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Staphylococcus aureus RESISTENTE À METICILINA: DETERMINAÇÃO DO PERFIL DE SENSIBILIDADE AOS ANTIMICROBIANOS, FATORES DE VIRULÊNCIA E DIVERSIDADE GENÉTICA

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Dissertação de Mestrado apresentada ao Programa de Pós-Graduação em Microbiologia da Universidade Estadual de Londrina como requisito parcial à obtenção do título de Mestre em Microbiologia.

Orientadora: Profa. Dra. Sueli Fumie Yamada Ogatta.
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RESUMO

Staphylococcus aureus é um patógeno oportunista que pode causar desde doenças de pele e tecidos moles a infecções disseminadas fatais. No início da década de 1940, as infecções causadas por esta bactéria foram tratadas com penicilina, logo após a sua introdução na terapia clínica. No entanto, o surgimento de isolados resistentes a este antimicrobiano levou ao desenvolvimento de penicilinas semissintéticas (meticilina). Crucialmente, em 1961, o primeiro relato de *S. aureus* resistente à meticilina (MRSA: *methicillin-resistant S. aureus*) foi descrito no Reino Unido. Atualmente, MRSA é uma das principais causas de infecções relacionadas à assistência à saúde (HA-MRSA: *healthcare-associated* MRSA) em todo o mundo, e em adição, isolados desta espécie bacteriana tem emergido como uma das principais causas de infecções em indivíduos saudáveis em ambientes comunitários (CA-MRSA: *community-associated* MRSA). Nos dias de hoje, as comunidades médica e científica são desafiadas com a emergência de *S. aureus* resistentes aos glicopeptídeos (vancomicina e teicoplanina), uma das últimas escolhas para o tratamento intravenoso de infecções causadas por cepas de MRSA. A partir deste novo cenário, observou-se o surgimento de VRSA (*vancomycin-resistantS. aureus*), VISA (*vancomycin-intermediate S. aureus*) e hVISA (*hetero*-VISA).MRSA constitui-se em um dos principais agentes de infecções em pacientes atendidos no Hospital Universitário de Londrina, PR. Assim, o objetivo deste trabalho foi determinaro perfil de sensibilidade a antimicrobianos, fatores de virulência e diversidade genética de isolados clínicos de MRSA. Foram utilizados 123 isolados clínicos de MRSA de pacientes assistidos no HU, entre janeiro de 2010 e junho de 2013. A maioria dos isolados foi obtida de amostras de sangue (43/123; 34,95%), seguido por fragmento de tecido (21/123; 17,07%). Todos os isolados foram sensíveis a linezolida e teicoplanina e resistentes à penicilina e cefoxitina. A maioria dos isolados apresentou resistência a oxacilina (116/123; 94,31%), eritromicina (116/123; 94,31%), ciprofloxacina (114/123; 92,68%) e clindamicina (113/123; 91,87%).A maioria dos isolados (73; 59,35%) foi sensível à vancomicina e destes, 62 (50,41%) apresentou CIM (Concentração Inibitória Mínima) de 2,0µg/mL.Todos os isolados apresentaram o gene *ica*A, e 112 isolados (91,05%) apresentaram os genes *hlb* e *hld*. Em relação à tipagem de SCC*mec*, o tipo II foi o mais freqüente entre os isolados (66; 53,66%), seguido pelo SCC*mec* tipo I (28; 22,76%). A partir da genotipagem por rep-PCR e análise do dendograma, os isolados foram reunidos em 17 grupos, denominados de A à Q, adotando-se 65% de similaridade. O grupo E reuniu a maioria dos isolados (50), seguido pelo grupo F (16) e H (14). Assim, observa-se que não há disseminação clonal de MRSA no HU, e que os isolados apresentam resistência heterogênea aos antimicrobianos. O trabalho contribuirá para adoção de medidas de controle da infecção hospitalar, assim como embasamento para o tratamento de infecções por *S. aureus*.

Palavras-chave: MRSA. SCC*mec*. Resistência a antimicrobianos. Virulência. Rep-PCR.

OLIVEIRA, Caio Ferreira. **Methicillin-resistant *Staphylococcus aureus*):** determination of profile antimicrobial susceptibility, virulence factors and genetic diversity. 2015. 49 p. Dissertação de Mestrado – Universidade Estadual de Londrina, Londrina – PR, 2015.

ABSTRACT

Staphylococcus aureus is an opportunistic pathogen that can cause since skin and soft tissue infections to fatal disseminated diseases. In the early 1940s, infections caused by this bacterium were treated with penicillin, after the introduction into clinical therapy. However, the appearance of isolates resistant to these antibiotics led to the development of semisyntheticspenicillins (methicillin). Crucially, in 1961, the first report of methicillin-resistant *S. aureus* (MRSA : Methicillin-resistant *S. aureus*) was described in the United Kingdom. Currently, MRSA is a major cause of health care-associated (HA - MRSA: healthcare -associated MRSA) infections worldwide, and in addition, isolates of this bacterial species has emerged as a major cause of infections in healthy individuals in community settings (CA - MRSA: community-associated MRSA). Actually, the medical and scientific communities has been challenged with the emergence of *S. aureus* resistant to glycopeptides (vancomycin and teicoplanin), one of the last choices for the intravenous treatment of infections caused by MRSA. In front of this fact, it was observed the emergence of VRSA (vancomycin-resistant *S. aureus*), VISA (vancomycin-intermediate *S. aureus*) and hVISA (hetero-VISA). MRSA constitutes one of the main agents of infections in treated patients at the University Hospital of Londrina, PR. The aim of this study was to determine the antimicrobial susceptibility profile, virulence factors and genetic diversity of clinical isolates of MRSA. Were used 123 clinical isolates of MRSA from watched patients to HU, between January 2010 and June 2013. The most of isolates were obtained from blood samples (43/123; 34,95%), followed by fragment tissue (21/123; 17,07%). All isolates were susceptible to linezolid and teicoplanin, and resistant to penicillin and cefoxitin. The most isolates were resistant to oxacillin (116/123; 94.31%), erythromycin (116/123; 94,31%), ciprofloxacin (114/123; 92,68%) and clindamycin (113/123; 91,87%). The most of isolates (73/123; 59,35%) were sensitive to vancomycin and of these, 62 (50,41%) showed MIC (Minimum Inhibitory Concentration) of 2,0µg /mL. All isolates had the gene *ica*A, and 112 isolates (91,05%) showed the *hlb* and *hld* genes. About of the SCC*mec* typing, type II was the most frequent among the isolates (66/123; 53,66%), followed by SCCmec type I (28/123; 22,76%). From the rep-PCR genotyping and analysis of the dendrogram, the isolates were divided into 17 groups, designated A to Q, adopting 65% similarity. The most of the isolates (50) was founded on the E group, followed by F (16) and H (14). Thus, it is observed that no clonal spread of MRSA in HU, and that the isolates have heterogeneous antimicrobial resistance. The work will contribute to the adoption of measures to control nosocomial infection, as basis for the treatment of *S. aureus* infections.

Keywords: MRSA. SCC*mec*. Antimicrobial resistance. Virulence. Rep-PCR .

LISTA DE ABREVIATURAS E SIGLAS

BEC	Clone Epidêmico Brasileiro (do inglês *BrazilianEpidemic Clone*)
CA-MRSA	*Staphylococcus aureus* resistentes à meticilina associados a infecções comunitárias (do inglês: *Community-acquiredmethicillin-resistantStaphylococcus aureus*)
CC	Complexo Clonal
CDC	Do inglês*Centers for Disease Control and Prevention*
CIM	Concentração Inibitória Mínima
CLSI	*Clinical and Laboratory Standards Institute*
CoNS	*Staphylococcus*Coagulase Negativos
CTAB	*CetylTrimethylAmmoniumBromide*
DNase	Desoxirribonuclease
EDTA	Ácido etilenodiamino tetra-acético (do inglês *Ethylenediaminetetraaceticacid*)
EUA	Estados Unidos da América
HA-MRSA	*Staphylococcus aureus* resistentes à meticilina de infecções relacionadas a assistência à saúde (do inglês *Hospital-acquiredmethicillin-resistantStaphylococcus aureus*)
hVISA	*heteroresistantVancomycin-intermediate Staphylococcus aureus*
IRAS	Infecções Relacionas à Assistência à Saúde
ISC	Infecções de Sítio Cirúrgico
MLST	*MultilocusSequenceTyping*
MR	Microrganismos Multirresistentes
MRSA	*Methicilin-resistantStaphylococcus aureus*
PAP	Perfil de Análise Populacional (do inglês *PopulationAnalysis Profile*)
PBP	Proteína ligadora à penicilina (do inglês *Penicillin-BindingProtein*)
PCR	Reação em cadeia da polimerase (do inglês *Polymerase Chain Reaction*)
PFGE	*Pulsed Field Gel Electrpphoresis*
PIA	Polissacarídeo de adesão intracelular (do inglês *PolysaccharideIntercellularAdhesion*)
PVL	Leucocidina de Panton-Valentine
SCC*mec*	Cassete cromossômico estafilocócico*mec* (do inglês S*taphylococcalCasseteChromosomemec*)
SDS	Dodecil sulfato de sódio (do inglês *Sodium Dodecyl Sulfate*)
ST	*SequenceType*
TBE	Tampão Tris-Borato-EDTA
TE	Tampão Tris-HCl-EDTA
TSB	Caldo triptona de soja (do inglês *TrypticSoyBroth*)
TSST	Toxina da Síndrome do Choque tóxico
UCI	Unidade de Cuidados Intermediários
UFC	Unidades Formadoras de Colônia
UTI	Unidade de Terapia Intensiva
VISA	Do inglês *Vancomycin-intermediateStaphylococcus aureus*
VRE	Do inglês *Vancomycin-resistantEnterococci*
VRSA	Do inglês *Vancomycin-resistantStaphylococcus aureus*

SUMÁRIO

1 INTRODUÇÃO

A resistência aos antimicrobianos é uma ameaça global em ascensão, sobretudo no ambiente hospitalar, onde microrganismos multirresistentes (MR)agravam os quadros de infecções e tornam os tratamentos mais caros e demorados (HOWDEN et al., 2014). Entre os MR classicamente descritos como agentes etiológicos das infecções relacionadas à assistência à saúde (IRAS), destaca-se *Staphylococcus aureus* resistente à meticilina (*Methicilin-resistant Staphylococcus aureus* – MRSA) (LOWY et al., 2003; HOWDEN et al., 2010; TAGLIETTI et al., 2012).

Atualmente, MRSA é o patógeno mais comumente identificado como agente de infecções nos hospitais em muitas partes do mundo, incluindo Europa, Américas, África do Norte, Oriente Médio e Leste da Ásia. Infecções por estas bactérias estão associadas à permanência prolongada em hospitais, aumentando os custos e complicações, sobretudo em pacientes internados em Unidades de Terapia Intensiva (UTI) e imunocomprometidos (CARVALHO et al., 2010).

Dados do *NationalNosocomialInfectionSurveillance System* (NNISS) mostram que entre todas as infecções causadas por *S. aureus* em UTIs de 300 hospitais nos EUA, 59,5% eram MRSA.Em 2008, a rede de saúde americana,*HealthcareSafety Network* (NHSN) do *Centers for DiseaseControlandPrevention* (CDC), publicou a frequência destes microrganismos em infecções hospitalares, referente ao período de 2006 a 2007 e comparou-as ao período anterior. Os microrganismos Gram-positivos foram os mais freqüentes, sendo MRSA responsável por 49,2% das infecções deste grupo (HIDRON et al., 2008).

Tentativas de desenvolver uma vacina contra este patógeno até o momento não resultou em resultados satisfatórios (BAGNOLI et al., 2012). Assim, a terapia com antimicrobianos é ainda a principal estratégia no combate a infecções por *S. aureus* (LOWY et al., 2003; HOLMES et al., 2012). No início dos anos 1940, estas infecçõeseram tratadas com penicilina. Contudo, já em 1942 foi relatado o primeiro caso de resistência a este fármaco, devido à aquisição do plasmídiocontendo o gene para a enzima penicilinase que degrada o anel β-lactâmico e impossibilita a ação do fármaco. A introdução das penicilinas semissintéticas resistentes a penicilinases, na década de 1960, possibilitou um avanço na terapêutica, porém, logo se detectou

resistência a meticilina, um representante desta classe, em *S. aureus*pela aquisição do gene *mec*A (LOWY et al., 2003; DUMITRESCU et al., 2010).

O gene*mec*A codifica uma proteína de ligação à penicilina (PBP) alterada, que possui baixa afinidade por β-lactâmicos eestá inserido em uma região de DNA conhecida como cassete cromossômico estafilocócico*mec* (SCC*mec* – *staphylococcal cassete chromosomemec*) (KATAYAMA, ITO, HIRAMATSU, 2000; HIRAMATSU et al.,2002). Diversos tipos de SCC*mec* surgiram por meio da transferência horizontal de *mec*A em eventos independentes e, até a presente data, onze tipos (I aoXI) têm sido descritos em MRSA isolados de humanos (IWG, 2009).
Devido a esta multirresistência, os antimicrobianos da classe dos glicopeptídeos, vancomicina e teicoplanina, foram utilizados no tratamento de infecções por MRSA, por mais de 40 anos, sem graves problemas com resistência ou sensibilidade diminuída (HOWDEN et al., 2014; HAL e FOWLER, 2013).Entretanto, Hiramatsu e colaboradores (1997) reportaram o isolamento de cepas de *S. aureus*, a partir de materiais clínicos, que apresentavam fenótipo de sensibilidade intermediária à vancomicina (VISA - *Vancomycin-intermediateStaphylococcus aureus*), denominada Mu50, e fenótipo de heterorresistência à vancomicina (hVISA – *heteroresistantVancomycin-intermediateStaphylococcus aureus*), denominada de Mu3. Posteriormente foi reportado, em 2002, nos EUA o primeiro isolado clínico de VRSA (*Vancomycin-resistantStaphylococcus aureus*) devido a transferência por conjugação do operon*van*A de *Enterococcusfaecalis*, com relatos subsequentes na Índia e Irã. A partir de então, a investigação de VISA e VRSA em outros países ao redor do mundo ganhou força e como consequência a ocorrência destas bactérias tem sido descritas em outros países, como nos EUA, Coréia do Sul, China e Reino Unido (HOWDEN et al., 2010).

A partir de ensaios utilizando PFGE (*Pulsed Field Gel Electrophoresis*) e MLST (*MultilocusSequenceTyping*) foiobservadoque isolados de VISA não são clonais (FRIDKIN et al., 2003; HOWE et al., 2004). Entretanto, recentemente foram descritos isolados de VISA e hVISA pertencentes aos complexos clonais 5 ou 8, em particular ST5 (CC5) e ST239 (CC8), refletindo o sucesso de adaptação ao ambiente hospitalarpor essas cepas (HOWDEN et al., 2014).

Além da capacidade de fácil aquisição de genes que conferem multirresistência aos antimicrobianos, *S. aureus* exibe extraordinários fatores de virulência, que podem ser classificados, basicamente, em: a) fatores relacionados

com a aderência às células do hospedeiro, matriz extracelularou adesão às superfícies de cateteres e próteses; b) fatores relacionados com a evasão da defesa do hospedeiro como proteína A e polissacarídeos capsulares; c) fatores relacionados com ainvasãoda célula do hospedeiro como diversas enterotoxinasestafilocócicas (SEs A-E, C-J, K, L, M, O e P), toxina da síndrome do choque tóxico (TSST) e hemolisinas(HOWDEN et al., 2010; PARK et al., 2012).

2 REVISÃO DA LITERATURA

2.1 *Staphylococcus aureus*: Características gerais, patogenicidade e fatores de virulência

S. aureus é uma bactéria esférica, do grupo dos cocos Gram positivos e produtora de catalase, com aproximadamente 0,5 a 1,5µm de diâmetro, formando um agrupamento irregular. *S. aureus* são imóveis e crescem em meios tradicionais, como ágar sangue, em caldo ou ágar simples, pH 7,0, na temperatura ótima de 37 °C. A coloração das colônias varia desde o acinzentado até o amarelo ouro, e em meio ágar-sangue observa-se um halo de hemólise em torno das colônias. Um importante meio para identificação de *S. aureus* é o ágar manitol salgado, seletivo para este gênero, uma vez que a bactéria é capaz de fermentar o manitol e produzir ácido. Além da produção de coagulase, enzima que caracteriza a espécie, esta bactéria também produz desoxirribonucleases (DNase) (MURRAY et al., 2003).

Espécies do gênero *Staphylococcus* vivem como comensais em diversos sítios anatômicos de humanos e outros animais, sendo *S. aureus* a espécie de maior importância médica. Estabactéria pode ser componente da microbiota normal da pele e membranas mucosas humanas, em especial a mucosa nasal, constituindo-se em um patógeno oportunista capaz de produzir infecções em diversos tecidos do corpo humano, associado a altas taxas de morbidade e mortalidade (KLUYTMANS et al., 1997; FRANCOIS et al., 2007).

A maioria das infecções provocadas por *S. aureus* resulta de portadores assintomáticos, em que o indivíduo pode ser colonizado por períodos curtos ou longos, causando doença quando há algum comprometimento do sistema imunológico (HOLMES et al., 2012). Aproximadamente 20% da população podem ser considerados portadores persistentes, sendo a maioria destes crianças. Por outro lado, uma grande parte da população (60%) alberga *S. aureus* de forma intermitente (KLUYTMANS et al., 1997).

A colonização persistente por *S. aureus* pode ter um efeito protetor contra a aquisição de outros patógenos, porém, esta barreira é reduzida ou eliminada quando os hospedeiros são submetidos à terapia com antimicrobianos (NOBLE et al., 1964). Assim, a aquisição e a transmissão de cepas resistentes de *S. aureus* é preocupante principalmente no ambiente hospitalar, uma vez que indivíduos colonizados

apresentam risco elevado de desenvolverem infecção invasiva por estes microrganismos (CALFEE, 2012).

O desenvolvimento das doenças causadas por *S. aureus* está associado, além das características do hospedeiro, com a expressão de fatores de virulência que permitem colonização persistente, disseminação no hospedeiro e a evasão do sistema imunológico. O conjunto de fatores de virulência necessários para causar a doença depende do local da infecção, e este pode ser determinante na disseminação da bactéria (VANDENESCH et al., 2012).

S. aureus produzem diversos fatores de virulência, muitos destes estão ligados (frequentemente covalentemente) à sua parede celular, e são geralmente mencionados como produtos ou fatores de superfície ou somáticos. Tais fatores são importantes, principalmente, na interação do microrganismo com o hospedeiro durante o processo inicial de colonização (adesão e invasão), nos mecanismos de evasão das defesas do hospedeiro e na modulação da resposta imune. Além disso, esses microrganismos podem secretar uma ampla variedade de proteínas conhecidas como exoproteínas. Estas, por sua vez, podem funcionar como agressinas (causando danos aos tecidos e órgãos do hospedeiro), evasinas (evadindo à ação das células de defesa do hospedeiro) ou modulinas, modulando a resposta imune (SALYERS e WHITT, 2002).

Particularmente, *S. aureus* secreta numerosas exotoxinas, incluindo um grupo de polipeptídios capazes de danificar a membrana plasmática da célula hospedeira. Entre esses polipeptídios estão toxinas formadoras de poros em hemácias (α, β, γ, δ hemolisinas), *Panton-ValentineLeukocidine* (PVL), que provoca a lise de leucócitos, e outras exotoxinas, como as PSMs (*phenolsolublemodulins*) (VANDENESCH, LINA, HENRY, 2012; PARK et al., 2012).

Tipicamente, isolados de MRSA apresentando SCC*mec* tipos IV, V e VI podem conter o gene que codifica PVL. Esta tem sido extensivamente estudada desde sua descoberta nos anos de 1970 por Panton e Valentine. Ela é composta por duas subunidades, LukS-PV e LukF-PV, codificadas pelos genes *luk*S-PV e *luk*F-PV, e são adquiridos por transferência genética horizontal. LukS-PV e LukF-PV são secretadas pela bactéria e ligando-seà membrana da célula hospedeira por receptores específicos onde se associam para formar poros nestaestrutura celular. É capaz de destruir leucócitos humanos e infligir grave dano tecidual, estando

relacionada com lesões necróticas de pele e grave pneumonia necrosante, tanto em crianças como em adultos (CHAMBERS, DE LEO, 2009).

Dentre as diferentes exotoxinas incluem-se pelo menos 4 citolisinas (α, β, δ, γ hemolisinas), toxinas esfoliativas (ETA, ETB, ETC e ETD), toxina-1 da síndrome do choque tóxico (*ToxicShockSyndrome Toxin*-1 – TSST-1) e várias enterotoxinas (*staphylococcalenterotoxins* – SE) como a SEA, SEB, SEC_{1-3}, SED, SEE, SEG-SEQ (DINGES, ORWIN e SCHLIEVERT, 2000; YAMAGUCHI et al., 2002; MURRAY et al., 2003). Essas toxinas são superantígenos, capazes de induzir a liberação maciça de citocinas por macrófagos e células T (SALYERS e WHITT, 2002).

Outro importante fator de virulência de *S. aureus* é a produção de biofilme, que pode ser fator determinante em infecções crônicas refratárias ao uso de antimicrobianos. Biofilmes são comunidades complexas de microrganismos aderidas a uma superfície por meio de uma matriz extracelular. Nesta, são encontradas proteínas, polissacarídeos e até mesmo DNA extracelular, além de outros componentes. A formação de biofilme é estabelecida por pelo menos dois eventos: aderência de células em uma superfície e acúmulo de células em camadas (YU et al., 2012).

O processo de adesão é favorecido pelo polissacarídeo de adesão intercelular (*PolysaccharideIntercellularAdhesion* – PIA), composto porresíduos de N-acetilglucosamina ligados por ligações β-1,6. O *locus* de adesão intercelular (*ica*) é um operon composto porquatro fases abertas de leitura (*Open Reading Frames* – ORFs): *ica*A, *ica*D, *ica*B e *ica*C, responsáveis pela síntesedo PIA, contribuindo para a formação, do biofilme de *S. aureus* (COSTERTON et al., 1999; RICE et al., 2007; OTTO, 2008; IZANO et al., 2008; YU et al., 2012).

S. aureus também é produtor de coagulase, outro importante fator de virulência. Esta enzima coagula o sangue ao transformar fibrinogênio em fibrina, formando coágulos que protegem a bactéria do reconhecimento e fagocitose por células dosistema imune. A coagulase é também utilizada no laboratório de microbiologia para diferenciar *S. aureus* das demais espécies do gênero *Staphylococcus*. Os que não produzem a coagulase, são chamados de *Staphylococcus*coagulase negativos (CoNS) (HOWDEN et al., 2010).

2.3 Resistência aos antimicrobianos

A descrição dos principais mecanismos de resistência aos β-lactâmicos e glicopeptídeos apresentados por *Staphylococcus aureus* será apresentada a seguir no artigo de revisão: "**Emergência de *Stapylococcus aureus* resistentes aos antimicrobianos: um desafio contínuo**", publicado na **Revista de Ciências Médicas e Biológicas**.

3 OBJETIVOS

3.1 Objetivo geral:

Determinar o perfilde sensibilidade a antimicrobianos, fatores de virulência e diversidade genética de isolados clínicos de MRSA, obtidos no Hospital Universitário de Londrina, PR, Brasil.

3.2 Objetivos específicos:

- Avaliar o perfil de sensibilidade aos antimicrobianos
- Determinar a concentração inibitória mínima (CIM) para vancomicina
- Realizar a tipagem de SCC*mec*
- Identificar genes que codificam fatores de virulência e resistência
- Avaliar a diversidade genética dos isolados

4 RESULTADOS

Os resultados desta dissertação serão apresentados na forma de artigo, intitulado “***Molecular and phenotypic characteristics of methicillin-resistant Staphylococcus aureus isolated from hospitalized patients***”, aceito para publicação na revista "***The Journal of Infection in Developing Countries***".

Molecular and phenotypic characteristics of methicillin-resistant *Staphylococcus aureus* isolated from hospitalized patients

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Abstract

Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the leading causes of infections acquired in both community and hospital settings. In this study, MRSA isolated from different sources of hospitalized patients was characterized by molecular and phenotypic methods.

Methodology: A total of 123 *S. aureus* isolates were characterized according to their genetic relatedness by repetitive element sequence based-PCR (REP-PCR), *in vitro* antimicrobial susceptibility profile, SCC*mec* typing and presence of seven virulence factor-encoding genes.

Results: REP-PCR fingerprinting showed low relatedness between the isolates, and the predominance of one specific lineage or clonal group was not observed. All isolates were susceptible to teicoplanin and linezolide. All isolates were resistant to cefoxitin and penicillin, and most were also resistant to one or more other antimicrobials. Fifty isolates (41.7%) were intermediately resistant to vancomycin. Most isolates harbored SCC*mec* typeII (53.7%), followed by type I (22.8%), type IV (8.1%) and type III (1.6%). All isolates harbored at least two virulence factor-encoding genes, and the prevalence was as follow: *coa*, 100%; *icaA*, 100%; *hla,* 13.0%; *hlb*, 91.1%; *hld*, 91.1%; *luk*S-PV and *luk*F-PV, 2.4%; and *tst*, 34.1%. A positive association with the presence of *hla* and SCC*mec* type II, and *tst* and SCC*mec* type I was observed.

Conclusion: This study showed the high virulence potential of multidrug-resistant MRSA circulating in a teaching hospital. A high prevalence of MRSA showing intermediate vancomycin resistance was also observed, indicating the urgent need to improve strategies for controlling the use of antimicrobials for appropriate management of *S. aureus* infections.

Keywords: antimicrobial resistance; intermediate vancomycin resistance; MRSA; SCC*mec* typing; virulence factors.

Introduction

*Staphylococcus aureus*can be found as a harmless colonizer of skin and mucosa, mainly the anterior nares, in 20 - 60% of the population [1-3]. Colonization by this bacterium is one important predisposing factor for staphylococcal infections [1]. In fact, as an opportunistic pathogen, *S. aureus* can cause diseases ranging from superficial skin and soft tissue infections to life-threatening disseminated infections [4]. Currently, *S.aureus* is one of the leading causes of healthcare-associated infection worldwide. Most importantly, a substantial proportion of staphylococcal infections are caused by methicillin-resistant *S. aureus* (MRSA), which also exhibits resistance to several other antimicrobials [5,6], contributing to its persistence as a human pathogen for decades.

The acquisition of the *mecA* gene, which encodes a penicillin-binding protein (PBP) with low affinity for the antimicrobial, called PBP2'or PBP2a, is the most common mechanism of methicillin-resistance. The gene *mecA* is inserted into a mobile genetic element (MGE) known as staphylococcal cassette chromosome *mec* (SCC*mec*), and currently, eleven types (SCC*mec*I to XI) have been described in MRSA strains isolated from various sources [7-10].

The virulence potential of *S. aureus* is extensive, with it being represented by both structural and secreted products, whose encoding genes are mostly located in MGEs, which also contribute to bacterial genome plasticity and evolution [11]. This diverse array of virulence factor-encoding genes facilitates the adhesion of bacterial cells to biotic or abiotic surfaces, resistance to host defenses, invasion and cell injury [12]. The differential expression of these genes may enhance *S.aureus* virulence, enabling the bacterium to cause specific clinical presentation [13]. Furthermore, the virulence of this bacterium may vary between isolates from different geographic regions [14].

Continuous efforts to understandthe biological basis of MRSA antimicrobial resistance and virulence are therefore necessary. This knowledge may contribute not only to the adoption of effective measures to control the infection, but also to the development of new anti-infective drugs that inhibit bacterial growth plus control virulence [12, 15].

In this study, MRSA strains isolated from different sources of patients seen at the University Hospital of Londrina, Paraná, Brazil were characterized by phenotypic and molecular methods. The *in vitro* antimicrobial susceptibility profile, genetic relatedness and occurrence of virulence genes *icaA* from intercellular adhesion *locus* (encodingN-acetylglucosaminyltransferase), *hla, hlb* and *hld* (encoding α-, β- and δ-hemolysin, respectively), *lukS*-PV and *lukF*-PV (encoding the β-pore-forming Panton-Valentine leukocidin) and *tst* (encoding toxic shock syndrome toxin) were also evaluated.

Material and Methods

Microorganisms

The University Hospital of Londrina, Paraná, Brazil is a 353-bed tertiary care center that serves the city of Londrina, in addition to several localities in the states of Paraná, São Paulo, and Mato Grosso do Sul. This is the major referral center for the Sistema Único de Saúde (a governmental healthcare assistance program) of northern Paraná. A total of 123 non-duplicate *S. aureus* isolates from patients seen in this hospital from June 2010 to June 2013 were randomly taken from the bacterial collection of the Laboratory of Clinical Microbiology of UniversidadeEstadual de Londrina (UEL). The isolates were classified according to CDC definitions of healthcare-associated infections [16]. The study protocol was approved by the Ethics Committee of UEL (CAAE no. 3346.0.000.268.09/protocol 186/09 CEP-UEL). All isolates were identified to the species level by standard phenotypic methods on the basis of colony morphology, Gram staining, catalase, DNase and mannitol fermentation after growing

in Columbia agar base (Oxoid) supplemented with 5% sheep blood at 37°C for 24 h. Bacteria were kept at -80°C intryptone soya broth (TSB, Oxoid) containing 30% glycerol. Species identification was also performed by a PCR-based method using specific primers for *coa* gene (encoding coagulase) regions according to Tiwari et al. (2008) [17].

Antimicrobial susceptibility pattern

Bacterial isolates were tested for antimicrobial susceptibility to cefoxitin (30 μg), ciprofloxacin (5 μg), clindamycin (2 μg), erythromycin (15 μg), gentamicin (10 μg), linezolide (10 μg), oxacillin (1 μg), penicillin (10 U), rifampicin (5 μg), sulfamethoxazole-trimethoprim (23.75/1.25 μg), teicoplanin (30 μg) and tetracycline (30 μg) using the disk-diffusion assay. Cefoxitin and oxacillinwere used to define methicillin-resistant *S. aureus* (MRSA) isolates. The minimal inhibitory concentration (MIC) for vancomycinwas determined by the broth-dilution method. MIC was determined as 100% growth inhibition. Both methods were performed and interpreted according to the Clinical Laboratory Standards Institute (CLSI, 2013) [18]. *S. aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 51299 were used as controls.

DNA extraction

A single bacterial colony was transferred to 3 mL TSB and incubated at 37°C for 24 h. The bacterial pellets obtained after centrifugation at 10,000 x *g* for 5 min were washed once with sterile 0.15 M phosphate-buffered saline (PBS), pH 7.2, resuspended in 300 μL sterile solution (10 mMTris-HCl and 1 mM EDTA, pH8.0, and 1.0 mg/mL lysozyme). Genomic DNA was extracted following the procedure described by Ausubel et al. (1991) [19], and a 2-μL aliquot was used in all amplification reactions.

MRSA typing

The identification of SCC*mec*type of all MRSA isolates was performed by multiplex PCR assay as described by Milheiriço et al. (2007) [20]. Non-typeable isolates were designated NT.NCTC10442 (type I), N315 (type II), 85/2082 (type III) and 81/108 (type IV) strains were used as control. The genetic relatedness of all MRSA was analyzed by repetitive element sequence based-PCR (REP-PCR) using the primer RW3A as described by Del Vecchio et al. (1995) [21]. Banding patterns were categorized using the UPGMA algorithm and Jaccard coefficient [22] of the Bionumerics v.4.6 software (Applied Mathematics, Kortrijk, Belgium), with the band tolerance set at 3% and the threshold cutoff value set at 85%.

Detection of virulence factor-encoding genes

The detection of nucleotide sequences corresponding to genes encoding virulence factors of *S. aureus* was performed by PCR. The genes *icaA*, *lukS*-PV and *lukF*-PV and *tst* (encoding toxic shock syndrome toxin) were amplified as described by Campbell et al. (2008) [23]. The genes *hla, hlb* and *hld* (encoding α-, β- and δ-hemolysin, respectively) were amplified as described by Jarraud et al. (2002) [24]. All PCRs were performedin a Veriti® 96-well thermal cycler (Applied Biosystems), and reactions without any template DNA were carried out simultaneously as negative control.

Statistical analysis

The categorical variables were compared with the chi-square test or Fisher's exact test using the BioEstat Software version 5.3. *p* values less than 0.05 were considered significant.

Results

Patients, MRSA identification and genotyping

The age of the patients enrolled in this study ranged from three months to 87 years (median of 50 years old), and the majority of them were men (*n*=86, 70.0%). The isolates were recovered from various clinical sources as follows: blood (*n*=43, 35.0%), tissue fragment (*n*=21, 17.1%), general discharge (n=18, 14.6%), tracheal aspirates (*n*=15, 12.2%), central venous catheter line (*n*=10, 8.1), urine (*n*=7, 5.7%) and general swab (*n*=9, 7.3%). In 17 patients (13%), MRSA infection was identified less than 48 h after hospital admission. All isolates harbored the gene *coa* and exhibited resistance tocefoxitin, although seven (5.7%) isolates were classified as susceptible to oxacillin.

Cluster analysis and visual observation of bands generated by REP-PCR typing revealed low relatedness between the isolates. By using a cutoff value of 95% and 85% similarity, a total of 94 and 62 different genotypes were respectively identified among the isolates, indicating their high diversity. To compare the REP-PCR pattern with other features of MRSA, those isolates showing 65% similarity were clustered in the same group, and the analysis of the dendrogram resulted in 17 different genotypes, named A to Q. The groups with similar REP-PCR profiles consisted of 50 (E, 40.7%), 16 (F, 13.0%), 14 (H, 11.4%), 13 (A, 10.6%), 8 (I, 6.5%), 5 (B, 4.1%) and 4 (G, 3.3%) isolates each. Three genotypes (D, M, N, 1.6% each) consisted of two isolates each. The other seven (5.6%) isolates had unique banding profiles.

Phenotypic and genotypic characterization of antimicrobial susceptibilities

The *in vitro* susceptibility patterns of MRSA to various antimicrobials are givenin Table 1. All isolates were susceptible to teicoplanin and linezolide. Besides being resistant to cefoxitin, all isolates showed resistance to penicillin. MRSA isolates were distributed intofour

SCC*mec* types, according to the multiplex PCR used in this study, and type II (66/123, 53.7%) was the most frequent, followed by type I (28/123, 22.8%), type IV (10/123, 8.1%) and type III (2/123, 1.6%). Seventeen (13.8%) isolates were classified as NT (Table 1).

Concerning the phenotypic resistance profile, the MRSA isolates were classified into 18 groups (Table 1). Three isolates were resistant only to β-lactam antimicrobials, and harbored the SCC*mec* type IV. Most isolates (50/123, 40.7%) displayed the group VIII phenotypic antimicrobial resistance profile (cefoxitin, oxacillin, penicillin, ciprofloxacin, clindamycin, and erythromycin) and harbored theSCC*mec* type I (3/50) and type II (47/50). Thirty (24.4%) out of 123 isolates were clustered in group X (cefoxitin, oxacillin, penicillin, ciprofloxacin, gentamycin, clindamycin, and erythromycin) and harbored the SCC*mec* types I (22/30), II (3/30), IV (3/30) and NT (2/30).Most isolates classified as NT were those with resistance to a greater number of antimicrobials.

Most isolates were susceptible to vancomycin (73/123, 59.4%), and among them 1.4% (1/73), 13.7% (10/73) and 84.9% (62/73) had MIC values of 0.25, 1.0 and 2.0 μg/mL, respectively. For all isolates, the MIC50 and MIC90 values were 2.0 and 4.0 μg/mL, respectively. Fifty (40.7%) isolates out of 123 isolates were intermediately resistant to vancomycin, according to CLSI (2013) criteria. Among these, 92% (46/50) and 8% (4/50) of isolates showed MIC values of 4 and 8 μg/mL, respectively. However, patients with intermediate MIC values did not have higher in-hospital mortality than those with susceptible MIC values.

Table 1: Relationship between phenotypic antimicrobial resistance profile and SCC*mec* types of MRSA isolates

Group	Antimicrobial resistance profile	SCC*mec* - Number of isolates (%*)					
		Type I	**Type II**	**Type III**	**Type IV**	**NT**	**Total (%#)**
I	FOX, P				2		2 (1.7)
II	FOX, OX, P				1		1 (0.8)
III	FOX, OX, P, E				2		2 (1.7)
IV	FOX, OX, P, SXT		1		1	1	3 (2.4)
V	FOX, P, CN, SXT		1				1 (0.8)
VI	FOX, P, CIP, DA, E		3				3 (2.4)
VII	FOX, OX, P, CIP, CN, E	1 (3.6)					1 (0.8)
VIII	FOX, OX, P, CIP, DA, E	3 (10.7)	47				50 (40.7)
IX	FOX, P, CIP, CN, DA, E				1		1 (0.8)
X	FOX, OX, P, CIP, CN, DA, E	22 (78.6)	3		3	2	30 (24.4)
XI	FOX, OX, P, CIP, DA, E, RD		8				8 (6.5)
XII	FOX, OX, P, CIP, DA, E, SXT		1				1 (0.8)
XIII	FOX, OX, P, CIP, DA, E, TE					1	1 (0.8)

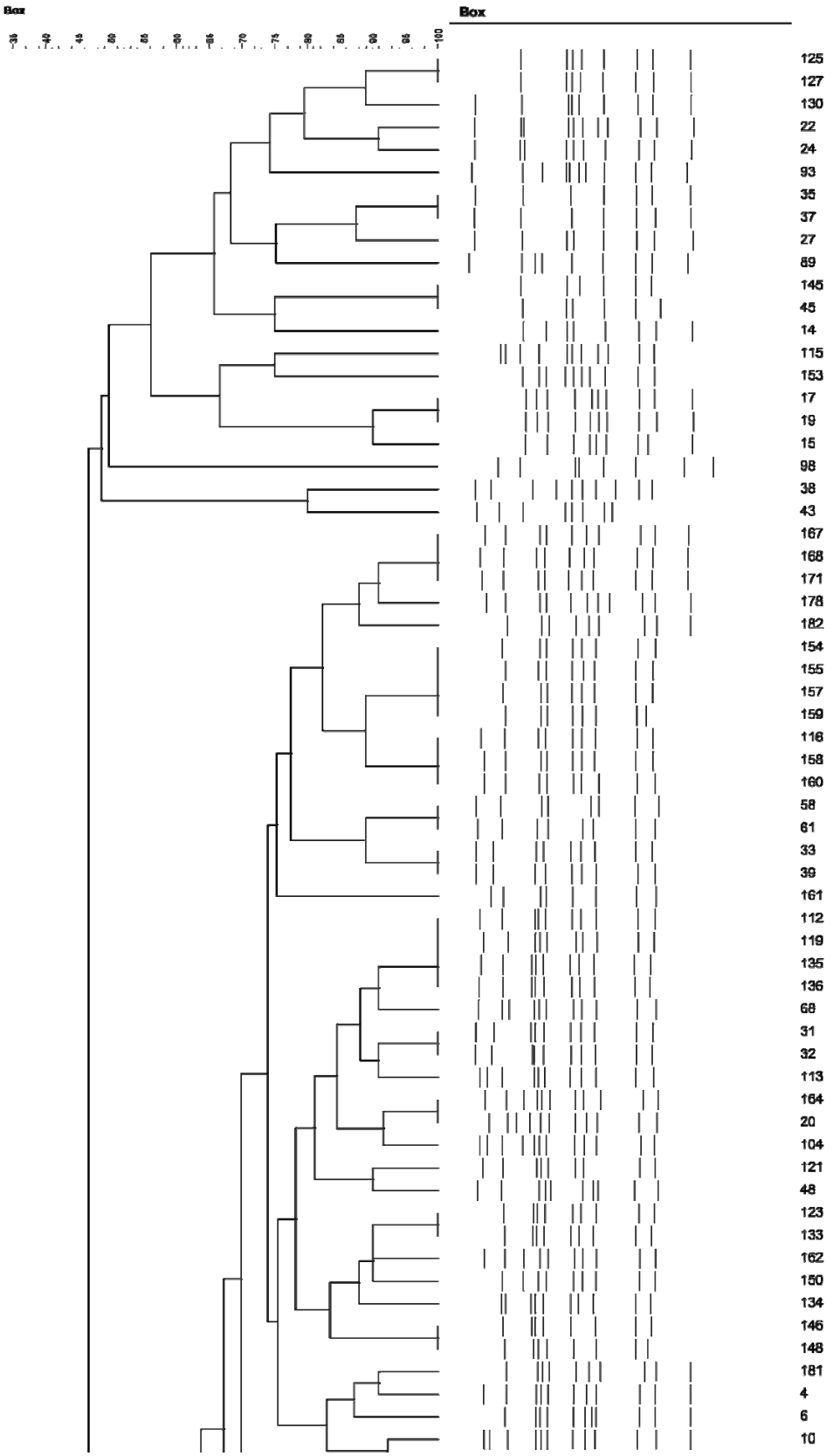
XIV	FOX, OX, P, CIP, CN, DA, E, RD	2 (7.1)					2 (1.7)
XV	FOX, OX, P, CIP, CN, DA, E, STX					3	3 (2.4)
XVI	FOX, OX, P, CIP, DA, E, TE, STX			1			1 (0.8)
XVII	FOX, OX, P, CIP, CN, DA, E, TE, STX		2			8	10 (8.1)
XVIII	FOX, OX, P, CIP, DA, E, TE, SXT, RD			1		2	3 (2.4)
	Total (%[#])	28 (22.8)	66 (53.7)	2 (1.6)	10 (8.1)	17 (13.8)	123 (100)

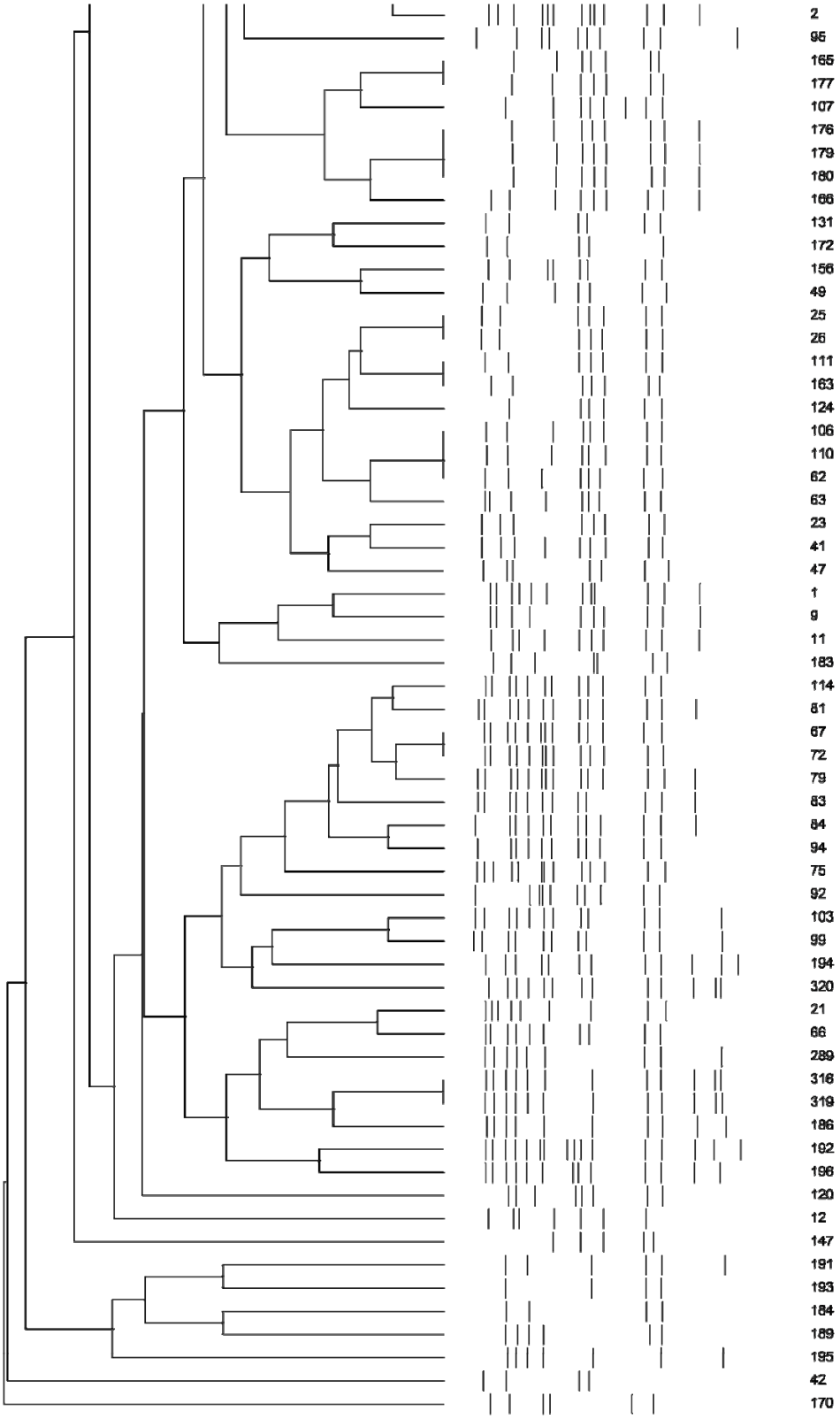
*Among the total isolates in each group; [#]Among all isolates analyzed. FOX, cefoxitin; OX, oxacillin; P, penicillin; E, erythromycin; STX, sulphamethoxazole-trimethoprim; CN, gentamycin; CIP, ciprofloxacin; DA, clindamycin; RD, rifampicin; TE, tetracyclin.

Relationship between SCC*mec *types and REP-PCR genotyping

Eighteen MRSA SCC*mec* type I (18/28, 64.3%) isolates were grouped in genotype E, and four (14.3%) and two (7.1%) in genotypes F and H, respectively. One isolate each (3.6%) was grouped in genotypes I, K, L, and P. The SCC*mec* type II isolates were mainly distributed into genotype E (28/66, 42.4%), followed by genotype H (11/66, 16.7%), F (8/66, 12.1%), I (6/66, 9.1%) and G (4/66, 6.0%). Two isolates each (3.0%) were grouped in genotypes M and N, and one isolate each (1.5%) in genotypes A, B, C, O and Q. One isolate each of SCC*mec* type III displayed the genotypes A and B. Three isolates each (30%) of SCC*mec* type IV were grouped in genotypes E and F, and one isolate each (10%) in genotypes A, B, H and J. Most NT-SCC*mec* (10/17, 58.8%) isolates were grouped in genotype A, and the other isolates were distributed into genotypes B (2/17, 11.8%), D (2/17, 11.8%), E (1/17, 5.9%), F (1/17, 5.9%) and I (1/17, 5.9%) (Figure 1).

Figure 1: REP-PCR fingerprinting pattern and SSC*mec* types obtained from 123 methicillin-resistant *Staphylococcus aureus* isolates from different sources of patients seen at the University Hospital of Londrina.





Cluster analysis was performed using UPGMA algorithm and Jaccard coefficient of the Bionumerics v. 4.6 software, with band tolerance set at 3% and threshold cutoff value set at 85%. SCC*mec* typing was performed by multiplex PCR assay as described by Milheiriço et al. (2007). All *S. aureus* isolates were resistant to cefoxitin. NT: non-typeable; BL: blood; TF:

tissue fragment; GD: general discharge; TA: tracheal aspirates; CVCL: central venous catheter line; UR: urine; GS: general swab.

Detection of virulence factor-encoding genes

The presence of the genes *coa*, *icaA*, *hla, hlb*, *hld*, *lukS*-PV, *lukF*-PV and *tst* in MRSA isolates was detected by PCR. The overall prevalence was as follows: *coa*, 100%; *icaA*, 100%; *hla,* 13.0%; *hlb*, 91.1%; *hld*, 91.1%; *lukS*-PV and *lukF*-PV, 2.4%; and *tst*, 34.1%, and no significant association with the origin of the isolates was observed (Table 2).All isolates harbored at least two virulence markers (Table 3), and a significant association with the presence of *hla* gene and the SCC*mec* type II ($p<0.005$), and *tst* and SCC*mec* type I ($p<0.001$) was observed. Although no statistical correlation was found, it was also observed that: a) most isolates (59/123, 48.0%) harbored the combination of *coa*, *ica*A, *hlb* and *hld* genes, which were distributed into SCC*mec* types II (46/66, 69.7%), III (2/2, 100%), IV (1/10, 10%) and NT (10/17, 58.8%); b) isolates with the gene *hla* harbored the SCC*mec* types I, II, IV and NT; c) the presence of PVL- encoding genes was detected only in isolates belonging to SCC*mec* type IV. A larger number of isolates may corroborate these findings.

Table 2: Distribution of virulence encoded genes according to the origin of 123 methicillin-resistant *Staphylococcus aureus* isolates from hospitalized patients of University Hospital of Londrina.

Clinical source	**Number of isolates harboring the virulence encoding gene (%)**						
	coa*	***icaA****	***hla****	***hlb****	***hld****	***tst****	**PVL***
Blood (*n*=43)	43 (100)	43 (100)	5 (11.6)	33 (76.7)	33 (76.7)	14 (32.6)	1 (2.3)
Tissue fragment (*n*=21)	21 (100)	21 (100)	3 (14.3)	20 (95.2)	20 (95.2)	4 (19)	-
General discharge (*n*=18)	18 (100)	18 (100)	3 (16.7)	18 (100)	18 (100)	7 (38.9)	-
Tracheal aspirates (*n*=15)	15 (100)	15 (100)	-	15 (100)	15 (100)	6 (40)	-
Venous central catheter line *(n*=10)	10 (100)	10 (100)	2 (20)	10 (100)	10 (100)	5 (50)	-
Urine (*n*=7)	7 (100)	7 (100)	2 (28.6)	7 (100)	7 (100)	1 (14.3)	-
General swab (*n*=9)	9 (100)	9 (100)	1 (11.1)	9 (100)	9 (100)	5 (55.6)	2 (22.2)
Total (%[#])	123 (100)	123 (100)	16 (13)	112 (91.1)	112 (91.1)	42 (34.1)	3 (2.4)

*Among the total isolates in eachgroup; [#]Among all isolates analyzed. *coa*: coagulase; *icaA*: intercellular adhesion *lócus* encoding N-acetylglucosaminyltransferase; *hla*: α-hemolysin*, hlb*: β-hemolysisn; *hld*: δ-hemolysin; *tst:* toxic shock syndrome toxin; PVL:*luk*S-PVand *luk*F-PV of the β-pore-forming Panton-Valentine leukocidin. -: absence

Table 3: Methicillin-resistant *Staphylococcus aureus* isolates harboring clusters of virulence encoded genes according to their SCC*mec* typing.

Virulence markers[¥]	**Number of isolates (%*)**					
	Type I	**Type II**	**Type III**	**Type IV**	**NT**	**Total (%[#])**
coa, *ica*A		10 (15.1)				10 (8.1)
coa, *ica*A, *hla*, *tst*	1 (3.6)					1 (0.8)
coa, *ica*A, *hlb*, *hld*		46 (69.7)	2 (100)	1 (10)	10 (58.8)	59 (48.0)
coa, *ica*A, *hlb*, *hld*, PVL				3 (30)		3 (2.4)
coa, *ica*A, *hla*, *hlb*, *hld*	1 (3.6)	3 (4.5)		1 (10)	4 (23.5)	9 (7.3)
coa, *ica*A, *hlb*, *hld*, *tst*	21 (75.0)	7 (10.7)		5 (50)	2 (11.8)	35 (28.5)
coa, *ica*A, *hla*, *hlb*, *hld*, *tst*	5 (17.8)				1 (5.9)	6 (4.9)
Total (%[#])	28 (22.8)	66 (53.7)	2 (1.6)	10 (8.1)	17 (13.8)	123 (100)

*Amongthe total isolates in eachgroup; [#]Amongallisolatesanalyzed.[¥]*coa*: coagulase; *icaA*: intercellularadhesion*locus*encoding N-acetylglucosaminyltransferase; *hla*: α-hemolysin*, hlb*: β-hemolysisn; *hld*: δ-hemolysin; *tst:* toxicshocksyndrometoxin; PVL:*luk*S-PVand*luk*F-PV oftheβ-pore-formingPanton-Valentineleucocidin

Discussion

In this study, MRSA isolates harboring four SCC*mec* types (I, II, III and IV) were identified. The predominance of one specific lineage or clonal group (as assigned by REP-PCR fingerprinting) was not observed, indicating that many different strains circulated in the hospital during the period analyzed here. These SCC*mec* types were also detected in MRSA surveys conducted in different Brazilian hospitals. However, the prevalence of SCC*mec* types varied between different regions [3, 25-31]. The Brazilian epidemic clone (BEC) carrying the SCC*mec* type III has been the predominant clone in several hospitals in Brazil [28, 29, 32]. However, in this study, a prevalence of SCC*mec* type II was observed, as by Caiaffa-Filho et al (2013) [30], who detected a high prevalence of this SCC*mec* type in a tertiary care teaching hospital in São Paulo, Brazil.

Not surprisingly, most MRSA strains harboring the SCC*mec* types I, II and III were resistant to more than three other non-β-lactam antimicrobial classes. Among SCC*mec* type IV isolates, most were susceptible to almost all non-β-lactam antimicrobials, which is a common feature of these strains. However, four isolates harboring SCC*mec* type IV showed resistance to more than three antimicrobial classes. This study also detected NT-SCC*mec* isolates, and except for one, all displayed resistance to a greater number of antimicrobial classes. Of note was a high proportion of MRSA strains showing intermediate resistance to vancomycin, one of the last therapeutic choices for the treatment of invasive infections. In fact, glycopeptide treatment failure and poor clinical outcomes have been reported in infections caused by vancomycin-intermediate *S. aureus* [33].

Besides being resistant to many antimicrobial agents, *S. aureus* possesses a number of virulence determinants, which makes it highly adaptive and versatile. The expression of the virulence factor-encoding genes seems likely to be dependent on the site of infection [34, 35]. In this study, the genes *coa*, *icaA*, *hlb* and *hld* were highly conserved among MRSA isolates,

independent of the clinical origin. Coagulase plays a role in intravascular coagulation, facilitating the aggregation of *S. aureus* in blood, which in turn promotes bacterial survival [35]. The gene *icaA* encodes the transmembrane protein *N*-acetylglucosaminyltransferase, which is involved in the biosynthesis of polysaccharide intercellular adhesin (PIA). This adhesin promotes cellular aggregation during the maturation stage of PIA-dependent biofilm formation by *S. aureus* [36]. The gene *hld* encodes the δ-Hemolysin (Hld or δ-toxin), a cytotoxic protein of phenol-soluble modulins family [37] that can also contribute in structuring and detachment stages of biofilm development [38]. The gene *hlb* encodes a magnesium-dependent sphingomyelin-specific phospholipase called β-hemolysin (Hlb), which can lyse erythrocytes, neutrophils and lymphocytes [39].

The pore-forming hemolysin-α (Hla or α-toxin), encoded by *hla*geneis virtually produced by all strains [34]. However, this gene was detected in only 13% of the isolates in this study, and its presence was strongly associated with the SCC*mec* type II-harboring isolates. The mechanism of cell lysis mediated by Hla is dependent on the initial interaction with a specific receptor on host membranes, often targeting a particular cell type [40]. It can damage epithelial cells, fibroblast, erythrocytes, lymphocytes, monocytes and macrophages, but not neutrophils [41]. The expression of Hla contributes to the pathogenesis of sepsis, skin infections and pneumonia in murine experimental infection [35, 40, 42]. Furthermore, the role of this exotoxin during biofilm formation *in vitro* and in a mucosal model of *S. aureus* infection has been shown elsewhere [43, 44].

It has been previously shown that SCC*mec* type IV harboring the gene encoding the bi-component PVL was characteristic of community-acquired MRSA, which is a highly virulent strain [26, 31]. However, PVL has also been detected in other SCC*mec* strains [45]. This pore-forming toxin, whose genes *lukF*-PV and *lukS*-PV are lysogenic bacteriophage-encoded (MGE), can lyse macrophages, monocytes and neutrophils and cause tissue necrosis

[46]. PVL has been associated with *S. aureus* skin and soft tissue infections, and necrotizing pneumonia and septic shock [35, 47]. In this study, the PVL-encoding genes were detected in three SCC*mec* type IV-harboring isolates, and except for one that showed resistance to erythromycin, the other two isolates were susceptible to all non-β-lactam antimicrobials analyzed here. In addition, in two patients who were outpatients, MRSA infection was identified less than 48 h after hospital admission, and none was using medical devices during the time of cultures. Altogether, these data indicate that these two cases may be epidemiologically characterized as community-associated MRSA infection [10].

The *tst* gene, which encodes the pyrogenic toxic shock-syndrome toxin 1 (TSST-1), is present in only a small number of *S. aureus* strains, and this can be explained by its genomic location in a pathogenicity island (MGE). In this study, a low prevalence of *tst* gene was also observed among MRSA isolates, and its presence was associated with SCC*mec* type I. TSST-1, a non-cytolytic toxin, is a member of superantigen (SAgs) family, which induces a potent activation of T cell and macrophages with large production of cytokines that can interfere with the host immune responses. In addition, the expression of *tst* or other Sags has been associated with enhanced susceptibility to endotoxic shock syndrome development, with high mortality rates [48].

Limitations of this study, which may reduce generalization of the results, are a) the number of isolates of each clinical source; b) the evaluation of a single hospital; and c) the presence or absence of a particular virulence factor-encoding gene being evaluated only by PCR. Despite these limitations, this study showed the high virulence potential of multidrug-resistant MRSA circulating in our hospital. Besides, a high prevalence of MRSA showing intermediate vancomycin resistance was observed. These results corroborate the importance of continuous monitoring of antimicrobial susceptibility profiles and potential virulence of *S.*

aureus.In addition, they will contribute to improving strategies for controlling the use of antimicrobials for appropriate management of infections caused by this bacterium.

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5 CONCLUSÃO

- A maioria dos isolados foi proveniente de pacientes do sexo masculino;
- A média de idade dos pacientes foi de 50 anos;
- Todos os isolados apresentaram provas fenotípicas e genotípicas para *S. aureus*;
- A maioria dos isolados foi proveniente de amostras de sangue;
- A maioria dos pacientes estava internada na enfermaria;
- A sensibilidade aos antimicrobianos foi heterogênea, sendo todos resistentes à penicilina e sensíveis à teicoplanina e linezolida, por disco difusão;
- A maioria dos isolados apresentou sensibilidade à vancomicina por microdiluição em caldo;
- O SCC*mec* tipo II foi o mais prevalente entre os isolados;
- Todos os isolados apresentaram ao menos 2 genes de virulência analisados;
- A genotipagem por rep-PCR mostrou-se eficiente e o grupo E reuniu o maior número de isolados, mostrando que não há disseminação clonal;
- O trabalho contribuirá para adoção de medidas de controle da infecção hospitalar, assim como embasamento no tratamento de infecções por *S. aureus*.

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