UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE – UFCSPA
CURSO DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE

Janine de Melo Rauber

Influência de fatores microbiológicos e de características clínicas no desfecho da terapia em infecções por *Staphylococcus* spp.

Porto Alegre
2016

Janine de Melo Rauber

Influência de fatores microbiológicos e de características clínicas no desfecho da terapia em infecções por *Staphylococcus* spp.

Tese submetida ao Programa de Pós-Graduação em Ciências da Saúde da Fundação Universidade Federal de Ciências da Saúde de Porto Alegre como requisito para a obtenção do grau de Doutor

Orientador: Dr. Pedro Alves d'Azevedo
Co-orientadora: Dra. Andréia Rosane de Moura Valim

Porto Alegre
2016

Catalogação na Publicação

Rauber, Janine de Melo
Influência de fatores microbiológicos e de características clínicas no desfecho da terapia em infecções por Staphylococcus spp. / Janine de Melo Rauber. -- 2016.
118 f. : 30 cm.

Tese (doutorado) -- Universidade Federal de Ciências da Saúde de Porto Alegre, Programa de Pós-Graduação em Ciências da Saúde, 2016.

Orientador(a): Pedro Alves d'Azevedo ; coorientador(a): Andréia Rosane de Moura Valim.

1. Staphylococcus spp.. 2. Infecções hospitalares. 3. Resistência microbiana. 4. Virulência. 5. Vancomicina. I. Título.

Sistema de Geração de Ficha Catalográfica da UFCSPA com os dados fornecidos pelo(a) autor(a).

Resumo

Os microrganismos do gênero *Staphylococcus* spp. são classificados como cocos gram-positivos e normalmente estão presentes na microbiota humana. Em ambientes hospitalares, no entanto, a exposição de pacientes debilitados a estes microrganismos pode ocasionar tanto infecções simples quanto patologias graves. A oxacilina é um dos antimicrobianos mais utilizados no tratamento de infecções por estafilococos, porém é bastante frequente o isolamento de cepas resistentes. A vancomicina, medicamento que pertence à classe dos glicopeptídeos, é a principal escolha na terapia de *Staphylococcus aureus* resistente à oxacilina/meticilina (MRSA), mas outras drogas alternativas também podem ser empregadas no caso de resistência, como a teicoplanina, daptomicina, tigeciclina e linezolida. Embora a maioria das cepas de MRSA demonstre sensibilidade *in vitro* a estes antimicrobianos alternativos, percebe-se que por vezes ocorre falha na ação do antibiótico e, sem explicação aparente, são detectados fracassos nas terapias em infecções estafilocócicas. O objetivo deste trabalho foi avaliar as variáveis relacionadas ao desfecho no tratamento de pacientes hospitalizados com infecções por *Staphylococcus* spp., analisando características clínicas do paciente bem como características microbiológicas e moleculares dos microrganismos isolados. O estudo foi observacional analítico e não intervencionista, com delineamento do tipo coorte prospectiva, com início em agosto de 2012 e término em julho de 2015, sendo um total de 232 amostras isoladas no período. Com relação às espécies, *S. aureus* esteve presente em 49,5% dos casos de infecção e *S. epidermidis* foi a principal espécie isolada dentre os estafilococos coagulase negativa (43,4%). Para as espécies de coagulase negativa, a resistência à meticilina foi significativamente maior ($p < 0.01$) se comparada à espécie *S. aureus* (75,7% vs. 17,6%). As amostras resistentes à meticilina apresentaram concentração inibitória mínima para vancomicina estatisticamente maior ($p < 0,001$) se comparado às sensíveis (1,11 vs. 1,56 µg/mL). Além disso, com relação à resistência, 36 amostras (15,5%) apresentaram hetero-resistência intermediária à vancomicina (hVIS). Não foi verificada transmissão vertical na maioria das amostras de hVIS uma vez que o perfil genético apresentou relação de similaridade em apenas quatro isolados. Após o tratamento da infecção, 23,8%

dos pacientes foram a óbito. A resistência à meticilina não foi relacionada à mortalidade, mas foi verificado um aumento significativo no tempo de internação hospitalar nestes casos. Não houve influência nas taxas de óbito em função da hetero-resistência intermediária e de valores elevados de MIC para vancomicina. A presença de leucocidina de Panton-Valentine (PVL) foi observada em 25 isolados e 46% das cepas de *S. aureus* foram consideradas fortes produtores de biofilme. A maioria dos isolados apresentou SCC*mec* IV (35%), SCC*mec* III (14%), *agr* I (46%), *agr* II (24%) e o *agr* esteve ausente em 11% das amostras. A ausência de *agr* foi associada ao aumento do MIC para vancomicina (1,93 vs. 1,59 µg/mL, p= 0,050). Após avaliação sérica, apenas 8,6% das dosagens de vancomicina estavam dentro da janela terapêutica, 51,4% estavam abaixo do especificado e 40% acima. Não foi verificada relação do óbito com os níveis séricos de vancomicina (p= 0,892), porém a nefrotoxicidade foi relacionada com níveis séricos de vancomicina superiores a 15 µg/mL.

Abstract

Microorganisms of the *Staphylococcus* spp. genus are classified as gram-positive cocci and usually are found in the human microbiota. In nosocomial environment, however, the exposure of debilitated patients to these microorganisms can cause both simple infections and serious diseases. Methicillin is one of antimicrobials used in the treatment of Staphylococcal infections, but is frequent the isolation of resistant strains. Vancomycin, a glycopeptide drug, is the first choice in the therapy of methicillin-resistant *Staphylococcus aureus* (MRSA), but alternative drugs can be employed in cases of resistance such as teicoplanin, daptomycin, tigecycline and linezolid. Although most MRSA strains are susceptible *in vitro* to these alternative antimicrobials, it is reported unexplained failure in antibiotic action and Staphylococcal infections therapy. The objective of this study was to evaluate the variables associated with outcome in treatment of *Staphylococcus* spp. infections, assessing clinical features of the patient as well as microbiological and molecular characteristics of microorganisms. The study was observational

and non-interventionist, a prospective cohort design, from August 2012 to July 2015, and 232 samples were isolated in the period. Regarding to species, *S. aureus* was found in 49.5% of cases of infection and *S. epidermidis* was the most frequent specie isolated from the coagulase negative *Staphylococci* (43.4%). For coagulase negative species, methicillin resistance was significantly higher ($p < 0.01$) compared to *S. aureus* (75.7% vs. 17.6%). Methicillin-resistant strains presented vancomycin minimum inhibitory concentration (MIC) statistically higher ($p < 0.001$) compared to susceptible strains (1.11 vs. 1.56 µg/mL). Moreover, with respect to resistance, 36 samples (15.5%) were classified as heterogeneous vancomycin-intermediate *Staphylococcus* spp. (hVIS). There was no vertical transmission in most of hVIS samples since the genetic profile showed similarity relation only in four isolates. After infection treatment, 23.8% of patients died. The methicillin resistance was not associated with mortality, but was a significant increase in length of hospital stay in these cases. There was no influence on mortality rates due to the heteroresistance or high levels of vancomycin MIC. The presence of Panton-Valentine Leucocidin (PVL) was observed in 25 isolates and 46% of *S. aureus* strains were found to be strong producers of biofilm. Most isolates were classified as SCC*mec* IV (35%), SCC*mec* III (14%), *agr* I (46%), *agr* II (24%) and *agr* was absent in 11% of samples. The absence of *agr* was associated with an increased vancomycin MIC (1.93 vs. 1.59 µg/mL, $p = 0.050$). After serum evaluation, only 8.6% of vancomycin doses were within the therapeutic window, 51.4% were below and 40% were above. There was no association of mortality with vancomycin troughs ($p = 0.892$), but nephrotoxicity was associated to vancomycin serum levels higher than 15 µg/mL.

SUMÁRIO

1 REVISÃO DA LITERATURA

1.1 Importância clínica dos *Staphylococcus* spp.

Staphylococcus spp. são cocos gram-positivos que, em análise microscópica, formam arranjos semelhantes a cachos de uvas. Parte da diferenciação entre as espécies do gênero é realizada através do teste da enzima coagulase: para *Staphylococcus aureus*, a espécie mais relevante e patogênica, o teste da coagulase é positivo(1); dentre os estafilococos coagulase-negativos (CoNS) mais comumente relacionados à infecção destacam-se o *S. epidermidis*, *S. haemolyticus* e *S. lugdunesis*(2, 3).

O gênero é frequentemente relacionado a infecções nosocomiais, devendo-se ter maior cuidado com pacientes internados, cujo sistema imunológico encontra-se debilitado. Assim, uma simples colonização pelo microrganismo pode evoluir para uma infecção grave, situação que ocorre normalmente com recém-nascidos, pacientes cirúrgicos, imunodeprimidos e imunocomprometidos(4). Nesse sentido, o gênero *Staphylococcus* spp. foi responsável por 27% das infecções hospitalares nos Estados Unidos da América (EUA) entre os anos de 2009 e 2010(5).

Componente da microbiota normal, o *S. aureus* encontra-se presente na pele e narinas de aproximadamente um terço da população(6-9). Assim como os demais microrganismos do gênero, são típicos patógenos oportunistas em ambientes hospitalares, e as cepas resistentes estão emergindo como agentes relevantes também em infecções adquiridas na comunidade(10).

Os indivíduos colonizados por *S. aureus* apresentam um risco aumentado de infecção por estas cepas. Muitos casos de infecções nosocomiais são adquiridas através da exposição às mãos de profissionais da saúde já colonizados pelo microrganismo ou que tiveram contato com pacientes infectados pelo estafilococo(11).

S. aureus é o primeiro na listagem dos patógenos associados às infecções de saúde relatados pela vigilância *National Healthcare Safety Network* (*Centers for Disease Control and Prevention*, CDC), especialmente responsáveis por infecções de sítio cirúrgico e pneumonia associada à

ventilação mecânica(5). Cerca de 14,5% das infecções em corrente sanguínea confirmadas em laboratório no Brasil são atribuídas a *S. aureus*(12).

Já os estafilococos coagulase-negativos são os patógenos oportunistas mais frequentemente relacionados a infecções de corrente sanguínea associada à cateter central(13) e a segunda maior causa de infecções de sítio cirúrgico(5). No Brasil, os CoNS são responsáveis por 17% das infecções da corrente sanguínea confirmadas em laboratório(12).

CoNS são parte da microbiota normal da pele humana e são comumente considerados como contaminantes de culturas microbiológicas(14). Realizar a diferenciação entre a verdadeira bacteremia e a contaminação é bastante difícil e deve envolver avaliação detalhada do paciente(15). Os pacientes imunocomprometidos, hospitalizados por longos períodos, pacientes criticamente doentes e recém-nascidos prematuros são os indivíduos mais vulneráveis à infecção por estafilococos coagulase-negativos(16), uma vez que o uso de dispositivos médicos, como cateteres está diretamente relacionado à capacidade do CoNS formar biofilme(17).

1.2 Resistência aos antimicrobianos

Nas últimas décadas, um importante problema de saúde pública tem causado alerta: o aumento da incidência de cepas resistentes aliado ao surgimento de bactérias multirresistentes(18). Muitos aspectos estão envolvidos no incremento da resistência, no entanto, o uso frequente e por vezes inadequado de antimicrobianos, tanto em ambiente hospitalar quanto domiciliar, tem sido determinante na emergência de infecções por cepas multirresistentes(19). Neste sentido, o tratamento de infecções causadas por *S. aureus* tem sido dificultado pela grande prevalência de cepas resistentes à meticilina (MRSA)(20).

1.2.1 Meticilina

Alguns anos após a descoberta da penicilina por Alexandre Fleming (1928), o índice de mortalidade por bacteremia causada por *S. aureus* reduziu de 70% para 30%. No entanto, em 1942 foi isolada a primeira cepa *S. aureus* produtora de penicilinase, enzima capaz de inativar o antimicrobiano. A incidência de resistência à penicilina aumentou significativamente após a

segunda guerra mundial devido ao uso intenso deste medicamento entre os soldados feridos. Em função destas cepas resistentes, a mortalidade causada pelo microrganismo aumentou para 50% em 1954(21).

Com o desenvolvimento do antibiótico meticilina (1959) para tratamento dos casos resistentes à penicilina, foi possível reduzir a mortalidade novamente para 30%. A meticilina foi introduzida como alternativa terapêutica para as bactérias produtoras de β-lactamases, uma vez que essa droga não sofre a ação destas enzimas. Entretanto, em 1961 relatou-se a primeira cepa de MRSA e, atualmente, estas cepas estão amplamente disseminadas(18). Nestes casos, o tratamento de infecções causadas por MRSA fica limitado à administração de vancomicina, daptomicina, linezolida, tigeciclina, sulfametoxazol + trimetoprima, substâncias que geralmente apresentam menor ação contra o microrganismo, custo mais elevado e/ou maior toxicidade(19).

A espécie *S. aureus* foi reportada em 16% das infecções hospitalares nos EUA e a prevalência de MRSA foi de 46 a 63% entre os isolados(5). Rosenthal e colaboradores (2012), em estudo realizado de 2004 a 2009 em 36 países da América Latina, Ásia, África e Europa pelo *International Nosocomial Infection Control Consortium* (INICC), registraram entre 71 e 84% de resistência à meticilina para *S. aureus*(22).

Os isolados de MRSA apresentam as proteínas ligadoras de penicilina com reduzida afinidade pela meticilina PBP2a (penicillin binding proteins), codificadas pelo gene *mec*A(23). Assim, não é possível que a meticilina se ligue às proteínas e realize a inibição da síntese da parede celular bacteriana. A presença do gene *mec*A é um dos fatores que caracteriza a resistência aos antimicrobianos β-lactâmicos e sua detecção é um dos métodos mais acurados para definição da resistência (CLSI, 2015).

O gene *mecA* é transportado em um elemento genético móvel chamado cassete cromossômico estafilocócico *mec* (SCC*mec*). Além do complexo *mec* que contém o gene *mecA*, o elemento SCC também pode transportar outros genes de resistência a antibióticos, plasmídeos e transposons(24). A fácil transferência deste elemento entre os estafilococos é uma das principais causas da rápida emergência de resistência(25).

A excisão e integração do elemento SCC*mec* no genoma dos

Staphylococcus é realizada por recombinases específicas *ccr* (*cassette chromosome recombinase*). Os elementos SCC*mec* podem ser classificados de acordo com a combinação de tipos de recombinases (*ccr*AB 1 a 6 e *ccr*C 1 a 2) e tipos de complexos *mec* (A – E)(26, 27). Para a formação dos diferentes tipos de SCC*mec*, algumas combinações possíveis de recombinases estão representadas na Figura 1. Atualmente, são conhecidos doze tipos de SCC*mec* (tipo I - XII) em isolados MRSA(28).

Em meados dos anos 90 foram relatadas as primeiras infecções causadas por *S. aureus* resistentes à meticilina associados à comunidade (CA-MRSA). Estes assim foram nomeados, pois esses isolados eram de pacientes que não tinham contato direto com sistemas de saúde que pudesse explicar tal infecção(29, 30).

A resistência mediada pelo gene *mec*A provinda da comunidade foi carreada por um SCC*mec* tipo IV, menor do que outros tipos de SCC*mec* com tipagem característica hospitalar (HA-MRSA)(31). Os SCC*mec* do tipo I ao III, originalmente nosocomiais, estão associados à maior resistência a antimicrobianos(32).

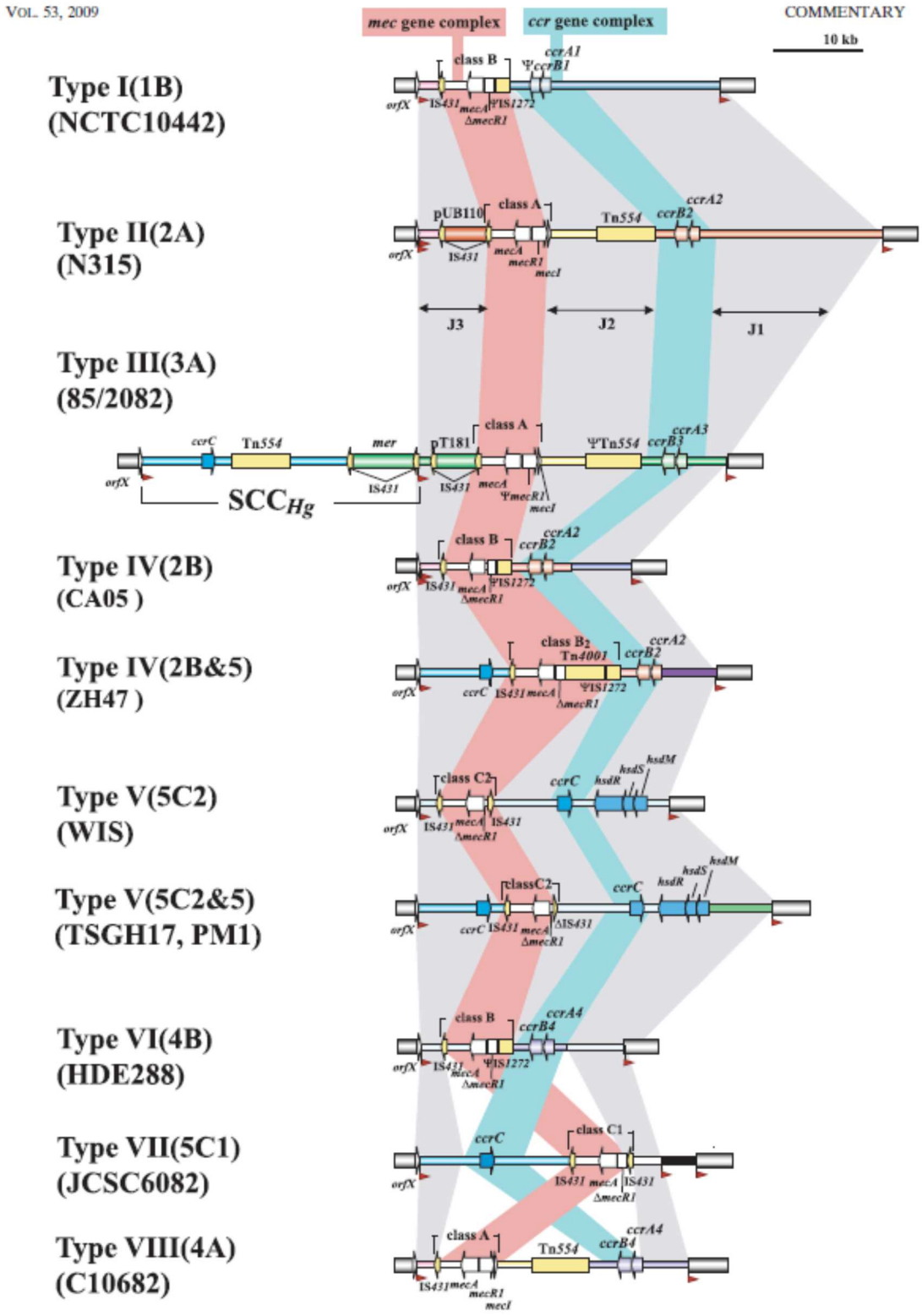
É importante enfatizar que os isolados MRSA eram quase inéditos em isolados de comunidades e, no entanto, nos últimos anos eles têm sido exaustivamente relatados. Isolados comunitários tem na sua maioria como destaque a produção de toxina, como a leucocidina de Panton-Valentine (PVL), que está associada à pneumonia necrotizante(33).

Características de linhagens SCC*mec* tipo IV, inicialmente relacionadas à comunidade, estão sendo evidenciadas em isolados MRSA nosocomiais, com maior resistência. Também foi verificado, que isolados hospitalares estariam distribuídos na comunidade, proporcionando recombinação entre isolados HA e CA-MRSA(34).

Nos últimos anos, está sendo definida uma nova classificação a respeito da origem em infecções por estafilococos. Considerando que estes microrganismos são os principais responsáveis por mastites bovinas, está sendo verificada a colonização por estafilococos em animais e, consequentemente, a transmissão de infecção para trabalhadores rurais e pecuaristas. O primeiro caso de LA-MRSA (liverstock-asssociated MRSA) foi publicado em 2005(35) e assim denominado por se diferenciar dos conhecidos

HA e CA-MRSA(36-38).

Figura 1. Representação dos diferentes tipos de SCC*mec*



Fonte: IWG-SCC(27)

Em análise molecular, os isolados LA-MRSA não são tipados em gel de eletroforese em campo pulsado (PFGE) utilizando a enzima *Sma*I, pertencem preferencialmente ao completo clonal (CC) 398, apresentam SCC*mec* tipo IV e V geneticamente diferenciado em relação ao CA-MRSA, além de não possuírem PVL e outras enterotoxinas(39). Assim, os isolados LA-MRSA parecem ser menos virulentos e menos transmissíveis do que os isolados HA e CA-MRSA. Outra característica importante é que a resistência aos β-lactâmicos pode ser conferida por um gene homólogo ao *mec*A, o gene *mec*C (*mec*A_{LGA251})(40, 41).

1.2.2 Vancomicina

A vancomicina, antibiótico da classe dos glicopeptídeos, é a principal escolha para o tratamento intravenoso de MRSA no caso de pacientes hospitalizados(42). Em ensaios clínicos, antimicrobianos lançados mais recentemente, como a daptomicina, linezolida e tigeciclina, não superam a atividade da vancomicina, medicamento desenvolvido e comercializado há mais de 50 anos(43).

Este antimicrobiano age inibindo a síntese da parede celular bacteriana, impedindo o acoplamento dos polímeros de peptidoglicano. A resistência ao antimicrobiano ocorre por meio de uma modificação no peptídeo da parede celular do microrganismo. Assim, há alteração do fragmento de D-alanil-D-alanina tanto para D-alanil-D-lactato quanto para D-alanil-D-serina, impossibilitando a ligação do fármaco. Esta resistência é mediada por loci gênicos, sendo que o mais frequente é o gene *van*A, carreado por plasmídeos e transposon, que conferem resistência ao antibiótico(44, 45).

Além da conhecida toxicidade deste antimicrobiano, falhas no tratamento de endocardites, bacteremias e pneumonias por cepas de *S. aureus* sensíveis e resistentes à meticilina (MSSA e MRSA) estão sendo relatadas desde 1977 até o momento(46).

Nesse sentido, segundo o consenso da *American Society of Health-System Pharmacist, Infectious Disease Society of America and Society of Infection Disease Pharmacists*, doses de vancomicina mais intensivas são

recomendadas para manter os níveis séricos entre 15 e 20 µg/mL, garantindo para a vancomicina uma área sob a curva (AUC) acima da MIC na razão ≥400(47). No entanto, estes regimes mais intensivos têm sido associados com um aumento nos eventos de nefrotoxicidade induzida por vancomicina(48).

A vancomicina é um antibiótico ainda seguro e de baixo custo, porém seu uso requer acompanhamento terapêutico adequado. A monitoração terapêutica de vancomicina tem a finalidade de alterar a dosagem quando esta estiver inadequada, impedindo a falha de tratamento, reduzindo o risco de resistência bacteriana e prevenindo a ocorrência de nefrotoxicidade(49).

As principais indicações para monitoração dos níveis séricos são pacientes que recebem doses elevadas de vancomicina, que fazem terapia prolongada, que têm maior probabilidade de desenvolver nefrotoxicidade temporária e/ou que são obesos. A frequência da dosagem da vancomicina no sangue será conforme a gravidade do estado do paciente(47).

Alguns estudos têm analisado a importância da mensuração dos níveis séricos do antimicrobiano e recentemente foram publicadas duas meta-análises nas quais apenas o parâmetro AUC/MIC, e não a variável níveis séricos de vancomicina, foi relacionado com falha terapêutica, persistência da bacteremia e mortalidade(50, 51).

Alguns estudos relatam dificuldades no tratamento de MRSA normalmente em casos de isolados com altos valores de concentração mínima inibitória (MIC) para vancomicina(52-54). Assim, a ineficácia poderia ser atribuída à menor proporção de AUC/MIC, sendo esta imprescindível para o sucesso da terapia com vancomicina(55). Entretanto, há controvérsia a respeito da real influência da MIC no desfecho da terapia com vancomicina.

Esta questão foi discutida em quatro meta-análises, não havendo consenso entre os autores(56-59). Ao contrário dos três estudos anteriores, de acordo com a mais recente meta-análise(59), não houve diferenças significativas no risco de morte quando comparados pacientes com alto MIC para vancomicina (≥1,5 µg/mL) para aqueles com baixo MIC em casos de bacteremia por *S. aureus*.

Outra dificuldade consiste no fato de que estão sendo relatados casos de *S. aureus* com resistência aos glicopeptídeos vancomicina e teicoplanina, principais alternativas em infecções causadas por MRSA. Mecanismos de

resistência intermediária (VISA) foram descobertos a partir da década de 80 através de mutações no cromossomo bacteriano(60). Em 2002, foi relatado o primeiro caso de resistência completa (VRSA) através da aquisição do gene *van*A. Embora publicadas, estes tipos de resistência à vancomicina ainda são pouco prevalentes(61).

Segundo o CLSI (*Clinical and Laboratory Standards Institute*), a sensibilidade à vancomicina é inferida através da análise da MIC. De acordo com critérios adotados a partir de 2006 (CLSI, 2006), os isolados com MIC menor ou igual a 2 µg/mL são considerados sensíveis à vancomicina; as cepas de MIC entre 4 e 8 µg/mL são ditas de sensibilidade intermediária (VISA); e culturas com MIC maior do que 16 µg/mL são resistentes ao antimicrobiano (VRSA).

Embora estas categorias sejam amplamente conhecidas na microbiologia (sensível, intermediário e resistente), para *S. aureus* está sendo definida uma nova classificação: a hetero-resistência intermediária à vancomicina (hVISA). Essencialmente, um isolado hVISA é um isolado de *S. aureus* com MIC para vancomicina dentro da faixa suscetível quando avaliado pelo métodos convencionais de rotina, mas neste isolado há uma proporção da população de células (de uma célula em cada milhão de células) que está na faixa de sensibilidade intermediária(62). Estima-se que aproximadamente 60% dos isolados de MRSA com MIC de 2 µg/mL são cepas de hVISA(60).

Os métodos utilizados para triagem de detecção hVISA mais populares são os Etest modificados, como Macro-método Etest e o Etest GRD. Neste caso, utiliza-se maior concentração de inóculo (6 $x10^8$, equivalente a escala 2 de McFarland), incubação prolongada (48h) ou meio enriquecido para crescimento. O método para confirmação de hVISA é realizado com base no crescimento da cultura, sendo conhecido como análise de perfil da população (PAP). Embora o PAP seja o padrão-ouro, trata-se de um teste laborioso e cuja demora no resultado (3 a 5 dias) não permite sua aplicação na rotina clínica(60).

A importância clínica das cepas hVISA deve-se ao fato de que sua presença parece estar relacionada a maiores chances de falha terapêutica com vancomicina, especialmente em casos de bacteremia(63-66). No entanto, embora muitos pesquisadores estejam estudando as razões da falha no

tratamento com vancomicina, não há ainda um consenso estabelecido e vários estudos são controversos.

1.3 Fatores de virulência

A virulência é definida quando características como a capacidade de adesão ao hospedeiro e síntese de compostos (enzimas, toxinas, e outros) estão presentes em um microrganismo. A expressão coordenada dos componentes de virulência e das proteínas de superfície é necessária para o estabelecimento de uma infecção(67, 68).

Considerando que os estafilococos são componentes da microbiota, as patologias causadas por estes microrganismos dependem da produção de toxinas e enzimas, sendo a expressão delas controlada por um regulador global, o *locus agr - accessory gene regulator*(68, 69).

Tsompanidou et al. (2011) descreve que a expressão de diversos genes de virulência do *S. aureus* é comandada pelo *locus agr* que é um agrupamento de genes com atividade de *quorum sensing*(70). Assim, trata-se de um processo coordenado de comunicação intra e interespécies dos microrganismos mediado por sua densidade populacional, permitindo que as bactérias tenham capacidade de se comportar como organismos complexos, se comunicando e agindo coordenadamente, respondendo a diferentes estímulos de modo unificado(71).

Os fatores de virulência dos *S. aureus* podem ser classificados em três categorias: (I) os fatores associados com a aderência à matriz extracelular ou às células do hospedeiro, como a produção de moléculas de fibrinogênio, fibronectina, colágeno ou da enzima coagulase; (II) os fatores relacionados com a evasão da defesa do hospedeiro, como a proteína A, diversas enterotoxinas estafilocócicas, a toxina da síndrome do choque tóxico (TSST-1), lipases e polissacarídeos capsulares; e (III) os fatores relacionados com a invasão na célula do hospedeiro e a penetração nos tecidos ou adesão de superfícies de cateteres e próteses, os quais incluem as proteínas (toxinas) α, β, γ, δ–hemolisinas e a leucocidina de Panton-Valentine (PVL)(72).

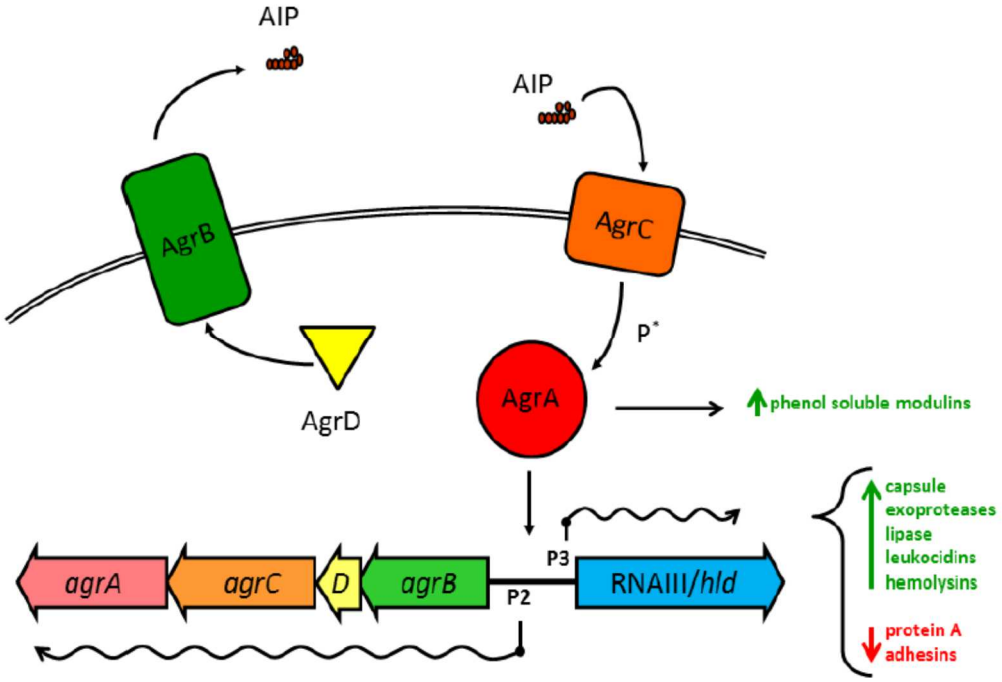
1.3.1 *locus agr* (*accessory gene regulator*)

O *locus agr* é um regulador transcricional que controla a expressão de

proteínas que estão associadas à virulência do *S. aureus*. Os fatores de virulência regulados pelo *agr* são divididos em duas classes: (a) fixação do microrganismo ao hospedeiro e evasão imune; (b) invasão e síntese de toxinas. A ativação do sistema *agr* muda as características do microrganismo, tornando-o um patógeno invasivo e agressivo(73).

Este sistema de regulação possui dupla ação: (a) inibição da transcrição de proteínas associadas a parede celular (proteína A, coagulase, fibronectina); (b) ativação de diversas exoproteínas (α-toxina, β-hemolisina, TSST-1, leucotoxina)(74). Através de uma reação em cadeia, envolvendo vários genes, o *agr* sintetiza o RNA III, o qual é molécula de RNA mensageiro (mRNA) que atua na transcrição e, em alguns casos, na tradução dos vários fatores de virulência, incluindo TSST-1 e hemolisinas(68).

Figura 2. Sistema de ativação do *agr*



Fonte: Gray et al., 2013(75)

O *locus agr* consiste em dois *operons* transcritos de forma divergente, a partir de dois promotores (P2 e P3), que produzem respectivamente, RNAII e RNAIII(69, 76). O promotor P2 faz a transcrição *agr* por meio do transcrito de RNAII, que engloba a*gr*A, *agr*B, *agr*C e *agr*D(73); em seguida, o *agr*A é

fosforilado e tem uma interação com o promotor P3, assim tendo início a transcrição de RNA III(77), conforme é mostrado na Figura 2.

O promotor P3, responsável pela síntese de RNAIII, é o regulador do gene alvo, ou seja, ativa o nível de transcrição dos genes e, também, está ligado com a codificação da δ-hemolisina, a qual é uma importante reguladora da expressão de um grande número de genes de virulência(78).

Os isolados clínicos possuem *agr* em sua maioria, entretanto, alguns isolados de *S. aureus* expressam pouco ou não expressam esses fatores, levantando uma questão sobre seu preciso papel(79). A disfunção do *agr* pode ser verificada pela ausência da produção da δ-hemolisina(80), sendo esta disfunção associada com prolongamento da duração de bacteremias. O estudo realizado por Schweizer e colaboradores (2011) comparou a disfunção do *agr* e a mortalidade de pacientes graves, verificando uma maior tendência de mortalidade no caso de disfunção no *agr*(81).

1.3.2 Toxina δ-hemolisina

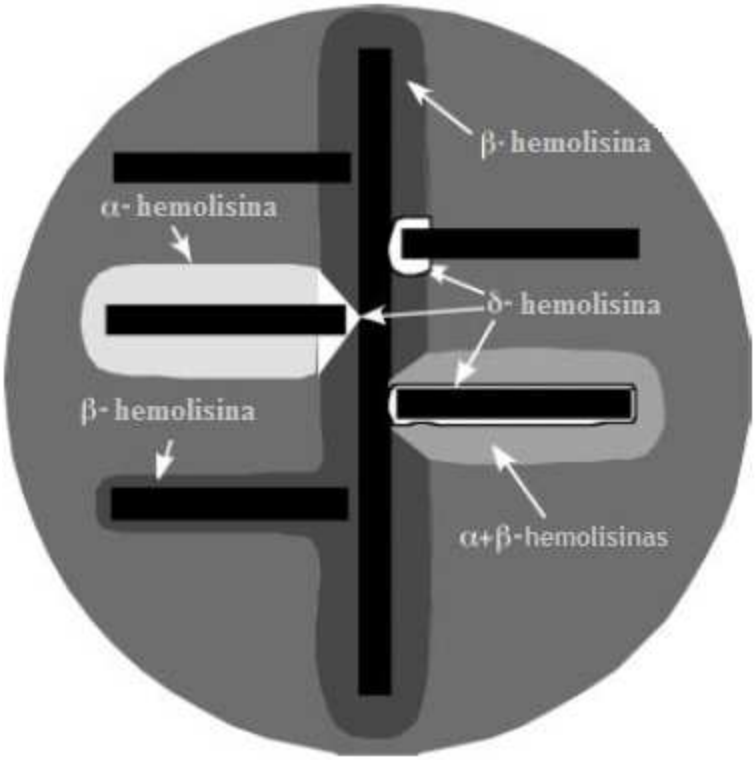
A maioria das espécies pertencentes ao gênero *Staphylococcus* produz a toxina δ-hemolisina, que também é conhecida como modulinas fenol solúveis γ (PSM γ)(82). É caracterizada como um peptídeo de 26 aminoácidos segregados, e a sua secreção ocorre no meio ou quase no final da fase exponencial de crescimento(83, 84). A toxina δ-hemolisina é codificada pelo gene *hld* que está localizado no RNAIII, sendo sua produção controlada pelo *agr*(75, 83, 85).

Há uma produção maior da toxina δ-hemolisina em isolados de *S. aureus* em cerca 97% em comparação aos SCN que é encontrada em 50 a 70% dos isolados. Embora a toxina δ-hemolisina cause efeitos citotóxicos, a sua importância na etiologia da doença não está totalmente elucidada (83, 86). Essa toxina é utilizada em muitos estudos através do método fenotípico pela hemólise sinérgica, para analisar a funcionalidade do *agr*, pois a produção da toxina δ-hemolisina é caracterizada pela funcionalidade do *agr*(81, 87, 88).

A identificação fenotípica da produção da toxina δ-hemolisina é determinada através de ágar sangue de carneiro utilizando uma cepa referência que produz apenas β-hemolisina em grande quantidade. Ocorre uma sinergia na lise de glóbulos vermelhos na atuação entre β e δ-hemolisinas,

portanto, quando há a produção a toxina δ-hemolisina ocorre um realce na zona de hemólise onde as hemolisinas coincidem(79) (Figura 3).

Figura 3. Determinação fenotípica da produção de δ-hemolisina em ágar sangue de carneiro



Fonte: Adaptada de Adhikari, Arvidson e Novick, 2007(89)

1.3.3 Leucocidina de Panton-Valentine (PVL)

As proteínas de superfície são um fator de grande relevância no que se refere a virulência dos microrganismos. A PVL tem sido relacionada como um fator de virulência em patologias como infecções graves de pele, tecidos moles, pneumonia necrotizante, entre outras(90).

Em 1932, a PVL foi descrita por Panton e Valentine como um fator de virulência e como uma toxina formadora de poros que causava morte celular, em especial de leucócitos(33, 72). Através de uma ação sinergética de dois componentes de secreção (LukS-PV e LukF-PV), permite a entrada de cátions, como o Ca^{+2}, resultando na desgranulação celular e induzindo a citólise, atacando os leucócitos polimorfonucleares e os macrófagos(72, 91).

Conforme Otto (2013), no componente S foram identificadas três

proteínas (proteínas que eluem vagarosamente) e no componente F, identificadas duas proteínas (proteínas que eluem ligeiramente). A PVL tem atividade leucotóxica, não tem atividade hemolítica (forma poros que aumentam permeabilidade a cátions e causam instabilidade osmótica)(33).

Em estudo realizado por Gillet e colaboradores (2002), com casos retrospectivos e prospectivos quanto à presença de PVL, concluíram que a sua presença pode complicar síndromes gripais em crianças e adultos jovens saudáveis, levando à rápida progressão para pneumonia com hemoptise e leucopenia. A comparação das manifestações da doença quando há presença ou ausência da PVL é complicada, devido à diferença de idade. Entretanto constataram que os mais jovens correm mais risco de morte do que os idosos e enfermos homólogos(92).

Há associação entre isolados comunitários e resistentes à meticilina (CA-MRSA) com cassete cromossômico (SCC*mec*) do tipo IV e a PVL. A aquisição de PVL por isolados CA-MRSA mudou a epidemiologia das infecções, tanto hospitalares quanto comunitárias(93, 94).

1.3.4 Biofilme

Todo o gênero *Staphylococcus*, mas principalmente os CoNS tem sua patogenicidade atribuída fundamentalmente à habilidade de formação de biofilme, o principal fator de virulência desses microrganismos. O biofilme é uma matriz polimérica formada de células, proteínas e polissacarídeos de adesão em que os microrganismos podem prosperar e se replicar. Esse fator de virulência dá aos microrganismos uma proteção contra os antimicrobianos, bem como as defesas imunológicas e facilita a incorporação nos tecidos do hospedeiro ou até mesmo no interior de dispositivos médicos(95, 96).

Durante grande parte da história da microbiologia, acreditava-se que os microrganismos viviam somente de maneira planctônica, ou seja, células suspensas que circulam isoladamente. Porém, atualmente, sabe-se que os microrganismos possuem capacidade de se agregar e crescer em superfícies expostas, formando os biofilmes. Estes podem ser formados por células de várias ou de uma única espécie bacteriana que se comunicam entre si, através do sistema *quorum sensing*(97).

Muito importante em ambiente hospitalar, o biofilme bacteriano está relacionado com infecções decorrentes do uso de cateteres e próteses, sendo assim, as bactérias conseguem se aderir aos biomateriais e se multiplicam para que ocorra a formação do biofilme, o qual depende da espécie bacteriana, tipo de tensão e condições do ambiente. Devido à formação do biofilme, estas bactérias ficam mais resistentes frente aos antimicrobianos e desinfetantes; também são capazes de resistir a fagocitose e a outros componentes da imunidade inata e adaptativa, bem como sistema de defesa inflamatória do hospedeiro(98).

Araújo e Freire (2013) descrevem os três mecanismos existentes e que justificam a capacidade dos microrganismos que estão presentes nos biofilmes possuírem maior resistência frente aos antimicrobianos. Estes mecanismos são: a capacidade da matriz de exopolissacarídeos impedir o contato do agente antibacteriano com os microrganismos, funcionando como um filtro; as bactérias apresentarem um metabolismo mais lento e a capacidade de modificar e/ou neutralizar o agente (principalmente em biofilmes com mais de uma espécie)(71).

De acordo com Prakash, Veeregowada e Krishnappa(99), estudos realizados no *Centers for Disease Control and Prevention* (CDC) estimaram que 65% das infecções bacterianas desenvolvidas em humanos foram decorrentes da formação de biofilme. Já Behlau e Gilmore(100) descreveram que os biofilmes desempenham papel de extrema importância nas infecções crônicas associados ao uso de dispositivos médicos implantados, pois 60% das infecções nosocomiais estão relacionadas com a formação de biofilmes nestes dispositivos.

1.4 Epidemiologia

A epidemiologia utiliza-se de métodos quantitativos para estudar a ocorrência de doenças nas populações humanas e para definir estratégias de prevenção e controle. Neste sentido, estudos epidemiológicos fornecem dados aos profissionais e aos órgãos de saúde para decidirem sobre a execução de ações de controle. A realização deste tipo de estudos permite a disponibilização de informações atualizadas sobre a ocorrência dessas doenças e agravos, bem

como dos fatores que a condicionam, numa área geográfica ou população definida(101, 102).

O laboratório e as instituições de pesquisa assumem um papel importante no diagnóstico do agente infeccioso e participam ativamente nas ações de vigilância epidemiológica. A epidemiologia molecular é o ramo da ciência médica que utiliza tecnologias da biologia molecular no suporte às investigações epidemiológicas(103-105). Considerando a importância de estudos de prevalência e ocorrência de surtos, epidemias e mesmo de mapeamento de reservatórios microbianos, o uso de métodos moleculares está, muitas vezes, associado à métodos convencionais de isolamento e identificação bacteriano baseados em cultura(106, 107).

Os métodos tradicionais de tipagem de amostras bacterianas como a sorotipagem têm sido substituídos por métodos moleculares baseados em reação em cadeia da polimerase (PCR) ou na análise dos padrões de restrição do DNA cromossomal por eletroforese em gel de campo pulsado (PFGE)(108-111). Além disso, novos métodos moleculares ainda não tão disseminados no Brasil, baseados no sequenciamento de material genômico (MLST), também vêm cada vez mais ganhando espaço no ramo da bacteriologia(112). Todos estes métodos apresentam importante aplicabilidade no monitoramento de espécies e na detecção de linhagens ou variantes de populações microbianas(106, 113).

1.4.1 Métodos moleculares

1.4.1.1 PCR

Através da técnica de PCR é possível amplificar, de maneira seletiva, uma sequência específica de DNA com a finalidade de avaliar sua presença em determinado organismo. Para tanto, são empregados oligonucleotídeos iniciadores da reação (*primer*s) que sejam específicos para região de interesse. O processo de replicação do DNA ocorre pela ação da enzima *Taq* DNA polimerase, sendo necessária a adição de co-fatores para reação. A presença

dos fragmentos de DNA amplificados é posteriormente verificada em gel de agarose acrescido brometo de etídio(114).

Mais de um conjunto de *primers* pode ser empregado em uma mesma reação, observando-se, entretanto, a temperatura ideal de anelamento para cada sequência. Este tipo de reação é chamada de *multiplex* PCR e, no caso dos estafilococos, tem aplicação na tipagem do SCC*mec* e do *locus agr*(115, 116). Considerando a variedade de tipos de SCC*mec* presentes nos *Staphylococcus*, este elemento também pode ser utilizado no estudo da epidemiologia destes microrganismos(117).

Outros métodos de tipagem molecular também utilizam a amplificação por PCR seguida de sequenciamento genético: tipagem da proteína A (*spa*) e tipagem por sequenciamento de *locus* diversos (MLST). A tipagem *spa* consiste no sequenciamento de um único *locus*, a região variável X do gene que codifica a proteína A em *S. aureus*. A diversidade do gene *spa* consiste no número variável de sequências de repetições com 24 pares de bases (pb) contidas na região X. Um código alfa numérico é atribuído a diferentes repetições, definindo a ordem de repetições específicas como tipos de *spa*(118).

O MLST é um método altamente discriminatório para tipagem de cepas e caracteriza isolados com base na sequência de fragmentos internos de aproximadamente 450 pb de sete genes constitutivos (*housekeeping*). Cada alelo recebe um número e através da combinação dos sete alelos (*multi locus*) é possível classificar cada isolado com um tipo de sequência (ST) que define a origem evolutiva dos isolados(119). Há uma boa correlação entre os grupos clonais determinados por MLST e pela tipagem *spa*(120).

1.4.1.2 PFGE

O desenvolvimento da técnica de PFGE foi realizado por Schwartz e Cantor (1984) originalmente para separação de cromossomos da levedura *Saccharomyces cerevisae*. Caracteriza-se por ser uma eletroforese, onde complexos de ácidos nucleicos migram em um gel de agarose sob um campo elétrico alternado disposto em diferentes ângulos(121, 122).

O DNA cromossômico bacteriano é clivado com uma enzima de restrição e os fragmentos de DNA resultantes são separados por eletroforese em gel num campo elétrico, com um gradiente de pulso alternado. Os padrões de fragmentos resultantes são analisados por meio de um software específico. O PFGE oferece a vantagem de possibilitar a comparação entre laboratórios desde que se estabeleçam os mesmos critérios e padrões(114, 123).

Devido à possibilidade de transmissão vertical e horizontal das infecções em hospitais, frequentemente há o questionamento a respeito da clonalidade dos microrganismos isolados. Para a grande maioria das espécies, é de consenso geral que a técnica de PFGE é o padrão ouro quando o objetivo é avaliar a clonalidade entre isolados clínicos e que apresentam história compatível de transmissibilidade em um breve intervalo de tempo(110, 117, 124).

Segundo critérios de interpretação descritos por Tenover e colaboradores (1995), diferentes isolados que apresentam o mesmo padrão de fragmentos de DNA são considerados a mesma cepa. Para perfis que diferem de um a três fragmentos, os isolados são considerados estreitamente relacionados. Isolados que diferem de quatro a seis fragmentos, sugerem a ocorrência de eventos genéticos independentes e são considerados como possivelmente relacionados. Isolados com mais de seis bandas de diferença refletem a ocorrência de três ou mais eventos genéticos e são classificados como epidemiologicamente não relacionados(110). A análise de perfis eletroforéticos mais complexos requerem a utilização de programas computacionais para a análise e interpretação da estrutura genética e da inter-relação entre os microrganismos(125, 126).

2 OBJETIVO

Avaliar o resultado da terapia antimicrobiana em pacientes com infecções por *Staphylococcus spp*. internados e relacionar este desfecho a fatores microbiológicos e moleculares dos isolados, bem como a características clínicas e epidemiológicas dos pacientes.

2.1 Objetivos específicos

- Identificar as espécies isoladas e determinar o perfil epidemiológico dos pacientes acometidos com infecções por *Staphylococcus* spp, avaliando as principais características clínicas e respectivas co-morbidades;
- Verificar o perfil de sensibilidade à meticilina/oxacilina dos isolados de *Staphylococcus* spp, estabelecer a concentração mínima inibitória (MIC) de vancomicina e identificar a presença de cepas de estafilococos com hetero-resistência intermediária à vancomicina (hVIS);
- Monitorar os níveis séricos de vancomicina dos pacientes em tratamento com este antimicrobiano, verificando a manutenção da concentração na faixa terapêutica;
- Analisar as características microbiológicas e moleculares das cepas de estafilococos isoladas em pacientes internados, verificando a presença de fatores de virulência e genes envolvidos com resistência, como formação de biofilme, presença de leucocidina Panton-Valentine – PVL, δ-hemolisina (gene *hld*), gene acessório regulador – *agr*; gene codificador de resistência à meticilina – *mec*A e tipo de cassete SCC*mec*;
- Avaliar o desfecho da terapia em infecções por estafilococos (alta ou óbito e tempo de internação hospitalar).

3 MANUSCRITOS

3.1 Associação entre desfecho e resistência antimicrobiana

Multidrug-resistant *Staphylococcus* spp. and its impact in patient outcome

Janine de Melo Rauber, Marcelo Carneiro, Guilherme Henrique de Oliveira Arnhold, Monica Basso Zanotto, Patrícia Raquel Wappler, Barbara Baggiotto, Andréia Rosane de Moura Valim, Pedro Alves d'Azevedo

Artigo aceito pelo periódico American Journal of Infection Control

Multidrug-resistant *Staphylococcus* spp. and its impact in patient outcome

Janine de Melo Rauber, PharmD, MSc;[1] Marcelo Carneiro, MD, PhD;[1,2,3] Guilherme Henrique de Oliveira Arnhold;[2] Monica Basso Zanotto;[2] Patrícia Raquel Wappler, PharmD;[2] Barbara Baggiotto;[2] Andréia Rosane de Moura Valim, PharmD, PhD;[2] Pedro Alves d'Azevedo, PharmD, PhD[1*]

Affiliations: 1. Universidade Federal de Ciências da Saúde, Porto Alegre, RS, Brazil; 2. Universidade de Santa Cruz do Sul, Santa Cruz do Sul, RS, Brazil; 3. Hospital Santa Cruz, Santa Cruz do Sul, RS, Brazil.

* Address correspondence: Universidade Federal de Ciências da Saúde de Porto Alegre, Rua Sarmento Leite, 245, CEP 90050-170, Porto Alegre, RS, Brazil. Phone: 55 51 3303-8742; Fax: 55 51 3303-8810 (pedro_dazevedo@yahoo.com.br).

Staphylococcal infections: resistance and outcome

Word count: 989 words

Abstract

To analyze the outcome, clinical features and resistance of *Staphylococcus* spp. infections, a prospective cohort study was conducted at a 234-bed, tertiary care teaching hospital. Multidrug-resistant (MDR) infections were associated with increased mortality and length of hospital stay (LOS), whereas methicillin resistance (MRS) was correlated with prolonged LOS after infection. These data highlight the importance of infection control and antibiotic stewardship to prevent the emergence and the spread of MRS and MDR healthcare-associated infections.

Introduction

Staphylococcus aureus is an important cause of community-acquired and health care-associated infections(1). Morbidity and mortality rates associated with *S. aureus* have increased in recent years, especially in cases of resistant strains(2). Coagulase-negative *Staphylococcus* (CoNS) is the most common opportunist pathogen associated with hospital-acquired central line infections and its pathogenicity is mainly due to the ability to form biofilms on medical devices(3).

The purpose of this study was to analyze the outcome (mortality and length of hospital stay), clinical features and resistance profile of *Staphylococcus* spp. infections. Furthermore, a secondary objective was to determine the independent risk factors for 30-day mortality in Staphylococcal infection cases.

Methods

A prospective cohort study was conducted at a 234-bed, tertiary care, teaching hospital located in southern Brazil. Adult inpatients admitted between July 2012 and June 2014 with a positive culture for *Staphylococcus* spp. were eligible for inclusion. Samples were excluded in cases of duplicates or recurrent infections. The study was reviewed

and approved by the Research Ethics Committee of UFCSPA (1779/12).

Healthcare-associated (HA) and community-acquired (CA) infections, type of infection and multidrug-resistance (MDR) were determined according to CDC and previously described criteria(4).

Antimicrobial susceptibility and minimum inhibitory concentration (MIC) through broth microdilution (BMD) were performed according to CLSI (2015). In order to confirm methicillin resistance *mec*A gene was assessed.

Variables were compared using Chi-square or Mann-Whitney U-test, as appropriate, by SPSS software, version 20.0 (SPSS Inc, Chicago, IL, USA). A p-value ≤ 0.05 was considered statistically significant. Multivariate logistic regression analysis was performed to identify independent risk factors for 30-day mortality.

Results

During the study period, 109 patients were included and 126 episodes of Staphylococcal infections were reported. Patients had a median Charlson comorbidity score of 2 (IQR, 1-3) and 69 patients (63%) had cardiovascular disease as comorbidity. Twenty-seven patients (25%) died and the median length of stay (LOS) was 30 days (IQR: 13-54). Ninety-one cases (72%) were considered as HA-infections and mortality was higher in comparison to CA-infections (25% vs. 10%, p=0.056).

The most common infection sites were skin and soft tissue (SST) or surgical site infections (SSI) (34%), bloodstream infections (BSI) (32%), pneumonia (PNEU) and lower respiratory tract infections (LRI) (31%). SSI and SST were more frequent for *S. aureus* (42%), while BSI represented 40% of CoNS infections.

S. aureus was the etiologic agent responsible for 73 cases (58%). The most recurrent

species in CoNS infections (n=53) were *S. epidermidis* (62%), *S. lugdunensis* (15%), *S. hominis* (8%), *S. warneri* (6%), *S. haemolyticus* (6%). Differences between *S. aureus* and CoNS infections are shown in Table 1.

The independent risk factors associated with 30-day mortality for Staphylococcal infections were MDR (OR= 3.92, 95% CI: 1.31-11.76, *p*= 0.015), age (OR= 1.03, 95% CI: 1.00-1.06, *p*= 0.032) and Charlson comorbidity score (OR= 1.36, 95% CI: 1.08-1.71, *p*= 0.009). No association with mortality was detected for methicillin-resistant isolates (*p*=0.330) and neither *S. aureus* nor CoNS were correlated with death (*p*=0.447).

MDR was associated with 30-day mortality for *S. aureus* infections (Table 2), whereas Charlson comorbidity index was correlated with the 30-day mortality for CoNS infections (OR= 1.56, 95% CI: 1.13-2.16, *p*= 0.006).

Discussion

A high mortality rate for *S. aureus* BSI and PNEU was reported in this study and the difference compared to a previous report(5) could be explained by a lower rate of CA-infections, which is usually associated with reduced mortality. Traditionally, lower mortality rates were associated to BSI caused by CoNS; however, in another previous study it was about 26%(6). A similar mortality rate was found in the current study and, surprisingly, there was no significant difference between mortality for *S. aureus* and CoNS infections. Considering the clinical features of CoNS infections, these data suggest the importance of hospital infection prevention and control programs, especially in ICU-admitted patients with invasive devices(3).

LOS is another important outcome, since it is responsible for an increase of in-hospital costs(7). Limited data were available for CoNS infections, but similar length of ICU

stay (24 days) was reported in a previous study(6). Unlike our findings, a long LOS (42 days) was previously verified for *S. aureus* infections(8) and the difference could probably be attributed to the low prevalence of methicillin resistance.

The influence of vancomycin MIC on outcome has been thoroughly investigated in four meta-analyses. According to the most recent one(9), there were no significant differences in the risk of death when comparing high-VAN MIC (≥1.5 mg/L) with low-VAN MIC, data similar to our findings.

Disagreements about the impact of methicillin resistance on mortality have been reported and meta-analyses including studies published until the year 2000 observed a 2-fold increased risk of mortality(2). Thereafter, in agreement with the present study, individual studies found no association with mortality after adjustments for confounders(1). Similarly to previous studies(1,8), LOS was prolonged in cases of methicillin resistance.

Regarding MDR, gram-negative pathogens have been of the most concern. In the present study, however, MDR *Staphylococcus* spp. and MDR *S. aureus* were independently associated with 30-day mortality and no previous study described this effect on the outcome. Differences between the impact of methicillin-resistant and MDR strains on outcome are probably related to the presence of distinct types of Staphylococcal cassette chromosome *mec*A (SCC*mec*), which encodes resistance to β-lactam and other antibiotic classes.

Considering the impact of methicillin-resistant and MDR isolates on outcome and therefore on hospital costs, antimicrobial stewardship is recommended to decrease the selective pressure produced by antibiotic prescribing(10).

Our study should be interpreted considering the fact that it is a single-center study with

a small sample size as limitation. However, despite of the limitation, our findings highlight the importance of developing guidelines aimed at the prevention of methicillin-resistant and MDR *Staphylococcus* spp. dissemination within healthcare settings. Thus, appropriate hand hygiene, contact isolation, cohorting patients and decrease in LOS are measures to prevent mainly the spread of opportunistic pathogens such as CoNS. Furthermore, antimicrobial stewardship is another essential tool to prevent the emergence of resistant strains, which were associated with increased mortality and LOS.

References

1. Cosgrove SE, Qi Y, Kaye KS, Harbarth S, Karchmer AW, Carmeli Y. The Impact of Methicillin Resistance in *Staphylococcus aureus* Bacteremia on Patient Outcomes: Mortality, Length of Stay, and Hospital Charges. *Infection Control and Hospital Epidemiology*. 2005;26(2):166-74.

2. Cosgrove SE, Sakoulas G, Perencevich EN, Schwaber MJ, Karchmer AW, Carmeli Y. Comparison of Mortality Associated with Methicillin-Resistant and Methicillin-Susceptible *Staphylococcus aureus* Bacteremia: A Meta-analysis. *Clinical Infectious Diseases*. 2003 January 1, 2003;36(1):53-9.

3. Rogers KL, Fey PD, Rupp ME. Coagulase-negative Staphylococcal infections. *Infectious Disease Clinics North America*. 2009 Mar;23(1):73-98.

4. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection*. 2012;18(3):268-81.

5. Kang C-I, Song J-H, Chung DR, Peck KR, Ko KS, Yeom J-S, et al. Clinical impact of

methicillin resistance on outcome of patients with *Staphylococcus aureus* infection: A stratified analysis according to underlying diseases and sites of infection in a large prospective cohort. *Journal of Infection*. 2010;61(4):299-306.

6. Olaechea PM, Álvarez-Lerma F, Palomar M, Insausti J, López-Pueyo MJ, Martínez-Pellús A, et al. Impact of primary and intravascular catheter-related bacteremia due to coagulase-negative *staphylococci* in critically ill patients. *Medicina Intensiva (English Edition)*. 2011;35(4):217-25.

7. Cohen B, Larson EL, Stone PW, Neidell M, Glied SA. Factors associated with variation in estimates of the cost of resistant infections. *Medical Care*. 2010 Sep;48(9):767-75.

8. Su C-H, Chang S-C, Yan J-J, Tseng S-H, Chien L-J, Fang C-T. Excess Mortality and Long-Term Disability from Healthcare-Associated *Staphylococcus aureus* Infections: A Population-Based Matched Cohort Study. *PLOS ONE*. 2013;8(8):e71055.

9. Kalil AC, Van Schooneveld TC, Fey PD, Rupp ME. Association between vancomycin minimum inhibitory concentration and mortality among patients with *Staphylococcus aureus* bloodstream infections: A systematic review and meta-analysis. *JAMA*. 2014;312(15):1552-64.

10. Henderson DK. Managing methicillin-resistant staphylococci: A paradigm for preventing nosocomial transmission of resistant organisms. *American Journal of Infection Control*. 2006;34(5):S46-S54.

Table 1. Comparison of patient demographics and outcome in *S. aureus* and Coagulase-negative *Staphylococcus* infections

Variables, n (%)	*S. aureus* (n= 73)	CoNS (n= 53)	*p*-value
Gender			
Male	42 (57)	28 (53)	0.840
Charlson comorbidity score, median (IQR)	2 (0-3)	2 (1-3)	0.430
Previous healthcare institution exposure	47 (64)	20 (38)	0.014
ICU admission	36 (49)	36 (68)	0.072
Invasive procedures / devices			
CVC	35 (48)	35 (66)	0.099
Mechanical ventilation	29 (40)	31 (58)	0.082
Urinary catheter	44 (60)	34 (64)	0.764
Surgery	49 (67)	33 (62)	0.731
Acquisition of infection			
Healthcare-associated	44 (60)	46 (85)	0.007
LOS (days), median (IQR)			
Hospital	25 (10-55)	33 (19-54)	0.068
Post-infection	12 (3-29)	18 (10-38)	0.026

ICU	8 (1-15)	15 (9-27)	0.004
Mortality			
30-day mortality	20 (27)	11 (21)	0.447
Antimicrobial resistance			
Methicillin-resistant	11 (15)	44 (83)	<0.001
Multidrug-resistant	25 (34)	38 (72)	<0.001
VAN MIC (mg/L), mean±SD			
BMD	1.19±1.06	1.47±0.69	0.003
Etest	1.71±1.15	1.96±0.66	0.002

IQR, interquartile range; LOS, length of stay; ICU, intensive care unit; CVC, Central venous catheter; VAN MIC, Vancomycin minimum inhibitory concentration; BMD, broth microdilution.

Table 2. Factors associated with 30-day all-cause mortality in cases *S. aureus* infections (n= 73)

	30-day all-cause mortality		Statistical analysis	
Variable, n (%)	Survival (n= 53)	Non-survival (n= 20)	Univariate *p*-value	Mutivariate *p*-value; OR (95% CI)
Pathogen				
MRSA	4 (8)	7 (33)	0.033	0.650; 1.58 (0.22-11.40)
MDR	12 (23)	13 (63)	0.004	0.028; 5.13 (1.20-22.05)
VAN MIC ≥ 1.5 mg/L (Etest)	36 (69)	13 (68)	1.000	-
Subject				
Male	31 (58)	11 (53)	0.880	-
Age (years), median (IQR)	67 (42-76)	66 (54-82)	0.237	-
Charlson comorbidity score, median (IQR)	1 (0-2)	3 (2-4)	0.003	0.314; 1.23 (0.82-1.85)
Sepsis/Shock	8 (15)	12 (60)	0.001	0.148; 3,75 (0.63-22.42)

Hospital-associated infection	29 (54)	16 (79)	0.110	-

OR, odds ratio; CI, confidence interval; MRSA, methicillin-resistant *S. aureus*; MDR, multidrug-resistant; VAN MIC, Vancomycin minimum inhibitory concentration; IQR, interquartile range; ICU, intensive care unit.

3.2 Relação do desfecho com fatores de virulência e moleculares

Staphylococcus aureus infections: the role of virulence factors and antimicrobial resistance on pathogenesis and patient outcome

Janine de Melo Rauber, Guilherme Henrique de Oliveira Arnhold, Patrícia Raquel Wappler, Gabriele de Moura Baierle, Barbara Bagiotto, Marcelo Carneiro, Pedro Alves d'Azevedo, Andréia Rosane de Moura Valim

Artigo a ser submetido ao periódico Virulence

Staphylococcus aureus infections: the role of virulence factors and antimicrobial resistance on pathogenesis and patient outcome

Janine M. Rauber[1], Guilherme H. O. Arnhold[2], Patrícia R. Wappler[2], Gabriele M. Baierle[2], Barbara Bagiotto[2], Marcelo Carneiro[2,3], Pedro A. d'Azevedo[1], Andréia R. M. Valim[2*]

Affiliations. [1] Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), Porto Alegre, RS, Brazil. [2] Universidade de Santa Cruz do Sul (UNISC), Santa Cruz do Sul, RS, Brazil. [3] Hospital Santa Cruz (HSC), Santa Cruz do Sul, RS, Brazil.

***Address correspondence.** Universidade de Santa Cruz do Sul, Departamento de Biologia e Farmácia, Avenida Independência, 2293, Universitário, CEP 96815-900, Santa Cruz do Sul, RS, Brazil. Phone: 55 51 3717-7399; Fax: 55 51 3717-7399 (avalim@unisc.br).

Keywords. *Staphylococcus aureus*; Virulence factors; Accessory gene regulator; Panton-Valentine leucocidin; Staphylococcal cassette chromosome *mec*; Molecular epidemiology.

Abbreviations. Vancomycin-resistant *Staphylococcus aureus* (VRSA); vancomycin-intermediate *S. aureus* (VISA); heterogeneous vancomycin-intermediate *S. aureus* (hVISA); community-acquired (CA); healthcare-associated (HA); Panton-Valentine

leucocidin (PVL); Staphylococcal cassette chromosome *mec* (SCC*mec*); accessory gene regulator (*agr*); minimum inhibitory concentration (MIC); penicillin-binding protein (PBP); skin and soft tissue (SST); surgical site infection (SSI); bloodstream infection (BSI); pneumonia (PNEU); lower respiratory tract infections (LRI); length of stay (LOS); intensive care unit (ICU); polymerase chain reaction (PCR); broth microdilution (BMD); brain heart infusion (BHI); population analysis profile / area under the curve (PAP/AUC); Clinical Laboratory Standards Institute (CLSI); Multidrug resistance (MDR).

Abstract

Staphylococcus aureus is an important cause of community-acquired (CA) and healthcare-associated (HA) infections. The success of *S. aureus* as pathogen is a result of the expression of several virulence factors combined with antimicrobial resistance. To determine molecular characteristics and virulence factors in *S. aureus* isolates and their association with resistance and patient outcome, 83 samples were evaluated to assess antimicrobial susceptibility, Panton-Valentine leucocidin (PVL) presence, Staphylococcal cassette chromosome *mec* (SCC*mec*) and accessory gene regulator (*agr*) typing, biofilm and δ-hemolysin production. PVL was detected in 25 isolates (30%), most of strains were typed as SCC*mec* IV (35%), SCC*mec* III (14%), *agr* I (46%), *agr* II (24%) and the absence of *agr* was verified in 11% (*agr*-defective). 30-day mortality was 28% and there was no influence of *agr*-defective or functional *agr* status. The *agr*-defective strains were associated to vancomycin minimum inhibitory concentration (MIC) increase and heterogeneous vancomycin-intermediate isolates (hVISA). The *agr* dysfunction was found in 28% of cases and was significantly higher in methicillin-resistant strains (MRSA). Although no association was found with outcome, epidemiological studies remain an essential tool for surveillance and have contributed to the knowledge about pathogenesis, adaptation to environment, antimicrobial resistance and virulence acquisition.

Introduction

Staphylococcus aureus is a common cause of infection, responsible for skin infections and serious invasive diseases[1]. Colonization by *S. aureus* is an important risk factor for infection[2] and the accessory gene regulator (*agr*) plays a significant role in Staphylococcal pathogenesis[3]. The *agr* quorum-sensing system upregulates secreted virulence factors and downregulates cell surface proteins, thereby managing invasiveness and cell dispersal from biofilms[4]. Mutations causing *agr* dysfunction influence disease development and the production of δ-hemolysin from RNAIII translation indicates a functional *agr* status[5, 6].

Biofilm production and Panton-Valentine leucocidin (PVL) are among the major virulence factors of *S. aureus* controlled by *agr*. Biofilm consists of sticky, surface-attached agglomerations of bacteria and it is difficult to treat, because the association of biofilm matrix and phenotypic characteristics of the bacteria confer resistance to the host immune response and antimicrobial drugs[7, 8]. In nosocomial infections, *S. aureus* ability to form biofilm in indwelling medical devices is essential to cause disease[9]. PVL, a bicomponent cytotoxin encoded by lukS-PV and lukF-PV genes, was associated with necrotic skin lesions including severe necrotizing pneumonia[10-12]. Although the exact role of PVL in *S. aureus* pathogenesis is unclear[13], in specific infection types and scenarios, PVL appears to significantly contribute to disease severity[14].

Due the outstanding ability to acquire resistance to antibiotics, the prevalence of methicillin-resistant *S. aureus* (MRSA) has increased over the last decades[15]. The Staphylococcal cassette chromosome *mec* (SCC*mec*) harbors the *mec*A gene, which is responsible for the methicillin resistance of *S.aureus* by encoding a novel penicillin-binding protein (PBP) designated PBP2a[16]. SCC*mec* is a mobile genetic element (MGE)

in MRSA and has been differentiated into at least 12 genetic types[17], among which types I to III are commonly found in healthcare-associated (HA)-MRSA, while type IV and V were reported to be frequently found in community-acquired (CA)-MRSA[18]. However, studies have suggested that CA-MRSA strains may be encroaching on nosocomial environment[19, 20], which makes distinction between HA and CA-MRSA strains more difficult in terms of SCC*mec* type.

In addition to methicillin resistance, strains with reduced susceptibility to vancomycin (VISA and hVISA) have arisen and reports of heteroresistance in *S. aureus* infections are increasingly frequent[21]. Interestingly, *van*A, *van*B, and *van*C genes that mediate resistance in vancomycin-resistant isolates (VRSA) were not found in VISA and hVISA strains[22]. Although the genetic basis has not been determined in general, the presence of a thickened cell wall with several more peptidoglycan layers appears to be responsible for reduced susceptibility to vancomycin[23]. The association between reduced susceptibility to vancomycin and dysfunctional *agr* can explain the VISA and hVISA phenotypes, since the *agr* system represses cell-wall proteins and activates exoprotein secretion[24].

Thus, the coordinated expression of several virulence factors coupled with antimicrobial resistance in adaptation to different environments has ensured the success of *S. aureus* as a pathogen. In order to increase the knowledge on *S. aureus* pathogenesis, the aim of the study was to determine molecular characteristics and virulence factors in *S. aureus* isolates and their association with antimicrobial resistance and patient outcome.

Results

During the study period, 83 episodes of *S. aureus* infections were reported. Fifty

samples (60%) were isolated from male and 74 (89%) from adult patients. Patients had a median Charlson comorbidity score of 2 (IQR, 0-3) and 56% had cardiovascular diseases, 18% endocrinological, 16% neurological and 15% of patients had respiratory diseases as comorbidities. Fifty-four (65%) of the events were classified as HA infections. Shock episodes were documented in 16 patients (19%) and 44 (53%) were admitted at the ICU. The most common infection sites were skin and soft tissue (SST) and surgical site infection (SSI) (39%), bloodstream infection (BSI) (31%), pneumonia (PNEU) and lower respiratory tract infections (LRI) (27%).

Strain characteristics are listed in Table 1. Most biofilm-producing strains were methicillin-susceptible (95 vs. 67%, p= 0.014). PVL was detected mainly in PNEU and LRI (39%), followed by SST and SSI (25%), and BSI (21%). Similar rates of PVL prevalence were observed in CA and HA infections (28 vs. 30%, p= 1.000). There was no difference in PVL frequency between MRSA and MSSA strains (28 vs. 43%, p= 0.330), and hVISA isolates (p= 0.888). However, PVL-positive strains showed a decrease in MDR phenotype (38 vs. 16%, p= 0.094). Six isolates of MRSA were PVL positive and SCC*mec* detected were type IVc (33%), type IVa (17%) and simultaneous detection of two types (50%). Regarding *agr*, all *agr* type IV samples showed PVL, whereas for other *agr* types (I, II and III) the PVL frequency range was between 30 to 40%.

Table 2 shows the association between *agr* status and resistance profile of strains or patients' outcome. Absence of *agr* (*agr*-defective) was associated to vancomycin MIC increase detected by Etest and the hVISA phenotype was more frequently observed in *agr*-defective strains. *agr* dysfunction was significantly higher in MRSA and MDR isolates (p= 0.002). The *agr* status (defective and dysfunctional) was similar in CA and HA infections and did not affect the frequency of shock in patients. There was no

influence of *agr*-defective or functional *agr* status on outcome (mortality and LOS). Biofilm production did not affect the mortality rate (p= 1.000) and there was no difference in 30-day mortality between PVL positive and negative strains (16 vs. 26%, p= 0.462).

Discussion

SCC*mec* and *agr* type prevalence varies considerably due to geographical location, as well the presence of virulence factors. Moreover, the frequency of these epidemiological markers is different according to the type of infection[12, 25]. Whereas the present study provides a molecular evaluation in different infections (BSI, PNEU, LRI, SST, SSI and UTI), a comparison with previous studies is difficult, since many studies evaluate only specific types of infection, e.g., bacteremia. However, similarly to our findings, a recent study in northeast Brazil[26] including different types of infection reported SCC*mec* types III and IV as more frequently isolated and Kimura et al.[27] found SCC*mec* type IV as predominant in blood, skin, and soft tissue isolates in Japan.

The SCC*mec* type IV is usually associated to CA-MRSA isolates and the high prevalence of this specific type can be explained by a successful combination of methicillin resistance with virulence verified in CA-MRSA infections[14]. Differences in fitness cost were detected comparing SCC*mec* types I and IV, proving that the presence of SCC*mec* type IV decreases the energetic cost in methicillin-resistant strains[28]. Furthermore, the expression of *mec*A gene is responsible for reducing the ability to secrete toxins. CA-MRSA expresses less PBP2a than HA-MRSA and, thus, it has a higher virulence expression combined with methicillin resistance, contributing to its success in the environment[29].

CA-MRSA is associated with an increased presence of virulence factors, including

PVL[14, 30]. However, Brust et al.[31] reported sixteen PVL-positive strains in an HA-MRSA outbreak. Moreover, in agreement with the current study, a high rate of HA-MRSA encoding PVL was described in a previous study[32] and there was no difference in PVL prevalence between CA and HA infections reported by Yu et al.[33].

Although poorly investigated, PVL is also encoded by MSSA isolates and a high frequency has been reported in Africa or Asia compared to the United States[34, 35]. The high frequency of PVL among MSSA strains is of special interest, since the most successful CA-MRSA clones share this genetic marker. A previous study assessing the genetic diversity indicated a probable dissemination of novel SCC*mec* elements among pre-existing PVL positive MSSA strains, rather than the spread of PVL phages among MRSA strains[36]. Similarly to our findings, Campbell et al.[37] found no difference in the PVL rates between MRSA and MSSA isolates.

In contrast to our study, Nurjadi et al.[38] described a higher MDR phenotype in PVL-positive strains. However, there is a significant difference in MDR definition between the current and the previous studies. Whereas for Nurjadi et al.[38] MDR means a resistance to any two or more antibiotics, for our study MDR means a resistance to at least three agents of different antimicrobial categories[39]. Thus, a greater number of isolates was defined as MDR in the previous study when compared with the current, explaining the contrast in results.

Biofilm is another important virulence factor in *S. aureus* infections. McCarthy et al.[40] described different mechanisms used by MSSA and MRSA isolates to form biofilm and how the acquisition or loss of methicillin resistance impacts on biofilm production. Clinical MSSA strains predominantly form biofilm dependent on the *icaADBC* operon and polysaccharide intercellular adhesin (PIA) production, whereas MRSA strains form

biofilms independent of PIA[41]. Regarding the acquisition of methicillin resistance, a high level of *mec*A gene expression is associated with repression of the *agr* system, leading to changes in cell wall, decrease in cytotoxin production and attenuated virulence[29, 42]. Our findings are in agreement with those, since we reported a lower biofilm production by MRSA isolates.

Most studies have evaluated *agr* dysfunction, rather than *agr* absence. *agr* dysfunction was associated with an elevated vancomycin MIC[43] and with hVISA phenotype[44], but in the present study a high MIC and heteroresistance were associated with *agr* absence. Results are similar, since a strain with *agr* absence will mandatorily not produce delta-hemolysin and will therefore be classified as dysfunctional *agr* isolate. In addition, our study has shown that *agr* dysfunction was significantly higher in MRSA and MDR isolates. According to Rudkin et al.[45], a high level of expression of PBP2a affects the responsiveness of the *agr* system and subsequently affects delta-hemolysin expression; therefore, the resistant strains have more *agr* dysfunction.

Paulander et al.[46] reported that the presence of the *agr* system imposes a fitness cost for *S. aureus* due the expression of RNAIII and indicated a possible adaptation against antibiotic pressure involving the loss of *agr*. Thus, the extensive use of antibiotics in hospitals can explain the reason for the high frequency of *agr*-negative isolation from nosocomial infections, when compared to community-acquired infections[47]. CA-MRSA strains probably require *agr* expression to evade innate host defenses and produce toxins, whereas HA-MRSA strains do not require high virulence expression to affect immunocompromised and critically-ill patients using medical devices such as intravascular catheters[48]. The activation of *agr* is suggested to be energetically expensive and, therefore, transition to a dysfunctional *agr* status is beneficial for long-term survival once innate host defenses have been evaded[49].

Antibiotic resistance genes are often located on MGEs such as transposons, plasmids, or genomic islands and SCC*mec* is an important element in *S. aureus*[50]. MGEs contain antibiotic resistance genes and usually do not contain genes for virulence determinants[51]. However, Kaito et al.[52] reported that the *psm-mec* gene, initially described in SCC*mec* types II and III, is responsible for virulence regulation, representing the first toxin gene of *S. aureus* co-localized on MGEs with antibiotic resistance genes[51]. The SCC*mec* of CA-MRSA does not contain the *psm-mec* gene, unlike the HA-MRSA SCC*mec*. Furthermore, some HA-MRSA strains carry mutated *psm-mec* or no *psm-mec* and produce higher amounts of extracellular toxins than HA-MRSA strains carrying intact *psm-mec*[52]. The transcription product of the *psm-mec* gene, located in the mobile genetic element SCC*mec* of HA-MRSA, but not in CA-MRSA, suppresses the expression of phenol-soluble modulin a (PSMa), a cytolytic toxin of *S. aureus*, increases biofilm formation and acts as a regulator by inhibiting AgrA translation[53]. In addition to the high frequency of *agr* loss or dysfunction, the presence of *psm-mec* gene in HA-MRSA strains can explain the reason of attenuated virulence compared to CA-MRSA isolates. The inhibition of the *agr* system by *psm-mec* might contribute to less energy-consuming and fitness to the antibiotic environment, since nosocomial strains do not require evading the host's defense barriers[53].

Similar to our findings, in a meta-analysis about the role of the PVL in Staphylococcal disease[12], PVL strains were not associated with increased mortality, prolonged hospital stay or increased disease severity, when assessed by the Acute Physiology and Chronic Health Evaluation II (APACHE) score. Poor evidence correlating biofilm production of *S. aureus* and outcome are available, but for *S. epidermidis* orthopedic device-related infections, the biofilm-forming ability affected the treatment outcome[54].

Previous studies have correlated *agr* dysfunction with higher mortality and prolonged

bacteremia, but the precise mechanism has yet to be established[55, 56]. According to Kang et al.[25], mortality attributed to MRSA bacteremia was less frequent in SCC*mec* type IV strains than types II and III. The present study found no association between outcome and *agr* function or SCC*mec* type; however, different types of infection were evaluated, instead of bacteremia. Pragman et al.[57] reported that *in vivo* expression of *S. aureus* virulence genes must occur in response to other virulence regulators rather than *agr* and, thus, *agr* system is not the master virulence regulator as described *in vitro*. The role of *agr* appears to be more related with pathogenesis than disease development or outcome, since down-regulation of *agr* is associated with colonization and *agr* activation with host invasion[25, 58].

Indeed, the success of *S. aureus* as a pathogen is a combination of virulence and resistance in environment; thus, the regulatory interdependence of the *mec* and *agr* systems may contribute to balancing these two important energy-consuming pathogenesis mechanisms. Although no association was identified between virulence factors and outcome in our study, a better understanding of the molecular epidemiology and the complex pathogen–host interactions can improve the design of strategies for prevention and treatment of *S. aureus* infections. Furthermore, epidemiological studies remain an essential surveillance tool and have contributed to the knowledge about pathogenesis, adaptation to environment, antimicrobial resistance and virulence acquisition.

Materials and Methods

Study Design and Population

A prospective cohort study was conducted at a 234-bed, tertiary care teaching hospital located in southern Brazil. Patients who had an inpatient positive culture for *S. aureus*

admitted between July 2012 and June 2014 were eligible for inclusion. Samples were excluded in cases of duplicates or relapse of current infections. The study was reviewed and approved by the Research Ethics Committee of Universidade Federal de Ciências da Saúde de Porto Alegre (1779/12).

Clinical Data and Definitions

The following information was collected from the patients' medical records using a standardized data collection form: demography characteristics, medical history (ICD – International Classification of Disease), previous healthcare institution exposure, comorbidities, Charlson comorbidity score[59], intensive care unit (ICU) admission, antimicrobial treatment, history of surgery or invasive devices, sepsis and/or shock[60], length of hospital stay, and outcome (discharge from hospital or 30-day all-cause mortality).

Definitions of clinical outcomes were adapted from clinical studies of patients with Staphylococcal infections[61, 62]. Healthcare-associated (HA), community-acquired (CA) infections and type of infection were determined according to previously described definitions and criteria[63, 64].

Multidrug resistance (MDR) was defined as non-susceptibility to at least one agent in three or more antimicrobial categories[39].

Microbiological and Molecular Methods

All isolates were identified according to standard methods[65]. Individual isolates were then stored in skim milk with 20% glycerol at -20°C until microbiological testing was performed. Genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega Corporation).

Antimicrobial susceptibility: Initial testing for methicillin resistance was performed according to the Clinical Laboratory Standards Institute (CLSI) guidelines, using a 30-µg cefoxitin disc and Mueller-Hinton agar. Susceptibility tests for other antimicrobials were assessed using the Kirby-Bauer disc diffusion method according to CLSI. Vancomycin minimum inhibitory concentration (MIC) was determined through the broth microdilution (BMD) and Etest (bioMérieux) methods, performed in agreement with the CLSI guidelines and the manufacturer's instructions, respectively. Screening test for hVISA was performed using brain heart infusion (BHI) agar plates containing 6 µg/mL of vancomycin (Sigma) and ATCC 700698 (Mu3), which was used as positive control. hVISA isolates were confirmed using the population analysis profile / area under the curve (PAP/AUC) method[66].

Biofilm: A microtiter plate assay was performed as described by Stepanovic et al.[67], with some modifications.

Delta-hemolysin: The expression of the *agr* gene cluster was determined by quantitating delta-hemolysin production, utilizing a previously described method[68].

Molecular analysis: *mec*A gene detection by polymerase chain reaction (PCR) was performed to confirm methicillin resistance of isolates[69]. The staphylococcal cassette chromosome *mec* (SCC*mec*) type and accessory gene regulator (*agr*) genotype were characterized using multiplex PCR, as previously described[70, 71]. PCR for the presence of the gene encoding Panton-Valentine Leucocidin (PVL) was performed as described by Lina et al.[10].

Statistical Analysis

Categorical variables were compared using Chi-square or Fisher's exact test and continuous variables were compared using the Mann-Whitney U-test, as appropriate.

All analyses were performed using the computer software SPSS version 20.0 (SPSS Inc, Chicago, IL, USA). A p-value ≤ 0.05 was considered statistically significant.

References

1. Lowy FD. Staphylococcus aureus Infections. New England Journal of Medicine 1998; 339:520-32.
2. von Eiff C, Becker K, Machka K, Stammer H, Peters G. Nasal carriage as a source of Staphylococcus aureus bacteremia. Study Group. The New England journal of medicine 2001; 344:11-6.
3. Yarwood JM, Schlievert PM. Quorum sensing in Staphylococcus infections. Journal of Clinical Investigation 2003; 112:1620-5.
4. Singh R, Ray P. Quorum sensing-mediated regulation of staphylococcal virulence and antibiotic resistance. Future Microbiol 2014; 9:669-81.
5. Dinges MM, Orwin PM, Schlievert PM. Exotoxins of Staphylococcus aureus. Clinical microbiology reviews 2000; 13:16-34.
6. Traber KE, Lee E, Benson S, Corrigan R, Cantera M, Shopsin B, Novick RP. agr function in clinical Staphylococcus aureus isolates. Microbiology (Reading, England) 2008; 154:2265-74.
7. Costerton JW, Cheng KJ, Geesey GG, Ladd TI, Nickel JC, Dasgupta M, Marrie TJ. Bacterial biofilms in nature and disease. Annu Rev Microbiol 1987; 41:435-64.
8. Otto M. Molecular basis of Staphylococcus epidermidis infections. Semin Immunopathol 2012; 34:201-14.
9. Vuong C, Saenz HL, Götz F, Otto M. Impact of the agr Quorum-Sensing System on Adherence to Polystyrene in Staphylococcus aureus. Journal of Infectious Diseases 2000; 182:1688-93.
10. Lina G, Piemont Y, Godail-Gamot F, Bes M, Peter MO, Gauduchon V, Vandenesch F, Etienne J. Involvement of Panton-Valentine leukocidin-producing Staphylococcus aureus in primary skin infections and pneumonia. Clin Infect Dis 1999; 29:1128-32.
11. Gillet Y, Issartel B, Vanhems P, Fournet J-C, Lina G, Bes M, Vandenesch F, Piémont Y, Brousse N, Floret D, et al. Association between Staphylococcus aureus strains carrying gene for Panton-Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. The Lancet 2002; 359:753-9.
12. Shallcross LJ, Fragaszy E, Johnson AM, Hayward AC. The role of the Panton-Valentine leucocidin toxin in staphylococcal disease: a systematic review and meta-analysis. The Lancet Infectious Diseases 2013; 13:43-54.
13. Ben Zakour NL, Guinane CM, Fitzgerald JR. Pathogenomics of the staphylococci: insights into niche adaptation and the emergence of new virulent strains. FEMS Microbiology Letters 2008; 289:1-12.
14. Otto M. Community-associated MRSA: what makes them special? International journal of medical microbiology : IJMM 2013; 303:324-30.
15. Chambers HF, Deleo FR. Waves of resistance: Staphylococcus aureus in the

antibiotic era. Nature reviews Microbiology 2009; 7:629-41.
16. Katayama Y, Ito T, Hiramatsu K. A New Class of Genetic Element, Staphylococcus Cassette Chromosome mec, Encodes Methicillin Resistance in Staphylococcus aureus. Antimicrobial Agents and Chemotherapy 2000; 44:1549-55.
17. Wu Z, Li F, Liu D, Xue H, Zhao X. Novel Type XII Staphylococcal Cassette Chromosome mec Harboring a New Cassette Chromosome Recombinase, CcrC2. Antimicrobial Agents and Chemotherapy 2015; 59:7597-601.
18. Naimi TS, LeDell KH, Como-Sabetti K, et al. COmparison of community- and health care–associated methicillin-resistant staphylococcus aureus infection. JAMA 2003; 290:2976-84.
19. Seybold U, Kourbatova EV, Johnson JG, Halvosa SJ, Wang YF, King MD, Ray SM, Blumberg HM. Emergence of Community-Associated Methicillin-Resistant Staphylococcus aureus USA300 Genotype as a Major Cause of Health Care—Associated Blood Stream Infections. Clinical Infectious Diseases 2006; 42:647-56.
20. Popovich KJ, Weinstein RA, Hota B. Are Community-Associated Methicillin-Resistant Staphylococcus aureus (MRSA) Strains Replacing Traditional Nosocomial MRSA Strains? Clinical Infectious Diseases 2008; 46:787-94.
21. Zhang S, Sun X, Chang W, Dai Y, Ma X. Systematic Review and Meta-Analysis of the Epidemiology of Vancomycin-Intermediate and Heterogeneous Vancomycin-Intermediate Staphylococcus aureus Isolates. PLOS ONE 2015; 10:e0136082.
22. Howden BP, Johnson PDR, Ward PB, Stinear TP, Davies JK. Isolates with Low-Level Vancomycin Resistance Associated with Persistent Methicillin-Resistant Staphylococcus aureus Bacteremia. Antimicrobial Agents and Chemotherapy 2006; 50:3039-47.
23. Howden BP, Davies JK, Johnson PDR, Stinear TP, Grayson ML. Reduced Vancomycin Susceptibility in Staphylococcus aureus, Including Vancomycin-Intermediate and Heterogeneous Vancomycin-Intermediate Strains: Resistance Mechanisms, Laboratory Detection, and Clinical Implications. Clinical microbiology reviews 2010; 23:99-139.
24. Bronner S, Monteil H, Prévost G. Regulation of virulence determinants in Staphylococcus aureus: complexity and applications. FEMS microbiology reviews 2004; 28:183-200.
25. Kang CK, Cho JE, Choi YJ, Jung Y, Kim N-H, Kim C-J, Kim TS, Song K-H, Choe PG, Park WB, et al. agr Dysfunction Affects Staphylococcal Cassette Chromosome mec Type-Dependent Clinical Outcomes in Methicillin-Resistant Staphylococcus aureus Bacteremia. Antimicrobial Agents and Chemotherapy 2015; 59:3125-32.
26. Andrade-Figueiredo M, Leal-Balbino TC. Clonal diversity and epidemiological characteristics of Staphylococcus aureus: high prevalence of oxacillin-susceptible mecA-positive Staphylococcus aureus (OS-MRSA) associated with clinical isolates in Brazil. BMC Microbiology 2016; 16:115.
27. Kimura Y, Morinaga Y, Akamatsu N, Matsuda J, Yamaryo T, Kawakami K, Matsuo H, Kosai K, Uno N, Hasegawa H, et al. Antimicrobial susceptibility and molecular characteristics of methicillin-resistant Staphylococcus aureus in a Japanese secondary care facility. Journal of Infection and Chemotherapy 2016; 22:14-8.
28. Lee SM, Ender M, Adhikari R, Smith JMB, Berger-Bächi B, Cook GM. Fitness

Cost of Staphylococcal Cassette Chromosome mec in Methicillin-Resistant Staphylococcus aureus by Way of Continuous Culture. Antimicrobial Agents and Chemotherapy 2007; 51:1497-9.
29. Rudkin JK, Edwards AM, Bowden MG, Brown EL, Pozzi C, Waters EM, Chan WC, Williams P, O'Gara JP, Massey RC. Methicillin Resistance Reduces the Virulence of Healthcare-Associated Methicillin-Resistant Staphylococcus aureus by Interfering With the agr Quorum Sensing System. The Journal of infectious diseases 2012; 205:798-806.
30. Vandenesch F, Naimi T, Enright MC, Lina G, Nimmo GR, Heffernan H, Liassine N, Bes M, Greenland T, Reverdy M-E, et al. Community-Acquired Methicillin-Resistant Staphylococcus aureus Carrying Panton-Valentine Leukocidin Genes: Worldwide Emergence. Emerging infectious diseases 2003; 9:978-84.
31. Brust T, da Costa TM, Amorim JC, Asensi MD, Fernandes O, Aguiar-Alves F. Hospital-associated methicillin-resistant Staphylococcus aureus carrying the PVL gene outbreak in a Public Hospital in Rio de Janeiro, Brazil. Brazilian Journal of Microbiology 2013; 44:865-8.
32. Mariem BJ-J, Ito T, Zhang M, Jin J, Li S, Ilhem B-BB, Adnan H, Han X, Hiramatsu K. Molecular characterization of methicillin-resistant Panton-valentine leukocidin positive staphylococcus aureus clones disseminating in Tunisian hospitals and in the community. BMC Microbiology 2013; 13:2-.
33. Yu F, Chen Z, Liu C, Zhang X, Lin X, Chi S, Zhou T, Chen X. Prevalence of Staphylococcus aureus carrying Panton—Valentine leukocidin genes among isolates from hospitalised patients in China. Clinical Microbiology and Infection 2008; 14:381-4.
34. Egyir B, Guardabassi L, Sørum M, Nielsen SS, Kolekang A, Frimpong E, Addo KK, Newman MJ, Larsen AR. Molecular Epidemiology and Antimicrobial Susceptibility of Clinical Staphylococcus aureus from Healthcare Institutions in Ghana. PLOS ONE 2014; 9:e89716.
35. Aung MS, Zi H, Nwe KM, Maw WW, Aung MT, Min WW, Nyein N, Kawaguchiya M, Urushibara N, Sumi A, et al. Drug resistance and genetic characteristics of clinical isolates of staphylococci in Myanmar: high prevalence of PVL among methicillin-susceptible Staphylococcus aureus belonging to various sequence types. New Microbes and New Infections 2016; 10:58-65.
36. Monecke S, Slickers P, Ellington MJ, Kearns AM, Ehricht R. High diversity of Panton–Valentine leukocidin-positive, methicillin-susceptible isolates of Staphylococcus aureus and implications for the evolution of community-associated methicillin-resistant S. aureus. Clinical Microbiology and Infection 2007; 13:1157-64.
37. Campbell SJ, Deshmukh HS, Nelson CL, Bae I-G, Stryjewski ME, Federspiel JJ, Tonthat GT, Rude TH, Barriere SL, Corey R, et al. Genotypic Characteristics of Staphylococcus aureus Isolates from a Multinational Trial of Complicated Skin and Skin Structure Infections. Journal of Clinical Microbiology 2008; 46:678-84.
38. Nurjadi D, Friedrich-Jänicke B, Schäfer J, Van Genderen PJJ, Goorhuis A, Perignon A, Neumayr A, Mueller A, Kantele A, Schunk M, et al. Skin and soft tissue infections in intercontinental travellers and the import of multi-resistant Staphylococcus aureus to Europe. Clinical Microbiology and Infection 2015; 21:567.e1-.e10.
39. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, et al. Multidrug-

resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical Microbiology and Infection 2012; 18:268-81.
40. McCarthy H, Rudkin JK, Black NS, Gallagher L, O'Neill E, O'Gara JP. Methicillin resistance and the biofilm phenotype in Staphylococcus aureus. Frontiers in Cellular and Infection Microbiology 2015; 5:1.
41. O'Neill E, Pozzi C, Houston P, Smyth D, Humphreys H, Robinson DA, O'Gara JP. Association between Methicillin Susceptibility and Biofilm Regulation in Staphylococcus aureus Isolates from Device-Related Infections. Journal of Clinical Microbiology 2007; 45:1379-88.
42. Pozzi C, Waters EM, Rudkin JK, Schaeffer CR, Lohan AJ, Tong P, Loftus BJ, Pier GB, Fey PD, Massey RC, et al. Methicillin Resistance Alters the Biofilm Phenotype and Attenuates Virulence in Staphylococcus aureus Device-Associated Infections. PLOS Pathogens 2012; 8:e1002626.
43. Holmes NE, Turnidge JD, Munckhof WJ, Robinson JO, Korman TM, O'Sullivan MVN, Anderson TL, Roberts SA, Warren SJC, Coombs GW, et al. Genetic and Molecular Predictors of High Vancomycin MIC in Staphylococcus aureus Bacteremia Isolates. Journal of Clinical Microbiology 2014; 52:3384-93.
44. Chong YP, Kim ES, Park S-J, Park K-H, Kim T, Kim M-N, Kim S-H, Lee S-O, Choi S-H, Woo JH, et al. Accessory Gene Regulator (agr) Dysfunction in Staphylococcus aureus Bloodstream Isolates from South Korean Patients. Antimicrobial Agents and Chemotherapy 2013; 57:1509-12.
45. Rudkin JK, Laabei M, Edwards AM, Joo H-S, Otto M, Lennon KL, O'Gara JP, Waterfield NR, Massey RC. Oxacillin Alters the Toxin Expression Profile of Community-Associated Methicillin-Resistant Staphylococcus aureus. Antimicrobial Agents and Chemotherapy 2014; 58:1100-7.
46. Paulander W, Nissen Varming A, Bæk KT, Haaber J, Frees D, Ingmer H. Antibiotic-Mediated Selection of Quorum-Sensing-Negative Staphylococcus aureus. mBio 2012; 3.
47. Shopsin B, Drlica-Wagner A, Mathema B, Adhikari RP, Kreiswirth BN, Novick R. Prevalence of agr Dysfunction among Colonizing Staphylococcus aureus Strains. Journal of Infectious Diseases 2008; 198:1171-4.
48. Gray B, Hall P, Gresham H. Targeting agr- and agr-Like Quorum Sensing Systems for Development of Common Therapeutics to Treat Multiple Gram-Positive Bacterial Infections. Sensors (Basel, Switzerland) 2013; 13:5130-66.
49. Cheung GYC, Wang R, Khan BA, Sturdevant DE, Otto M. Role of the Accessory Gene Regulator agr in Community-Associated Methicillin-Resistant Staphylococcus aureus Pathogenesis. Infection and immunity 2011; 79:1927-35.
50. Ito T, Okuma K, Ma XX, Yuzawa H, Hiramatsu K. Insights on antibiotic resistance of Staphylococcus aureus from its whole genome: genomic island SCC. Drug Resistance Updates 2003; 6:41-52.
51. Queck SY, Khan BA, Wang R, Bach T-HL, Kretschmer D, Chen L, Kreiswirth BN, Peschel A, DeLeo FR, Otto M. Mobile Genetic Element-Encoded Cytolysin Connects Virulence to Methicillin Resistance in MRSA. PLOS Pathogens 2009; 5:e1000533.
52. Kaito C, Saito Y, Ikuo M, Omae Y, Mao H, Nagano G, Fujiyuki T, Numata S, Han X, Obata K, et al. Mobile Genetic Element SCC<italic>mec</italic>-encoded <italic>psm-mec</italic> RNA Suppresses Translation of <italic>agrA</italic> and Attenuates MRSA Virulence. PLoS Pathog 2013; 9:e1003269.

53. Ikuo M, Nagano G, Saito Y, Mao H, Sekimizu K, Kaito C. Inhibition of Exotoxin Production by Mobile Genetic Element SCCmec-Encoded psm-mec RNA Is Conserved in Staphylococcal Species. PLOS ONE 2014; 9:e100260.
54. Morgenstern M, Post V, Erichsen C, Hungerer S, Bühren V, Militz M, Richards RG, Moriarty TF. Biofilm formation increases treatment failure in Staphylococcus epidermidis device-related osteomyelitis of the lower extremity in human patients. Journal of Orthopaedic Research 2016:n/a-n/a.
55. Fowler VG, Sakoulas G, McIntyre LM, Meka VG, Arbeit RD, Cabell CH, Stryjewski ME, Eliopoulos GM, Barth Reller L, Ralph Corey G, et al. Persistent Bacteremia Due to Methicillin-Resistant Staphylococcus aureus Infection Is Associated with agr Dysfunction and Low-Level In Vitro Resistance to Thrombin-Induced Platelet Microbicidal Protein. Journal of Infectious Diseases 2004; 190:1140-9.
56. Schweizer ML, Furuno JP, Sakoulas G, Johnson JK, Harris AD, Shardell MD, McGregor JC, Thom KA, Perencevich EN. Increased mortality with accessory gene regulator (agr) dysfunction in Staphylococcus aureus among bacteremic patients. Antimicrob Agents Chemother 2011; 55:1082-7.
57. Pragman AA, Schlievert PM. Virulence regulation in Staphylococcus aureus: the need for in vivo analysis of virulence factor regulation. FEMS Immunology & Medical Microbiology 2004; 42:147-54.
58. Novick RP. Autoinduction and signal transduction in the regulation of staphylococcal virulence. Mol Microbiol 2003; 48:1429-49.
59. Charlson M, Szatrowski TP, Peterson J, Gold J. Validation of a combined comorbidity index. Journal of Clinical Epidemiology 1994; 47:1245-51.
60. Levy M, Fink M, Marshall J, Abraham E, Angus D, Cook D, Cohen J, Opal S, Vincent J-L, Ramsay G. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. Intensive Care Med 2003; 29:530-8.
61. Hermsen ED, Hanson M, Sankaranarayanan J, Stoner JA, Florescu MC, Rupp ME. Clinical outcomes and nephrotoxicity associated with vancomycin trough concentrations during treatment of deep-seated infections. Expert Opin Drug Saf 2010; 9:9-14.
62. Park K-H, Kim ES, Kim HS, Park S-J, Bang KM, Park HJ, Park S-Y, Moon SM, Chong YP, Kim S-H, et al. Comparison of the clinical features, bacterial genotypes and outcomes of patients with bacteraemia due to heteroresistant vancomycin-intermediate Staphylococcus aureus and vancomycin-susceptible S. aureus. Journal of Antimicrobial Chemotherapy 2012; 67:1843-9.
63. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care–associated infection and criteria for specific types of infections in the acute care setting. American journal of infection control 2008; 36:309-32.
64. Klevens R, Morrison MA, Nadle J, et al. Invasive methicillin-resistant staphylococcus aureus infections in the united states. JAMA 2007; 298:1763-71.
65. Tenover FC, Tickler IA, Goering RV, Kreiswirth BN, Mediavilla JR, Persing DH, for the MC. Characterization of Nasal and Blood Culture Isolates of Methicillin-Resistant Staphylococcus aureus from Patients in United States Hospitals. Antimicrobial Agents and Chemotherapy 2012; 56:1324-30.
66. Wootton M, Howe RA, Hillman R, Walsh TR, Bennett PM, MacGowan AP. A modified population analysis profile (PAP) method to detect hetero-resistance to vancomycin in Staphylococcus aureus in a UK hospital. The Journal of antimicrobial chemotherapy 2001; 47:399-403.
67. StepanoviĆ S, VukoviĆ D, Hola V, Bonaventura GD, DjukiĆ S, ĆIrkoviĆ I,

Ruzicka F. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. APMIS : acta pathologica, microbiologica, et immunologica Scandinavica 2007; 115:891-9.

68. Sakoulas G, Eliopoulos GM, Moellering RC, Wennersten C, Venkataraman L, Novick RP, Gold HS. Accessory Gene Regulator (agr) Locus in Geographically Diverse Staphylococcus aureus Isolates with Reduced Susceptibility to Vancomycin. Antimicrobial Agents and Chemotherapy 2002; 46:1492-502.
69. Murakami K, Minamide W, Wada K, Nakamura E, Teraoka H, Watanabe S. Identification of methicillin-resistant strains of staphylococci by polymerase chain reaction. J Clin Microbiol 1991; 29:2240-4.
70. Zhang K, McClure J-A, Elsayed S, Louie T, Conly JM. Novel Multiplex PCR Assay for Characterization and Concomitant Subtyping of Staphylococcal Cassette Chromosome mec Types I to V in Methicillin-Resistant Staphylococcus aureus. Journal of Clinical Microbiology 2005; 43:5026-33.
71. Gilot P, Lina G, Cochard T, Poutrel B. Analysis of the Genetic Variability of Genes Encoding the RNA III-Activating Components Agr and TRAP in a Population of Staphylococcus aureus Strains Isolated from Cows with Mastitis. Journal of Clinical Microbiology 2002; 40:4060-7.

Table 1. Microbiological and molecular characteristics of *S. aureus* isolates in community-acquired and healthcare-associated infections

Characteristics, n (%)	*S. aureus* (n= 83)	CA (n= 29)	HA (n= 54)	p-value
Resistant to				
Methicillin	14 (17)	2 (7)	12 (22)	0.124
Ciprofloxacin	18 (22)	4 (14)	14 (26)	0.039
Clindamycin	34 (41)	7 (24)	27 (50)	0.034
Erythromycin	42 (51)	11 (38)	31 (57)	0.212
Gentamicin	10 (12)	1 (3)	9 (17)	0.151
Penicillin	66 (80)	25 (86)	41 (76)	0.545
Rifampicin	6 (7)	2 (7)	4 (7)	1.000
Sulfametoxazole-trimethoprim	10 (12)	0	10 (19)	0.008
Multidrug-resistant	26 (31)	8 (28)	18 (33)	0.845
VAN MIC (mg/L), mean±SD				
BMD	1.15±0.98	1.03±0.51	1.22±1.18	0.948
Etest	1.61±1.06	1.51±0.77	1.69±1.23	0.637
hVISA	8 (10)	2 (7)	6 (10)	0.992
SCC*mec* type[a]	(n= 14)	(n= 2)	(n= 12)	0.389
I	0	0	0	-
II	1 (7)	0	1 (10)	-
III	2 (14)	0	2 (20)	-
IVa	2 (14)	0	2 (20)	-
IVb	0	0	0	-
IVc	2 (14)	1 (50)	1 (10)	-
IVd	1 (7)	0	1 (10)	-
V	0	0	0	-
NT	2 (14)	0	2 (20)	-
2 types	4 (30)	1 (50)	3 (30)	-
3 types	0	0	0	-
Virulence factors				
Biofilm producers	75 (90)	27 (93)	48 (89)	0.918
Strong biofilm	38 (46)	15 (52)	23 (43)	0.583
Delta-hemolysis	59 (71)	18 (62)	41 (76)	0.224
PVL	25 (30)	8 (28)	16 (30)	1.000
agr type				0.016
I	38 (46)	9 (32)	29 (54)	-
II	20 (24)	9 (32)	11 (23)	-
III	14 (17)	9 (32)	5 (9)	-
IV	2 (2)	0	2 (4)	-

Absent / none	9 (11)	2 (7)	7 (13)	0.794

[a] evaluated only for methicillin resistant isolates (n= 14); CA, community-acquired infection; HA, healthcare-associated infection; VAN MIC, Vancomycin minimum inhibitory concentration; BMD, broth microdilution; SD, standard deviation; hVISA, heterogeneous vancomycin-intermediate *S. aureus*; SCC*mec*, Staphylococcal cassette chromosome *mec*; NT, nontypable; PVL, Panton-Valentine leucocidin; *agr*, accessory gene regulator.

Table 2. Association between *agr* status and resistance or outcome in *S. aureus* infections

Variable, n (%)	*agr* status			
	Genotype		Phenotype	
	Absent (n= 9)	Present (n= 74)	Dysfunctional (n= 23)	Functional (n= 60)
Methicillin-resistant	5 (56)	9 (12)	9 (39)	5 (9)
	p= 0.002		p= 0.002	
Multidrug-resistant	7 (75)	19 (26)	11 (48)	15 (25)
	p= 0.013		p= 0.077	
Vancomycin MIC				
BMD (mean±SD)	1.43±0.73	1.08±0.96	1.41±1.59	1.00±0.53
	p= 0.068		p= 0.099	
Etest (mean±SD)	1.93±0.61	1.59±1.12	1.68±1.58	1.59±0.86
	p= 0.050		p= 0.767	
hVISA	3 (34)	5 (7)	3 (13)	5 (9)
	p= 0.037		p= 0.916	
30-day mortality	5 (56)	15 (20)	8 (35)	12 (20)
	p= 0.146		p= 0.364	
LOS (days), median (IQR)	37 (9-60)	13 (7-33)	10 (8-29)	16 (7-35)
	p= 0.152		p= 0.654	

agr, accessory gene regulator; MIC, minimum inhibitory concentration (mg/L); BMD, broth microdilution; SD, standard deviation; hVISA, heterogeneous vancomycin-intermediate *S. aureus*; LOS, length of hospital stay; IQR, interquartile range.

3.3 Hetero-resistência intermediária à vancomicina e desfecho da terapia

Heterogeneous vancomycin-intermediate Staphylococcal infections: molecular epidemiology, clinical features and outcome

Janine de Melo Rauber, Guilherme Henrique de Oliveira Arnhold, Gabriela Rosa da Cunha, Barbara Baggiotto, Marcelo Carneiro, Pedro Alves d'Azevedo, Andreia Rosane de Moura Valim

Artigo a ser submetido ao periódico International Journal of Antimicrobial Agents

Heterogeneous vancomycin-intermediate Staphylococcal infections: molecular epidemiology, clinical features and outcome

Janine M. Rauber[1], Guilherme H. O. Arnhold[2], Gabriela R. Cunha[1], Barbara Bagiotto[2], Marcelo Carneiro[2,3], Pedro A. d'Azevedo[1], Andréia R. M. Valim[2*]

Affiliations. [1] Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), Porto Alegre, RS, Brazil. [2] Universidade de Santa Cruz do Sul (UNISC), Santa Cruz do Sul, RS, Brazil. [3] Hospital Santa Cruz (HSC), Santa Cruz do Sul, RS, Brazil.

***Address correspondence.** Universidade de Santa Cruz do Sul, Departamento de Biologia e Farmácia, Avenida Independência, 2293, Universitário, CEP 96815-900, Santa Cruz do Sul, RS, Brazil. Phone: 55 51 3717-7399; Fax: 55 51 3717-7399 (avalim@unisc.br).

Keywords. Staphylococcal infections; heterogeneous vancomycin-intermediate *Staphylococcus* spp.; pulsed-field gel electrophoresis; molecular epidemiology.

Abbreviations. Vancomycin-resistant *Staphylococcus aureus* (VRSA); vancomycin-intermediate *S. aureus* (VISA); heterogeneous vancomycin-intermediate *S. aureus* (hVISA); community-acquired (CA); healthcare-

associated (HA); Panton-Valentine leucocidin (PVL); Staphylococcal cassette chromosome *mec* (SCC*mec*); accessory gene regulator (*agr*); minimum inhibitory concentration (MIC); skin and soft tissue (SST); surgical site infection (SSI); bloodstream infection (BSI); pneumonia (PNEU); lower respiratory tract infections (LRI); length of stay (LOS); intensive care unit (ICU); polymerase chain reaction (PCR); broth microdilution (BMD); brain heart infusion (BHI); population analysis profile / area under the curve (PAP/AUC); Clinical Laboratory Standards Institute (CLSI); multidrug resistance (MDR).

Abstract

Heterogeneous vancomycin-intermediate *Staphylococcus* spp. (hVIS) phenotype has been associated with increased mortality and vancomycin failure. A prospective cohort was performed to identify hVIS strains in a teaching hospital of southern Brazil and to evaluate the epidemiology of infections caused by hVIS for 36 months (July 2012 to June 2015). Antimicrobial susceptibility was assessed using disc diffusion and vancomycin minimum inhibitory concentration (MIC) was analyzed by broth microdilution (BMD) and Etest. Molecular assays were used to evaluate *mec*A and *hld* genes, Panton-Valentine leucocidin (PVL), Staphylococcal chromosomal cassette *mec* (SCC*mec*) and accessory gene regulator (*agr*) typing. hVIS isolates were characterized by pulsed-field gel electrophoresis (PFGE). Population analysis profile - area under the curve (PAP-AUC) was performed for each isolate to be compared with the reference strain Mu3 (ATCC700698). 200 isolates were analyzed and none of the isolates was characterized as vancomycin-intermediate *Staphylococcus* (VIS). 34 samples were confirmed as hVIS (17.0%) and 70.6% of these strains were methicillin-resistant. Absence of *agr* was more frequent in hVIS isolates than vancomycin-susceptible isolates (p=0.031), MIC higher than 1 µg/mL by BMD (p=0.018) and higher than 1.5 µg/mL by Etest (p=0.007). No association with outcome was found related to hVIS strains. Two clones including a total of four samples were identified by PFGE. These results suggest that hVIS isolates from our hospital had a different genetic pattern, indicating a possible strain evolution, rather than spread. Therefore, the suitable use of vancomycin and therapeutic drug monitoring are recommended in order to avoid the emergence of

heteroresistance in susceptible strains.

Introduction

Staphylococcus aureus is an important cause of both community-acquired (CA) and health care-associated (HA) infections and *coagulase-negative Staphylococcus* (CoNS) is the most common opportunist pathogen associated with HA central-line infections[1, 2]. Antibiotic resistance is a major public-health problem worldwide and the association between inappropriate use of antimicrobials and incidence of resistance has been extensively reported[3-5]. Thus, the treatment of infections caused by *Staphylococcus* spp. has been a challenge due to the high prevalence of methicillin-resistant strains (MRSA)[6]. The glycopeptide antibiotic vancomycin has been traditionally used as a first-line agent for treating MRSA infections[7]. Although the drug has been in the market since the 50s, there have been few reports of vancomycin-resistant *S. aureus* (VRSA)[8]. However, although the majority of isolates are susceptible, there have been many descriptions of treatment failure with vancomycin in studies published from the 1970s[9].

Since Hiramatsu et al.[10] reported the first MRSA clinical strain with reduced vancomycin susceptibility from Japan in 1997, vancomycin-intermediate *S. aureus* (VISA) isolates were described in different countries, including Brazil[11]. Different from VRSA, VISA strains do not have the vancomycin resistance genes (*van*A, *van*B or *van*C); however, a vancomycin minimum inhibitory concentration (MIC) between 4 and 8 µg/mL is verified. The intermediate resistance mechanism seems to be associated with cell-wall thickening, which difficults antibiotic action[12].

Heterogeneous vancomycin-intermediate *Staphylococcus* (hVIS) is defined as an isolate with a vancomycin MIC within the susceptible range when tested by routine methods, but a subpopulation of cells (1 in 10^6) are in the vancomycin-intermediate range[13]. It is estimated that approximately 60% of the MRSA isolates with MIC of 2 µg/mL are hVISA strains[12]. Some screening methods employed for hVISA detection are the macro-Etest method, glycopeptide resistance detection (GRD) Etest and brain heart infusion (BHI) agar containing vancomycin, but the confirmation of heteroresistance is carried out based on culture growth through the method of population analysis profiling - area under the curve (PAP-AUC). Although the PAP-AUC is the gold standard, it is a laborious test and of which delay in the result (3-5 days) does not allow its use in clinical routine[12].

The clinical importance of hVISA strains is due to the fact that their presence seems to be related to a higher chance of treatment failure, especially in cases of bacteremia[14-17]. However, although many researchers have studied the reasons for treatment failure, there is no consensus among authors. Additionally, the real influence of heteroresistance on the outcomes mortality and length of hospital stay (LOS) is still widely debated.

The objective of this study was to evaluate the prevalence of heterogeneous vancomycin-intermediate isolates in the *Staphylococcus* genus in a teaching hospital in southern Brazil, to investigate the influence of this phenotype on clinical characteristics and outcome, as well as analyze the molecular epidemiology of hVIS infections.

Materials and methods

1. Study Design and Population

A prospective cohort study was designed to evaluate the prevalence rates and to analyze the phenotypic and molecular characteristics of hVIS isolates. All patients with a positive culture for *Staphylococcus* spp. admitted between July 2012 and June 2015 at a 234-bed, tertiary care, teaching hospital located in southern Brazil were included. Cases of duplicates or recurrent infections were excluded. The study was reviewed and approved by the Research Ethics Committee of Universidade Federal de Ciências da Saúde de Porto Alegre (1779/12).

2. Clinical Data and Definitions

Clinical information was collected from the patients' medical records including demography characteristics, medical history (ICD – International Classification of Disease), previous healthcare institution exposure, comorbidities, Charlson comorbidity score[18], intensive care unit (ICU) admission, antimicrobial treatment, length of hospitalization and outcome (discharge from hospital and 30-day all-cause mortality).

Definitions for clinical outcomes were adapted from clinical studies of patients with Staphylococcal infections[19, 20]. Healthcare-associated (HA) and community-acquired (CA) infections were determined according to previously described criteria[21]. The type of infection was determined according to the definition and criteria of the Centers for Disease Control and Prevention (CDC): bloodstream infections (BSI), pneumonia (PNEU), lower respiratory tract

infections (LRI), skin and soft tissue infections (SST), surgical site infections (SSI) and urinary tract infection (UTI)[22]. Multidrug-resistant (MDR) microorganism was defined as non-susceptibility to at least one agent in three or more antimicrobial categories[23].

3. Microbiological Evaluation

Antimicrobial susceptibility was assessed by the Kirby-Bauer disc diffusion method according to Clinical Laboratory Standards Institute (CLSI). Vancomycin MIC was determined by the broth microdilution (BMD) and Etest (bioMérieux, Durham, NC) methods, performed according to the CLSI guidelines and the manufacturer's instructions, respectively.

Microtiter plate assay was performed as described by Stepanovic et al.[24] to determine biofilm formation. The expression of the *agr* gene was evaluated by quantitating delta-hemolysin production, utilizing a previously described method[25].

Brain heart infusion (BHI) agar containing 4 and 6 µg/mL of vancomycin was used to screening the presence of the hVIS phenotype[26]. 100 µL of 0.5 McFarland standard bacterial suspension was inoculated and the plates were incubated for 48 hours at 35°C. The culture was considered positive if the growth of 1 or more colonies was observed. All positive strains with hVIS phenotype were confirmed using the PAP-AUC method[27]. After incubation on solid medium, samples were diluted in sterile saline at concentrations ranging of 10^{-1} to 10^{-7} from a 0.5 McFarland bacterial suspension. 10 µL of these dilutions were spotted on BHI agar plates containing 0.5, 1, 2, 3, 4, 6 and 8 µg/mL of vancomycin. After 48 hours of incubation at 35°C, colonies were counted to

determine the log CFU/mL. Results of the counting were plotted against the concentration of vancomycin and the AUC was determined by using GraphPad Prism 6 software. Isolates were confirmed as hVISA if the ratio of AUC of the sample was ≥0.9, compared to AUC of the reference strain Mu3 (ATCC700698).

4. Molecular Analysis

Genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega Corporation, Madison, WI, USA) and *mec*A gene detection was performed by polymerase chain reaction (PCR), confirming methicillin resistance of isolates[28]. The Staphylococcal cassette chromosome *mec* (SCC*mec*) type and accessory gene regulator (*agr*) genotype were characterized using multiplex PCR, as previously described[29, 30]. PCR was performed for the presence of the gene encoding Panton-Valentine Leucocidin (PVL) and delta-hemolysin (*hld*) gene, as described by Lina et al.[31] and Marconi et al.[32], respectively.

Chromosomal DNA analysis by pulsed field gel electrophoresis (PFGE) was performed using a practical adaptation of literature methodology described by McDougal et al.[33] and Pinto et al.[34]. The fragments were analyzed by PFGE using 1% agarose gel (Pulsed Field Certified Agarose, Bio-Rad Laboratories) in 0.5X Tris-borate-EDTA buffer in a CHEF-DR III system (Bio-Rad Laboratories, Hercules, CA). Gels were stained with 0.5 µg/mL ethidium bromide, visualized under UV light and photographed using GelDoc XM system (Bio-Rad Laboratories, Hercules, CA). Circulating epidemic Latin American clones were used as PFGE controls: Brazilian Epidemic Clone (BEC), Pediatric, Cordobean/Chilean, New York/Japan, Oceania Southwest Pacific Clone (OSPC), E-MRSA 15, E-MRSA 16, USA 300 and USA 400.

5. Statistical analysis

Categorical variables were compared using Chi-square or Fisher's exact test and continuous variables were compared using the Mann-Whitney U-test, as appropriate. All analyses were performed with the computer software SPSS version 20.0 (SPSS Inc, Chicago, IL, USA). A p-value ≤ 0.05 was considered statistically significant. PFGE analysis was performed using BioNumerics software version 6.0 (Applied Maths, Kortrijk, Belgium). Banding pattern was analyzed using Dice coefficient and the unweighted pair group method with arithmetic means (UPGMA), tolerance and optimization were adjusted to 1.5 and 1.0%, respectively. Clusters were defined as isolates with a similarity above 80% in the dendrogram (Tenover et al., 1995).

Results

During the study period, 200 episodes of Staphylococcal infections were reported. One hundred and twelve samples (56%) were isolated from male and 71.5% (n=143) from adult patients. Patients had a median Charlson comorbidity score of 2 (IQR, 0-3) and 63% had cardiovascular disease, 24% respiratory and 15% of patients had neurological diseases as comorbidities. The most common infection sites were BSI (46%), followed by SSI and SST (29%), PNEU and LRI (21.5%). *S. aureus* was the etiologic agent responsible for 50% of cases. The most recurrent species in CoNS infections (n=100) were *S. epidermidis* (65%), *S. lugdunensis* (8%), *S. haemolyticus* (7%), *S. hominis* (7%) and *S. warneri* (7%).

Ninety-three samples (47.5%) were considered as methicillin-resistant and none

of the isolates was characterized as vancomycin-intermediate or vancomycin-resistant *Staphylococcus* (VIS or VRS). Eighteen isolates (18%) were identified as MRSA and 75 samples (75%) were methicillin-resistant in CoNS infections ($p<0.001$). After screening for the presence of the hVIS phenotype, 64 samples (32%) were submitted to PAP-AUC analysis and 34 isolates (17%) were confirmed as hVIS.

hVIS isolates were more frequently observed in CoNS samples compared to *S. aureus* strains (24% vs. 10%, $p=0.003$). Furthermore, detection of hVIS phenotype was more recurrent in methicillin-resistant strains than susceptible isolates (67% vs. 42%, $p=0.013$). The prevalence of heterogeneous vancomycin-intermediate isolates in MRSA strains was about 17%. hVIS was more often isolated from children and neonates than adults (27% vs. 14%, $p=0.058$) and preterm newborns ($p=0.041$).

Twenty-two of 34 hVIS strains (64.7%) were BSI isolates, 6 (17.6%) were SSI and SST samples, 5 (14.7%) were PNEU and LRI and one (3%) was obtained from the ICU. Ten of 34 hVIS strains (29.4%) were identified as *S. aureus*, 18 (52.9%) as *S. epidermidis*, 5 (14.7%) as *S. lugdunensis* and one (3%) was recognized as *S. haemolyticus*.

Antimicrobial resistance and microbiological characteristics of isolates are shown in Table 1. All samples were susceptible to linezolid and tigecycline and *mec*A gene was detected in all methicillin-resistant isolates. VAN MIC was higher for hVIS in comparison to vancomycin-susceptible *Staphylococcus* (VSS) using both methods (Table 1). MIC distribution determined by BMD and Etest compared to hVIS frequency are shown in Figures 1, indicating that high MIC

was associated to the hVIS phenotype. A lower ratio of hVIS strains was biofilm and delta-hemolysin-producing when compared to VSS isolates, as shown in Table 1. SCC*mec* detected for 3 MRSA isolates were type III, type IVc and simultaneous detection of types II and IVc in a same sample. The most common SCC*mec* for CoNS were type IVa (50%) and type V (18%).

PVL was detected in 32 of *S. aureus* isolates (32%) and 4 (40%) of hVIS strains. The *agr* typing for *S. aureus* and CoNS detected *agr* I as the most frequent type (44% and 43%, respectively), followed by *agr* II (23% and 16%) and *agr* III (20 and 5%). Most strains of hVIS showed *agr* I or absence of *agr* for both species. The absence of *agr* was verified in 10% of *S. aureus* and in 36% of CoNS isolates ($p<0.001$). For heterogeneous vancomycin-intermediate identified as *S. aureus*, the absence of *agr* was significantly higher than VSSA (38% vs. 7%, $p=0.031$).

Thirty-eight patients died (19%) and 128 (64%) were admitted at the ICU. There was no difference in the mortality rate or ICU admission between hVIS and VSS isolates (Table 2). Most of non-survivors patients were admitted in the ICU (22% vs. 10%, $p=0.061$) and had MDR infections (26% vs. 12%, $p=0.024$). As show in Table 2, hVIS phenotype was associated to prolonged LOS and vancomycin therapy. Glycopeptide was used in 76 patients (38%) and vancomycin treatment was more frequent in non-survivors (63% vs. 33%, $p=0.002$). Charlson score was similar for patients receiving vancomycin and in patients treated with other antimicrobials ($p=0.537$), but was higher when comparing non-survivors to survivors (3.0 vs. 1.0, $p<0.001$).

Table 1. Microbiological characteristics of *Staphylococcus* spp isolates

Characteristics, n (%)	*Staphylococcus* spp. (n= 200)	VSS (n= 166)	hVIS (n= 34)	p-value
Resistant to				
Methicillin	93 (48)	70 (42)	23 (67)	0.013
Ciprofloxacin	84 (42)	70 (42)	14 (42)	0.725
Clindamycin	96 (48)	78 (47)	18 (54)	0.570
Erythromycin	122 (61)	96 (58)	26 (73)	0.293
Gentamicin	66 (33)	46 (28)	20 (58)	0.001
Penicillin	172 (86)	142 (86)	30 (89)	0.784
Rifampicin	12 (6)	11 (7)	1 (3)	0.578
Sulfamethoxazole/ trimethoprim	74 (37)	60 (36)	14 (42)	0.554
Multidrug-resistant	102 (51)	81 (49)	21 (61)	0.234
Vancomycin MIC, mean±SD				
BMD	1.32±0.81	1.25±0.79	1.53±0.74	0.016
Etest	1.74±0.82	1.71±0.88	2.02±0.57	<0.001
Virulence factors				
Biofilm	160 (80)	138 (83)	22 (64)	0.033
Delta-hemolysin	110 (55)	94 (57)	16 (47)	0.418

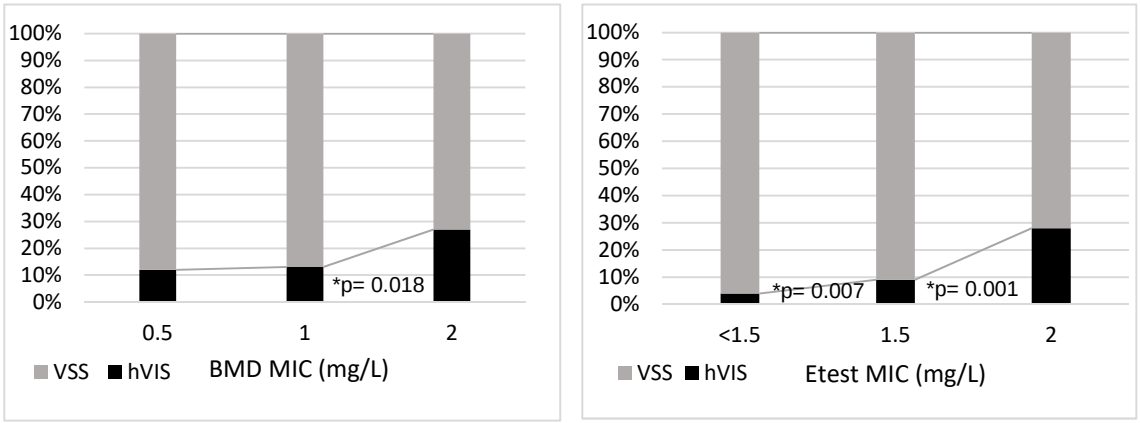
hVIS, heterogeneous vancomycin-intermediate *Staphylococcus* spp.; VSS, vancomycin-susceptible *Staphylococcus* spp.; MIC, Minimum inhibitory concentration; SD, Standard deviation; BMD, Broth microdilution.

Table 2. Comparison of clinical features and outcome in hVIS and vancomycin-susceptible *Staphylococcus* spp.

Variables, n (%)	VSS (n= 166)	hVIS (n= 34)	p-value
Previous healthcare institution exposure	75 (45)	9 (26)	0.055
Infection acquisition			
Health-care associated	120 (72)	29 (85)	0.230
Vancomycin therapy	55 (33)	19 (56)	0.025
Treatment duration (days)	3.74	6.48	0.025
(95% CI)	(2.2-5.3)	(3.6-9.4)	
ICU admission	105 (63)	23 (68)	0.660
LOS (days), median (IQR)			
Hospital	19 (9-41)	36 (14-56)	0.018
Mortality			
30-day mortality	32 (19)	8 (23)	0.883

hVIS, heterogeneous vancomycin-intermediate *Staphylococcus* spp.; VSS, vancomycin-susceptible *Staphylococcus* spp.; CI, confidence interval; ICU, intensive care unit; IQR, interquartile range; LOS, length of stay.

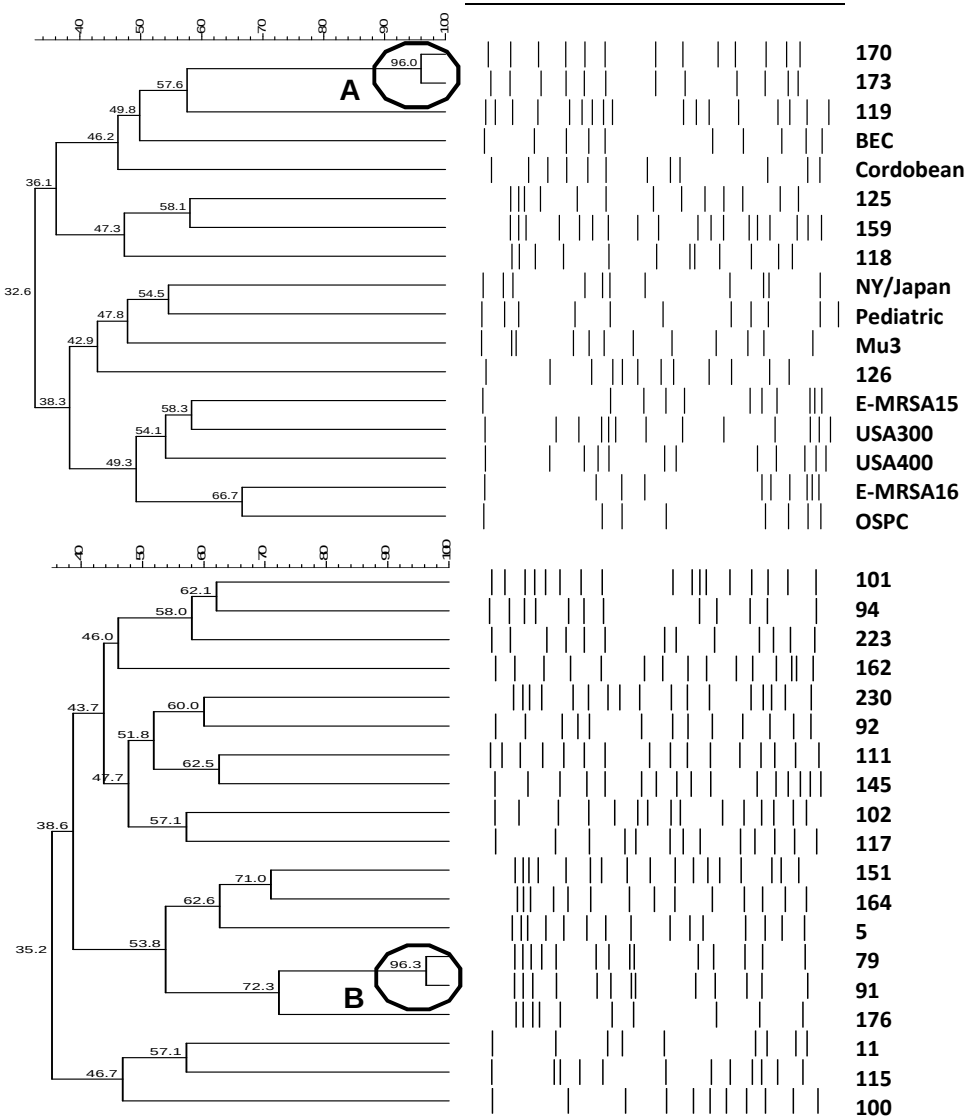
Figure 1. Vancomycin MIC distribution and heterogeneous vancomycin-intermediate *Staphylococcus* spp. frequency



hVIS, heterogeneous vancomycin-intermediate *Staphylococcus* spp.; VSS, vancomycin-susceptible *Staphylococcus* spp.; BMD, broth microdilution; MIC, minimum inhibitory concentration.

PFGE was used to assess the degree of genetic similarity and the results are shown in Figure 2. Two clones were observed, including four isolates: clone A, comprising isolates 170 and 173; and clone B, comprising isolates 79 and 91. No clonal association was verified between hVIS isolates and methicillin-resistant *S. aureus* epidemic international clones.

Figure 2. PFGE dendrogram of heterogeneous vancomycin-intermediate *Staphylococcus* spp isolates and methicillin-resistant *S. aureus* epidemic international clones



Species	MR	SCC*mec*	*agr*	PVL
S. aureus	+	NT	-	-
S. aureus	-	NA	I	-
S. aureus	+	IVc	III	+
S. aureus	+	*	*	*
S. aureus	+	*	*	*
S. aureus	+	II/IVc	-	-
S. aureus	-	NA	III	-
S. aureus	-	NA	-	-
S. aureus	+	*	*	*
S. aureus	+	*	*	*
S. aureus	+	*	*	*
S. aureus	-	NA	IV	+
S. aureus	+	*	*	*
S. aureus	+	*	*	*
S. aureus	+	*	*	*
S. aureus	+	*	*	*
S. aureus	+	*	*	*
S. epidermidis	+	IVa	I	NA
S. epidermidis	+	NT	-	NA
S. epidermidis	-	NA	III	NA
S. epidermidis	+	V	-	NA
S. epidermidis	+	NT	II	NA
S. epidermidis	+	IVa	I	NA
S. epidermidis	-	NA	I	NA
S. haemolyticus	+	V	-	NA
S. epidermidis	+	IVa	III	NA
S. epidermidis	+	II	I	NA
S. epidermidis	+	IVa	-	NA
S. epidermidis	+	IVa	I	NA
S. epidermidis	+	IVa	-	NA
S. lugdunensis	+	III	-	NA
S. lugdunensis	+	III	-	NA
S. lugdunensis	+	IVc	I	NA
S. epidermidis	+	IVa	I	NA
S. epidermidis	-	NA	I	NA
S. epidermidis	+	IVa	I	NA

MR, methicillin resistance; NA, not applicable; NT, nontypable;
* not available

Discussion

The prevalence rates of hVISA vary worldwide depending on the region. A recently published meta-analysis[35] indicated a hVISA prevalence of 6.05% among 99.042 MRSA strains. According to the authors, this rate has increased over the years, especially in blood culture samples, being higher in Asian countries when compared to countries in Europe and America. Some studies indicate a prevalence rate similar to that described by Zhang et al.[35]: approximately 4 to 5% in MRSA samples from Argentina[36], Japan[37], Singapore[38] and other countries in Asia[39]. However, a 10% rate was described in Taiwan[40] and 12% in Australia[41], rates similar to that found in the present study. Other studies show very high levels of hVISA, with rates varying between 26 and 29%[42-44].

No attention from researchers around the world has been given to the heteroresistance phenomenon in the case of infections by CoNS. Considering this group of microorganisms has high methicillin-resistance rates, which are even higher than MRSA rates, vancomycin becomes an important antimicrobial agent in the treatment of infections caused by CoNS. Thus, studies reporting the prevalence of heteroresistance in CoNS are important and can assist prescribers in choosing the appropriate antimicrobial agent, considering the possibility of treatment failure with vancomycin. Additionally, the frequency of 23% of hVIS phenotype in coagulase-negative *Staphylococci* is therefore a matter of concern and, to date, only one description of their prevalence has been published, derived from a study carried out in our own country that included blood samples, indicating 8.5% of CoNS isolates with this phenotype[45].

Most studies evaluated the hVIS phenotype in methicillin-resistant strains and few authors investigated heteroresistance in susceptible isolates[36, 46, 47]. However, methicillin-susceptible samples can also disclose the hVIS phenotype and this phenomenon has increased in recent years, according to Hu et al.[47]. Although the frequency of heteroresistance in MSSA isolates is lower than that of MRSA strains (9% vs. 17%), this fact is probably associated with a significant increase in vancomycin MICs among MSSA clinical isolates[46, 48]. Thus, it becomes important to detect reduced vancomycin susceptibility, both in methicillin-susceptible and resistant strains.

High vancomycin MIC has also been associated with hVIS phenotype in previous literature studies[41, 42, 49, 50] and is consistent with our findings indicating that the frequency of hVIS increases with vancomycin MIC. In this case, for isolates containing high vancomycin MIC, although the strains are classified as sensitive, prescribers should consider other treatment options such as ceftaroline, linezolid, daptomycin and tigecycline. Some studies have reported an increase in resistance levels to other antimicrobial for hVIS samples[20, 40], a fact that was not observed in this study, with the exception of resistance to gentamicin, which was significantly higher in hVIS cases.

Regarding the SCC*mec* type of hVISA isolates, the meta-analysis of Zhang et al.[35] indicates the predominance of SCC*mec* types II (48%), IV (18%) and III (18%) in 685 identified MRSA strains. Moreover, other studies not mentioned in this meta-analysis reported a higher frequency of these three types of SCC*mec*, which is in agreement with our results[42, 51]. Similarly to our study, heteroresistance was mainly found in samples of *agr* types I and II, a fact described by previous studies[42, 43, 52, 53]. Studies evaluating the presence

of PVL in hVIS isolates show very variable results, such as the absence of this virulence factor in China[54], 4% in Israel[55] and 30% in Argentina[36], with the latter being a similar result to that of the current study.

The impact of heteroresistance on biofilm formation is still a controversial subject among different authors. According to Voung et al.[56] and Sakoulas et al.[57], the surfactant action of delta-hemolysin can prevent hydrophobic interactions of cell-surface with polystyrene, reducing biofilm formation. Thus, samples with dysfunctional *agr* (i.e., non-delta-hemolysin-producing) show more pronounced biofilm formation. Howden et al.[58] and Maor et al.[55] did not verify this increase in clinical samples of hVISA, generally characterized as dysfunctional *agr*. Similar to this study, the authors verified a reduction in biofilm formation, a fact probably related to cell wall changes due to this phenotype.

Although authors[25, 44, 46] described the association between loss of *agr* functionality and hVIS phenotype, other studies also found no difference in *agr* dysfunctional activity, represented by the production of delta-hemolysin between hVISA and VSSA strains[17]. Though the dysfunctional *agr* rate found was lower (albeit not significantly) in the hVIS isolates, it is noteworthy that *agr* absence was associated with heteroresistance in our study. This type of association between *agr* absence and hVIS phenotype in clinical samples was not previously described in the literature. The study by Paulander et al.[59] suggests that the fitness cost of carrying *agr* is enhanced by the presence of some antibiotics and that treatment with those antibiotics will select *agr*-negative mutants. Additionally, according to Shopin et al.[60], carrying an *agr*-negative strain was associated with hospitalization, raising the possibility that such strains may be selected in a nosocomial setting.

The influence of the hVISA phenotype has been the subject of many studies and a published meta-analysis[61] grouped these studies to assess the impact on treatment failure and patient mortality. According to the author, the probability of treatment failure was higher in hVISA infections (OR: 2.37, 95%CI: 1.53-3.67), but there was no difference in mortality between infections caused by hVISA and VSSA. Results published after the meta-analysis confirmed these data[17], as well as the results of our study. A possible explanation for the lack of a difference in mortality between hVIS and VSS strains is that heteroresistance has been associated with virulence factor reduction, leading to a decrease in host immune response, resulting in reduced mortality, but increased infection persistence[62-64].

LOS is another important outcome, but unfortunately it is not evaluated in most of the studies. Prolonged LOS is responsible for an increase of in-hospital costs[65] and hVIS infections were associated with longer LOS in the current study, similarly to previous studies[17, 38].

Sakoulas et al.[57] assessed the effects of prolonged vancomycin administration on MRSA in a patient with recurrent bacteremia and although they did not verify the development of heteroresistance when vancomycin toughs were maintained above 10 g/L. Previous studies associated prior exposure to vancomycin and the development of heteroresistance[66, 67], data similar to those of the current study. The majority of hVISA and VISA strains seem to arise through *in vivo* evolution during failed therapy with vancomycin for seemingly VSSA infections[68]. However, previous reports indicated that hVISA could also be generated by exposing MRSA to imipenem[69] or be induced by common rifampicin resistance mutations[70, 71].

The molecular analysis indicated the presence of clones A and B. In Clone A, the samples were isolated from prosthesis secretion due to osteomyelitis with a 3-day interval between the isolation; both patients were males (24 to 59 years old) who underwent surgery and were treated by the same physician during hospitalization. There is a suggestion of vertical transmission, as there is an association between the patients, who were treated by similar medical and nursing staffs. As for Clone B, the samples were isolated from tracheal aspirate and blood with a 10-day interval; one patient was a preterm newborn, but the other patient was 39 years old and had been hospitalized for three months at the institution. The evidence of transmission is not so clear; however, an assessment of the staff disclosed the existence of a substitute nursing technician who alternated care in the adult and pediatric units, according to hospitalization demand. In both clones (A and B), the probable absence of adequate hand hygiene and presence of fomites could explain the transmission of strains. Regarding the transmission of isolates with intermediate resistance, de Lassence et al.[72] reported the first description of a large outbreak of colonization and infection with a single VISA strain that affected 21 patients in a medical ICU at a tertiary care teaching hospital.

Our study should be interpreted in the context of some limitations. First, this was a single-center study with a small sample size. Second, there was no enzymatic cleavage in some hVIS isolates due to technical limitations and, therefore, these isolates were not assessed by PFGE. Finally, considering that sample isolation occurred during the course of infection, it is not possible to categorically affirm that the heteroresistant phenotype is indeed a consequence of treatment.

Despite of its limitations, this is the first description of an association between *agr* absence and hVIS phenotype. In addition to *agr* dysfunction, which is widely correlated with heteroresistance, the *agr* system loss can alternatively explain the reason for virulence decrease. Furthermore, our findings are a matter of concern, since high prevalence rates of heteroresistance in CoNS were described, unlike previously reported rates in the only study available to date. Considering the genetic diversity observed in the present study, which indicates strain evolution rather than transmission between patients, the suitable use of vancomycin and therapeutic drug monitoring are recommended in order to avoid the emergence of heteroresistance in susceptible strains.

References

[1] Sievert DMP, Ricks PP, Edwards JRMS, Schneider AMPH, Patel JP, Srinivasan AMD, et al. Antimicrobial-Resistant Pathogens Associated with Healthcare-Associated Infections: Summary of Data Reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2009–2010. Infection Control and Hospital Epidemiology. 2013;34:1-14.

[2] Chitnis ASMDMPH, Edwards JRM, Ricks PMP, Sievert DMP, Fridkin SKMD, Gould CVMDM. Device-Associated Infection Rates, Device Utilization, and Antimicrobial Resistance in Long-Term Acute Care Hospitals Reporting to the National Healthcare Safety Network, 2010. Infection Control and Hospital Epidemiology. 2012;33:993-1000.

[3] Costelloe C, Metcalfe C, Lovering A, Mant D, Hay AD. Effect of antibiotic prescribing in primary care on antimicrobial resistance in individual patients: systematic review and meta-analysis. BMJ. 2010;340.

[4] Fishman N. Policy Statement on Antimicrobial Stewardship by the Society for Healthcare Epidemiology of America (SHEA), the Infectious Diseases Society of America (IDSA), and the Pediatric Infectious Diseases Society

(PIDS). Infection Control & Hospital Epidemiology. 2012;33:322-7.

[5] Morris AM. Antimicrobial Stewardship Programs: Appropriate Measures and Metrics to Study their Impact. Current Treatment Options in Infectious Diseases. 2014;6:101-12.

[6] Calfee DP. Methicillin-resistant Staphylococcus aureus and vancomycin-resistant enterococci, and other Gram-positives in healthcare. Curr Opin Infect Dis. 2012;25:385-94.

[7] Avent ML, Vaska VL, Rogers BA, Cheng AC, van Hal SJ, Holmes NE, et al. Vancomycin therapeutics and monitoring: a contemporary approach. Internal Medicine Journal. 2013;43:110-9.

[8] Askari E, Tabatabai SM, Arianpoor A, Nasab MN. VanA-positive vancomycin-resistant staphylococcus aureus: Systematic search and review of reported cases. Infectious Diseases in Clinical Practice. 2013;21:91-3.

[9] Kollef MH. Limitations of vancomycin in the management of resistant staphylococcal infections. Clin Infect Dis. 2007;45 Suppl 3:S191-5.

[10] Hiramatsu K, Hanaki H, Ino T, Yabuta K, Oguri T, Tenover FC. Methicillin-resistant Staphylococcus aureus clinical strain with reduced vancomycin susceptibility. Journal of Antimicrobial Chemotherapy. 1997;40:135-6.

[11] Oliveira GAMD, Dell'Aquila AMMD, Masiero RLP, Levy CEP, Gomes MSP, Cui LP, et al. Isolation in Brazil of Nosocomial Staphylococcus aureus with Reduced Susceptibility to Vancomycin • Infection Control and Hospital Epidemiology. 2001;22:443-8.

[12] Howden BP, Davies JK, Johnson PDR, Stinear TP, Grayson ML. Reduced Vancomycin Susceptibility in Staphylococcus aureus, Including Vancomycin-Intermediate and Heterogeneous Vancomycin-Intermediate Strains: Resistance Mechanisms, Laboratory Detection, and Clinical Implications. Clinical microbiology reviews. 2010;23:99-139.

[13] Hiramatsu K. Vancomycin-resistant Staphylococcus aureus: a new model of antibiotic resistance. The Lancet Infectious Diseases. 2001;1:147-55.

[14] Ward PB, Johnson PD, Grabsch EA, Mayall BC, Grayson ML. Treatment failure due to methicillin-resistant Staphylococcus aureus (MRSA) with reduced susceptibility to vancomycin. Med J Aust. 2001;175:480-3.

[15] Charles PG, Ward PB, Johnson PD, Howden BP, Grayson ML. Clinical features associated with bacteremia due to heterogeneous vancomycin-intermediate Staphylococcus aureus. Clin Infect Dis. 2004;38:448-51.

[16] Howden BP, Ward PB, Charles PG, Korman TM, Fuller A, du Cros P, et al. Treatment outcomes for serious infections caused by methicillin-resistant Staphylococcus aureus with reduced vancomycin susceptibility. Clin Infect Dis. 2004;38:521-8.

[17] Casapao AM, Leonard SN, Davis SL, Lodise TP, Patel N, Goff DA, et al.

Clinical Outcomes in Patients with Heterogeneous Vancomycin-Intermediate Staphylococcus aureus Bloodstream Infection. Antimicrobial Agents and Chemotherapy. 2013;57:4252-9.

[18] Charlson M, Szatrowski TP, Peterson J, Gold J. Validation of a combined comorbidity index. Journal of Clinical Epidemiology. 1994;47:1245-51.

[19] Hermsen ED, Hanson M, Sankaranarayanan J, Stoner JA, Florescu MC, Rupp ME. Clinical outcomes and nephrotoxicity associated with vancomycin trough concentrations during treatment of deep-seated infections. Expert Opin Drug Saf. 2010;9:9-14.

[20] Park K-H, Kim ES, Kim HS, Park S-J, Bang KM, Park HJ, et al. Comparison of the clinical features, bacterial genotypes and outcomes of patients with bacteraemia due to heteroresistant vancomycin-intermediate Staphylococcus aureus and vancomycin-susceptible S. aureus. Journal of Antimicrobial Chemotherapy. 2012;67:1843-9.

[21] Klevens R, Morrison MA, Nadle J, et al. Invasive methicillin-resistant staphylococcus aureus infections in the united states. JAMA. 2007;298:1763-71.

[22] Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care–associated infection and criteria for specific types of infections in the acute care setting. American journal of infection control. 2008;36:309-32.

[23] Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical Microbiology and Infection. 2012;18:268-81.

[24] StepanoviĆ S, VukoviĆ D, Hola V, Bonaventura GD, DjukiĆ S, ĆIrkoviĆ I, et al. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. APMIS : acta pathologica, microbiologica, et immunologica Scandinavica. 2007;115:891-9.

[25] Sakoulas G, Eliopoulos GM, Moellering RC, Wennersten C, Venkataraman L, Novick RP, et al. Accessory Gene Regulator (agr) Locus in Geographically Diverse Staphylococcus aureus Isolates with Reduced Susceptibility to Vancomycin. Antimicrobial Agents and Chemotherapy. 2002;46:1492-502.

[26] Hiramatsu K, Aritaka N, Hanaki H, Kawasaki S, Hosoda Y, Hori S, et al. Dissemination in Japanese hospitals of strains of Staphylococcus aureus heterogeneously resistant to vancomycin. The Lancet. 1997;350:1670-3.

[27] Wootton M, Howe RA, Hillman R, Walsh TR, Bennett PM, MacGowan AP. A modified population analysis profile (PAP) method to detect hetero-

resistance to vancomycin in Staphylococcus aureus in a UK hospital. The Journal of antimicrobial chemotherapy. 2001;47:399-403.

[28] Murakami K, Minamide W, Wada K, Nakamura E, Teraoka H, Watanabe S. Identification of methicillin-resistant strains of staphylococci by polymerase chain reaction. J Clin Microbiol. 1991;29:2240-4.

[29] Zhang K, McClure J-A, Elsayed S, Louie T, Conly JM. Novel Multiplex PCR Assay for Characterization and Concomitant Subtyping of Staphylococcal Cassette Chromosome mec Types I to V in Methicillin-Resistant Staphylococcus aureus. Journal of Clinical Microbiology. 2005;43:5026-33.

[30] Gilot P, Lina G, Cochard T, Poutrel B. Analysis of the Genetic Variability of Genes Encoding the RNA III-Activating Components Agr and TRAP in a Population of Staphylococcus aureus Strains Isolated from Cows with Mastitis. Journal of Clinical Microbiology. 2002;40:4060-7.

[31] Lina G, Piemont Y, Godail-Gamot F, Bes M, Peter MO, Gauduchon V, et al. Involvement of Panton-Valentine leukocidin-producing Staphylococcus aureus in primary skin infections and pneumonia. Clin Infect Dis. 1999;29:1128-32.

[32] Marconi C, Cunha MLRS, Araújo Jr JP, Rugolo LMSS. Standardization of the PCR technique for the detection of delta toxin in Staphylococcus spp. Journal of Venomous Animals and Toxins including Tropical Diseases. 2005;11:117-28.

[33] McDougal LK, Steward CD, Killgore GE, Chaitram JM, McAllister SK, Tenover FC. Pulsed-Field Gel Electrophoresis Typing of Oxacillin-Resistant Staphylococcus aureus Isolates from the United States: Establishing a National Database. Journal of Clinical Microbiology. 2003;41:5113-20.

[34] Pinto TCA, Souza ARV, de Pina SECM, Costa NS, Borges Neto AA, Neves FPG, et al. Phenotypic and Molecular Characterization of Optochin-Resistant Streptococcus pneumoniae Isolates from Brazil, with Description of Five Novel Mutations in the atpC Gene. Journal of Clinical Microbiology. 2013;51:3242-9.

[35] Zhang S, Sun X, Chang W, Dai Y, Ma X. Systematic Review and Meta-Analysis of the Epidemiology of Vancomycin-Intermediate and Heterogeneous Vancomycin-Intermediate Staphylococcus aureus Isolates. PLOS ONE. 2015;10:e0136082.

[36] Di Gregorio S, Perazzi B, Ordoñez AM, De Gregorio S, Foccoli M, Lasala MB, et al. Clinical, Microbiological, and Genetic Characteristics of Heteroresistant Vancomycin-Intermediate Staphylococcus aureus Bacteremia in a Teaching Hospital. Microbial Drug Resistance. 2015;21:25-34.

[37] Yamakawa J, Aminaka M, Okuzumi K, Kobayashi H, Katayama Y, Kondo S, et al. Heterogeneously vancomycin-intermediate Staphylococcus aureus

(hVISA) emerged before the clinical introduction of vancomycin in Japan: a retrospective study. J Infect Chemother. 2012;18:406-9.

[38] Fong RKC, Low J, Koh TH, Kurup A. Clinical features and treatment outcomes of vancomycin-intermediate Staphylococcus aureus (VISA) and heteroresistant vancomycin-intermediate Staphylococcus aureus (hVISA) in a tertiary care institution in Singapore. European Journal of Clinical Microbiology & Infectious Diseases. 2009;28:983-7.

[39] Song J-H, Hiramatsu K, Suh JY, Ko KS, Ito T, Kapi M, et al. Emergence in Asian Countries of Staphylococcus aureus with Reduced Susceptibility to Vancomycin. Antimicrobial Agents and Chemotherapy. 2004;48:4926-8.

[40] Huang S-H, Chen Y-C, Chuang Y-C, Chiu S-K, Fung C-P, Lu P-L, et al. Prevalence of vancomycin-intermediate Staphylococcus aureus (VISA) and heterogeneous VISA among methicillin-resistant S. aureus with high vancomycin minimal inhibitory concentrations in Taiwan: A multicenter surveillance study, 2012–2013. Journal of Microbiology, Immunology and Infection. 2015.

[41] van Hal SJ, Jones M, Gosbell IB, Paterson DL. Vancomycin Heteroresistance Is Associated with Reduced Mortality in ST239 Methicillin-Resistant <italic>Staphylococcus aureus</italic> Blood Stream Infections. PLOS ONE. 2011;6:e21217.

[42] Bae I-G, Federspiel JJ, Miro JM, Woods CW, Park L, Rybak MJ, et al. Heterogeneous Vancomycin-Intermediate Susceptibility Phenotype in Bloodstream Methicillin-Resistant Staphylococcus aureus Isolates from an International Cohort of Patients with Infective Endocarditis: Prevalence, Genotype, and Clinical Significance. The Journal of infectious diseases. 2009;200:1355-66.

[43] Campanile F, Borbone S, Perez M, Bongiorno D, Cafiso V, Bertuccio T, et al. Heteroresistance to glycopeptides in Italian meticillin-resistant Staphylococcus aureus (MRSA) isolates. International journal of antimicrobial agents. 2010;36:415-9.

[44] Hu H-C, Kao K-C, Chiu L-C, Chang C-H, Hung C-Y, Li L-F, et al. Clinical outcomes and molecular typing of heterogenous vancomycin-intermediate Staphylococcus aureus bacteremia in patients in intensive care units. BMC infectious diseases. 2015;15:1-7.

[45] Nunes APF, Schuenck RP, Bastos CCR, Magnanini MMF, Long JB, Iorio NLP, et al. Heterogeneous resistance to vancomycin and teicoplanin among Staphylococcus spp. isolated from bacteremia. Brazilian Journal of Infectious Diseases. 2007;11:345-50.

[46] Harigaya Y, Ngo D, Lesse AJ, Huang V, Tsuji BT. Characterization of heterogeneous vancomycin-intermediate resistance, MIC and accessory gene regulator (agr) dysfunction among clinical bloodstream isolates of staphyloccocus aureus. BMC infectious diseases. 2011;11:287-.

[47] Hu J, Ma XX, Tian Y, Pang L, Cui LZ, Shang H. Reduced Vancomycin Susceptibility Found in Methicillin-Resistant and Methicillin-Sensitive Staphylococcus aureus Clinical Isolates in Northeast China. PLOS ONE. 2013;8:e73300.

[48] Holmes NE, Turnidge JD, Munckhof WJ, Robinson JO, Korman TM, O'Sullivan MVN, et al. Genetic and Molecular Predictors of High Vancomycin MIC in Staphylococcus aureus Bacteremia Isolates. Journal of Clinical Microbiology. 2014;52:3384-93.

[49] Musta AC, Riederer K, Shemes S, Chase P, Jose J, Johnson LB, et al. Vancomycin MIC plus Heteroresistance and Outcome of Methicillin-Resistant Staphylococcus aureus Bacteremia: Trends over 11 Years. Journal of Clinical Microbiology. 2009;47:1640-4.

[50] Chen H, Liu Y, Sun W, Chen M, Wang H. The incidence of heterogeneous vancomycin-intermediate Staphylococcus aureus correlated with increase of vancomycin MIC. Diagnostic microbiology and infectious disease. 2011;71:301-3.

[51] Mendes RE, Deshpande LM, Smyth DS, Shopsin B, Farrell DJ, Jones RN. Characterization of Methicillin-Resistant Staphylococcus aureus Strains Recovered from a Phase IV Clinical Trial for Linezolid versus Vancomycin for Treatment of Nosocomial Pneumonia. Journal of Clinical Microbiology. 2012;50:3694-702.

[52] Sun W, Chen H, Liu Y, Zhao C, Nichols WW, Chen M, et al. Prevalence and Characterization of Heterogeneous Vancomycin-Intermediate Staphylococcus aureus Isolates from 14 Cities in China. Antimicrobial Agents and Chemotherapy. 2009;53:3642-9.

[53] Casapao AM, Davis SL, McRoberts JP, Lagnf AM, Patel S, Kullar R, et al. Evaluation of Vancomycin Population Susceptibility Analysis Profile as a Predictor of Outcomes for Patients with Infective Endocarditis Due to Methicillin-Resistant Staphylococcus aureus. Antimicrobial Agents and Chemotherapy. 2014;58:4636-41.

[54] Liu C, Chen Z-j, Sun Z, Feng X, Zou M, Cao W, et al. Molecular characteristics and virulence factors in methicillin-susceptible, resistant, and heterogeneous vancomycin-intermediate Staphylococcus aureus from central-southern China. Journal of Microbiology, Immunology and Infection. 2015.

[55] Maor Y, Hagin M, Belausov N, Keller N, Ben-David D, Rahav G. Clinical Features of Heteroresistant Vancomycin-Intermediate Staphylococcus aureus Bacteremia versus Those of Methicillin-Resistant S. aureus Bacteremia. Journal of Infectious Diseases. 2009;199:619-24.

[56] Vuong C, Saenz HL, Götz F, Otto M. Impact of the agr Quorum-Sensing System on Adherence to Polystyrene in Staphylococcus aureus. Journal of Infectious Diseases. 2000;182:1688-93.

[57] Sakoulas G, Gold HS, Cohen RA, Venkataraman L, Moellering RC, Eliopoulos GM. Effects of prolonged vancomycin administration on methicillin-resistant Staphylococcus aureus (MRSA) in a patient with recurrent bacteraemia. Journal of Antimicrobial Chemotherapy. 2006;57:699-704.

[58] Howden BP, Johnson PDR, Ward PB, Stinear TP, Davies JK. Isolates with Low-Level Vancomycin Resistance Associated with Persistent Methicillin-Resistant Staphylococcus aureus Bacteremia. Antimicrobial Agents and Chemotherapy. 2006;50:3039-47.

[59] Paulander W, Nissen Varming A, Bæk KT, Haaber J, Frees D, Ingmer H. Antibiotic-Mediated Selection of Quorum-Sensing-Negative Staphylococcus aureus. mBio. 2012;3.

[60] Shopsin B, Drlica-Wagner A, Mathema B, Adhikari RP, Kreiswirth BN, Novick R. Prevalence of agr Dysfunction among Colonizing Staphylococcus aureus Strains. Journal of Infectious Diseases. 2008;198:1171-4.

[61] van Hal SJ, Paterson DL. Systematic Review and Meta-Analysis of the Significance of Heterogeneous Vancomycin-Intermediate Staphylococcus aureus Isolates. Antimicrobial Agents and Chemotherapy. 2011;55:405-10.

[62] Soriano A, Marco F, Martinez JA, Pisos E, Almela M, Dimova VP, et al. Influence of vancomycin minimum inhibitory concentration on the treatment of methicillin-resistant Staphylococcus aureus bacteremia. Clin Infect Dis. 2008;46:193-200.

[63] Howden BP, Smith DJ, Mansell A, Johnson PD, Ward PB, Stinear TP, et al. Different bacterial gene expression patterns and attenuated host immune responses are associated with the evolution of low-level vancomycin resistance during persistent methicillin-resistant Staphylococcus aureusbacteraemia. BMC Microbiology. 2008;8:1-14.

[64] Peleg AY, Monga D, Pillai S, Mylonakis E, Moellering RC, Eliopoulos GM. Reduced Susceptibility to Vancomycin Influences Pathogenicity in Staphylococcus aureus Infection. The Journal of infectious diseases. 2009;199:532-6.

[65] Cohen B, Larson EL, Stone PW, Neidell M, Glied SA. Factors associated with variation in estimates of the cost of resistant infections. Medical care. 2010;48:767-75.

[66] Fridkin SK, Hageman J, McDougal LK, Mohammed J, Jarvis WR, Perl TM, et al. Epidemiological and Microbiological Characterization of Infections Caused by Staphylococcus aureus with Reduced Susceptibility to Vancomycin, United States, 1997–2001. Clinical Infectious Diseases. 2003;36:429-39.

[67] Moise PA, Smyth DS, El-Fawal N, Robinson DA, Holden PN, Forrest A, et al. Microbiological effects of prior vancomycin use in patients with

methicillin-resistant Staphylococcus aureus bacteraemia. The Journal of antimicrobial chemotherapy. 2008;61:85-90.

[68] Howden BP, Peleg AY, Stinear TP. The evolution of vancomycin intermediate Staphylococcus aureus (VISA) and heterogenous-VISA. Infection, Genetics and Evolution. 2014;21:575-82.

[69] Katayama Y, Murakami-Kuroda H, Cui L, Hiramatsu K. Selection of Heterogeneous Vancomycin-Intermediate Staphylococcus aureus by Imipenem. Antimicrobial Agents and Chemotherapy. 2009;53:3190-6.

[70] Matsuo M, Hishinuma T, Katayama Y, Cui L, Kapi M, Hiramatsu K. Mutation of RNA Polymerase β Subunit (rpoB) Promotes hVISA-to-VISA Phenotypic Conversion of Strain Mu3. Antimicrobial Agents and Chemotherapy. 2011;55:4188-95.

[71] Watanabe Y, Cui L, Katayama Y, Kozue K, Hiramatsu K. Impact of rpoB Mutations on Reduced Vancomycin Susceptibility in Staphylococcus aureus. Journal of Clinical Microbiology. 2011;49:2680-4.

[72] de Lassence A, Hidri N, Timsit J-F, Joly-Guillou M-L, Thiery G, Boyer A, et al. Control and Outcome of a Large Outbreak of Colonization and Infection with Glycopeptide-Intermediate Staphylococcus aureus in an Intensive Care Unit. Clinical Infectious Diseases. 2006;42:170-8.

3.4 Influência dos níveis séricos de vancomicina no desfecho

The importance of therapeutic drug monitoring of vancomycin in cases of suspected or confirmed Staphylococcal infections

Janine de Melo Rauber, Barbara Baggiotto, Monica Basso Zanotto, Marcelo carneiro, Andréia Rosane de Moura Valim, Pedro Alves d'Azevedo

Artigo submetido ao periódico Journal of Clinical Pharmacy and Therapeutics

The importance of therapeutic drug monitoring of vancomycin in cases of suspected or confirmed Staphylococcal infections

Janine de Melo Rauber, PharmD, MSc;[1] Bárbara Baggiotto;[2] Mônica Basso Zanotto;[2] Marcelo Carneiro, MD, PhD;[1,2,3] Andréia Rosane de Moura Valim, PharmD, PhD;[2] Pedro Alves d'Azevedo, PharmD, PhD[1*]

Affiliations: 1. Universidade Federal de Ciências da Saúde, Porto Alegre, RS, Brazil; 2. Universidade de Santa Cruz do Sul, Santa Cruz do Sul, RS, Brazil; 3. Hospital Santa Cruz, Santa Cruz do Sul, RS, Brazil.

* Address correspondence: Universidade Federal de Ciências da Saúde de Porto Alegre, Rua Sarmento Leite, 245, CEP 90050-170, Porto Alegre, RS, Brazil. Phone: 55 51 3303-8742; Fax: 55 51 3303-8810 (pedro_dazevedo@yahoo.com.br).

Conflict of interest. All authors report no conflicts of interest relevant to this article.

Source of funding. CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), FAPERGS (Fundação de Amparo à Pesquisa do Rio Grande do Sul), both located in Brazil.

SUMMARY

What is known and objective: Vancomycin is a glycopeptide antibiotic effective for severe gram-positive infections. For therapeutic effectiveness of vancomycin treatment, it is necessary to achieve appropriate trough levels, which requires constant monitoring, since it can cause toxicity. The aim of this study was to analyze the impact of vancomycin serum levels on patient outcomes and occurrence of adverse effects.

Methods: A prospective cohort study was carried out at a 234-bed, tertiary care, teaching hospital located in southern Brazil between July 2012 and June 2014. Data collected from the patients' medical records included: demographic information, medical history, comorbidities, Charlson index, vancomycin troughs, creatinine serum levels and outcome.

Results and discussion: During the study period, 145 therapeutic drug monitoring tests were performed and only 8.6% of vancomycin troughs were considered appropriate. No association was verified between vancomycin serum levels and mortality or length of hospital stay. Vancomycin troughs were significantly higher in patients that reported nephrotoxicity.

What is new and conclusion: Although no association was verified between vancomycin troughs and outcome, the importance of this parameter measurement is proven, since a small number of doses remained within the appropriate range. Furthermore, drug-induced nephrotoxicity was associated to vancomycin serum levels higher than 15 mg/liter. Therefore, vancomycin troughs should be monitored to avoid the occurrence of adverse events.

Keywords. Vancomycin, Therapeutic Drug Monitoring (TDM), Nephrotoxicity, *Staphylococcus* spp.

WHAT IS KNOWN AND OBJECTIVE

Vancomycin has traditionally been used as a first-line agent for treating methicillin-resistant *Staphylococcus aureus* (MRSA) and other Gram-positive bacteria including Coagulase-negative *Staphylococcus* spp. (CoNS), *Enterococcus faecalis* and *Enterococcus faecium* (1, 2). The drug acts inhibiting the synthesis of bacterial cell wall by binding to the terminal D-alanine-D-alanine and preventing the coupling of the peptidoglycan polymers (3, 4).

In recently published clinical trials with antimicrobials, such as daptomycin, linezolid and tigecycline, superiority in relation to vancomycin activity was not observed, a drug developed and marketed for over 50 years (5). However, there are many controversies regarding the appropriate and safe use of this agent (6). The interchangeability between the reference product and the generic drug, for instance, was questioned by Vesga et al. (7), which found that pharmaceutical equivalence does not imply in therapeutic equivalence for vancomycin.

The American Society of Health-System Pharmacist, Infectious Disease Society of America and Society of Infectious Disease Pharmacists guidelines recommend more-intensive vancomycin doses to maintain troughs between 15 and 20 mg/liter, warranting an area under the concentration time curve/minimum inhibitory concentration ratio of ≥400 for vancomycin (8). Previous studies have associated inadequate serum levels of vancomycin with treatment failure, development of nephrotoxicity and bacterial heteroresistance (9-14). However, recent meta-analyzes reported no significant difference between low and high trough regimen in terms of treatment failure and mortality (15, 16).

Length of hospital stay (LOS) is another important outcome responsible for an increase of in-hospital costs (17), but unfortunately the impact of vancomycin troughs is not evaluated in studies.

Considering the controversial results of the published studies and the lack of data regarding the influence on the LOS, the objective of this study was to analyze the impact of vancomycin troughs on patient outcomes (mortality and LOS) and occurrence of drug-induced nephrotoxicity.

METHODS

A prospective cohort study was conducted at a 234-bed, tertiary care teaching hospital located in southern Brazil, between July 2012 and June 2014. The study was reviewed and approved by the Research Ethics Committee of Universidade Federal de Ciências da Saúde de Porto Alegre (1779/12).

The study included all patients who were treated with the antibiotic vancomycin for at least 72 hours. Data collected from the patients' medical records included: demographic information, medical history, comorbidities, Charlson index (18), vancomycin troughs, creatinine serum levels and outcome.

The vancomycin assay was performed using the immunoassay method of the microparticles by chemiluminescence on Architect i2000SR equipment (Abbott®, USA). The collection of biological samples to evaluate serum levels was performed 72 hours after start of the treatment, immediately before the administration of the vancomycin dose (trough concentration). The creatinine assay was performed using the kinetic test without deproteinization according to the method described by Jaffe in 1886 (19).

Drug-induced nephrotoxicity was assessed using the RIFLE criteria, which consists in three levels (risk, injury and failure) based on the magnitude of serum creatinine elevation and two outcome measures (20).

Categorical variables were compared using Chi-square or Fisher's exact test and continuous variables were compared using the Mann-Whitney U-test, as appropriate. All analyses were performed using the computer software SPSS version 20.0 (SPSS Inc, Chicago, IL, USA). A p-value ≤ 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

During the study period, 145 therapeutic drug monitoring tests were performed for 86 patients (66% males and 62% adults), with an average dose of 1.6 per patient. The International Classification of Diseases (ICD) through which patients were hospitalized consisted mainly of infectious (39.5%), heart (14%) and neurological diseases (11.6%), with a mean Charlson comorbidity score of 1.0 (basal comorbidities). Fifty-six (65%) patients treated with vancomycin had a confirmed infection. About 37% of infections were caused by coagulase-negative *Staphylococcus*, 10% by *S. aureus* and 16% by other gram-positive microorganisms. The main sites of microorganism isolation were blood (39.7%), respiratory tract (39.7%) and skin or soft-tissue secretions (20.6%).

Only 8.6% of vancomycin troughs were considered appropriate within the therapeutic window, 51.4% were considered as low and 40% as high levels. In a hospital in Canada, Bollinger et al. (21) evaluated 108 patients, of which 72.2% had serum suboptimal concentrations of vancomycin.

Vancomycin is an antibiotic agent very often employed in clinical practice, but its use requires appropriate therapeutic monitoring. Vancomycin therapeutic monitoring aims to change the dose when serum levels are inadequate, preventing treatment failure, reducing the risk of bacterial resistance and preventing the occurrence of nephrotoxicity (22).

Most of the vancomycin is excreted by the kidney, so patients with renal dysfunction require dose adjustment to prevent drug accumulation (23). Patients who are already on dialysis are at greater risk of the toxic effects of vancomycin in the bloodstream. In patients with normal renal function, the half-life is 6 to 12 hours, while for patients with impaired renal function, the clearance time increases to 100 to 200 hours (24).

The main indications for serum level monitoring are patients receiving high vancomycin doses, which are on prolonged therapy and are most likely to develop reversible nephrotoxicity, patients with concomitant use of other nephrotoxic drugs and/or obese ones. The frequency of vancomycin measurement in blood will be carried out according to the severity of the patient's condition (8).

Loading doses in vancomycin therapy may more rapidly attain troughs of 15 to 20 mg/L in adults (25); however, drug monitoring must be evaluated prior to the next dose at steady-state condition (just before the fourth dose) (8).

As shown in Table 1, high levels were observed in adults (23.42 mg/liter) when compared to neonates (15.06 mg/liter) and children (14.06 mg/liter) ($p = 0.044$ and $p < 0.001$, respectively). Serum levels in neonates and children were more adequate when compared to those in adults, a fact that is probably due to calculation of the antibiotic dose based on the weight of newborns and children.

A study by De Waelen et al. (26) determined the efficacy of a dose protocol based on body weight in adult ICU patients. Of the total 227 patients, 126 (55.5%) received doses according to the protocol. The mean serum levels achieved in the first and second day were respectively 19.32 mg/liter and 21.08 mg/liter and most patients achieved adequate levels of vancomycin.

Prescribers do not usually choose the measurement of vancomycin serum levels due to the high cost however employ nomograms (ratio of creatinine clearance, vancomycin clearance and weight) to obtain the amount of drug to be administered (4, 27).

The use of a nomogram has been proven to be more cost-effective than conventional vancomycin measurement. However, published nomograms in clinical use have been proven to be inaccurate and most have not been clinically validated. Furthermore, no published nomogram to date has been constructed to achieve vancomycin serum trough levels of 15–20 mg/L (8, 28).

Several parameters have been proposed for the monitoring of vancomycin pharmacodynamics, such as the concentration time above the minimum inhibitory concentration and the area under the curve above the MIC (AUC/MIC). Clinical efficacy is achieved when the result of AUC is ≥400 (8). For less severe infections, the recommended serum levels are above 10 mg/liter, while values between 15 and 20 mg/liter are indicated for severe infections (meningitis, bacteremia, endocarditis, among others). Serum levels of 15 mg/liter are recommended in case of infections by microorganisms with MIC of 1 mg/liter (8, 29). The difficulty in using vancomycin therapy occurs mainly in cases with high MIC, as there is a reduced chance to reach the parameter of AUC ≥400.

The comparison of serum vancomycin levels and mortality is described in Table 1. 27% of the patients died and no association was verified with serum vancomycin levels (p=0.892). Hospital length of stay was not affected by vancomycin troughs (p=0.152 and p=0.401) as shown in Table 2. As shown in Table 3, nephrotoxicity was not associated with mortality, but Charlson score was correlated with death. Similarly to present study, the Charlson index was considered a suitable predictor of death in patients with severe Staphylococcal infections in a previous analysis (30).

Many studies found an association between vancomycin serum levels and outcome. According to Kullar et al. (31), treatment failure predictors were endocarditis, nosocomial infection, initial vancomycin levels < 15 mg/liter, MIC > 1 mg/liter and AUC < 421. Albur et al. (10) associated mortality with serum levels < 10 mg/liter. The study by Forstner et al. (11) also reported similar results, with higher treatment failure observed in patients with vancomycin levels < mg/liter. However, similarly to the present study, Cao et al. (32) were not able to associate vancomycin levels to patient clinical response, suggesting the need for further studies to establish the influence of serum levels on clinical efficacy.

Nephrotoxicity was verified in 30.2% of patients, and of these patients, 15.3% showed risk, 53.8% reported injury and 30.9% had failure. Vancomycin levels were higher (16.71 vs. 39.97 mg/liter, p<0.001) in patients that reported nephrotoxicity (Table 1).

Lodise et al. (33) associated exposure to vancomycin with nephrotoxicity onset in patients infected with gram-positive microorganisms. Moreover, van Hal et al. (34) associated this event with serum levels higher than 15 mg/liter, while Ragab et al. (35) associated it with levels higher than 10 mg/liter in chil-

dren. However, Elyasi et al. (14) demonstrated that the renal injury is not associated with the therapeutic range, i.e., the patient may have renal injury even when drug dose is within the recommended range. Factors such as low body weight, dose, duration of therapy and concomitant use of other nephrotoxic medications increase the susceptibility to nephrotoxicity development (36).

Our study should be interpreted in the context of some limitations. First, this was a single-center study with a small sample size. Second, clinical data were collected from the patients' medical records and unfortunately some information could be missing. Finally, were included all patients treated with vancomycin in the study period irrespective of confirmation of infection.

Table 1. Comparison of vancomycin troughs according to age and outcomes

Variable		Vancomycin troughs (mg/liter)	p-value
Demographics			
Neonates (n=41)		15.06	0.044
Adults (n=93)		23.42	
Children (n=11)		14.06	<0.001
Outcomes			
Mortality	No	20.87	0.892
	Yes	18.46	
Nephrotoxicity	No	16.71	<0.001
	Yes	39.97	

Table 2. Association between vancomycin serum levels and length of hospital stay

Vancomycin troughs (mg/liter)	Length of stay (days)	p-value
<15	44	0.152
>15	54	
10-20	44	0.401
<10 or >20	45	

Table 3. Comparison of clinical features and outcome in patients treated with vancomycin

Variables, n (%)	Survivors (n= 63)	Non-survivors (n= 23)	p-value
Male	39 (62)	18 (79)	0.090
Adult	39 (62)	14 (61)	0.924
Confirmed infection	40 (64)	16 (70)	0.731
Charlson index, median (IQR)	0 (0-1)	1 (1-3)	0.001
LOS, mean±SE (days)	35.8±3.4	43.2±12.7	0.654
Nephrotoxicity	21 (33)	5 (22)	0.427
Creatinine, mean±SE (mg/dL)	1.79±0.21	1.55±0.27	0.497
Vancomycin troughs, mean±SE (mg/liter)	20.87±1.95	18.46±2.73	0.892
Duration of therapy, mean±SE (days)	15.1±1.7	55.1±11.5	0.005

IQR, Interquartile range; LOS, Length of hospital stay; SE, Standard error.

WHAT IS NEW AND CONCLUSION

Even vancomycin troughs have not been associated with mortality in this study, the therapeutic drug monitoring remains an important tool since the most of serum levels measured were outside the appropriate range. Furthermore, the drug-induced nephrotoxicity was associated to vancomycin troughs higher than 15 mg/liter and monitoring is required in order to prevent the occurrence of this adverse event. More appropriate vancomycin troughs were observed in neonates and children, when compared to adults, because physicians' prescriptions are based on patient weight, while for adults, a standard dose is normally prescribed. Empirical doses may result in serum levels outside the recommended therapeutic range, compromising the effectiveness of the treatment and increasing the chances of toxicity.

REFERENCES

1. Álvarez R, López Cortés LE, Molina J, Cisneros JM, Pachón J. Optimizing the Clinical Use of Vancomycin. Antimicrobial Agents and Chemotherapy. 2016 May 1, 2016;60(5):2601-9.
2. Willems RJ, Hanage WP, Bessen DE, Feil EJ. Population biology of Gram-positive pathogens: high-risk clones for dissemination of antibiotic resistance. FEMS microbiology reviews. 2011 Sep;35(5):872-900.
3. Périchon B, Courvalin P. Heterologous Expression of the Enterococcal vanA Operon in Methicillin-Resistant Staphylococcus aureus. Antimicrobial Agents and Chemotherapy. 2004 November 1, 2004;48(11):4281-5.
4. Levine DP. Vancomycin: A History. Clinical Infectious Diseases. 2006 January 1, 2006;42(Supplement 1):S5-S12.
5. DeLeo FR, Otto M, Kreiswirth BN, Chambers HF. Community-associated meticillin-resistant Staphylococcus aureus. Lancet. 2010 May 1;375(9725):1557-68.
6. Holmes NE, Tong SY, Davis JS, van Hal SJ. Treatment of methicillin-resistant Staphylococcus aureus: vancomycin and beyond. Semin Respir Crit Care Med. 2015 Feb;36(1):17-30.
7. Vesga O, Agudelo M, Salazar BE, Rodriguez CA, Zuluaga AF. Generic Vancomycin Products Fail In Vivo despite Being Pharmaceutical Equivalents of the Innovator. Antimicrobial Agents and Chemotherapy. 2010;54(8):3271-9.

8. Rybak M, Lomaestro B, Rotschafer JC, Moellering R, Jr., Craig W, Billeter M, et al. Therapeutic monitoring of vancomycin in adult patients: a consensus review of the American Society of Health-System Pharmacists, the Infectious Diseases Society of America, and the Society of Infectious Diseases Pharmacists. American journal of health-system pharmacy : AJHP : official journal of the American Society of Health-System Pharmacists. 2009 Jan 1;66(1):82-98.
9. Park K-H, Kim ES, Kim HS, Park S-J, Bang KM, Park HJ, et al. Comparison of the clinical features, bacterial genotypes and outcomes of patients with bacteraemia due to heteroresistant vancomycin-intermediate Staphylococcus aureus and vancomycin-susceptible S. aureus. Journal of Antimicrobial Chemotherapy. 2012 August 1, 2012;67(8):1843-9.
10. Albur MS, Bowker K, Weir I, MacGowan A. Factors influencing the clinical outcome of methicillin-resistant Staphylococcus aureus bacteraemia. European Journal of Clinical Microbiology & Infectious Diseases. [journal article]. 2012;31(3):295-301.
11. Forstner C, Dungl C, Tobudic S, Mitteregger D, Lagler H, Burgmann H. Predictors of clinical and microbiological treatment failure in patients with methicillin-resistant Staphylococcus aureus (MRSA) bacteraemia: a retrospective cohort study in a region with low MRSA prevalence. Clinical Microbiology and Infection. 2013;19(7):E291-E7.
12. Sakoulas G, Gold HS, Cohen RA, Venkataraman L, Moellering RC, Eliopoulos GM. Effects of prolonged vancomycin administration on methicillin-resistant Staphylococcus aureus (MRSA) in a patient with recurrent bacteraemia. Journal of Antimicrobial Chemotherapy. 2006 April 1, 2006;57(4):699-704.
13. Barriere SL, Stryjewski ME, Corey GR, Genter FC, Rubinstein E. Effect of vancomycin serum trough levels on outcomes in patients with nosocomial pneumonia due to Staphylococcus aureus: a retrospective, post hoc, subgroup analysis of the Phase 3 ATTAIN studies. BMC infectious diseases. [journal article]. 2014;14(1):1-5.
14. Elyasi S, Khalili H, Dashti-Khavidaki S, Mohammadpour A. Vancomycin-induced nephrotoxicity: mechanism, incidence, risk factors and special populations. A literature review. Eur J Clin Pharmacol. 2012 2012/09/01;68(9):1243-55.
15. Men P, Li H-B, Zhai S-D, Zhao R-S. Association between the AUC(0-24)/MIC Ratio of Vancomycin and Its Clinical Effectiveness: A Systematic Review and Meta-Analysis. PLOS ONE. 2016;11(1):e0146224.
16. Prybylski JP. Vancomycin Trough Concentration as a Predictor of Clinical Outcomes in Patients with Staphylococcus aureus Bacteremia: A Meta-analysis of Observational Studies. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy. 2015;35(10):889-98.
17. Cohen B, Larson EL, Stone PW, Neidell M, Glied SA. Factors associated with variation in estimates of the cost of resistant infections. Medical care. 2010 Sep;48(9):767-75.
18. Charlson M, Szatrowski TP, Peterson J, Gold J. Validation of a combined comorbidity index. Journal of Clinical Epidemiology. 1994;47(11):1245-51.

19. Delanghe JR, Speeckaert MM. Creatinine determination according to Jaffe—what does it stand for? NDT Plus. 2011;4(2):83-6.
20. Bellomo R, Ronco C, Kellum JA, Mehta RL, Palevsky P, the Aw. Acute renal failure – definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. Critical Care. 2004;8(4):R204-R12.
21. Bollinger M, Hamilton M, Schroeder K, Link S, Nguyen J, Chu D, et al. Vancomycin use in a rural hospital: a 3-year retrospective study. Can J Rural Med. 2015 Spring;20(2):56-62.
22. Horne KC, Howden BP, Grabsch EA, Graham M, Ward PB, Xie S, et al. Prospective Comparison of the Clinical Impacts of Heterogeneous Vancomycin-Intermediate Methicillin-Resistant Staphylococcus aureus (MRSA) and Vancomycin-Susceptible MRSA. Antimicrobial Agents and Chemotherapy. 2009;53(8):3447-52.
23. Avent ML, Vaska VL, Rogers BA, Cheng AC, van Hal SJ, Holmes NE, et al. Vancomycin therapeutics and monitoring: a contemporary approach. Internal Medicine Journal. 2013;43(2):110-9.
24. Vandecasteele SJ, De Bacquer D, De Vriese AS. Implementation of a Dose Calculator for Vancomycin to Achieve Target Trough Levels of 15–20 μg/mL in Persons Undergoing Hemodialysis. Clinical Infectious Diseases. 2011 July 15, 2011;53(2):124-9.
25. Reardon J, Lau TT, Ensom MH. Vancomycin loading doses: a systematic review. Ann Pharmacother. 2015 May;49(5):557-65.
26. De Waele JJ, Danneels I, Depuydt P, Decruyenaere J, Bourgeois M, Hoste E. Factors associated with inadequate early vancomycin levels in critically ill patients treated with continuous infusion. International journal of antimicrobial agents. 2013;41(5):434-8.
27. Lima TdM, Elias SC, Estrela RdCE, Cardoso FLL. Implementation of vancomycin dosing nomogram in an electronic prescribing system: an innovative tool in antibiotic stewardship. Brazilian Journal of Pharmaceutical Sciences. 2014;50:567-72.
28. Elyasi S, Khalili H. Vancomycin dosing nomograms targeting high serum trough levels in different populations: pros and cons. Eur J Clin Pharmacol. [journal article]. 2016:1-12.
29. Broome L, So T-Y. An Evaluation of Initial Vancomycin Dosing in Infants, Children, and Adolescents. International Journal of Pediatrics. 2011;2011:470364.
30. Lesens OMD, Methlin CMD, Hansmann YMD, Remy VMD, Martinot MMD, Bergin CMD, et al. Role of Comorbidity in Mortality Related to Staphylococcus aureus Bacteremia: A Prospective Study Using the Charlson Weighted Index of Comorbidity • Infection Control and Hospital Epidemiology. 2003;24(12):890-6.
31. Kullar R, Davis SL, Levine DP, Rybak MJ. Impact of vancomycin exposure on outcomes in patients with methicillin-resistant Staphylococcus aureus bacteremia: support for consensus guidelines suggested targets. Clin Infect Dis. 2011 Apr 15;52(8):975-81.
32. Cao G, Liang X, Zhang J, Zhou Y, Wu J, Zhang Y, et al. Vancomycin serum trough concentration vs. clinical outcome in patients with gram-

positive infection: a retrospective analysis. Journal of Clinical Pharmacy and Therapeutics. 2015;40(6):640-4.

33. Lodise TP, Graves J, Evans A, Graffunder E, Helmecke M, Lomaestro BM, et al. Relationship between vancomycin MIC and failure among patients with methicillin-resistant Staphylococcus aureus bacteremia treated with vancomycin. Antimicrob Agents Chemother. 2008 Sep;52(9):3315-20.
34. van Hal SJ, Paterson DL, Lodise TP. Systematic Review and Meta-Analysis of Vancomycin-Induced Nephrotoxicity Associated with Dosing Schedules That Maintain Troughs between 15 and 20 Milligrams per Liter. Antimicrobial Agents and Chemotherapy. 2013 February 1, 2013;57(2):734-44.
35. Ragab AR, Al-Mazroua MK, Al-Harony MA. Incidence and Predisposing Factors of Vancomycin-Induced Nephrotoxicity in Children. Infectious Diseases and Therapy. 2013;2(1):37-46.
36. Rostas SE, Kubiak DW, Calderwood MS. High-Dose Intravenous Vancomycin Therapy and the Risk of Nephrotoxicity. Clinical therapeutics. 2014;36(7):1098-101.

4 CONSIDERAÇÕES FINAIS

A análise microbiológica e molecular dos isolados, bem como a avaliação das características clínicas dos pacientes constituem importantes ferramentas no estudo epidemiológico em infecções por estafilococos. O estudo da influência destes fatores no desfecho contribui para estabelecimento de diretrizes de tratamento e medidas para prevenção destas infecções.

De acordo com o presente estudo, não houve diferença na mortalidade dos pacientes em função das espécies, isto é, *S. aureus* em comparação com os estafilococos coagulase negativa. As características clínicas relacionadas ao perfil de co-morbidades dos pacientes, sumarizadas no índice de Charlson, influenciaram significativamente na mortalidade.

Os isolados multirresistentes foram relacionados a índices superiores de óbito e prolongamento do tempo de internação. No entanto, a resistência à meticilina foi associada apenas ao aumento do tempo de permanência hospitalar. As variáveis concentração mínima inibitória e formação de biofilme não apresentaram relação com a taxa de mortalidade dos pacientes. Estes resultados evidenciam a necessidade do controle de infecção para elaboração e implementação de rotinas de prevenção da emergência e disseminação de cepas resistentes, amplamente relacionadas à desfechos negativos.

Não houve influência da hetero-resistência intermediária à vancomicina no desfecho da terapia, porém a elevada prevalência desta característica nos estafilococos coagulase negativa causa preocupação uma vez que a maioria destas cepas apresenta resistência à meticilina, sendo a vancomicina ainda a principal escolha terapêutica. A ausência de clonalidade entre a maioria dos isolados hetero-resistentes sugere uma adaptação frente ao tratamento ao invés da transmissão entre pacientes. A investigação do impacto dos níveis séricos de vancomicina revelou ausência de relação com a mortalidade, porém foi verificada associação entre níveis superiores a 15 µg/mL e nefrotoxicidade induzida. Portanto, o monitoramento terapêutico deste antimicrobiano torna-se uma prática importante a fim de evitar a possível indução de hetero-resistência

intermediária à vancomicina além da ocorrência de eventos indesejáveis como a nefrotoxicidade.

Considerando as características moleculares, não se verificou influência do tipo de SCC*mec* e *locus agr* no desfecho do tratamento. No entanto, a ausência de *locus agr* e a disfunção do *agr*, analisada através do teste fenotípico de δ-hemolisina, foram relacionadas com a resistência à meticilina, aumento da concentração mínima inibitória e hetero-resistência intermediária à vancomicina. A presença de PVL também não foi associada à mortalidade. Embora não tenha sido verificado impacto dos fatores moleculares no desfecho da terapia, estudos de avaliação molecular colaboram para a melhor compreensão do mecanismo de patogênese e adaptação do microrganismo de acordo com o ambiente.

5 REFERÊNCIAS BIBLIOGRÁFICAS

1. Casey AL, Lambert PA, Elliott TSJ. Staphylococci. International journal of antimicrobial agents. 2007;29, Supplement 3(0):S23-S32.
2. Rogers KL, Fey PD, Rupp ME. Coagulase-negative staphylococcal infections. Infect Dis Clin North Am. 2009 Mar;23(1):73-98.
3. Silveira ACdO, d'Azevedo PA. Staphylococcus lugdunensis: um olhar diferenciado no laboratório clínico. Jornal Brasileiro de Patologia e Medicina Laboratorial. 2011;47:151-6.
4. Piette A, Verschraegen G. Role of coagulase-negative staphylococci in human disease. Veterinary microbiology. 2009 Feb 16;134(1-2):45-54.
5. Sievert DMP, Ricks PP, Edwards JRMS, Schneider AMPH, Patel JP, Srinivasan AMD, et al. Antimicrobial-Resistant Pathogens Associated with Healthcare-Associated Infections: Summary of Data Reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2009–2010. Infection Control and Hospital Epidemiology. 2013;34(1):1-14.
6. von Eiff C, Becker K, Machka K, Stammer H, Peters G. Nasal carriage as a source of Staphylococcus aureus bacteremia. Study Group. The New England journal of medicine. 2001 Jan 4;344(1):11-6.
7. Peacock SJ, de Silva I, Lowy FD. What determines nasal carriage of Staphylococcus aureus? Trends in microbiology. 2001 Dec;9(12):605-10.
8. Nulens E, Gould I, MacKenzie F, Deplano A, Cookson B, Alp E, et al. Staphylococcus aureus carriage among participants at the 13th European Congress of Clinical Microbiology and Infectious Diseases. Eur J Clin Microbiol Infect Dis. 2005 2005/02/01;24(2):145-8.
9. Gorwitz RJ, Kruszon-Moran D, McAllister SK, McQuillan G, McDougal LK, Fosheim GE, et al. Changes in the prevalence of nasal colonization with Staphylococcus aureus in the United States, 2001-2004. The Journal of infectious diseases. 2008 May 1;197(9):1226-34.
10. Willems RJ, Hanage WP, Bessen DE, Feil EJ. Population biology of Gram-positive pathogens: high-risk clones for dissemination of antibiotic resistance. FEMS microbiology reviews. 2011 Sep;35(5):872-900.
11. Lowy FD. Staphylococcus aureus Infections. New England Journal of Medicine. 1998;339(8):520-32.
12. ANVISA. Boletim de Segurança do Paciente e Qualidade em Serviços de Saúde. Rede Nacional de Monitoramento da Resistência Microbiana em Seviços de Saúde. 2015;12.
13. Chitnis ASMDMPH, Edwards JRM, Ricks PMP, Sievert DMP, Fridkin SKMD, Gould CVMDM. Device-Associated Infection Rates, Device Utilization, and Antimicrobial Resistance in Long-Term Acute Care Hospitals Reporting to the National Healthcare Safety Network, 2010. Infection Control and Hospital Epidemiology. 2012;33(10):993-1000.
14. von Eiff C, Peters G, Heilmann C. Pathogenesis of infections due to coagulase negative staphylococci. The Lancet Infectious Diseases. 2002;2(11):677-85.
15. Rahkonen M, Luttinen S, Koskela M, Hautala T. True bacteremias caused by coagulase negative Staphylococcus are difficult to distinguish from blood culture contaminants. European Journal of Clinical Microbiology &

Infectious Diseases. 2012 2012/10/01;31(10):2639-44.
16. Ziebuhr W, Hennig S, Eckart M, Kränzler H, Batzilla C, Kozitskaya S. Nosocomial infections by Staphylococcus epidermidis: how a commensal bacterium turns into a pathogen. International journal of antimicrobial agents. 2006;28, Supplement 1(0):14-20.
17. Otto M. Molecular basis of Staphylococcus epidermidis infections. Semin Immunopathol. 2012;34(2):201-14.
18. Uekotter A, Peters G, Becker K. Is there any rationale for treatment of Staphylococcus aureus infections with antimicrobials that are determined to be ineffective in vitro? Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2011 Aug;17(8):1142-7.
19. Gould IM. Clinical activity of anti-Gram-positive agents against methicillin-resistant Staphylococcus aureus. The Journal of antimicrobial chemotherapy. 2011 May;66 Suppl 4:iv17-iv21.
20. Calfee DP. Methicillin-resistant Staphylococcus aureus and vancomycin-resistant enterococci, and other Gram-positives in healthcare. Curr Opin Infect Dis. 2012 Aug;25(4):385-94.
21. Brumfitt W, Hamilton-Miller J. Methicillin-Resistant Staphylococcus aureus. New England Journal of Medicine. 1989;320(18):1188-96.
22. Rosenthal VD, Bijie H, Maki DG, Mehta Y, Apisarnthanarak A, Medeiros EA, et al. International Nosocomial Infection Control Consortium (INICC) report, data summary of 36 countries, for 2004-2009. American journal of infection control. 2012;40(5):396-407.
23. Chambers HF. Methicillin-resistant staphylococci. Clinical microbiology reviews. 1988 April 1, 1988;1(2):173-86.
24. Ito T, Katayama Y, Asada K, Mori N, Tsutsumimoto K, Tiensasitorn C, et al. Structural Comparison of Three Types of Staphylococcal Cassette Chromosome mec Integrated in the Chromosome in Methicillin-Resistant Staphylococcus aureus. Antimicrobial Agents and Chemotherapy. 2001;45(5):1323-36.
25. Shore AC, Coleman DC. Staphylococcal cassette chromosome mec: Recent advances and new insights. International Journal of Medical Microbiology. 2013;303(6–7):350-9.
26. Peacock SJ, Paterson GK. Mechanisms of Methicillin Resistance in Staphylococcus aureus. Annual Review of Biochemistry. 2015;84(1):577-601.
27. International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements Corresponding author: Teruyo Ito. Mailing address: Juntendo University DoBHB-kTJPFE. Classification of Staphylococcal Cassette Chromosome mec (SCCmec): Guidelines for Reporting Novel SCCmec Elements. Antimicrobial Agents and Chemotherapy. 2009;53(12):4961-7.
28. Wu Z, Li F, Liu D, Xue H, Zhao X. Novel Type XII Staphylococcal Cassette Chromosome mec Harboring a New Cassette Chromosome Recombinase, CcrC2. Antimicrobial Agents and Chemotherapy. 2015 December 1, 2015;59(12):7597-601.
29. Herold BC, Immergluck LC, Maranan MC, Lauderdale DS, Gaskin RE, Boyle-Vavra S, et al. Community-acquired methicillin-resistant Staphylococcus aureus in children with no identified predisposing risk.

JAMA. 1998 Feb 25;279(8):593-8.
30. Four pediatric deaths from community-acquired methicillin-resistant Staphylococcus aureus - Minnesota and North Dakota, 1997-1999. MMWR Morb Mortal Wkly Rep. 1999 Aug 20;48(32):707-10.
31. Daum RS, Ito T, Hiramatsu K, Hussain F, Mongkolrattanothai K, Jamklang M, et al. A Novel Methicillin-Resistance Cassette in Community-Acquired Methicillin-Resistant Staphylococcus aureus Isolates of Diverse Genetic Backgrounds. Journal of Infectious Diseases. 2002 November 1, 2002;186(9):1344-7.
32. Yamamoto T, Nishiyama A, Takano T, Yabe S, Higuchi W, Razvina O, et al. Community-acquired methicillin-resistant Staphylococcus aureus: community transmission, pathogenesis, and drug resistance. J Infect Chemother. 2010 Aug;16(4):225-54.
33. Otto M. Community-associated MRSA: what makes them special? International journal of medical microbiology : IJMM. 2013;303(0):324-30.
34. Rodríguez-Noriega E, Seas C. The changing pattern of methicillin-resistant staphylococcus aureus clones in Latin America: implications for clinical practice in the region. Brazilian Journal of Infectious Diseases. 2010;14:87-96.
35. Voss A, Loeffen F, Bakker J, Klaassen C, Wulf M. Methicillin-resistant Staphylococcus aureus in Pig Farming. Emerging Infectious Disease journal. 2005;11(12):1965.
36. Graveland H, Duim B, van Duijkeren E, Heederik D, Wagenaar JA. Livestock-associated methicillin-resistant Staphylococcus aureus in animals and humans. International Journal of Medical Microbiology. 2011;301(8):630-4.
37. Smith TC, Pearson N. The emergence of Staphylococcus aureus ST398. Vector Borne Zoonotic Dis. 2011 Apr;11(4):327-39.
38. Kock R, Becker K, Cookson B, van Gemert-Pijnen JE, Harbarth S, Kluytmans J, et al. Methicillin-resistant Staphylococcus aureus (MRSA): burden of disease and control challenges in Europe. Euro Surveill. 2010 Oct 14;15(41):19688.
39. Garcia-Graells C, Antoine J, Larsen J, Catry B, Skov R, Denis O. Livestock veterinarians at high risk of acquiring methicillin-resistant Staphylococcus aureus ST398. Epidemiology and infection. 2012 Mar;140(3):383-9.
40. Becker K, Ballhausen B, Köck R, Kriegeskorte A. Methicillin resistance in Staphylococcus isolates: The “mec alphabet” with specific consideration of mecC, a mec homolog associated with zoonotic S. aureus lineages. International Journal of Medical Microbiology. 2014;304(7):794-804.
41. García-Álvarez L, Holden MTG, Lindsay H, Webb CR, Brown DFJ, Curran MD, et al. Meticillin-resistant Staphylococcus aureus with a novel mecA homologue in human and bovine populations in the UK and Denmark: a descriptive study. The Lancet Infectious Diseases. 2011;11(8):595-603.
42. Avent ML, Vaska VL, Rogers BA, Cheng AC, van Hal SJ, Holmes NE, et al. Vancomycin therapeutics and monitoring: a contemporary approach. Internal Medicine Journal. 2013;43(2):110-9.
43. DeLeo FR, Otto M, Kreiswirth BN, Chambers HF. Community-associated meticillin-resistant Staphylococcus aureus. Lancet. 2010 May 1;375(9725):1557-68.
44. Périchon B, Courvalin P. Heterologous Expression of the Enterococcal vanA

Operon in Methicillin-Resistant Staphylococcus aureus. Antimicrobial Agents and Chemotherapy. 2004 November 1, 2004;48(11):4281-5.
45. Levine DP. Vancomycin: A History. Clinical Infectious Diseases. 2006 January 1, 2006;42(Supplement 1):S5-S12.
46. Kollef MH. Limitations of vancomycin in the management of resistant staphylococcal infections. Clin Infect Dis. 2007 Sep 15;45 Suppl 3:S191-5.
47. Rybak M, Lomaestro B, Rotschafer JC, Moellering R, Jr., Craig W, Billeter M, et al. Therapeutic monitoring of vancomycin in adult patients: a consensus review of the American Society of Health-System Pharmacists, the Infectious Diseases Society of America, and the Society of Infectious Diseases Pharmacists. American journal of health-system pharmacy : AJHP : official journal of the American Society of Health-System Pharmacists. 2009 Jan 1;66(1):82-98.
48. van Hal SJ, Paterson DL, Lodise TP. Systematic Review and Meta-Analysis of Vancomycin-Induced Nephrotoxicity Associated with Dosing Schedules That Maintain Troughs between 15 and 20 Milligrams per Liter. Antimicrobial Agents and Chemotherapy. 2013 February 1, 2013;57(2):734-44.
49. Pereira Alves ML, Melo GdAN, Yamada SS, Nishiyama P. Therapeutic monitoring of vancomycin. Acta Scientiarum Health Sciences. 2012;34(2).
50. Prybylski JP. Vancomycin Trough Concentration as a Predictor of Clinical Outcomes in Patients with Staphylococcus aureus Bacteremia: A Meta-analysis of Observational Studies. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy. 2015;35(10):889-98.
51. Men P, Li H-B, Zhai S-D, Zhao R-S. Association between the AUC(0-24)/MIC Ratio of Vancomycin and Its Clinical Effectiveness: A Systematic Review and Meta-Analysis. PLOS ONE. 2016;11(1):e0146224.
52. Soriano A, Marco F, Martinez JA, Pisos E, Almela M, Dimova VP, et al. Influence of vancomycin minimum inhibitory concentration on the treatment of methicillin-resistant Staphylococcus aureus bacteremia. Clin Infect Dis. 2008 Jan 15;46(2):193-200.
53. Lodise TP, Graves J, Evans A, Graffunder E, Helmecke M, Lomaestro BM, et al. Relationship between vancomycin MIC and failure among patients with methicillin-resistant Staphylococcus aureus bacteremia treated with vancomycin. Antimicrob Agents Chemother. 2008 Sep;52(9):3315-20.
54. Yoon YK, Kim JY, Park DW, Sohn JW, Kim MJ. Predictors of persistent methicillin-resistant Staphylococcus aureus bacteraemia in patients treated with vancomycin. The Journal of antimicrobial chemotherapy. 2010 May;65(5):1015-8.
55. Holland TL, Fowler VG. Vancomycin Minimum Inhibitory Concentration and Outcome in Patients With Staphylococcus aureus Bacteremia: Pearl or Pellet? Journal of Infectious Diseases. 2011 August 1, 2011;204(3):329-31.
56. van Hal SJ, Lodise TP, Paterson DL. The Clinical Significance of Vancomycin Minimum Inhibitory Concentration in Staphylococcus aureus Infections: A Systematic Review and Meta-analysis. Clinical Infectious Diseases. 2012 March 15, 2012;54(6):755-71.
57. Mavros MN, Tansarli GS, Vardakas KZ, Rafailidis PI, Karageorgopoulos DE, Falagas ME. Impact of vancomycin minimum inhibitory concentration on clinical outcomes of patients with vancomycin-susceptible Staphylococcus aureus infections: a meta-analysis and meta-regression. International journal of antimicrobial agents. 2012;40(6):496-509.

58. Jacob JT, DiazGranados CA. High vancomycin minimum inhibitory concentration and clinical outcomes in adults with methicillin-resistant Staphylococcus aureus infections: a meta-analysis. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases. 2013;17(2):e93-e100.
59. Kalil AC, Van Schooneveld TC, Fey PD, Rupp ME. Association between vancomycin minimum inhibitory concentration and mortality among patients with staphylococcus aureus bloodstream infections: A systematic review and meta-analysis. JAMA. 2014;312(15):1552-64.
60. Howden BP, Davies JK, Johnson PDR, Stinear TP, Grayson ML. Reduced Vancomycin Susceptibility in Staphylococcus aureus, Including Vancomycin-Intermediate and Heterogeneous Vancomycin-Intermediate Strains: Resistance Mechanisms, Laboratory Detection, and Clinical Implications. Clinical microbiology reviews. 2010 January 1, 2010;23(1):99-139.
61. Sievert DM, Rudrik JT, Patel JB, McDonald LC, Wilkins MJ, Hageman JC. Vancomycin-resistant Staphylococcus aureus in the United States, 2002-2006. Clin Infect Dis. 2008 Mar 1;46(5):668-74.
62. Hiramatsu K. Vancomycin-resistant Staphylococcus aureus: a new model of antibiotic resistance. The Lancet Infectious Diseases. 2001;1(3):147-55.
63. Ward PB, Johnson PD, Grabsch EA, Mayall BC, Grayson ML. Treatment failure due to methicillin-resistant Staphylococcus aureus (MRSA) with reduced susceptibility to vancomycin. Med J Aust. 2001 Nov 5;175(9):480-3.
64. Charles PG, Ward PB, Johnson PD, Howden BP, Grayson ML. Clinical features associated with bacteremia due to heterogeneous vancomycin-intermediate Staphylococcus aureus. Clin Infect Dis. 2004 Feb 1;38(3):448-51.
65. Howden BP, Ward PB, Charles PG, Korman TM, Fuller A, du Cros P, et al. Treatment outcomes for serious infections caused by methicillin-resistant Staphylococcus aureus with reduced vancomycin susceptibility. Clin Infect Dis. 2004 Feb 15;38(4):521-8.
66. Casapao AM, Leonard SN, Davis SL, Lodise TP, Patel N, Goff DA, et al. Clinical Outcomes in Patients with Heterogeneous Vancomycin-Intermediate Staphylococcus aureus Bloodstream Infection. Antimicrobial Agents and Chemotherapy. 2013 September 1, 2013;57(9):4252-9.
67. Parker D, Prince A. Immunopathogenesis of Staphylococcus aureus pulmonary infection. Semin Immunopathol. 2012;34(2):281-97.
68. Yarwood JM, Schlievert PM. Quorum sensing in Staphylococcus infections. Journal of Clinical Investigation. 2003;112(11):1620-5.
69. Peng HL, Novick RP, Kreiswirth B, Kornblum J, Schlievert P. Cloning, characterization, and sequencing of an accessory gene regulator (agr) in Staphylococcus aureus. Journal of bacteriology. 1988;170(9):4365-72.
70. Tsompanidou E, Sibbald MJJB, Chlebowicz MA, Dreisbach A, Back JW, van Dijl JM, et al. Requirement of the agr Locus for Colony Spreading of Staphylococcus aureus. Journal of bacteriology. 2011 March 1, 2011;193(5):1267-72.
71. Araujo LVd, Freire DMG, Nitschke M. Biossurfactantes: propriedades anticorrosivas, antibiofilmes e antimicrobianas. Química Nova. 2013;36:848-58.

72. Santos ALd, Santos DO, Freitas CCd, Ferreira BLA, Afonso IF, Rodrigues CR, et al. Staphylococcus aureus: visitando uma cepa de importância hospitalar. Jornal Brasileiro de Patologia e Medicina Laboratorial. 2007;43:413-23.
73. Antunes LC, Ferreira RB, Buckner MM, Finlay BB. Quorum sensing in bacterial virulence. Microbiology (Reading, England). 2010 Aug;156(Pt 8):2271-82.
74. Bronner S, Monteil H, Prévost G. Regulation of virulence determinants in Staphylococcus aureus: complexity and applications. FEMS microbiology reviews. 2004 2004-05-01 00:00:00;28(2):183-200.
75. Gray B, Hall P, Gresham H. Targeting agr- and agr-Like Quorum Sensing Systems for Development of Common Therapeutics to Treat Multiple Gram-Positive Bacterial Infections. Sensors (Basel, Switzerland). 2013;13(4):5130-66.
76. Morfeldt E, Taylor D, von Gabain A, Arvidson S. Activation of alpha-toxin translation in Staphylococcus aureus by the trans-encoded antisense RNA, RNAIII. The EMBO Journal. 1995;14(18):4569-77.
77. Koenig RL, Ray JL, Maleki SJ, Smeltzer MS, Hurlburt BK. Staphylococcus aureus AgrA Binding to the RNAIII-agr Regulatory Region. Journal of bacteriology. 2004;186(22):7549-55.
78. Montgomery CP, Boyle-Vavra S, Daum RS. Importance of the Global Regulators <italic>Agr</italic> and <italic>SaeRS</italic> in the Pathogenesis of CA-MRSA USA300 Infection. PLOS ONE. 2010;5(12):e15177.
79. Traber KE, Lee E, Benson S, Corrigan R, Cantera M, Shopsin B, et al. agr function in clinical Staphylococcus aureus isolates. Microbiology (Reading, England). 2008 Aug;154(Pt 8):2265-74.
80. Traber K, Novick R. A slipped-mispairing mutation in AgrA of laboratory strains and clinical isolates results in delayed activation of agr and failure to translate delta- and alpha-haemolysins. Mol Microbiol. 2006 Mar;59(5):1519-30.
81. Schweizer ML, Furuno JP, Sakoulas G, Johnson JK, Harris AD, Shardell MD, et al. Increased mortality with accessory gene regulator (agr) dysfunction in Staphylococcus aureus among bacteremic patients. Antimicrob Agents Chemother. 2011 Mar;55(3):1082-7.
82. Cogen AL, Yamasaki K, Muto J, Sanchez KM, Crotty Alexander L, Tanios J, et al. <italic>Staphylococcus epidermidis</italic> Antimicrobial ?-Toxin (Phenol-Soluble Modulin-?) Cooperates with Host Antimicrobial Peptides to Kill Group A <italic>Streptococcus</italic>. PLOS ONE. 2010;5(1):e8557.
83. Dinges MM, Orwin PM, Schlievert PM. Exotoxins of Staphylococcus aureus. Clinical microbiology reviews. 2000 January 1, 2000;13(1):16-34.
84. Vanderpool CK, Balasubramanian D, Lloyd CR. Dual-function RNA regulators in bacteria. Biochimie. 2011 Nov;93(11):1943-9.
85. Marconi C, Cunha MLRS, Araújo Jr JP, Rugolo LMSS. Standardization of the PCR technique for the detection of delta toxin in Staphylococcus spp. Journal of Venomous Animals and Toxins including Tropical Diseases. 2005;11:117-28.
86. Otto M. Phenol-soluble modulins. International journal of medical microbiology : IJMM. 2014;304(2):164-9.
87. Cheung GYC, Duong AC, Otto M. Direct and synergistic hemolysis caused

by Staphylococcus phenol-soluble modulins: implications for diagnosis and pathogenesis. Microbes and Infection / Institut Pasteur. 2012;14(4):380-6.
88. Sakoulas G, Eliopoulos GM, Moellering RC, Wennersten C, Venkataraman L, Novick RP, et al. Accessory Gene Regulator (agr) Locus in Geographically Diverse Staphylococcus aureus Isolates with Reduced Susceptibility to Vancomycin. Antimicrobial Agents and Chemotherapy. 2002;46(5):1492-502.
89. Adhikari RP, Arvidson S, Novick RP. A Nonsense Mutation in agrA Accounts for the Defect in agr Expression and the Avirulence of Staphylococcus aureus 8325-4 traP::kan. Infection and immunity. 2007 September 1, 2007;75(9):4534-40.
90. Lina G, Piemont Y, Godail-Gamot F, Bes M, Peter MO, Gauduchon V, et al. Involvement of Panton-Valentine leukocidin-producing Staphylococcus aureus in primary skin infections and pneumonia. Clin Infect Dis. 1999 Nov;29(5):1128-32.
91. Melles DC, van Leeuwen WB, Boelens HAM, Peeters JK, Verbrugh HA, van Belkum A. Panton-Valentine Leukocidin Genes in Staphylococcus aureus. Emerging infectious diseases. 2006;12(7):1174-5.
92. Gillet Y, Issartel B, Vanhems P, Fournet J-C, Lina G, Bes M, et al. Association between Staphylococcus aureus strains carrying gene for Panton-Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. The Lancet. 2002;359(9308):753-9.
93. Sola C, Saka HA, Group tCMCS, Vindel A, Bocco JL. Emergence and Dissemination of a Community-Associated Methicillin-Resistant Panton-Valentine Leucocidin-Positive Staphylococcus aureus Clone Sharing the Sequence Type 5 Lineage with the Most Prevalent Nosocomial Clone in the Same Region of Argentina. Journal of Clinical Microbiology. 2008 May 1, 2008;46(5):1826-31.
94. Shallcross LJ, Fragaszy E, Johnson AM, Hayward AC. The role of the Panton-Valentine leucocidin toxin in staphylococcal disease: a systematic review and meta-analysis. The Lancet Infectious Diseases. 2013;13(1):43-54.
95. Babu E, Oropello J. Staphylococcus lugdunensis: the coagulase-negative staphylococcus you don't want to ignore. Expert review of anti-infective therapy. 2011 Oct;9(10):901-7.
96. Vuong C, Otto M. Staphylococcus epidermidis infections. Microbes and Infection. 2002;4(4):481-9.
97. Fey PD. Modality of bacterial growth presents unique targets: how do we treat biofilm-mediated infections? Current Opinion in Microbiology. 2010;13(5):610-5.
98. Arciola CR, Campoccia D, Speziale P, Montanaro L, Costerton JW. Biofilm formation in Staphylococcus implant infections. A review of molecular mechanisms and implications for biofilm-resistant materials. Biomaterials. 2012;33(26):5967-82.
99. Prakash B, Veeregowda BM, Krishnappa G. Biofilms: A survival strategy of bacteria. Current Science. 2003 nov 2003;85(9):1299-307.
100. Behlau I, Gilmore MS. Microbial biofilms in ophthalmology and infectious disease. Archives of Ophthalmology. 2008;126(11):1572-81.
101. Waldman EA. Usos da vigilância e da monitorização em saúde pública.

Informe Epidemiológico do Sus. 1998;7:7-26.
102. Glass RI. New prospects for epidemiologic investigations. Science. 1986;234:951-5.
103. van Belkum A, Struelens M, de Visser A, Verbrugh H, Tibayrenc M. Role of Genomic Typing in Taxonomy, Evolutionary Genetics, and Microbial Epidemiology. Clinical microbiology reviews. 2001;14(3):547-60.
104. Blanc DS. The use of molecular typing for epidemiological surveillance and investigation of endemic nosocomial infections. Infect Genet Evol. 2004 2004/09//;4(3):193-7.
105. Gilbert GL. Molecular diagnostics in infectious diseases and public health microbiology: cottage industry to postgenomics. Trends Mol Med. 2002 Jun;8(6):280-7.
106. Hasnain SE. Molecular epidemiology of infectious diseases: a case for increased surveillance. Bulletin of the World Health Organization. 2003;81:474-.
107. Song T, Toma C, Nakasone N, Iwanaga M. Sensitive and rapid detection of Shigella and enteroinvasive Escherichia coli by a loop-mediated isothermal amplification method. FEMS Microbiology Letters. 2005 2005-02-01 00:00:00;243(1):259-63.
108. van Belkum A. DNA fingerprinting of medically important microorganisms by use of PCR. Clinical microbiology reviews. 1994;7(2):174-84.
109. Arbeit RD, Arthur M, Dunn R, Kim C, Selander RK, Goldstein R. Resolution of Recent Evolutionary Divergence among Escherichia coli from Related Lineages: The Application of Pulsed Field Electrophoresis to Molecular Epidemiology. Journal of Infectious Diseases. 1990 February 1, 1990;161(2):230-5.
110. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. Journal of Clinical Microbiology. 1995;33(9):2233-9.
111. Goering RV. Pulsed field gel electrophoresis: A review of application and interpretation in the molecular epidemiology of infectious disease. Infection, Genetics and Evolution. 2010;10(7):866-75.
112. Maiden MCJ. Multilocus Sequence Typing of Bacteria. Annual Review of Microbiology. 2006;60(1):561-88.
113. Li W, Raoult D, Fournier P-E. Bacterial strain typing in the genomic era. FEMS microbiology reviews. 2009 2009-09-01 00:00:00;33(5):892-916.
114. Trindade PA, McCulloch JA, Oliveira GA, Mamizuka EM. Molecular techniques for MRSA typing: current issues and perspectives. Brazilian Journal of Infectious Diseases. 2003;7:32-43.
115. Zhang K, McClure J-A, Elsayed S, Louie T, Conly JM. Novel Multiplex PCR Assay for Characterization and Concomitant Subtyping of Staphylococcal Cassette Chromosome mec Types I to V in Methicillin-Resistant Staphylococcus aureus. Journal of Clinical Microbiology. 2005;43(10):5026-33.
116. Gilot P, Lina G, Cochard T, Poutrel B. Analysis of the Genetic Variability of Genes Encoding the RNA III-Activating Components Agr and TRAP in a Population of Staphylococcus aureus Strains Isolated from Cows with Mastitis. Journal of Clinical Microbiology. 2002;40(11):4060-7.
117. Miragaia M, Carriço JA, Thomas JC, Couto I, Enright MC, de Lencastre H.

Comparison of Molecular Typing Methods for Characterization of Staphylococcus epidermidis: Proposal for Clone Definition. Journal of Clinical Microbiology. 2008;46(1):118-29.
118. Shopsin B, Gomez M, Montgomery SO, Smith DH, Waddington M, Dodge DE, et al. Evaluation of Protein A Gene Polymorphic Region DNA Sequencing for Typing of Staphylococcus aureus Strains. Journal of Clinical Microbiology. 1999;37(11):3556-63.
119. Urwin R, Maiden MC. Multi-locus sequence typing: a tool for global epidemiology. Trends in microbiology. 2003 Oct;11(10):479-87.
120. Harmsen D, Claus H, Witte W, Rothgänger J, Claus H, Turnwald D, et al. Typing of Methicillin-Resistant Staphylococcus aureus in a University Hospital Setting by Using Novel Software for spa Repeat Determination and Database Management. Journal of Clinical Microbiology. 2003;41(12):5442-8.
121. Simske JS, Scherer S. Pulsed-field gel electrophoresis of circular DNA. Nucleic Acids Research. 1989;17(11):4359-65.
122. Schwartz DC, Cantor CR. Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. Cell. 1984 May;37(1):67-75.
123. Murchan S, Kaufmann ME, Deplano A, de Ryck R, Struelens M, Zinn CE, et al. Harmonization of Pulsed-Field Gel Electrophoresis Protocols for Epidemiological Typing of Strains of Methicillin-Resistant Staphylococcus aureus: a Single Approach Developed by Consensus in 10 European Laboratories and Its Application for Tracing the Spread of Related Strains. Journal of Clinical Microbiology. 2003;41(4):1574-85.
124. Cookson BD, Robinson DA, Monk AB, Murchan S, Deplano A, de Ryck R, et al. Evaluation of Molecular Typing Methods in Characterizing a European Collection of Epidemic Methicillin-Resistant Staphylococcus aureus Strains: the HARMONY Collection. Journal of Clinical Microbiology. 2007;45(6):1830-7.
125. Lukinmaa S, Nakari U-M, Eklund M, Siitonen A. Application of molecular genetic methods in diagnostics and epidemiology of food-borne bacterial pathogens. APMIS : acta pathologica, microbiologica, et immunologica Scandinavica. 2004;112(11-12):908-29.
126. Sintchenko V, Gallego B. Laboratory-Guided Detection of Disease Outbreaks: Three Generations of Surveillance Systems. Archives of Pathology & Laboratory Medicine. 2009;133(6):916-25.
127. Álvarez R, López Cortés LE, Molina J, Cisneros JM, Pachón J. Optimizing the Clinical Use of Vancomycin. Antimicrobial Agents and Chemotherapy. 2016 May 1, 2016;60(5):2601-9.
128. Holmes NE, Tong SY, Davis JS, van Hal SJ. Treatment of methicillin-resistant Staphylococcus aureus: vancomycin and beyond. Semin Respir Crit Care Med. 2015 Feb;36(1):17-30.
129. Vesga O, Agudelo M, Salazar BE, Rodriguez CA, Zuluaga AF. Generic Vancomycin Products Fail In Vivo despite Being Pharmaceutical Equivalents of the Innovator. Antimicrobial Agents and Chemotherapy. 2010;54(8):3271-9.
130. Liu C, Bayer A, Cosgrove SE, Daum RS, Fridkin SK, Gorwitz RJ, et al. Clinical Practice Guidelines by the Infectious Diseases Society of America for the Treatment of Methicillin-Resistant Staphylococcus aureus Infections in Adults and Children. Clinical Infectious Diseases. 2011

February 1, 2011;52(3):e18-e55.
131. Park K-H, Kim ES, Kim HS, Park S-J, Bang KM, Park HJ, et al. Comparison of the clinical features, bacterial genotypes and outcomes of patients with bacteraemia due to heteroresistant vancomycin-intermediate Staphylococcus aureus and vancomycin-susceptible S. aureus. Journal of Antimicrobial Chemotherapy. 2012 August 1, 2012;67(8):1843-9.
132. Albur MS, Bowker K, Weir I, MacGowan A. Factors influencing the clinical outcome of methicillin-resistant Staphylococcus aureus bacteraemia. European Journal of Clinical Microbiology & Infectious Diseases. [journal article]. 2012;31(3):295-301.
133. Forstner C, Dungl C, Tobudic S, Mitteregger D, Lagler H, Burgmann H. Predictors of clinical and microbiological treatment failure in patients with methicillin-resistant Staphylococcus aureus (MRSA) bacteraemia: a retrospective cohort study in a region with low MRSA prevalence. Clinical Microbiology and Infection. 2013;19(7):E291-E7.
134. Sakoulas G, Moellering RC, Jr., Eliopoulos GM. Adaptation of methicillin-resistant Staphylococcus aureus in the face of vancomycin therapy. Clin Infect Dis. 2006 Jan 1;42 Suppl 1:S40-50.
135. Barriere SL, Stryjewski ME, Corey GR, Genter FC, Rubinstein E. Effect of vancomycin serum trough levels on outcomes in patients with nosocomial pneumonia due to Staphylococcus aureus: a retrospective, post hoc, subgroup analysis of the Phase 3 ATTAIN studies. BMC infectious diseases. [journal article]. 2014;14(1):1-5.
136. Elyasi S, Khalili H, Dashti-Khavidaki S, Mohammadpour A. Vancomycin-induced nephrotoxicity: mechanism, incidence, risk factors and special populations. A literature review. Eur J Clin Pharmacol. 2012 2012/09/01;68(9):1243-55.
137. Charlson M, Szatrowski TP, Peterson J, Gold J. Validation of a combined comorbidity index. Journal of Clinical Epidemiology. 1994;47(11):1245-51.
138. Delanghe JR, Speeckaert MM. Creatinine determination according to Jaffe—what does it stand for? NDT Plus. 2011;4(2):83-6.
139. Bellomo R, Ronco C, Kellum JA, Mehta RL, Palevsky P, the Aw. Acute renal failure – definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. Critical Care. 2004;8(4):R204-R12.
140. Bollinger M, Hamilton M, Schroeder K, Link S, Nguyen J, Chu D, et al. Vancomycin use in a rural hospital: a 3-year retrospective study. Can J Rural Med. 2015 Spring;20(2):56-62.
141. Horne KC, Howden BP, Grabsch EA, Graham M, Ward PB, Xie S, et al. Prospective Comparison of the Clinical Impacts of Heterogeneous Vancomycin-Intermediate Methicillin-Resistant Staphylococcus aureus (MRSA) and Vancomycin-Susceptible MRSA. Antimicrobial Agents and Chemotherapy. 2009;53(8):3447-52.
142. Vandecasteele SJ, De Bacquer D, De Vriese AS. Implementation of a Dose Calculator for Vancomycin to Achieve Target Trough Levels of 15–20 μg/mL in Persons Undergoing Hemodialysis. Clinical Infectious Diseases. 2011 July 15, 2011;53(2):124-9.
143. Reardon J, Lau TT, Ensom MH. Vancomycin loading doses: a systematic review. Ann Pharmacother. 2015 May;49(5):557-65.

144. De Waele JJ, Danneels I, Depuydt P, Decruyenaere J, Bourgeois M, Hoste E. Factors associated with inadequate early vancomycin levels in critically ill patients treated with continuous infusion. International journal of antimicrobial agents. 2013;41(5):434-8.
145. Lima TdM, Elias SC, Estrela RdCE, Cardoso FLL. Implementation of vancomycin dosing nomogram in an electronic prescribing system: an innovative tool in antibiotic stewardship. Brazilian Journal of Pharmaceutical Sciences. 2014;50:567-72.
146. Wesner AR, Brackbill ML, Coyle LL, Kidd RS. Prospective Trial of a Novel Nomogram to Achieve Updated Vancomycin Trough Concentrations. Interdisciplinary Perspectives on Infectious Diseases. 2013;2013:8.
147. Elyasi S, Khalili H. Vancomycin dosing nomograms targeting high serum trough levels in different populations: pros and cons. Eur J Clin Pharmacol. [journal article]. 2016:1-12.
148. Broome L, So T-Y. An Evaluation of Initial Vancomycin Dosing in Infants, Children, and Adolescents. International Journal of Pediatrics. 2011;2011:470364.
149. Lesens OMD, Methlin CMD, Hansmann YMD, Remy VMD, Martinot MMD, Bergin CMD, et al. Role of Comorbidity in Mortality Related to Staphylococcus aureus Bacteremia: A Prospective Study Using the Charlson Weighted Index of Comorbidity • Infection Control and Hospital Epidemiology. 2003;24(12):890-6.
150. Kullar R, Davis SL, Levine DP, Rybak MJ. Impact of vancomycin exposure on outcomes in patients with methicillin-resistant Staphylococcus aureus bacteremia: support for consensus guidelines suggested targets. Clin Infect Dis. 2011 Apr 15;52(8):975-81.
151. Cao G, Liang X, Zhang J, Zhou Y, Wu J, Zhang Y, et al. Vancomycin serum trough concentration vs. clinical outcome in patients with gram-positive infection: a retrospective analysis. Journal of Clinical Pharmacy and Therapeutics. 2015;40(6):640-4.
152. Ragab AR, Al-Mazroua MK, Al-Harony MA. Incidence and Predisposing Factors of Vancomycin-Induced Nephrotoxicity in Children. Infectious Diseases and Therapy. 2013;2(1):37-46.
153. Rostas SE, Kubiak DW, Calderwood MS. High-Dose Intravenous Vancomycin Therapy and the Risk of Nephrotoxicity. Clinical therapeutics. 2014;36(7):1098-101.

ANEXOS

ANEXO A – Parecer do Comitê de Ética em Pesquisa (UFCSPA)

ANEXO B – Formulário de coleta de dados

1. ***Staphylococcus spp.*** **( )**	2. **Vancomicina ( )**	**Registro paciente**: n.º _____
3. **Nome**:		4. **Atendimento:** n.º _____
5. **Idade**: ____	**Nasc.:** _____/_____/_____	6. **Sexo**: (1) Feminino (2) Masculino
7. **Tipo de internação**: (1) Clínica (2) Cirúrgica eletiva (3) Cirúrgica de urgência		
8. **Peso internação:**		**Peso alta:**
9. **CID (Classificação Internacional de Doença):**		
10. **Data de internação**: _____/_____/_____		11. **Tempo de internação pré-UTI:** _____dias
12. **Data de internação na UTI**: _____/_____/_____		13. **Tempo de internação em UTI:** _____dias
14. **Indicação de UTI**: Monitorização (1) hemodinâmica (2) cardíaca (3) respiratória (4) neurológica		
15. **Co-morbidades**: (1) Cardíacas (2) Pulmonares (3) Neurológicas (4) Malignidade (5) Endocrinológica (6) Renal Aguda (creatinina maior_____) (7) Renal Crônica (creatinina basal_____) (8) Hepática (9) Imunodeficiência (10) Prematuridade **Observação:**_____		
16. Índice **Charlson**: _____		
17. **Cultura positiva**: (0) Não (1) Sim _____/_____/_____		18. **Nova cultura positiva:** (0) Não (1) Sim ____/____/____
19. **Laudo(s) da(s) cultura** (s):_____ _____		
20. **Local de isolamento**: (1) Sangue (2) Urina (3) Escarro (4) Ferida operatória ( ) Outro:_____		
Procedimentos invasivos: 21. **Ventilação Mecânica**: (0) Não (1) Sim. Início____/_____/_____Final____/_____/_____ 22. Retirada temporária da ventilação mecânica: (0) Não (1) Sim. Início_____/_____/_____Final_____/_____/_____ 23. **Cateter venoso central**: (0) Não (1) Sim. Início_____/_____/_____Final_____/_____/_____ **Tentativas:** _____ 24. Retirada do cateter venoso central durante o tratamento: (0) Não (1) Sim Data:_____/_____/_____ 25. **Sondagem vesical:** (0) Não (1) Sim. Início_____/_____/_____Final_____/_____/_____ 26. **Procedimento cirúrgico:** (0) Não (1) Sim. Data:_____/_____/_____ Início: _____ h Final: _____ h		
27. **Vancocinemia:** Data: ____/____/____ - _____ Concentração sérica: _____		
Data: ____/____/____ - _____ Concentração sérica: _____		Data: ____/____/____ - _____ Concentração sérica: _____
Data: ____/____/____ - _____ Concentração sérica: _____		Data: ____/____/____ - _____ Concentração sérica: _____
Medicações: 28. **Inibidor bomba de prótons:** (0) Não (1) Sim 29. **Bloqueador H2:** (0) Não (1) Sim 30. **Benzodiazepínico:** (0) Não (1) Sim 31. **Opióides:** (0) Não (1) Sim 32. **Curares:** (0) Não (1) Sim 33. **Anti-retrovirais:** (0) Não (1) Sim **Observações:** _____ 34. **Cefalosporina 1ª G:** (0) Não (1) Sim. ___/___/___ até ___/___/___ Dose:_____ / _____ 35. **Cefalosporina 2ª G:** (0) Não (1) Sim. ___/___/___ até ___/___/___ Dose:_____/_____ 36. **Cefalosporina 3ª G:** (0) Não (1) Sim. ___/___/___ até ___/___/___ Dose:_____/_____ 37. **Cefalosporina 4ª G:** (0) Não (1) Sim. ___/___/___ até ___/___/___ Dose:_____/_____ 38. **Beta-lactâmico:** (0) Não (1) Sim. ___/___/___ até ___/___/___ Dose:_____/_____ 39. **Beta-lact./inibidor:** (0) Não (1) Sim. ___/___/___ até ___/___/___ Dose:_____/_____ 40. **Carbapenem:** (0) Não (1) Sim. ___/___/___ até ___/___/___ Dose:_____/_____ 41. **Aminoglicosídeo:** (0) Não (1) Sim. ___/___/___ até ___/___/___ Dose:_____/_____ (0) Não (1) Sim. ___/___/___ até ___/___/___ Dose:_____/_____ 42. **Lincosamina:** (0) Não (1) Sim. ___/___/___ até ___/___/___ Dose:_____/_____ 43. **Glicopeptídeo:** (0) Não (1) Sim. ___/___/___ até ___/___/___ Dose:_____/_____ 44. **Linezolida:** (0) Não (1) Sim. ___/___/___ até ___/___/___ Dose:_____/_____ 45. **Tigeciclina:** (0) Não (1) Sim. ___/___/___ até ___/___/___ Dose:_____/_____ 46. **Oxacilina:** (0) Não (1) Sim. ___/___/___ até ___/___/___ Dose:_____/_____ 47. **Quinolona:** (0) Não (1) Sim. ___/___/___ até ___/___/___ Dose:_____/_____ 48. **Anaerobicida:** (0) Não (1) Sim. ___/___/___ até ___/___/___ Dose:_____/_____ 49. **Polimixina:** (0) Não (1) Sim. ___/___/___ até ___/___/___ Dose:_____/_____ 50. **Sulfametoxazol/trimetropim:** (0) Não (1) Sim. ___/___/___ até ___/___/___ Dose:_____		
51. **Alta**: (1) Não (2) Sim. Data: _____/_____/_____		52. **Óbito**: (1) Não (2) Sim. Data: _____/_____/_____
53. **Quadro de piora clínica**: Data: _____/_____/_____ Motivo:_____ Troca ATB: (0) Não (1) Sim Data: _____/_____/_____ Motivo:_____ Troca ATB: (0) Não (1) Sim Data: _____/_____/_____ Motivo:_____ Troca ATB: (0) Não (1) Sim Data: _____/_____/_____ Motivo:_____ Troca ATB: (0) Não (1) Sim Data: _____/_____/_____ Motivo:_____ Troca ATB: (0) Não (1) Sim		

ANEXO C – Carta de aceite: American Journal of Infection Control

ANEXO D – Resumos publicados em eventos científicos

1. RAUBER, J.M. ; BAGGIOTTO, B. ; SOARES, R. O. ; ZANOTTO, M. B. ; CARNEIRO, M. ; VALIM, A. R. M. ; d'AZEVEDO, P. A. . The importance of therapeutic drug monitoring of vancomycin in cases of empirical treatment and Staphylococcal infections. In: 26th European Congress of Clinical Microbiology, 2016, Amsterdã. Anais 26th European Congress of Clinical Microbiology, 2016. p. 164-164.

2. BAGGIOTTO, B. ; ARNHOLD, G. H. O. ; D'AZEVEDO, P. A. ; RAUBER, J.M. ; VALIM, A. R. M. . Influência dos níveis séricos de vancomicina na resposta clínica do paciente. In: XXI Seminário de Iniciação Científica da UNISC, 2015, Santa Cruz do Sul - RS. XXI Seminário de Iniciação Científica da UNISC, 2015.

3. RAUBER, J.M.; ARNHOLD, G. H. O. ; BAGGIOTTO, B. ; CUNHA, G. R. ; VALIM, A. R. M. ; D'AZEVEDO, P. A. . Genetic variability of *Staphylococcus* spp. with vancomycin intermediate heteroresistance (hVIS) in a hospital in south of Brazil. In: 28º Congresso Brasileiro de Microbiologia, 2015, Florianópolis - SC. 28º Congresso Brasileiro de Microbiologia.

4. RAUBER, J.M.; ARNHOLD, G. H. O. ; WAPPLER, P. R. ; BAIERLE, G. M. ; MORAES, L. B. ; CARNEIRO, M. ; VALIM, A. R. M. ; D'AZEVEDO, P. A. . Molecular characteristics and virulence factors of community-acquired and healthcare-associated *Staphylococcus aureus* isolates. In: 115th General Meeting of American Society for Microbiology, 2015. 115th General Meeting of American Society for Microbiology, 2015.

5. BAGGIOTTO, B. ; ARNHOLD, G. H. O. ; CARNEIRO, M. ; D'AZEVEDO, P. A. ; RAUBER, J.M. ; VALIM, A. R. M. . Identificação de parâmetros que influenciam na falha do tratamento de infecções causadas por *Staphylococcus aureus*. In: XX Seminário de Iniciação Científica da UNISC, 2014, Santa Cruz do Sul - RS. XX Seminário de Iniciação Científica da UNISC, 2014.

6. ARNHOLD, G. H. O. ; RAUBER, J.M. ; MORAES, L. B. ; BAGGIOTTO, B. ; VALIM, A. R. M. . Desenvolvimento de metodologia alternativa para triagem de *Staphylococcus* spp. heterorresistentes à vancomicina em amostras clínicas. In: III Seminário Integrador de Iniciação Científica Tecnológica e Inovação, 2014, Lajeado - RS. III Seminário Integrador de Iniciação Científica Tecnológica e Inovação.

7. MORAES, L. B. ; RAUBER, J.M. ; D'AZEVEDO, P. A. ; ARNHOLD, G. H. O. ; BAGGIOTTO, B. ; VALIM, A. R. M. . Análise genotípica de fatores de virulência e resistência em amostras hospitalares de *Staphylococcus* spp.. In: III Seminário Integrador de Iniciação Científica Tecnológica e

Inovação, 2014, Lajeado - RS. III Seminário Integrador de Iniciação Científica Tecnológica e Inovação.

8. WAPPLER, P. R. ; ARNHOLD, G. H. O. ; D'AZEVEDO, P. A. ; RAUBER, J.M. ; VALIM, A. R. M. . Avaliação da resistência a antimicrobianos em cepas de *Staphylococcus* spp de origem hospitalar através de métodos fenotípicos. In: IV Salão de Ensino e de Extensão e XIX Seminário de Iniciação Científica, 2013, Santa Cruz do Sul. Anais IV Salão de Ensino e de Extensão e XIX Seminário de Iniciação Científica, 2013. p. 64-64.

9. TRINDADE, N. S. ; WAPPLER, P. R. ; D'AZEVEDO, P. A. ; RAUBER, J.M. ; VALIM, A. R. M. . Influência dos fatores epidemiológicos no desfecho de infecções por *Staphylococcus* spp.. In: IV Salão de Ensino e de Extensão e XIX Seminário de Iniciação Científica, 2013, Santa Cruz do Sul. Anais IV Salão de Ensino e de Extensão e XIX Seminário de Iniciação Científica, 2013. p. 104.

10. BAIERLE, G. M. ; ARNHOLD, G. H. O. ; WAPPLER, P. R. ; D'AZEVEDO, P. A. ; RAUBER, J.M. ; VALIM, A. R. M. . Formação de biofilme em isolados de *Staphylococcus* spp em amostras de pacientes internados em um hospital de ensino no sul do Brasil. In: IV Salão de Ensino e de Extensão e XIX Seminário de Iniciação Científica, 2013, Santa Cruz do Sul. Anais IV Salão de Ensino e de Extensão e XIX Seminário de Iniciação Científica, 2013. p. 105.

11. ZANOTTO, M. B. ; WAPPLER, P. R. ; D'AZEVEDO, P. A. ; RAUBER, J.M. ; VALIM, A. R. M. . Avaliação dos níveis séricos de vancomicina em pacientes internados em um hospital de ensino da região central do Rio Grande do Sul. In: IV Salão de Ensino e de Extensão e XIX Seminário de Iniciação Científica, 2013, Santa Cruz do Sul. Anais IV Salão de Ensino e de Extensão e XIX Seminário de Iniciação Científica, 2013. p. 106.

12. RAUBER, J.M.; WAPPLER, P. R. ; KRUMMENAUER, E. C. ; MACHADO, JANETE APARECIDA ALVES ; CARNEIRO, M. ; VALIM, A. R. M. ; D'AZEVEDO, P. A. . Perfil de sensibilidade de amostras de *Staphylococcus* spp isoladas em um hospital de ensino do sul do Brasil. In: III Congresso Latino Americano de Resistência Microbiana e X Sul Encontro de Controle de Infecção, 2013, Gramado, RS. Journal of Infection Control, 2013. v. 2. p. 89.