have been carried out in several countries to investigate practices to control infection and compliance with universal precautions in dental procedures. [4]

Contamination of surfaces and instruments in dental procedures by hepatitis B surface antigen and hepatitis C virus has been related. [12] However, data on microbial contamination of surfaces or instruments in dental surgeries are scarce. Specific environmental microorganisms found in dental settings have been suggested as an indicator of infection control. *Viridans* group streptococci, which are prevalent in human saliva, have been proposed as a biological indicator of contamination of dental equipment surfaces. [3]

The aim of the present study was, therefore, to evaluate the contamination level of dental equipment surfaces in a dental school (undergraduate clinic of the Dental School of Piracicaba). Three different periods - prior to, during, and after clinical procedures - were evaluated. The most prevalent microorganisms were also determined.

Methods

The present study was carried out in the undergraduate Clinic of the Dental School of Piracicaba, University of Campinas (UNICAMP), São Paulo, Brazil. Samples were obtained from surfaces of equipment and objects involved in dental treatment. Surfaces were sampled considering six groups as follows:

Group 1 – Dental chair push buttons (n=10);

Group 2 – Light device handles (n=10);

Group 3 - 3-in-1 syringe buttons (n=10);

Group 4 - X-ray tubes (n=2);

Group 5 - Door handle of the main entrance (n=2);

Group 6 - Computer “enter” keys (n=4).

Sampling was carried out prior to clinical activities (5:30 am), during clinical activities (between 2:00 and 3:00 pm) and one hour after clinical procedures (6:30 pm). The cleaning procedures in the clinic were performed at the end of the day (7:00-9:00 pm) by a specialized team. The first procedure before the start of clinical activities was the samples collection. After that, the usual cleaning and disinfecting procedures were carried out by the students.

Three different environments were evaluated: adults' clinic, pediatric clinic, and emergency service clinic. All equipment analyzed in the present study complied with the symmetric distribution of the clinic's air-conditioning system (Figure 1). Since the door handle and the x-ray tubes are the same for all clinical settings, they were not considered for the emergency clinic. "Enter keys" were important to the present study because they are frequently used to register all clinical procedures and data.

Sample Collection

Samples were collected by rubbing the selected surfaces with sterile cotton swabs immersed in 0.1 ml of 0.9% NaCl sterilized solution. Controls for each group were collected by simple exposing the swabs in the air at same time in order to simulate the same procedures of the samples collection. The swabs were rubbed (without twirling) against the equipment surfaces in a three centimetres line through double shuttle movements by just one operator. After that, the swabs were cut into sterile tubes containing 0.9 ml of 0.9% NaCl and immediately assayed.

Microorganism Counting

Five minutes after collection, samples were sonicated at 5% amplitude and 5-second intervals for 60 seconds (Vibra Cell 400W, Sonics & Materials Inc - 5% amplitude,

9.9 second cycle, 6 pulses – Newtown, USA). The resulting solution (10 µL) was inoculated in Petri dishes with 10 mL of BHI agar (Brain Heart Infusion – Difco Co. – Michigan, USA) and incubated at 37°C during 24 h (Fanem Model 002 – Sao Paulo, Brazil). Other dishes containing the same material were incubated at 37°C in 10% CO_2 during 48 h (Jovan IG 150 – Winchester, USA).

After incubation, colonies were quantified with a stereoscopic microscope (Stemi SV6, ZEISS – Thornwood, USA) and their macroscopic characteristics recorded. Results were expressed as cfu x 10^3/mL. Pure cultures collected from isolated colonies were Gram-stained and examined at 1000x magnification (OLYMPUS – Tokyo, Japan). All colonies were submitted to biochemical tests for species identification. [13,14,15]

Statistical Analysis

Microorganism counts, considering each period (prior to, during and following clinical procedures) and each clinical environment (adults, pediatric, and emergency clinics), were submitted to the Kruskal-Wallis and multiple comparison tests at a significance level of 5% (p<0.05).

Results

All surfaces revealed microorganisms' contamination in all periods and clinical environments. Absence of growth was verified in all control groups of every period.

Comparison among periods showed the greatest microorganism counts in the samples collected during clinical activities (p<0.05). Figure 2 shows the microorganism counts means of the groups at different time periods, regardless of clinical environment. No

statistically significant differences were observed among groups evaluated prior to clinical activities ($p>0.05$).

Figure 3 shows the microorganism counts means observed in Group 1 (dental chair push buttons). This group presented the greatest microorganism counts ($p<0.05$) among all groups, which is valid particularly for the emergency clinic.

Groups 2 and 3 showed high microorganism counts during clinical activities ($p<0.05$). Again, the emergency clinic environment presented the highest microorganism counts considering these two groups ($p<0.05$). These results are presented in figures 4 and 5, respectively.

Group 4 presented no statistically significant difference prior to clinical activities when adults and pediatric clinics were compared ($p>0.05$). However, a significant increase in the microorganism counts was observed during and following clinical procedures, regardless of the clinical environment ($p<0.05$) (Fig. 6).

Considering Group 5, no statistically significant differences were observed among periods and environments, except for the microorganism counts at the adults' clinic before activities, which presented the lowest number (Fig. 7).

The pediatric clinic showed the greatest microorganism counts considering Group 6 ($p<0.05$). No statistically significant differences were observed between samples collected prior to or after clinical activities ($p>0.05$), as shown in figure 8.

In general, *Staphyloccocus epidermidis* and viridans streptococci were the most observed microorganisms. Among bacilli genus, *Bacillus subtillis* were the most commonly found. Clinical activities induced more viridans streptococci compared to the other studied periods. Streptococci species emerged in greater number during and after clinical activities

when compared to staphylococci species. Table 1 shows the percentage of microorganisms identified in the three different periods.

Discussion

As previously observed, the present study showed an increase in microbial contamination on surfaces during clinical activities.[5,16] The patients' oral hygiene, aerosols, and the presence of a great number of people (professionals, students and patients) could be a cause of the clinical environment contamination observed in the present study.[6,16,17,18] According to Bentley *et al*., the dentist's position in relation to the patient, and the position of the treated teeth in the mouth may also influence clinical environment contamination.[17]

As expected, the surfaces most frequently handled during treatment showed the highest levels of contamination.[19] Results observed in Group 1 at the emergency clinic during clinical activities could be probably caused by the large number of patients (approximately 12) treated at the same day period. At the same period, approximately three patients were treated in the adults' clinic.

The frequency of use of dental equipment or the kind of the treatment might directly contribute to microbial contamination. Invasive procedures, such as abscess drainage, which is carried out at the emergency clinic probably result in higher contamination than a simple clinical procedure in any clinical environment. [17,20,21]

A high contamination in light handles and 3-in-1 syringes was also observed by McColl *et al*..[22] Adequate routine decontamination procedures of dental equipment are extremely important to remove bacterial deposits, caused by clinical procedures.[23] However, cleaning 3-in-1 syringe buttons is practically impossible, due to a protection overlapping the buttons.

It has been shown that the internal chambers of 3-in-1 syringes are contaminated during routine dental procedures. Sterilized syringes (autoclave-safe) have been suggested in order to avoid any risk of cross-infection.[22,24] However, cross-contamination risk still exists due to mineral and bioburden deposits on the roughness of internal surfaces of metal tips. Thus, it is strong recommended the use of disposable 3-in1 syringe tips in order to decrease this risk.[25,26] The presence of contamination on handles and switches following treatment was anticipated, and the use of protection barriers such as cling film to cover these areas and which can be replaced between patients has been previously suggested.[27]

The contamination of X-ray tubes shows a great possibility of cross-contamination between patients. White & Glaze have reported that 77% of patients were contaminated with *Streptococcus pyogenes* and *S. aureus* after radiograph taking.[28] Another relevant study has shown patients' contamination with pathogenic yeasts and bacterial respiratory pathogens after radiographic examination.[29]

The door handle of the main entrance (Group 5) presented similar contamination in all periods tested in the present study. Although the contamination level observed for group 6 ("enter" keys) was low, it clearly denotes the need for better cross-infection control procedures, especially when involving dental students.[17]

The results observed in the present study were in agreement to Hackney *et al.*, who observed that the presence of *Viridans* streptococci on dental equipment surfaces could be considered a indicator of potential sources of biological contamination.[3] Besides *Viridans* streptococci, a great prevalence of non-oral microorganisms, such as *S. epidermidis*, *S. aureus* and *B. subtilis,* was verified in the present study.

Since the dental environment should be free of oral bacteria after dental procedures, microorganisms should not be found on any of the equipment surfaces. Stricter

decontamination procedures should be implemented in order to control cross-contamination. [17,18,21] Current concepts of cross-infection control such as the use of discardable plastic covers (barriers) over surfaces contaminated during treatment, and sterilization of all other equipment that cannot be protected in another fashion have been advocated. [3,30,31]

The present study concluded that clinical activity increases the number of microorganisms in dental environments, where streptococci were the most prevalent contaminant on equipment surfaces.

Acknowledgments

The authors thank to CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for financial support and Mr. Jorge Valério for his assistance in manuscript preparation.

References

1. Horiba N, Yoshida T, Suzuki K, Maekawa Y, Ito M, Matsumoto T, Nakamura H. Isolation of methicillin-resistant staphylococci in the dental operatory. *J Endod* 1995; 21:21-25.
2. Warren D K, Fraser V J. Infection control measures to limit antimicrobial resistance. *Crit Care Med* 2001;29, n.4 Suppl: N128-N134.
3. Hackney Jr RW, Crawford J J, Tulis JJ. Using a biological indicator to detect potential source of cross-contamination in the dental operatory. *J Am Dent Ass* 1998; 129: 1567-1577.

4. Monarca S, Grottolo M, Renzi D, Paganelli C, Sapelli P, Zerbini I, Nardi G. Evaluation of environmental bacterial contamination and procedures to control cross infection in a sample of Italian dental surgeries. *Occup Environ Med.* 2000; 57: 721-6.
5. Legnani P, Checchi L, Pelliccioni GA, D'Achille C. Atmospheric contamination during dental procedures. *Quintessence Int* 1994; 25: 435-439.
6. Osorio R, Toledano M, Liebana J, Rosales JI, Lozano JA. Environmental microbial contamination. Pilot study in a dental surgery. *Int Dent J* 1995; 45:352-357.
7. Grenier D. Quantitative analysis of bacterial aerosols in two different dental clinic environments. *Appl Environ Microbiol.* 1995;61: 3165-8.
8. Atlas RM, Williams JF, Huntington MK. Legionella contamination of dental-unit waters. *Appl Environ Microbiol.* 1995;61:1208-13.
9. Pankhurst CL, Philpott-Howard JN. The microbiological quality of water in dental chair units. *J Hosp Infect.* 1993; 23:167-74.
10. Palenik C J, Burke F J, Miller CH. Strategies for dental clinic infection control. *Dent Update* 2000; 27: 7-15.
11. Noro A, Suyama Y,Takahashi E, Chattin BR, Hirai Y, Takahashi K, Ishitawa T. Effectiveness of the "clean-area-system" for infection control in the dental clinic. *Bull Tokyo Dent Coll* 1998; 39:15-24.
12. Piazza M, Guadagnino V, Picciotto L, Borgia G, Nappa S. Contamination by hepatitis B surface antigen in dental surgeries. *Br Med J (Clin Res Ed)*. 1987; 22: 473-4.
13. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn Jr. WC. *Color atlas and textbook of diagnostic microbiology*, 5th. ed. Philadelphia: J.B. Lippincott Company; 1997:1465.

14. Hardie JM. Genus Streptococcus. In: Sneath PHA, Mair NS, Sharpe E. *Bergey's Manual Systematic Bacteriology*, 1st ed. Baltimore, MD: Williams & Wilkins; 1986: 1043-1071.

15. Sneath PHA. Endospore-forming Gram-Positive Rods and Cocci. In: Sneath PHA, Mair NS, Sharpe E. *Bergey's Manual Systematic Bacteriology*, 1st ed. Baltimore, MD: Williams & Wilkins; 1986: 1104-1139.

16. Kedjarune U, Kukiattrakoon B, Yapong B, Chowanadisai S, Leggat P. Bacterial aerosols in the dental clinic: effect of time, position and type of treatment. *Int Dent J* 2000; 50:103-107.

17. Bentley CD, Burkhart NW, Crawford JJ. Evaluating spatter and aerosol contamination during dental procedures. *J Am Dent Assoc* 1994; 125:579-584.

18. Kedjarune U, Leggat PA. Bacterial aerosols in the dental clinic: a review. *Int Dent J* 2001; 51:39-44.

19. Shearer B G. Biofilm and the dental office. Review. *J Am Dent Assoc*. 1996; 127:181-189.

20. Checchi L, Montebugnoli L, Samaritani S. Contamination of the turbine air chamber: a risk of cross infection. *J Clin Periodontol* 1998; 25:607-611.

21. Bennett AM, Fulford MR, Walker JT, Bradshaw DJ, Martin MV, Marsh PD. Microbial aerosols in general dental practice. *Br Dent J* 2000; 189: 664-667.

22. McColl E, Bagg J, Winning S. The detection of blood on dental surgery surfaces and equipment following dental hygiene treatment. *Br Dent J* 1994; 176:65-67.

23. Vickery K, Pajkos A, Cossart Y. Evaluation of the effectiveness of decontamination of dental syringes. *Br Dent J* 2000; 189:620-624.

24. Merchant VA, Molinari JA. Study on adequacy of sterilization of air-water syringe tips. *Clin Prev Dent* 1991; 13: 20-22.

25. Martin MV. The air/water syringe: a potential source of microbial contamination. *Br Dent J.* 1998; 28:278-279.

26. Puttaiah R, Cottone JA, Guildersleeve J, Azmoudeh A, Tenney J. Rationale for using single-use disposable air/water syringe tips. *Compend Contin Educ Dent.* 1999;20:1056-1058, 1060, 1063-1064.

27. BDA Advisory Service. The control of cross-infection in dentistry. *British Dental Association Advice Sheet* 1991; A12.

28. White SC, Glaze S. Interpatient microbiological cross-contamination after dental radiographic examination. *J Am Dent Assoc* 1978; 96:801-804.

29. Autio KL, Rosen S, Reynolds NJ, Bright JS. Studies of cross-contamination in the dental clinic. *J Am Dent Assoc* 1980; 100:358-362.

30. Crawford JJ, Broderius C. Control of cross-infection risks in the dental operatory: prevention of water retraction by bur cooling spray systems. *J Am Dent Assoc* 1988; 116:685-687.

31. Kohn WG, Collins AS, Cleveland JL, Harte JA, Eklund KJ, Malvitz DM; Centers for Disease Control and Prevention (CDC). Guidelines for infection control in dental health-care settings – 2003. *M M W R* 2003; 52:1-61.

Tables and Figures

	Prior to	*During to*	*After to*
Staphylococcus epidermidis	***37%***	***8%***	***31%***
Staphylococcus aureus	***8%***	***8%***	***5%***
Bacillus subtilis	***10%***	***10%***	***7%***
Streptococcus pneumoniae	***1%***	***8%***	***8%***
Bacillus cereus	***2%***	***1%***	***1%***
Streptococcus pyogenes	***8%***	***4%***	***4%***
Streptococcus mitis	***13%***	***18%***	***12%***
Streptococcus salivarius	***2%***	***3%***	***1%***
Streptococcus mutans	***0%***	***4%***	***1%***
Streptococcus sanguis	***3%***	***8%***	***8%***
Streptococcus sobrinus	***0%***	***3%***	***0%***
Streptococcus sanguis II	***2%***	***7%***	***4%***
Others	***14%***	***18%***	***18%***
Total number of CFU/period	***3,864***	***14,760***	***10,968***

Table 1 - Percentage of microorganisms identified from all surfaces evaluated in the three different periods. The bottom line refers to total number of microorganisms found in the three different periods, regardless the surfaces evaluated.

Figures

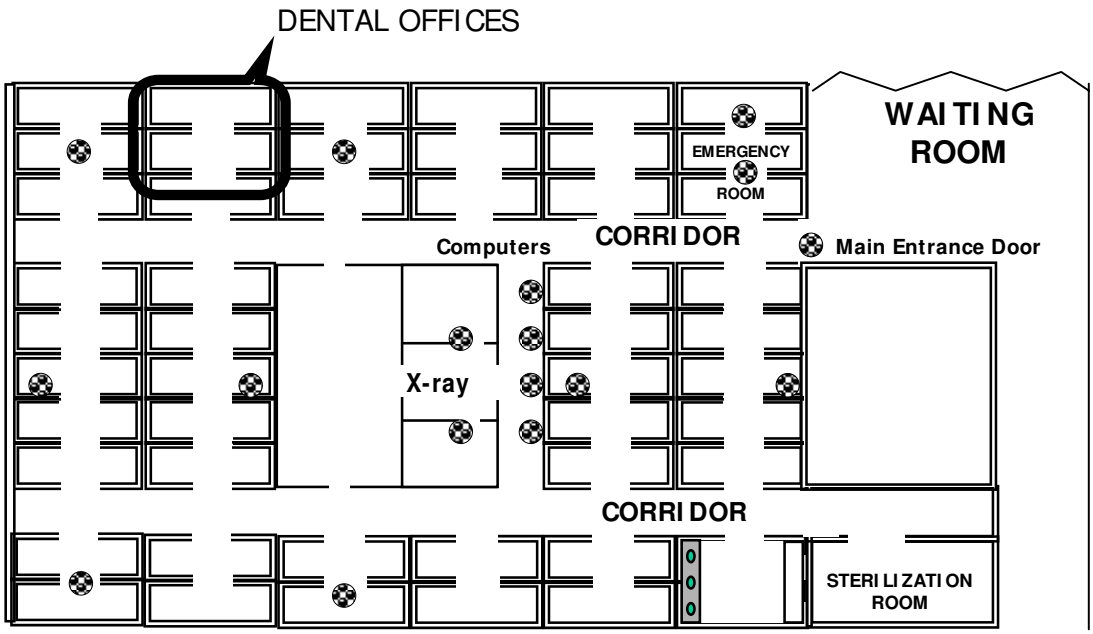


Figure 1 – Disposition of sites where the samples were obtained.

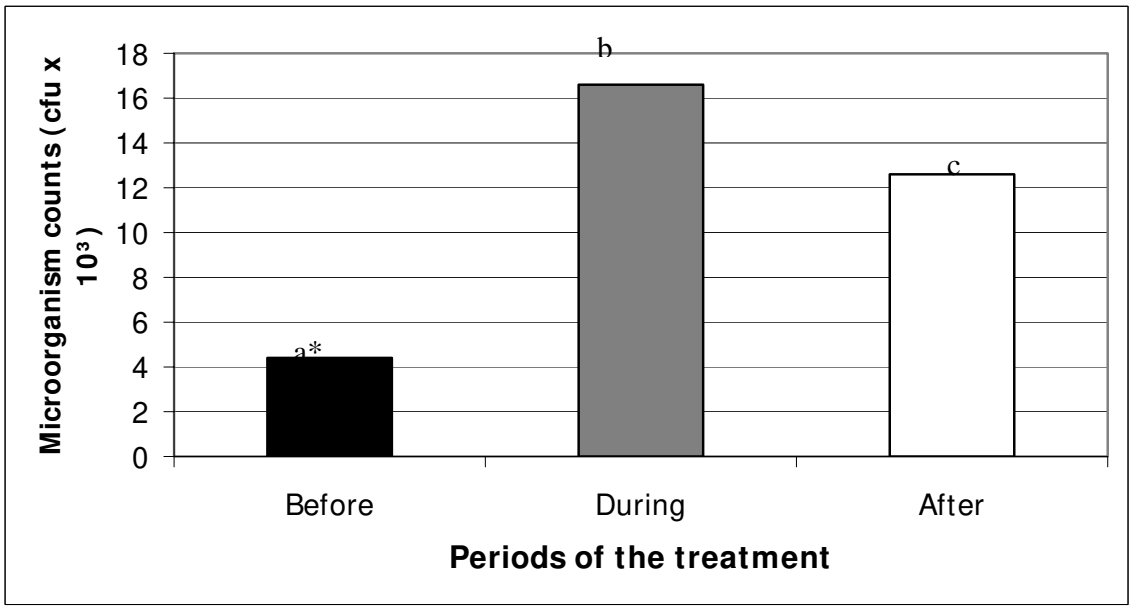


Figure 2 - Means of the microorganism counts for the groups evaluated at different time periods, regardless of clinical environment. *different letters denote statistically significant differences.

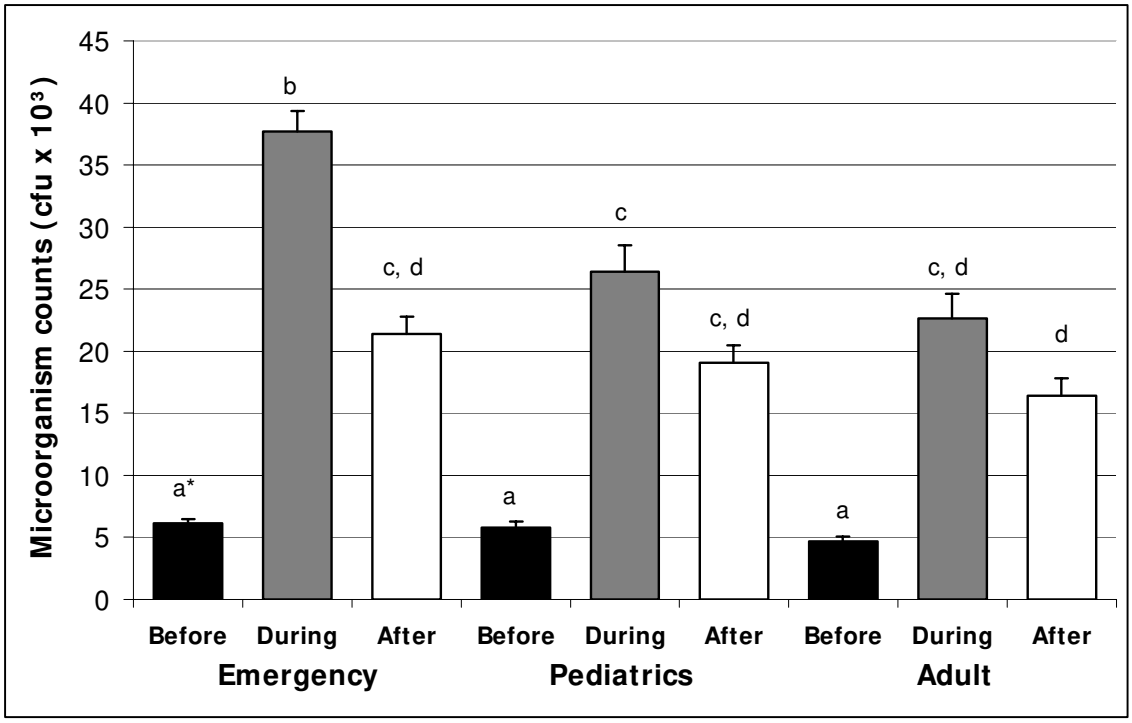


Figure 3 – Colony forming units collected from push buttons, before, during and after clinical procedures. *different letters denote statistically significant differences.

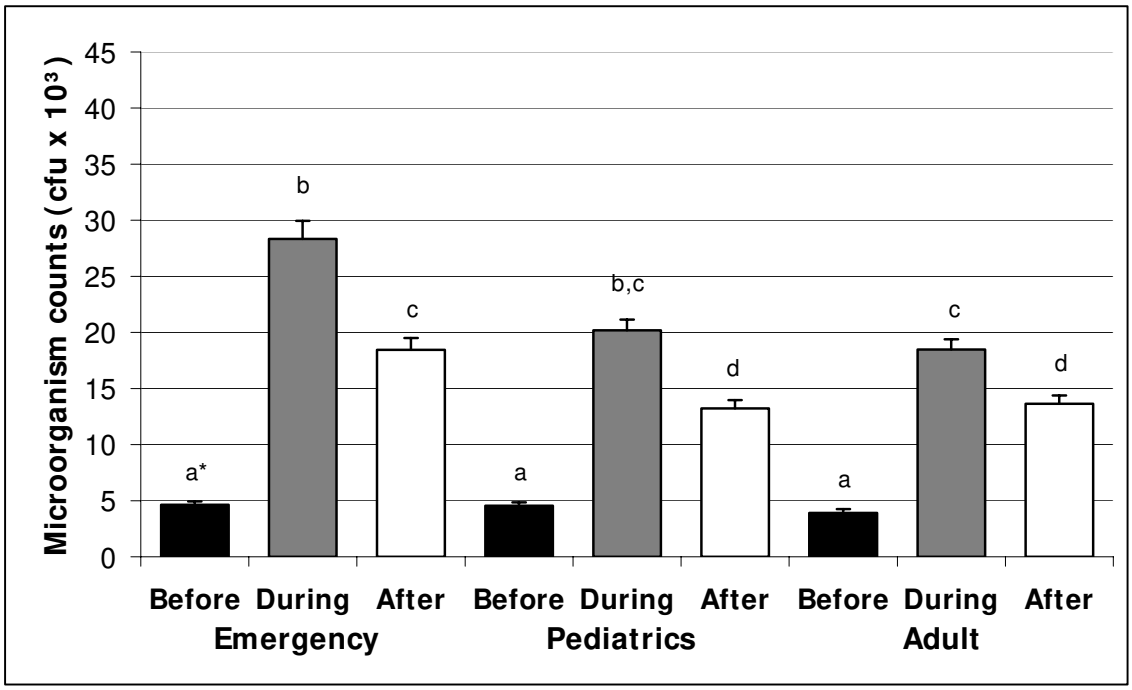


Figure 4 - Colony forming units collected from reflector handles, before, during and after clinical procedures. *different letters denote statistically significant differences.

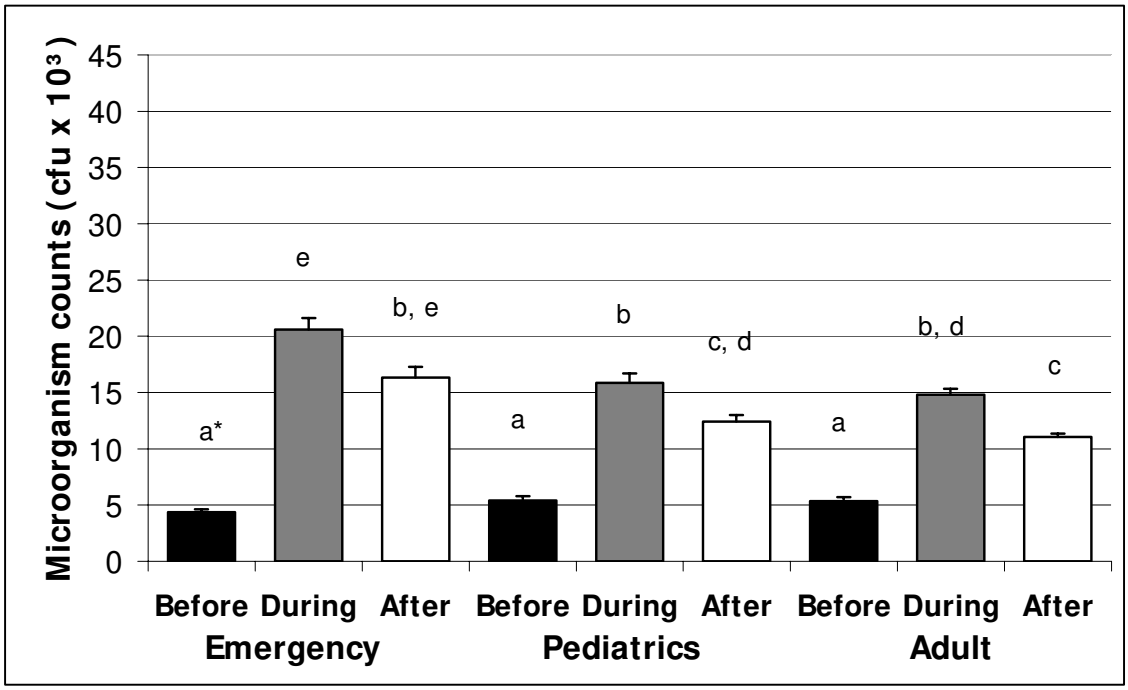


Figure 5 – Colony forming units collected from air-water syringes, before, during and after clinical procedures. *different letters denote statistically significant differences.

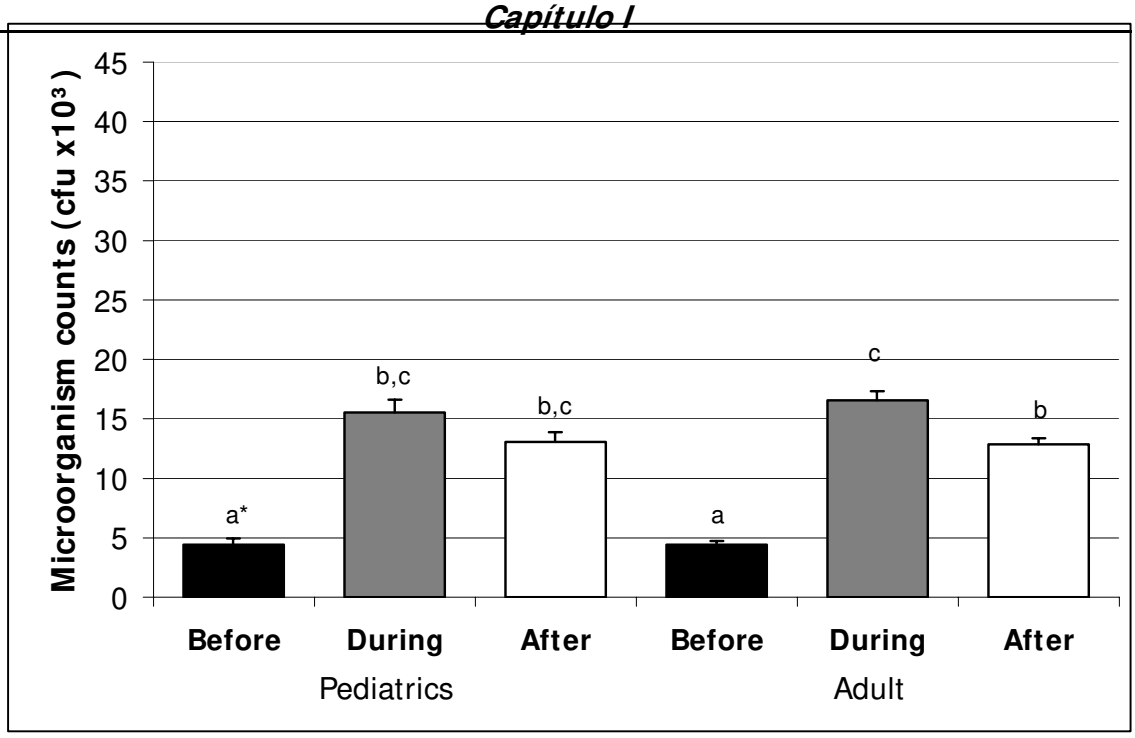


Figure 6 – Colony forming units collected from x-ray devices, before, during and after clinical procedures. *different letters denote statistically significant differences.

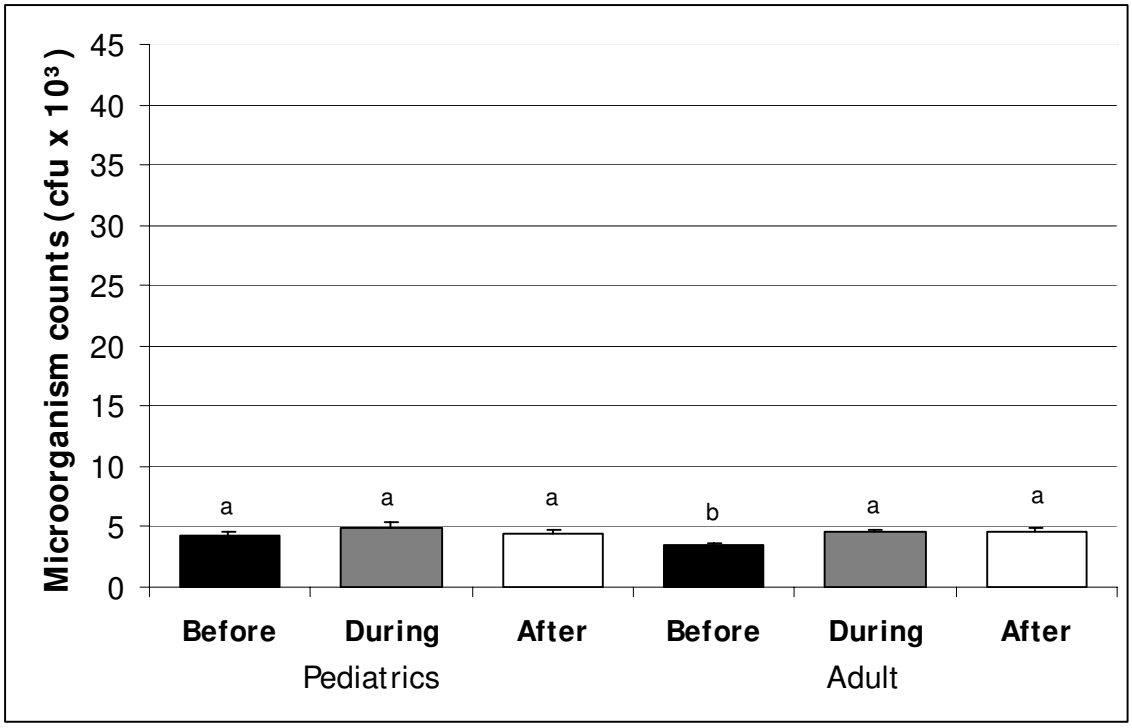


Figure 7 – Colony forming units collected from door handle of the main entrance door, before, during and after clinical procedures.

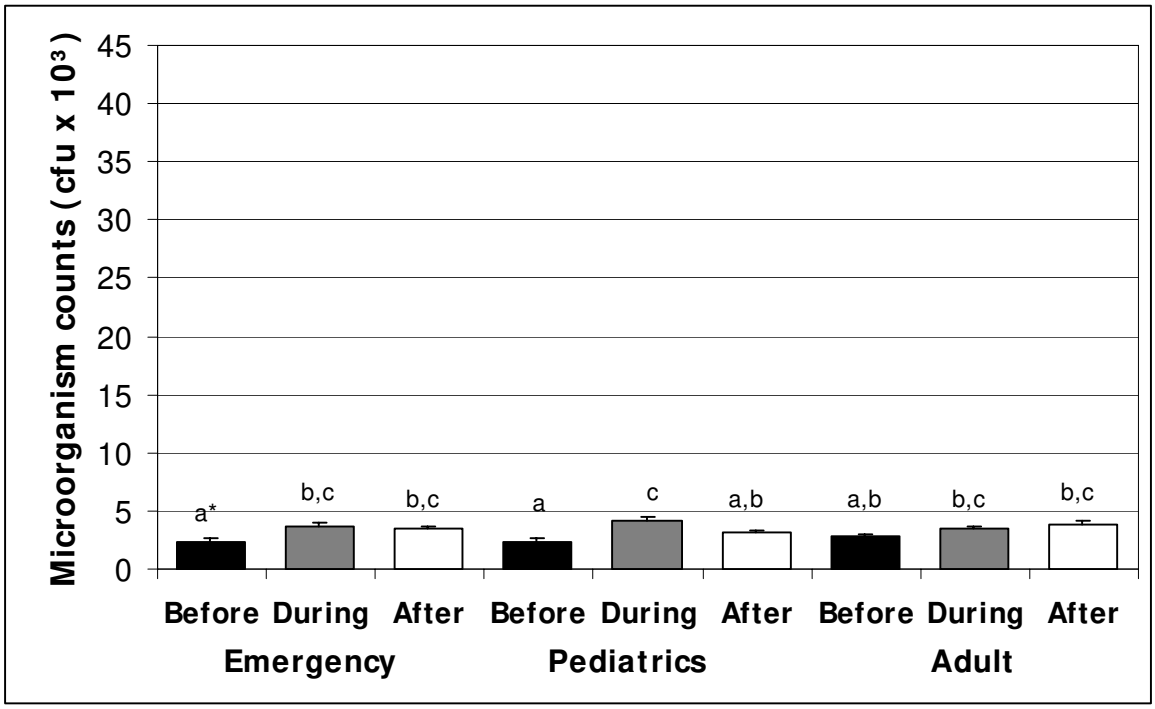


Figure 8 – Colony forming units collected from "Enter" Keys, before, during and after clinical procedures. *different letters denote statistically significant differences.

3.2. Title: **Isolation of Methicilin-resistant *Staphylococcus aureus* in a Dental Clinic Environment**

Rogério Heládio Lopes Motta[a] *, DDS, MSc, PhD

Cristiane de Cássia Bergamaschi, Pharm, MSc Student

Juliana Cama Ramacciato[b] , DDS, MSc, PhD, Assistant Professor

Sinvaldo Baglie[c] , Pharm, MSc, Assistant Professor

Francisco Carlos Groppo[a] , DDS, MSc, PhD, Associate Professor

Thales Rocha de Mattos-Filho[a] , DDS, MSc, PhD, Chairman

[a]*Department of Physiological Sciences, Area of Pharmacology, Dentistry School of Piracicaba - UNICAMP, Piracicaba, Brazil*

[b]*Department of Physiological Sciences, Area of Pharmacology, São Leopoldo Mandic, Campinas, Brazil*

[c] *Department of Pharmaceutical Sciences, Ponta Grossa University, Paraná, Brazil*

*Corresponding author:

Rogério Heládio Lopes Motta - Department of Physiologic Sciences

Dental School of Piracicaba, University of Campinas,

Av Limeira # 901, Areião – CEP (zip code)13.414-903

Piracicaba, São Paulo, Brazil. - Phone: 55 1934125310 Fax: 55 1934210144

e-mail:rogeriomotta@fop.unicamp.br

De: prs_staff@slackinc.com
Enviado em: qui 18/11/2004 17:12
Para: rogeriomotta@fop.unicamp.br
Assunto: Manuscript Submission

You have successfully submitted your manuscript, Isolation of Methicilin-resistant Staphylococcus aureus in a Dental Clinic Environment. Your Manuscript Number is: ICHE-2004-304 Version 1.

Take note of this number for future reference. You can log on at any time to see the current status of your manuscript(s). The web address is http://www.rapidreview.com and your username is rogeriom.

If you wish to contact the journal office, here's how:

Infection Control and Hospital Epidemiology SLACK, Incorporated 6900 Grove Road

Thorofare, NJ 08086 USA

Phone: 856-848-1000 329

Fax: 856-848-6091

Thank you for your submission.

Shirley Strunk, ELS

Managing Editor

Infection Control and Hospital Epidemiology

Isolation of Methicilin-resistant *Staphylococcus aureus* in a Dental Clinic Environment

Abstract

Objective: To determine the number and the susceptibility of *Staphylococcus aureus* collected in a dental clinical environment against antimicrobial agents commonly used in Dentistry. **Setting:** Undergraduate clinic of the Dental School of Piracicaba, University of Campinas, Brazil. **Material and Methods:** Cotton sterile swabs were used to collect the samples from dental-chair push buttons, 3-in-1 syringes, X-ray tubes, computer keys, doorknobs, and light handles before (P1), during (P2) and after (P3) clinical procedures. These samples were spread on BHI agar and incubated at 37°C for 24 hours. The resulting *S. aureus* were counted and classified using the Gram staining and biochemical tests. The counts among the periods and groups were analyzed by Kruskal-Wallis and Dunn tests (alpha=5%). Commercial paper disks containing widely prescribed antimicrobial agents (beta-lactams, macrolides, clindamycin and vancomycin) were used to perform the antimicrobial susceptibility tests. **Results:** An increase in the number of microorganisms was observed during clinical procedures ($p<0.05$). The highest bacterial resistance rates were observed for beta-lactam group. All isolated strains were sensitive to vancomycin and 2% of the samples were resistant to methicillin. **Conclusions:** Clinical activities increased the number and the proportion of resistant *S. aureus* dispersed in dental clinical environment. The present study highlights the need to establish strategies to prevent emergence of resistant bacterial strains in dental settings.

Key words: environmental contamination, *Staphylococcus aureus*, cross-contamination, dentistry, infection control.

Introduction

Cross-contamination in dental settings caused by viruses, bacteria, and fungi present in fluids such as blood and saliva has been of great concern to dental practitioners in the last decades. [1] It is a well known fact that air, surfaces, dental materials, instruments, and water in dental units could be vehicles for cross contamination in dental settings; therefore, more research on microbial contamination by aerosols involving this environment is still necessary.[2]

Some studies have reported that the aerosols provided during dental treatment may contain microorganisms with low pathogenicity and are not a source of infection disease among dental personnel and patients.[3] However, this cannot be ignored, since the number of unrecognized infective patients seeking dental care is increasing.[4] Furthermore, microorganisms which cause respiratory and blood diseases are found in dental equipment and can be hazardous for dental patients and staff when infection-control routines are done inadequately. [5]

Of the many species of oral microorganisms, *Staphylococcus aureus* has been considered one of the most dangerous and versatile human pathogens.[6] Beyond the capacity to survive on dried surfaces for a mean of five days, *Staphylococcus aureus* has been more resistant to antimicrobial agents.[7]

Benzylpenicillin (penicillin G), introduced in the early 1940s, could briefly eliminate infections by *Staphylococcus aureus*, once its continuous use induced resistant strains that produce β-lactamase.[8] Among these resistant microorganisms, methicillin-

resistant *Staphylococcus aureus* (*MRSA*) has become a prevalent pathogen throughout the world.[9]

The importance of nosocomial *MRSA* as a pathogen is associated with its resistance to antimicrobial agents and its increased prevalence.[10] Moreover, although it is much less common and seldom fatal for younger patients, *MRSA* shows a significant morbidity and mortality rate among the elderly or patients with an impaired health.[11]

The prevention of horizontal transmission of *MRSA* has become increasingly important as the prevalence of this pathogen increases. Oral carriage of *MRSA* may induce re-colonization in other body sites or even cause cross-infection to other patients and dental professionals.[12] *MRSA* can sometimes survive on instruments and object surfaces for two or three days and on hands for up to three hours.[13]

Many studies were carried out to investigate *MRSA* contamination of various items, such as computers, pagers, television sets, stethoscopes, uniforms and gowns, blood-pressure cuffs, mattresses, pillows, chairs, bedframes, and over-bed tables.[14,15] However, more research in environmental contamination involving these microorganisms in dental settings is needed.

The aim of this study was, therefore, to assess the level of contamination of dental equipment surfaces by *Staphylococcus aureus* strains, as well as to investigate the antimicrobial susceptibility of these microorganisms against methicillin, vancomycin and the most commonly used antimicrobial agents in Dentistry.

Materials and Methods

The present study was carried out in the undergraduate multichair clinic of Piracicaba Dental School, University of Campinas (UNICAMP), São Paulo, Brazil. This dental clinic has 86 dental chair units, one urgency room (3 dental chairs) and one sterilization room. Samples were collected from surfaces of equipment involved in dental treatment. Surfaces were assigned into six groups as follows:

Group 1 – Dental chair push buttons (n=10);

Group 2 – Light device handles (n=10);

Group 3 - 3-in-1 syringes (n=10);

Group 4 - Computer "enter" keys (n=4);

Group 5 - Door handle of the main entrance (n=2);

Group 6 - X-ray tubes (n=2).

Sampling was carried out prior to (5:30 am), during (between 2:00 and 3:00 pm) and one hour after clinical activities (6:30 pm). Each of the six collects was performed bimonthly in a one-year period. The environment in three different sites–adults' clinic, paediatrics clinic, and urgency service clinic–was evaluated. All dental chair units analyzed in the present study complied with the symmetric distribution of the clinic's air-conditioning system (Figure 1). Since the door handle and the x-ray tubes are the same for all clinical settings, they were not considered for the urgency service clinic. Computer enter keys were important to the present study since they are frequently used to register all clinical procedures and data.

The routinely infection-control procedures used during this study were recommended by Brazilian Sanitary Vigilance Agency, which are similar to the protocol suggested by Center for Disease Control and Prevention (CDC).

Sample Collection

Samples were collected by rubbing the surfaces of the items previously described with sterile cotton swabs immersed in 0.1 ml of 0.9% NaCl sterilized solution. Controls for each group were performed by simply exposing the swabs in the air at the time of collection. All swabs were cut, placed into sterile tubes containing 0.9 ml of 0.9% NaCl and immediately assayed.

Five minutes after collection, samples were sonicated at 5% amplitude and 5-second intervals for 60 seconds (Vibra Cell 400W, Sonics & Materials Inc - 5% amplitude, 9.9 second cycle, 6 pulses – Newtown, USA). The resulting solution (10 µL) was inoculated in Petri dishes with 10 mL of BHI agar (Brain Heart Infusion – Difco Co. – Michigan, USA) and incubated at 37^{o}C for 24 h (Fanem Model 002 – São Paulo, Brazil).

After incubation, colonies were quantified with a stereoscopic microscope (Stemi SV6, ZEISS – Thornwood, USA) and their macroscopic characteristics recorded. Results were expressed as cfu x 10^3/mL. Pure cultures collected from isolated colonies were Gram-stained and examined at 1000x magnification (OLYMPUS – Tokyo, Japan). All colonies were submitted to biochemical tests in order to identify the species.[16,17]

Antimicrobial susceptibility test

The isolates were subcultured in brain heart infusion agar (BHI - Difco Co. – Michigan, USA), Gram-stained, examined at 1000x magnification (OLYMPUS – Tokyo, Japan) and their purity was checked. For the susceptibility tests, a spectrophotometer (Spectronic 20 – Bausch & Lomb, Rochester, USA), at 550 nm of wavelength, was used to adjust the optical density of overnight cultures. A standardized inoculum (10^8cfu/mL) was obtained between 75 to 80% of transmittance. Each suspension (600 μL) was mixed into 60mL of Mueller-Hinton agar (MHA - Difco Co. – Michigan, USA), which was previously supplemented with 1.5% sterile sheep blood, and poured onto a Petri dish (150mm x 20mm). The final inoculum concentration was 10^6cfu/mL.[18]

Antimicrobial agents commonly used in Dentistry, methicillin, and vancomycin were assayed. Commercial paper disks, 6.5 mm diameter (Cefar Diagnostics Co – São Paulo, Brazil) with 10 μg ampicillin (Ap), 10 μg amoxicillin (Ax), 20 μg amoxicillin/10 μg clavulanic acid (Amc), 15 μg azithromycin (Azi), 30 μg cefazolin (Cef), 15 μg clarithromycin (Cla), 2 μg clindamycin (Cl), 30 μg chloramphenicol (Chlo), 15 μg erythromycin (Ery), 5 μg of methicillin (Met), 10 units of penicillin G (Pen), and 30 μg of vancomycin (Van) were placed onto agar and incubated at 35ºC for 18 hours. For methicillin and vancomycin, the incubation time was 24 hours. After incubation (Fanem Model 002 – Sao Paulo, Brazil), the inhibition zones were measured and recorded according to the National Committee for Clinical Laboratory Standards (NCCLS). All microorganisms were classified as sensitive or resistant.[18]

Statitical Analysis

Microorganism counts, considering each period (prior to, during and following clinical procedures) and each clinical environment (adults, paediatrics, and urgency clinics), were submitted to the Kruskal-Wallis and Dunn tests at a significance level of 5% (p<0.05).

Results

Considering all periods of collect, not all surfaces in the different sites tested revealed *Staphylococcus aureus* contamination. No bacterial growth was observed in the controls concerning all periods tested. Most groups, especially groups 5 and 6, revealed insignificant growth regarding the samples collected before clinical activities. Table 1 shows data for different sites, periods, and surfaces tested.

Comparison among periods showed the greatest *Staphylococcus aureus* counts for the samples collected during clinical activities (p<0.05). The paediatrics clinic, considering the three different sites tested, showed the greatest *Staphylococcus aureus* counts. No statistically significant differences were observed for the sites tested prior to clinical activities (p>0.05). Figure 2 shows the *S. aureus* count means regarding the different periods and clinical sites tested.

Group 1 (dental chair push buttons) revealed the greatest *Staphylococcus aureus* counts (p<0.05) among all groups, which is valid particularly for the paediatrics and urgency clinics. Groups 2 and 3 showed high contamination by *Staphylococcus aureus* during clinical activities for all sites (p<0.05). The paediatrics clinic environment

demonstrated the highest *Staphylococcus aureus* counts considering these two groups ($p<0.05$).

Group 4 presented no statistically significant difference among the three different sites tested prior to clinical activities ($p>0.05$). However, a significant increase in the *Staphylococcus aureus* counts was observed during and after clinical activities, considering all sites tested ($p<0.05$).

No statistically significant differences were observed among periods and sites in group 5, except for the *Staphylococcus aureus* counts at the adults' clinic after activities and paediatrics clinic during activities, which presented the highest counts.

Group 6 showed the greatest *Staphylococcus aureus* counts for the samples collected in the paediatrics clinic ($p<0.05$). No statistically significant differences were observed for samples collected prior to clinical activities ($p>0.05$).

The *S. aureus* isolates showed high antimicrobial resistance rates, especially against beta-lactams. The microorganisms collected from the urgency service clinic revealed the greatest resistance rates against the antimicrobial agents tested. *MRSA* was observed only for the samples collected in the urgency service clinic. No resistance to vancomycin was detected concerning all clinical sites and surfaces tested in the present study. Figure 3 shows the antimicrobial susceptibility profile of the *S. aureus* isolates regarding the three different clinical sites tested.

Discussion

There was an increase in microbial contamination during dental procedures, agreeing with results of previous studies.[19,20] It has been reported that aerosols may

contribute to the contamination of clinical environments.[2,20,21] The patients' oral hygiene and the presence of a great number of people (professionals, students and patients) might have caused the clinical environment contamination observed in the present study.[5,19,22] All these factors can account for the high contamination observed during clinical procedures.

Staphylococcus aureus is rarely encountered in the dental community. However, a growing number of studies suggest that these microorganisms can be isolated more frequently from the oral cavity of particular groups of patients, especially of children.[12,23,24] This can explain the high *S. aureus* contamination observed in the paediatrics clinic in the present study.

Direct skin/hand/finger contact is known as the most common route for *S. aureus* transmission. [11,13] Many previous studies have evaluated *S. aureus* contamination regarding hand contact involving different objects, such as pens, stethoscopes, uniforms, and chairs.[14,15] Hence, as expected, the surfaces most frequently handled during dental treatment showed the highest levels of contamination, especially those in Group 1, which showed the highest *S. aureus* count. Moreover, the frequency of dental equipment use or the kind of treatment might directly contribute to microbial contamination. Invasive procedures might result in a higher contamination than that observed in simple clinical procedures in any clinical environment.[22, 25]

Microbial contamination in light handles and 3-in-1 syringes was also investigated by McColl *et al*.[26] Adequate dental equipment decontamination is extremely important to remove bacterial deposits, caused by clinical procedures.[27] However, cleaning 3-in-1 syringe buttons is quite difficult, due to a protection overlapping the buttons.

It has been shown that the internal chambers of 3-in-1 syringes are contaminated during routine dental procedures. Sterilized 3-in-1 syringes (autoclave-safe) have been suggested to reduce the cross-contamination risk.[26] Such contamination is possible due to mineral and bioburden deposits on internal walls of syringe metal tips. To decrease this risk, disposable 3-in-1 syringe tips are strongly recommended.[28]

Contamination levels observed for group 4 ("enter" keys) were low, but adequate cross-contamination control is still needed, especially when dental students are involved.[22] Furthermore, computer keys, a relevant microorganism reservoir, may also be responsible for nosocomial infections.[29]

The door handle of the main entrance (Group 5) presented low contamination in all periods tested in the present study. However, this contamination cannot be ignored once door handles are considered as reservoirs of *S. aureus* colonization, including *MRSA*. Therefore, regular disinfection of door handles is necessary.[14]

X-ray tubes show a great cross-contamination risk between patients. White & Glaze have reported that 77% of patients were contaminated with *Staphylococcus aureus* after radiograph taking.[30] Another relevant study revealed patients' contamination by pathogenic yeasts and bacterial respiratory pathogens after radiographic examination. [31]

Of all the strains isolated in the present study, 82% were resistant to penicillin G, 78% to ampicillin, 74% to amoxicillin, 41% to amoxicillin/clavulanate and 7% to cefazolin. This high antimicrobial resistance against beta-lactam antibiotics is warning, once these agents are the largest and most widely used in Dentistry.[32] These results are in agreement with those in previous studies showing an alarming reduction in the *S. aureus* susceptibility to beta-lactams.[33,34]

The *S. aureus* isolated also showed the following resistance rates: 42% to clarithromycin; 37% to erythromycin; 24% to azithromycin; 17% to chloramphenicol and 12% to clindamycin. These rates are similar to or slightly higher than those observed in studies investigating staphylococci isolated from dental clinical environments. These results suggest that stricter decontamination procedures are needed to control cross-contamination, which can be unsafe to both professional and patient.[1,35]

All isolated strains were sensitive to vancomycin. Although all samples showed low resistance to methicillin, the isolation of *MRSA* in a dental clinic environment is of great concern, once this microorganism is a major clinical environmental problem.[10] These microorganisms have become a common and serious microbial contamination of medical equipment and other environmental surfaces in hospital settings; however, there is no data on the isolation of *MRSA* from equipment or environmental surfaces in dental settings.[36]

The spread of pathogens such as *MRSA* during dental treatment justifies the use of suitable means of infection control. At least two cases of cross-infection by *MRSA*, from a general dental practitioner to patients, have been reported.[37] Therefore, identifying cross-infection risk involving dental healthcare workers and patients is of great importance.[38]

Current concepts of cross-infection control like the utilization of disposable plastic covers over contamination-risk surfaces during treatment and equipment sterilization or decontamination have been advocated.[39] In a previous study, a comparison of surface bacterial contamination levels in a large dental clinic showed that the contamination level reduced significantly when equipment and new infection control procedures were improved.[40]

A pre-procedural use of an antiseptic mouth rinse is another method for reducing overall bacterial counts produced during dental activities. Chlorhexidine or essential oils for one-minute mouthwash before a dental procedure has been shown to significantly reduce the bacterial contamination dispersed by aerosols during dental treatment.[41]

The Center for Disease Control and Prevention (CDC) has recently revised and published new guidelines on infection control regarding dental health care settings. [39] All of these guidelines must be applied considering barriers (to decrease the risk of contamination) and a careful anamnesis of patients, allowing an accurate diagnosis and an adequate clinical treatment. These measures are highly recommended for patients having symptoms or a recent history of infection and undergoing antibiotic therapy.[1,42] A "targeted spectrum" antibiotic, when needed, should be carefully selected.[43]

In conclusion, clinical activities might increase *S. aureus* contamination in dental settings, suggesting the need for adequate post-operative decontamination procedures, once these microorganisms showed high antimicrobial resistance rates. Moreover, the isolation of *MRSA* highlights the need to establish strategies to prevent emergence of resistant bacterial strains in dental settings. There is a great risk of cross-contamination in dental settings caused by microorganisms dispersed with the aerosols; therefore, further studies are needed to investigate clinical environmental contamination.

Acknowledgments

The authors thank to CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for financial support and Mr. Jorge Valério for his assistance in manuscript preparation.

References

1. Horiba N, Yoshida T, Suzuki K, Maekawa Y, Ito M, Matsumoto T, Nakamura H. Isolation of methicillin-resistant staphylococci in the dental operatory. *J Endod* 1995; 21:21-25.
2. Monarca S, Grottolo M, Renzi D, Paganelli C, Sapelli P, Zerbini I, Nardi G. Evaluation of environmental bacterial contamination and procedures to control cross infection in a sample of Italian dental surgeries. *Occup Environ Med.* 2000; 57: 721-6.
3. Hardie J. Concerns regarding infection control recommendations for dental practice. *J Can Dent Assoc.* 1992;58:377-8, 382-6.
4. DePaola LG, Carpenter WM. Bloodborne pathogens: current concepts. *Compend Contin Educ Dent*. 2002;23:207-10, 212, 214.
5. Hackney Jr RW, Crawford J J, Tulis JJ. Using a biological indicator to detect potential source of cross-contamination in the dental operatory. *J Am Dent Ass* 1998; 129: 1567-1577.
6. Honma K, Tawara Y, Okuda K. Detection of methicillin-resistant staphylococcus aureus in human saliva and on denture surfaces. *Bull Tokyo Dent Coll.* 1994; 35 :217-20.
7. Getchell-White SI, Donowitz LG, Groschel DH. The inanimate environment of an intensive care unit as a potential source of nosocomial bacteria: evidence for long survival of *Acinetobacter calcoaceticus*. *Infect Control Hosp Epidemiol.* 1989;10:402-7.

8. Chambers HF. Methicillin-resistant staphylococci. *Clin Microbiol Rev.* 1988;1:173-86.
9. Boyce JM, Havill NL, Kohan C, Dumigan DG, Ligi CE. Do infection control measures work for methicillin-resistant *Staphylococcus aureus*? *Infect Control Hosp Epidemiol.* 2004;25:395-401.
10. Cesur S, Cokca F. Nasal carriage of methicillin-resistant *Staphylococcus aureus* among hospital staff and outpatients. *Infect Control Hosp Epidemiol.* 2004;25:169-71.
11. Bradley S. Methicillin-resistant Staphylococcus aureus: long-term care concerns. *Am J Med* .1999;106:2S-10S.
12. Smith AJ, Jackson MS, Bagg J. The ecology of Staphylococcus species in the oral cavity. *J Med Microbiol*. 2001;50:940-6.
13. Mulligan ME, Murray-Leisure KA, Ribner BS. Methicillin resistant *Staphylococcus aureus*: a consensus review of the microbiology, pathogenesis, and epidemiology with implications for prevention and management. *Am J Med* .1993;94:313-28,.
14. Oie S, Hosokawa I, Kamiya A. Contamination of room door handles by methicillin-sensitive/methicillin-resistant *Staphylococcus aureus*. *J Hosp Infect.* 2002;51:140-3.
15. Singh D, Kaur H, Gardner WG, Treen LB. Bacterial contamination of hospital pagers. *Infect Control Hosp Epidemiol.* 2002;23:274-6.
16. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn Jr. WC. *Color atlas and textbook of diagnostic microbiology*, 5th. ed. Philadelphia: J.B. Lippincott Company; 1997:1465.

17. Sneath PHA. Endospore-forming Gram-Positive Rods and Cocci. In: Sneath PHA, Mair NS, Sharpe E. *Bergey's Manual Systematic Bacteriology*, 1st ed. Baltimore, MD: Williams & Wilkins; 1986: 1104-1139.

18. NCCLS - National Committee For Clinical Laboratory. Standards Performance standards for antimicrobial susceptibility testing 2002; M100 – S12, NCCLS 22: 50-69.

19. Kedjarune U, Kukiattrakoon B, Yapong B, Chowanadisai S, Leggat P. Bacterial aerosols in the dental clinic: effect of position, time and type of treatment. *Int Dent J.* 2000;50:103-107.

20. Legnani P, Checchi L, Pelliccioni GA, D'Achille C. Atmospheric contamination during dental procedures. *Quintessence Int* 1994; 25: 435-439.

21. Kedjarune U, Leggat PA. Bacterial aerosols in the dental clinic: a review. *Int Dent J* 2001; 51:39-44.

22. Bentley CD, Burkhart NW, Crawford JJ. Evaluating spatter and aerosol contamination during dental procedures. *J Am Dent Assoc* 1994; 125:579-584.

23. Miyake Y, Iwai T, Sugai M, Miura K, Suginaka H, Nagasaka N. Incidence and characterization of *Staphylococcus aureus* from the tongues of children. *J Dent Res*. 1991;70:1045-7.

24. Suzuki J, Komatsuzawa H, Sugai M, *et al*. A long-term survey of methicillin-resistant *Staphylococcus aureus* in the oral cavity of children. *Microbiol Immunol.* 1997;41:681-6.

25. Bennett AM, Fulford MR, Walker JT, Bradshaw DJ, Martin MV, Marsh PD. Microbial aerosols in general dental practice. *Br Dent J* 2000; 189: 664-667.

26. McColl E, Bagg J, Winning S. The detection of blood on dental surgery surfaces and equipment following dental hygiene treatment. *Br Dent J* 1994; 176:65-67.
27. Vickery K, Pajkos A, Cossart Y. Evaluation of the effectiveness of decontamination of dental syringes. *Br Dent J* 2000; 189:620-624.
28. Puttaiah R, Cottone JA, Guildersleeve J, Azmoudeh A, Tenney J. Rationale for using single-use disposable air/water syringe tips. *Compend Contin Educ Dent.* 1999;20:1056-1058, 1060, 1063-1064.
29. Hartmann B, Benson M, Junger A, et al. Computer keyboard and mouse as a reservoir of pathogens in an intensive care unit. *J Clin Monit Comput*. 2004;18:7-12.
30. White SC, Glaze S. Interpatient microbiological cross-contamination after dental radiographic examination. *J Am Dent Assoc* 1978; 96:801-804.
31. Autio KL, Rosen S, Reynolds NJ, Bright JS. Studies of cross-contamination in the dental clinic. *J Am Dent Assoc* 1980; 100:358-362.
32. Handal T, Olsen I. Antimicrobial resistance with focus on oral beta-lactamases. *Eur J Oral Sci*. 2000;108:163-74.
33. Frere JM. Beta-lactamases and bacterial resistance to antibiotics. *Mol Microbiol* 1995; 16: 385-395.
34. Slavkin HC. Emerging and re-emerging infectious diseases: a biological evolutionary drama. *J Am Dent Assoc* 1997; 128:108-113.
35. Mattos Filho TR, Groppo FC, Pacheco ABND, Motta RHL, Napimoga MH. Antimicrobial susceptibility of air-dispersed microorganisms in dental settings. *Gen Dent***.** 2005;(Jan-Feb):In press.

36. Molinari JA, Palenik CJ. Environmental surface infection control, 2003. *Compend Contin Educ Dent* 2004;25:30-37.

37. Martin MV, Hardy P. Two cases of oral infection by methicillin-resistant *Staphylococcus aureus*. *Br Dent J.* 1991 19;170:63-4.

38. Prospero E, Savini S, Annino I. Microbial aerosol contamination of dental healthcare workers' faces and other surfaces in dental practice. *Infect Control Hosp Epidemiol*. 2003;24:139-41.

39. Kohn WG, Collins AS, Cleveland JL*, et al.*. Guidelines for infection control in dental health-care settings – 2003. *M M W R* 2003; 52:1-61.

40. Williams HN, Singh R, Romberg E. Surface contamination in the dental operatory: a comparison over two decades.*J Am Dent Assoc*. 2003; 134:325-330.

41. Harrel SK, Molinari J. Aerosols and splatter in dentistry: a brief review of the literature and infection control implications. *J Am Dent Assoc*. 2004;135:429-37.

42. Miller CH, Cottone J A. The basic principles of infectious diseases as related to dental practice. *Dent Clin North Am* 1993; 37: 1-20.

43. Lieberman JM. Appropriate antibiotic use and why it is important: the challenges of bacterial resistance. *Pediatr Infect Dis J*. 2003 ; 12:1143-1151.

Figures and Tables

		Dental-chair push buttons	Light handles	3-in-1 syringes	Computer keys	Door knobs	X-ray tubes
Pediatrics	before	0.87 (±1.31) **a,d (A)***	0.47 (±1.02) **a,e (A,B)**	0.17 (±0.67) **a (B)**	0.23 (±0.64) **a,f (A,B)**	0.17 (±0.57) **a (A,B)**	0.00 (±0.00) **a (B)**
	during	3.49 (±2.53) **b,e (A)**	2.83 (±2.23) **b (A)**	2.52 (±1.71) **b,f (A)**	2.64 (±1.29) **b (B)**	1.14 (±1.06) **b (C)**	4.31 (±1.37) **b (A)**
	after	2.71 (±3.24) **c (A)**	1.69 (±2.12) **c (B)**	0.90 (±1.59) **c,e (C)**	0.66 (±1.45) **a,c,e (C)**	0.09 (±0.37) **a (C)**	1.20 (±2.27) **a (B,C)**
Adult	before	0.56 (±1.00) **a (A)**	0.30 (±0.81) **a (A)**	0.31 (±0.93) **a,c (A)**	0.23 (±0.64) **a,f (A)**	0.00 (±0.00) **a (A)**	0.43 (±0.98) **a (A)**
	during	1.99 (±1.57) **c (A,C)**	1.87 (±1.61) **c,d (A,C)**	1.58 (±1.33) **d,f (C)**	2.36 (±0.94) **b,d (A)**	0.66 (±1.06) **a,b (B)**	2.51 (±0.61) **c (A)**
	after	1.26 (±1.23) **d (A)**	1.42 (±1.36) **c (A)**	1.02 (±1.14) **d,e (A)**	1.21 (±1.15) **e,f (A)**	1.43 (±1.36) **b (A)**	2.40 (±0.55) **c (B)**
Emergency unit	before	1.00 (±1.13) **a,d (A,B)**	1.11 (±1.04) **c,e (A)**	0.46 (±0.84) **a,c,e (B)**	1.03 (±1.01) **f (A,B)**		
	during	4.14 (±1.69) **b (A)**	2.60 (±1.73) **b,d (B)**	2.04 (±1.54) **f (B)**	2.26 (±1.2) **b,e (B)**		
	after	2.33 (±1.36) **e,c (A)**	1.60 (±1.25) **c,d (B)**	0.92 (±1.14) **c,d (C)**	1.46 (±1.12) **d,e,f (B,C)**		

Table 1 – Means of colonies forming units of *S. aureus* (± s.e.m.) considering each group. Capital letters show comparisons among each surface tested considering each period evaluated (horizontal lines). Small letters show comparisons among each surface tested considering all periods evaluated (vertical lines). *different letters mean statistically significant differences ($p< 0.05$).

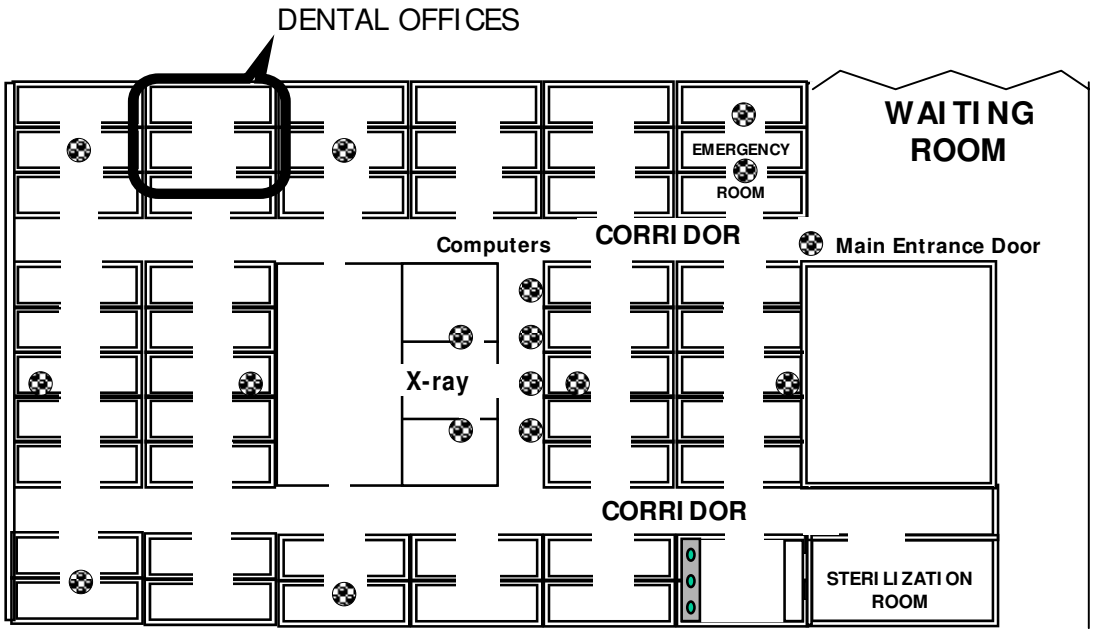


Figure 1 – Disposition of sites where the samples were obtained.

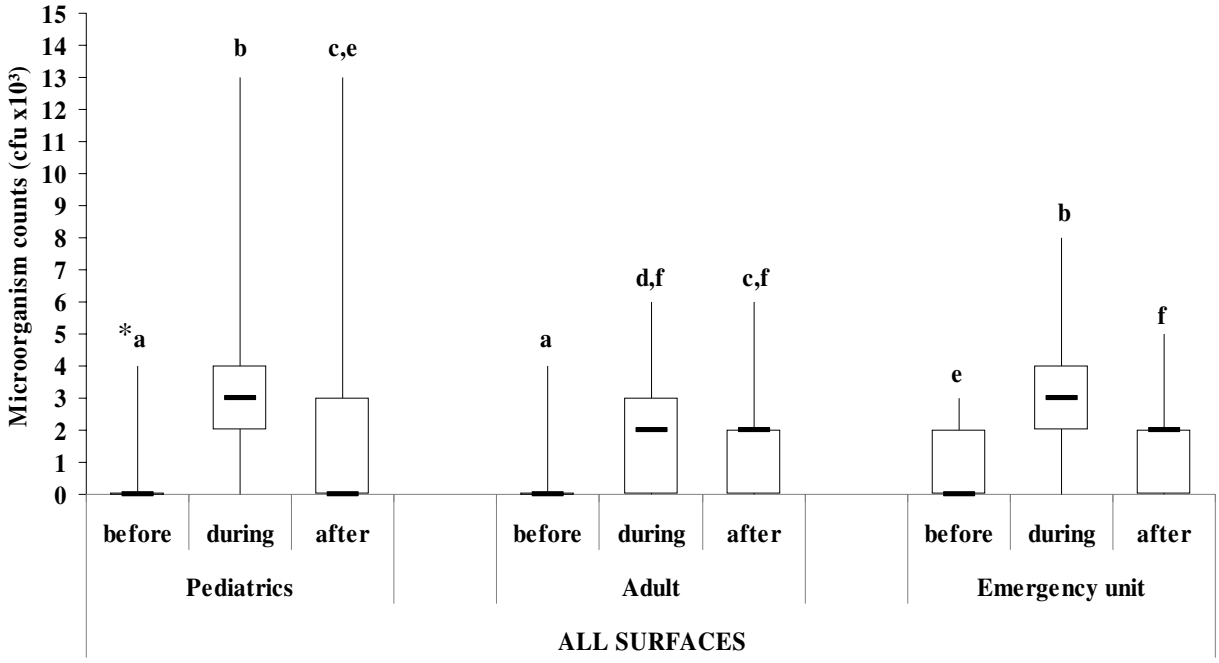


Figure 2 – Box-whisker plot for comparison among groups considering all surfaces for each clinical site and period evaluated (Central line: median; Box: lower and upper quartiles; Whisker: maximum and minimum values). *different letters denote statistically significant differences.

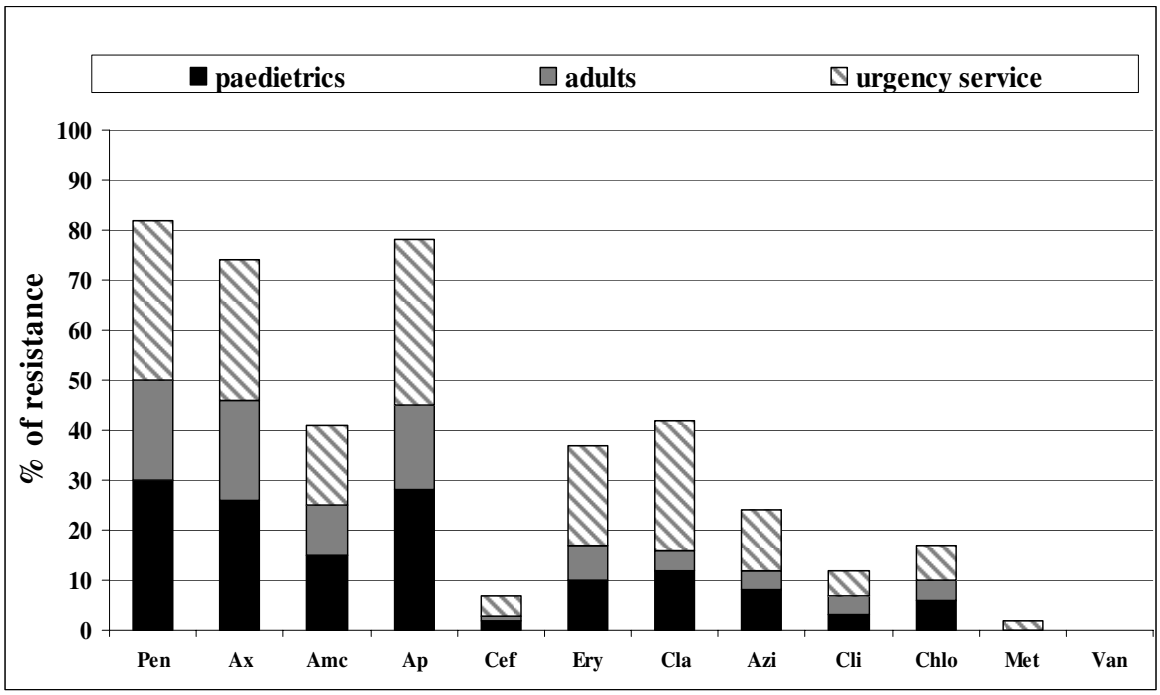


Figure 3 - Antimicrobial resistance profile of the *S. aureus* isolates regarding the three different clinical sites tested.

3.3.Title: **The effect of amoxicillin and vancomycin against MRSA infection. *In vivo* study in rats.**

Rogério Heládio Lopes Motta[a] *, DDS, MSc, PhD Student

Sinvaldo Baglie[c], Pharm, MSc, Assistant Professor

Juliana Cama Ramacciato[b], DDS, MSc, PhD, Assistant Professor

Thales Rocha de Mattos-Filho[a], DDS, MSc, PhD, Chairman

Francisco Carlos Groppo[a], DDS, MSc, PhD, Associate Professor

[a]*Department of Physiological Sciences, Area of Pharmacology, Dentistry School of Piracicaba - UNICAMP, Piracicaba, Brazil*

[b]*Department of Physiological Sciences, Area of Pharmacology, São Leopoldo Mandic, Campinas, Brazil*

[c] *Department of Pharmaceutical Sciences, Ponta Grossa University, Paraná, Brazil*

*Corresponding author:

Rogério Heládio Lopes Motta - Department of Physiologic Sciences

Dental School of Piracicaba, University of Campinas,

Av Limeira # 901, Areião – CEP (zip code)13.414-903

Piracicaba, São Paulo, Brazil. - Phone: 55 1934125310 Fax: 55 1934210144

e-mail: rogeriomotta@fop.unicamp.br

Abstract

The aim of this study was to evaluate the course of both methicillin-resistant *Staphylococcus aureus* (MRSA) and penicillin-sensitive *S. aureus* (PSSA) strains by using the granulomatous-tissue infected model in rats. The effect of amoxicillin and vancomycin was also observed. Four polyurethane sponges were placed in the back of 180 rats. After 14 days, two granulomatous tissues received 0.5 ml of 10^8 cfu/ml of *S. aureus* ATCC 25923 (PSSA) or MRSA (previously isolated from a dental clinic environment). Two days later, the rats were divided into six groups according drugs and microorganisms inoculated: G1- Amoxicillin 50 mg/kg/p.o. and PSSA; G2 - Amoxicillin 50 mg/kg/p.o. and MRSA; G3 - Vancomycin 50 mg/kg/i.p. and PSSA; G4 - Vancomycin 50 mg/kg/i.p. and MRSA; G5 – saline (0.9% NaCl 1ml/p.o.) and PSSA; and G6 – saline and MRSA. After 6, 24, and 48 hours of drug administration, 10 rats per period of each group were killed. Both infected and non-infected granulomatous tissues were removed, placed in eppendorfs with 1 ml of 0.9% NaCl and weighed. Infected tissues were dispersed in a sonicator and spread (10 µl) on salt mannitol agar and the colonies were counted after aerobic incubation (24h at 37°C). Microorganism counts and granulomatous-tissue's weight results were submitted to the Multifactorial ANOVA (alpha=0.05). A HPLC system with UV detection was used to quantify the concentration of the antimicrobial agents in the serum and tissue. No statistically significant differences ($p>0.05$) were observed among the weights of non-infected tissues. The groups 2 and 6 showed the higher weight values ($p<0.05$). G3, G4 and G5 showed reduction of microorganism counts after 6h ($p<0.05$). G1, G2 and G6 did not show significant reduction among their periods. Both drugs were quantified only at 6-hours after their administration. The strains (PSSA or MRSA) did not affect the serum or the non-

infected tissue concentrations ($p>0.05$) but significantly reduced the amoxicillin concentration in the infected tissue ($p<0.05$). We concluded that a single dose of amoxicillin did not affect the curse of infection induced by a penicillin-susceptible strain. The MRSA strain isolated from a clinical environment showed high pathogenicity in comparison to a penicillin-susceptible *S. aureus* strain and it was not eradicate by a single dose of vancomycin.

Key words: Methicillin resistance, *Staphylococcus aureus*, staphylococcal infection, vancomycin, amoxicillin.

Introduction

Many studies have reported that aerosols related to dental treatment may contain low-pathogenic microorganisms, which are not considered as source of infection diseases among dental personnel and patients.[1] However, these microorganisms cannot be ignored, since the number of unrecognized infective or immunocompromised patients seeking dental care is increasing.[2] Furthermore, microorganisms which cause respiratory and blood diseases are found in dental equipment and can be hazardous for dental patients and staff when infection control is carried out inadequately.[3]

The possible routes for the spread of oral microorganisms in a dental office are closely related to the body fluids of an infected patient, the environmental surfaces or instruments that have been contaminated and the airborne infectious particles from the patient.[4] However, the potential of aerosol transmission of pathogenic agents during dental treatment to cause an infection disease is not well known.[5]

In a busy practice, the time between patients for thorough cleaning and disinfection is often inadequate, making the cross-contamination control a more difficult challenge. In addition, very pathogenic microorganisms, such as *Streptococcus pyogenes*, *Streptococcus pneumoniae*, hepatitis B virus and *S. aureus,* are able to survive in dental setting surfaces for longer periods.[1]

S. aureus has been considered one of the most dangerous and versatile human pathogens.[6] Beyond the capacity to survive on dried surfaces in dental offices for at least for five days, some strains, such as methicillin-resistant (MRSA)*,* are markedly resistant to many antimicrobial agents. Moreover, MRSA isolated from several items (stethoscopes, uniforms, blood-pressure cuffs, mattresses, computer keys, chairs, doorknobs) are considered vectors for cross-contamination in clinical environments.[7,8]

MRSA strains cause a significant morbidity and mortality rate among elderly patients and those with impaired health. It is much less prevalent and seldom fatal for younger patients. *MRSA* colonization is uncommon in the healthy host, and its carriage may be a marker of increased debility.[9] Furthermore, community-acquired MRSA infection is becoming more prevalent.[10,11]

Due to the high clinical relevance of MRSA strains, some recent studies have accessed the *in vivo* effects of antimicrobial agents against these microorganisms by using infection models in animals.[12-14]

The aim of this study was to evaluate the pathogenicity of methicillin-resistant *S. aureus* isolated from a dental clinic environment and *S. aureus* ATCC 25923 in granulomatous tissues and to observe the efficacy of amoxicillin and vancomycin against these microorganisms in an infection model in rats.

Material and Methods

Bacterial strains. A PSSA type culture (ATCC 25923) and a clinical isolate of MRSA (CAT5; isolated from the dental clinics of Piracicaba Dental School, Sao Paulo, Brazil), were used. The isolates were stored at -80°C in BHI broth (Merck, Darnstadt, Germany) with 20% (v/v) glycerol (Sigma Chemical Co., St Louis, MO, U.S.A.). Antibiogram assays (data not shown) showed that both PSSA and MRSA strains were vancomycin susceptible. MRSA was penicillin, methicillin and oxacillin resistant.

Antimicrobial agents. Amoxicillin trihydrate and vancomycin were obtained from Sigma Chemical Co. (St Louis, MO, U.S.A.). Physiological saline solution (0.9% NaCl) was administered to the control animals and was also used to dilute both antimicrobial agents.

Animals. One hundred and eighty adult male *Wistar* rats (*Rattus norvegicus*- albinus), 60 days of age and weighing 175±25g, were obtained from CEMIB-UNICAMP (Centro de Bioterismo-ICLAS Monitoring/Reference Center, Campinas, Brazil) where they were maintained under aseptic conditions. The Institutional Committee for Ethics in Animal Research, University of Campinas – Unicamp approved all procedures (protocol # 423-1).

Granulomatous tissue model. Granulomatous tissue was induced as previously described.[15,16] Briefly, four sterilized polyurethane sponge discs (density 35 kg/m^3) were subcutaneously implanted in the back of all rats. These sponge discs (Proespuma Com. & Ind. Ltd., Sao Paulo, Brazil) were 12 mm in diameter and 5 mm thick, weighing 12.1±0.81mg. Figure 1 shows the position of implanted sponges. After 14 days of sponge positioning, a careful antisepsis was carried out in the back of all animals and PSSA was injected (0.5 ml of suspension of 10^8cfu/ml) into the two granulomatous tissue samples at

the tail position of 90 animals. MRSA was injected under the same conditions mentioned above in the other 90 animals.

Experimental groups. Two days after infection, all animals were assigned into six groups of thirty animals each considering the drugs and bacterial strains tested: G1- amoxicillin 50 mg/kg/p.o. and PSSA; G2 - amoxicillin 50 mg/kg/p.o. and MRSA; G3 - vancomycin 50 mg/kg/i.p. and PSSA; G4 - vancomycin 50 mg/kg/i.p. and MRSA; G5 - physiological saline (0.9% NaCl) 1.0 ml/p.o. and PSSA; G6 - physiological saline (0.9% NaCl) 1.0 ml/p.o. and MRSA. All drugs were administered in single dose. Subsequently, each group was subdivided into three different periods of time (10 animals each) in order to collect the samples.

Surgical and sampling procedures. After 6, 24, and 48 hours of drugs administration, 10 rats of each group were killed via inhalation of CO_2 followed by cervical dislocation. Each infected granulomatous tissue sample was then removed and separately placed in eppendorfs with 1 ml of 0.9% NaCl solution. These tubes were weighed before and after tissue insertion. Their content was dispersed using an ultrasonic system (Vibra Cell 400W, Sonics & Materials Inc., Danbury, CT, U.S.A.) and diluted 100 and 1000 times in saline solution. Ten microliters of the resulting suspensions were spread on salt mannitol agar (Merck, Darnstadt, Germany) in triplicate and incubated (Fanem Model 002 – Sao Paulo, Brazil) at 37°C during 18 hours. After incubation, resulting colonies were counted using a manual colony counter and submitted to the Multifactorial ANOVA (software Systat 10.2®). In addition, weight results were submitted to the Multifactorial ANOVA (software Systat 10.2®). Tissue and serum concentrations obtained in each period of time were

submitted to the impaired *t* test (software Bioestat 1.0® for Windows©) in order to compare the PSSA and MRSA groups considering each antibiotic.

Instrumentation and chromatographic conditions. The liquid chromatography equipment consisted of a Varian Model 9012 HPLC pump and a manual Rheodyne 7125 injection valve equipped with a 50µl loop (Varian Inc. Corporate, Palo Alto, USA). The chromatographic separations were carried out using a Lichrospher column 100 RP18 (125mm x 4mm x 5µm, Merck, Darnstadt, Germany). The separated components were detected using a Varian Model 9050 with UV-VIS detector (Varian Inc. Corporate, Palo Alto, USA). The detection signals were obtained using the Star 9050 integrator software (Varian Inc. Corporate, Palo Alto, USA). Amoxicillin and vancomycin analyses were performed by using adapted and validated methods.[17-19] All analyses were performed at room temperature.

Preparation of standard solutions for HPLC analysis. The standard stock solutions of amoxicillin and vancomycin were diluted ranging from 0.1µg/mL to 100µg/mL, and stored in the dark at 4°C. Drug-free serum and tissue samples were prepared by adding the amounts of amoxicillin and vancomycin to achieve the calibration curves.

Results

The weight values (mean ± s.e.m.) of the granulomatous tissue considering each group are shown in Figure 2. No statistically significant difference ($p>0.05$) was observed among the weights of non-infected tissues. According to the Multifactorial ANOVA, the weight of the infected tissues was determined by the strains (MRSA or PSSA). MRSA

infection induced higher weight values when compared to the ones infected by PSSA ($p<0.05$) regardless the treatment used. In addition, infected tissues exhibited higher weights when compared to non-infected tissues ($p<0.05$) and the period of time did not affect the weights, except for control group of MRSA and amoxicillin and control groups of PSSA-infected tissues.

Figures 3 and 4 show microorganism counts (cfu/g ± s.e.m.) of granulomatous tissues infected with PSSA and MRSA, respectively. Considering PSSA infected tissues, vancomycin significantly reduced ($p<0.05$) the microorganism counts after 6 hours when compared to all other periods and treatments. At 6h-period control group showed the highest level of bacteria, which significantly decreased ($p<0.05$) after this period. Amoxicillin did not have marked effect against the strains along the time ($p>0.05$).

A different profile was verified in the microorganism levels in the tissues infected by the MRSA strain. Neither saline solution nor amoxicillin were able to reduce MRSA counts ($p<0.05$). Statistically significant reduction in MRSA counts ($p<0.05$) was observed only after 6 hours of the administration of vancomycin.

The quantification limits of amoxicillin and vancomycin of HPLC assays were 0.39µg/mL and 0.78µg/mL, respectively, which allowed the quantification of the antimicrobial agents only at the 6hour period.

Figures 5 and 6 show the serum and tissue concentration of amoxicillin and vancomycin, respectively, according to the strain used to induce the infection. Amoxicillin and vancomycin serum concentrations were not influenced ($p>0.05$) by the strain used to induce the infection. However, the strains had influence over the amoxicillin tissue concentration, since tissues inoculated with MRSA revealed a lower concentration of

amoxicillin ($p<0.05$). The tissue concentration of vancomycin was also not influenced by the strain used to induce the infection ($p>0.05$).

Discussion

Adherence is one of the most important phenomena for infection establishment.[20] The ability of *S. aureus* to adhere on extracellular matrix proteins is thought to be responsible for its colonization and ability to cause infection.[21] Except for the later groups where vancomycin were administered (24 h and 48h of PSSA strain), all tissues inoculated with bacteria showed visible infection, which was characterized by purulent secretion (eye examination) confirmed by bacterial counting. Thus, the granulomatous tissue used in the present study could provide a good surface to bacterial adherence. Some important characteristics for an infection model, such as simplicity of the technique, reproducibility, measurability, and tissue involvement[22] are found in the model used in the present study.

The weight values of non-infected tissues in the present study were also used to verify the standardization of surgical procedures. The absence of differences among them (Figure 2) indicated that the surgical phases could not be considered as a source of variability, probably because only one investigator was responsible for all procedures.[23]

When compared to PSSA infected tissues, the ones infected with MRSA showed higher weight values suggesting a major pathogenicity of MRSA strain. The pathogenesis of MRSA is attributed to the combined effects of extracellular factors and toxins, together with increased invasive properties such as adherence, biofilm formation, and resistance to phagocytosis.[24]

Usually a large inoculum is required to establish a reproducible infection in animals, especially in rats. This large inoculum may be needed to overcome host defences, but may result in a fulminant course of infection.[22] The host defence could be responsible for the reduction of the bacterial number and, as a result, the reduction on weight of infected tissues (PSSA and MRSA) of animals submitted to saline solution (control groups).

After two days and six hours of infection course, both control groups (MRSA and PSSA) showed a lower levels of colony forming units (approximately $4x10^4$cfu/g) in comparison to the large initial inoculum utilized ($5x10^7$cfu). This finding is in compliance with those observed in previous studies, reporting infection establishment in granulomatous tissues two days after inoculation.[15,16] Although the host defense system of the rats certainly had influenced the number of bacteria and the tissue weight, MRSA strain appear to be lower sensible to the host than PSSA (Figure 2).

Amoxicillin levels in serum or tissue at 6 hours after drug administration exceeded the minimum inhibitory concentration (MIC) for penicillin-susceptible *S. aureus* strains.[25] However, this level was not enough to keep a significant reduction in the PSSA number in the later periods. Even at large doses used in the present study, amoxicillin was not able to eradicate the susceptible strain. A slow bacterial growth at the infectious site and the biofilm structure might affect the efficacy of amoxicillin.[26] In addition, the reduction of amoxicillin concentration between 6 and 24 hours could greatly contributed to the eradication failure.

The serum and tissue concentrations of amoxicillin found in the present study were similar to or slightly lower than those observed in previous studies.[15,16] However, a significant difference was observed considering infected and non-infected tissues,

especially for MRSA-infected tissues. The inoculum effect (loss of efficacy against dense microbial populations) affects the ability of an antimicrobial agent to penetrate to the core of the infection.[27] Besides, a larger mass of bacteria results in more glycocalyx production and reduced pH, which might explain the low amoxicillin concentrations observed in infected tissues.[28]

As expected, the MRSA strain was also resistant to amoxicillin in the *in vivo* model. The ability to reduce the antimicrobial concentration on the site (Figure 5), probably due to antibiotic lysis by beta-lactamase, could be responsible for the survival of MRSA strain against the high dose of amoxicillin.

Virtually all *S. aureus* strains, including most coagulase-negative staphylococci, are susceptible to vancomycin.[29] In the present study, a single dose of vancomycin killed all bacteria growing in the PSSA-infected tissue between 6h and 24h. Probably, the capacity of injuring protoplasts by altering the permeability of their cytoplasmic membrane and selectively inhibiting RNA synthesis [30] could contributed to the observed effect against PSSA cells. In addition, vancomycin exhibits minimal concentration-dependent killing, but a moderately long in vitro postantibiotic effect, which maybe has supplementary effect. [30,31]

There is no cross-resistance between the beta-lactams, which inhibit cell wall biosynthesis in the third phase, and vancomycin, which prevent cross-linking of the cell wall peptidoglycan during the second stage. In addition, there is no competition for binding sites between both drugs.[29] Thus, it is expected that vancomycin could eradicate even penicillin-resistant bacteria.

A significant reduction induced by vancomycin was observed in the present study, considering the tissue infected with the MRSA strain. Although significant when compared

to the absence of effect of the other treatment, the observed bacterial reduction was not expressive. Many reasons could be responsible for this observation. First, just one dose was administered and it was clearly not enough to kill all bacteria. Besides, like penicillins, vancomycin requires actively growing bacteria to exert its effect.[30] The biofilm induced by the present model did not stimulate the bacterial growth. In fact, Figure 3 evidences the decline of bacterial population even when saline solution was administered. Second, vancomycin retains activity between local pH 6.5 and 8 and it is possible that the resulting abscesses had lower values of pH. Finally, although it is known that the concentrations achieved in abscess fluid approach those obtained in serum, vancomycin is eliminated by glomerular filtration, with 80% to 90% of dose appearing in the urine within 24 hours. [32] Probably, in the second period of the present study, the single dose of vancomycin was almost all excreted.

Because most infections do not occur in plasma but rather in tissue sites (extracellular fluid), the ability of antibiotics to reach the target sites is a key determinant of clinical outcome. It is very important to realize that the free (unbound) antibiotic in the interstitial fluid at the target site is the real responsible for the antibacterial activity. Free antimicrobial concentrations in tissue are more relevant than serum concentrations in predicting therapeutic efficacy.[33] The differences between tissue and serum levels of vancomycin could be explained by the poor tissue diffusion of vancomycin and the plasmatic half-life (5-6 hours).[34] Moreover, the poor tissue diffusion may also explain the similarity of vancomycin concentrations between infected and non-infected tissues in this period.

It remains unclear whether vancomycin levels need to be maintained constantly above the MIC for clinical efficacy. A previous study stated that vancomycin levels should constantly exceed the MIC when a MRSA strain is the pathogen.[35] Furthermore, MRSA has the ability to remain alive and to spread inside polymorphonuclear (PMN) cells, where it is prevented from being killed and are then released when PMN are disrupted during apoptosis.[36] These facts might contribute to explain the low sensitivity of vancomycin observed in group 4.

A positive correlation between minimal inhibitory concentrations or other *in vitro* tests and successful resolution of infection process *in vivo* has not been uniformly demonstrated.[37] The nature and virulence of the infection, exponential versus stationary microbial growth phases, local factors (pH, inoculum size and blood supply) antibiotic pharmacokinetics and host resistance can all significantly affect antimicrobial efficacy.[27] The present study confirms the difficulty to establish a good *in vitro/in vivo* correlation. Both strains used showed differences between their *in vitro/in vivo* antimicrobial resistance profile, since PSSA strain was considered susceptible to amoxicillin but the drug failed to eradicate the bacteria. MRSA was considered susceptible against vancomycin but it also failed. Probably a complete scheme including more doses distributed during larger periods will produce different results.

Many previous studies have been shown the high pathogenicity of MRSA in different infection models.[38] It becomes more evident that these microorganisms use very efficient strategies to circumvent and misguide the host defenses in order to colonize and invade tissues.[39] The spread of pathogens such as MRSA justifies the use of suitable means of infection control and continuous surveillance on its antimicrobial resistance.[40] At least

two cases of cross-infection caused by MRSA, from a general dental practitioner to patients, have been reported.[41]

In conclusion, a single dose of amoxicillin did not affect the curse of infection induced by a penicillin-susceptible strain. The MRSA strain isolated from a clinical environment showed high pathogenicity in comparison to a penicillin-susceptible *S. aureus* strain and it was not eradicate by a single dose of vancomycin.

Acknowledgements

The authors thank CAPES for financial support and Mr. Jorge Valério for his assistance in the manuscript preparation.

References

1. Hardie J. Concerns regarding infection control recommendations for dental practice. *J Can Dent Assoc.* 1992;58:377-8, 382-6.
2. DePaola LG, Carpenter WM. Bloodborne pathogens: current concepts. *Compend Contin Educ Dent*. 2002;23:207-10, 212, 214.
3. Hackney Jr RW, Crawford J J, Tulis JJ. Using a biological indicator to detect potential source of cross-contamination in the dental operatory. *J Am Dent Ass* 1998; 129: 1567-1577.
4. Harrel SK, Molinari J. Aerosols and splatter in dentistry: a brief review of the literature and infection control implications. *J Am Dent Assoc*. 2004;135:429-37.
5. Bennett AM, Fulford MR, Walker JT, Bradshaw DJ, Martin MV, Marsh PD. Microbial aerosols in general dental practice. *Br Dent J* 2000; 189: 664-667.

6. Honma K, Tawara Y, Okuda K. Detection of methicillin-resistant staphylococcus aureus in human saliva and on denture surfaces. *Bull Tokyo Dent Coll.* 1994; 35 :217-20.
7. Oie S, Hosokawa I, Kamiya A. Contamination of room door handles by methicillin-sensitive/methicillin-resistant *Staphylococcus aureus*. *J Hosp Infect.* 2002;51:140-3.
8. Singh D, Kaur H, Gardner WG, Treen LB. Bacterial contamination of hospital pagers. *Infect Control Hosp Epidemiol.* 2002;23:274-6.
9. Bradley S. Methicillin-resistant Staphylococcus aureus: long-term care concerns. *Am J Med* .1999;106:2S-10S.
10. Chambers HF. The changing epidemiology of *Staphylococcus aureus*? *Emerg Infect Dis*. 2001;7:178-82.
11. Dufour P, Gillet Y, Bes M, et al. Community-acquired methicillin-resistant Staphylococcus aureus infections in France: emergence of a single clone that produces Panton-Valentine leukocidin. *Clin Infect Dis*. 2002;35:819-24.
12. Balaban N, Gov Y, Giacometti A, *et al.*. A chimeric peptide composed of a dermaseptin derivative and an RNA III-inhibiting peptide prevents graft-associated infections by antibiotic-resistant staphylococci. *Antimicrob Agents Chemother*. 2004;48:2544-50.
13. Jabes D, Candiani G, Romano G, *et al*. Efficacy of dalbavancin against methicillin-resistant Staphylococcus aureus in the rat granuloma pouch infection model. *Antimicrob Agents Chemother*. 2004;48:1118-23.
14. Miyazaki S, Okazaki K, Tsuji M, Yamaguchi K. Pharmacodynamics of S-3578, a novel cephem, in murine lung and systemic infection models. *Antimicrob Agents Chemother*. 2004;48:378-83.

15. Groppo FC, Simões RP, Ramacciato JC *et al*. Effect of sodium diclofenac on serum and tissue concentration of amoxicillin and on staphylococcal infection. *Biol Pharm Bull.* 2004;27:52-5.
16. Baglie S, Groppo FC, Filho TR. Tissue pharmacokinetics of amoxicillin. An experimental design in rats. Braz J Infect Dis. 2000;4:197-203.
17. Pires de Abreu LR, Ortiz RM, de Castro SC, Pedrazzoli J Jr. HPLC determination of amoxicillin comparative bioavailability in healthy volunteers after a single dose administration. *J Pharm Pharm Sci.* 2003;6:223-30.
18. Saito M, Santa T, Tsunoda M, Hamamoto H, Usui N. An automated analyzer for vancomycin in plasma samples by column-switching high-performance liquid chromatography with UV detection. *Biomed Chromatogr*. 2004;18:735-8.
19. Carlqvist J, Westerlund D. Automated determination of amoxycillin in biological fluids by column switching in ion-pair reversed-phase liquid chromatographic systems with post-column derivatization. *J Chromatogr.* 1985;344:285-96.
20. Lorian V. In vitro simulation of in vivo conditions: physical state of the culture medium. *J Clin Microbiol.* 1989;27:2403-6.
21. Goerke C, Wolz C. Regulatory and genomic plasticity of Staphylococcus aureus during persistent colonization and infection. *Int J Med Microbiol.* 2004;294:195-202.
22. Zak O, O'Reilly T. Animal infection models and ethics -- the perfect infection model. *J Antimicrob Chemother*. 1993;31:193-205.
23. Groppo FC, Mattos-Filho TR, Del-Fiol FS. Bioassay of amoxicillin in rats. *Biol Pharm Bull.* 2000;23:1033-5.

24. Ando E, Monden K, Mitsuhata R, Kariyama R, Kumon H. Biofilm formation among methicillin-resistant Staphylococcus aureus isolates from patients with urinary tract infection. *Acta Med Okayama.* 2004;58:207-14.

25. Turnidge JD. The pharmacodynamics of beta-lactams. *Clin Infect Dis.* 1998;27:10-22.

26. Montgomery EH, Kroeger DC. Principles of anti-infective therapy. Dent Clin North Am. 1984;28:423-32.

27. Pallasch TJ. Pharmacokinetic principles of antimicrobial therapy. *Periodontol 2000*. 1996;10:5-11.

28. Gerber AU, Greter U, Segessenmann C, Kozak S. The impact of the pre-treatment interval on antimicrobial efficacy in a biological model. *J Antimicrob Chemother.* 1993;31 Suppl D:29-39.

29. Tammy S. Lundstrom, MD, Jack D. Sobel, MD. Antibiotics for gram-positive bacterial infections: vancomycin, quinupristin-dalfopristin, linezolid, and daptomycin. *Infect Dis Clin N Am.* 2004; 18: 651–668.

30. Cunha BA, Quintiliani R, Deglin JM, et al. Pharmacokinetics of vancomycin in anuria. *Rev Infect Dis*. 1981; Suppl 3:269.

31. Craig WA. Basic pharmacodynamics of antibacterials with clinical applications to the use of beta-lactams, glycopeptides, and linezolid. *Infect Dis Clin North Am.* 2003;17:479-501.

32. Torres JR, Sanders CV, Lewis AC. Vancomycin concentration in human tissues: preliminary report. *J Antimicrob Chemother*. 1979;5:475.

33. Liu P, Derendorf H. Antimicrobial tissue concentrations. *Infect Dis Clin North Am.* 2003;17:599-613.

34. Ackerman BH, Vannier AM, Eudy EB. Analysis of vancomycin time-kill studies with *Staphylococcus* species by using a curve stripping program to describe the relationship between concentration and pharmacodynamic response. *Antimicrob Agents Chemother.* 1992;36:1766-9.

35. Houlihan HH, Mercier RC, Rybak MJ. Pharmacodynamics of vancomycin alone and in combination with gentamicin at various dosing intervals against methicillin-resistant *Staphylococcus aureus*-infected fibrin-platelet clots in an in vitro infection model. *Antimicrob Agents Chemother.* 1997;41:2497-501.

36. Hebert A, Sayasith K, Senechal S, Dubreuil P, Lagace J. Demonstration of intracellular Staphylococcus aureus in bovine mastitis alveolar cells and macrophages isolated from naturally infected cow milk. *FEMS Microbiol Lett.* 2000;193:57-62.

37. Peterson LR, Shanholtzer CJ. Tests for bactericidal effects of antimicrobial agents: technical performance and clinical relevance. *Clin Microbiol Rev.* 1992;5:420-32.

38. Weiss WJ, Lenoy E, Murphy T *et al*. Effect of srtA and srtB gene expression on the virulence of Staphylococcus aureus in animal models of infection. *J Antimicrob Chemother.* 2004;53:480-6.

39. Hornef MW, Wick MJ, Rhen M, Normark S. Bacterial strategies for overcoming host innate and adaptive immune responses. *Nat Immunol.* 2002;3:1033-40.

40. Kohn WG, Collins AS, Cleveland JL*, et al.*. Guidelines for infection control in dental health-care settings – 2003. *M M W R* 2003; 52:1-61.

41. Martin MV, Hardy P. Two cases of oral infection by methicillin-resistant *Staphylococcus aureus*. *Br Dent J.* 1991 19;170:63-4.

Figures

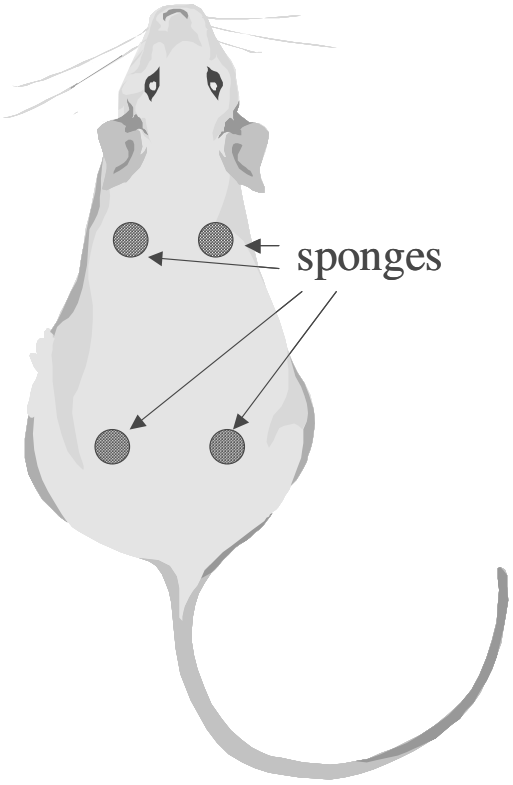


Figure 1. Schematic representation of sponge positioning.

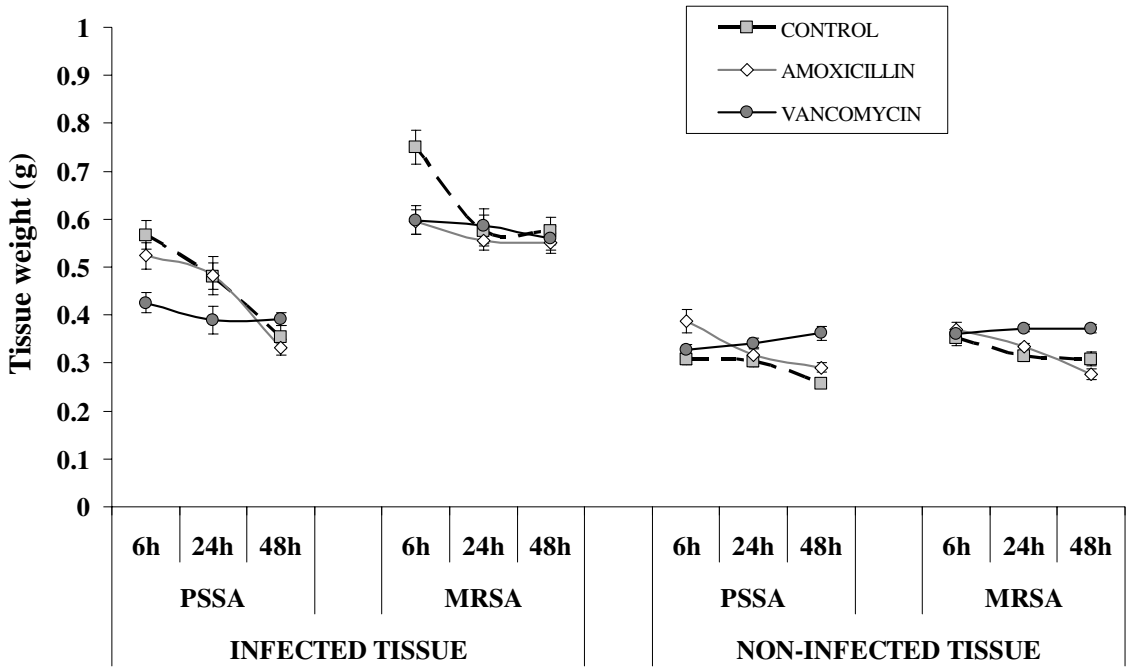


Figure 2 - The weight values (mean ± s.e.m.) of the granulomatous tissue considering each group.

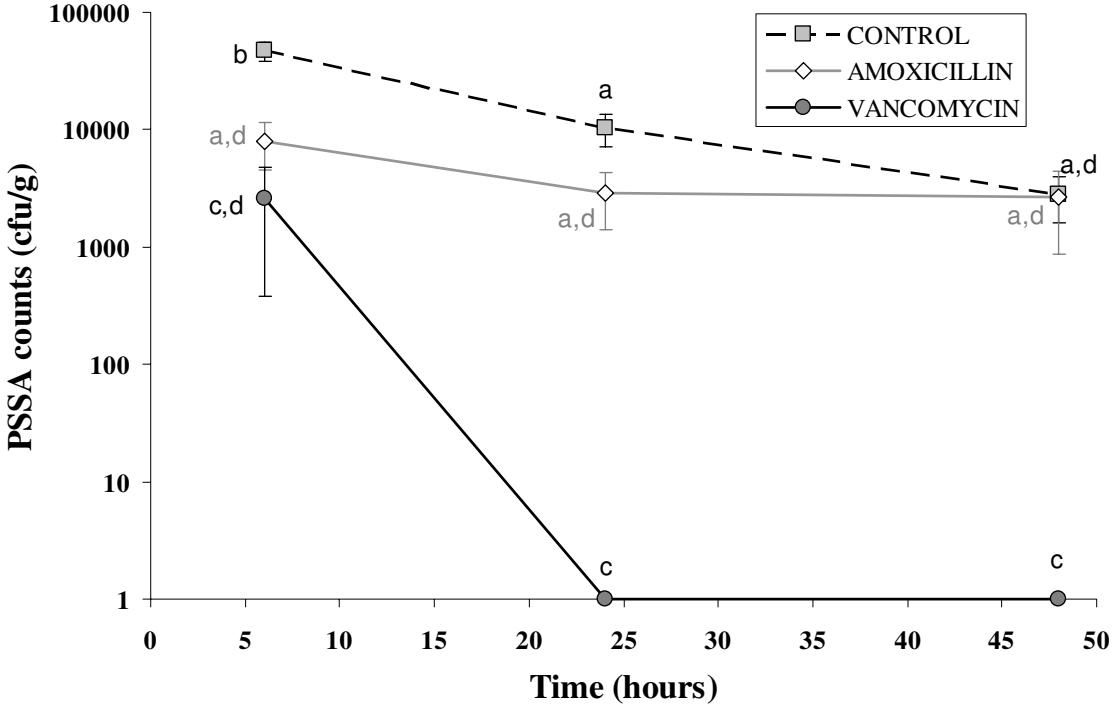


Figure 3 – Mean values (± s.e.m.) of microorganism counts (cfu/g in log scale) of groups 1, 3 and 5 (inoculated with PSSA strain). Different letters mean statistically significant differences ($p<0.05$) among groups.

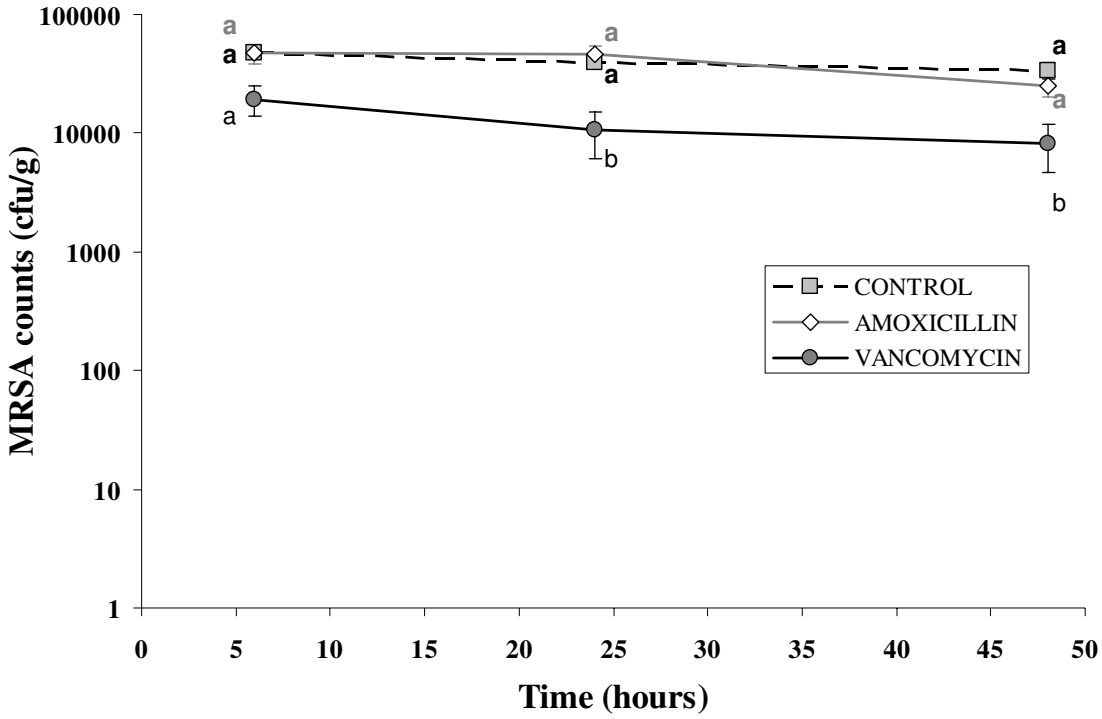
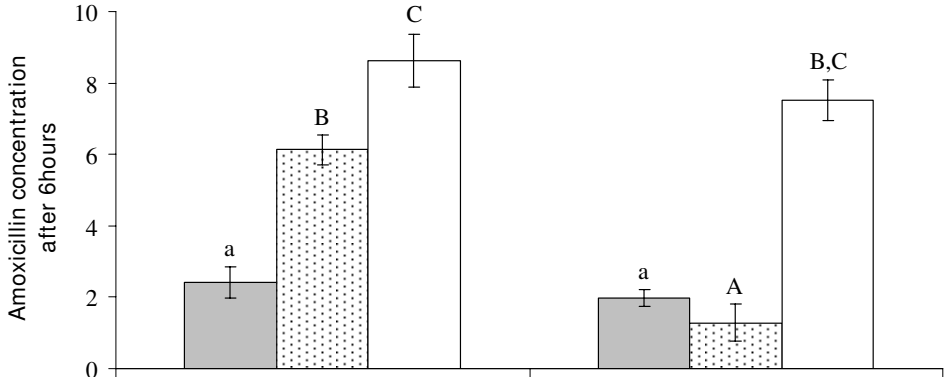
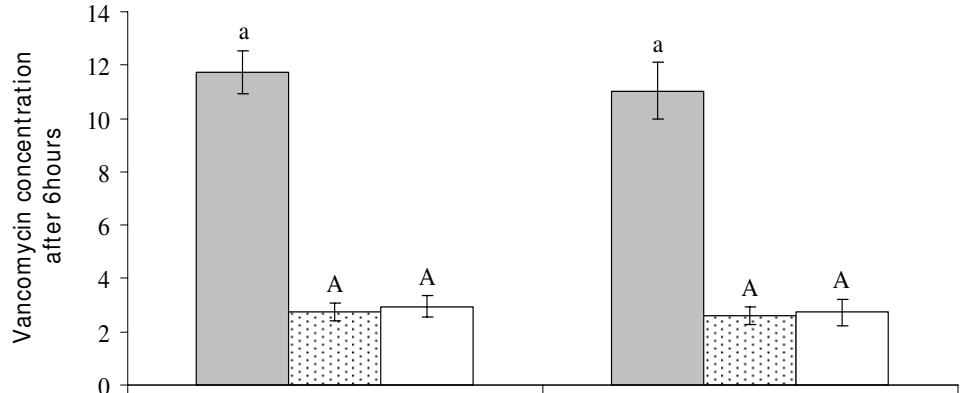


Figure 4 – Mean values (± s.e.m.) of microorganism counts (cfu/g in log scale) of groups 2, 4 and 6 (inoculated with MRSA strain). Different letters mean statistically significant differences ($p<0.05$) among groups.



	PSSA	MRSA
Serum (µg/mL)	2.41	1.97
Infected tissue (µg/g)	6.13	1.29
Non-infected tissue (µg/g)	8.62	7.52

Figure 5 – Serum and tissue concentrations (mean ± s.e.m.) of amoxicillin in the different periods considering groups 1 and 2. Different letters (capital letters for tissue and small letters for serum) mean statistically significant differences ($p < 0.05$).



	PSSA	MRSA
Serum (µg/mL)	11.73	11.02
Infected tissue (µg/g)	2.74	2.62
Non-infected tissue (µg/g)	2.95	2.72

Figure 6 – Serum and tissue concentrations (mean ± s.e.m.) of vancomycin in the different periods considering groups 1 and 2. Different letters (capital letters for tissue and small letters for serum) mean statistically significant differences ($p < 0.05$).

4. Conclusões Gerais

4**.1. – Artigo 1** - A atividade clínica aumenta a contaminação em ambientes clínicos odontológicos, onde os estreptococos foram os microrganismos mais prevalentes.

4**.2. – Artigo 2 –** A atividade clínica aumenta a contaminação por *Staphylococcus aureus* no ambiente clínico odontológico, demonstrando a necessidade de um adequado controle de contaminação cruzada nestes ambientes uma vez que os microrganismos isolados demonstraram alta resistência aos antimicrobianos testados.

4.3. – Artigo 3 – A amoxicilina em dose única não foi capaz de interferir na infecção induzida pela cepa de *S. aureus* sensível à penicilina. A cepa de *S. aureus* resistente à meticilina, isolada do ambiente clínico, mostrou uma alta patogenicidade em comparação com a cepa sensível à penicilina e, além disso, a mesma não foi erradicada por uma dose única de vancomicina.

Referências Bibliográficas*

1. Baglie S, Groppo FC, Filho TR. Tissue pharmacokinetics of amoxicillin. An experimental design in rats. ***Braz J Infect Dis***. 2000;4(4):197-203.
2. Bentley CD, Burkhart, NW, Crawford, JJ. Evaluating spatter and aerosol contamination during dental procedures. ***J Am Dent Assoc***. 1994; 125(5): 579-84.
3. Bradley S. Methicillin-resistant *Staphylococcus aureus*: long-term care concerns. ***Am J Med*** .1999;106(5A):2S-10S.
4. Cottone JA, Terezhalmy GT, Molinari JA. ***Pratical infection control in dentistry***. Philadelphia: Lea & Febiger;1991.
5. Crawford JJ. Sterilization, disinfection, and aseps in dentistry. *In*: Block SS. ***Disinfection, sterilization and preservation***. Philadelphia: Lea & Febige;1983. 3ª ed. p.505-23.
6. de Sá Del Fiol F, Rocha De Mattos Filho T, Groppo FC. Evaluation in an animal model and in vitro of the combination clavulanic acid and cephalosporins against beta-lactamase producing and nonproducing *Staphylococcus aureus* strains. ***Braz J Infect Dis***. 2000;4(1):36-42.
7. Gerber AU, Greter U, Segessenmann C, Kozak S. The impact of the pre-treatment interval on antimicrobial efficacy in a biological model. ***J Antimicrob Chemother***. 1993;31 Suppl D:29-39.

* De acordo com a norma da FOP/Unicamp, baseada no modelo de Vancouver. Abreviatura dos periódicos em conformidade com o Medline.

8. Groppo FC, Simões RP, Ramacciato JC *et al*. Effect of sodium diclofenac on serum and tissue concentration of amoxicillin and on staphylococcal infection. ***Biol Pharm Bull***. 2004;27(1):52-5.

9. Guimarães Júnior J. ***Biossegurança e controle de infecção cruzada em consultórios odontológicos***. São Paulo: Santos; 2001.

10. Honma K, Tawara Y, Okuda K. Detection of methicillin-resistant staphylococcus aureus in human saliva and on denture surfaces. ***Bull Tokyo Dent Coll***. 1994; 35(4): 217-20.

11. Kedjarune U, Kukiattrakoon B, Yapong B, Chowanadisai S, Leggat P. Bacterial aerosols in the dental clinic: effect of position, time and type of treatment. ***Int Dent J***. 2000;50(2):103-107.

12. Kedjarune U, Leggat PA. Bacterial aerosols in the dental clinic: a review**.** ***Int Dent J*** .2001; 51(1):39-44.

13. Mattos Filho TR, Groppo FC, Pacheco ABND, Motta RHL, Napimoga MH. Antimicrobial susceptibility of air-dispersed microorganisms in dental settings. ***Gen Dent*.** 2005;(Jan-Feb):In press.

14. Miller RC, Abbott KA, Collard EW, Duncanson MG Jr, Willer RD. Potential for cross-contamination within a dental school enviroment. ***J Dent Educ***. 1990; 54(2): 160-62.

15. Oie S, Hosokawa I, Kamiya A. Contamination of room door handles by methicillin-sensitive/methicillin-resistant *Staphylococcus aureus*. ***J Hosp Infect***. 2002;51(2):140-3.

16. Pires de Abreu LR, Ortiz RM, de Castro SC, Pedrazzoli J Jr. HPLC determination of amoxicillin comparative bioavailability in healthy volunteers after a single dose administration. ***J Pharm Pharm Sci***. 2003;6(2):223-30.
17. Saito M, Santa T, Tsunoda M, Hamamoto H, Usui N. An automated analyzer for vancomycin in plasma samples by column-switching high-performance liquid chromatography with UV detection. ***Biomed Chromatogr***. 2004;18:735-8.
18. Samaranayake LP, Scheutz F, Cottone JA. ***Controle da infecção para a equipe odontológica***. São Paulo: Santos; 1995.
19. Singh D, Kaur H, Gardner WG, Treen LB. Bacterial contamination of hospital pagers. ***Infect Control Hosp Epidemiol***. 2002;23(5):274-6.
20. Vaudaux P, Francois P, Bisognano C ,Schrenzel J, Lew DP. Comparison of levofloxacin, alatrofloxacin, and vancomycin for prophylaxis and treatment of experimental foreign-body-associated infection by methicillin-resistant *Staphylococcus aureus*. ***Antimicrob Agents Chemother***. 2002;46(5):1503-9.
21. Warren DK, Fraser VJ. Infection control measures to limit antimicrobial resistance. ***Crit Care Med.*** 2001;29(4):N128-34.

Anexo – Certificado do Comitê de Ética

INSTITUTO DE BIOLOGIA
UNICAMP

CEEA-IB-UNICAMP

Comissão de Ética na Experimentação Animal
CEEA-IB-UNICAMP

CERTIFICADO

Certificamos que o Protocolo nº 423-1, sobre "Efeito da Vancomici
e da Amoxicilina sobre a Infecção Estafilocó
Estudo em Ratos",
sob a responsabilidade de Rogério Heládio Lopes Motta
Depto. Ciências Fisiológicas/FOP-UNICAMP, está de acordo
com os Princípios Éticos na Experimentação Animal adotados pelo Colégio Brasileiro de Experimentação Animal (COBEA), tendo sido aprovado pela Comissão de Ética na Experimentação Animal (CEEA)-IB-UNICAMP em reunião de 05/07/2002

Campinas, 05 de julho de 2002.

CERTIFICATE

We certify that the protocol nº 423-1, entitled "Effects of Vancomyc
and Amoxicillin against Staphylococcus au
in Rats",
is in agreement with the Ethical Principles in Animal Research established by the Brazilian College for Animal Experimentation (COBEA). This project was approved by the institutional Committee for Ethics in Animal Research (State University of Campinas – UNICAMP) on 05/07/2002

Campinas 05 de julho de 2002.

Profa. Dr(a) Alba R.M. Souza Brito
Presidente – CEEA/IB/UNICAMP

UNIVERSIDADE ESTADUAL DE CAMPINAS
INSTITUTO DE BIOLOGIA
CIDADE UNIVERSITÁRIA ZEFERINO VAZ
CAMPINAS - SP - BRASIL

TELEFONE (019) 788 7126
FAX (019) 289.3124