UNIVERSIDADE ESTADUAL DE CAMPINAS

FACULDADE DE ODONTOLOGIA DE PIRACICABA

ROBERTA CRISTIANE CATELLI BAGLIE

FARMACÊUTICA

AVALIAÇÃO DA RESISTÊNCIA A ANTIMICROBIANOS DE *Staphylococcus aureus* E ESTREPTOCOCOS GRUPO *VIRIDANS* DE PACIENTES COM RISCO A ENDOCARDITE INFECCIOSA.

Tese apresentada à Faculdade de Odontologia de Piracicaba, Universidade Estadual de Campinas, para obtenção do título de Doutora em Odontologia, Área de Farmacologia, Anestesiologia e Terapêutica.

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DEDICATÓRIA

A **DEUS**, Pai todo poderoso e misericordioso, por sempre estar ao meu lado guiando meus passos e iluminando minha vida.

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Meu sincero e carinhoso agradecimento!

EPÍGRAFE

É PRECISO SABER VIVER

Composição: Roberto Carlos e Erasmo Carlos

Quem espera que a vida
Seja feita de ilusão
Pode até ficar maluco
Ou morrer na solidão
É preciso ter cuidado
Pra mais tarde não sofrer
É preciso saber viver
Toda pedra do caminho
Você deve retirar
Numa flor que tem espinhos
Você pode se arranhar
Se o bem e o mal existem
Você pode escolher
É preciso saber viver

É preciso saber viver
É preciso saber viver
É preciso saber viver

Toda pedra do caminho
Você deve retirar
Numa flor que tem espinhos
Você pode se arranhar
Se o bem e o mal existem
Você pode escolher
É preciso saber viver

É preciso saber viver...

SUMÁRIO

RESUMO

A endocardite infecciosa (EI) é uma infecção grave das válvulas cardíacas, do endocárdio mural e de septos defeituosos, provocada principalmente pelos *Staphylococcus aureus* e pelos estreptococos grupo *viridans.* O objetivo deste trabalho foi verificar a resistência aos antimicrobianos de cepas de *Staphylococcus aureus* e estreptococos grupo *viridans* provindas de 100 voluntários, sendo 60 adultos (16 a 75 anos) que foram divididos em três grupos: saudáveis (grupo 1), cardiopatas de baixo risco (grupo 2) e cardiopatas de alto risco (grupo 3) para EI e 40 crianças (6 a 14 anos) que foram divididas em dois grupos: saudáveis (grupo 1) e cardiopatas de alto risco (grupo 2) para EI. Além disso, os voluntários dos grupos 2 e 3 e os responsáveis pelas crianças do grupo 2 responderam a um formulário sobre os riscos da EI e sua profilaxia. Amostras de saliva (diluídas 1:1000) foram inoculadas em *Mitis Salivarius* agar e incubadas em microaerofilia durante 48 h. Amostras de pele (swab) foram inoculadas em *Sal Manitol* agar e incubadas em aerobiose durante 24 h. Após testes bioquímicos, os estreptococos grupo *viridans* e os *Staphylococcus aureus* foram identificados e submetidos a testes de susceptibilidade microbiológica empregando-se agentes antimicrobianos. Dos voluntários adultos, foram isoladas 186 cepas, 58 do grupo 1, 62 do grupo 2 e 66 do grupo 3. Dentre os estreptococos a espécie *S. mitis* foi a mais comum. A cepa mais resistente contra as penicilinas (amoxicilina, ampicilina, meticilina e oxacilina) foi a do *S. aureus* isolada dos grupos 2 (57,9%) e 3 (80%). Considerando os grupos 2 e 3: 21 voluntários (52,5%) tinham conhecimento sobre sua doença, entretanto, somente 12,5% sabiam o significado de EI. Dezessete voluntários (42,5%) sabiam sobre a antibioticoprofilaxia antes de algum procedimento dental e 10 voluntários nomearam a amoxicilina como o principal antibiótico para a profilaxia. Trinta e cinco voluntários não receberam informações dos médicos ou dentistas sobre a importância da saúde oral para prevenir a EI. A maior parte (60%) disse ter visitado o dentista pela última vez há mais de 1 ano.

RESUMO

Trinta e cinco porcento afirmou apresentar algum tipo de doença oral (gengivite, cárie, etc.). Dos voluntários pediátricos, foram isoladas 98 colônias, 51 do grupo 1 e 47 do grupo 2. A espécie mais isolada foi *Streptococcus pneumoniae*: 23,4% e 25,5% nos grupos 1 e 2, respectivamente. *S. aureus* corresponderam a 36,2% e 31,9% nos grupos 1 e 2, respectivamente. Quatorze responsáveis (70%) tinham conhecimento sobre a doença de suas crianças; entretanto, somente 15% sabia o significado de "endocardite infecciosa". Onze responsáveis (55%) sabiam sobre a antibioticoprofilaxia antes de algum procedimento dental, e 10 responsávies nomearam a amoxicilina como o principal antibiótico para a profilaxia. Dezesseis responsáveis (80%) não receberam informações dos médicos ou dentistas sobre a importância da saúde oral para prevenir a EI. A maioria (40%) disse que a criança visitou o dentista pela última vez há 6 meses. Concluímos que, de acordo com o perfil de resistência das cepas de estreptococos orais, a amoxicilina é o agente antimicrobiano de primeira escolha para a profilaxia da EI. É clara a necessidade de oferecer informações sobre a EI para os pacientes afetados por doenças cardíacas ou para seus responsáveis.

Palavras chave: endocardite, resistência antimicrobiana, *S. aureus* e estreptococos *viridans.*

ABSTRACT

The aim of this study was to verify the antimicrobial resistance of *Staphylococcus aureus* and *viridans* streptococci from 100 volunteers, 60 adults (16 to 75 years-old), which were divided into three groups: healthy (Group 1), low risk (Group 2) and high risk (Group 3) for infective endocarditis (IE) and 40 children (6 to 14 years-old) which were divided into two groups: healthy (Group 1) and high risk (Group 2) for IE. In addition, volunteers were submitted to a structured formulary about IE risks and prophylaxis. Saliva samples were inoculated on *Mitis Salivarius* agar and incubated in microaerophilia during 48h. Skin samples were inoculated on *Mannitol-Salt* agar and incubated in aerobiosis during 24h. After biochemical tests *viridans* streptococci and *S. aureus* were identified and submitted to antimicrobial susceptibility tests against antimicrobial agents. Considering adults, *Streptococcus mitis* was the most common strain isolated among streptococci. 186 strains were isolated, being 58 from group 1, 62 from group 2 and 66 from group 3. The most resistant strain against penicillins (amoxicillin, ampicillin, methicillin and oxacillin) was *S. aureus* isolated from groups 2 (57.9%) and 3 (80%). Considering groups 2 and 3: twenty-one volunteers (52.5%) were aware about their disease however, only 12.5% were aware of the meaning of "infective endocarditis". Seventeen volunteers (42.5%) knew about antibiotic prophylaxis before some dental procedures, and 10 volunteers named amoxicillin as the main antibiotic for prophylaxis. Thirty-five volunteers did not receive information from physicians or dentists about importance of oral health to prevent IE. The majority (60%) reported the last visit to the dentist more than 1 year ago. Thirty-five percent reported some kind of oral disease (gingivitis, caries, etc.). Considering children, 98 strains were isolated, being 51 from group 1 and 47 from group 2. *Streptococcus pneumoniae* was the most common streptococci isolated: 23.4% and 25.5% in groups 1 and 2, respectively. *S. aureus* corresponded to 36.2% and 31.9% in groups 1 and 2, respectively. Fourteen guardians (70%) were aware

about their child's disease; however, only 15% were aware of the meaning of "infective endocarditis". Eleven guardians (55%) knew about antibiotic prophylaxis before some dental procedures, and 10 volunteers named amoxicillin as the main antibiotic for prophylaxis. Sixteen guardians (80%) did not received information from physicians or dentists about importance of oral health to prevent IE. The most part (40%) reported the last visit to the dentist at least six months ago. We concluded that the resistant profile of the oral streptococci strains supports amoxicillin as the first choice antimicrobial agent for IE prophylaxis. There was a clear need for more information regarding IE among patients affected by cardiac disease or their guardians.

Key words: endocarditis, antimicrobial resistance, *S. aureus* and *viridans streptococci*.

1. INTRODUÇÃO

A endocardite infecciosa continua sendo uma complicação séria e letal em pacientes de risco, pois causa destruição valvar, mural, septal e insuficiência cardíaca (ROMAN *et al.*, 1997), embora não seja um evento raro (COWPER, 1996).

A doença caracteriza-se pela colonização do coração, geralmente as válvulas, por qualquer bactéria ou, mais raramente, por fungos e vírus. A etiologia bacteriana é a mais comum, daí o termo endocardite bacteriana ser considerado como sinônimo de endocardite infecciosa. Os focos de infecção iniciam a formação de trombos friáveis, carregados de microrganismos, criando as chamadas vegetações infectantes. Este processo é mais comum em locais cardíacos lesados, principalmente nas cúspides das válvulas cardíacas (BAYER *et al.*, 1998).

Defeitos cardíacos em conjunto com bacteremias provocadas por patologias orais elevam o risco à endocardite, embora um pequeno, mas ascendente número de condições não cardíacas e padrões ambientais seja também capaz de induzir a susceptibilidade. A hipótese dos procedimentos médicos ou odontológicos como indutores diretos da endocardite infecciosa não está bem estabelecida (COWPER, 1996; STRON, 2000).

Os sintomas clínicos incluem: febre (em 88% dos casos), falência cardíaca (39%), problemas neurológicos (20%) e fenômenos embólicos (22%) (BITAR *et al.*, 2000).

Atualmente, as cepas de *Staphylococcus aureus* são responsabilizadas pela maior incidência de endocardite infecciosa, seguidas pelos estreptococos grupo *viridans* (BENN *et al.*, 1997; YAMAUCHI *et al.*, 1997; DUDKIEWICZ *et al.*, 1996), ainda que outros microrganismos como fungos e vírus possam ser causadores (BAYER *et al.*, 1998).

No Brasil, as taxas de prevalência no fim da década de 80 eram de 31% para os estreptococos grupo *viridans* e de 20% para o *S. aureus* (MANSUR *et al.*, 1990). Entretanto, no fim da década de 90 esta taxa inverteu-se, sendo o *S.*

aureus responsável por 27,2% e os estreptococos grupo *viridans* por 15,9% (RUIZ JR *et al*., 2000). Esta tendência tem sido mostrada em outros trabalhos (FIGUEIREDO *et al*., 2001), mesmo em adolescentes (AOUN *et al*., 1997).

HALL *et al*. (1996) estudaram a bacteremia provocada após extrações dentais. Uma hora antes da extração, os pacientes recebiam eritromicina (1g) ou clindamicina (0,6g), ou seja, a posologia recomendada pela *American Heart Association* na época. Os resultados mostraram incidência de bacteremia de estreptococos grupo viridans de 79% para a eritromicina e de 74% para o grupo da clindamicina. Resultados similares foram encontrados também com as penicilinas (HALL *et al*., 1993).

MARTIN *et al*. (1997) revisaram os episódios de endocardite infecciosa envolvendo procedimentos odontológicos na Inglaterra. Os procedimentos implicados na endocardite infecciosa foram a exodontia (23), medição de bolsa periodontal (21), endodontia com instrumentação extra-canal (7) e cirurgia oral menor (2). Os cirurgiões-dentistas envolvidos falharam em aplicar o regime profilático (48), prescreveram antibióticos incorretos (2) ou utilizaram posologia incorreta (2). Os autores observaram, ainda, um caso de endocardite infecciosa, apesar da profilaxia com a amoxicilina administrada corretamente.

Em 1997, a *American Heart Association* revisou as suas diretrizes para a prevenção da endocardite infecciosa. Os resultados apontaram que, para os procedimentos odontológicos que envolvam riscos aos pacientes nos quais é recomendada a profilaxia, o regime passou a ser considerado como sendo dose única de 2 g de amoxicilina para adultos ou 50 mg/Kg em crianças, 1 hora antes do procedimento odontológico, não sendo necessária qualquer dose adicional. A eritromicina passou a não ser mais recomendada como alternativa a pacientes alérgicos à penicilina, mas a clindamicina e outras alternativas são possíveis. Enfatizaram ainda que a maior parte dos casos registrados não foi atribuída a procedimentos invasivos (DAJANI *et al*., 1997; TAUBERT & DAJANI, 1998).

A profilaxia da endocardite tem sido amplamente discutida (GRINBERG, 1997; HARBARTH *et al*., 2000; CABELL *et al*., 2003), bem como o preocupante aumento da resistência aos antimicrobianos dos microrganismos envolvidos nestes casos (TENG *et al*., 1998; LEVY *et al*., 2001; BRYSKIER, 2002).

As infecções hospitalares causam uma significante mortalidade e morbidade nos pacientes. As 2,5 milhões de infecções que ocorrem a cada ano custam ao sistema de saúde americano de 5 a 10 milhões de dólares. Os *Staphylococcus aureus* têm sido reconhecidos como importantes patógenos e são os causadores mais comuns destas infecções. Cerca de 30% de todas as infecções causadas pelo *S. aureus* são devidas à microbiota (PERL & GOLUB, 1998).

ROMAN *et al*. (1997) relataram que em maio de 1993, ocorreu um surto de *Staphylococcus aureus* meticilina-resistentes (MRSA) no seu centro de atendimento. A tipagem genética da cepa mostrou, mais uma vez, a propensão de certas cepas de *S. aureus* em produzir doenças epidêmicas, uma rápida difusão dentro das instituições de saúde e o problema de natureza global que é a resistência aos antimicrobianos.

KAMIYA (1997) argumentou que o uso de drogas antimicrobianas no Japão é extremamente alto. O uso intensivo de drogas de amplo-espectro, especialmente no tratamento de um crescente número de pacientes idosos e imunocomprometidos, resultou numa emergencial e expansiva resistência dos microrganismos. Um dos principais patógenos identificados dentro desta característica vem sendo as cepas de *Staphylococcus aureus* meticilina-resistentes.

FARIAS *et al*. (1997) avaliaram o padrão de susceptibilidade de cepas de *Staphylococcus aureus* oxacilina-susceptíveis e oxacilina-resistentes a agentes antimicrobianos comumente utilizados em infecções causadas por estes patógenos. As cepas (117) foram isoladas de vários hospitais em São Paulo, Campinas e João Pessoa. Obtiveram a concentração inibitória mínima para 24 agentes antimicrobianos, incluindo beta-lactâmicos (penicilinas e cefalosporinas) e

macrolídeos. Os resultados mostraram uma alta e preocupante taxa de resistência entre os *S. aureus* obtidos nos hospitais brasileiros. Poucas drogas foram efetivas contra infecções por este patógeno.

Os estreptococos grupo *viridans* são os principais envolvidos na endocardite provocada por procedimentos odontológicos (DAJANI, 1997). Na realidade, estes são "subgrupos", tais como o grupo do *Streptococcus mitis* (*S. oralis, S. sanguis, S. gordonii, S. mitis* e *S. pneumoniae*), do *Streptococcus milleri* (*S. anginosus, S. constellatus* e *S. intermedius*), do *Streptococcus mutans* (*S. mutans, S. rattus, S. cricetus, S. sobrinus, S. ferus, S. macacae* e *S. downeii*) e ainda *S. salivarius* (*S. salivarius* e *S. vestibularis*) (SLOTS & TAUBMAN, 1992).

A prevalência de estreptococos como causa de bacteremias, que são potencialmente fatais, em hospitais tem aumentado, o que requer que os agentes usados na terapia empírica incluam em seu espectro de atividade os estreptococos grupo *viridans* e beta-hemolíticos (PFALLER *et al*., 1997).

A incidência de infecções causadas por microrganismos facultativos gram-positivos (especialmente os estreptococos grupo *viridans*) multi-resistentes a drogas está aumentando, apesar dos avanços da terapia antibacteriana nos últimos 20 anos. Estes estreptococos em particular, são comumente resistentes aos beta-lactâmicos e aminoglicosídeos (BAQUERO, 1997).

TUOHY & WASHINGTON (1997) obtiveram a susceptibilidade antimicrobiana de 68 estreptococos grupo *viridans* (31 variedades de *Streptococcus sanguis*, 12 variedades de *S. mitis*, 3 variedades de *S. salivarius* e 8 variedades de *S. milleri*) colhidos do sangue humano e 14 variedades de *S. milleri* colhidos de abscessos. Foram testados contra penicilina G, amoxicilina, cefazolina, ceftriaxona, meropenem, clindamicina, quinupristina/dalfopristina, rifampicina, levofloxacina, ofloxacina, vancomicina e gentamicina através do método da microdiluição. As taxas de susceptibilidade foram determinadas em termos de porcentagem das cepas susceptíveis. Para o *S. sanguis* os valores encontrados

foram: penicilina, 74%; amoxicilina, 84%; clindamicina, 87%; cefatriaxona, 94% e vancomicina, 100%. Para o *S. mitis* foram: penicilina, 42%; cefatriaxona, 58%; amoxicilina, 67%; clindamicina, 100% e vancomicina, 100%. Para o *S. milleri* foram: penicilina, 100%; amoxicilina, 100%; cefatriaxona, 100%, clindamicina, 100% e vancomicina, 100%. Duas das três cepas isoladas do *S. salivarius* foram susceptíveis à penicilina, amoxicilina e cefatriaxona; as 3 foram susceptíveis à clindamicina e vancomicina. Levofloxacina, quinupristina/dalfopristina e rifampicina foram altamente ativas contra todas as cepas.

Assim, avaliar a resistência bacteriana frente a antimicrobianos nos pacientes com risco à endocardite infecciosa, é particularmente importante, pois mesmo para microrganismos que há décadas têm sido sensíveis a determinados antimicrobianos, pode surgir inesperadamente resistência (LEVY *et al.*, 2001).

2. PROPOSIÇÃO

1. Colher, isolar e identificar cepas de *Staphylococcus aureus* e estreptococos grupo *viridans* provindas de pacientes cardiopatas classificados como sendo de alto risco para endocardite infecciosa, de pacientes cardiopatas de baixo risco para endocardite infecciosa e de voluntários sadios.

2. Verificar e comparar a sensibilidade destas cepas bacterianas aos antimicrobianos.

3. Avaliar o grau de conhecimento dos pacientes ou de seus responsáveis sobre a endocardite, seus riscos e profilaxia.

3. CAPÍTULOS

Esta tese está baseada na Deliberação CCPG/001/98/Unicamp e na aprovação pela Congregação da Faculdade de Odontologia de Piracicaba em sua 105ª Reunião Ordinária em 17/12/2003, que regulamenta o formato alternativo para tese de Doutorado e permite a inserção de artigos científicos de autoria do candidato.

Assim sendo, esta tese é composta de dois capítulos contendo artigos que serão submetidos à publicação em revistas científicas, conforme descrito a seguir:

Capítulo 1

Artigo "***Antimicrobial resistance of Staphylococcus aureus and viridans streptococci of adults at risk for infective endocarditis***"

Este artigo foi submetido ao periódico: Infection Control and Hospital Epidemiology. Comprovante de submissão no anexo 2.

Capítulo 2

Artigo "***Viridans streptococci of children at risk for infective endocarditis are penicillin susceptible***"

Este artigo será submetido ao periódico: Journal of Paediatric and Child Health.

3.1 Capítulo 1

Title: *Antimicrobial resistance of Staphylococcus aureus and viridans streptococci of adults at risk for infective endocarditis.*

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ABSTRACT

Objective: to verify the antimicrobial resistance of *Staphylococcus aureus* and *viridans* streptococci from 60 volunteers, divided into three groups: healthy (Group 1), low-risk (Group 2) and high-risk (Group 3) for infective endocarditis (IE). In addition, volunteers were submitted to a structured formulary about IE. **Design:** saliva samples were inoculated on MSA and incubated in microaerophilia (48h). Samples from ear-skin were inoculated on SMA and incubated in aerobiosis (24h). After biochemical tests *viridans* streptococci and *S. aureus* were identified and submitted to antimicrobial susceptibility tests against antimicrobial agents. **Results:** *Streptococcus mitis* was the most common strain isolated among streptococci. 186 strains were isolated, being 58 from group 1, 62 from group 2 and 66 from group 3. The most resistant strain against penicillins was *S. aureus* isolated from groups 2 (57.9%) and 3 (80%). Considering groups 2 and 3: twenty-one volunteers (52.5%) were aware about their disease however, only 12.5% were aware of the meaning of "IE". Seventeen volunteers (42.5%) knew about antibiotic prophylaxis before some dental procedures, and 10 volunteers named amoxicillin as the main antibiotic for prophylaxis. Thirty-five volunteers did not receive information from dentists about importance of oral health to prevent IE. Sixty percent reported the last visit to the dentist more than 1 year ago. Thirty-five percent reported some kind of oral disease. **Conclusion:** the resistant profile of the oral streptococci strains supports amoxicillin as the first choice antimicrobial

agent for IE prophylaxis. There was clear need for more information regarding IE among patients affected by cardiac disease.

Key words: endocarditis, antimicrobial resistance, *S. aureus* and *viridans streptococci.*

INTRODUCTION

Infective endocarditis (IE) is an infection involving the endocardium in valvular, mural, and septal defects, as well as in arteriovenous and arterioarterial short-circuits[1].

Although relatively uncommon, IE is a life-threatening disease that usually affects individuals with underlying structural cardiac defects who develop bacteremia caused by microorganisms likely to cause endocarditis. Bacteremia may occur spontaneously or may complicate from a focal infection (e.g., urinary tract infection, pneumonia, or cellulitis)[2].

The most common microorganisms associated with endocarditis are viridans streptococci and *Staphylococcus aureus*[3]. The microorganisms of viridans group are the alpha-hemolytic streptococci: *Streptococcus oralis*, *S. sanguis*, *S. parasanguis*, *S. gordonii*, *S. mitis*, *S. mutans*, *S. salivarius*, *S. sobrinus*, and *milleri* group (*S. intermedius*, *S. constellatus*, and *S. adjacens*)[4].

Viridans streptococci are responsible for about 40% of the cases of endocarditis[5]. The high levels of resistance in the viridans streptococci have been demonstrated and it is a cause for concern[6].

Groppo *et al*. (2005)[7] observed moderate resistance levels (up to 30%) against beta-lactams and macrolides of salivary streptococci from volunteers classified as high-risk for endocarditis. *S. aureus* strains collected from forearm

skin of the same subjects, however, showed high levels of antimicrobial resistance (particularly against beta-lactams antibiotics).

Antibiotic prophylaxis for at risk patients is recommended for dental and oral procedures likely to cause bacteremia. Since 1997, The American Heart Association has been recommended 2g amoxicillin, one hour before certain dental procedures[2]. However, protective efficacy of antimicrobial prophylaxis has never been definitively proven[8]. In addition, reports of antimicrobial prophylaxis failure related to resistant viridans strains, mainly *S. mitis*, has been increasing[8,9].

Despite the morbidity and mortality of IE, many of patients at risk have inadequate knowledge of their disease. At least a half of these patients did not show appropriate knowledge about the potential risks of the disease in a previous study[10].

The aim of this study was to observe the antimicrobial susceptibility of *Staphylococcus aureus* and *viridans* streptococci group obtained from healthy and endocarditis-risk volunteers. In addition, a survey tested the knowledge about IE risks and prophylaxis of these volunteers.

MATERIAL AND METHODS

Sixty patients from the Cardiology Ambulatory of Clinics Hospital at State University of Campinas (Sao Paulo, Brazil) were included: Group 1 - 20 healthy, 10 women and 10 men (26.4±4.0 years-old); Group 2 – 20 volunteers at low-risk for

endocarditis, 8 women and 12 men (59.4±16.9 years-old) and Group 3 - 20 volunteers at high-risk for endocarditis, 12 women and 8 men (46.5±10.5 years-old). The present study was approved by Ethical Committee of the Faculty of Medical Sciences – UNICAMP. A write-informed consent was obtained from volunteers.

All volunteers were previously diagnosed by the medical staff according to the American Heart Association guidelines for endocartitis prophylaxis. Exclusion criteria included other disease condition than cardiac (diabetes, hypertension, etc.) and use of antimicrobial agents, including mouthwashes, in the previous two weeks before the beginning of study.

Inclusion criteria for Group 2 included any cardiac conditions related with low-risk for endocarditis, such as isolated secundum atrial septal defect; surgical repair of atrial septal defect, ventricular septal defect, or patent ductus arteriosus; previous coronary artery bypass graft surgery; mitral valve prolapse without valvar regurgitation; physiologic, functional, or innocent heart murmurs; previous Kawasaki disease without valvar dysfunction; previous rheumatic fever without valvar dysfunction; and cardiac pacemakers (intravascular and epicardial) and implanted defibrillators[2]. None of volunteers reported biological prosthetic valves.

Inclusion criteria for Group 3 included individuals who have prosthetic heart valves; a previous history of endocarditis (even in the absence of other heart disease); complex cyanotic congenital heart disease, or surgically constructed

systemic pulmonary shunts or conduits[2]. Fourteen (70%) of the volunteers of the group 3 reported biological prosthetic valve and six (30%) of mechanical prosthetic valve. Two volunteers also reported previous history of infective endocarditis.

Sterilized swabs soaked with 0.1 mL of sterilized saline solution (0.9% NaCl) were rubbed against the volunteers' ear skin. The swabs were placed into sterilized tubes with Stuart's transport medium. Each sample (in duplicates) was then (until 3 hours) spread on Petri dishes (9.0 cm x 2.0 cm) containing *Salt Mannitol* Agar (Difco Laboratories, Detroit, MI). All Petri dishes were incubated in aerobiosis during 24 hours at 37ºC (Fanem Model 002 – Sao Paulo, Brazil)[7].

Subjects also deposited 1.0 mL of mixed saliva directly in sterilized Eppendorf tubes, using sterilized straws to avoid labial contamination. Each sample (in duplicates) was 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) and diluted (1:1000). Each dilution (in duplicates) was spirally plated (Spiral Plater System, Don Whitley Scientific, Shipley, UK) automatically in a logarithmic distribution on Petri dishes (9.0 cm x 2.0 cm) containing *Mitis Salivarius* agar (Difco Laboratories). These dishes were incubated either with 10% CO_2 at 37ºC (Jovan IG 150 – Winchester, USA) for 48 hours[7].

The morphology of each colony on the *Salt Mannitol* agar and *Mitis Salivarius* agar was characterized by using a stereoscopic microscope (Stemi SV 6, Carl Zeiss Microimaging, Thornwood, NY) and Gram stain. All colonies were

submitted to catalase and coagulase tests. The staphylococci strains that showed both positive tests were classified as *S. aureus*[11].

The streptococci colonies growing on *Mitis Salivarius* agar were submitted to biochemical tests by using thioglycolate agar medium base added by 0.0016% bromocresol purple and the following carbohydrates (1%) separately: mannitol, sorbitol, amylum, rafinose, inulin, lactose and arginine. In addition, all streptococci were submitted to adherence and hemolysis tests. Depending on biochemical tests the different species of streptococci were determinate[11].

For sensitivity tests involving all microorganisms, paper discs (6.5 mm diameter – Cefar Diagnostics Co – Sao Paulo, Brazil) containing 30 µg vancomycin, 10 µg ampicillin, 10 µg amoxicillin, 15 µg azithromycin, 15 µg clarithromycin, 30 µg cephazolin, 30 µg cephalexin, 30 µg cephalotine, 30 µg cephadroxil, 2 µg clindamycin, or amoxicillin (20 µg) plus potassium clavulanate (10 µg) were used. In addition, *S. aureus* strains were tested against 1 µg oxacillin and 5µg methicillin[7].

These paper discs were placed on *Mueller-Hinton* agar (Merck, Whitehouse Station, NJ) plus 1.5% sheep blood. The inoculum of each strain was adjusted to 10^8 CFU/mL by a spectrophotometer (Spectronic 20 – Bausch & Lomb, Rochester, USA). The *S. aureus* strains were incubated under aerobic condition at 37°C during 18 hours. Streptococci strains were incubated with 10% of CO_2 (Jovan IG 150 – Winchester, USA) for 18 hours[11].

Inhibition zone diameters were measured and classified according to the National Committee for Clinical Laboratory Standards (NCCLS), as resistant, intermediate or susceptible depending upon the diameter of the zone of inhibition produced[12].

A penicillin-sensible *S. aureus* strain (ATCC 25923) was used as a quality control for the susceptibility tests described above.

The volunteers of groups 2 and 3 were submitted to a structured formulary (adapted from Stucki et al., 2003)[10] in order to evaluate their knowledge about infective endocarditis. Table 1 shows the multiple-choice questions observed.

Statistical analysis

Chi-square test was used for statistical analysis of the data, when appropriate, considering a significant level of 5%.

RESULTS

The total number of isolated strains was 186, being 58 from group 1, 62 from group 2 and 66 from group 3. Figure 1 shows the distribution of isolated species in each group. All streptococci were considered as viridans group. The *S. pneumoniae* strain was also alpha-hemolytic. *Streptococcus mitis* was the most common streptococci isolated from 34.5%, 29% and 30.3% of volunteers of

groups 1, 2 and 3, respectively. There were no statistical significant difference among the three groups (Chi-square, p=0.7678). *S. aureus* was the second most isolated (8.6%, 30.6% and 30.3% of volunteers of groups 1, 2 and 3, respectively), being less isolated from healthy subjects (Chi-square, p=0.001).

Figure 2 shows the resistance profile of the studied strains. The antibiotics that produced zero values and the sensible bacteria were omitted. All bacteria, including *S. aureus* strains, were sensible to vancomycin, cephazolin, cephalexin, cephalotine, cephadroxil, clindamycin, and amoxicillin plus potassium clavulanate. All *S. aureus* strains were also sensible to oxacillin and methicillin.

The three groups also showed an expressive percentage (up to 40%) of intermediary resistant streptococci strains.

None of the evaluated strains showed resistance for more than two antimicrobial agents. Two *S. mitis* strains (groups 1 and 3) showed resistance against two antibiotics (ampicillin and azithromycin) at same time. However, these two strains did not show resistance against amoxicillin or clarithromycin. None of the streptococci strains could be considered truly resistant against amoxicillin. Only macrolide resistance could be observed particularly in streptococci strains of group 1.

On contrary, *S. aureus* strains were markedly resistant against amoxicillin and ampicillin and less resistant against macrolides. The highest number of resistant *S. aureus* was observed in group 3. Two *S. aureus* strains

(group 3) were resistant against macrolides and beta-lactams (ampicillin and amoxicillin). The *S. aureus* strains resistant against ampicillin were also resistant against amoxicillin.

Table 2 shows the answers of each question of the formulary.

DISCUSSION

The present study investigated some of the antimicrobial agents recommended since 1997 by the American Heart Association (AHA) for endocarditis prophylaxis. In addition, other antibiotics were also studied in order to characterize resistance profile of microorganisms, although they are not indicated for endocarditis prophylaxis[13,14].

Sensitivity tests are very effective in evaluating the susceptibility and resistance of isolated strains of bacteria[15]. Among these tests, the disk diffusion method used in the present study is considered very reliable[16,17]. Resistant strains are considered in this test as bacteria that are not inhibited or killed by usual blood antimicrobial concentrations and the intermediary resistant ones are the strains which will be probably inhibited by high antibiotic blood concentrations.

Staphylococcus aureus and *viridans* group streptococci are the most prevalent bacteria in cases of infective endocarditis[18]. *S. aureus* is a human-skin commensal microorganism or nosocomial pathogen. Steckelberg et al. (1990)[19] reported that only 1.3% of infective endocarditis cases caused by *S. aureus* strains

could be related to dental treatment while *viridans* streptococci were responsible for 30% of these cases.

Nevertheless, *S. aureus* has been isolated from the mouths of elderly patients and those suffering from periodontitis[20]. In the present study, *S. aureus* strains were not found in saliva samples, probably due to the volunteers profile and the methodology of saliva collection, which avoided skin contaminants. However, a high incidence of antimicrobial resistance considering amoxicillin and ampicillin, similar to that reported in previous studies, was observed especially for *S. aureus* strains collected from skin [7,21,22].

Curiously, the resistance rates of *S. aureus* strains were higher in groups 2 and 3. The *S. aureus* resistance against amoxicillin observed in the present study is important because this drug is the first choice for endocarditis prophylaxis[23].

Viridans group streptococci have been surpassed by staphylococci as the leading cause of infective endocarditis[24], but they have been frequently associated with infective endocarditis[1].

Fortunately, the antimicrobial resistance rates of streptococci observed in the present study in all groups were lower than those previously reported, especially considering amoxicillin and ampicillin[25,26].

This may be due to differences between methodologies, since these previous studies collected strains from blood or other sites (including pleural infusion, ascites and abscess aspirate) in infected patients. Another study[7] using

similar methodology found higher resistant rates (approximately 16%) for amoxicillin/ampicillin considering salivary streptococci. However, the authors did not specify which streptococci species were studied.

The rate of penicillin-resistant streptococci found in the present study is in agreement with previous observations that these microorganisms are usually sensitive[27]. Other study showed that 94% of oral *viridans* streptococci isolated from high-risk endocarditis patients were susceptible to penicillin, which is very similar to the rates found in the present study[28].

The resistance rates of oral *viridans* streptococci found in the present study suggests that amoxicillin could be still effective in high-risk endocarditis patients. However, the frequency of resistance and partial resistance to macrolides among species of *viridans* streptococci may limit the use of these drugs as prophylactic agents for infections caused by these microorganisms[26,28].

Penicillin-resistant streptococci are classified as having either intermediate (MIC 0.1–1 mg/L) or high resistance (MIC >1 mg/L). Intermediately resistant streptococci might respond to standard therapy because serum concentrations of beta-lactam are usually much greater than the MIC for these bacteria[29]. Thus, the results of *in vitro* tests must be carefully considered.

Despite the low resistance rates observed for viridans streptococci, it is important to keep in mind that they came from *in vitro* tests. There is an increasing number of reports about infectivel endocarditis after dental treatment

caused by amoxicillin-resistant *S. mitis* strain[30] and in at least two cases the patients were submitted to previous antibiotic prophylaxis with 2g amoxicillin[8,9].

Inadequate understanding of IE and prophylaxis among patients with valvular heart disease and artificial valves is notorious, in spite of education efforts[10]. In agreement with other studies[31,32], the present data also showed insufficient knowledge of endocarditis in high and low-risk patients.

Almost all volunteers could not define endocarditis and only 10-15% was aware of the precautions necessary to prevent it. The most part of the volunteers did not ever have information from physicians and dentists about IE. Data from formulary are alarming and measures to improve patient's knowledge are urgent. However, a previous study showed that even patients at risk who had been carefully trained by a standardized protocol showed only slightly improvement of knowledge of IE[10].

Maybe the most preoccupying data is that in both groups the volunteers were not aware of the need for continuous dental follow-up, since the majority of the volunteers had not seen a dentist in the previous year.

The most part of the volunteers in groups 2 and 3 did not informe the dentist about their heart condition, probably performing dental treatment without antibiotic prophylaxis. However, these volunteers did not report endocarditis development after the dental treatment.

The validity of antibiotic prophylaxis for endocarditis has been recently reviewed[33]. The risks of antibiotic therapy include anaphylaxis in 1:5,000 patients[34] which could potentially cause more deaths than endocarditis[35,36].

Various strategies have been proposed to modify antibiotic use in an attempt to improve outcomes, including reducing the rates of resistance. These strategies include education, automatic stop orders, prior approval before an antibiotic is dispensed, computer-guided prescribing, and antibiotic cycling[37].

Resistance against antimicrobial agents that have been recommended to prevent bacterial endocarditis could result in a therapy failure. Indeed, the incidence of infective endocarditis has not changed since the introduction of antibiotic prophylaxis[38]. The resistant microorganisms are probably just part of the problem.

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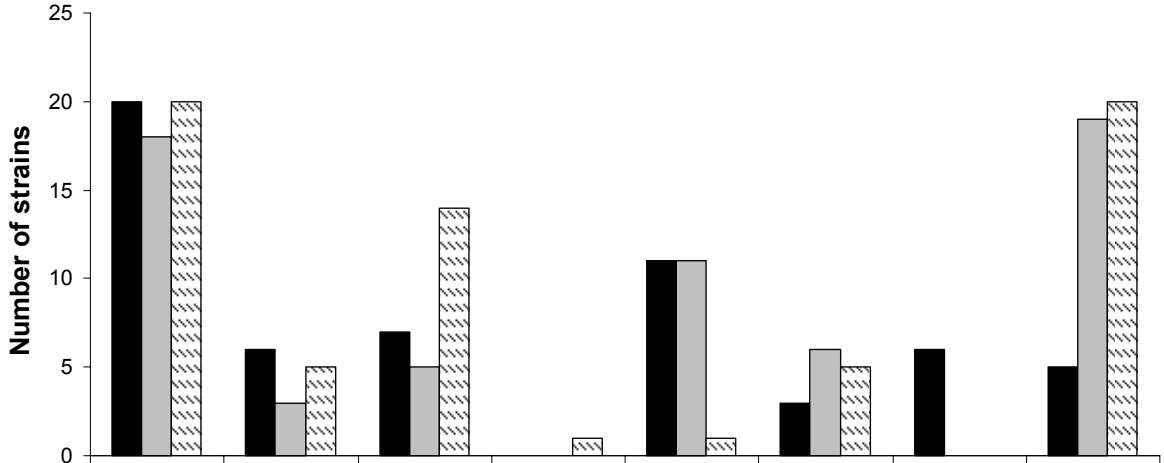
Table 1. Multiple-choice questions answered by groups 2 and 3.

Questions	Possible answer
Could you name your heart condition?	Yes or No
Do you have prosthetic heart valve?	Yes or No
Do you know what infective endocarditis is?	Yes or No
How serious do you think infective endocarditis is?	( ) minor disease ( ) intermediate disease ( ) life threatening disease
Do you think you are at risk for infective endocarditis?	Yes or No
Do you know whether you are in low, moderate or high risk group for infective endocarditis?	( ) low ( ) moderate ( ) high
How can you prevent infective endocarditis?	a) Regular dental checkups b) Good dental hygiene c) a and b d) None alternative
Do you think that you need additional drugs during dental treatment?	Yes or No. If yes, what kind?
Which of the following parameters increase infective endocarditis risk?	a) Poor oral hygiene/infecctions in the oral cavity b) Dental procedure c) a and b d) None
When was your last dental checkup?	( ) 3 months ( ) 6 months ( ) 1 year ( ) 1 ½ year ( ) I don't remember
When did you last receive information about infecctive endocarditis and prophylaxis from your doctor?	( ) I don't remember ( ) I receive regular information in infective endocarditis and prophylaxis ( ) I never receive information in infective endocarditis and prophylaxis
Have you informed your dentist about your heart condition?	Yes or No
Do you have any current dental disease?	Yes or No
Do you still have your own teeth?	Yes or No
Do you use over-the-counter medicines?	Yes or No

Table 2. Proportions of the answers of each question of the questionnaire according to the groups 2 and 3.

Answers	Group 2 (n=20)	Group 3 (n=20)
Cannot name their heart condition	9 (45%)	10 (50%)
Do not know what infective endocarditis is	18 (90%)	17 (85%)
He/she thinks that infective endocarditis is a serious disease*	2 (10%)	3 (15%)
He/she thinks that he/she is not at risk for infective endocarditis	17 (85%)	10 (50%)
He/she thinks that he/she is in the high risk group for infective endocarditis **	2 (10%)	4 (20%)
Do not know how to prevent infective endocarditis	18 (90%)	17 (85%)
Do you think that you need additional drugs during dental treatment?		
No	17 (85%)	6 (30%)
Amoxicillin	2 (10%)	9 (45%)
Ampicillin	0 (0%)	1 (5%)
Antibiotic	1 (5%)	1 (5%)
Yes. I cannot remember which medicine	0 (0%)	3 (15%)
Which of the following parameters increase infective endocarditis risk?		
Poor oral higiene and mouth infeccions	4 (20%)	12 (60%)
Poor oral higiene	2 (10%)	3 (15%)
Mouth infeccions	2 (10%)	4 (20%)
Did not answer	12 (60%)	1 (5%)
When was your last dental checkup?		
one year ago	2 (10%)	5 (25%)
more than one year ago	13 (65%)	10 (50%)
three months ago	1 (5%)	2 (10%)
Six months ago	3 (15%)	2 (10%)
I can not remember	1 (5%)	1 (5%)
Did you ever receive information about IE and prophylaxis from your doctor?		
I cannot remember	1 (5%)	0 (0%)
I never received information	17 (85%)	17 (85%)
I always receive information	2 (10%)	3 (15%)
Did not informed the dentist about his/her heart condition	13 (65%)	10 (50%)
Do you have any current dental disease?		
No	16 (80%)	11 (55%)
Gengivitis	1 (5%)	5 (25%)
Bad breath	2 (10%)	2 (10%)
Bleeding and caries	3 (15%)	2 (10%)
Do not have his/her own teeth	12 (60%)	7 (35%)
Do you use over-the-counter medicines?		
No	9 (45%)	6 (30%)
Aspirin	2 (10%)	1 (5%)
Analgesics	4 (20%)	8 (40%)
Analgesics and non-steroidal anti-inflammatory drugs	2 (10%)	3 (15%)
Non-steroidal anti-inflammatory drugs	2 (10%)	3 (15%)

* The rest of volunteers do not know if IE is a serious disease. ** Most volunteers did not answer.



	S. mitis	*S. mutans*	*S. oralis*	*S. pneumoniae*	*S. salivarius*	*S. sobrinus*	*S. vestibularis*	*S. aureus*
Group 1	20	6	7	0	11	3	6	5
Group 2	18	3	5	0	11	6	0	19
Group 3	20	5	14	1	1	5	0	20

Figure 1 – Number of strains according to species isolated from each group.

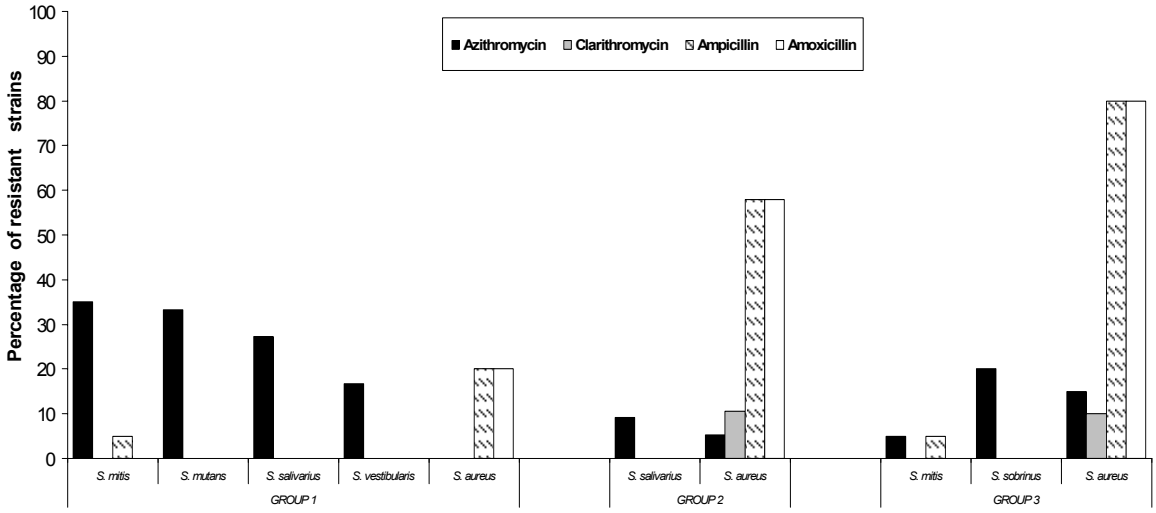


Figure 2. Resistance profile of the strains according to each group.

3.2 Capítulo 2

Title: *Viridans streptococci of children at risk for infective endocarditis are penicillin susceptible.*

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SUMMARY

Aim: to verify the antimicrobial resistance of *Staphylococcus aureus* and *viridans* streptococci of children at high risk for endocarditis.

Design: 40 children (6 to 14 years-old), which were divided into two groups: healthy (Group 1) and high-risk (Group 2) for infective endocarditis (IE). In addition, children's guardians were submitted to a structured formulary about IE risks and prophylaxis. Saliva samples were inoculated on *Mitis Salivarius* agar and incubated in microaerophilia during 48h. Skin samples were inoculated on *Mannitol-Salt* agar and incubated in aerobiosis during 24h. After biochemical tests *viridans* streptococci and *S. aureus* were identified and submitted to antimicrobial susceptibility tests against antimicrobial agents.

Results: 98 strains were isolated, being 51 from group 1 and 47 from group 2. *Streptococcus pneumoniae* was the most common streptococci isolated: 23.4% and 25.5% in groups 1 and 2, respectively. *S. aureus* corresponded to 36.2% and 31.9% in groups 1 and 2, respectively. Fourteen guardians (70%) were aware about their child's disease; however, only 15% were aware of the meaning of "infective endocarditis". Eleven guardians (55%) knew about antibiotic prophylaxis before some dental procedures, and 10 named amoxicillin as the main antibiotic for prophylaxis. Sixteen guardians (80%) did not received information from physicians or dentists about the importance of oral health to prevent IE. The most part (40%) reported the last visit to the dentist at least six months ago.

Conclusions: The resistant profile of the oral streptococci strains supports amoxicillin as the first choice antimicrobial agent for IE prophylaxis. There was a clear need for more information regarding IE among children´s guardians affected by cardiac disease.

INTRODUCTION

Infective endocarditis (IE) is an infection of the endocardium in valvular, mural, septal defects and arteriovenous or arterioarterial short-circuits[1]. Congenital heart defects (CHD), such as ventricular septal defect, patent ductus arteriosus, aortic valve abnormalities and Fallot's tetralogy, are common underlying conditions for IE in children, and it is greatly correlated with previous corrective or palliative surgery for CHD[2].

IE is relatively uncommon, but it is still considered as a life-threatening disease and it usually affects individuals who develop bacteremia, which may occur spontaneously or may complicate from a focal infection, such as urinary tract infection, pneumonia, or cellulitis. Poor dental hygiene may also produce bacteremia directly proportional to the degree of oral inflammation. Individuals who are at risk for IE should establish and maintain the best possible oral health to reduce potential sources of bacteremia[3].

The microorganisms most commonly associated with IE in children are the alpha-hemolytic (viridans) streptococci especially oral strains (*Streptococcus oralis*, *S. sanguis*, *S. parasanguis*, *S. gordonii*, *S. mitis*, *S. mutans*, *S. salivarius*, *S. sobrinus*, *S. intermedius*, and *S. constellatus*) and *Staphylococcus aureus*[4]. The significant levels of antimicrobial resistance of these bacteria is becoming a matter of concern[5].

A previous study showed at least one penicillin-resistant viridans streptococci strain isolated from oral cavity of 19 of 31 (61.3%) children at risk for IE[6].

Since 1997, the American Heart Association has been recommended antibiotic prophylaxis (2 g amoxicillin) for dental and oral procedures likely to cause bacteremia. However, the efficacy of antimicrobial prophylaxis has never been definitively proven. Reports of failure of prophylaxis caused by resistant viridans strains, mainly *S. mitis*, have been increasing[7].

Despite the morbidity and mortality of IE, many of patients at risk did not show appropriate knowledge about the potential risks of the disease in a previous study[8]. The guardians of children at risk for IE basically showed the same profile[2].

The aim of this study was to observe the antimicrobial susceptibility of *Staphylococcus aureus* and *viridans* streptococci group obtained from both healthy and IE-risk children. In addition, a survey tested the knowledge about endocarditis risks and prophylaxis of the guardians of IE-risk children.

MATERIAL AND METHODS

The present study was approved by Ethical Committee of the Faculty of Medical Sciences – UNICAMP. A write-informed consent was obtained from childrens' guardians.

Forty patients, aging 6 to 14 years-old (10.5±2.9), from the Cardiology Ambulatory of Clinics Hospital at State University of Campinas (Sao Paulo, Brazil) were included into two groups: Group 1 - 20 healthy children (10 female) and Group 2 – 20 children at high-risk for IE (9 female).

All volunteers were previously diagnosed by the medical staff according to the American Heart Association guidelines for endocartitis prophylaxis. Exclusion criteria included other disease condition than cardiac (diabetes, hypertension, etc.) and use of antimicrobial agents, including mouthwashes, in the previous two weeks before the beginning of study.

Inclusion criteria for Group 2 included individuals who have prosthetic heart valves; a previous history of endocarditis (even in the absence of other heart disease); complex cyanotic congenital heart disease, or surgically constructed systemic pulmonary shunts or conduits[3].

Sterilized swabs soaked with 0.1 mL of sterilized saline solution (0.9% NaCl) were rubbed against the volunteers' ear skin. The swabs were placed into sterilized tubes with Stuart's transport medium. Each sample (in duplicates) was then (until 3 hours) spread on Petri dishes (9.0 cm x 2.0 cm) containing *Salt Mannitol* Agar (Difco Laboratories, Detroit, MI). All Petri dishes were incubated in aerobiosis during 24 hours at 37ºC[9].

Subjects also deposited 1.0 mL of mixed saliva directly in sterilized eppendorf tubes, using sterilized straws to avoid labial contamination. Each

sample (in duplicates) was sonicated until 3 hours (Vibra-Cell 400W, Sonics & Materials, Inc., Newtown, CT) and diluted (1:1000). Each dilution (in duplicates) was spirally plated (Spiral Plater System, Don Whitley Scientific, Shipley, UK) automatically in a logarithmic distribution on Petri dishes (9.0 cm x 2.0 cm) containing *Mitis Salivarius* agar (Difco Laboratories). These dishes were incubated either with 10% CO_2 at 37ºC for 48 hours[9].

The morphology of each colony on the *Salt Mannitol* agar and *Mitis Salivarius* agar was characterized by using a stereoscopic microscope (Stemi SV 6, Carl Zeiss Microimaging, Thornwood, NY) and Gram stain. All colonies were submitted to catalase and coagulase tests. Positive tests staphylococci strains were classified as *S. aureus*[10].

The streptococci colonies growing on *Mitis Salivarius* agar were submitted to biochemical tests by using thioglycolate agar medium base added by 0.0016% bromocresol purple and the following carbohydrates (1%) separately: mannitol, sorbitol, amylum, rafinose, inulin, lactose and arginine. In addition, all streptococci were submitted to adherence and hemolysis test. Depending on biochemical tests the different species of streptococci were determinate[10].

For sensitivity tests involving all microorganisms, paper discs containing 30 µg vancomycin, 10 µg ampicillin, 10 µg amoxicillin, 15 µg azithromycin, 15 µg clarithromycin, 30 µg cephazolin, 30 µg cephalexin, 30 µg cephalotine, 30 µg cephadroxil, 2 µg clindamycin, or amoxicillin (20 µg) plus potassium clavulanate

(10 µg) were used. In addition, *S. aureus* strains were tested against 1 µg oxacillin and 5 µg methicillin[9].

These paper discs were placed on *Mueller-Hinto*n agar (Merck, Whitehouse Station, NJ) plus 1.5% sheep blood. The inoculum of each strain was adjusted to 10^8 CFU/mL by a spectrophotometer. The *S. aureus* strains were incubated under aerobic condition at 37°C during 18 hours. Streptococci strains were incubated with 10% of CO_2 (Jovan IG 150 – Winchester, USA) for 18 hours[10].

Inhibition zone diameters were measured and classified according to the National Committee for Clinical Laboratory Standards (NCCLS), as resistant, intermediate or susceptible depending upon the diameter of the zone of inhibition produced[11].

A penicillin-sensible *S. aureus* strain (ATCC 25923) was used as a quality control for the susceptibility tests described above.

The guardians of group 2 were submitted to a structured formulary (adapted from Stucki et al., 2003)[8] in order to evaluate their knowledge about infective endocarditis. Table 1 shows the multiple-choice questions observed.

Statistical analysis

Chi-square test was used for statistical analysis of the data, when appropriate, considering a significant level of 5%.

RESULTS

The total number of isolated strains was 98, being 51 from group 1 and 47 from group 2. Figure 1 shows the distribution of species isolated in each group. All streptococci belonged to the viridans group. The *S. pneumoniae* strain was also alpha-hemolytic. *Streptococcus pneumoniae* was the most common streptococci isolated: 23.4% and 25.5% in groups 1 and 2, respectively. There were no statistically significant difference (Chi-square, p=0.6669) among both groups regarding streptococci. *S. aureus* corresponded to 36.2% and 31.9% in groups 1 and 2, respectively (Chi-square, p=0.6932).

Figure 2 shows the resistance profile of the studied strains. The antibiotics that produced zero values and the sensible bacteria were omitted. All bacteria, including *S. aureus* strains, were sensible to vancomycin, cephazolin, cephalexin, cephalotine, cephadroxil, clindamycin, and amoxicillin plus potassium clavulanate. All *S. aureus* strains were also sensible to oxacillin and methicillin. Both groups 1 and 2 also showed some intermediary-resistant streptococci strains, especially *S. pneumoniae* and *S. mitis*.

All strains which showed resistance against amoxicillin were also resistant against ampicillin. However, the three azithromycin-resistant strains were not resistant against clarithromycin.

None of the evaluated strains showed resistance against more than two antimicrobial classes. Two *S. aureus* strains (groups 1 and 2) showed resistance

against ampicillin, amoxicillin and azithromycin at same time. None of the streptococci strains could be considered truly resistant against both amoxicillin and ampicillin. Only one streptococci strain from group 2 showed resistance against azithromycin.

On contrary, all *S. aureus* strains were markedly resistant against amoxicillin and ampicillin.

Table 2 shows the answers of each question of the questionnaire.

DISCUSSION

Resistant strains are considered in the disk diffusion test as bacteria that are not inhibited or killed by usual blood concentrations and the intermediary resistant ones are the strains which will probably inhibited just by high antibiotic blood concentrations.

Penicillin-resistant streptococci are classified as having either intermediate (MIC 0.1–1 mg/L) or high resistance (MIC > 1 mg/L). Intermediately resistant streptococci might respond to standard therapy because serum concentrations of beta-lactams are usually much greater than the MIC for these bacteria, especially during antibiotic prophylaxis[4]. Thus, the results of *in vitro* tests of the present study must be carefully interpreted. Despite the absence of resistance observed in streptococci, some strains of *S. pneumoniae* and *S. mitis* were "intermediary resistant".

S. pneumoniae accounts for 3% to 5% of IE cases in children. There was a worldwide explosion of multi-drug resistance among clinical isolates of pneumococci during the 1990s[12]. Considering the in vitro data of the present study and the *S. pneumoniae* characteristics, antibiotic prophylaxis in IE high-risk children must be carefully followed by clinicians.

Staphylococcus aureus and viridans group streptococci were the most prevalent bacteria in cases of infective endocarditis in adults and in children[13]. While viridans streptococci have becoming less frequent, the incidence of staphylococcal IE has increasing[14,15]. In addition, a significant rate of occult IE in patients with staphylococcal bacteremia has been reported, being *S. aureus* the most common cause of acute IE in children[12].

Despite *S. aureus* strains in the present study were all resistant against amoxicillin, Steckelberg *et al*. (1990)[16] reported that only 1.3% of IE cases caused by this microorganism could be related to dental treatment while *viridans* streptococci were responsible for 30% of these cases. *S. aureus* has been isolated from elderly-patients mouths and those suffering from periodontitis[17], but not from children. In the present study, *S. aureus* strains were not found in saliva samples, probably due to the volunteers profile and the methodology of saliva collection, which avoided skin contaminants. The high incidence of antimicrobial resistance considering amoxicillin and ampicillin was similar to that reported in previous studies[9].

Fortunately, the antimicrobial resistance rates of streptococci observed in the present study in both groups were lower than those previously reported, especially considering amoxicillin and ampicillin[18]. This may be due to differences between methodologies, since these previous studies collected strains from blood or other sites (pleural infusion, ascites and abscess aspirate) in infected patients.

The rate of penicillin-resistant streptococci found in the present study is in agreement with previous observations that these microorganisms are usually sensitive[19]. Other study showed that 94% of oral *viridans* streptococci isolated from high-risk endocarditis patients were susceptible to penicillin, which is very similar to the rates found in the present study[20]. However, Nishi et al. (1999)[6] were able to isolate at least one penicillin-resistant viridans streptococci strain from oral cavity of 19 (61.3%) children at risk for IE.

Despite the low resistance rates observed for viridans streptococci, it is important to keep in mind that they came from *in vitro* tests. The resistance rates of oral *viridans* streptococci found in the present study suggest that amoxicillin could be still effective in high-risk endocarditis patients.

There is an increasing number of reports about bacterial endocarditis after dental treatment caused by amoxicillin-resistant *S. mitis* strain[21] and at least in two cases the patients were submitted to previous antibiotic prophylaxis with 2g amoxicillin[7]. Again, it is a clear indication that antibiotic prophylaxis in IE high-risk children must be carefully followed by clinicians.

Inadequate understanding of IE and prophylaxis among children´s guardians with valvular heart disease and artificial valves is notorious, in spite of education efforts[9]. In agreement with other studies[2,22,23], the present data also showed insufficient knowledge of endocarditis among high-risk children´s guardians.

Almost all guardians could not define IE, but most of them could correctly name the heart condition of the children. They were not aware of the precautions necessary to prevent it, but a half of them know that an antibiotic (the majority correctly named it) is necessary prior dental treatment.

The most part of the guardians did not ever have information from physicians and dentists about IE. Data from formulary are alarming and measures to improve children´s guardians knowledge are urgent. However, a previous study showed that even patients at risk who had carefully been trained by standardized protocol showed only slightly improvement of knowledge of IE[8].

Fortunately, the majority of the guardians are aware of the need for dental follow-up and the most part of guardians informed the dentist about the heart condition of their child. Curiously, at least 20% of the children were probably submitted to dental treatment without antibiotic prophylaxis but they did not develop IE. The lack of adherence on dental care of children with congenital heart disease has been previously mentioned. Franco et al. (1996)[24] observed that 19% of these children had never visited a dentist.

The validity of antibiotic prophylaxis for endocarditis has been recently reviewed[25]. The risks of antibiotic therapy include anaphylaxis in 1:5,000 patients[26] which could potentially cause more deaths than the ones caused by endocarditis[27,28].

Various strategies have been proposed to modify antibiotic use in an attempt to improve outcomes, including reducing the rates of resistance. These strategies include education, automatic stop orders, prior approval before an antibiotic is dispensed, computer-guided prescribing, and antibiotic cycling[29].

Resistance against antimicrobial agents that usually are recommended to prevent IE could result in a therapy failure. Indeed, the prevalence of IE has not changed since the introduction of antibiotic prophylaxis[30]. The resistant microorganisms are probably part of the problem.

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Table 1. Multiple-choice questions answered by group 2.

Question	Possible answer
Could you name your child's heart condition?	Yes or No
Does your child have prosthetic heart valve?	Yes or No
Do you know what infective endocarditis is?	Yes or No
How serious do you think infective endocarditis is?	( ) minor disease ( ) intermediate disease ( ) life threatening disease
Do you think your child is at risk for infective endocarditis?	Yes or No
Do you know whether your child is in low, moderate or high risk group for infective endocarditis?	( ) low ( ) moderate ( ) high
How can you prevent infective endocarditis?	a) Regular dental checkups b) Good dental hygiene c) a and b d) None alternative
Do you think that your child needs additional drugs during dental treatment?	Yes or No. If yes, what kind?
Which of the following parameters increase infective endocarditis risk?	a) Poor oral hygiene/infecctions in the oral cavity b) Dental procedure c) a and b d) None
When was your child's last dental checkup?	( ) 3 months ( ) 6 months ( ) 1 year ( ) 1 ½ year ( ) I don't remember
When did you last receive information about infecctive endocarditis and prophylaxis from your child's doctor?	( ) I don't remember ( ) I receive regular information in infective endocarditis and prophylaxis ( ) I never receive information in infective endocarditis and prophylaxis
Have you informed your child's dentist about his/her heart condition?	Yes or No
Does your child have any current dental disease?	Yes or No
Does your child use over-the-counter medicines?	Yes or No

Table 2. Proportions of the answers of each question of the questionnaire.

Answers	Group 2 (n=20)
Cannot name their child's heart condition	6 (30%)
Do not know what infective endocarditis is	17 (85%)
He/she thinks that infective endocarditis is a serious disease*	3 (15%)
He/she thinks that her/his child is not at risk for infective endocarditis	10 (50%)
He/she thinks that her/his child is in the high risk group for infective endocarditis **	8 (40%)
Do not know how to prevent infective endocarditis	17 (85%)
Do you think that your child needs additional drugs during dental treatment?	
No	9 (45%)
Amoxicillin	10 (50%)
Antibiotic	1 (5%)
Which of the following parameters increase infective endocarditis risk?	
Poor oral higiene and mouth infeccions	13 (65%)
Poor oral higiene	2 (10%)
Mouth infeccions	5 (25%)
When was your child's last dental checkup?	
one year ago	4 (20%)
more than one year ago	5 (25%)
three months ago	3 (15%)
six months ago	8 (40%)
Did you ever receive information about infecctive endocarditis and prophylaxis from your child's doctor?	
I cannot remember	1 (5%)
I never received information	16 (80%)
I always receive information	3 (15%)
Did not informed the dentist about his/her child's heart condition	4 (20%)
Does your child have any current dental disease?	
No	18 (90%)
Bleeding and caries	2 (10%)
Does your child use over-the-counter medicines?	
No	3 (15%)
Penicillin	2 (10%)
Analgesics	13 (65%)
Analgesics and non-steroidal anti-inflammatory drugs	2 (10%)

* The rest of volunteers do not know if IE is a serious disease. ** Most volunteers did not answer.

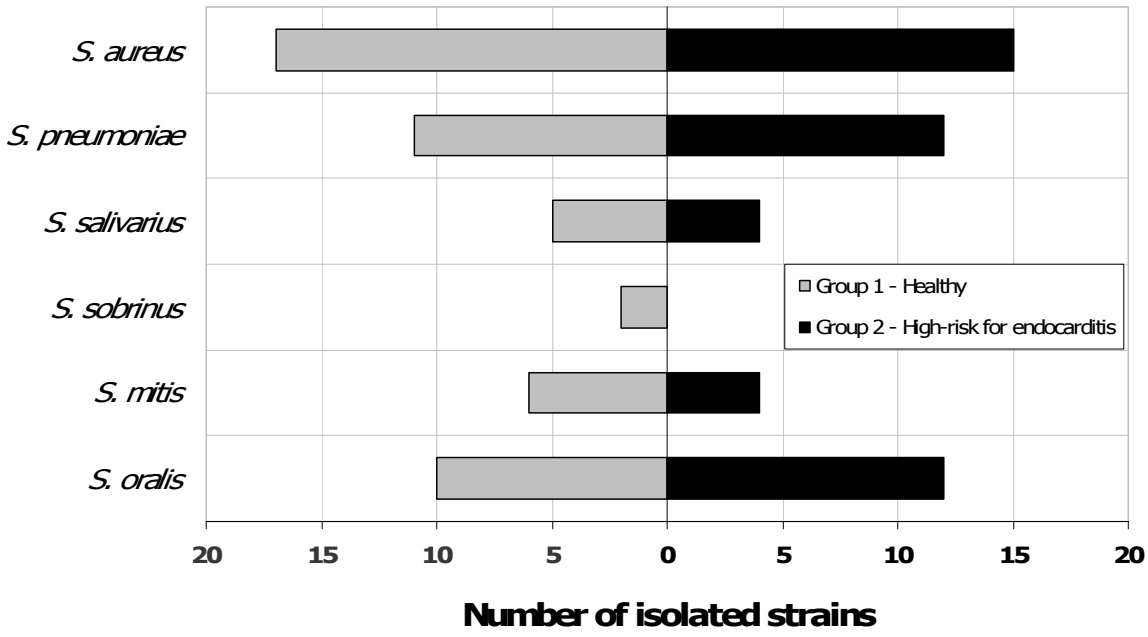

Figure 1 – Number of strains according to species isolated from each group.

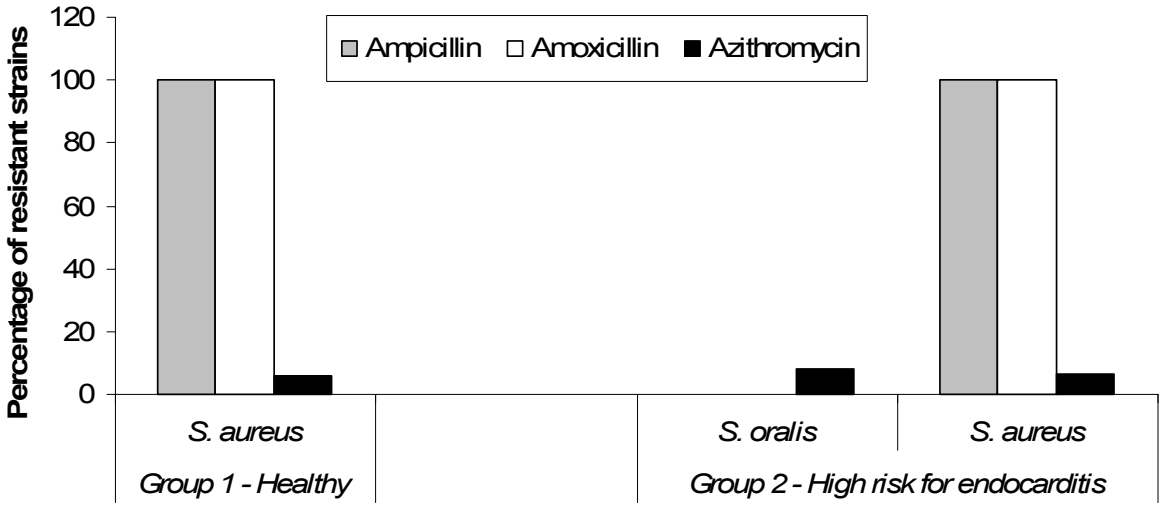

Figure 2. Antimicrobial resistance of the strains according to each group.

4. CONCLUSÃO

1. A amoxicilina continua sendo o antimicrobiano adequado para primeira escolha na profilaxia da endocardite infecciosa, em crianças e adultos, para procedimentos odontológicos que causam bacteremia.

2. Há a necessidade de oferecer mais informações sobre a endocardite infecciosa tanto para os pacientes portadores de doenças cardíacas com risco para esta cardiopatia e para seus responsáveis, no caso de crianças, quanto para os profissionais da área médica e odontológica.

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

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ANEXO 1 – CERTIFICADO DO COMITÊ DE ÉTICA EM PESQUISA

FACULDADE DE CIÊNCIAS MÉDICAS
COMITÊ DE ÉTICA EM PESQUISA
Caixa Postal 6111, 13083-970 Campinas, SP
(0_19) 3788-8936
FAX (0_19) 3788-8925
www.fcm.unicamp.br/pesquisa/etica/index.html
cep@fcm.unicamp.br

CEP, 20/07/04.
(Grupo III)

PARECER PROJETO: N° 227/2004

I-IDENTIFICAÇÃO:

PROJETO: **"AVALIAÇÃO DA RESISTÊNCIA ANTIMICROBIANA DE STAPHYLOCOCCUS AUREUS E ESTREPTOCOCOCOS DO GRUPO VIRIDANS DE PACIENTES COM E SEM RISCO À ENDOCARDITE BACTERIANA"**
PESQUISADOR RESPONSÁVEL: Roberta Cristiane Catelli Baglie
INSTITUIÇÃO: Hospital das Clínicas/UNICAMP
APRESENTAÇÃO AO CEP: 17/05/2004
APRESENTAR RELATÓRIO EM 15/06/05

II - OBJETIVOS

Colher, isolar e identificar cepas de *Staphylococcus aureus* e Estreptococos do grupo *viridans* provindas de pacientes com cardiopatia de alto risco para endocardite bacteriana, de pacientes com cardiopatias de baixo ou nenhum risco para endocardite bacteriana e de voluntários sadios.

Verificar e comparar a sensibilidade a antimicrobianos destas cepas bacterianas.

III - SUMÁRIO

O estudo será aberto, não randomizado e não cruzado. Serão colhidas cepas de microorganismos provindas de saliva e da derme de 80 voluntários de ambos os sexos, com idade entre 10-60 anos, os quais (ou seus responsáveis). Os voluntários serão triados nos ambulatórios de especialistas da Faculdade de Ciências Médicas da UNICAMP. Os voluntários serão divididos em 4 grupos: Grupo 1- vinte indivíduos cardiopatas de valvas protéticas, sendo classificados como pacientes com alto risco a endocardite bacteriana os quais fazem uso frequentede antimicrobianos; Grupo 2- Vinte indivíduos cardiopata, portadores de doenças congênitas cardíacas cianóticas complexas, classificados como pacientes com alto risco a endocardite bacteriana os quais fazem uso frequentes deantimicrobianos; Grupo 3- Vinte indivíduos cardiopatas classificados como pacientes com baixo ou nenhum risco a endocardite bacteriana; Grupo 4- Vinte indivíduos sadios. Cada grupo terá seu critério primário de inclusão próprio.

IV - COMENTÁRIOS DOS RELATORES

O estudo está justificado, com metodologia adequada. O orçamento da pesquisa está descrito. Não há riscos para os participantes, e não há procedimento invasivo. O TCLE é longo e

- 1 -

está completo.

Recomendação: Sugere ao pesquisador abreviar o TCLE.

V - PARECER DO CEP

O Comitê de Ética em Pesquisa da Faculdade de Ciências Médicas da UNICAMP, após acatar os pareceres dos membros-relatores previamente designados para o presente caso e atendendo todos os dispositivos das Resoluções 196/96 e complementares, bem como ter aprovado o Termo do Consentimento Livre e Esclarecido, assim como todos os anexos incluídos na Pesquisa, resolve aprovar sem restrições o Protocolo de Pesquisa supracitado.

O conteúdo e as conclusões aqui apresentados são de responsabilidade exclusiva do CEP/FCM/UNICAMP e não representam a opinião da Universidade Estadual de Campinas nem a comprometem.

VI - INFORMAÇÕES COMPLEMENTARES

O sujeito da pesquisa tem a liberdade de recusar-se a participar ou de retirar seu consentimento em qualquer fase da pesquisa, sem penalização alguma e sem prejuízo ao seu cuidado (Res. CNS 196/96 – Item IV.1.f) e deve receber uma cópia do Termo de Consentimento Livre e Esclarecido, na íntegra, por ele assinado (Item IV.2.d).

Pesquisador deve desenvolver a pesquisa conforme delineada no protocolo aprovado e descontinuar o estudo somente após análise das razões da descontinuidade pelo CEP que o aprovou (Res. CNS Item III.1.z), exceto quando perceber risco ou dano não previsto ao sujeito participante ou quando constatar a superioridade do regime oferecido a um dos grupos de pesquisa (Item V.3.).

O CEP deve ser informado de todos os efeitos adversos ou fatos relevantes que alterem o curso normal do estudo (Res. CNS Item V.4.). É papel do pesquisador assegurar medidas imediatas adequadas frente a evento adverso grave ocorrido (mesmo que tenha sido em outro centro) e enviar notificação ao CEP e à Agência Nacional de Vigilância Sanitária – ANVISA – junto com seu posicionamento.

Eventuais modificações ou emendas ao protocolo devem ser apresentadas ao CEP de forma clara e sucinta, identificando a parte do protocolo a ser modificada e suas justificativas. Em caso de projeto do Grupo I ou II apresentados anteriormente à ANVISA, o pesquisador ou patrocinador deve enviá-las também à mesma junto com o parecer aprovatório do CEP, para serem juntadas ao protocolo inicial (Res. 251/97, Item III.2.e)

Relatórios parciais e final devem ser apresentados ao CEP, de acordo com os prazos estabelecidos na Resolução CNS-MS 196/96.

VII - DATA DA REUNIÃO

Homologado na VII Reunião Ordinária do CEP/FCM, em 20 de julho de 2004.

Profa. Dra. Carmen Silvia Bertuzzo
PRESIDENTE DO COMITÊ DE ÉTICA EM PESQUISA
FCM / UNICAMP

- 2 -

ANEXO 2 – COMPROVANTE DE SUBMISSÃO À PUBLICAÇÃO DO CAPÍTULO 1

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Thank you for your submission.

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Infection Control and Hospital Epidemiology