UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO"
FACULDADE DE MEDICINA DE BOTUCATU

Letícia Chamma Lastoria

COLONIZAÇÃO POR *Staphylococcus aureus* EM PESSOAS VIVENDO COM HIV/AIDS ACOMPANHADAS EM UM SERVIÇO AMBULATORIAL DE REFERÊNCIA EM BOTUCATU (SP): PREVALÊNCIA, RESISTÊNCIA À METICILINA E EPIDEMIOLOGIA MOLECULAR.

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Orientador:
Prof. Dr. Carlos Magno C. B. Fortaleza

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Palavras-chave: AIDS; Colonização; HIV; MRSA; *Staphylococcus aureus*.

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SUMÁRIO

RESUMO

Staphylococcus aureus resistente à meticilina (*Methicillin-resistant S.* aureus, MRSA) é cada vez mais reconhecido como uma ameaça para pessoas vivendo com HIV/AIDS (PVHA). No entanto, a magnitude da colonização por MRSA varia entre diferentes países e regiões geográficas. Nós realizamos um estudo que teve por objetivo identificar a prevalência e os fatores de risco para colonização por *S. aureus* como um todo e MRSA em PVHA residindo em cidades de pequeno porte do interior do Estado de São Paulo. Isolados de MRSA foram caracterizados por Eletroforese em Gel de Campo Pulsado (*Pulsed-Field Gel Electrophoresis*, PFGE) e tiveram o Cassete Cromossômico Estafilocóccico (*Staphylococcal Chromosome Cassete*, SCC) *mec* tipado. Análise espacial foi realizada para identificar agregados geográficos e correlação com indicadores socioeconômicos. No primeiro momento, realizamos um estudo de prevalência pontual coletando *swab* nasal e de orofaringe de 368 PVHA atendidas em ambulatório de referência em Botucatu, SP. Sessenta e sete sujeitos residentes na cidade sede foram seguidos com coletas em dois outros momentos, e tiveram seus contactantes domiciliares também investigados para colonização. As taxas de prevalência de *S. aureus* e MRSA no primeiro levantamento foram 25,8% e 2,7%. A colonização por *S. aureus* foi negativamente associada com o uso de antibióticos beta-lactâmicos e drogas ilícitas. Por outro lado, fatores de risco para MRSA incluíam uso de crack e internação hospitalar recente. Inquéritos repetidos identificaram novos casos de colonização por MRSA, mas nenhum sujeito apresentou positividade em mais de uma ocasião. Quatro *clusters* foram identificados na PFGE, agrupando sujeitos em

diferentes níveis – domicílio, cidade, região. Dos 19 isolados caracterizados, apenas um não carreava o SCC*mec* tipo IV. Análise espacial identificou *hot spots* par sujeitos colonizados com *S. aureus*, mas não conseguimos ligar esse padrão a indicadores sócio-econômicos. Em conclusão, nós idenficamos baixa – mas relevante – prevalência de MRSA em PVHA. Foram identificados tanto fatores de risco tradicionalmente associados a aquisição na comunidade quanto outros ligados a exposição a hospitais, de modo que as rotas predominantes de transmissão não puderam ser determinadas com base epidemiológica.

Palavras-chave: *Staphylococcus aureus*, MRSA, HIV, AIDS, Colonização, Epidemiologia molecular, Epidemiologia espacial

ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is increasingly recognized as a threat for people living with HIV/AIDS (PLWHA). However, the magnitude of asymptomatic MRSA colonization in that group varies among different countries and geographic regions. We conducted a study that aimed at identifying the prevalence and risk factors for both overall *S. aureus* and MRSA colonization among PLWHA attending in small cities from inner São Paulo State, Brazil. MRSA isolates were characterized using Pulsed-Field Gel Electrophoresis (PFGE), and submitted to typing of the Staphylococcal Chromosome Cassete (SCC)*mec*. Spatial analysis was performed to search for geographical clusters and correlation with socioeconomic indicators. In a first point prevalence survey, nasal and oropharyngeal swabs of 368 people were collected. Sixty-seven subjects from the main city (Botucatu) were surveyed for colonization in two other occasions, and had swabs collected from household members. The prevalence rates for *S. aureus* and MRSA in the first survey were 25.8% and 2.7%. The overall *S. aureus* colonization was negatively associated with the use of beta-lactams and of illicit drugs. On the other hand, MRSA colonized subjects were more likely to use crack and to have been admitted to a hospital during the past year. Repeated surveys found additional cases of MRSA colonization, but all subjects were positive in only one occasion. Four PFGE clusters were characterized, grouping subjects in household, city and region level. Of 19 total MRSA isolates, only one did not harbor SCC*mec* type IV. Spatial analysis detected hot spots of *S. aureus* colonized subjects from Botucatu, but that finding could not

be linked to socio-economic indicators. In conclusion, we found small but relevant prevalence of MRSA among PLWHA. Community and healthcare-associated risk factors were identified, so that predominant routes of transmission could not be determined on epidemiological grounds.

Keywords: *Staphylococcus aureus*, MRSA, HIV, AIDS, Colonization, Molecular epidemiology, Spatial Epidmeiology.

1. INTRODUÇÃO

1.1. A interseção de duas pandemias

Desde sua emergência, arbitrariamente datada no início da década de 1980, a aids desenvolveu uma dinâmica de transmissão complexa, afetando de forma desigual a população mundial.[1] De fato, à medida que a epidemia atingiu novos continentes, populações especialmente vulneráveis foram desproporcionalmente acometidas.[2,3,4] Um recente estudo ecológico buscou indicadores relacionados à prevalência de aids nos diversos países. Os achados mostram relação direta com a taxa de fertilidade em adolescentes e inversa com escolaridade e densidade de médicos no país.[5] Não resta dúvida de que, apesar da possibilidade de acometer qualquer grupo social, a aids ainda representa - para citar um artigo recente - uma "pandemia para pobres".[6] Este aspecto – e a consequente negligência de populações sob risco – contribuem para as dificuldades de uma ação global de erradicação e/ou controle.[7]

No Brasil, são bem conhecidas as tendências epidemiológicas da aids nas décadas de 1990 e 2000 – feminização, pauperização e interiorização.[8] Esta última é particularmente importante. Estudos mostram que a proporção de Pessoas Vivendo com HIV/Aids (PVHA) que residem em cidades de pequeno porte vem crescendo de forma significante nos últimos anos.[9,10] Em consequência disso, o número de clínicas especializadas para atendimento a essa população sofreu aumento exponencial. Só para termos uma ideia, esse número

saltou de 33 em 1996 para 540 em 2002 e 663 em 2007.[11,12] Esse aumento dá uma medida dos esforços realizados pelo ministério da saúde do Brasil para promover acesso universal e equitativo das PVHA à terapêutica apropriada.[13,14]

As estafilococcias – e mais especificamente aquelas causadas pelos *Staphylococcus aureus* resistentes à meticilina (MRSA) – dão a impressão de não pertencer ao mesmo universo.[15] No entanto, a infecção por MRSA é também uma condição de morbidade que emergiu há algumas décadas, apresentou mudanças importantes em sua epidemiologia e afeta desigualmente as populações do mundo.[16,17] O MRSA, e recentemente sua variante associada a (ou adquirida na) comunidade, o *Community associated*[CA-]MRSA, são causa reconhecida de doença grave.[15] No entanto, sua epidemiologia complexa, a difícil interpretação da relação entre carreamento assintomático (colonização) e doença invasiva e as múltiplas manifestações clínicas dificultam o entendimento do MRSA como um risco global à saúde pública.[18] Nesse ponto, o MRSA contrasta de forma importante com o HIV.

1.2. *Staphylococcus aureus:* características, patogenicidade e importância clínica

Staphylococcus (do grego, *staphilé* = cachos de uva)[19] são microorganismos não formadores de esporos e que dão origem a células arredondadas de aproximadamente um micron de diâmetro e que, quando observados à microscopia óptica, têm aparência semelhante a cacho de uva, morfologicamente cocos Gram positivos (**Figura 1**). Em meio de cultura sólida, as colônias de *Staphylococcus aureus* são elevadas, brilhantes e de forma arredondada[20,21]: em placa de ágar sangue, observa-se halo de hemólise em torno das colônias. O nome da espécie *S. aureus* decorre da cor dourada das colônias, em função de pigmentos carotenoides formados durante seu crescimento.

É a única espécie encontrada em seres humanos que pode produzir a enzima coagulase, sendo que esta no plasma pode se ligar a fatores séricos com potencial para converter o fibrinogênio em fibrina podendo desencadear processo de coagulação. As espécies não produtoras de coagulase são ditas coagulase negativas.[19]

O meio de cultura ágar sal manitol é seletivo para o *S. aureus* e importante para sua identificação já que o mesmo fermenta o manitol, produzindo ácido lático.[22]

O gênero *Staphylococcus* engloba 51 espécies e 27 subespécies[23] – mas entre elas o *S. aureus* se destaca pela virulência e relevância clínica.

O *S. aureus* é amplamente disseminado em populações humanas, podendo colonizar assintomaticamente pessoas saudáveis. A colonização – presença do

microorganismo sem repercussão clínica- tem início logo após o nascimento, podendo recorrer ao longo da vida.[24] Em indivíduos saudáveis o *S. aureus* pode integrar a microbiota da pele e mucosas, sendo a mucosa nasal sítio primário de sua colonização.[25] Apesar de ser, na maior parte das vezes, inócua, a colonização é fator de risco para o desenvolvimento da doença invasiva em função de falhas de barreiras físicas e imunológicas, podendo produzir infecções oportunistas importantes e graves.[25,26]

A capacidade do *S. aureus* de causar doença invasiva está relacionada a diversos fatores de virulência, envolvidos na adesão, destruição tecidual e indução de resposta inflamatória sistêmica.[27]

As estafilococcias são diversas e apresentam amplo espectro de gravidade, incluindo desde infecções de pele e tecidos moles a quadros infecciosos invasivos e potencialmente fatais como sepse, pneumonia e endocardite. (Tabela 1)[19,20,27]

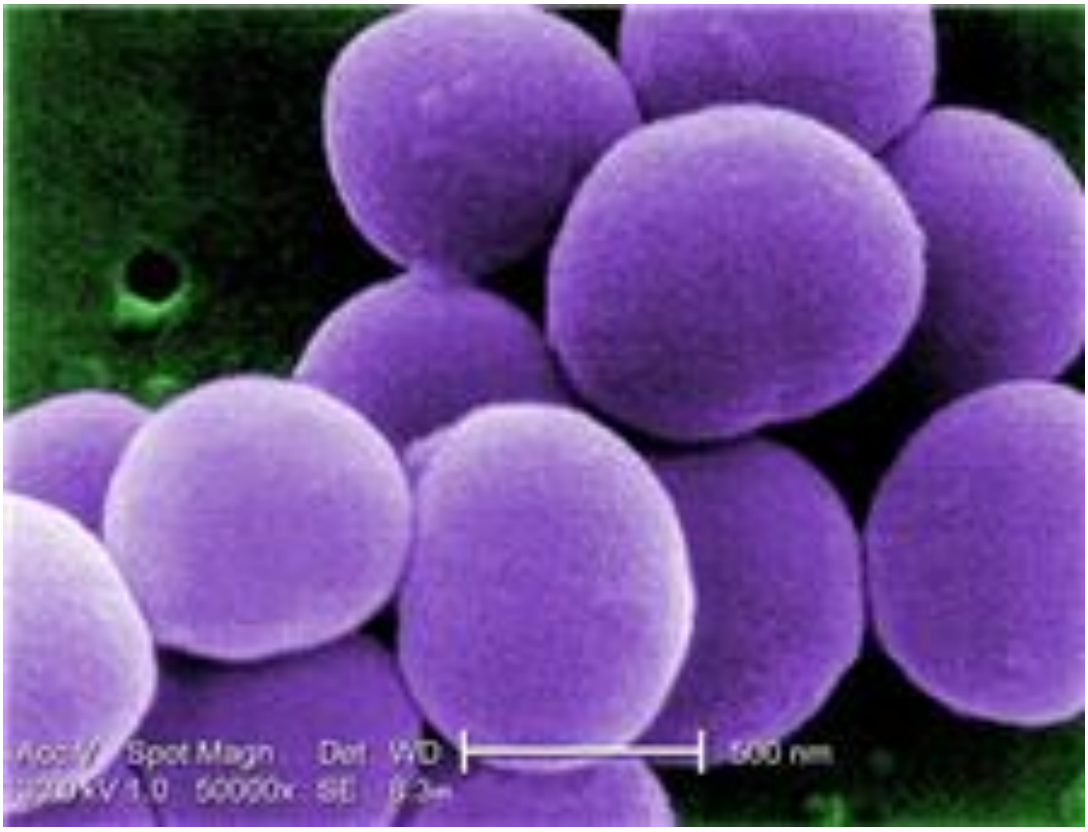

Figura 1*:* Representação colorizada de imagem de microscopia eletrônica de colônia de *Staphylococcus aureus* (Fonte: *Centers for Diseases Control and Prevention,* www.cdc.gov).

Tabela 1. Principais síndromes infecciosas causadas por *Staphylococcus aureus*.[27]

Origem	Síndromes
Adquiridas na comunidade	Toxi-infecções alimentares
	Infecções de pele e partes moles: foliculites, erisipelas, celulites
	Síndrome do choque tóxico
	Pneumonias
	Endocardites
	Osteomielites
	Abscessos de órgãos sólidos
Adquiridas em hospitais ou em serviços de saúde	Infecções de sítio cirúrgico
	Infecções da corrente sangüínea
	Pneumonias
	Infecções de pele e partes moles

Tanto nas infecções adquiridas na comunidade quanto naquelas adquiridas nos hospitais e serviços de saúde, o *S. aureus* pode determinar quadros graves, pondo em risco a vida das pessoas acometidas.

As infecções por *S. aureus* mantém-se como causa importante de morbidade e mortalidade.[19,27] Paradoxalmente, essa bactéria foi um dos primeiros alvos da terapia antimicrobiana – já na década de 1940.[28] A relevância continuada do *S.* aureus contraria o otimismo inicial da era antimicrobiana, quando diversos autores que previram o fim das doenças infecciosas com o

advento da antibioticoterapia.[28,29] O fator preocupante é que o *S. aureus* tem mostrado ao longo das décadas tendência consistente de desenvolver resistência aos agentes antimicrobianos usualmente empregados na prática clínica, sendo que ao final da década de 1940 surgiram as primeiras cepas resistentes.[29]

1.3. Resistência à meticilina.

Pouco depois da introdução da penicilina na prática clínica, ainda na década de 1940, foram observados os primeiros isolados de *S. aureus* com resistência a esse antimicrobiano.[29] Estes estavam inicialmente restritos ao ambiente hospitalar. O mecanismo responsável por essa resistência era a produção de uma enzima da classe das beta-lactamases, inicialmente denominada penicilinase. Para tratar isolados de *S. aureus* resistente à penicilina (*Penicillin-resistant S. aureus*, PRSA), foram desenvolvidas as penicilinas penicilinase-resistentes (como a meticilina e a oxacilina) e as cefalosporinas de primeira geração.

Em 1961 foi identificado na Inglaterra o primeiro isolado de *S. aureus* resistente à meticilina (*Methicillin-resistant S. aureus,* MRSA). Essa resistência era mediada pela alteração do sítio ativo da penicilina na célula bacteriana. Este sítio é uma enzima responsável pela integridade da parede bacteriana, e foi denominado Proteína Ligadora da Penicilina (*Penicillin-bindingProtein*, PBP). A PBP alterada por mutação (PBP2a) não tem afinidade por antimicrobianos da

classe dos beta-lactâmicos. Em consequência, o isolado se torna resistente a todas as penicilinas, cefalosporinas, monobactams e carbapenêmicos.[30]

Nas décadas seguintes, o MRSA tornou-se predominante entre as cepas de *S. aureus* de pacientes hospitalizados. Ao mesmo tempo, isolados comunitários perderam quase completamente a suscetibilidade à penicilina, mantendo-se em sua maioria meticilina-sensíveis (*Methicillin-susceptible S. aureus,* MSSA).[31]

Após 1990 foram relatadas de forma crescente infecções por MRSA em pessoas sem antecedente de contato com serviços de saúde. Esse fenômeno foi epidemiologicamente relacionado a alguns grupos, como aborígenes australianos, atletas, militares, presidiários e homens que fazem sexo com homens.[32] No entanto, observou-se ocorrência crescente de doença invasiva grave por MRSA de origem comunitária mesmo em pessoas que não pertenciam a esses grupos. Esse quadro renovou o interesse em estudos voltados à elucidação da epidemiologia molecular do *S. aureus* e dos mecanismos relacionados à resistência à meticilina.

O gene codificador da resistência à meticilina (*mecA*) está localizado em uma "ilha genômica" de resistência, conhecida como cassete cromossômico estafilocócico *mec* (*Staphylococicalchromosome cassete mec*, SCC*mec*). Pelo menos onze tipos de SCCmec foram descritos até o momento. A hipótese mais aceita atualmente é de que esses cassetes foram adquiridos em diferentes ocasiões a partir de espécies de estafilococos coagulase-negativa, gerando linhagens não relacionadas de MRSA[33]. SCC*mec* dos tipos I, II e III são encontrados em isolados de MRSA circulantes em hospitais e outros serviços de saúde (*Hospital-associated*[HA]-MRSA). Esses cassetes são maiores e albergam diversos determinantes de resistência. Por essa razão, isolados hospitalares de

MRSA costumam apresentar resistência simultânea a várias classes de antimicrobianos. Por outro lado, SCC*mec* IV, V, VI são encontrados isolados de MRSA de origem comunitária (*Community-associated*[CA]-MRSA). Estes cassetes cromossômicos têm tamanho menor e carreiam menos genes de resistência.[34]Essa é uma das razões pelas quais os isolados de CA-MRSA mantém suscetibilidade a diversas classes de antimicrobianos (sulfas, clindamicina, quinolonas), enquanto os HA-MRSA são via de regra multirresistentes.[35]

O contexto atual da resistência de *S. aureus* inclui a emergência de isolados intermediariamente sensíveis ou resistentes aos glicopeptídeos. Essa é uma classe tradicionalmente utilizada para terapia de MRSA em hospitais, e a emergência de resistência a ela reduz de forma ainda mais drástica as opções terapêuticas.[36]

1.3. CA-MRSA na população geral

Casos de infecção por CA-MRSA são frequentemente graves. Entre os fatores associados a essa gravidade está a presença frequente de um fator de virulência, denominado Leucocidina de Panton Valentine (*PantonValentineLeukocidin,* PVL). A PVL é uma toxina capaz de induzir a destruição de leucócitos humanos e causar grande dano tecidual, tem sido descrita em casos de fasciíte e pneumonias necrotizantes oriundos da comunidade.[32]

A crescente incidência de quadros graves e de isolados multirresistentes de *S. aureus* motivou uma série de estudos que tinham por objetivo quantificar a carga (*burden*) da colonização e infecção estafilocóccicas na população.

Uma dessas iniciativas, capitaneada pelo Centro para Prevenção e Controle de Doenças (Centers for Disease Control and Prevention, CDC), procurou descrever a incidência e distribuição de doenças invasivas por MRSA nos Estados Unidos.[38] Em nove cidades norte-americanas foi realizada vigilância ativa dessas infecções durante os anos de 2004 e 2005. Foram identificadas 8.987 infecções. Destas, 85,0% haviam sido adquiridas em hospitais e outros serviços de saúde, enquanto 13,7% eram de origem comunitária.

Outra pesquisa recente utilizou dados secundários do sistema de saúde britânico para estimar o comportamento epidemiológico das infecções estafilocócicas durante as décadas de 1990 e 2000.[39] Os autores apresentaram achados alarmantes: um aumento de cinco vezes nas hospitalizações por sepse, pneumonia estafilocócica, síndrome da pele escaldada e impetigo; aumento de três vezes das internações por abscessos e celulites, e de 150% nas admissões por infecções de ossos e articulações.

Outros estudos procuraram quantificar o carreamento de *S. aureus* na população. Nos anos de 2001 e 2002, incluiu-se no estudo NHANES (National Health and Nutrition Examination Survey) a coleta de amostras de nasofaringe. O estudo NHANES é realizado em âmbito nacional e corresponde a uma amostra representativa da população civil não institucionalizada dos Estados Unidos. A triagem para carreamento nasal de S. aureus foi realizada em todos os indivíduos com idade acima de um ano participantes do estudo. Os resultados foram descritos e analisados em três publicações.[40,41,42] Em 9.622 participantes, a

prevalência de colonização por S. aureus foi de 32,4%. Colonização por MRSA estava presente em 0,8%. Fatores significativamente associados ao carreamento de S. aureus foram: idade menor que 6 anos, adultos que não haviam completado o ensino médio, e diagnóstico de asma. O carreamento de MRSA estava associado a idade maior que 65 anos, sexo feminino, diabetes e estadia recente em casas de repouso (mas não em hospitais). Um acompanhamento desse estudo em anos posteriores demonstrou que a prevalência do carreamento de S. aureus sofreu redução estatisticamente significante, caindo para 28,6% em 2003-2004. Nesse mesmo período, porém, taxas de carreamento de MRSA quase duplicaram (0,8% para 1,5%, $p<0.05$).[43]

Os dados do NHANES são bastante abrangentes, embora seu significado seja limitado pelo longo período decorrido desde o estudo. Outras publicações apresentam estimativas populacionais de carreamento de S. aureus ou MRSA em espaços mais restritos. Furuya et al investigaram o carreamento nasal de MRSA em uma amostra de 739 habitantes de Manhattan, identificando prevalência de 0,3%.[44] Outro estudo, realizado em Birmingham (Inglaterra), investigou 274 pacientes, identificando taxas de carreamento de 33,0% para S. aureus e 1,5% para MRSA.[45] Em Taiwan, Lu et al realizaram triagem para carreamento nasal de MRSA em 1.838 pessoas da comunidade e 393 indivíduos com contato com serviços de saúde.[45] No primeiro grupo, a prevalência de colonização foi de 3,6%. No segundo, os resultados foram: 5,0% (profissionais da saúde), 5,8% (pacientes em enfermaria de agudos), 5,9% (pacientes de hemodiálise) e 11,0% (indivíduos em instituições de longa permanência).

Em estudo realizado pelo nosso grupo foram testados 686 moradores de área urbana de Botucatu-SP-Brasil (122.000 habitantes) para colonização nasal

por *S. aureus* e MRSA. Foram identificadas prevalências de 32,7% e 0,9%, respectivamente.[46]

Ainda é incerto o significado desses dados para abordagens terapêuticas em pacientes oriundos da comunidade. Esse dilema é ainda mais intenso quando falamos de populações especialmente suscetíveis, como as pessoas vivendo com HIV/Aids (PVHA).

1.4. MRSA e HIV/Aids

HIV/aids representa um dos mais importantes problemas de saúde no mundo, estimando-se que 2 milhões de indivíduos se infectam anualmente (OMS). Ainda de acordo com a Organização Mundial de Saúde (OMS) havia aproximadamente 36,9 milhões de pessoas em todo o mundo vivendo com HIV/aids (PVHA) até o final de 2014. Desse total 2,6 milhões eram crianças. No Brasil, de acordo com o boletim epidemiológico HIV/aids 2015, foram registrados desde o início da epidemia de aids até junho de 2015 798.366 casos de aids.

Infecções estafilocócicas são especialmente frequentes em PVHA[47], inclusive com altas taxas de recorrências. Diversos estudos demonstram que esta população apresenta também elevada prevalência de colonização nasal persistente por *S. aureus*[48], sendo que pessoas saudáveis podem ser carreadoras e funcionarem como reservatórios dentro da comunidade.[25]

Segundo Crum-Cianflone et al, mesmo na era HAART, PVHA continuam a ter alta incidência de colonização por *S.aureus* não relacionada a imunossupressão, mas sim a comportamentos específicos como o uso de drogas ilícitas.[49]

Além da frequente colonização e tendência ao desenvolvimento de doença invasiva, as PVHA apresentam diversos fatores associados a maior risco de carreamento de MRSA,[50] entre eles estão: grande exposição ao ambiente e a procedimentos intrahospitalares ou em outros serviços de saúde[31] e uso frequente de antimicrobianos. Outros preditores de carreamento de MRSA em PVHA têm sido descritos, como baixa contagem de células CD4,[51] uso de drogas ilícitas[52,53], comportamento sexual de alto risco[54] e encarceramento prévio.[55,56] .Entretanto, em relação a baixa contagem de CD4+, alguns estudos não mostraram associação entre esta e risco aumentado para CA-MRSA.[49] Em que medida a imunossupressão, avaliada pela contagem absoluta de CD4+, contribui para o risco de CA-MRSA é um aspecto que não está ainda bem estabelecido.[57]

Há ainda outras lacunas no conhecimento da epidemiologia de CA-MRSA em PVHA, sendo poucos os dados de revisão de literatura sobre a colonização e infecção por MRSA em PVHA na era HAART (highly active antiretroviral therapy).[58] Estudos mostram que as PVHA têm de 6 a 18 vezes maior risco de infecção por MRSA quando comparadas à população geral.[58,59] Em estudo realizado nos Estados Unidos da América, observou-se incidência de 12,3/1.000 pessoas ano comparado com 1-2/1.000 pessoas ano[60] respectivamente. Embora infecções, inclusive graves, por CA-MRSA tenham sido relatadas com frequência em PVHA, poucos autores abordaram o carreamento nasal de *S. aureus* e MRSA em indivíduos sem antecedentes de internação recente. Em estudo norte

americano recente, encontrou-se 8% de prevalência de colonização ou infecção em 900 PVHA.[60] O estudo incluiu pacientes ambulatoriais, mas não excluiu aqueles que relatavam internação no último ano, devendo-se destacar que em estudo de metanálise internação prévia, no último ano, mostrou-se significantemente associada a colonização por MRSA.[61] Padoveze et al,[62] estudando PVHA atendidos em Campinas - São Paulo e que não tinham antecedentes de internação, não encontraram colonização por MRSA. Zervou et al, em estudo de metanálise, publicado em 2014, relatam prevalência de colonização por MRSA em PVHA estimada em 6,9%, sendo a prevalência estimada em 8,8% quando analisada para a América do Norte separadamente.[61]

A localização da residência e a rede social comunitária podem contribuir para a manutenção de reservatórios de MRSA na comunidade[38,42] indicando a necessidade de avaliação da circulação de isolados MRSA em PVHA, especialmente no contexto da atenção à Aids no Brasil.

1.5. Considerações sobre a contribuição da epidemiologia espacial

No Brasil o uso do georreferenciamento tem história recente, porém tem sido de grande valia na área da saúde ao oferecer a visão da distribuição espacial de fatores de risco ambientais e, principalmente, facilitando o trabalho de associá-los aos determinantes sociais de saúde locais. Tem sido utilizado no monitoramento, planejamento e na avaliação das ações de saúde. É recurso muito importante na análise dinâmica de difusão espacial das doenças e suas relações com o ambiente, propiciando alta resolução gráfica para avaliação da saúde populacional e também na identificação de regiões e grupos com risco potencial de adoecer.[63,64,65]

Considerando a relevância de *S. aureus* e MRSA e seu impacto sobre a saúde em PVHA e as lacunas ainda existentes no conhecimento da epidemiologia de MRSA em população de PVHA no Brasil, estudos voltados a esse tema são prementes. Nosso estudo pretendeu abranger esse conjunto temático, incluindo alguns aspectos específicos como, determinação da prevalência de colonização de *S. aureus* e MRSA em familiares residentes no domicílio das PVHA, incluindo georreferenciamento.

O estudo portanto reflete a tentativa de conhecer o comportamento de *S. aureus* em uma população de risco especial e em seu núcleo familiar, com a finalidade de contribuir para a discussão sobre medidas e esquemas terapêuticos que poderão ser adotadas nesses casos.

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3. OBJETIVOS

3.1. Objetivos gerais:

- Identificar prevalência e preditores de colonização nasal e orofaríngea por *Staphylococcus aureus* sensível e/ou resistente à meticilina em pessoas vivendo com HIV/aids (PVHA) atendidas no Serviço de Ambulatórios Especializados de Infectologia "Domingos Alves Meira" (SAEi/DAM) próprio da Fundação para o Desenvolvimento Médico e Hospitalar (FAMESP).
- Determinar a prevalência de colonização nasal e orofaríngea por *Staphylococcus aureus* sensível e/ou resistente à meticilina dos familiares residentes no mesmo domicílio das pessoas vivendo com HIV/aids (PVHA) atendidas no Serviço.
- Estudar a distribuição espacial de PVHA colonizados ou não por *S. aureus* na área urbana do município de Botucatu (SP).

3.2. Objetivos específicos:

- Caracterizar a resistência à meticilina em isolados colonizantes nasais e orofaríngeos de PVHA e de seus contactantes domiciliares, por meio de testes fenotípicos e genotípicos.
- Identificar fatores de risco associados à colonização por *S. aureus* e MRSA em PVHA.
- Identificar padrões de colonização transitória e persistente na população do estudo.
- Caracterizar a aquisição de MRSA quanto ao local de aquisição: comunidade ou serviços de saúde.
- Caracterizar cadeias de transmissão de MRSA entre PVHA e seus contatos domiciliares.
- Caracterizar o cassete cromossômico SCC*mec* presente em isolados de MRSA do estudo.
- Identificar agrupamentos espaciais de sujeitos da pesquisa (PVHA) residentes em Botucatu, em especial aqueles colonizados por *S. aureus* ou MRSA.

4. ARTIGO

Conforme normas do Programa de Pós-Graduação em Doenças Tropicais da FMB-UNESP, os resultados serão apresentados em artigo.

O presente artigo foi escrito segundo as normas do periódico PLoS ONE.

Title Page

Title: Carriage of Methicillin-resistant *Staphylococcus aureus* among people living with HIV-AIDS in inner São Paulo State, Brazil: molecular and spatial epidemiology.

Authors:

Letícia Chamma Lastoria, MD. Department of Tropical Diseases, Faculdade de Medicina de Botucatu, UNESP – Univ Estadual Paulista. City of Botucatu, Brazil.

Maria de Lourdes Ribeiro de Souza da Cunha, PhD. Department of Microbiology and Immunology, Instituto de Biociências de Botucatu, UNESP – Univ Estadual Paulista. City of Botucatu, Brazil.

Camila Sena Martins de Souza, MSc. Department of Microbiology and Immunology, Instituto de Biociências de Botucatu, UNESP – Univ Estadual Paulista. City of Botucatu, Brazil.

Lenice do Rosário de Souza, MD, PhD. Department of Tropical Diseases, Faculdade de Medicina de Botucatu, UNESP – Univ Estadual Paulista. City of Botucatu, Brazil.

Cassiano Victória, PhD. Department of Veterinary Hygiene, Faculdade de Medicina Veterinária e Zootecnia, UNESP – Univ Estadual Paulista. City of Botucatu, Brazil.

Carlos Magno Castelo Branco Fortaleza, MD, PhD. Department of Tropical Diseases, Faculdade de Medicina de Botucatu, UNESP – Univ Estadual Paulista. City of Botucatu, Brazil.

Corresponding author:
Carlos Magno Castelo Branco Fortaleza
Departamento de Doenças Tropicais
Faculdade de Medicina de Botucatu
Distrito de Rubião Junior, S/N
Botucatu, São Paulo State, Brazil
ZIP CODE: 18618-970
Phone: +55 14 3880 1284
Email: cmfortaleza@uol.com.br

Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is increasingly recognized as a threat for people living with HIV/AIDS (PLWHA). However, the magnitude of asymptomatic MRSA colonization in that group varies among different countries and geographic regions. We conducted a study that aimed at identifying the prevalence and risk factors for both overall *S. aureus* and MRSA colonization among PLWHA attending in small cities from inner São Paulo State, Brazil. MRSA isolates were characterized using Pulsed-Field Gel Electrophoresis (PFGE), and submitted to typing of the Staphylococcal Chromosome Cassete (SCC)*mec*. Spatial analysis was performed to search for geographical clusters and correlation with socioeconomic indicators. In a first point prevalence survey, nasal and oropharyngeal swabs of 368 people were collected. Sixty-seven subjects from the main city (Botucatu) were surveyed for colonization in two other occasions, and had swabs collected from household members. The prevalence rates for *S. aureus* and MRSA in the first survey were 25.8% and 2.7%. The overall *S. aureus* colonization was negatively associated with the use of beta-lactams and of illicit drugs. On the other hand, MRSA colonized subjects were more likely to use crack and to have been admitted to a hospital during the past year. Repeated surveys found additional cases of MRSA colonization, but all subjects were positive in only one occasion. Four PFGE clusters were characterized, grouping subjects in household, city and region level. Of 19 total MRSA isolates, only one did not harbor SCC*mec* type IV. Spatial analysis detected hot spots of *S. aureus* colonized subjects from Botucatu, but that finding could not be linked to socio-economic indicators. In conclusion, we found small but relevant prevalence of MRSA among PLWHA. Community and healthcare-associated risk factors were identified, so that predominant routes of transmission could not be determined on epidemiological grounds.

Introduction

The impact of colonization and infection with Methicillin-resistant *Staphylococcus aureus* (MRSA) among people living with HIV/AIDS (PLWHA) has been extensively studied in the late 1990s and early 2000s [1], [2]. However, the changing epidemiology of both HIV and MRSA in the past decades produced a shift in major concerns. Early studies focused on the risks of acquiring multidrug-resistant organisms during hospital admission [3], [4]. But since then the advent of highly active antiretroviral therapy (HAART) made hospital admissions less common for PLWHA in many countries [5]. On the other hand, the emergence of community-associated (CA)-MRSA – often much virulent – posed a new threat for that group [6].

In Brazil, the governmental AIDS program provides universal access to diagnosis, therapy and routine laboratory tests (including CD4 lymphocytes count, measures of viral load and genotyping of viral resistance) [7]. This approach reduced mortality and slowed the growth of the epidemics [8], [9]. On the other hand, it failed to interrupt a relevant epidemiological trend, the interiorization. Ever since the late 1990s, the proportion of PLWHA who live in small cities - often far from great urban centers – is continuously increasing [10]. In response to this picture, there was a major increase in the number of public clinics providing care to PLWHA., That number rose from 33 to 540 in 2002 and 663 in 2007[11], [12].

On a separate – but related – topic, the global emergence of CA-MRSA still puzzles experts and public health authorities [13]. There are reports of relevant incidence of CA-MRSA infection (or prevalence of asymptomatic colonization)

involving populations as diverse as USA residents [14], [15], Maltese people [16], Australian indigenous communities [17] and Pygmies in Gabon [18]. In this setting, some questions arise, concerning the burden of colonization, patterns of transmission, the risk of invasive infection and the impact on vulnerable populations – including PLWHA.

Recent studies emphasize the intersection of HIV and CA-MRSA pandemics [19], [20], [21] Prevalence of colonization in this group is variable – with studies reporting rates ranging from similar to those reported for the general population [22] up to more than 16% [23]

In previous studies, we documented CA-MRSA infection and colonization in small cities from inner São Paulo State, Brazil [24], [25]. This pattern of "interiorization" has similarities with that described for AIDS, so that both epidemics intermingle in a new setting.

Our study was designed to address this intersection. We aimed to identify the prevalence of overall *Staphylococcus aureus* and MRSA colonization among PLWHA attending a reference outpatient clinic in inner São Paulo State, Brazil. We were particularly interested in identifying predictors for colonization, as well as clonal pattern of isolates and the spatial distribution of urban cases.

Materials and methods

Ethical issues

This study was conducted according to the principles expressed in the Declaration of Helsinki. It was approved by the reference Committee for Ethics in

Research ("Comitê de Ética em Pesquisa" from "Faculdade de Medicina de Botucatu". City of Botucatu, São Paulo State, Brazil). A written informed consent was obtained from all study subjects or their legal guardians.

Study setting and design

The study was conducted in the "Specialized Care Center for Infectious Diseases" (SCCID) from Faculdade de Medicina de Botucatu (Botucatu Medical Faculty). It is located in Botucatu city (130,000 inhabitants; 22º 53' 09" S, 48º 26' 42" W), and is the single referral center for surrounding municipalities – and area comprising 500,000 people. Presently, the SCCID cares for over 500 PLWHA.

The study had a cross-sectional design, with serial prevalence surveys. We included all PLWHA aged 15 years or more who were attending regularly the SCCID and agreed to participate the study. There were no specific exclusion criteria. However, due to ethical legislation constraints, we could not include prisoners in our study.

Point prevalence survey

In the first phase of the study, a point prevalence survey was carried out, including subjects cared for in the SCCID. All those subjects had nasal and oropharyngeal (throat) swabs collected, on the day they attended the clinic to perform routine laboratory tests (CD4+ lymphocyte counts and measure of viral load).

831

832 **Serial surveys**

833

834 All subjects who lived in the city of Botucatu were invited to participate the
835 further phases of the study. Briefly, a home visit was scheduled for days after
836 inclusion in the study. In this visit, we performed: (a) collection of second-time
837 samples of nasal and oropharyngeal swab; (b) collection of swabs from the same
838 sites in all household communicants who were not infected by HIV; and (c)
839 georreferencing of households. The third survey was performed in the patients
840 scheduled return for routine medical appointments (in average, 3 months after
841 the first survey).

842

843 **Microbiology and molecular methods**

844

845 Specimens were transported in Stuart medium and cultured in Baird Parker
846 agar. Species identification was performed through phenotypic and genotypic
847 techniques, as previously described [25].
848 Susceptibility tests followed guidelines from the Clinical Laboratory Standards
849 Institute (CLSI), using disks for oxacillin and cefoxitin [26]. However, our
850 definition of methicillin-resistance for practical purposes was based on the
851 detection of the *mec*A gene by Polymerase Chain Reactions (PCR), as described
852 by Murakami et al [27]. The multiplex-PCR protocol described by Milheiriço et al
853 [28] was used for the characterization of the staphylococcal cassette
854 chromosome *mec* (SCC*mec*).

Molecular strain typing of MRSA isolates was performed with Pulsed-Field Gel Electrophoresis (PFGE). We applied a protocol of DNA digestion with the enzyme *sma*I, modified from McDoughal et al. [29]. The analysis of similarity was performed using the Dice coefficient. Clusters were defined on the basis of similarity values over 80%. Dendrograms were drawn based on Unweighted Pair Group Method Using Arithmetic Averages (UPGMA) in the BioNumerics 6.1 software (Applied Maths, Belgium). International MRSA clones and isolates from a general population-based survey from the city of Botucatu [25] were included as controls in the dendrogram.

Epidemiological analysis

Data were collected by application of a questionnaire in the moment of inclusion to the study and complemented with the review of medical charts and laboratory files. The issues assessed included: (a) demographics and socio-economic data; (b) behavioral factors (e.g., sexual orientation, practice of sports, smoking, alcoholism, use of illicit drugs); (c) comorbidities and HIV related disorders (e.g., opportunistic infections); (d) time since HIV diagnosis, CD4 lymphocyte counts, viral load, use of antiretrovirals; (e) recent hospital admission, surgical procedures; (f) recent bacterial infections and use of antimicrobials.

Data were analyzed in EPI INFO 7 (Centers for Disease Control and Prevention, Atlanta, GA, USA) and SPSS 20.0 (IBM, Armonk, NY, USA). Descriptive statiscial methods were applied for overall data. For results of the first survey (including subjects from all cities in the region), we performed analysis of factors predictive both for overall *S. aureus* and MRSA colonization.

The first analytical step involved bivariate analysis. Dichotomous variables were analyzed using the Chi-square or Fisher Exact test. For continuous variables, we used the Mann-Whitney U test. Multivariable analysis was performed using logistic regression methods. A forward selection process was applied to include variables in the models [30]. The thresholds for inclusion and removal of variables in the models were $P<0.05$ and $P>0.1$, respectively. The Hosmer & Lemeshow test was applied to analyze goodness-of-fit in the multivariable models [31]. Whenever colinearity of two or more variables was detected, one of them was selected on the basis of the goodness-of-fit of the resulting model.

Spatial epidemiology

The household georreferencing was performed for subjects living in urban area of the city of Botucatu, using a high precision handheld Global Positioning System (GPS) device, Montana 650 (Garmin, Olathe, KS, USA). Data were subsequently transferred to a Geographic Information System (GIS) in the software ArcGIS 10 (ESRI, Redlands, CA, USA) and superimposed to the map of Botucatu municipality, provided by the city health department.

The steps of database modeling and map plotting were perfomed in the Department of Veterinary Hygiene from the "Faculdade de Medicina Veterinária e Zootecnia", "Universidade Estadual Paulista" – Campus of Botucatu.

The inicial descriptive phase involved plotting subjects (overall PLWHA, *S. aureus* positive) and applying the Kernel density estimation for each group [32]. Moran's I was calculated to estimate the correlation of subjects coordinates with georreferenced information of families' income and average people living in

905 households [32]. All the maps were generated in the coordinate system SIRGAS
906 2000 UTM Zone 22, with the Transverse Mercator projection and datum
907 Planimetric SIRGAS 2000.
908
909 **Results**
910
911 *Results of the first survey*
912
913 A total of 368 subjects were included – 112 of whom lived in the city of Botucatu.
914 The proportion of patients included (among all those cared for in SCCID) was
915 74.5% (80.5% for those living in Botucatu).
916 The total prevalence of *S. aureus* colonization in the first (whole sample) survey
917 was 25.8% (95% Conficence interval [CI], 21.5%-30.7%). Rates of nasal and
918 oropharyngeal carriage were 20.9% and 3.5%, respectively. Ten subjects were
919 colonized with MRSA (8 in the nares, 2 in the throat) – a prevalence rate of 2.7%
920 (95%CI, 1.4%-5.1%).
921 **Tables 1** and **2** present results of models for predictors of carriage of *S. aureus*
922 and MRSA, respectively. Briefly, overall colonization with *S. aureus* was
923 negatively associated with the recent use of beta-lactams (Odds Ratio[OR], 0.19;
924 95%CI, 0.30-0.98, *P*=0.04) and - surprisingly - with current use of illicit drugs
925 (OR, 0.19; 95%CI, 0.06-0.62; *P*=0.006). In the analysis of MRSA we adopted two
926 strategies. Given the extensive colinearity and the small number of MRSA
927 subjects, we avoided including both variables in the same model. Therefore, in
928 the model not including "hospital admission", neurocryptococosis (OR, 13.37;
929 95%CI, 2.20-81.21; *P*=0.005) and a history of use of crack (OR, 8.26; 95%CI, 1.88-

930 37.52, *P*=0.006). In the alternative model, excluding neurocryptoocosis, recent
931 hospital admission was the single predictor identified (OR, 4.04; 95%CI, 1.14-
932 14.34; *P*=0.04).
933
934 *Serial surveys and household contactants*
935
936 Only 67 subjects (59.8% of those living in Botucatu) could be followed in both
937 second and third surveys. The proportions of subjects colonized with overall *S.*
938 *aureus* in one, two or three surveys were 20.9%, 13.4% and 7.5% - and 41.8%
939 had at least one positive sample. A total of 7 subjects (10.4%) were positive for
940 MRSA in a single sample, and none among them had this agent recovered more
941 than once.
942 At the time of the second survey, we tested 76 household contactants of research
943 subjects for nasal or oropharyngeal colonization. The prevalence of *S. aureus* in
944 this group was 30.6% (23.6% for nasal colonization, 16.7% for oropharyngeal
945 colonization). We found MRSA in three people (3,9%), of whom 2 were colonized
946 in the nares and 2 in the throat. We found association between colonization in
947 the contactant and in the index subject for both overall *S. aureus* (OR, 2.95;
948 95%CI, 1.03-8.52; *P*=0.04) and MRSA (OR, 17.71; 95%CI, 1.42-221.16; *P*=0.03).

949
950 **Table 1.** Predictors of colonization with *Staphylococcus aureus* among people living with HIV/AIDS in inner São Paulo State, Brazil.
951

Predictors	*S. aureus* (95)	Other (273)	OR (95%CI)	*P*	OR (95%CI)	*P*
Demographic and socio-economic data						
Female gender	39 (4.1)	107 (39.9)	1.08 (0.62-1.74)	0.75		
Age, median years (quartiles)	43 (33-49)	43 (33-51)	...	0.89		
White	66 (69.5)	206 (75.5)	0.74 (0.44-1.24)	0.25		
Living in Botucatu	28 (29.5)	84 (30.8)	0.94 (0.56-1.57)	0.81		
Number of people in the household, median (range)	3 (0-9)	3 (1-10)	...	0.18		
Monthly income in US$, median (quartiles)	375 (250-550)	375 (250-625)	...	0.49		
Rural worker	9 (9.5)	15 (5.5)	1.80 (0.76-4.26)	0.18		
Healthcare worker	6 (6.3)	11 (4.0)	1.61 (0.58-4.47)	0.40		
Behavior data						
Men that has sex with men	18 (18.9)	64 (23.5)	0.76 (0.42-1.36)	0.36		
Tattoo	23 (24.2)	77 (28.2)	0.81 (0.48-1.39)	0.45		
Piercing	4 (4.2)	19 (7.0)	0.59 (0.19-1.77)	0.34		
Current use of illict drugs	**3 (3.2)**	**39 (14.3)**	**0.20 (0.06-0.65)**	**0.003**	**0.19 (0.06-0.62)**	**0.006**
Past or present use of marijuana	**10 (10.5)**	**55 (21.6)**	**0.43 (0.21-0.87)**	**0.02**		
Past or present use of crack	11 (11.6)	28 (10.3)	1.14 (0.55-2.40)	0.72		
Past or present use of inhalatory cocaine	13 (13.7)	41 (15.0)	0.90 (0.46-1.70)	0.75		
Past or present use of intravenous cocaine	2 (2.1)	8 (2.9)	0.71 (0.15-3.42)	1.00		
Smoking	33 (34.7)	103 (37.7)	0.88 (0.51-1.43)	0.60		
Alcoholism*	6 (6.3)	22 (8.1)	0.77 (0.30-1.96)	0.58		

Practice of sports	27 (28.4)	91 (33.3)	0.79 (0.48-1.33)	0.38
Collective sports	5 (5.3)	14 (5.1)	1.13 (0.36-2.93)	1.00
Comorbidities				
Neoplasia (solid tumor oh hematological)	3 (3.2)	17 (6.2)	0.49 (0.14-1.71)	0.31
Heart disease	4 (4.2)	9 (3.3)	1.30 (0.39-4.29)	0.75
Chronic lung disease	2 (2.1)	4 (1.5)	1.45 (0.26-8.03)	0.65
Renal disease	5 (5.3)	5 (1.8)	2.98 (0.84-10.52)	0.13
Liver disease (chronic hepatitis or other)	13 (13.7)	31 (11.4)	1.24 (0.62-2.48)	0.55
Diabetes mellitus	5 (5.3)	11 (4.0)	1.32 (0.45-3.91)	0.57
Disease of the CNS (except infections)	0 (0.0)	5 (1.8)	0.00 (undefined)	0.33
Systemic arterial hypertension	18 (18.9)	51 (18.7)	1.02 (0.56-1.85)	1.00
Dislipidemia	34 (35.8)	87 (31.9)	1.19 (0.73-1.95)	0.48
HIV/AIDS related data				
Years since diagnosis, median (quartiles)	6 (2-12)	7 (3-14)	...	0.16
Years of clinical follow-up, median (quartilhes)	4 (1-10)	5 (1-10)	...	0.38
CD4 lymphocite count, median (quartiles)	462 (274-709)	442 (265-676)	...	0.87
Lowest past CD4 count, median (quartiles)	247 (99-373)	193 (69-337)	...	0.20
Viral load bellow detection limit	62 (66.0)	180 (66.2)	0.96 (0.60-1.63)	0.97
Peak previous viral load (logarythm), media (quartiles)	4.48 (2.84-5.26)	4.47 (3.77-5.22)	...	0.57
Current use of HAART	86 (90.5)	257 (94.1)	0.60 (0.25-1.40)	0.23
Opportunistic disease**	43 (45.3)	107 (39.2)	1.28 (0.80-2.06)	0.30
Pnemocystis pneumonia**	14 (14.7)	39 (14.3)	1.04 (0.54-2.01)	0.91
Neurotoxoplasmosis**	6 (6.3)	35 (12.8)	0.46 (0.19-1.13)	0.08

Neurocryptococosis**	4 (4.2)	8 (2.9)	1.46 (0.43-4.95)	0.52		
Tuberculosis**	13 (13.7)	23 (8.4)	1.72 (0.54-3.56)	0.14		
Cryptosporidiasis**	4 (4.2)	9 (3.3)	1.29 (0.39-4.29)	0.75		
Zoster**	7 (7.4)	8 (2.9)	1.46 (0.43-4.95)	0.52		
Exposure to health care in the past year						
Hospital admission	17 (17.9)	99 (21.6)	0.79 (0.43-1.44)	0.47		
Surgical procedure	10 (10.5)	32 (11.7)	0.89 (0.42-1.88)	0.75		
Presumed bacterial pneumonia	10 (10.5)	29 (8.8)	1.22 (0.56-2.67)	0.62		
Skin infection	7 (7.4)	27 (9.9)	0.73 (0.31-1.72)	0.47		
Use of antimicrobial (except antivirals)	44 (46.3)	141 (51.6)	0.81 (0.51-1.29)	0.37		
Number of antimicrobials used, median (range)	0 (0-5)	1 (0-6)	...	0.28		
Use of beta-lactams	17 (17.9)	75 (27.5)	0.58 (0.32-1.04)	0.06	**0.55 (0.30-0.99)**	**0.04**
Use of quinolones	5 (5.3)	21 (7.7)	0.66 (0.24-1.82)	0.43		
Use of macrolides	4 (4.2)	12 (4.4)	0.90 (0.30-3.04)	1.00		
Use of other antimicrobials	32 (33.7)	86 (31.5)	1.10 (0.67-1.81)	0.70		
Current use of TMP/SMX	15 (15.8)	56 (20.5)	0.73 (0.39-1.58)	0.32		

Note. All data in number (%), except otherwise specified. Statistically significant results presented in boldface.

* Define as daily ingestion of alcoholic beverages. ** Recorded in medical charts, regardless of the time of occurrence.

OR, Odds Ratio. CI, Confidence interval. CNS, Central Nervous System. HAART, Highly active antiretroviral therapy. TMP/SMX, Trimethoprim-Sulfamethoxazole.

957

958 **Table 2.** Predictors of colonization with Methicillin-resistant *Staphylococcus aureus* (MRSA) among people living with HIV/AIDS in inner
959 São Paulo State, Brazil.

960

Predictors	MRSA (10)	Other (358)	OR (95%CI)	*P*	OR (95%CI)	*P*
Demographic and socio-economic data						
Female gender	4	142 (39.7)	1.01 (0.28-3.66)	1.00		
Age, median years (quartiles)	41.5 (34-51)	43 (35-51)	...	0.88		
White	5	267 (74.6)	0.34 (0.10-1.20)	0.14		
Living in Botucatu	3	109 (30.4)	0.98 (0.25-3.86)	1.00		
Number of people in the household, median (range)	3 (1-10)	3 (0-9)	...	0.49		
Monthly income in US$, median (quartiles)	375 (195-375)	375 (250-625)	...	0.13		
Rural worker	1	23 (6.4)	1.62 (0.20-13.33)	0.50		
Healthcare worker	0	17 (4.7)	0.00 (undefined)	1.00		
Behavior data						
Men that has sex with men	1	81 (22.6)	0.38 (0.05-3.04)	0.47		
Tattoo	0	100 (27.9)	0.00 (undefined)	0.07		
Piercing	0	23 (6.4)	0.00 (undefined)	1.00		
Current use of illict drugs	0	42 (11.7)	0.00 (undefined)	0.61		
Past or present use of marijuana	1	68 (19.0)	0.47 (0.06-3.80)	0.69		
Past or present use of crack	3	36 (10.1)	3.83 (0.95-15.48)	0.07	**8.26 (1.88-37.52)[a]**	**0.006**
Past or present use of inhalatory cocaine	1	53 (14.8)	0.64 (0.08-5.15)	1.00		
Past or present use of intravenous cocaine	1	9 (2.5)	4.31 (0.49-37.71)	0.24		

Smoking	6	130 (36.3)	2.63 (0.73-9.49)	0.18
Alcoholism*	1	27 (7.5)	1.36 (0.17-11.16)	0.55
Practice of sports	1	117 (32.7)	0.23 (0.30-1.82)	0.18
Collective sports	0	19 (5.3)	0.00 (undefined)	1.00
Comorbidities				
Neoplasia (solid tumor oh hematological)	0	20 (5.6)	0.00 (undefined)	1.00
Heart disease	1	12 (3.4)	3.20 (0.37-27.35)	0.31
Chronic lung disease	0	6 (1.7)	0.00 (undefined)	1.00
Renal disease	1	9 (2.5)	4.31 (0.49-37.71)	0.24
Liver disease (chronic hepatitis or other)	1	43 (12.0)	0.81 (0.10-6.58)	1.00
Diabetes mellitus	1	15 (4.2)	2.54 (0.30-21.37)	0.36
Disease of the CNS (except infections)	0	5 (1.4)	0.00 (undefined)	1.00
Systemic arterial hypertension	1	68 (19.0)	0.47 (0.06-3.80)	0.70
Dislipidemia	2	119 (33.2)	0.50 (0.11-2.40)	0.51
HIV/AIDS related data				
Years since diagnosis, median (quartiles)	7 (1-13)	7 (3-14)	...	0.67
Years of clinical follow-up, median (quartiles)	3 (1-7)	6 (1-11)	...	0.27
CD4 lymphocite count, median (quartiles)	260 (195-618)	498 (308-612)	...	0.11
Lowest past CD4 count, median (quartiles)	170 (27-302)	220 (69-352)	...	0.36
Viral load bellow detection limit	6	236 (65.9)	0.78 (0.22-2.80)	0.74
Peak previous viral load (logarythm), media (quartiles)	3.43 (1-4.63)	4.18 (2.70-4.91)	...	0.19
Current use of HAART	8	335 (93.6)	0.28 (0.06-1.37)	0.14
Opportunistic disease**	5	145 (40.5)	1.47 (0.42-5.17)	0.54

Pnemocystis pneumonia**	1	52 (14.5)	0.65 (0.81-5.27)	1.00		
Neurotoxoplasmosis**	0	41 (11.5)	0.00 (undefined)	0.61		
Neurocryptococosis**	**2**	**10 (2.8)**	**8.70 (1.63-46.32)**	**0.04**	**13.37 (2.20-81.21)[a]**	**0.005**
Tuberculosis**	2	34 (9.5)	2.38 (0.49-11.67)	0.26		
Cryptosporidiasis**	0	12 (3.6)	0.00 (undefined)	1.00		
Zoster**	1	17 (4.7)	2.23 (0.27-18.62)	0.40		
Exposure to health care in the past year						
Hospital admission	**5**	**71 (19.8)**	**4.04 (1.14-14.34)**	**0.04**	**4.04 (1.14-14.34)[b]**	**0.04**
Surgical procedure	0	42 (11.7)	0.00 (undefined)	0.61		
Presumed bacterial pneumonia	0	34 (9.5)	0.00 (undefined)	0.61		
Skin infection	2	32 88.9)	2.55 (0.52-12.51)	0.23		
Use of antimicrobial (except antivirals)	5	182 (50.8)	0.981 (0.28-3.40)	1.00		
Number of antimicrobials used, median (range)	0.5 (0-3)	1 (0-6)	...	0.86		
Use of beta-lactams	3	89 (24.9)	1.30 (0.33-5.12)	0.72		
Use of quinolones	0	26 (7.3)	0.00 (undefined)	1.00		
Use of macrolides	0	16 (4.5)	0.00 (undefined)	1.00		
Use of other antimicrobials	3	115 (32.1)	0.91 (0.23-3.57)	1.00		
Current use of SMT/TMP	3	68 (19.0)	1.83 (0.46-7.25)	0.41		

Note. All data in number (%), except in the MRSA column (absolute data) or if otherwise specified. Statistically significant results presented in boldface.

* Define as daily ingestion of alcoholic beverages. ** Recorded in medical charts, regardless of the time of occurrence.

[a] Final model not including the variable "hospital admission". [b] Final model not including the variable "neurocryptococosis".

OR, Odds Ratio. CI, Confidence interval. CNS, Central Nervous System. HAART, Highly active antiretroviral therapy. TMP/SMX, Trimethoprim-Sulfamethoxazole.

Molecular epidemiology

Regardless of the history of exposure to healthcare, all but one of the 19 MRSA isolates from subjects and contactants harbored SCC*mec* type IV, and the remaining strain could not be typed with the Milheiriço multiplex PCR technique. PFGE typing three clusters (A, B, C) grouping isolates from more than one study subject and an additional cluster (D) grouping an isolate from this sample with one from the population-based survey conducted in 2011 in Botucatu [25]. The dendrogram is shown in **Figure 1,** and **Table 3** describes the clusters identified. Interestingly, the only isolate for which we could not assign a SCC*mec* type (345) did not belong to any cluster and was the least similar to other isolates from our study.

Figure in additional file

Figure 1. Dendrogram including 19 MRSA isolates from this study, alongside with six MRSA isolates from the Botucatu population-based survey (year 2011) and several international controls.

Note. Arrows and letters in red represent isolates from subjects living in Botucatu. * Isolates from the population-based survey. All isolates from the present study harbor SCC*mec* type IV, except number 345 (NT=not typable).

Table 3. Summary of clusters grouping study subjects

Cluster	Total PLWHA	PLWHA from Botucatu	Household contacts	*2011 survey*
A	3	1	2	*2*
B	3	1	1	*0*
C	3	2	0	*0*
D	0	0	1	*1*

It is worth noting that subject number 254 has one contactant colonized with MRSA from the same cluster (A). On the other hand, subject 139 had an isolate not belonging to any cluster. Curiously, his contactant was co-colonized with two MRSA strains that were not related to index case nor among themselves, and belonged to clusters B and D.

Spacial analysis

We georreferenced the households of study subjects (PLWHA) who lived in the urban area of Botucatu. For the purpose of this analysis, we took in account the positivity for *S. aureus* or MRSA in any culture in the serial surveys.

Figure 2 presents the point distribution of subjects, highlighting those who were positive for overall *S. aureus* or MRSA. The Kernel density is shown inf **Figure 3.**

Figure in additional file

Figure 2. Study subject addresses ploted over a map of the urban area o Botucatu, São Paulo State, Brazil.

(A) Subjects positive (red) for *S. aureus* in at least one of the surveys, plotted among subjects negative in all surveys (green). (B) Subjects positive (yellow) for *S. aureus* in at least one of the surveys, plotted among subjects negative in all surveys (blue).

Figure in additional file

Figure 3. Kernel density maps for special distribution of overall subjects (A) and those colonized with (B) *Staphylococcus aureus* as a whole or (C) MRSA.

Figure 4 presents subjects' addresses plotted over a map showing average values of family income and number of people living in dwellings. Results from regression models of spatial correlation for average number of people per dwellings were not significant for the whole sample (P=0.64) or for carriers of *S. aureus* (P=0.64). Similarly, we did not find special correlation with monthly income either for overall PLWHA (P=0.76) or *S. aureus* (P=0.30).

Figure in additional file

Figure 4. Study subjects plotted in maps with distribution of censitary socioeconomical data.

(A) and (B) present maps of average people in dwellings, with georreference of overall subjects (green circles) and of those colonized with *S. aureus* (red triangles). (C) and (D) present similar plots (overall subjects and *S. aureus* carrirers) over a map of average family income.

Discussion

Our findings can be interpreted in many ways. First, we documented colonization with MRSA among PLWHA from inner São Paulo State. The point prevalence rate (2.4%) is not particularly high when compared to the international literature – where prevalence of up to 16.8% are reported [23]. In fact, our rate is lower than the pooled prevalence of 6.9% reported in a recent meta-analysis [33]. On the other hand, a survey performed in the early 2000s including HIV-positive outpatients from inner São Paulo did not find any subject harboring MRSA [34]. Even though that study was conducted in another city, it is reasonable to infer that prevalence of MRSA colonization may be growing. It is also worth noting that, among our subjects who were studied in three surveys, the cumulative prevalence (i.e., MRSA positive in any of the surveys) was higher than 10%.

The emergence of CA-MRSA was a turning point both in the epidemiology and in the clinical relevance of staphylococcal infections [35]. However, authors have

recently reported a blurring of the definitions of “community-associated” and “healthcare-associated” infections [36]. This is especially the case for special groups. PLWHA are a heterogeneous population that includes both seemingly “healthy” persons (asymptomatic, most achieving viral control with proper therapy) and others with poor compliance to therapy and a history of several opportunistic infections. Often, this latter group presents variable amounts of social vulnerability, including poverty, alcoholism and addiction to illicit drugs [38]. In countries – such as Brazil – where the poorest people have access to public health, this group is more often admitted to acute care hospitals. Therefore the same population may be exposed to risk factors associated with CA-MRSA (illicit drugs, poor hygiene practices) and HA-MRSA (frequent admissions), making it difficult to ascertain the origin of isolates on epidemiological grounds [37], [38].

Our results are exemplary. Eighteen out of 19 isolates tested positive for SCC*mec* type IV, usually found in CA-MRSA. However, recent hospital admission was epidemiologically associated with MRSA carriage in one of the logistic regression models. An alternative model associated MRSA with previous neurocryptococosis (an infection that invariably requires hospital admission) and the use of crack. Neurocryptococosis may be a proxy that suggests an association of MRSA to admissions that took place more than a year before the survey (and therefore did not meet the “recent admission” criteria). On the other hand, the use of crack has become epidemic in Brazil, and is part of a common milieu that combines poverty, violence and sexually transmitted diseases [39].

It is worth noting that, despite the small number of subjects colonized with MRSA in the first survey, the factors associated with this outcome were more

meaningful than those associated with overall *S. aureus*. Indeed, subjects colonized with *S. aureus* were less likely to have received beta-lactams antimicrobials and less likely to use illicit drugs. This latter finding is puzzling, but it may reflect changes in microbial ecology of nares and throat that favor overgrowth of competing microorganisms. One should notice that the use of intravenous drugs – a reported risk factor for MRSA – is rare in Brazil, a pattern reflected in our sample. [40]

There is now sufficient evidence for international spread of specific MRSA clones [41]. However – and contrary to the case for HIV – the routes for this dissemination are far less clear [42]. Therefore, studies that approach networks of transmission – such as households and neighborhoods - are required [43], [44]. Miller et al [45] carried out a survey selecting subjects in a household level, and found that, in dwellings with more than one member colonized with *S. aureus*, 50% carried that same strains. Sexual transmission has been demonstrated [46], but it obviously does not explain all events of spread among household members. We addressed this issue by performing a survey among HIV-negative household contactants of study subjects. As presented above, a positive index subject was associated with greater risk of colonization of household members in analysis for both overall *S. aureus* and MRSA. However, only one patient had a household contact colonized with the same strain as his. We did not type methicillin-susceptible *S. aureus*, and therefore could not detect possible transmission of those strains among family members.

Interesing insights arise from comparison of this study with the population-based survey of nasal *S. aureus* colonization, conducted in Botucatu in 2011. In that study, we identified two instances of MRSA transmission among family

members [25]. Also, two subjects living in the same street harbored similar isolates. It is worth noting that 3 out of 6 isolates from that study grouped with strains from the present investigation. This finding suggests the long-term persistence of specific clones in the population. The fact that clusters grouped isolates from people living in Botucatu and in other neighboring cities points out either to regional spread or to cross-transmission during outpatients appointments.

The clinical significance of MRSA colonization among PLWHA is not completely clear. While for some authors colonization is a major risk factor for invasive infections [47], others believe this association does not apply to the dinamics of CA-MRSA [48]. Our study was not designed to address those issues, and we found no association between MRSA (or *S. aureus*) colonization and presumed bacterial infections. But, interestingly, in all subjects submitted to serial collections of swabs, MRSA was found in only one occasion. Furthers research is necessary in order to clarify if MRSA carriage among PLWHA in Brazil is generally transient. This issue – transient versus persistant *S. aureus* colonization in PLWHA – was addressed previously, but in that study no subject carried MRSA. [34] In that study, advanced HIV disease was associated with persistent colonization.

In order to assess both the distribution of PLWHA and of colonization, we georreferenced dwellings of subjects who lived in Botucatu. This allowed us to identify “hot spots” concentrating cases of HIV/Aids and colonized subjects. In a first attempt of interpretation, we thought that peripheral distribution of colonization could be related to socio-economic conditions. In Botucatu, as in many other cities in Brazil, poorer people live in the periphery of urban areas.

However, we failed to demonstrate special correlations with two important measures of social vulnerability – the average number of people in dwellings and the average family monthly income. Unfortunately, the number of MRSA-positive subjects was too low to warrant a specific analysis.

Our study has some limits, which regard the relatively small sample (especially in serial surveys), not collecting swabs from other body sites (e.g., groin) and the fact that typing of methicillin-susceptible strains was not performed. However, it also has strengths, including an effort to address extensively the subjects vulnerability and the combined use of classical, molecular and spatial epidemiologic methods.

In conclusion, we documented small but relevant prevalence of MRSA among PLWHA from small cities in inner São Paulo State, Brazil. Despite the small number of MRSA-colonized subjects, we found association of this carriage to previous hospital admission and use of crack. Findings from PFGE typing point out to spread in different levels – household, city, neighboring municipalities. While more research is needed to fully aknowledge the threat posed by MRSA to PLWHA, it is clear that any policy directed at preventing and/or controlling that agent must not be restricted to great urban centers.

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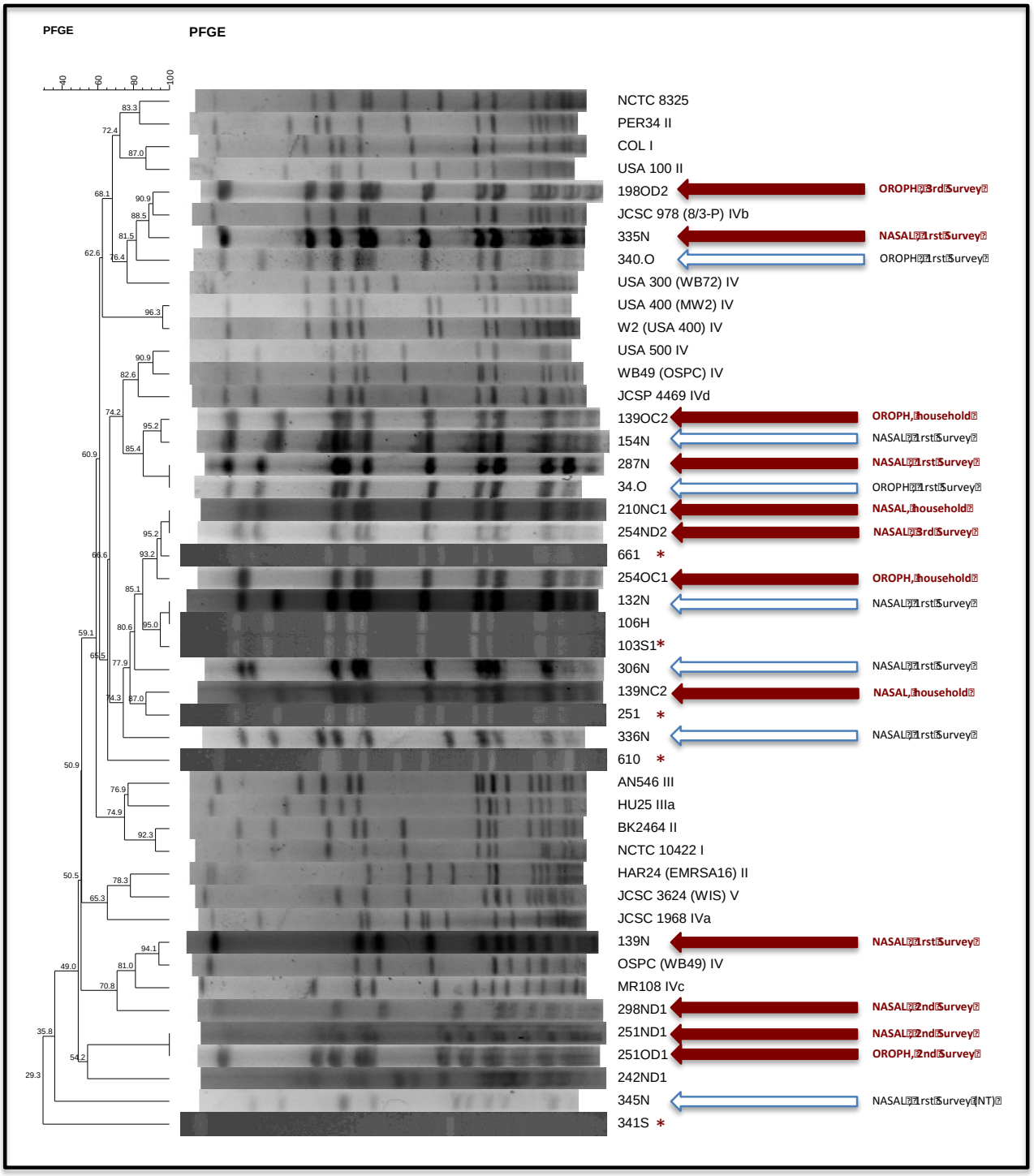

Figure 1. Dendrogram including 19 MRSA isolates from this study, alongside with six MRSA isolates from the Botucatu population-based survey (year 2011) and several international controls.

Note. Arrows and letters in red represent isolates from subjects living in Botucatu. * Isolates from the population-based survey. All isolates from the present study harbor SCC*mec* type IV, except number 345 (NT=not typable).

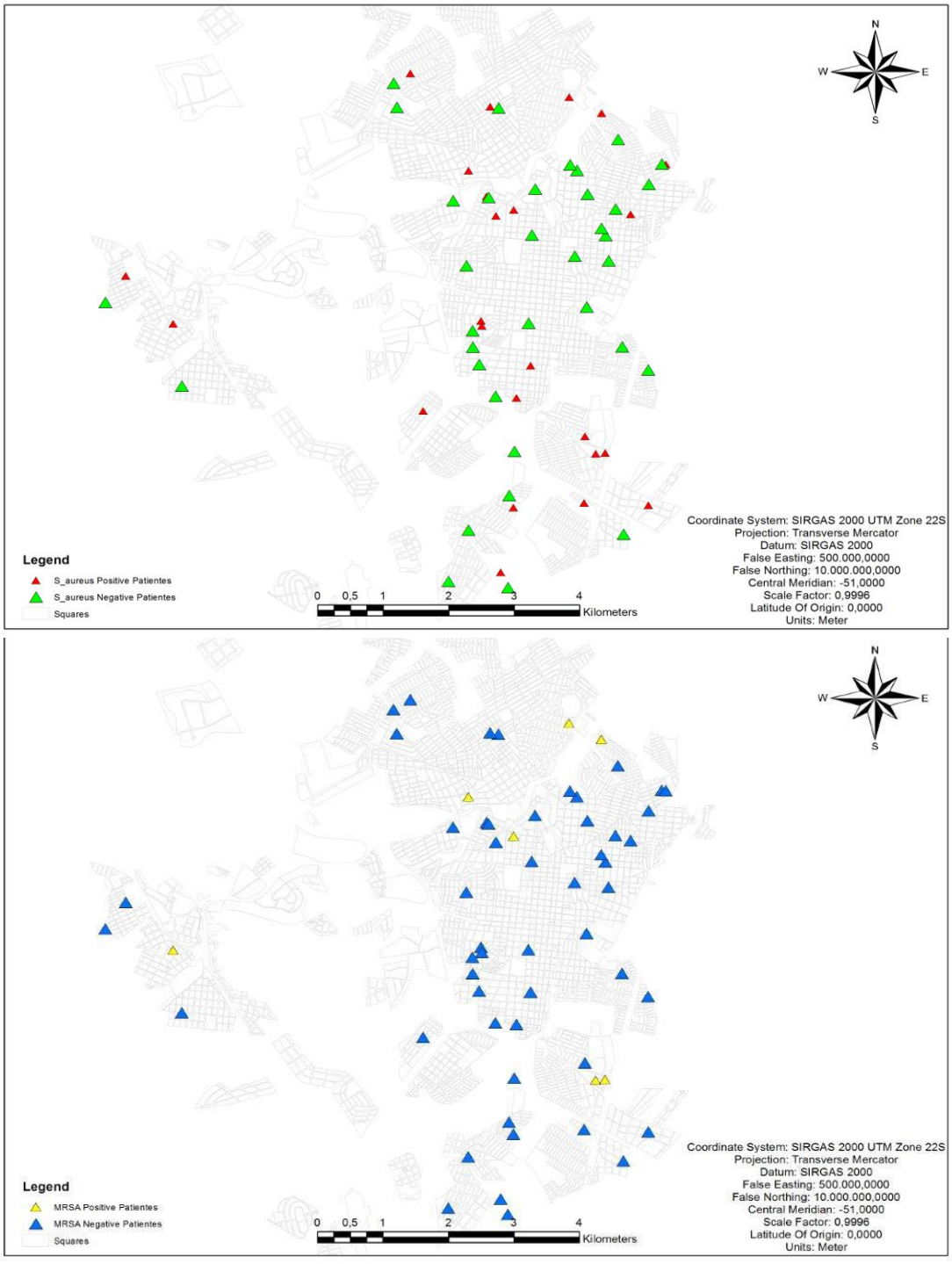

Figure 2. Study subject addresses ploted over a map of the urban area o Botucatu, São Paulo State, Brazil.

(A) Subjects positive (red) for *S. aureus* in at least one of the surveys, plotted among subjects negative in all surveys (green). (B) Subjects positive (yellow) for MRSA in at least one of the surveys, plotted among subjects negative in all surveys (blue).

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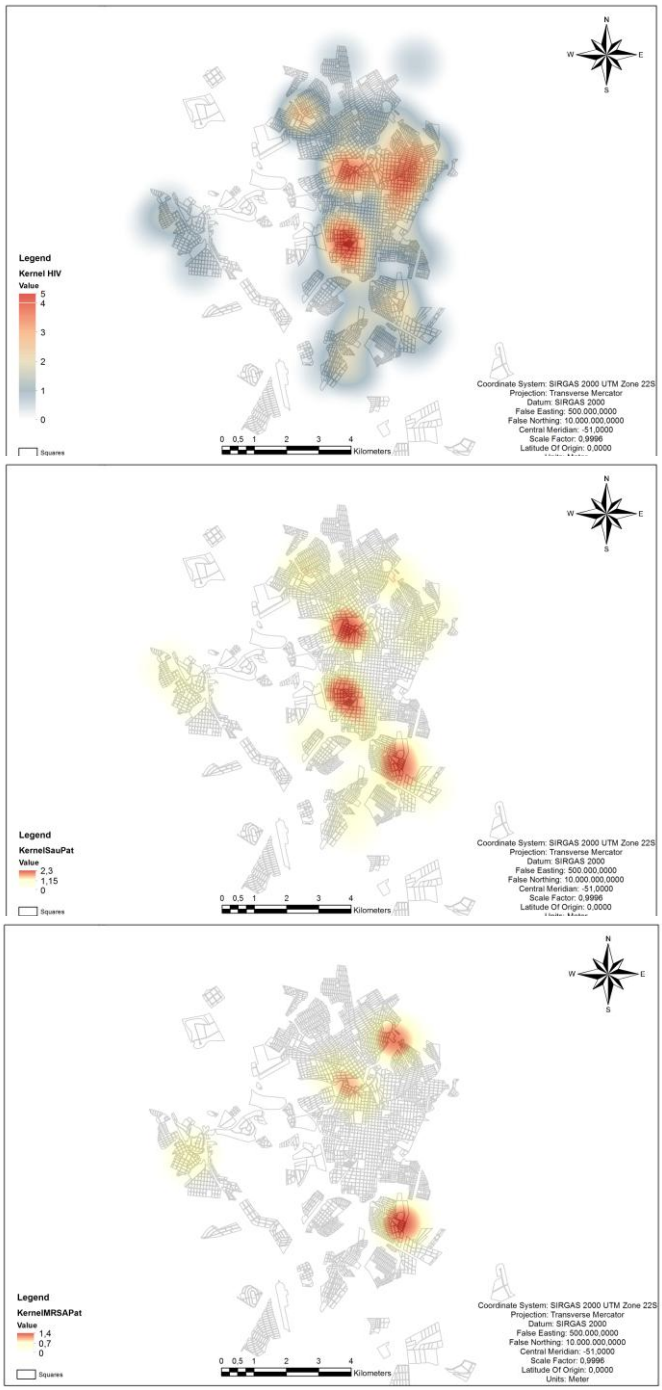

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1342 **Figure 3.** Kernel density maps for special distribution of overall subjects (A) and

1343 those colonized with (B) *Staphylococcus aureus* as a whole or (C) MRSA.

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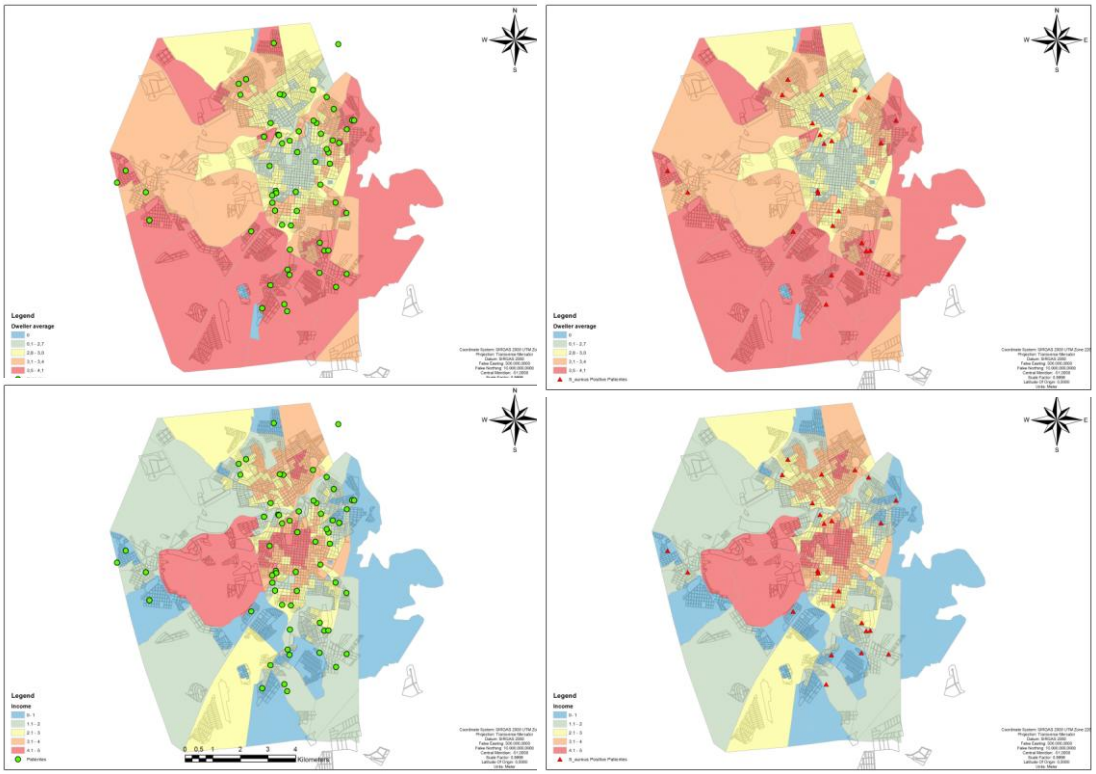

Figure 4. Study subjects plotted in maps with distribution of censitary socioeconomical data.

(A) and (B) present maps of average people in dwellings, with georreference of overall subjects (green circles) and of those colonized with *S. aureus* (red triangles). (C) and (D) present similar plots (overall subjects and *S. aureus* carrirers) over a map of average family income.

5. CONCLUSÃO

- As taxas de prevalência de *S. aureus* e MRSA no primeiro levantamento foram 25,8% e 2,7%. A colonização por *S. aureus* foi negativamente associada com o uso de antibióticos beta-lactâmicos e drogas ilícitas. Por outro lado, fatores de risco para MRSA incluíam uso de crack e internação hospitalar recente.
- Inquéritos repetidos identificaram novos casos de colonização por MRSA, mas nenhum sujeito apresentou positividade em mais de uma ocasião.
- Houve associação estatística entre a presença de *S. aureus* ou MRSA no paciente índice e colonização pelo mesmo agente em pessoas do seu domicílio.
- Quatro *clusters* foram identificados na PFGE, agrupando sujeitos em diferentes níveis – domicílio, cidade, região.
- Dos 19 isolados caracterizados, apenas um não carreava o SCC*mec* tipo IV.
- Análise espacial mostrou *hot spots* para sujeitos colonizados com *S. aureus*, mas não conseguimos ligar esse padrão a indicadores sócio-econômicos.
- Em nosso estudo, idenficamos baixa – mas relevante – prevalência de MRSA em PVHA. Foram encontrados tanto fatores de risco tradicionalmente associados a aquisição na comunidade quanto outros ligados a exposição a hospitais, de modo que as rotas predominantes de transmissão não puderam ser determinadas com base epidemiológica.