Faculdade de Ciências Médicas

PROGRAMA DE PÓS-GRADUAÇÃO EM CIENCIAS MEDICAS

Perfil de Susceptibilidade aos Antimicrobianos e Genotipagem de *Staphylococcus aureus e Staphylococcus* spp Coagulase Negativos Isolados de Hemoculturas de Pacientes Hospitalizados em três Hospitais da cidade de Porto Alegre, RS

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Biblioteca Paulo Lacerda de Azevedo

2006

Dissertação de Mestrado

FUNDAÇÃO FACULDADE FEDERAL DE CIÊNCIAS MÉDICAS DE PORTO ALEGRE

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PEREZ, Leandro Reus Rodrigues

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Dissertação (Mestrado - Ciências Médicas) Fundação Faculdade Federal de Ciências Médicas de Porto Alegre.

1. *Staphylococcus aureus*

2. *Staphylococcus* coagulase-negativos

3. gene *mec*A

4. oxacilina

5. cefoxitina

“A mente é como um pára-quedas”,

só faz sentido quando pode abrir-se”

James Dewar, físico escocês

AGRADECIMENTOS

Ao Prof. Dr. Pedro Alves d'Azevedo pela orientação, oportunidade de aprimoramento científico e pessoal durante o desenvolvimento deste trabalho.

Ao Prof. Dr. Afonso Luís Barth pela co-orientação segura, por ter propiciado crescimento profissional e pessoal através deste e de outros trabalhos e pela sua participação desde minha formação acadêmica na graduação.

Ao Prof. Cícero Armídio Gomes Dias pela oportunidade de crescimento técnico-científico, pelo exemplo de caráter e de profissionalismo e pelo acompanhamento sempre seguro durante a realização deste trabalho.

À Ana Lúcia Souza Antunes pelo carinho, amizade, coleguismo, pelos muitos ensinamentos profissionais, pessoais e de caráter.

Às colegas da pós-graduação, em especial à Carina Secchi, Silvana Vargas Superti e Juliana Caierão, pela amizade, incentivo, auxílio técnico e ensinamentos.

Às colegas de laboratório Maria Beatriz, Rosângela e Sandra pela amizade, carinho, dedicação e auxílio durante a realização deste trabalho.

Aos monitores Filipe Dari Krüger e Ananda Cristine Galvão pelo empenho e seriedade dedicados ao desenvolvimento deste trabalho.

Aos colegas de graduação, Mário Lettieri Teixeira e Alexandre Meneghello Fuentefria pelo incentivo e amizade.

À Aline Reolon pelo carinho, compreensão, apoio e paciência.

Aos meus familiares, em especial meus pais, que sempre incentivaram a busca pelo conhecimento e que, sempre presentes com seu amor incondicional, têm facilitado a trilha deste caminho.

Às instituições que contribuíram para a execução deste trabalho: Hospital de Clínicas de Porto Alegre, Hospital Nossa Senhora da Conceição e Irmandade Santa Casa de Misericórdia de Porto Alegre.

À Fundação Faculdade Federal de Ciências Médicas de Porto Alegre (FFFCMPA) por acreditar na capacidade científica e intelectual daqueles que ingressam em algumas de suas áreas de atuação.

A Universidade Federal do Rio Grande do Sul (UFRGS) pela parceria e incentivo científico.

A todos que auxiliaram na execução deste trabalho e que estiveram presentes neste momento: muito obrigado.

Este trabalho foi parcialmente financiado pela Coordenação de Aperfeiçoamento Pessoal de Ensino Superior (CAPES), Conselho Nacional de Pesquisa Científica e Tecnológica (CNPq), Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS) e Fundação Faculdade Federal de Ciências Médicas de Porto Alegre (FFFCMPA).

SUMÁRIO

RESUMO

O gênero *Staphylococcus* está entre os mais microrganismos comumente isolados em laboratórios de microbiologia clínica e têm uma grande importância, especialmente como causa de infecções hospitalares. *Staphylococcus aureus* é um dos mais importantes agentes de infecções hospitalares e também das adquiridas na comunidade, ao passo que *Staphylococcus* coagulase-negativos (SCoN) estão entre as principais causas de bacteremias, especialmente em pacientes com fatores predisponentes como a presença de implantes protéticos, uso de cateteres intravasculares e imunodepressão. A multiresistência antimicrobiana é uma das principais características observadas entre amostras hospitalares de estafilococos, destacando-se a resistência à oxacilina. Clinicamente, estafilococos resistentes à oxacilina são problemáticos porque esses microrganismos vão apresentar resistência cruzada a virtualmente todos os agentes β-lactâmicos e também à outras classes de antimicrobianos. A detecção laboratorial da resistência à oxacilina entre isolados de estafilococos é bastante conflitante, uma vez que alguns isolados podem apresentar hetero-resistência, isto é, variação fenotípica na expressão da resistência. Com a finalidade de caracterizar adequadamente a resistência a oxacilina entre isolados de estafilococos, estudos que definam técnicas com maior sensibilidade e especificidade, além de rapidez e custo, são de grande interesse para o diagnóstico clínico-laboratorial e para o controle de infecções hospitalares. Em vista disso, os objetivos desse trabalho foram: caracterizar fenotipicamente isolados clínicos de *S. aureus* e SCoN; analisar a resistência à meticilina

comparando a ação de dois antimicrobianos, cefoxitina e oxacilina, pelas técnicas de disco difusão, diluição em agar e triagem em agar e a confirmação dos resultados foram realizados com a Reação em Cadeia da Polimerase (PCR) para a detecção do gene *mecA*. Entre os métodos fenotípicos utilizados, em disco difusão com oxacilina 1μg mostrou uma maior correlação com a resistência entre as espécies de estafilococos e o genótipo *mec*A-positivo, principalmente para *S. aureus*, apesar das recentes recomendações do uso de disco de cefoxitina 30 μg como melhor preditor desta resistência. Disco de cefoxitina, utilizando os novos "breakpoints" do *Clinical and Laboratory Standard Institute* (CLSI), demonstrou ser acurado para detecção de resistência entre SCoN. Adicionalmente, o método de triagem em agar com cefoxitina 4 μg/mL mostrou desempenho igual ou superior aos demais métodos aplicados, podendo ser utilizada a mesma concentração para isolados de *S. aureus* e SCoN, sem a necessidade de suplementação de sal ao meio de cultura.

Palavras-chave: *Staphylococcus aureus*, *Staphylococcus* coagulase-negativos, *mec*A, oxacilina, cefoxitina

ABSTRACT

The genus Staphylococus is among the most frequent microrganisms isolated in clinical microbiology laboratories and has a great importance, especially as cause of hospital infections. *Staphylococcus aureus* has predominated as an important agent of hospital and acquired-community infections and coagulase-negative *Staphylococcus* (CoNS) are between the main cause of bacteremias, especially in patients with predisponent factors as presence of prosthetic implanted, use of catheters intravasculars and imunocompromited. The antimicrobial multiresistance is a main characteristics observed among staphylococci isolates, mainly the resistance for oxacillin. Clinically, oxacillin-resistant staphylococci are problematic because these microrganisms presented crossed resistance virtually with all β-lactamics agents and others antimicrobials. Laboratorial detection of the resistance to oxacillin among staphylococci isolates is conflicting, due to heteroresistance, i. e., phenotypic variation in the expression of resistance. With the purpose of to characterize adequately the oxacillin resistance among staphylococci isolates, studies that define techniques with major sensitivity and specificity, beyond rapidity and low cost, are great interesting for laboratorial diagnostic and infection control surveillance. Hence, the objectives of this work is: to characterize phenotipically *S. aureus* and CoNS; to analyze the oxacillin resistance with cefoxitin in comparison with oxacillin by disk-diffusion, agar dilution and agar screening methods with Polimerase Chain Reaction (PCR) for *mecA* gene. Among phenotypic methods, diffusion disk with oxacillin 1µg showed to greater correlation with resistance among staphylococci species with genotype *mec*A-positive

mainly for *S. aureus* despite the recent recommendations of cefoxitin disk 30 μg as better predictor for resistance than oxacillin. Cefoxitin disk, using the new *breakpoints* of the Clinical and Laboratory Standard Institute (CLSI) proved to be accurate in determining resistance among CoNS. In addition, agar screening method with cefoxitin 4 μg/mL showed performance equal or superior to the other screening methods applied, without the need of supplementing salt to the culture medium and, additionally, the same antimicrobial concentration can be used for both *S. aureus* and CoNS.

Keywords: *Staphylococcus aureus*, coagulase-negative *Staphylococcus*, *mec*A gene, oxacillin, cefoxitin

INTRODUÇÃO

Entre a grande diversidade de microrganismos de importância clínica, os cocos Gram-positivos predominam como um dos principais causadores de infecções nosocomiais e adquiridas na comunidade (Jeljaszewicz, Mlynarczyk & Mlynarczyk, 1998). *Staphylococcus aureus* é o mais comum agente causador de infecções no mundo (Chang et al, 2003), ao passo que *Staphylococcus* spp coagulase negativos são os mais comuns em infecções nosocomiais da corrente circulatória e àquelas devido a implantes, tais como cateteres e próteses (Kloos & Bannerman, 1994; Oplustil, et al, 1994; Kloos & Bannerman, 1999). Os cocos Gram positivos são mais sensíveis aos antimicrobianos que os bacilos Gram negativos por causa da ausência de membrana externa. Entretanto, muitos destes cocos Gram positivos, entretanto, freqüentemente têm natureza de resistência intrínseca elevada a antimicrobianos. Adicionalmente, estes microrganismos são capazes de adquirir resistência rápida às drogas utilizadas (Jeljaszewicz, Mlynarczyk & Mlynarczyk, 1998).

O histórico das infecções hospitalares tem relação íntima com o desenvolvimento de resistência pelos microrganismos. As investigações em relação a agentes etiológicos responsáveis por infecções hospitalares se intensificaram na década de 50. Nesta época, cepas de *Staphylococcus aureus* resistentes à penicilina começaram a ser isolados, indicando que as bactérias podiam desenvolver resistência aos antimicrobianos. Novos

antimicrobianos β-lactâmicos foram desenvolvidos, e na década seguinte, com o uso em larga escala destes agentes, começaram a ser isolados outros microrganismos resistentes. A partir da década de 70, o uso indiscriminado de antimicrobianos β-lactâmicos levou ao surgimento de cepas de *S. aureus* resistentes à meticilina (MRSA – "Methicilin Resistent *Staphylococcus aureus"*) , em diversas partes do mundo, assim como um aumento na incidência de infecções causadas por microrganismos oportunistas resistentes como *Staphylococcus epidermidis* e *Enterococcus* spp (Weber & Rutala, 1989). Mais grave do que a resistência aos β-lactâmicos semi-sintéticos, é o isolamento de cepas com reduzida susceptibilidade e/ou resistência plena aos antimicrobianos da classe dos glicopeptídeos, tais como *Enterecoccus* spp e *Staphylococcus* spp (Hiramatsu, 2001; Oliveira et al, 2001; Baiocchi et al, 2003; Lutz et al, 2003).

A disseminação de genes de resistência, ou ainda, a transferência de elementos genéticos móveis tais como transposons, é a principal preocupação no controle e monitoramento da resistência antimicrobiana. Na maioria dos casos, a resistência bacteriana é mediada por genes, cuja detecção constitui método preciso de determinação de resistência. Entre os genes mais estudados está o gene *mecA*, um fragmento genético do cassete cromossômico estafilocócico *mec*, que determina resistência para meticilina e, virtualmente, a todos antimicrobianos da classe dos β-lactâmicos. A resistência à meticilina codificada pelo gene *mecA* é amplamente disseminada entre as espécies de *Staphylococcus* (Pierre et al, 1990; Archer & Niemeyer, 1994). Esta distribuição difundida do gene *mecA* pode ser devido às transmissões horizontais entre isolados de *Staphylococcus* spp coagulase negativos e de *S. aureus* (Archer & Scott, 1991; Archer & Niemeyer, 1994). O mecanismo de resistência à meticilina é devido a produção de uma proteína de ligação à penicilina,

codificada pelo gene *mecA*, denominada "Penicilin Binding Protein" 2a ou 2' (PBP 2a ou PBP 2'). Esta proteína apresenta baixa afinidade de ligação para os antimicrobianos β-lactâmicos ao contrário da proteína ligante de penicilina constitutiva, que se liga covalentemente aos β - lactâmicos (Archer & Niemeyer, 1994). A detecção molecular do gene *mecA* é o método de referência para determinar a resistência à meticilina (Hussain et al, 2000).

As infecções nosocomiais causadas por estes *Staphylococcus* meticilina-resistentes requerem freqüentemente a administração de antimicrobianos não β-lactâmicos e as opções tornam-se ainda mais limitadas (Archer & Niemeyer, 1994).

Recentemente, o *Clinical and Laboratory Standards Institute / National Comitee for Clinical Laboratory Standards* (CLSI/NCCLS, 2005) padronizou novas metodologias para predizer a resistência do gênero *Staphylococcus* spp mediada pelo gene *mecA*, para a classe dos β-lactâmicos, e gene *erm*, para a classe dos macrolídeos. Os resultados obtidos pelo teste de difusão em disco utilizando disco de cefoxitina (30 μg) e os novos "breakpoints" podem ser utilizados para determinar a resistência em *Staphylococcus* spp mediada pelo gene *mecA*. Muitos estudos vêm apontando a utilização da cefoxitina, pelo método da difusão em agar, como método de melhor predição da resistência mediada pelo gene *mecA* (Felten et al, 2002; Skov et al, 2003; Cauvelier et al, 2004; Pottumarthy, Fritsche & Jones, 2005; Sharp, Warren & Jr, 2005; Swenson et al, 2005). Contudo, algumas espécies de *Staphylococcus* spp podem não apresentar uma boa correlação entre a resistência devido ao *mecA* e este agente antimicrobiano. Com isso, a grande dificuldade de detecção laboratorial da resistência, principalmente em isolados de *Staphylococcus* spp resistentes à meticilina. Porque estes isolados podem apresentar um fator de declínio da efetividade clínica de

muitos agentes antimicrobianos, o aperfeiçoamento e/ou desenvolvimento de métodos laboratoriais mais acurados deste padrão de resistência e suas implicações clínicas tornam-se cada vez mais relevantes.

Descrição do Gênero *Staphylococcus*

A denominação *Staphylococcus* (do grego *staphylé*, "cacho de uvas"), primeiramente descrita por Ogston em 1881, foi utilizada para descrever células bacterianas de forma circular (cocos) em arranjos irregulares, dispostos em forma de "cachos de uva", que foram observados num abcesso. Contudo, foi Rosenbach, em 1884, quem descreveu adequadamente o gênero *Staphylococcus* (Cookson, 1997).

O gênero *Staphylococcus* é fortemente relacionado ao gênero *Micrococcus*, conforme estudos da composição de bases do DNA. No entanto, estudos mais recentes de composição do DNA, hibridização DNA-rRNA e análise comparativa de oligonucleotídeos da porção 16S do rRNA, demonstraram que o gênero *Staphylococcus* é mais estreitamente relacionado com o mais recente gênero descrito *Macrococcus* que, por sua vez, tem uma relação muito forte com os gêneros *Bacillus*, *Salinococcus*, *Gamella*, *Listeria*, *Planococcus* and *Brochothrix*. Na atualidade, estes gêneros estão sendo agrupados, juntamente com o gênero *Staphylococcus* e muitos outros gêneros, na Família *Bacillaceae* (Bannerman, 2003).

Os membros do gênero *Staphylococcus* são cocos Gram-positivos (0.5 a 1.5 μm em diâmetro) que aparecem em cadeias simples, aos pares, tétrades, cadeias curtas (três a quatro células) ou em grupamentos irregulares. *Staphylococcus* são imóveis, não-esporulados, e usualmente catalase positivos e não-encapsulados ou apresentam formação

limitada de cápsula. A maioria das espécies é anaeróbia facultativa e seu conteúdo genômico varia, em tamanho, de 2000 a 3000 kb (Bannerman, 2003).

O gênero *Staphylococcus* é, atualmente, composto por 35 espécies e 17 subspécies que estão distribuídas na natureza, embora sejam principalmente encontrados na microbiota da pele e membrana mucosa de mamíferos e aves. *Staphylococcus* geralmente apresentam uma relação simbiótica com seu hospedeiro, entretanto, se as barreiras cutâneas naturais forem danificadas por traumas ou implantes de próteses médicas, estes microrganismos podem entrar no tecido do hospedeiro e/ou colonizar corpo estranho e passar a tornar-se um patógeno (Bannerman, 2003).

Staphylococcus encontrados em humanos e outros primatas incluem *S. aureus*, *S. epidermidis*, *S. capitis*, *S. caprae*, *S. sacharolyticus*, *S. warneri*, *S. pasteuri*, *S. haemolyticus*, *S. hominis*, *S. lugdunensis*, *S. auricularis*, *S. saprophyticus*, *S. cohnii*, *S. xylosus* e *S. simulans*. Entretanto, algumas espécies, como *S. xylosus* e *S. simulans* são somente transitórias e primariamente adquiridos de animais domésticos e seus produtos (Bannerman, 2003).

Os membros do gênero *Staphylococcus* possuem um conteúdo de G+C em seu DNA de 30 a 39 mol%. Isto o diferencia do gênero *Micrococcus* que possui um conteúdo G+C que varia entre 66 e 75 mol% (Cookson, 1997; Bannerman, 2003).

A parede celular dos *Staphylococcus* contem ácido teicóico e peptideoglicano. O diaminoácido presente no peptideoglicano é a L-lisina e a ligação interpeptídeos do peptídeoglicano consiste de peptídeos oligoglicinas (susceptível a ação de lisostafina). Os ácidos teicóicos da parede celular de *Staphylococcus* podem ser poli (poliol-fosfato), poli (glicerol-fosfato e glicosil-fosfato), ou poli (glicosil-fosfato), dependendo da espécie (Bannerman, 2003).

Importância Clínica dos *Staphylococcus* spp

Staphylococcus aureus

Staphylococcus spp são, reconhecidamente, uma importante causa de infecções humanas nosocomiais e adquiridas na comunidade (Chambers, 2001; Foster, 2004).

S. aureus é bem descrito como um patógeno humano oportunista (Bannerman, 2003). Infecções nosocomiais causadas por *S. aureus* têm sido a maior causa de morbi-mortalidade (Jeljaszewicz, Mlynarczyk & Mlynarczyk, 1998). Suas infecções são principalmente agudas e piogênicas e, se não tratadas, pode disseminar-se ao redor de tecidos ou via bacteremia a sítios metastáticos, envolvendo outros órgãos. Algumas das infecções mais comuns causadas por *S. aureus* envolvem a pele e estes incluem furúnculos, celulites, impetigo e infecções pós-operatórias de vários sítios (Kloos & Bannerman, 1999).

A porção nasal anterior é o mais freqüente sítio de colonização humana, embora a pele (especialmente quando danificada), vagina, axila, períneo e orofaringe podem, também, serem colonizadas. Aproximadamente 25 a 50% das pessoas saudáveis podem ser colonizados persistente ou transitoriamente pelo *S. aureus* (Kloos & Bannerman, 1999). A razão da colonização é alta entre diabéticos insulino-dependentes, pacientes infectados com a Síndrome da Imunodeficiência Humana (SIDA), usuários de drogas injetáveis, pacientes submetidos a hemodiálise e indivíduos com lesões de pele. Os sítios de infecções servem como reservatórios de microrganismos de *S. aureus* que poderão causar futuras infecções, e pessoas colonizadas com *S. aureus* apresentam um grande risco de infecções subseqüentes que indivíduos não colonizados. Muitos indivíduos que desenvolvem infecções com *S. aureus* é devido a seu próprio isolado colonizante, mostrando-se como fonte endógena de infecção. Entretanto, *S. aureus* pode ser adquirido de outras pessoas e ou ainda de fontes

ambientais (Bannerman, 2003). A transmissão mais freqüente resulta da colonização transitória das mãos de pessoas que trabalham em hospitais, as quais transferem isolados de *S. aureus* de um paciente para outro. A disseminação de *Staphylococcus* de aerossóis de secreções nasais e respiratórias de pessoas saudáveis colonizadas também tem sido descritos (Connolly, Noble & Phillips, 1993).

S. aureus é a causa mais comum de infecções cirúrgicas e é o segundo, atrás apenas de SCoN, como causa de bacteremia primária (Sader et al, 2004). De modo crescente, os isolados nosocomiais de *S. aureus* são resistentes a múltiplos antimicrobianos. Na comunidade, *S. aureus* permanece como uma importante causa de infecções de pele e tecidos, infecções respiratórias e endocardites infecciosas.

Com o crescente número de pacientes que recebem terapia de infusão residencial, isto pode ter aumentado o número de infecções estafilocócicas adquiridas na comunidade. Diversos relatos de infecções adquiridas na comunidade, tanto em regiões urbana e rural, causadas por *S. aureus* resistentes a meticilina (MRSA) em indivíduos sem exposição prévia a medicamentos vem sendo descritos (Layton, Hierholzer & Patterson, 1995; Herold et al, 1998; Jones et al, 2002; Salmenlinna, Lyytikainen & Vuopio-Varkila, 2002; Ribeiro et al, 2005). Em contraste aos MRSA adquiridos no hospital, estes isolados comunitários tem permanecido susceptíveis a muitos antimicrobianos não β-lactâmicos (Herold et al, 1998; Salmenlinna, Lyytikainen & Vuopio-Varkila, 2002). O interesse tem sido a aparente capacidade de isolados MRSA-adquiridos na comunidade causar sérias doenças em indivíduos imunocompetentes. Esta habilidade pode ser devido a presença de diferentes genes produtores de toxinas nestes isolados bem como o uso de agentes β-lactâmicos para tratamento empírico de pacientes infectados com estes isolados.

S. aureus é um patógeno piogênico que apresenta a capacidade de induzir a formação de abscessos em sítios locais ou ainda em infecções metastáticas (Bannerman, 2003). Esta clássica resposta patológica ao *S. aureus* define o segmento no qual progride a doença. A bactéria induz uma resposta inflamatória caracterizada por uma intensa resposta inicial de leucócitos polimorfonucleares (PMN) e a subseqüente infiltração de macrófagos e fibroblastos. Em qualquer uma das respostas da célula hospedeira (incluindo a deposição de fibrina e colágeno) está a infecção local ou disseminada a tecidos adjacentes ou a corrente circulatória (Kloos & Bannerman, 1999).

Nas doenças estafilocócicas devido a toxinas, a infecção clínica não está, invariavelmente, presente. *S. aureus* produz tipos de toxinas: citotoxinas, toxinas pirogênicas (superantígenos) e toxinas esfoliativas (Kloos & Bannerman, 1999; Bannerman, 2003).

Staphylococcus spp coagulase-negativos

Como um grupo, os *Staphylococcus* spp coagulase-negativos (SCoN) estão entre os microrganismos mais freqüentemente isolados no laboratório de microbiologia clínica e estão cada vez mais tendo importância, especialmente como infecções nosocomiais (Von Eiff, Peters & Heilmann, 2002). Estes microrganismos são habitantes normais da pele humana e membranas mucosas e, por isso, um dos maiores desafios do trabalho diagnóstico para distinguir cepas infectantes das contaminantes (Von Eiff, Peters & Heilmann, 2002). Entretanto, observando-se a patogênese das infecções devido SCoN e considerando, particularmente, os fatores de virulência, nota-se que as espécies de SCoN tem sido identificadas como uma das maiores causas de infecções nosocomiais, especialmente em

pacientes com fatores predisponentes tais como implantes protéticos e uso de cateteres (Von Eiff, Peters & Heilmann, 2002).

Dados do *National Nosocomial Infections Surveillance System* (NNIS 1999) de janeiro de 1990 a até maio de 1999 mostraram que SCoN estão entre os patógenos mais freqüentemente isolados (37,3%, comparados com 12,6% para *S. aureus*) de infecções da corrente circulatória em pacientes internados na unidade de terapia intensa. A grande dificuldade, no entanto, é a distinção de infecções causadas por estes microrganismos, uma vez que SCoN tem sido extensivamente disseminado como contaminante de culturas. O tratamento também é difícil, porque muitos SCoN carreiam resistência múltipla aos antimicrobianos, incluindo a classe dos glicopeptídeos (Diekema et al, 2001).

As manifestações clínicas das infecções devido a maioria dos SCoN diferem marcantemente das infecções ocasionadas por *S. aureus*. Normalmente, o quadro clínico é discreto e não-específico, e o curso clínico mais sub-agudo ou até crônico, sem sinais fulminantes de infecção (Von Eiff, Peters & Heilmann, 2002). Bacteremias devido a SCoN são raramente perigosas, especialmente quando tratadas pronta e adequadamente. Entretanto, síndromes sépticas e fatais podem ocorrer, principalmente em pacientes imunocomprometidos e/ou se uma das espécies altamente virulenta, como *Staphylococcus lugdunenesis*, estiver envolvido (Von Eiff, Peters & Heilmann, 2002).

A importância de SCoN como patógenos nosocomiais tem incitado maior interesse em sua detalhada caracterização. Pesquisas sobre SCoN vêm sendo realizadas, incluindo o desenvolvimento de métodos mais acurados para identificação das espécies, para distinção entre cepas infectantes das contaminantes, tipagem epidemiológica das espécies e detecção da resistência à meticilina (Rowe et al, 2002; Ferreira et al, 2003; Caierão et al, 2004).

A capacidade dos SCoN em colonizar e desenvolver processos infecciosos está relacionada, principalmente, com o seu poder de aderência aos biomateriais que compõem os aparelhos e dispositivos médicos (Bannerman, 2003). Os principais fatores de virulência descritos para a espécie *S. epidermidis* são: a capacidade de formação de biofilme / produção de "slime", a presença de enzimas extracelulares e produção de toxinas, além de adesinas capsulares polissacarídicas (Von Eiff, Peters & Heilmann, 2002; Bannerman, 2003).

A patogênese das infecções associadas a "corpos estranhos" com *S. epidermidis* é caracterizada pela capacidade desta espécie em colonizar a superfície de polímeros pela formação de uma multicamada densa denominada biofilme. A formação de biofilme ocorre em duas etapas: na primeira, a bactéria rapidamente adere ao material polimérico. Na segunda, durante a fase de acumulação, a bactéria prolifera para formar multicamadas celulares sobre a superfície do polímero, as quais são embebidas em material extracelular (Von Eiff, Peters & Heilmann, 2002). Em associação com o biofilme, outras proteínas tem sido descritas, tais como uma adesina polissacarídica intracelular (PIA) e um antígeno associado ao "slime" (SAA), os quais desempenham papel importante no acúmulo de células bacterianas e na formação do biofilme, respectivamente (Christensen et al, 1982; Kloos & Bannerman, 1994). Estudos mostraram que o "slime" formado promove um decréscimo na atividade de importantes drogas como os glicopeptídeos (teicoplanina e vancomicina) utilizadas contra infecções causadas por microrganismos resistentes a meticilina (Souli & Giamarellou, 1998).

Além da produção de "slime", outros fatores de virulência estão envolvidos com a capacidade infecciosa dos SCoN, principalmente na evasão do sistema imune do hospedeiro. Assim, atuam, embora menos toxigênico que as de *S. aureus*, as exotoxinas e

enzimas dos SCoN (Von Eiff, Peters & Heilmann, 2002; Bannerman, 2003). A produção de lantibióticos, que são bacteriocinas e/ou antimicrobianos peptídeos produzidas por *S. epidermidis* e outros cocos Gram-positivos, explica a colonização efetiva da pele, uma vez que possui papel substancial na colonização bacteriana sobre a pele e membranas mucosas pela exclusão competitiva de microrganismos que são sensíveis a suas atividades bactericidas (Von Eiff, Peters & Heilmann, 2002). Ainda, a capacidade de proliferação do microrganismo e conseqüente infecção estão associadas à habilidade destes microrganismos em adquirir elementos essenciais das células hospedeiras para seu crescimento. Estes microrganismos capturam íon ferro de transferrinas e lactoferrinas do hospedeiro, o mecanismo de aquisição de ferro pelos estafilococos não está bem esclarecido, embora dois princípios são sugeridos: o primeiro a produção de queladores de ferro chamados sideróforos e o segundo, um mecanismo pelo qual a bactéria assimila ferro pela dependência de um contato direto entre a transferrina do hospedeiro e a célula bacteriana (Von Eiff, Peters & Heilmann, 2002).

Epidemiologia das infecções estafilocócicas

A ocorrência das infecções estafilocócicas está associada a um grande nímero de fatores de risco:

♦ Fatores relacionados ao estado do hospedeiro, tais como: quebra da barreira cutâneo-mucosa, deficiência no sistema imune e idade (principalmente em idosos e neonatos) (Kloos & Bannerman, 1994);

♦ Fatores relacionados a materiais de natureza física, como uso e/ou implantação de dispositivos médicos, tais como: cateteres, próteses, marcapassos transvenosos, lentes intra-oculares, entre outros (Kloos & Bannerman 1994; Von Eiff, Peters & Heilmann, 2002);

♦ Fatores relacionados a procedimentos e protocolos de manuseio do paciente, como a utilização de procedimentos invasivos, que aumentam a susceptibilidade do indivíduo às infecções (Kloos & Bannerman, 1994). As infecções estafilocócicas são principalmente de natureza hospitalar, embora mais recentemente tenham sido descritas infecções ocasionadas por *S. aureus* comunitários denominados de *Community Acquired Methicillin Resistance S. aureus* (CA-MRSA) em diversos países do mundo (Crum, 2005; Weber, 2005), inclusive no Brasil (Ribeiro et al, 2005).

As principais infecções ocasionadas por *S. aureus* e SCoN incluem infecções do sistema sangüíneo, infecções de sítio cirúrgico, infecções do trato urinário, artrite séptica, secreções intra-abdominais e de mama, osteomielite, otite média, entre outros (Kloos & Bannerman, 1999). Em estudo realizado pelo *Antimicrobial Surveillance Program* SENTRY durante o período de 1997 a 2002, *S. aureus* foi o principal patógeno associado às infecções da corrente circulatória na América do Norte, América Latina, e o segundo mais freqüentemente isolado na Europa, atrás somente da *Escherichia coli*. Além disso, no mesmo estudo, SCoN ocuparam o terceiro lugar como patógenos mais freqüentemente isolados na América do Norte, América Latina e Europa (Biedenbach, Moet & Jones, 2004; Sader et al, 2004). Em outro estudo, que verificou a freqüência de isolamento de patógenos oriundos de diferentes infecções, como da corrente circulatória, pneumonia hospitalar, pele e tecidos moles e do trato genito-urinário de pacientes europeus, observou-se que o gênero *Staphylococcus* era o mais prevalente em bacteremias, sendo que *S. aureus* foi o mais predominante, seguido de *S. epidermidis*, *S. haemolyticus* e *S. hominis*.

Na América Latina, durante o período de janeiro de 1997 a dezembro de 2001, SCoN surgem como o terceiro maior causador de bacteremias, sendo *S. aureus* o agente mais prevalente (Biedenbach, Moet & Jones, 2004).

No Brasil, um estudo realizado pelo SENTRY no qual avaliou a ocorrência dos mais importantes patógenos isolados entre 1997 e 1999, *S. aureus* foi o mais predominante, seguido por SCoN, em infecções da corrente circulatória (Diekema et al, 2001).

Entre os SCoN, a espécie mais prevalente em bacteremias é *S. epidermidis*, embora outras espécies, tais como *S. lugdunensis*, *S. warneri*, *S. sciuri*, *S. hominis* e *S. haemolyticus*, também podem estar envolvidas (Diekema et al, 2001; Caierão et al, 2004). Além das infecções da corrente circulatória, *S. epidermidis* também é a principal espécie envolvida na maioria das demais infecções causadas por este grupo de microrganismos (Kloos & Bannerman, 1994).

S. haemolyticus é a segunda espécie mais isolada em infecções nosocomiais, tendo sido relacionada com endocardites de válvula cardíaca, septicemias, peritonites, infecções do trato urinário e de sítio cirúrgico (Kloos & Bannerman, 1994).

S. saprophyticus é o segundo agente etiológico das infecções do trato urinário comunitárias, sendo muito comum em mulheres jovens sexualmente ativas (Huebner & Goldmann, 1999). Sua patogenicidade está relacionada com a capacidade de colonizar o trato urogenital, tendo maior capacidade de aderência às células urogenitais do que o *S. epidermidis* (McTaggart, Rigby & Elliot, 1990).

A distribuição das espécies de SCoN isoladas de diferentes espécimes clínicos pode ser conseqüência da presença destes microrganismos colonizando diferentes sítios do corpo. Portanto, considerar uma amostra de SCoN como agente responsável pela infecção

ou como contaminante, ainda continua um desafio para os microbiologistas (Nafziger & Wenzel, 1989; Topeli et al, 1997).

Resistência dos *Staphylococcus* spp à meticilina

Logo que a penicilina foi introduzida, cepas de *S. aureus* resistentes à penicilina começaram a surgir. Esta resistência foi conseqüência da indução da produção de uma enzima, β-lactamase de pequeno espectro de ação, eficiente na hidrólise das penicilinas (Foster, 2004). O número de cepas estafilocócicas produtoras de penicilinase continua a aumentar e são predominantes nos hospitais. Devido a não efetividade das penicilinas, antimicrobianos da classe das cefalosporinas foram introduzidas. A grande utilização de cefalosporinas levou a seleção de clones de *Staphylococcus* resistentes à meticilina (MRS, do inglês *Methicillin Resistance Staphylococcus*). Estes possuem resistência a todos os antimicrobianos β-lactâmicos, incluindo cefalosporinas e carbenêmicos. Esta resistência também pode ser apresentada em bactérias que produzem uma proteína de ligação a penicilina (PBP, do inglês *Penicillin Binding Protein*) alternativa que tem baixa afinidade para todos antimicrobianos β-lactâmicos (Foster, 2004). Exemplos deste mecanismo são os *S. aureus* resistentes à meticilina (MRSA) e SCoN resistentes à meticilina (MR-CNS, do inglês methillin-resistant coagulase-negative *Staphylococci*).

Resistência a meticilina é um efeito da mutação nos genes *pbp* e *abc* ou resultado da aquisição de um gene exógeno que codifica para a resistência (gene *mec*). O primeiro tipo de resistência ocorreu em cepas com PBPs modificadas devido à alteração da capacidade de ligação a penicilina ou hiperprodução de PBPs, as quais foram obtidas em laboratório e superexpressam PBP 4. O segundo tipo de aquisição de resistência é o mais comum entre

os isolados clínicos. Esta surgiu primeiramente num único clone de MRSA disseminado no mundo, mas agora um grande número de cepas tem adquirido o determinante *mec* (Domanski & Bayles, 1995; Henze & Berger-Bacchi, 1996). A aquisição dos genes de resistência pode ser devido a plasmídios (DNA extracromossômico) ou através de elementos genéticos móveis (transposons). A região *mec* contém diversos genes.

Alguma alteração na biossíntese do peptideoglicano e/ou composição da membrana celular bacteriana podem influenciar no nível de resistência à meticilina a despeito da correlação deste gene na expressão da resistência (Berger-Bacchi, 1996; Berger-Bacchi, 1997). A prevalência de resistência à meticilina é bastante variada entre os diferentes locais, mas, independentemente disso, é sempre de grande relevância para os programas de controle epidemiológico.

Para a determinação da susceptibilidade à meticilina, através de testes fenotípicos recomendados pelo CLSI/NCCLS 2005, utilizam-se oxacilina e cefoxitina como preditores da resistência à meticilina. Entre os testes fenotípicos recomendados estão:

♦ triagem em agar Mueller Hinton (screening) suplementado com 4% de cloreto de sódio (NaCl) e 6 µg/mL de oxacilina, sendo a leitura realizada após 24 horas de incubação a 35 ºC, somente para amostras de MRSA;

♦ teste de difusão em agar (método de Kirby-Bauer) com a utilização de discos de oxacilina 1 µg e cefoxitina 30 µg, com leitura após 24 horas de incubação a 35 ºC e interpretação conforme "breakpoints" padronizados para *S. aures* e SCoN;

♦ determinação da concentração inibitória mínima (CIM), utilizando oxacilina, através da técnica de microdiluição ou diluição em agar, padronizados para S. *aureus* e SCoN conforme "breakpoints" do CLSI.

Técnicas moleculares para a detecção do gene *mecA* em *Staphylococcus* spp vêm sendo utilizadas (Archer & Pennell, 1990; Kolbert et al, 1998; Schmitz et al, 1998). A técnica da Reação em Cadeia da Polimerase ou *Polimerase Chain Reaction* (PCR), para a detecção do gene *mecA* a partir de seqüências de oligonucleotídeos complementares específicos, tem sido considerado como padrão na detecção da resistência à meticilina em amostras de *Staphylococcus* spp. Alternativamente, o método de aglutinação em látex para detecção da PBP 2a também pode ser utilizado como referência na detecção da resistência à meticilina em *Staphylococcus* (CLSI, 2005). Contudo, o alto custo destas técnicas tem estimulado a pesquisa de métodos fenotípicos, menos onerosos, para a acurada caracterização da resistência à meticilina.

Em 2005, o CLSI preconizou a utilização de cefoxitina, através do método de difusão em disco, para detectar a resistência à meticilina entre as cepas de *Staphylococcus*. Cefoxitina, uma cefamicina, seria melhor preditora da presença do gene *mecA*, por ter uma maior atividade indutora da expressão gênica do que oxacilina (Darini, Palazzo & Felten, 2004). Diversos estudos realizados apontaram sua superioridade na detecção da resistência à meticilina (Felten et al, 2002; Skov et al, 2003; Cauvelier et al, 2004; Pottumarthy, Fritsche & Jones, 2005; Sharp, Warren & Jr, 2005; Swenson et al, 2005). No entanto, Frigatto et al (2005) mostraram haver falha na correta caracterização da resistência à meticilina, com uso de de disco de cefoxitina. Além disso, outros métodos que não a difusão em disco, para cefoxitina, não têm sido padronizados. Desta maneira, a importância da avaliação de novos métodos, propondo novos "breakpoints", na utilização da cefoxitina para correlacionar a resistência com a presença do gene *mecA* em *Staphylococcus* são necessários.

1. Archer GL, Pennell E. 1990. Detection of methicillin resistance in staphylococci by using a DNA probe. *Antimicrobial Agents and Chemotherapy* 34: 1720-1724.

2. Archer GL, Scott J. 1991. Conjugative transfer genes in staphylcoccal isolates from United States. *Antimicrobial Agents and Chemotherapy* 35:2500–2504.

3. Archer GL, Niemeyer DM. 1994. Origin and evolution of DNA associated with resistance to methicillin in *staphylococci*. *Trends in Microbiology* 2: 325–347.

4. Baiocchi SA, Tognim MCB, Baiocchi OCG et al. 2003. Endocarditis due to glycopeptide-intermediate *Staphylococcus aureus*: case report and strain characterization. *Diagnostic Microbiology and Infections Disease* 45: 149-152.

5. Bannerman TL. 2003. *Staphylococcus*, *Micrococcus*, and other catalase-positive cocci that grow aerobically. In PR Murray, EJ Baron, JH Jorgensen, MA Pfaller, RH Yolken (eds), *Manual of Clinical Microbiology*, American Society Microbiology, Washington, 384-404.

6. Berger-Bacchi B. 1997. Resistance not mediated by beta-lactamase (methicillin resistance). In: Crossley KB, Archer GL, editors. The Staphylococci in Human Disease. New York: Churchill Livingston, 158-74.

7. Berger-Bacchi B. 1996. Update on methicillin resistance mechanisms in staphylococci. *Chemotherapy* 42: 19-26.

8. Biedenbach DJ, Moet GJ, Jones RN. 2004. Occurrence and antimicrobial resistance pattern comparisons among bloodstream infection isolates from the SENTRY

Antimicrobial Surveillance Program (1997–2002). *Diagnostic Microbiology and Infectious Disease* 50: 59-69.

9. Caierão J, Musskopf M, Superti S et al. 2004. Evaluation of phenotypic methods for methicillin resistance characterization in coagulase-negative staphylococci (CNS). *Journal of Medical Microbiology* 53: 1195-1199.

10. Cauwelier B, Gordts B, Descheemaecker P et al. 2004. Evaluation of a disk diffusion method with cefoxitin (30µg) for detection of methicillin-resistant *Staphylococcus aureus*. *European Journal of Clinical Microbiology and Infectious Diseases* 23: 389-392.

11. Chambers HF. 2001. The changing epidemiology of *Staphylococcus aureus*? *Emerging Infectious Diseases* 7, 178-82.

12. Chang S, Sievert DM, Hageman JC et al. 2003. Infection with vancomycin-resistant *Staphylococcus aureus* containing the vanA resistance gene. *New England Journal of Medicine* 348: 1342– 1347.

13. Christensen GD, Simpson WA, Bisno AL et al. 1982. Adherence of slime-producing strains of *Staphylococcus epidermidis* to smooth surfaces. *Infection and Immunity* 37: 318-326.

14. Clinical and Laboratory Standards Institute (CSLI)/NCCLS. 2005. *Performance Standards for Antimicrobial Susceptibility Testing – Fifteenth Informational Supplement M100-S15*. (CSLI)/NCCLS, Wayne, PA, USA.

15. Connolly S, Noble WC, Phillips I. 1993. Mupirocin resistance in coagulase-negative staphylococci. *Journal of Medical Microbiology* 39: 450-453.

16. Cookson BD. 1997. *Staphylococcus aureus*. In: Emmerson Am, Hawkey PM, Gillspie SH, editors. Principles and pratice of clinical bacteriology. West Sussex, England: John Wiley & Sons, 109-129.

17. Crum NF. 2005. The emergence of severe, community-acquired methicillin-resistant *Staphylococcus aureus* infections. *Scandinavian Journal of Infectious Diseases* 37: 651-656.

18. Darini ALC, Palazzo ICV & Felten A. 2004. Cefoxitin does not induce production of Penicillin Binding Protein 2a in methicillin-susceptible *Staphylococcus aureus* strains. *Journal of Clinical Microbiology* 42: 4412-4413

19. Diekema DJ, Pfaller MA, Schmitz FJ et al. 2001. Survey of infections due to *staphylococcus* species: frequence of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the western pacific region for the SENTRY antimicrobial surveillance program, 1997-1999. *Clinical Infectious Diseases* 32 (2): 114-32.

20. Domanski TL, Bayles KW. 1995. Analisys of *Staphylococus aureus* genes encoding penicillin-binding protein 4 and an ABC-type transporter. *Gene* 167: 111-114.

21. Felten A, Grandry B, Lagrange PH et al. 2002. Evaluation of three techniques for detection of low-level methicillin-resistant *Staphylococcus aureus* (MRSA): a disk diffusion method with cefoxitin and moxalactam, the vitek 2 system, and the MRSA-screen latex agglutination test. *Journal of Clinical Microbiology* 40: 2766-2771.

22. Ferreira RBR, Iorio NLP, Malvar KL et al. 2003. Coagulase-negative staphylococci: comparison of phenotypic and genotypic oxacillin susceptibility tests and evaluation of the agar screening test by using different concentrations of oxacillin. *Journal of Clinical Microbiology* 41: 3609-3614.

23. Ferguson KP, Lambe DW, Keplinger. 1990. Comparison of the pathogenicity of three species of coagulase-nagative Staphylococcis in a mouse model with and without a foreign body. *Canadian Journal of Microbiology* 37: 722-724.

24. Foster TJ. 2004. The *Staphylococcus aureus* "superbug". *The Journal of Clinical Investigation* 114: 1693-1696.

25. Frigatto EAM, Machado AMO, Piganatari ACC et al. 2005. Is the Cefoxitin Disk Test Reliable Enough To Detect Oxacillin Resistance in Coagulase-Negative Staphylococci? *Journal of Clinical Microbiology* 43: 2028-2029.

26. Henze UU, Berger-Bacchi B. 1996. Penicillin-binding protein 4 overproduction increases beta-lactam resistance in *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy* 40: 2121-2126.

27. Herold BC, Immergluck LC, Maranan MC et al. 1998. Communityacquired methicillin-resistant *Staphylococcus aureus* in children with no identified predisposing risk. JAMA 279: 593–598.

28. Hiramatsu K. 1998. Vancomycin resistance in staphylococci. *Drug Resistance Updates* 1: 135-150.

29. Huebner J, Goldmann DA. 1999. Coagulase-negative staphylococci: role as pathogens. *Annals of Review Medical* 50: 223-236.

30. Hussain Z, Stoakes L, Massey V et al. 2000. Correlation of oxacilin MIC with *mec*A gene carriage in coagulase-negative staphylococci. *Journal of Clinical Microbiology* 38: 752-54.

31. Jeljaszewicz J, Mlynarczyk G, Mlynarczyk A. 1998. Current threats of antibiotic resistance in bacteria. *Blok Operacyjny* 3-4: 49-55.

32. Jones RN. 1996. Impact of changing pathogens and antimicrobial susceptibility patterns in the treatment of serious infections in hospitalized patients. *American Journal of Medicine* 100: 1-12.

33. Jones TF, Kellum ME, Porter SS et al. 2002. An outbreak of community-acquired foodborne illness caused by methicillinresistant *Staphylococcus aureus. Emerging Infectious Diseases* 8: 82–84.

34. Kloos WE, Bannerman TL. 1994. Update on clinical significance of coagulase-negative staphylococci. *Clinical Microbiology Reviews* 7: 117-140.

35. Kloos WE, Bannerman TL. 1999. *Staphylococcus* and *Micrococcus*, In Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C. and Yolken, R.H. (ed). Manual of clinical Microbiology 7th Ed. ASM Press. Washington DC, p 264-282.

36. Kolbert CP, Arruda J, Varga-Delmore P et al. 1998. Branched-DNA assay for detection of the *mecA* gene in oxacillin-resitant and oxacillin-sensitive staphylococci. *Journal of Clinical Microbiology* 36: 2640-2644.

37. Lambe DW, Ferguson KPJ, Keplinger JL et al. 1990. Pathogenecity of *Staphylococcus lugdunensis*, *Staphylococcus schleiferi* and three other coagulase-negative staphylococci in a mouse model and possible virulence factors. *Canadian Journal of Microbiology* 36: 455-463.

38. Layton MC, Hierholzer Jr. WJ, Patterson JE. 1995. The evolving epidemiology of methicillin-resistant *Staphylococcus aureus* at a university hospital. *Infection Control and Hospital Epidemiology* 16: 12–17.

39. Lutz L, Machado A, Kuplich N et al. 2003. Clinical failure of vancomycin treatment of *Staphylococcus aureus* infection in a tertiary hospital in southern Brazil. *The Brazilian Journal of Infectious Diseases* 7(3): 227-231.

40. Marshall SA, Wilk WW, Pfaller MA et al. 1998. *Staphylococcus aureus* and coagulase-negative staphylococci from blood stream infections: frequency of occurrence, antimicrobial susceptibility and molecular (*mecA*) characterization of oxacillin resistance in the SCOPE Program. *Diagnostic Microbiology and Infectious Diseases* 30, 205-214.

41. Mcdonnell RW, Sweeney HM, Cohen S. 1983. Conjugational transfer of gentamicin resistance plasmids intra- and interspecifically in *Staphylococcus aureus* and S*taphylococcus epidermidis. Antimicrobial Agents and Chemotherapy* 23: 151-60.

42. McTaggart LA, Rigby RC, Elliot TSJ. 1990. The pathogenesis of urinary tract infections associated with *Escherichia coli*, *Staphylococcus saprophyticus* and *Staphylococcus epidermidis*. *Journal of Medical Microbiology* 32: 135-141.

43. Nafziger DA, Wenzel RP. 1989. Coagulase-negative staphylococci: epidemiology, evaluation and therapy. *Infectious Diseases and Clinical North American* 3: 915-929.

44. National Nosocomial Infectious Surveillance (NNIS) System report: data summary from January 1990-May 1999, issued June 1999. *American Journal of Infection Control* 27: 520-32.

45. Oliveira GA, Dell'Aquila AM, Masiero RL et al. 2001. Isolation in Brazil of nosocomial *Staphylococcus aureus* with reduced susceptibility to vancomycin. *Infection Control and Hospital Epidemiology* 22(7): 443-448.

46. Oplustil CP, Sakamoto TN, Mendes CMF. 1994. Distribuição das diferentes espécies de estafilococos coagulase negativa em um hospital universitário. *Revista Brasileira de Patologia Clínica* 30: 173-177.

47. Pierre J, Williamson R, Bornet M et al. 1990. Presence of an additional penicillin-binding protein in methicillin-resistant *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus homonis*, and *Staphylococcus simulans* with a low affinity

for methicillin, cephalothin, and cefamandole. *Antimicrobial Agents and Chemotherapy* 34: 1691–1694.

48. Pottumarthy S, Fritsche TR, Jones RN. 2005. Evaluation of alternative disk diffusion methods for detecting *mecA*-mediated oxacillin resistance in an international collection of staphylococci: Validation report from the SENTRY Antimicrobial Surveillance Program. *Diagnostic Microbiology and Infectious Diseases* 51: 57-62.

49. Ribeiro A, Dias C, Carvalho MCS et al. 2005. First report of infection with Community-Acquired Methicillin-Resistant *Staphylococcus aureus* in South America. *Journal of Clinical Microbiology* 43: 1985-1988.

50. Rowe F, Superti SV, Scheibe RM et al. 2002. Agar diffusion, agar dilution, Etest ang agar screening test in the detection of methicillin resistance in staphylococci. *Diagnostic Microbiology and Infection Disease* 43: 45-48.

51. Sader HS, Jones RN, Gales AC et al. 2004. SENTRY antimicrobial surveillance program report: Latin American and Brazilian results for 1997 through 2001. *The Brazilian Journal of Infectious Diseases* 8: 25-79.

52. Salmenlinna S, Lyytikainen O, Vuopio-Varkila J. 2002. Community-acquired methicillin-resistant *Staphylococcus aureus*, Finland. *Emerging Infectious Diseases* 8: 602–607.

53. Sharp SE, Warren JA, Jr RBT. 2005. Cefoxitin disk diffusion screen for confirmation of oxacillin-resistant *Staphylococcus aureus* isolates and utility in the clinical laboratory. *Diagnostic Microbiology and Infectious Diseases* 51: 69-71.

54. Schmitz FJ, Steiert M, Hofmann B et al. 1998. Detection of staphylococcal genes directly from cerebrospinal and peritoneal fluid samples using a multiplex polymerase

chain reaction. *European Journal of Clinical Microbiology and Infection Diseases* 17: 272-274.

55. Skov R, Smyth R, Clausen M et al. 2003. Evaluation of a cefoxitin 30µg disc on Iso-Sensitest agar for detection of methicillin-resistant *Staphylococcus aureus*. *Journal of Antimicrobial Chemotherapy* 52, 204-207.

56. Souli M, Giamarellou H. 1998. Effects of slime produced by clinical isolates of coagulase-negative staphylococci on activities of various antimicrobial agents. *Antimicrobial Agents and Chemotherapy* 42: 100-107.

57. Swenson JM, Tenover FC, Cefoxitin Disk Study Group. 2005. Results of disk diffusion testing with cefoxitin correlate with presence of *mecA* in *Staphylococcus* spp. *Journal of Clinical Microbiology* 43: 3818-3823.

58. Topeli A, Unal S, Hayran M et al. 1997. Septic Shock as a predictor of mortality in bacteremia caused by oagulase-negative staphylococci. *European Journal of Clinical Microbiology and Infections Diseases* 16: 411-416.

59. Von Eiff C, Peters G, Heilmann C. 2002. Pathogenesis of infections due to coagulase-negative staphylococci. *Lancet Infectious Disease* 2 (11): 677-685.

60. Walsh TR, Howe RA. 2002. The prevalence and mechanisms of vancomycin resistance in *Staphylococcus aureus*. *Annal Review in Microbiology* 56: 657– 675.

61. Weber DJ, Rutala WA. 1989. Changing trends in infection control and hospital epidemiology. *Infection Diseases of Clinical North American* 3: 671-683.

62. Weber JT. 2005. Community-associated methicillin-resistant *Staphylococcus aureus*. *Clinical of Infectious Diseases* 41: 5269-5272.

♦ Melhor avaliação do perfil de susceptibilidade à cefoxitina, em comparação à presença do gene *mecA*, por diferentes técnicas fenotípicas, como marcador de resistência à meticilina em isolados de *Staphylococcus aureus* e *Staphylococcus* coagulase negativos.

♦ Avaliar e propor "breakpoints" para as técnicas fenotípicas de screening e diluição em agar, com uso da cefoxitina, para isolados clínicos de *Staphylococcus* spp.

Geral

♦ Detectar, através da Reação em cadeia da Polimerase (PCR) o gene *mecA*, responsável pela expressão da PBP 2a, em isolados clínicos de *Staphylococcus* spp obtidos de hemoculturas de pacientes internados em três hospitais de Porto Alegre, no período de agosto a dezembro de 2004.

Específicos

♦ Descrever a prevalência do gene *mecA* entre isolados de *Staphylococcus* spp obtidos de hemoculturas de pacientes hospitalizados;

♦ Caracterizar a resistência à meticilina pelos métodos de disco-difusão, triagem em agar e diluição em agar em comparação com PCR/*mecA* (padrão-ouro).

♦ Verificar os melhores "breakpoints" para aplicação de técnicas fenotípicas com uso da cefoxitina para caracterizar a resistência à meticilina entre isolados de *Staphylococccus* spp.

Estudo transversal (teste diagnóstico).

HIPÓTESES DO ESTUDO

♦ A resistência à cefoxitina é um marcador acurado da presença do gene *mecA* (determinante genético da resistência à meticilina).

♦ O método de triagem (s*creening*) com cefoxitina tem melhor correlação com o gene *mecA* do que os demais métodos fenotípicos.

ARTIGOS PRINCIPAIS

IS CEFOXITIN A BETTER PREDICTOR FOR THE *mecA* GENE THAN OXACILLIN?

Running title: Predictors of the *mecA* gene in staphylococci

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Keywords: staphylococci, *mecA* gene, cefoxitin, oxacillin

ABSTRACT

Aims: To compare cefoxitin and oxacillin by different phenotypic methods with polimerase chain reaction (PCR) as markers for detection of methicillin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci (CoNS) isolates.

Methods: A total of 343 consecutive *Staphylococcus* isolates were tested through: disk diffusion (cefoxitin 30μg and oxacillin 1μg); agar dilution with cefoxitin and oxacillin (using concentrations since 0.25 to 256 μg/mL); screening with oxacillin (6 μg/mL and NaCl 4% for *S. aureus*; 4 μg/mL and NaCl 4% for CoNS) and cefoxitin (4 μg/mL with and without NaCl 2% for *S. aureus* and CoNS); and PCR for the *mecA* gene as the gold standard.

Results: The *mecA* gene was detected in 41.3% of *S. aureus* and in 78.4% of CoNS. Of the phenotypic methods, oxacillin and cefoxitin disks presented better correlation with *mecA* gene for *S. aureus* and CoNS, respectively. The screening method with cefoxitin 4 μg/mL presented 100% sensitivity for all isolates. Of the 343 isolates, 3 *S. aureus* and 5 CoNS isolates showed discrepant results between some phenotypic method and the PCR.

Conclusion: Cefoxitin may be used as a surrogate marker for predict resistance due *mecA* gene, although oxacillin has showed great correlation with *mecA*-positive genotype, particularly in *S. aureus*. Also, the screening method with cefoxitin 4 μg/mL showed performance equal or superior to the other screening methods applied and can be used to characterize *mecA* gene-related resistance among staphylococci species.

INTRODUCTION

The genus *Staphylococcus* is one of the main agents of human infections in the world and is responsible for high morbidity and mortality rates. *Staphylococcus aureus* has predominated as one of the main agents of hospital infections as well as community-acquired [1] [2] while coagulase-negative staphylococci (CoNS) are among the most common agents of hospital bacteremias, especially those relating to prosthetic implants and intravascular catheters.[3]

Methicillin resistance in these organisms arose shortly after the therapeutic practice with semi-synthetic β-lactam agents and, since then, treatment of staphylococcal infections has become increasingly more restricted and problematic.[4] Monitoring methicillin resistance is crucially important because some microrganisms, or even genetic components of resistance, are highly transmissible, leading to dissemination of resistance and increase of infections.[1] [2] [4] In a study carried out by antimicrobial surveillance program SENTRY in Latin America, 35.0% of the *S. aureus* and 80.4% of the CoNS, involved in bacteremias, were methicillin-resistant. In samples of blood stream infections from Brazil, methicillin resistance was detected in 35.2% of *S. aureus* and in 84.6% of CoNS.[5]

In order to obtain more accurate results in the detection of methicillin resistance, the Clinical and Laboratory Standards Institute 2005 [6] advises the use of cefoxitin as a better predictor of *mecA* gene-mediated resistance in staphylococci species. Accordingly, the aim of this study was to assess the accuracy of cefoxitin and oxacillin by phenotypic methods as compared to PCR (gold-standard) for detection of *mecA* gene in *S. aureus* and CoNS.

MATERIAL AND METHODS

Bacterial Samples. Consecutive staphylococci isolates were analyzed. They were collected between Aug to Dec 2004 from blood of patients committed from three hospital (Hospital de Clínicas of Porto Alegre, Hospital Nossa Senhora da Conceição and Complexo Hospitalar Irmandade Santa Casa of Porto Alegre) located in Porto Alegre city, South Brazil. Repeat isolates from the same patient were excluded. The identification of the species was performed by the

conventional methods as per standard protocol.[7] All samples were frozen and stored at -20°C in skim milk with 10% glycerol.

Preparation of the Bacterial Inoculum for the Susceptibility Tests. The isolates were cultivated on tryptic soy agar (Difco Laboratories, Detroit, USA) with 5% defibrinated sheep blood and a 0.5 McFarland standard suspension was prepared for each sample.

Disk Diffusion Test. We evaluated the disk diffusion test by using cefoxitin (30 μg) and oxacillin (1 μg) discs (Oxoid, Hampshire, UK) on Mueller-Hinton agar (Difco Laboratories, Detroit, USA) according to CLSI guidelines.[7]

Determination of Minimum Inhibitory Concentrations by Agar Dilution Test. Minimum Inhibitory Concentrations (MIC) was determined using a range of 0.25-256 μg/mL for cefoxitin and oxacillin. For oxacillin was add 2% NaCl in the medium. The results were read after 24 h of incubation at 35 °C.

Screening Method. Using a Steers replicator, the bacterial isolates were inoculated on Mueller-Hinton agar plates (Difco Laboratories, Detroit, USA), in the following conditions:

- oxacilin 6 μg/mL and 4% NaCl, for *S. aureus*;[6]
- oxacilin 4 μg/mL and 4% NaCl, for CoNS [8] and
- cefoxitin 4 μg/mL (with or without addition of 2% NaCl), for *S. aureus* and CoNS.

The reading of the results was carried after 24 h of incubation at 35 °C. The growth of more than one colony was taken as a positive result.

Detection of the mecA Gene by Polymerase Chain Reaction (PCR). A polymerase chain reaction (PCR) procedure was used to verify the presence of the *mecA* gene. Primers ($mecA_1$: 5'-TGG CTA TCG TGT CAC AAT CG and $mecA_2$: 5'-CTG GAA CTT GTT GAG CAG AG) and PCR reaction followed the procedures reported by Bignardi *et al*.[9]

Quality Control. In the susceptibility tests and PCR we used the American Type Culture Collection (ATCC): *S. aureus* ATCC 25923 (methicillin-susceptible) and *S. aureus* ATCC 33591 (methicillin-resistant).

RESULTS

A total of 343 staphylococci samples were analyzed, 167 *S. aureus* and 176 CoNS. The presence of the *mecA* gene was detected in 41.3% of *S. aureus* and 78.4% of CoNS. Table 1 shows the distribution of the *mecA* gene among the species.

Cefoxitin and oxacillin MIC were determined for all samples and the results are presented in Table 2. All 98 *S. aureus mecA*$^{-}$ were inhibited in cefoxitin 8 μg/mL and oxacillin 4 μg/mL. Also, one CoNS *mecA*$^{-}$ presented MIC 16 μg/mL for cefoxitin. All 69 *S. aureus mecA*$^{+}$ presented MIC 256 μg/mL for cefoxitin and oxacillin. Among CoNS *mecA*$^{+}$, resistance to elevated concentrations of cefoxitin and oxacillin was observed. The results of disk diffusion and agar screening tests are shown in Table 3.

For *S. aureus*, cefoxitin 30 μg disk presented 98.5% sensitivity and 100% specificity. One *S. aureus mecA*$^{+}$ isolate was susceptible to cefoxitin. Among CoNS, sensitivity and specificity were 100%. With use of oxacillin 1μg disk, sensitivity and specificity for CoNS were 100% and 92.1%, respectively. For *S. aureus*, however, oxacillin 1μg disk was 100% susceptible and specific. Among screening tests, agar screening with cefoxitin 4 μg/mL presented 100% sensitivity for all species and 99.0% and 97.4% specificity for *S. aureus* and CoNS, respectively. Agar screening with oxacillin 6 μg/mL for *S. aureus* presented 98.5% and 100% of sensitivity and specificity, respectively.

Table 4 shows samples that presenting some discrepance between phenotypic methods and PCR for *mecA* gene.

DISCUSSION

The transcription of the *mecA* gene is regulated by two chromosomal different regions with high protein sequence homology, the regulatory genes mecR1-mec1 and blaR1-bla1. MecR1 and blaR1 are promoters while mec1 and bla1 are repressors of the transcription of the *mecA* and *blaZ* genes, respectively.[4] [10] The difficulty in characterizing methicillin resistance in staphylococci is due to heterogeneity of repressor-promoter expression.[11] Cefoxitin, a cephamycin, would be a

stronger inducer of PBP 2a than oxacillin by inducing more effectively the promoting region mecR1.[4] [10]

In our study, three different methods were evaluated – disk diffusion, agar dilution and agar screening – using cefoxitin and oxacillin antimicrobials in order to predict *mecA* gene in staphylococci species.

Although oxacillin 1μg disk proved to be a convenient method for all *S. aureus* isolates analyzed here, it provided discrepant results with PCR for three CoNS isolates: *S. epidermidis*, *S. warneri* and *S. saprophyticus* (MIC oxacillin values of 4, 0.25 and 4 μg/mL, respectively). In a recent study, cefoxitin disk presented an excellent correlation for *mecA* gene in *S. aureus* and only 3% of major error among CoNS.[12] Using cefoxitin disk, all *S. aureus* $mecA^-$ were adequately characterized. However, one isolate $mecA^+$ (zone diameter 22 mm) was mischaracterized as susceptible after confirmation with PCR. The others phenotypic methods characterized this isolate as methicillin-resistant. This finding disagree with several literature studies showing that cefoxitin 30μg disk is a better predictor for the *mecA* gene than oxacillin 1μg disk for *S. aureus*.[13] [14][15][16][17][18] Among the CoNS, all were correctly characterized with cefoxitin disk. It should be noted that the region flanked by primers $mecA_1$ and $mecA_2$, used in the PCR reaction, was sequenced and the result no showed alteration in nucleotide sequence of DNA (data no shown). Perhaps total or partial sequencing of the *mec* gene, particularly at positions Ser-403, Lys-597 and Tyr-446, which are antimicrobial anchorage regions, were not encompassed by flanked interval with the primers used and could show a genetic mutation.[19][20]

Among CoNS, our findings showed that cefoxitin 30μg disk was 100% sensitive and specific for the *mecA* detection, while oxacillin 1μg disk presented 1.7% (3/176) of major error.

Among screening tests evaluated, agar screening with cefoxitin 4 μg/mL proved to be the best predictor for methicillin resistance, more sensitive than that oxacillin 6 μg/mL. Caierão *et al*,[8] showed that screening with oxacillin 4 μg/mL presented 100% accuracy among *S.*

epidermidis, *S. haemolyticus* and *S. hominis*. In our study, however, two *mecA*$^{-}$ samples, *S. epidermidis* and *S. capitis* subsp. *urealyticum*, were characterized as resistant (1.13% of major error). Screening with cefoxitin 4 μg/mL plus 2% NaCl presented excellent sensitivity (100%), but results mischaracterized, 3% (5/167) for *S. aureus* and 1.13% (2/176) for CoNS were verified. The addition of salt to the culture medium is recommended for the determination of oxacillin MIC. For cefoxitin, however, our results showed that there was no improvement in the performance of the method. Also, the presence of salt in the screening method using cefoxitin seems to have conditioned an increase in the MIC values, favoring a misinterpretation of false resistance.

Finally, our results show that cefoxitin may be used as a surrogate marker for predict resistance due *mecA* gene, although oxacillin be show great correlation with *mecA*-positive genotype, particularly in *S. aureus*. Between the most sensitive methods, agar screening with cefoxitin 4 μg/mL show performance equal or superior to the others screening methods applied, without the need of supplementing salt and the same antimicrobial concentration can be used for *S. aureus* and CoNS. Besides, it may be facilely applied in the routine clinical laboratories.

ACKNOWLEDGMENTS

The authors are grateful to the bacteriology laboratories of each hospital for their collaboration and to Cícero Armídio Gomes Dias for his review of the paper. This work was supported in part by CAPES, CNPq and FFFCMPA.

COMPETING INTEREST STATEMENT: None to declare.

REFERENCES

1. Chambers HF. The changing epidemiology of *Staphylococcus aureus*? *Emerg Infect Dis* 2001;**7**: 178-82.

2. Foster TJ. The *Staphylococcus aureus* "superbug". *J Clin Invest* 2004;**114**: 1693-1696.

3. Marshall SA, Wilk WW, Pfaller MA *et al*. *Staphylococcus aureus* and coagulase-negative staphylococci from blood stream infections: frequency of occurrence, antimicrobial susceptibility and molecular (*mecA*) characterization of oxacillin resistance in the SCOPE Program. *Diagn Microbiol Infect Dis* 1998;**30**: 205-214.

4. Lowy FD. Antimicrobial resistance: the example of *Staphylococccus aureus*. *J Clin Invest* 2003;**111**: 1265-1273.

5. Sader HS, Jones RN, Gales AC *et al*. SENTRY Antimicrobial Surveillance Program Report: Latin American and Brazilian Results for 1997 through 2001. *Braz J Infect Dis* 2004;**8**: 25-79.

6. Clinical and Laboratory Standards Institute (CSLI)/NCCLS. *Performance Standards for Antimicrobial Susceptibility Testing – Fifteenth Informational Supplement M100-S15*. (CSLI)/NCCLS, Wayne, PA, USA, 2005.

7. Bannerman TL. *Staphylococcus*, *Micrococcus*, and other catalase-positive cocci that grow aerobically. In PR Murray, EJ Baron, JH Jorgensen, MA Pfaller, RH Yolken (eds), *Manual of Clinical Microbiology*, American Society Microbiology, Washington, 2003;384-404.

8. Caierão J, Musskopf M, Superti S *et al*. Evaluation of phenotypic methods for methicillin resistance characterization in coagulase-negative staphylococci (CNS). *J Med Microbiol* 2004; **53**:1195-1199.

9. Bignardi GE, Woodford N, Chapman A *et al*. Detection of the *mecA* gene and phenotypic detection of resistance in *Staphylococcus aureus* isolates with borderline or low-level methicillin resistance. *J Antimicrob Chemother* 1996;**37**: 53-63.

10. McKinney TK, Sharma VK, Craig WA *et al*. Transcription of the gene mediating methicillin resistance in *Staphylococcus aureus* (*mecA*) is corepressed but not coinduced by cognate *mecA* and β-lactamase regulators. *J Bacteriol* 2001;**183**: 6862-6868.

11. Berger-Bächi B, Rohrer S. Factors influencing methicillin resistance in staphylococci. *Arch Microbiol* 2002;**178**: 165-171.

12. Pottumarthy S, Fritsche TR, Jones RN. Evaluation of alternative disk diffusion methods for detecting *mecA*-mediated oxacillin resistance in an international collection of staphylococci: Validation report from the SENTRY Antimicrobial Surveillance Program. *Diagn Microbiol Infect Dis* 2005;**51**: 57-62.

13. Felten A, Grandry B, Lagrange PH *et al*. Evaluation of three techniques for detection of low-level methicillin-resistant *Staphylococcus aureus* (MRSA) a disk diffusion method with cefoxitin and moxalactam, the Vitek 2 system, and the MRSA-screen latex agglutination test. *J Clin Microbiol* 2002;**40**: 2766-71.

14. Fernandes CJ, Fernandes LA, Collignon P. Cefoxitin resistance as a surrogate marker for the detection of methicillin-resistant *Staphylococcus aureus*. *J Antimicrob Chemother* 2005;**55**: 506-510.

15. Sharp SE, Warren JA, Jr, RBT. Cefoxitin disk diffusion screen for confirmation of oxacillin-resistant Staphylococcus aureus isolates and utility in the clinical laboratory. *Diagn Microbiol Infect Dis* 2005;**51**: 69-71.

16. Velasco D, Tomas MM, Cartelle M *et al*. Evaluation of different methods for detecting methicillin (oxacillin) resistance in *Staphylococcus aureus*. *J Antimicrob Chemother* 2005;**55**: 379-382.

17. Cauwelier B, Gordts B, Descheemaecker P *et al*. Evaluation of a disk diffusion method with cefoxitin (30μg) for detection of methicillin-resistant *Staphylococcus aureus*. *Eur J Clin Microbiol Infect Dis* 2004;**23**: 389-392.

18. Skov R, Smyth R, Clausen M *et al*. Evaluation of a cefoxitin 30μg disc on Iso-Sensitest agar for detection of methicillin-resistant *Staphylococcus aureus*. *J Antimicrob Chemother* 2003;**52**: 204-207.

19. Fuda C, Suvorov M, Vakulenko SB *et al*. The basis for resistance to β-lactam antibiotics by penicillin-binding protein 2a of methicillin-resistant *Staphylococcus aureus*. *J Biol Chem* 2004;**39**: 40802-40806.

20. Chambers HF. Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications. *Clin Microbiol Rev* 1997;**10**: 781-91.

Table 1. Occurrence of the *mecA* gene among staphylococci isolates

Species	No. (%)	*mecA*	
		positive	negative
S. aureus	167 (48.7)	69 (41.3)	98 (58.7)
S. epidermidis	120 (35.0)	100 (83.3)	20 (16.7)
S. haemolyticus	22 (6.4)	19 (86.3)	3 (13.6)
S. capitis subsp. *urealyticum*	12 (3.5)	5 (41.6)	7 (58.4)
Other CoNS*	22 (6.4)	14 (4.1)*	8 (2.33)
Total	343 (100)	207 (60.3)	136 (39.7)

*Species (nº/n° *mecA* positive): *S. hominis* (6/6), *S. lugdunensis* (5/2), *S. sciuri* (3/2), *S. simulans* (2/1), *S. warneri* (2/1), *S. saprophyticus* (1/0), *S. auricularis* (1/1), *S. cohnni* (1/1), *S. capitis* subsp. *capitis* (1/0).

Table 2. Cefoxitin and oxacillin MIC for methicillin-resistant (*mecA*$^+$) and methicillin-susceptible (*mecA*$^-$) *S. aureus* and CoNS

Organisms (No.)	No. of Isolates with MICs (μg/mL) for cefoxitin/oxacillin											
	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
S. aureus mecA$^+$ (69)						6/7	7/2	3/1	3/22	34/19	16/18	
S. aureus mecA$^-$ (98)	0/15	1/12	23/36	68/34	5/1	1/0						
CoNS *mecA*$^+$ (138)						24/31	42/15	14/6	8/26	19/15	24/25	7/20
CoNS *mecA*$^-$ (38)	0/28	2/5	16/0	15/1	4/2	0/2	1/0					

Table 3. Sensitivity and specificity of variations of approved agar screen tests for detection of methicillin resistance using the presence of the *mecA* gene as the gold standard

Species*	Cefoxitin disk diffusion 30 µg		Oxacillin disk diffusion 1 µg		Agar screening oxacillin 6µg/mL + 4% NaCl	
	Sens†	**Spec†**	**Sens†**	**Spec†**	**Sens†**	**Spec†**
S. aureus	98.5	100	100	100	98.5	100
CoNS	100	100	100	92.1	NA‡	NA‡
Total	99.5	100	100	97.8	98.5	100

Species*	Agar screening oxacillin 4µg/mL + 4% NaCl		Agar screening cefoxitin 4µg/mL		Agar screening cefoxitin 4µg/mL + 2% NaCl	
	Sens†	**Spec†**	**Sens†**	**Spec†**	**Sens†**	**Spec†**
S. aureus	NA‡	NA‡	100	99	100	94.9
CoNS	100	94.7	100	97.4	100	94.7
Total	100	94.7	100	98.5	100	94.8

*Number of isolates and *mecA* status are given in Table 1

†Sensitivity (Sens), the percentage of *mecA*-positive strains correctly categorized; specificity (Spec), the percentage of *mecA*-negative strains correctly categorized.

‡Not applied (NA), tests no were applied for this species determined.

Table 4. Species that showed a discrepant result between some phenotypic method and PCR

Sample nº	Species	PCR	Phenotypic methods discrepants
		mecA	
73	*S. saprophyticus*	negative	Oxacillin disk diffusion
86	*S. epidermidis*	negative	Oxacillin disk diffusion
138	*S. warneri*	negative	Oxacillin disk diffusion
150	*S. aureus*	positive	Cefoxitin disk diffusion
120	*S. aureus*	positive	Agar screening oxacillin 6µg/mL
143	*S. aureus*	negative	Agar screening cefoxitin 4µg/mL with and without salt
176	*S. capitis* subsp. *urealyticum*	negative	Agar screening oxacillin 4µg/mL
179	*S. epidermidis*	negative	Agar screening cefoxitin 4µg/mL with and without salt oxacillin screen agar 4µg/mL

Which are the best disc diffusion and MIC breakpoints to predict the presence of the *mecA* gene in CoNS?

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Keywords: coagulase-negative staphylococci, *mecA*, cefoxitin

The methicillin resistance in coagulase-negative staphylococci (MR-CoNS) is due the expression of an altered protein, called penicillin-binding protein 2a (PBP 2a), encoded by the *mecA* gene, with low binding affinities to virtually all beta-lactam agents.[1] The heterogeneous resistance for these antimicrobials is a problem frequently sees in the routine laboratories clinical.

Cefoxitin, a cephamycin, has been used for characterization of the methicillin resistance in staphylococci species, with particular success mainly for *Staphylococcus aureus* isolates. Among CoNS species, controversial results have been obtained, in part due the difficulties of individual characterization of the species and borderline-resistant isolates.[2]

We evaluated cefoxitin in order to predict the presence of the genetic determinant of the methicillin resistance, the *mecA* gene, through the disc diffusion and agar dilution methods. The disc diffusion test was realized in accordance to CLSI guidelines,[3] using cefoxitin 30µg disc (Oxoid, Hampshire, UK) and Mueller-Hinton agar (Difco Laborartories, Detroit, USA). The minimum inhibitory concentration (MIC) was determined by agar dilution test using a range of 0.25-256 mg/L of cefoxitin. The plates were inoculated using Steer's replicator, incubated at 35ºC and screened after 24 h incubation. The growth of more than one colony on the plates was utilized as criterion for establishment of the MIC values.

We evaluated samples consecutive of a collection obtained between August to December 2004 from blood cultures of inside patients of the hospitals of the Porto Alegre city, Brazil. A total of 176 CoNS isolates were evaluated, included 120 *Staphylococcus epidermidis* (68.2%), 23 *Staphylococcus haemolyticus* (13%), 12 *Staphylococcus capitis* (7%), 6 *Staphylococcus hominis* (3.4%), 5 *Staphylococcus lugdunensis* (2.8%) and 10 others CoNS (5.6%). The presence of the *mecA* gene was detected in 78.4% (138/176) of this isolates.

In the CLSI 2005 guidelines for testing CoNS except *S. lugdunensis* with cefoxitin 30µg disc recommend that isolates exhibiting halo diameter of ≤24 and ≥25 mm are classified as resistant (*mecA*-positive) and susceptible (*mecA*-negative), respectively. In study by Swenson *et al,*[2] sensibility and specificity of 99 and 96%, respectively, were obtained for cefoxitin disc among

CoNS isolates. Frigatto *et al*[4] showed that five *S. epidermidis mecA*-positive strains were incorrectly characterized using cefoxitin 30μg disc, but cefoxitin MIC wasn't evaluated. In our study, cefoxitin 30μg disc, using CLSI guidelines (≤24 mm/≥25 mm for S/R among CoNS except *S. lugdunensis*), was 100% accurate for characterization of the resistance due *mecA* gene among *S. epidermidis* specie. Besides, 100% of accuracy was obtained for characterization of methicillin-susceptible isolates, where 26 mm was the minor halo diameter obtained. For isolates *mecA*-positive, o major halo diameter obtained was of 24 mm. For *S. lugdunensis*, that had breakpoints equal to *S. aureus* (≤19 mm/≥20 mm for R/S), the five strains were correctly classified.

Of 38 MS-CoNS (*mecA*-negative) isolates, 5.26% had cefoxitin MICs of 0.5 mg/L , 42.1% had cefoxitin MICs of 1 mg/L, 39.5% had cefoxitin MICs of 2 mg/L, 10.5% had cefoxitin MICs of 4 mg/L and 2.6% (only 1 isolate) had cefoxitin MIC of 16 mg/L. With a cefoxitin MIC proposed of ≤4 mg/L for characterization of the *mecA* gene presence, 97.4 % (37/38) are correctly classified as susceptible and only one isolate, *S. epidermidis mecA*-negative, was mischaracterized as resistant (Fig 1). Fernandes *et al* showed that cefoxitin MIC of ≤4 mg/L also can be used for clearly distinguished methicillin-susceptible *S. aureus* isolates.[5] On the other hand, of 138 MR-CoNS (*mecA*-positive) isolates, 17.4% had cefoxitin MIC of 8 mg/L, 30.4% had cefoxitin MIC of 16 mg/L, 10.1% had cefoxitin MIC of 32 mg/L, 5.8% had cefoxitin MIC of 64 mg/L and 13.7%, 17.4% and 5.1% had cefoxitin MIC of 128, 256 and >256 mg/L, respectively. With a cefoxitin MIC of ≥8 mg/L, all MR-CoNS isolates are correctly classified as resistant (Fig 1).

We determined the correlation between halo diameter cefoxitin 30μg disc and cefoxitin MIC by coefficient of Pearson's correlation. The results obtained show a strong correlation (r=0.71) between diameter halo and MICs values for cefoxitin among CoNS species (except *S. lugdunensis*), showed that the application of these two phenotypic tests has strong association when using cefoxitin as surrogate market for *mecA* gene.

Finally, we proposed MIC breakpoints for cefoxitin of ≤4 mg/L and ≥8 mg/L for characterization of MS-CoNS (*mecA*-negative) and MR-CoNS (*mecA*-positive) isolates. With these

cut-offs, sensibility and specificity are of 100% and 97.4%, respectively. Besides, we agree with breakpoints by CLSI for testing CoNS species with cefoxitin disc.

Transparency declarations: None to declare

REFERENCES

1. **Kuwara-Arai K, Kondo N, Hori S et al.** Suppression of methicillin resistance in a *mecA*-containing pre-methicillin-resistant Staphylococcus aureus strain is caused by the *mecI*-mediated repression of PBP 2' production. *Antimicrob Agents Chemother* 1996; **40**:2680-2685.

2. **Swenson JM, Tenover FC and the Cefoxitin Disk Study Group.** Results a disk diffusion testing with cefoxitin correlate with presence of *mecA* in *Staphylococcus* spp. *J Clin Microbiol* 2005; **43**:3818-3823.

3. **Clinical and Laboratory Standard Institute.** Performance standards for antimicrobial susceptibility testing. Fifteenth informational supplement M100-S15. 2005. CLSI, Wayne, PA.

4. **Frigatto EAM, Machado AMO, Pignatari ACC et al.** Is the Cefoxitin Disk Test Reliable Enough To Detect Oxacillin Resistance in Coagulase-Negative Staphylococci? *J. Clin. Microbiol* 2005; **43**:2028-2029.

5. **Fernandes CJ, Fernandes LA and Collignon P on behalf of the Australian Group on Antimicrobial Resistance.** Cefoxitin resistance as a surrogate marker for the detection of methicillin-resistant *Staphylococcus aureus*. *J Antimicrob Chemother* 2005; **55**:506-510.

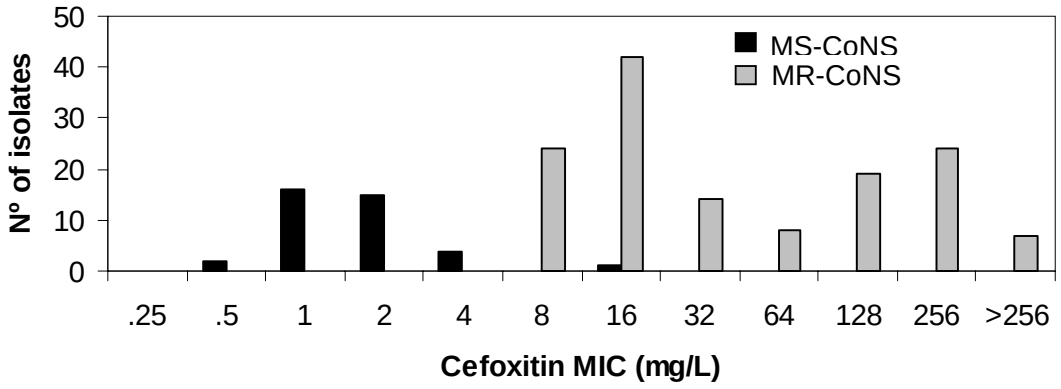

Figure 1. Frequency distribution of cefoxitin MICs in CoNS strains.

CONCLUSÕES

Após a realização deste estudo, nossos resultados permitem concluir que:

♦ As taxas de resistência à meticilina entre as amostras analizadas, 41,3% e 78,4% para *S. aureus* e SCoN, respectivamente, são semelhantes as taxas de resistência descritas na literatura, tanto no Brasil quanto na Europa.

♦ Embora nosso estudo possa ter incluído apenas amostras representantes de um clone endêmico de MRSA presente no Brasil, o teste de difusão a partir do disco de oxacilina 1μg mostrou-se adequado para correta caracterização desta resistência entre os isolados de *S. aureus*, a despeito das recentes recomendações do CSLI para utilização do disco de cefoxitina 30 μg.

♦ O teste de difusão em disco com cefoxitina 30 μg mostrou-se uma técnica adequada para caracterizar resistência à meticilina entre isolados de SCoN e também *S. aureus*, apesar de uma amostra de *S. aureus* deste estudo (amostra 150) ter sido erroneamente caracterizada como susceptível.

♦ Entre os métodos de triagem realizados, a triagem em agar contendo cefoxitina 4 μg/mL sem a suplementação de sal mostrou performance igual ou superior aos demais métodos de

triagem para a detecção de amostras de *Staphylococcus* meticilina-resistentes, podendo ser considerada para a aplicação na rotina laboratorial.

♦ Para isolados de SCoN, nossos resultados indicam que os "breakpoints" de ≤ 4 μg/mL e de ≥ 8 μg/mL de cefoxitina são os melhores para caracterizar isolados sensíveis e resistentes à meticilina, respectivamente (100% de sensibilidade e 97,4% de especificidade).

ANTIMICROBIALS USED AS ANIMAL GROWTH PROMOTERS AND THEIR ROLE IN THE DEVELOPMENT OF BACTERIAL RESISTANCE

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ABSTRACT

It is indisputable that inadequate use of antimicrobials leads to selection of resistant microorganisms. This happens whether antimicrobials are used to treat infections in humans, animals or plants or whether as prophylactics or promoters of animal growth. The extent to which the use of antimicrobials in animals contributes to the emergence of resistant microorganisms remains a controversial issue. However, the selection of resistant bacteria in animal foods by growth promoter antimicrobials (GPAs) and subsequent dissemination among farm animals (and/or other rustic environments) are major factors in the propagation of bacterial resistance in the animal reservoir. The ratio of dissemination of bacterial resistance from animals to the environment and, more importantly, in the food production chain are key determinants for dissemination to human beings.

Keywords: growth promoters; antimicrobials; bacterial resistance.

1. INTRODUCTION

In order to obtain high productivity coupled with quality of the products of animal origin, food additives such as antimicrobials are used to promote rapid animal growth [1]. The indiscriminate use of antimicrobials in animal feeding since the early 1950s may have resulted in the selection of resistant bacterial populations, determining an imbalance in the symbiosis between the desirable microbiota and the animal [1]. In addition, all antimicrobials used for prophylaxis of animals are administered directly in their food or drinking water, and all antimicrobials used to promote growth are administered as food additives. The concentration of antimicrobials in foods for prophylaxis and therapy are usually greater than the concentration for growth promotion [1, 2, 3]. Antimicrobials used in human or animal therapy cannot be used simultaneously as growth promoters. The necessary control for this, however, does not include an opportune withdrawal of growth promoter antimicrobials (GPAs), especially members of a few classes of antimicrobials that were recently brought into human use [4]. When glycopeptides and streptogramins which are naturally used as growth promoters (avoparcin and virginiamycin, respectively) became major drugs – as well as vancomycin and quinopristin-dalfopristin – for treatment of nosocomial infections by multiresistant microorganisms, this at first had no impact on their use as GPAs [5].

The first warning sign came in 1994, when it was recognized that glycopeptide-resistant enterococci (GRE) could be isolated from animal food in Great Britain and, subsequently, in Germany and Denmark [6]. It was suggested that animal foods served as a reservoir for the infections by GRE that were rapidly disseminating in European and American hospitals, causing considerable alarm as they are resistant to all commonly used drugs and, thus, virtually intractable. Subsequent investigations into isolated enterococci derived from animal faeces and foods of animal origin, carried out in different continents and in many countries, have confirmed the strict association between the use of antimicrobials as growth promoters and high levels of resistance to important antimicrobials drugs, primarily in enterococci. This association has been much studied in the avoparcin-GRE pair [6, 7] but it has also been demonstrated for GPAs of other classes, notably macrolides (tylosin and spiramycin) [6], evernimicins (avilamicin) [6], streptogramins (virginiamycin) [6, 7] and bacitracin [6].

The aim of this study is to review the indiscriminate use of antimicrobial agents as promoters of animal growth, to describe the main agents of the different classes used for this purpose, and to determine which mechanisms of resistance are involved and their potential consequences.

2. ANTIMICROBIALS USED AS ANIMAL GROWTH PROMOTERS

2.1 Avoparcin

2.1.1 The antimicrobial

This is a glycopeptide antimicrobial produced by *Streptomyces candidus*, active against Gram-positive bacteria and not is used in human medical care [8]. The glycopeptides exert bactericidal activity against sensitive microorganisms by inhibiting the cell wall synthesis by acting as competitive antagonists in the polymerization of the peptidoglycan chain that forms this wall [8]. In reproducing microorganisms, which are forming new cell walls, the drugs of this group interrupt the polymerization process (or transglycolysation) in the disaccharide-peptide subunits, which will compose the peptidoglycan in the new wall, by binding and forming complexes with these subunits [8]. Avoparcin also attaches to the cytoplasmic membrane of sensitive bacteria, altering it selective permeability.

2.1.2 Genes and Resistance Mechanisms

The mechanism of action consists in the inhibition of the cell wall synthesis by binding to the peptidoglycan precursor and inhibiting transglycolysation by modification of the glycopeptide target [9]. Studies have demonstrated that there is cross-resistance between avoparcin and vancomycin in enterococci of animal origin, and this resistance appears to be related to the *vanA* genes group which is present in strains of human origin [9]. The resistance to glycopeptides is inducible and transferable, the genes of resistance being located in the chromosome, in plasmids and transposons. The biochemical mechanism of this resistance consists in structural modifications, and enterococci with high resistance to glycopeptides were found to use altered, genetically coded precursors of the peptidoglycan, so that precursors with D-alanine-D-Lactate terminations instead of D-alanyl-D-alanine are produced. These modified precursors are thus not recognized by vancomycin and other antimicrobials of the group [10].

2.2 Spiramycin

2.2.1 The antimicrobial

It is a macrolide characterized by the presence of a lactone macrocyclic ring in its molecule, to which bind in position 5 of a disaccharide. This antimicrobial is produced by *Streptomyces ambofaciens* [11, 12, 13]. Spiramycin has three components (named as spiramycin I, II and III) which present similar antimicrobial and pharmacodynamic properties. These three substances are organic bases, spiramycin II and III being derived from the first one. Spiramycin II is a monoacetate ester and spiramycin III is a monoproprionate ester. Spiramycin I is the main component of the antimicrobial. Like the other macrolides, spiramycin is primarily bacteriostatic by acting on protein synthesis by binding to the 50S ribosomal subunit, preventing the translocation of the amino acids that form peptides [11].

2.2.2 Genes and Resistance Mechanisms

The mode of action of this macrolide of 16 members has not been clarified, although it is clear that it binds to a disaccharide in position 5' of the lactone ring [14]. The occurrence of mutations, with gain or loss of amino acids, disrupt the structure of the binding site of the drug in the ribosome, reducing the ability to interact and inhibit the bacterial ribosomal synthesis [15, 16].

Simple basis mutations in this area confer cross-resistance. Methylations of rRNA subunits by methyl transferases can confer a similar resistance mechanism [16]. Genes conferring macrolide resistance and whose products belong to the superfamily of ATP-dependent transport proteins have been found in pathogenic bacteria such as *Staphylococcus aureus*. Another mechanism of resistance described for spiramycin is the efflux of the antimicrobial from the inside of the bacterial wall by a system of active transport pump [16]. Among the resistance determinants, the *srmB* gene is supposed to be involved in antibiotic export [16].

2.3 Tylosin

2.3.1 The Antimicrobial

Tylosin is an antimicrobial used as a prophylactic agent, GPA added to animal feeding, as well as in the treatment of diseases [17]. Tylosin has 16 members attached to the lactone ring with an additional disaccharide ring of mycarose in position 5, which provides tylosin with the ability to inhibit the reaction of peptyl transferase and of protein synthesis. Tylosin has an action spectrum that is similar to that of spiramycin [13, 16].

2.3.2 Genes and Resistance Mechanisms

Resistance to macrolides can be develop in bacteria by several mechanisms. One of the main mechanisms is efflux. This mechanism is encoded by determinant *msrA* (macrolide streptogramin type B resistance) gene [14]. Another mechanism of resistance is due to modifications in subunit 23S of rRNA through adenine-N-6-methyltransferase, which prevent macrolides attachment by methylation [17, 18]. This methylation leads to a conformational change in the ribosome which results in cross-resistance to different macrolides. This is the most frequent mechanism of resistance [14]. In general, those genes that code for these methylases are called *erm* (erythromycin ribosome methylation), with phenotype of macrolide-lincosamine-estreptogramin (MLS) resistance that can be expressed in constitutive or inducible form [15].

2.4. Bacitracin

2.4.1. The antimicrobial

Bacitracin belongs to the group of cyclic polypeptides and is produced by *Bacillus licheniformis*. It acts by interfering with the synthesis of peptidoglycans, resulting in the inhibition of synthesis of the bacterial cell wall. It is active especially against Gram-positive bacteria, its action spectrum being similar to that of penicillins [19].

2.4.2. Genes and Resistance Mechanisms

Mechanisms of resistance to bacitracin have been described in Gram-negative bacteria, and only very recently in Gram-positive ones. In *Escherichia coli* the *bacA* gene was identified that codified for a protein that enhances the activity of isoprenol kinase, suggesting that this gene, which has a chromosomal location, confers resistance by a phosphorylation mechanism. Gene homologue of *bac A* have been found in *S. aureus* and *S. pneumoniae*. It is yet unclear the extent to these genes are involved in acquired resistance, since they are present in a great variety of bacterial species, many of the susceptible to the bacitracin. Actually, in Gram-positive bacteria, the mechanism of resistance must be clarified [19]. Bcr proteins are components of an ATP

transporting system. A new gene of bacitracin resistance, *bcr C,* coding for a homologue of the resistance gene of *Bacillus licheniformis*, was recently described in *E. coli* [20].

2.5. Virginiamycin

2.5.1. The antimicrobial

Virginiamycin belongs to the group of streptogramins. They are part of the MLS group of antimicrobials [13]. So far only three streptogramins have been marketed as therapeutic drugs in humans or GPAs: virginiamycin, pristinamicin, and quinopristin-dalfopristin. Virginiamycin is produced by *Streptomyces virginiae* [19]. It acts by binding to bacterial 50S ribosomal subunit to form an active complex able to inhibit protein synthesis and cause the death of the bacterial cell. The antimicrobials of the group of streptogramins have a broad spectrum of activity, acting against such Gram-positive bacteria as staphylococci, streptococci, and enterococci as well as against some Gram-negative cocci. However, most Gram-negative bacteria are naturally resistant to these antimicrobials due to the impermeability of their cell walls [20].

2.5.2 Genes and Resistance mechanisms

Resistance to streptogramins can occur through three different mechanisms: inactivation of the antimicrobial by the bacterial cell, efflux mechanism, or by alteration in binding target of the drug [10]. The alteration in binding target of the drug is mediated by gene *erm*, affecting the binding of streptogramin to the bacterial ribosome [15]. Another possible mechanism in enterococci can be mediated by an acetyl transferase which inactivates streptogramin, an enzyme that is coded for by the *vat* gene *(D)* [15]. The last mechanism of resistance to streptogramins, active efflux, is coded for by the *vga A* or *vga B* genes in staphylococci. Another gene may be involved, is *mrs A*, that code for an active transport of streptogramin in staphylococci [15]. Assessing the resistance of enterococci to virginiamycin through phenotypic methods is quite complicated, since interpretative criteria have not been established so far.

3. DISCUSSION

Suspicion on the security of the use of antibiotics in the form of growth promoter exist since its discovery. The main doubt inhabits if the antibiotic use in the diets of the animals contributes for the resistance of bacteria enteric, capable to transfer its resistance to pathogenic bacteria, causing risk the public health. The use of a given antimicrobial as animal growth promoter can lead to an increase in bacterial resistance by selective pressure, including cross-resistance to other classes of antimicrobial agents [9]. Although the association of resistance with its transference to the human population is not clear, the intake of animal derivatives may be a potential transmission route of resistant bacteria. In fact, tylosin and spiramycin confer cross resistance to the macrolide erythromycin, which is an important antimicrobial drug for humans. In 1969, the Swann [21] committee recommended that tylosin should not be available as a growth promoter. With a consequence of the widespread use of spiramycin and tylosin for growth promotion as well as for treatment of animal diseases, macrolide resistance is prevalent in *Campylobacter* spp, which are important zoonotic bacteria transferred from animals to humans through the food chain. Microbiologic and clinical evidence accumulated so far suggest the existence of an association between the use of antimicrobials in animal production and the increase in the prevalence of resistant bacteria [9]. Virginiamycin confers cross resistance to streptogramins used in human medicine [22]. This was the background behind the ban the virginiamycin as a growth promoter in Denmark from January 1998. Bacteria of simple or multiple resistance, such as salmonelas, that

they are pathogenic for human beings can be transferred of animals or animal products to the man. Despite the different opinions and abovementioned scientific evidences, some authors mention cases of crossed resistance. Thus, Pedersen [23] points the crossed resistance of the tilosyn and the espiramycin in relation the estreptogramin and of the avilamycin in relation the evernomycin, antibiotics of the line human being.

The transmission of bacterial resistance arising from animal feeding to man leads to the formation of human carriers, which come to harbor this bacterial resistance. This has been clearly documented for GRE. Several studies have demonstrated a high proportion of resistant bacteria in human health, both in the community and in hospital settings [3]. Similarly, high levels of resistance have not been shown when antimicrobials agents are not used as animal growth promoters [9]. Studies by Willems et al. [24] have showed that a substantial proportion of GRE isolates from hospitals (26-86%) are indistinguishable to the isolates obtained from foods of animal origin by molecular typing. Although the ratio of non-homogeneous – and thus controversial – resistance among animal residues is still obscure, it can provide strong additional support to the hypothesis that GREs are transmitted primarily from animals to man [1]. The reduction (or non-selection) of resistance has been repeatedly shown in studies of enterococci in animal feeding. These studies show a reduced ratio of enterococci with resistance to glycopeptides, quinopristin-dalfopristin, macrolides, and evernimicin after the banish of use of avoparcin, virginiamycin, tylosin, spiramycin, and bacitracin [9]. Although the restriction and/or ban of the use of GPA has resulted in marked reductions in the occurrence of bacterial resistance in animal food, these reductions have not amounted to a complete disappearance of these strains. Several investigations have shown that, although the likelihood of fortuitous residues of GPA resistant enterococci in animals or in food products was reduced, resistant strains are silently present in the environment, animals foods, and food products at low levels [5, 8]. In this case, by removing selective pressure we will have reduced the number of viable resistant bacteria, which can be transfer from animals to humans by the feeding route, but the resistance seeds remain firmly planted in the agricultural environment for many years. On the other hand, if we readmit the use of these antimicrobials for promotion of animal growth, the resistant strains can acquire even higher levels of resistance [25]. In Brazil, does not have studies on the consequence of the antimicrobials used as animal growth promoters. The prohibition of many growth promoters in european, has alterated the routine of country of exportation, as Brazil, by increase in cost price of the products of animal source.

In conclusion, the use of antimicrobials in animal feeding to promote growth is a serious problem of public health, especially in the cases where some classes of antimicrobials are also used in humans. The use of GPAs creates a greater reservoir of bacterial resistance in animal food, with potential dissemination to man by the feeding route or by animal contact itself.

4. REFERENCES

[1] Bates J., Jordens J. Z., Griffiths D. T. Farm animals as a putative reservoir for vancomycin-resistant enterococcal infection in man. *Journal of Antimicrobial Chemotherapy* 1994, 34: 507-514.

[2] Aarestrup F. M. Occurrence of glycopeptide resistance among *Enterococcus faecium* isolates from conventional and ecological poultry farms. *Microbial Drug Resistance* 1995, 1: 255-257.

[3] Klare I., Heier H., Claus H., Bohme G., Marin S., Seltmann G., Hakenbeck R., Antanassova V., Witte W. *Enterococcus faecium* strains with vanA-mediated high-level glycopeptide resistance isolated from animal foodstuffs and fecal samples of humans in the community. *Microbial Drug Resistance* 1995, 1: 265-272.

[4] Sengelov G., Agerso Y., Halling-Sorensen B., Baloda S. B., Andersen J. S., Jensen L. B. Bacterial antibiotic resistance levels in Danish farmland as a result of treatment with pig manure slurry. *Environment International* 2003, 28: 587-595.

[5] Middelbo H: Weaning pigs without antibiotic growth promoters: strategies to improving health and performance. In Nutritional Biotechnology in the Feed and Food Industries. Edited by Lyons T. P. and Jacques K. A. Nottingham: *Nottingham University Press*. 2003, p170-184.

[6] Aarestrup F. M., Kruse H., Tast E., Hammerum A. M., Jensen L. B. Associations between the use of antimicrobial agents for growth promotion and the occurrence of resistance among *Enterococcus faecium* from broilers and pigs in Denmark, Finland and Norway. *Microbial Drug Resistance* 2000, 6:63-70.

[7] Robredo B., Singh K. V., Baquero F., Murray B. E., Torres C. Vancomycin-resistant enterococci isolated from animals and food. *International Journal of Food Microbiology* 2000, 54: 197-204.

[8] Cormican M. G., Erwin M. E., Jones R. N. Avoparcin, a glycopeptide used in animal foods: antimicrobial spectrum and potency tested against human isolates from the United States. *Diagnostic Microbiology of Infectious Diseases* 1997, 29: 241-248.

[9] Casewell M., Friis C., Marco E., McMullin P., Phillips I. The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. *Journal of Antimicrobial Chemotherapy* 2003, 52: 159-161.

[10] Tavares W. Bactérias Gram-positivas problemas: resistência do estafilococo, do enterococo e do pneumococo aos antimicrobianos. *Revista da Sociedade Brasileira de Medicina Tropical* 2000, 33 (3): 281-301.

[11] Pernodet J. L., Alegre M. T., Blondelet-Rouault M. H., Guerineau M. Resistance to spiramycin in *Streptomyces ambofaciens*, the producer organism, involves at least two different mechanisms. *Journal of Genetic Microbiology* 1993, 139: 1003-11.

[12] Pernodet J. L., Gourmelen A., Blondelet-Rouault M. H., Cundliffe E. Dispensable ribosomal resistance to spiramycin conferred by *srmA* in the spiramycin producer *Streptomyces ambofaciens*. *Microbiology* 1999, 145: 2355-2364.

[13] Tavares W. (2001). *Manual de antibióticos e quimioterápicos antiinfecciosos*. São Paulo: Editora Atheneu: 636; 674-677.

[14] Poulsen S. M., Kofoed C., Vester B. Inhibition of the ribosomal peptidyl transferase reaction by the mycarose moiety of the antibiotics Carbomycin, Spiramycin and Tylosin. *Journal of Molecular Biology* 2000, 304: 471-481.

[15] Fluit A. D. C., Visser M. and Schmitz F. Molecular Detection of Antimicrobial Resistance. *Clinical Microbiology Review* 2001, 14: 836-871.

[16] Vester B., Douthwaite S. Macrolide resistance conferred by base substituitions in 23S rRNA. *Antimicrobial Agents and Chemotherapy* 2001, 45: 1-12.

[17] Onan L., LaPara T. M. Tylosin-resistant bacteria cultivated from agricultural soil. *FEMS Microbiology Letters* 2003, 220: 15-20.

[18] Murray J. M., Baron E. J., Pfaller M. A., Tenover F. C., Yolken R. H. *Manual of Clinical Microbiology*. 7ed., Washington, D.C., American Society for Microbiology, 1999.

[19] Butaye P., Devriese L. A., Haesebrouck F. Antimicrobial Growth Promoters Used in Animal Feed: Effects of Less Known Antibiotics on Gram-Positive Bacteria. *Clinical Microbiology Reviews* 2003, 16: 175-188.

[20] Dutta G. N., Devriese L. A. Observations in the vitro sensitivity of Gram –positive intestinal bacteria of farms animals to growth promotions antibacterials. *Journal of Applied Bacteriology* 1984, 56: 117-123.

[21] Swann M. M. *Joint Committee on the use of Antibiotics in Animal Husbandry and Veterinary Medicine.* London: HMSO , 1969.

[22] Welton L. A., Thal L. A., Perri M. B., Donabedian S., McMahon J., Chow J. W., Zervos M. J. Antimicrobial resistance in enterococci isolated from turkey flocks fed virginiamycin. *Antimicrobial Agents Chemotherapy* 1998, 42: 705-708.

[23] Pedersen K. B. Some growth promoters in animals do confer antimicrobial resistance in humans. *British Medical Journal* 1999, 318: 1076.

[24] Willems R. J., Top J., van Den Braak N., van Belkum A., Endtz H., Mevius D., Stobberingh E., van Den Bogaard A., van Embden J. D. Host specificity of vancomycin-resistant *Enterococcus faecium*. *Journal of Infectious Diseases* 2000, 182: 816-823.

[25] Wegener H. C. Antibiotics in animal feed and their role in resistance development. *Current Opinion in Microbiology* 2003, 6: 439-445.

Use of the D Test Method to Detect Inducible Clindamycin Resistance in Coagulase Negative Staphylococci (CoNS)

Running title: **Inducible Clindamycin Resistance in CoNS**

Manuscript category: Research note

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Abstract

According to National Committee for Clinical Laboratory Standards (NCCLS, 2004), a method to evaluate the inducible clindamycin resistance in accordance with an approach of the disks of erythromycin and clindamycin – the D test – has been reported. We analyzed the performance of this method in two hundred coagulase negative staphylococci (CoNS) strains obtained from blood cultures of hospitalized patients at a general hospital from Southern Brazil. Twenty-seven clinical isolates with suitable profile (erythromycin-resistance and clindamycin-susceptible) were evaluated for realization of D test. Thus, only five CNS showed D test positive. The D test method show be simple and an important technic in the detection of inducible clindamycin resistance. However, it is a method of easy adaptation to the laboratorial routine.

***Key words*:** clindamycin, resistance, D test.

INTRODUÇÃO

The determination of antimicrobial susceptibility of a clinical isolate is often crucial for the optimal antimicrobial therapy of infected patients. This is particularly important considering the increasing of resistance and the emergence of multidrug-resistant microorganisms [1-3]. Several authors have screened clinical isolates of erythromycin-resistant *Staphylococcus aureus* and coagulase-negative staphylococci (CoNS) for genes encoding resistance to macrolides, lincosamides and streptogramins type B (MLSB) [4-10].

Resistance to macrolides (e.g. erythromycin) can occur by two different mechanisms: efflux due to macrolide streptogramin resistance (*msrA* gene) and ribosome alteration due to erythromycin ribosome methylase (*erm* gene) [11, 12]. Macrolide resistance due to efflux encoded by *msrA* has been more prevalent in CNS than in *S. aureus* [13].

Different mechanisms of acquired MLSB resistance have been found in gram-positive bacteria [11, 12]. The first mechanism of macrolide resistance described was due to posttranscriptional modifications of the 23S rRNA by the adenine-*N*-6-methyltransferase. Target modification alters a site in 23S rRNA common to the binding of MLSB antibiotics. Modification of the ribosomal target confers cross-resistance to MLSB antibiotics (MLSB resistant phenotype) and remains the most frequent mechanism of resistance. In general, genes encoding of these methylases are designated *erm*. Expression of MLSB resistance in staphylococci may be

constitutive (MLSBc) or inducible (MLSBi). When expression is constitutive, the strains are resistant to all MLSB type antibiotics. When expression is inducible, the strains are resistant to 14- and 15-membered macrolides only [11, 12].

For MLSBi strains, erythromycin will induce production of the methylase, which allows clindamycin resistance to be expressed. Inducible clindamycin resistance can be detected with a simple disk approximation test, commonly referred as D test [14]. For this test, an erythromycin disk is placed 15 mm to 26 mm (edge to edge) from a clindamycin disk in a standard disk diffusion test. Following incubation, a flattening of the zone in the area between the disks where both drugs have diffused indicates that the organism has inducible clindamycin resistance [14, 15, 16].

The purposes of this study was to characterize the antimicrobial susceptibility patterns (erythromycin, clindamycin and oxacillin) and to evaluate, according to D test [17], all coagulase negative staphylococci from a collection of 200 clinical isolates from blood cultures that had the correct characterization: resistance to erithromycin and susceptibility to clindamycin.

We analyzed 200 consecutives clinical isolates of CNS obtained from patients admitted in a general hospital in Porto Alegre city, in Southern Brazil, between January and June 2002. The samples were identified (only for species-level identification) through MicroScan, panel Pos-Combo 13 (Dade Behring – Deerfield, Illinois, USA). For selection criteria, the method for determining clindamycin susceptibility (disk diffusion) was perfomed separated on the plate and so it is not confused with the performance of the D test itself.

The susceptibility tests - disks for the following agents at the concentrations specified: 15 µg erythromycin, 2 µg clindamycin and 1µg oxacillin (Difco Laboratories, Detroit, Mich.) - were performed by the agar disk diffusion (Kirby-Bauer) method according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS 2004).

D test Method - For this test, the erythromycin disks were placed 15 mm and 26 mm (edge to edge) from clindamycin disks, as recommended (NCCLS, 2004) on Muller-Hinton agar plate (Oxoid – Hampshire, England), while the disks also were placed to 10 mm of distance. According to evaluation criterious of NCCLS 2004, the flattened (positive test) or not (negative test) clindamycin zone between a erythromycin and clindamycin disks was verified.

We performed the clindamycin induction test on CNS that had the following profile: test resistant or intermediate to erythromycin and susceptible to clindamycin using routine antimicrobial susceptibility test. Twenty seven CNS of our collection (n=200) had this profile. *S. aureus* ATCC 25923 were used for quality control (QC) of the clindamycin and erythromycin disks, according to

the standard disk diffusion QC procedure. The susceptibility patterns for CoNS isolates are showed in the Table 1. One handred tirty three (66.5%) of the isolates were oxacillin-resistant. The resistance and intermediate resistance was 63% and 3.5%, respectively, for erithromycin and 53.5% and 1% respectively, for clindamycin. The phenotypic pattern compatible for realization of the D test was obtained for 27 CoNS isolates (13.5% of the all). Thus, only five (18.5%) were positive for inducible clindamycin resistance. The test was more visible when the erythromycin disk was placed 15 mm or 10 mm from the clindamycin disk. Positive reactions D test were showed when test intermediate to erythromycin were included as well as test resistante to erythromycin. In total, five positive reactios were observed, three (14.3%) for resistance and two (33.4%) for intermediate resistance to erithromycin. We observed distinct species in this five CoNS isolates D test positive: two *Staphylococcus epidermidis*, two *Staphylococcus haemolyticus* and one *Staphylococcus simulans*. Both *S. epidermidis* (2) and *S. haemolyticus* (2) are carrier of the *mecA* gene – oxacillin-resistant, whereas *S. simulans* is not (data no showed). The distance between disks more suitable to detection of the induction of resistance were 15 mm (standard) and 10 mm (no standard). The Figure 1 showed the induction of the resistance in 10 mm distance.

Resistance in Gram-positive bacteria not only increases morbidity and mortality, but also the costs of management of hospitalized patients. Studies have indicated a great increase in the ratio of resistance of staphylococci to MLS group and failure in the treatment with clindamycin in infections for microorganisms with inducible resistance to MLS group [17]. Reporting clindamycin as susceptible for *Staphylococcus* spp. that test erythromycin resistant and clindamycin susceptible without checking for inducible clindamycin resistance may result in inappropriate clindamycin therapy. How caution, add comment of resistance based on detection of inducible clindamycin resistance has been proposed [18]. On the other hand, negative results for inducible clindamycin resistance, report clindamycin susceptible and to add comment that this *Staphylococcus* spp does not demonstrate inducible clindamycin resistance *in vitro* [18].

The D test is acceptable for all *Staphylococcus* spp including oxacillin susceptible or oxacillin resistant *S. aureus* or coagulase-negative staphylococci [18]. Many of the recently recognized methicillin-resistence *Staphylococcus aureus* (MRSA) that cause community-associated infections have the *msr*A gene and the oral clindamycin may be a treatment option for these patients. In this case, these *S. aureus* strains are susceptibles to clindamycin and do not present inducible resistance to this antimicrobial agent. Although the clindamycin can be effective in some patients, did not recommend it used without before to realize the D test [18].

An important fact in our study, was that we incorporate clinical isolated of CNS that presented an profile of intermediate resistance to clindamycin. In fact, two isolated that showed compatible profile with the realization of the D test (erythromycin-intermediate and clindamycin-susceptible), resulted in resistance to clindamycin and positive D test. This isolates were identified as *Staphylococcus epidermidis* and *Staphylococcus haemolyticus*. Outbreaks caused by multiresistents and offensives *S. epidermidis* and *S. haemolyticus* have been reported in various nosocomial settings, such as in individual intensive care units (ICU) or other units within a hospital [19]. Save this results, ours *Staphylococcus* spp isolates have resistance levels less than in others countries (data no publish showed in 104th General Meeting of the American Society for Microbiology, New Orleans, LA, 2004). Until now, there is a not study what report this test in brazilian clinical isolates.

This disk approximation test proved to be a good method to detect staphylococci strains with inducible clindamycin resistance. As demonstrated in the effectued analyses, the method revealed to be adequate and viable for the evaluation of this phenotype of resistance, when was used 15 mm (standard) and 10 mm (no standard) of distance between the disks. With 26 mm of distance between the disks, macrolide resistance was not detected in two isolates (*S. epidermidis* and *S. haemolyticus*). In summary, the D test method revealed to be practical in the established conditions, being able to be used in the qualitative determination (phenotyping) of the resistance in coagulase-negative staphylococci, mainly when the minor standardized distance (15 mm) between disks was used. Additional advantages include decreased managements costs of treating in resistant infections (by diagnostic confirmation), more rapidity in the results and its easy adaptation in the laboratorial routine.

The D test can be used as a tool auxiliary and alternative method to inducible clindamycin resistance detection in the routine of clinical laboratories. However, the confirmation of the *erm* gene in staphylococci strain with D test positive would assist in the standardization of the test (suitable distance between disks, sensitivity and specificity of the test). Moreover, the present study verified only 5 positive tests and a greater number would be required for validation of the interpretation of the distance between the disks.

Acknowledgements

The authors wish to thanks Maria Beatriz and Rosângela for their support, bacteriology of the Irmandade Santa Casa de Misericórdia de Porto Alegre (ISCMPA), Fundação Faculdade Federal de Ciências Médicas de Porto Alegre (FFFCMPA), Conselho Nacional de Desenvolvimento

Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brasília, Brazil.

References

1. Fluit, A. C., F.-J. Schmitz, M. E. Jones, J. Acar, R. Gupta, and J. Verhoef for the SENTRY Participants Group. (1999). Antimicrobial resistance among community-acquired pneumoniae isolates in Europe: first results from the SENTRY antimicrobial surveillance program 1997. *Int. J. Infect. Dis.* 3:153–156.

2. Fluit, A. C., M. E. Jones, F.-J. Schmitz, J. Acar, R. Gupta, and J. Verhoef for the SENTRY Participants Group. (2000a). Bacteremia in European hospitals, incidence and antimicrobial susceptibility. *Clin. Infect. Dis*. 30:454–460.

3. Fluit, A. C., M. E. Jones, F. J. Schmitz, J. Acar, R. Gupta, and J. Verhoef for the Sentry Participants Group. (2000b). Antimicrobial resistance among Urinary Tract Infection (UTI) isolates in Europe: results from the SENTRY Antimicrobial Surveillance Program 1997. *Antonie Leeuwenhoek*. 77:147–152.

4. Jenssen, W. D., S. Thakker-Varia, D. T. Dubin, and M. P. Weinstein. (1987). Prevalence of macrolides-lincosamides-streptogramin B resistance and *erm* gene classes among clinical strains of staphylococci and streptococci. *Antimicrob. Agents Chemother*. 31:883– 888.

5. Arthur, M., C. Molinas, C. Mabilat, and P. Courvalin. (1990). Detection of erythromycin resistance by the polymerase chain reaction using primers in conserved regions of *erm* rRNA methylase genes. *Antimicrob. Agents Chemother*. 34:2024–2026.

6. Eady, E. A., J. I. Ross, J. L. Tipper, C. E. Walters, J. H. Cove, and W. C. Noble. (1993). Distribution of genes encoding erythromycin ribosomal methylases and an erythromycin efflux pump in epidemiologically distinct groups of staphylococci. *J. Antimicrob. Chemother*. 31:211–217.

7. Allignet, J., and N. el Solh. (1995). Diversity among the gram-positive acetyltransferases inactivating streptogramin A and structurally related compounds and characterization of a new staphylococcal determinant, *vatB*. *Antimicrob. Agents Chemother*. 39:2027–2036.

8. Allignet, J., S. Aubert, A. Morvan, and N. el Sohl. (1996). Distribution of genes encoding resistance to streptogramin A and related compounds among staphylococci resistant to these antibiotics. *Antimicrob. Agents Chemother*. 40:2523–2528.

9. Allignet, J., N. Liassine, and N. el Sohl. (1998). Characterization of a staphylococcal plasmid related to pUB110 and carrying two novel genes, *vatC* and *vgbB*, encoding resistance to streptogramins A and B and similar antibiotics. *Antimicrob. Agents Chemother*. 42:1794–1798.

10. Jensen, L. B., N. Frimondt-Moller, and F. M. Aarestrup. (1999). Presence of *erm* gene classes in gram-positive bacteria of animal and human origin in Denmark. *FEMS Microbiol. Lett.* 170:151–158.

11. Leclercq, R., R. B. Giannattasio, H. J. Jin, and B. Weisblum. (1991). Bacterial resistance to macrolide, lincosamide and streptogramin antibiotics by target modification. *Antimicrob. Agents Chemother*. 35:1267–1272.

12. Weisblum, B. (1999). Resistance to macrolide-lincosamide-streptogramin antibiotics, p. 682–698. *In* V. A. Fischetti (ed.), Gram-positive pathogens. American Society for Microbiology, Washington, D.C.

13. Eady, E. A., J. I. Ross, J. L. Tipper, C. E. Walters, J. H. Cove, and W. C. Noble. (1993). Distribution of genes encoding erythromycin ribosomal methylases and an erythromycin efflux pump in epidemiologically distinct groups of staphylococci. *J. Antimicrob. Chemother*. 31:211–217.

14. Fiebelkorn, K. R., Crawford, S. A., McElmeel M. L., and Jorgensen, J. H. (2003). Practical disk diffusion method for detection of inducible clindamycin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci. *J Clin Microbiol*. 41:4740- 44.

15. Sanchez, M. L., K. K. Flint, and R. N. Jones. (1993). Occurrence of macrolidelincosamide-streptogramin resistances among staphylococcal clinical isolates at a university medical center. Is false susceptibility to new macrolides and clindamycin a contemporary clinical and in vitro testing problem? *Diagn Microbiol Infect Dis*. 16:205-13.

16. Fiebelkorn, K. R., Crawford, S. A., McElmeel, M. L. and Jorgensen, J. H. (2003) Practical Disk Diffusion Method for Detection of Inducible Clindamycin Resistance in *Staphylococcus aureus* and Coagulase-Negative Staphylococci. *J Clin Microbiol*, 41: 4740-4744.

17. Panagea, S., Perry, J. D., Gould, F. K. (1999). Should clindamycin be used as treatment of patients with infections caused by erythromycin-resistant staphylococci? *J Antimicrob Chemother*, 44: 581-582.

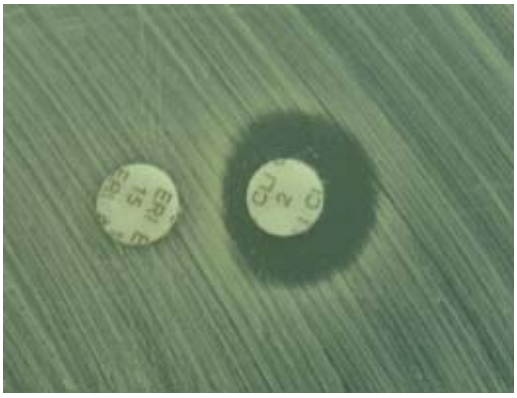
18. National Committee for Clinical Laboratory Standards - NCCLS. (2004). Performance Standards for Antimicrobial Disk Susceptibility Tests. M100-S14. Wayne, PA: National Committee for Clinical Laboratory Standards.

19. National Nosocomial Infections Surveillance (NNIS) System. (1999). National Nosocomial Infections Surveillance (NNIS) System Report, data summary from January 1990–May 1999, issued June 1999. *Am J Infect Control*, 27:520–32.

Table 1. Antimicrobial susceptibility patterns among coagulase negative staphylococci isolates

Pattern	Antimicrobials		
	Oxacillin (%)	Erithromycin (%)	Clindamycin (%)
Resistant	133 (66.5)	126 (63)	107 (53.5)
Intermediate	0 (0)	07 (3.5)	02 (1)
Susceptible	67 (33.5)	67 (33.5)	91 (45.5)
Total	200 (100)	200 (100)	200 (100)

Figure 1.



Legend of the Figure 1.

Figure 1. D test positive result for *S. epidermidis* strain (distance of 10 mm between disks. Left, eritromycin disk and right, clindamycin disk.

FINGERPRINT OF COAGULASE-NEGATIVE *STAPHYLOCOCCUS* SPP BY SENSITIVITY TO A PANEL OF DIFFERENT KILLER YEASTS

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Running head: Fingerprint of *Staphylococcus* by killer yeasts

Keywords: killer yeast; fingerprint, Coagulase negative *Staphylococcus* spp

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Abstract

A panel initially composed of 60 clinical isolates of Coagulase-negative *Staphylococcus* spp (SCN) and *Staphylococcus aureus* multi resistant (SAU) was used to evaluate the discriminatory power of three different fingerprint methods based on sensitivity towards of 11 selected killer yeast obtained from different environmental sources. The short panel of eleven killer yeasts allowed to differentiate the two species of *Staphylococcus* and to discriminate sensitive strains of Coagulase-negative *Staphylococcus* spp in 90%, 97% and 100%, using the respective Binary data matrix (BDM), Triplet data matrix (TDM) and Quantitative data matrix (QDM) fingerprint methods. The QDM presented the highest discriminatory power, insured through the Simpson's and Hunter and Gaston's indices for the measurement of diversity. Furthermore, this quantitative procedure fingerprint showed be sensitive, accurate and of methodological simplicity, allowed a routine use even in microbiological laboratories with minimal resources.

Introduction

The first use of the yeast killer system for inter- and intraspecific discrimination of microorganism was reported for *Candida albicans* isolates, proving to be an epidemiological tool of great value in identifying presumptive cases of nosocomial fungal infections (Polonelli *et al*., 1983). Later, the killer system also proved to be very valuable when applied to the differentiation of others opportunistic yeasts and molds, as *Candida glabrata, C. kefyr, C. parapsilosis, C. tropicalis, Cryptococcus neoformans, Saccharomyces cerevisiae, Pseudallescheria boydii* , *Penicilium camemberti* and *Aspergillus niger* (Fanti *et al*., 1989; Polonelli *et al*., 1987; Buzzini & Martini, 2000; Buzzini & Martini, 2001; Buzzini *et al*., 2003; Buzzini *et al*., 2004). Following initial descriptions that the killer phenomenon posses activity against others eukaryotic and also prokaryotic microorganisms led to a utilization of this feasible and reproducible method of differentiation in etiological agents of nocardiosis, as well as slow-growing bacterials, such as the mycobacteria (Morace *et al*., 1988; Morace *et al*., 1989).

Many phenotypic and genotypic methods, such as resistotyping, enzyme production, bacteriocin susceptibility, phage typing, ribotyping and DNA hybridization have been used for biotyping bacterial species (Catalano, 1994; Jarlov, 1999; Muller *et al*., 1999). The killer system, when adapted to the growth factors of the susceptible bacterial in test, may represent a simple,

sensible and reproducible tool for studying epidemiological cases in microbiological laboratories with minimal resources.

In this report, mathematical models for differentiate the two species of *Staphylococcus* and to discriminate sensitive strains of Coagulase-negative *Staphylococcus* spp through of the toxics effects of an short panel of yeasts killer strains, were used as a sensitive, accurate and simple fingerprint methods. Different data matrices were applied to describe of discriminatory power, verifying which better choice, in accordance with specific indices for the measurement of diversity.

Materials and Methods

Strains

The killer panel composed of 11 strains (Table 1), labelled with killer reference numbers in agreement with your isolation source, were selected of a previous screening of 48 killer strains obtained from phylloplane of *Hibiscus rosa-sinensis* (KYHB), phylloplane of Bromeliads (KYBI), raw milk (KYLV), raw goat milk (KYLC), cheese (KYQU) and fruits (KYF), all in *Brazilian* territory. YEPG (yeast extract 10 g/l, peptone 10 g/l, glucose 20 g/l) agar slants were used to maintain cultures at 4 °C.

Sixty gram-positive cocci human pathogens isolated from two hospitals (H1 and H2) in Porto Alegre city, Brazil, were included in the sensitive panel. Thirty classified as SCN are Coagulase-negative *Staphylococcus* spp. multi resistant and, the remaining thirty isolates labelled as SAU represent *Staphylococcus aureus* multi resistent . Cultures were maintained at –20°C in double-strength skim milk (Difco Laboratories, Detroit, MI, USA).

Killer assay

Assessment of killer activity against gram-positive cocci human pathogens was performed in Mueller Hinton Agar (Difco Laboratories, Detroit, MI, USA). Overnight grown target cells from gram-positive isolates were suspended in distilled water to a density of 0.5 Mac Farland standard, and spread on the assay Petri dishes. Inoculum of potential killer yeasts were prepared as suspensions (10^8 cells/mL) of 24h cells, imprinted onto the solidified agar surface by a multipoint inoculator or Steer's replicator, and incubated at at 37ºC / 48h. The killer activity (KA) was considered if there was an evident zone of inhibition of the pathogenic gram-positive isolate around its inoculum, and as not possessing killer activity if a clear inhibition zone was absent. There were no discrepant results in repeated experiments.

Statistical assessment of growth inhibition zone

Using a metric ruler, zone inhibition were measured in millimeter for each sensitivity strain. All these quantitative data represents the average value of three separate determinations ($P < 0.01$). Basis statistic evaluation of data was obtained by ANOVA.

The presence (KA) or absence (0) of the killer in each one of the tested strains were plotted in a first matrix of data, denominated binary data matrix (BDM). The BDM was then converted in a triplet data matrix (TDM) by grouping binary data in triplets. Conventional numerical activity codes from 1 to 8 were denominate for each triplet, combining sequence of 0 or KA arranged in all possible ways (Polonelli *et al*. 1983; Buzzini and Martini, 2004). Finally, the quantitative data matrix (QDM) was obtained by substituting halo values in four capital letter, according with the size of inhibition halo in millimeters reported in Table 1 (i.e. A: 0 - 4mm; B: 4 - 8mm; C: 8 -12mm; D: >12mm) (Buzzini *et al*., 2003; Buzzini and Martini, 2004).

Results

All coagulase-negative *Staphylococcus* spp isolates were sensitive to at least one of the killer yeasts included in the panel (Table 1), unlike *Staphylococcus aureus* strains that only three isolated presented sensitivity. Although is just suggested for the interaction with yeasts (Magliani *et al*., 1997), the diverse sensitivity for the eleven killer yeasts of the panel, may be consequence of presence or ausence of specific receptors or active sites in the cell walls of coagulase-negative *Staphylococcus* spp isolates. The short panel of eleven killer yeasts allowed to discriminate sensitive strains of Coagulase-negative *Staphylococcus* spp in 90%, 97% and 100%, using the respective Binary data matrix (BDM), Triplet data matrix (TDM) and Quantitative data matrix (QDM) fingerprint methods. Although five integral of the panel of killer yeasts showed activity against three *Staphylococcus aureus* strains, using the panel without these five yeast was possible to differentiate the two species of *Staphylococcus* whit 98% of accuracy.

Data given in Fig.1, esteeming the relationshipds between the number of killer yeasts combined (KY) and the number of discriminated strains (DS) of coagulase-negative *Staphylococcus* spp, demonstrated that the use of quantitative data matrix (QDM) possess an effective amplification of its fingerprint efficacy, when compared to the other methods of fingerprint data matrices. The discriminatory power o BDM, TDM and NDM was proved by using specific indices for the measurement of diversity: Simpson's index (λ) and Hunter and Gaston's index (D). The relantionships between λ, D and KY are reported in Fig. 2a,b.

Discussion

With this study was possible to assure the sensitivity and accuracy of three different fingerprint methods in a group of thirty coagulase-negative *Staphylococcus* spp clinical isolates selected from two hospitals, in order to determine which method would be best suited for epidemiological studies with this specie.

The discriminatory power (*D*), based on the probability that two unrelated strains sampled from the test population will be placed into different typing groups, was calculated in accordance with two indices for measurement the diversity, to avoid subjective conclusions not based in discriminatory analysis. Simpson's index (λ) is applicable to situations in which all strains can be placed into mutually exclusive groups, i.e. correlates the number types with total population in test; neverthelless Hunter and Gaston index (*D*) overcome this limitation and correlates the number of strains wich are indistinguishable in the population with your total *n* (Hunter, 1990). In agreement with these concepts of statistical literature, the most powerful fingerprint method is the one that can correctly place all *n* strains in *n* different groups (Hunter, 1990; Buzzini *et al*., 2004). As well as the results obtained for Buzzini *et al*. (2004) in 44 *Saccharomyces cerevisiae* strains the discriminatory power expressed by the BDM, TDM and QDM fingerprint methods in coagulase-negative *Staphylococcus* spp clinical isolates differed (Figure 2a, b): QDM showed values of λ and *D* higher than that exhibited by frequency of BDM or TDM, particularly when in KY increases, although the effectiveness of TDM is only 6% smaller. This small, but significant difference can be explained, as already suggested in fingerprint of yeasts, may be outcome of the different specific receptors present in the walls of sensitivity bacterial cells determining differential quantitative effectiveness. There was not any statistical correlation between the origins of the clinical isolates (hospital H1 and H2) and the sensitivity profile to the presented panel, either whit the diversity of origin of the killer yeasts.

One of the great advantages of this panel, besides your precision, is the small number of strains that is composed. This brings benefits in the easiness of your execution and also, as already told by other authors in killer systems for firngerprint methods, it reduces the problem of loss of discriminatory power (Polonelli *et al*., 1983; Buzzini and Martini; 2001; Buzzini *et al*., 2004). The use of the multipoint inoculator or Steer's replicator in this technique brought, besides precision in the inoculum, an execution speed could be easily reproduced in any laboratory.

Although the panel killer has not had 100% of specificity for the coagulase-negative *Staphylococcus* spp, on the other hand we cannot stop notifying your antagonistic profile against three clinical isolates of *Staphylococcus aureus* resistent at tetracycline, penicilin, rifampicin, chloramphenicol and sulfamethoxazole. Besides, with few exceptios, the killer yeasts studied to date lose your activity at or near human body temperature (37ºC) (Hodgson *et al*., 1995), opens news perspectives for their exploitation as topical application of concentrated purified toxin preparations in therapy against this bacterial pathogen.

As proves the statistical indices for the measurement of diversity, the phenotypic fingerprint method based in quantitative data is the most sensitive and accurate that BDM and TDM. This characteristic associated your methodological easiness and low cost may allowed a routine use even in microbiological laboratories with minimal resources to apply in techniques of molecular fingerprint.

References

Buzzini, P. and Martini, A. (2000) Discrimination between Candida albicans and others pathogenic species of the genus Candida by their differential sensitivities to toxins of a panel of killer yeasts. Journal of Clinical Microbiology, 39, 3362-3364.

Buzzini, P. and Martini, A. (2001) Differential growth inhibition as a tool to increase the discriminating power of killer toxin sensitivity in fingerprint of yeasts. FEMS Microbiology Letters 193, 31-36.

Buzzini, P. and Martini, A. (2001) Large-scale screening of selected Candida maltosa, Debaryomyces hansenii and pichia anomala killer toxin activity against pathogenic yeasts. Medical Mycology, 39, 479-482.

Buzzini, P.; Berardinelli, S.; Turchetti, B.; Cardinali, G. and Martini, A. (2003) Fingerprint of yeasts at the strains level by differential sensitivity responses to a panel of selected killer toxins. Systematic and Applied Microbiology, 26, 466-470.

Buzzini, P.; Turchetti, B. and Martini, A. (2004) Assesment of discriminatory power of three different fingerprint methods based on killer toxin sensitivity for the differentation of Saccharomyces cerevisiae strains, 96, 1194-1201.

Catalano, M. (1994) Bacterial genotyping in nosocomial infections. Medicina, 54, 596-604.

Fanti, F.; Conti, S.; Campani, L.; Morace, G.; Dettori, G. and Polonelli, L. (1989) Studies on the epidemiology of *Aspergillus fumigatus* infections in a university hospital. Eur. J. Epidemiol. 5, 8-14.

Hodgson, V.J.; Button, D.; Walker, G.M. (1995) Anti-Candida activity of a novel killer toxin from the yeast *Williopsis mrakii*. Microbiology, 141, 2003-2012.

Hunter, P. (1990) Reproducibility and indices of discriminatory power of microbial typing methods. Journal of Clinical Microbiology, 28, 1903-1905.

Jarlov, J. (1999) Phenotypic characteristics of coagulase-negative staphylococci: typing and antibiotic suscetibility. APMIS Suppl. 91, 1-42.

Magliani, W.; Conti, S.; Gerloni, M.; Bertolotti, D. and Polonelli, L. (1997) Yeast Killer Systems. Clinical Microbiology Reviews, 10, 369-400.

Morace, G.;. Archibusacci, C; Sestito, M. and Polonelli, L. (1984) Strain differentiation of pathogenic yeasts by the killer system. Mycopathologia 84, 81–85.

Morace, G.; Dettori, G.; Sanguinetti, M.; Manzara, S. and Polonelli., L. (1988) Biotyping of aerobic actinomycetes by modified killer system. Eur. J. Epidemiol. 4, 99–103.

Morace, G.; Manzara, S.; Dettori, G.; Fanti, F.; Conti, S.; Campani, L.; Polonelli, L. and Chezzi., C. (1989) Biotyping of bacterial isolates using the yeast killer system. Eur. J. Epidemiol. 5, 303–310.

Muller, F.M., Lischewski, A., Harmsen, D. and Hacker, J. (1999) Standardized molecular typing. Mycoses 42, 69-72.

Polonelli, L., C. Archibusacci, M. Sestito, and G. Morace. 1983. Killer system: a simple method for differentiating *Candida albicans* strains. J. Clin. Microbiol. 17, 774–780.

Polonelli, L.; Dettori, G.; Cattel, C. and Morace, G. (1987). Biotyping of mycelial fungus cultures by the killer system. Eur. J. Epidemiol. 3, 237–242.

Table 1. Quantitative Data Matrix (QDM) referent at differential growth inhibition halos of Coagulase-negative *Staphylococcus* spp and *Staphylococcus aureus* multi resistent

		Collection number of the 11 killer yeasts										
Source	Sensitivity strains	KYQU72	KYLC60	KYQU45	KYLC83	KYLV102	KYQU127	KYQU89	KYQU88	KYQU83	KYHB55	KYQU100
H1	SCN156	D	D	D	D	C	D	B	D	D	A	B
H1	SCN167			B			B			A		
H1	SCN131			B	B		D		A			B
H1	SCN176				B		B					
H1	SCN136				A		B					
H1	SCN165	D			B	B		C	A			A
H1	SCN120			B	B		D	B	B		B	B
H1	SCN179				D					B		
H1	SCN147	D	B	C	D	B		D	D	D	B	D
H1	SCN128				B		A	B	B			B
H1	SCN166	A		D			B	B	B	B	B	A
H1	SCN181		A	B	D		D	B	B			B
H1	SCN180				B	B	B	D				
H1	SCN138	A		B	B		B	B	B	B		B
H1	SCN173				D		D	B	B	B		B
H1	SCN132				B		D	B	B	B		

H1	SCN172			A	B		B	B	B	D	B	B
H1	SCN168	A	A		D	A	B	D	D	D		A
H2	SCN1				C		D	B	B	B		B
H2	SCN 2	B		C			D	C	B	B	A	
H2	SCN 3				D	C		D		D		B
H2	SCN 4		C		B		D		D		B	
H2	SCN 5		D	C	C		A	B	A			
H2	SCN 6	B				B	D	D	B		C	A
H2	SCN 7		D	B	B			C		B		B
H2	SCN 8				D	B	D		D	A		
H2	SCN 9	A	B			A				A		B
H2	SCN 10			B	B	D	A		B		B	
H2	SCN 11		D	A	C	D	D	D		B		B
H2	SCN 12	B			D	B			C		B	
H2	SAU152											
H2	SAU151											
H1	SAU162											
H2	SAU154											
H1	SAU163											
H1	SAU159											
H1	SAU183											
H1	SAU179											
H1	SAU157			C			B	B	C	C		

H1	SAU158					
H1	SAU161	C	B	C	C	C
H2	SAU155					
H2	SAU156					
H1	SAU159					
H1	SAU143					
H1	SAU153					
H1	SAU167					
H1	SAU164					
H1	SAU61					
H1	SAU51					
H2	SAU91					
H1	SAU160					
H1	SAU 60		B		B	B
H1	SAU 1					
H1	SAU 2					
H1	SAU 3					
H1	SAU 4					
H1	SAU 5					

A: 0 - 4mm; B: 4 - 8mm; C: 8 -12mm; D: >12mm

Figure 1. Relationship between the number of discriminated strains (DS) and the number of killer yeasts (KY).

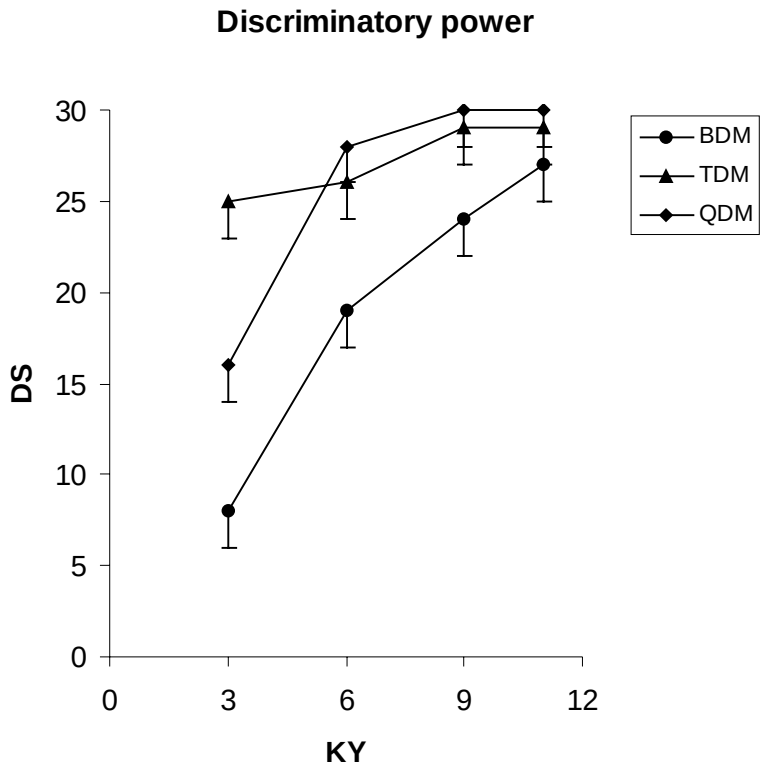
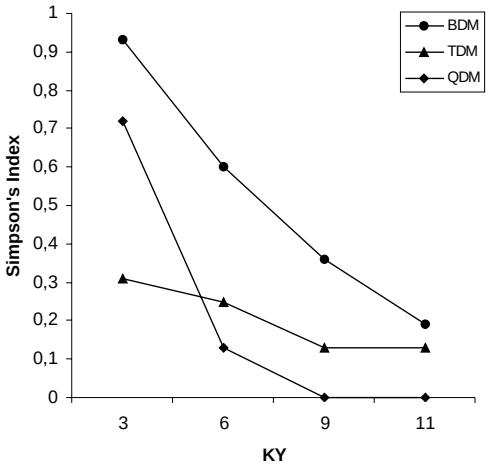
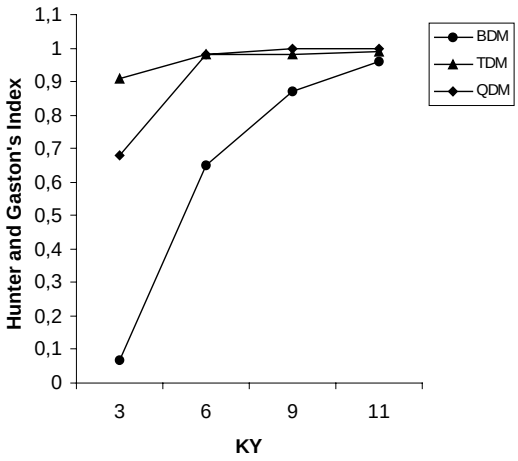


Figure 2. Variability of the indices of Simpson's index (λ) (A) and Hunter and Gaston index (*D*) (B) in relation to combination of killer yeasts (KY) in Binary data matrix (BDM), Triplet data Matrix (TDM) and Quantitative data Matrix (QDM).

A)



B)



ACTIVITY OF FLUOROQUINOLONES AGAINST UROPATHOGENICS *ENTEROCOCCUS* spp AT A GENERAL HOSPITAL IN SOUTHERN BRAZIL DURING 1996 - 2003

***Running title*: Activity of Fluoroquinolones Against *Enterococcus* spp**

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ABSTRACT

Introduction: The determination of antimicrobial susceptibility of clinical isolate is often crucial for the optimal antimicrobial therapy of infected patients. This need is essential with increasing resistance and the emergence of multi-resistant microorganisms. Moreover, nosocomially acquired urinary tract infections are common and *Enterococcus* spp is one of the most common causes of nosocomial infection. In the cases of urinary tract infections caused by *Enterococcus* spp, the fluoroquinolones can be considered agents of choice, because of their broad antimicrobial spectrum and their favourable pharmacodynamic properties. **Objective:** To investigate the *in vitro* antimicrobial susceptibility of the uropathogenics *Enterococcus* spp against fluoroquinolones commonly tested during 1996-2003. **Materials and Methods:** The susceptibility profile to fluoroquinolones was determined by disk diffusion method conform NCCLS guidelines. **Results:** The susceptibility rates was calculated for each year and showed range of 0.4-6.5% to ciprofloxacin, 0.9-10.0% to norfloxacin, 1.1-11.5% to levofloxacin and 0.0-24.0% to gatifloxacin. When intermediate resistance was added, the susceptibility rates ranging from 0.1-2.6% to ciprofloxacin, 0.8-4.0% to norfloxacin, 1.1-11.5% to levofloxacin and 1.3-24 to gatifloxacin. **Conclusion:** Of 200 isolates of *Enterococcus* spp collected and tested between 1996-2003, all showed apparent increase of the levels of resistance, although this increase no have been statistically signicant ($p<0.05$). However, the results demonstrate a rapidity in the acquisition of resistance to fluoroquinolone-class antimicrobials agents in enterococci uropathogenics strains.

Key-words: Uropathogenics *Enterococcus* spp, Fluoroquinolones, Antimicrobial Resistance.

1. Introduction

Urinary tract infection (UTI) is a common infection that usually occurs by invasion of tissues by one or several species microrganisms of that ingress the opening of the urethra and multiply in the urinary tract, inducing an inflammatory response as well as signs and symptoms whose nature and intensity vary according to the terrain. Men, women, and children develop UTIs (1).

The fluoroquinolone antimicrobials have a long history of use in the treatment of urinary tract infections UTIs. New fluoroquinolones have large in vitro activity against uropathogens (2). The fluoroquinolones has been considered agents of choice in the case of nosocomially acquired UTI and complicating factors, such as obstruction, stones, splints and catheters with some kind of biofilm infection, because of their broad antibacterial spectrum and their favourable pharmacodynamic properties. But should be used only agents with high bioavailability and mainly renal excretion. Fluoroquinolone antimicrobials exert their antibacterial effects by inhibition of certain bacterial topoisomerase enzymes, namely, DNA gyrase (bacterial topoisomerase II) and topoisomerase IV. These essential bacterial enzymes alter the topology of double-stranded DNA within the cell (3, 4).

Ciprofloxacin and norfloxacin were introduzed in 1980s and denoted advancement in the treatment of infections due to multi-resistance microorganisms, particulary in ITU. However, several studies showed an increase of resistance to fluoroquinolones (5, 6, 7). Consequently, new antimicrobial agents have been used to combat microorganisms with very high rates of resistance. Levofloxacin and gatifloxacin are new-generation of fluoroquinolone-class antimicrobials and have United States Food and Drug Administration (FDA) indications for treatment of uncomplicated and complicated UTI. Moreover, levofloxacin is the only newer fluoroquinolone indicated for the treatment of *Pseudomonas aeruginosa* and *Enterococcus faecalis* ITUs (8, 9).

In this study, we analysed the susceptibility profile by disk diffusion method to ciprofloxacin, norfloxacin, levofloxacin and gatifloxacin in *Enterococcus* spp isolates what were collected during the period 1996-2003 from UTI at a general hospital in the Porto Alegre city, Southern Brazil.

2. Material and methods

Bacterial samples. Two hundred (N=200) clinical isolates of *Enterococcus* spp recovered from patients admitted at a general hospital in Porto Alegre, Brazil, were analyzed in this study. Twenty five (n=25) clinical isolates of each year since 1996 until 2003. They were retrieved from our culture collections located at Fundação Faculdade Federal de Ciências Médicas de Porto Alegre.

Strains were tested for their phenotypic characteristics by conventional biochemical tests as recommended (10, 11, 12). Most strains were also identified by analysis of electrophoretic whole-cell protein profiles (13).

Antimicrobial susceptibility test. The antimicrobial susceptibility tests were performed by disk diffusion method according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS), 2004 (14). The *Enterococcus* spp isolates were tested for susceptibility to ciprofloxacin (5μg), norfloxacin (10μg), levofloxacin (5μg) and gatifloxacin (5μg) (BBL-Becton Dickinson Microbiology, Cockeysville, Franklin Lakes, NJ, USA).

Statistical analysis. The statistical analyses was done througt the Chi-square test (χ^2). Statistical significance was accepted when the *P*-value was <0.05.

3. Results

Among the 200 *Enterococcus* spp isolates the profile of susceptibility range was 60% to ciprofloxacin, 36% to norfloxacin, 40% to levofloxacin and 40% to gatifloxacin in the period valued. In a previou study of Southern Brazil (15), the resistance levels in enterococci species for ciprofloxacin and norfloxacin, at Hospital de Clínicas de Porto Alegre (HCPA) and Complexo Hospitalar Santa Casa de Misericórdia (CHSCM), in Porto Alegre city was 20.9% for both drugs. We only evaluate in this study the resistance levels to fluoroquinolones in the CHSCM. In this institution, the resistance levels to ciprofloxacin and norfloxacin were 30.5% (61/200) and 26.5% (53/200), respectively. According to the results of disk diffusion tests, gatifloxacin proved to be more efficient against *Enterococcus* spp clinical isolates (resistance minor). The resistance levels to levofloxacin and gatifloxacin were 23.5% (47/200) and 13% (26/200). The Table 1 showed the antibiotype of the enterococci isolates for the fluoroquinolones.

We are able in calculated the susceptibility rates to enterococci strains (Table 2). The susceptibility rates, when considered only the resistance, range was 0.4-6.5% to ciprofloxacin, 0.9-10% to norfloxacin, 1.1-11.5% to levofloxacin and 1.7-24% to gatifloxacin. When evaluate resistance and intermediate resistance, the susceptibility rates range was 0.1-2.6% to ciprofloxacin, 0.8-4% to norfloxacin, 1.1-11.5% to levofloxacin and 1.3-24% to gatifloxacin. To little variation for ciprofloxacin and gatifloxacin is due to low detection of strains with intermediate profile to fluoroquinolones.

4. Discussion

Urinary tract infections are treatable with different antibiotics, including ampicillin, amdinocillin, fosfomycin, trimethoprim and several different fluoroquinolones. Fluoroquinolones are the most widely used antibiotics for the treatment of UTIs in most countries of Western Europe and North America (16).

Between pathogenics microrganisms of the urinary tract, *Enterococcus* spp is an of the biggest caused of UTIs and has been strongly associated with urethral catheterization (17). Microrganisms can be introduced into the bladder at the time of catheterization and, depending on host resistance, may at times induce a UTI. Bladder infection, called cystitis, is by far the most common UTI. In the bladder there is a favorable environment for the bacterial reproduction because of the presence of nutrients, temperature of incubation and absence of fagocitose.

The excessive use of antimicrobial and the seletive pressure have occasionad an easy adaptation to antimicrobial agents and increase of resistance in a short space of time. Evidence here of the increase of resistance in the new-generation fluoroquinolone-class antimicrobials – levofloxacin and gatifloxacin – showing the adaptation rapidly acquired of this microorganisms. The difference, although not statistically significant, in the range of the profile of susceptibility among the enterococci strains could be attributed to acquired resistance.

Expanded-spectrum quinolones such as ciprofloxacin and norfloxacin are highly effective against gram-negative bacteria and eradicate bacteriuria in >90% of the cases of UTIs (18). Nevertheless, we see a decrease of the resistance to ciprofloxacin and norfloxacin in *Enterococcus* spp (Gram-positive cocci) by disk diffusion method (*in vitro*), with a variation of the percentage of the resistance of 44% and 40% , respectively.

The disk diffusion method in agar is poor for the detection of microbial resistance, being necessary the application of techniques that are more sensible. Moreover, many of antimicrobials used in clinical conduct have high concentration in the bladder due to retention. Besides, the minimum inibitory concentration (MIC) get reaches bigger levels than MIC serum get with the dosage normally managed of the antimicrobials.

The susceptibility profiles to antimicrobials agent, between which fluoroquinolones, shows variations that are dependents of the environment and are influenced by use antimicrobials that may to acquire resistance. Hence, the performance of a control service of infection and monitoring is essential.

In our study, the most active fluoroquinolone-class antimicrobial agent against *Enterococcus* spp was gatifloxacin in all years (1996-2003). Gatifloxacin displayed a >18-fold

increased susceptibility rates that ciprofloxacina (major increase) in 2000 and a >1.6-fold increased susceptibility rates that levofloxacin (minor increase) in 2002. When compared itself, gatifloxacin showed a >13-fold decreased of the susceptibility rates (24.0 in 1996 – major susceptibility rates – and 1.75 in 2002 – minor susceptibility rates). The profile of susceptibility should be interpreted in accordance to environment of the period, regard previous and predominant use of an specific antimicrobial, which increase the level of resistance for the antimicrobial applied whereas others agents increase his performance against the microrganisms. This is the reason because an antimicrobial agent at present increase and decrease of activity for the same microrganism. The activity of the ciprofloxacin (1999-2001) is representative of this effect.

5. Conclusion

The optimisation of the use of antimicrobial agent has emerged as one critical factor in the control of urinary tract infections in nosocomial environment since the bacterial resistance advances in major ratio of that the development of new terapeutic class agents against these uropathogenic microorganisms, mainly enterococci species. However, the disk diffusion method applied, at this occasion, is weak to evaluate the action of the antimicrobials. Our results showed an apparent increase of the levels of resistance enterococcal to fluoroquinolones by disk diffusion method. But this method is able only how screening method due his accuracy poor, being necessary the application of the confirmatory methods to evaluate the degree of the bacterial resistance. Moreover, this study used only 25 isolates of each year and a greater number would be required for an interpretation more refined.

Acknowledgements

This study was supported in part by Fundação Faculdade Federal de Ciências Médicas de Porto Alegre, CNPq and CAPES programs (Brasília – Brazil). The authors thank to Laboratório Central of the Irmandade Santa Casa de Porto Alegre, Brazil, for their help to collect the *Enterococcus* spp isolates.

Table 1. Antimicrobial activity of fluoroquinolone-class antimicrobials tested against *Enterococcus* spp collected during 1996-2003.

Year	Ciprofloxacin			Norfloxacin			Levofloxacin			Gatifloxacin		
	R	I	S	R	I	S	R	I	S	R	I	S
1996	20% (5)	8% (2)	72% (18)	20% (5)	-	80% (20)	12% (3)	-	88% (22)	4% (1)	-	96% (24)
1997	16% (4)	16% (4)	68% (17)	16% (4)	8% (2)	76% (19)	12% (3)	-	88% (22)	4% (1)	12% (3)	84% (21)
1998	8% (2)	40% (10)	52 (13)	8% (2)	12% (3)	80% (20)	8% (2)	-	92% (23)	-	8% (2)	92% (23)
1999	20% (5)	48% (12)	32% (8)	20%(5)	24% (6)	66% (14)	12% (3)	-	88% (22)	4% (1)	4% (1)	92% (23)
2000	32% (8)	56% (14)	12% (3)	20%(5)	8% (2)	72% (18)	20% (5)	-	80% (20)	12% (3)	4% (1)	84% (21)
2001	48% (12)	12% (3)	40%(10)	36% (9)	8% (2)	66% (14)	36% (9)	-	64% (16)	24% (6)	12% (3)	64% (16)
2002	52 (13)	28% (7)	20% (5)	48% (12)	8% (2)	44% (11)	48% (12)	-	52% (13)	32% (8)	12% (3)	56% (14)
2003	48% (12)	8% (2)	44% (11)	44% (11)	8% (2)	48% (12)	40% (10)	4% (1)	56% (14)	24% (6)	16% (4)	60% (15)

R, resistant; I, intermediate and S susceptibility.

Table 2. Susceptibility rates of the fluoroquinolones tested against *Enterococcus* spp during 1996-2003.

Year	ciprofloxacin		norfloxacin		Levofloxacin		gatifloxacin	
	S/R (%)	S/R+I (%)	S/R (%)	S/R+I (%)	S/R (%)	S/R+I (%)	S/R (%)	S/R+I (%)
1996	3.6	2.6	4	4	7.3	7.3	24	24
1997	4.2	2.1	4.7	3.2	7.3	7.3	21	5.2
1998	6.5	1.1	10	4	11.5	11.5	-	11.5
1999	1.6	0.5	3.3	1.5	7.3	7.3	23	11.5
2000	0.4	0.1	3.6	2.6	4	4	7	5.2
2001	0.8	0.7	1.8	1.5	1.8	1.8	2.7	1.8
2002	0.4	0.2	0.9	0.8	1.1	1.1	1.7	1.3
2003	0.9	0.8	1.1	0.9	1.4	1.3	2.5	1.5

S/R is calculated by ratio: susceptibility (S) / resistance (R).

S/R+I is calculated by ratio: susceptibility (S) / resistance (R) + intermediate (I).

References

1. Members of the Jury of the Consensus Conference on nosocomial urinary tract infections (NUTI) in adult patients (2003). Nosocomial urinary tract infections (NUTI) im adult patients: Consensus conference 2002, short text. *Médecine et maladies infectieuses* **33**: 218s-222s.

2. Naber K.G. (2001). Which fluoroquinolones are suitable for the treatment of urinary tract infections. *International Journal of Antimicrobial Agents* **17**: 331–41.

3. Drlica K. & Zhao X.L. (1997). DNA gyrase, topoisomerase IV, and the 4-quinolones. *Microbiology Reviews* **61**: 377–392.

4. Everett M.J. & Piddock L.J.V. (1998). Mechanisms of resistance to fluoroquinolones. *In* J. Kuhlmann, A. Dahlhoff, and H. J. Zeiler (ed.), Quinolone antibacterials 1998. Springer-Verlag KG, Berlin, Germany, pp. 259–297.

5. Carratala J., Fernandez-Sevilla A., Tubau F., Callis M. & Gudiol F. (1995). Emergence of quinolone-resistant *Escherichia coli* bacteremia in neutropenic patients with cancer who have received prophylactic norfloxacin. *Clinical Infectious Diseases* **20**: 557-60.

6. Cometta A., Calandra T., Bille J. & Glauser M.P. (1994). *Escherichia coli* resistant to fluoroquinolones in patients with cancer and neutropenia. *New England Journal of Medicine* **330**: 1.240-1.

7. Hooper D.C. & Wolfson J.S. (1989). Bacterial resistance to the quinolone antimicrobial agents. *The American Journal of Medicine* **87**: 17S-23S.

8. Tarshis G.A., Miskin B.M., Jones T.M., Champlin J., Wingert K.J., Breen J.D. & Brown M.J. (2001). Once-daily oral gatifloxacin versus oral levofloxacin in treatment of uncomplicated skin and soft tissue infections: double-blind, multicenter, randomized study. *Antimicrobial Agents and Chemotherapy* **45**: 2358-62.

9. Stein G.E. & Schooley S. (2004). Urinary concentrations and bactericidal activities of newer fluoroquinolones in healthy volunteers. *International Journal of Antimicrobial Agents* (In press).

10. Facklam R.R. & Collins M.D. (1989). Identification of *Enterococcus* species isolated from human infections by a conventional test scheme. *Journal of Clinical Microbiology* **27**: 731–734.

11. Facklam R.R. & Teixeira L.M. (1997). *Enterococcus* In: *Topley and Wilson's Microbiology and Microbial Infections.* 9th ed. L. Collier, A. Balows, M. Edward Sussman Arnold, London, United Kingdom, pp. 669–682.

12. Facklam R.R., Sahm D. & Teixeira L.M. (1999). *Enterococcus* In: *Manual of Clinical Microbiology.* 7th ed. P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, R. H. Yolken, Washington, D.C.: ASM Press, pp. 297–305.

13. Merquior V.L.C., Peralta J.M., Facklam R.R. & Teixeira L.M. (1994). Analysis of electrophoretic whole-cell protein profiles as a tool for characterization of *Enterococcus* species. *Current in Microbiology* **28**: 149–153.

14. NCCLS - National Committee for Clinical Laboratory Standards. (1999). *Performance Standards for Antimicrobial Disk Susceptibility Tests*. Wayne, PA, USA.

15. d'Azevedo P.A., Dias C.A.G., Lemos S.K., Bittencourt J.A.F. & Teixeira L.M. (2004). Antimicrobial susceptibility among *Enterococcus* strains isolated in Porto Alegre city, RS. *Brazilian Journal of Microbiology* (In press).

16. Naber K.G. (2000). Survey on antibiotic usage in the treatment of urinary tract infections. *Journal of Antimicrobial Chemotherapy* **46** (Suppl. 1): 49–52.

17. Oliveira L.C., Lucon A.M., Nahas W.C., Ianhez L.E. and Arapi S. (2001). Catheter-associated urinary infection in kidney post-transplant patients. *São Paulo Medical Journal* **119**: 165-8.

18. Naber, K. G. (2000). Treatment options for acute uncomplicated cystitis in adults. *Journal of Antimicrobial Chemotherapy* **46** (Suppl. 1): 23–27.

♦ Avaliar e caracterizar genotipicamente isolados de *S. aureus* resistentes à meticilina obtidas neste estudo, através da técnica de Eletroforese em gel de Campo Pulsado (PFGE).

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