Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

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

Leticia Chamma Lastória
Universidade Estadual Paulista Julio de Mesquita Filho Faculdade de Medicina Campus de Botucatu
Maria de Lourdes Ribeiro de Souza da Cunha
Universidade Estadual Paulista Julio de Mesquita Filho Instituto de Biociencias Campus de Botucatu
Camila Sena Martins de Souza
Universidade Estadual Paulista Julio de Mesquita Filho Faculdade de Medicina Campus de Botucatu
Lenice do Rosário de Souza
Universidade Estadual Paulista Julio de Mesquita Filho Faculdade de Medicina Campus de Botucatu
Cassiano Victória
Universidade Estadual Paulista Julio de Mesquita Filho
Carlos Magno Castelo Branco Fortaleza (cmfortaleza@uol.com.br)
São Paulo State University (UNESP), Botucatu Medical School https://orcid.org/0000-0003-4120-1258

Research

Keywords: Methicillin-resistant Staphylococcus aureus, HIV-AIDS, MRSA, São Paulo State, Brazil

Posted Date: January 15th, 2020

DOI: https://doi.org/10.21203/rs.2.20865/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Abstract

Background: 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.

Methods: We conducted a study that aimed at identifying the prevalence, risk factors and spatial epidemiology of both overall *S. aureus* and MRSA colonization among PLWHA from 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.

Results: In a first point prevalence survey, nasal and oropharyngeal swabs of 368 people were collected. Sixty-seven subjects from the city of 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 SCCmec type IV. Spatial analysis of households of subjects living in the city of Botucatu found significant overdispersion of cases, but no association with socio-economic indicators.

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.

Methods

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 School). 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 conducted in 2014–2016. 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 incarcerated persons 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).

Serial surveys

All subjects who lived in the city of Botucatu were invited to participate the further phases of the study. Briefly, a home visit was scheduled for 30 days after inclusion in the study. In the first visit, we performed: (a) collection of second-time samples of nasal and oropharyngeal swab; (b) collection of swabs from the same sites in all household communicants who were not infected by HIV; and (c) georreferencing of households. The third survey was performed in the patients scheduled return for routine medical appointments (in average, 3 months after the first survey).

Microbiology and molecular methods

Specimens were transported in Stuart medium and cultured in Baird Parker agar. Species identification was performed through phenotypic and genotypic techniques, as previously described [25].

Susceptibility tests followed guidelines from the Clinical Laboratory Standards Institute (CLSI), using disks for oxacillin and cefoxitin [26]. However, our definition of methicillin-resistance for practical purposes was based on the detection of the mecA gene by Polymerase Chain Reactions (PCR), as described by Murakami et al [27]. The multiplex-PCR protocol described by Milheiriço et al [28] was used for the characterization of the staphylococcal cassette chromosome mec (SCCmec).

Molecular strain typing of MRSA isolates was performed with Pulsed-Field Gel Electrophoresis (PFGE). We applied a protocol of DNA digestion with the enzyme smaI, 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]. A second step of exploratory analysis was performed for three groups (PLWHA, S. aureus positive, S. aureus negative), who were integrated in separate to identify coincidental points. We applied the “integrate” ArcGIS tool, using a standard distance of fifteen meters between the points. Afterwards, the “collect events” tool was applied for counting overlapping points. Moran’s I was calculated to estimate global spatial autocorrelation [32]. A similar approach was used to estimate the correlation of subjects coordinates with georreferenced information of families’ income and average people living in households.

All the maps were generated in the coordinate system SIRGAS 2000 UTM Zone 22, with the Transverse Mercator projection and datum Planimetric SIRGAS 2000.

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.

Results

Results of the first survey

A total of 368 subjects were included – 112 of whom lived in the city of Botucatu. The proportion of patients included (among all those cared for in SCCID) was 74.5% (80.5% for those living in Botucatu).

The total prevalence of S. aureus colonization in the first (whole sample) survey was 25.8% (95% Conficence interval [CI], 21.5%-30.7%). Rates of nasal and oropharyngeal carriage were 20.9% and 3.5%, respectively. Ten subjects were colonized with MRSA (8 in the nares, 2 in the throat) – a prevalence rate of 2.7% (95%CI, 1.4%-5.1%).

Tables 1 and 2 present results of models for predictors of carriage of S. aureus and MRSA, respectively. Briefly, overall colonization with S. aureus was negatively associated with the recent use of beta-lactams (Odds Ratio[OR], 0.19; 95%CI, 0.30–0.98, P = 0.04) and - surprisingly - with current use of illicit drugs (OR, 0.19; 95%CI, 0.06–0.62; P = 0.006). In the analysis of MRSA we adopted two strategies. Given the extensive colinearity and the small number of MRSA subjects, we avoided including both variables in the same model. Therefore, in the model not including “hospital admission”, neurocryptococosis (OR, 13.37; 95%CI, 2.20-81.21; P = 0.005) and a history of use of crack (OR, 8.26; 95%CI, 1.88–37.52, P = 0.006). In the alternative model, excluding neurocryptoocosis, recent hospital admission was the single predictor identified (OR, 4.04; 95%CI, 1.14–14.34; P = 0.04).

Table 1

Predictors of colonization with Staphylococcus aureus among people living with HIV/AIDS in inner São Paulo State, Brazil.

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		

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.

Predictors	S. aureus (95)	Other (273)	OR (95%CI)	P	OR (95%CI)	P
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						

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.

Predictors	S. aureus (95)	Other (273)	OR (95%CI)	P	OR (95%CI)	P
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		

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.

Predictors	S. aureus (95)	Other (273)	OR (95%CI)	P	OR (95%CI)	P
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		

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.

Predictors	S. aureus (95)	Other (273)	OR (95%CI)	P	OR (95%CI)	P
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		

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.

Predictors	S. aureus (95)	Other (273)	OR (95%CI)	P	OR (95%CI)	P
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.

Table 2

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

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		

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.

Predictors	MRSA (10)	Other (358)	OR (95%CI)	P	OR (95%CI)	P
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						

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.

Predictors	MRSA (10)	Other (358)	OR (95%CI)	P	OR (95%CI)	P
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		

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.

Predictors	MRSA (10)	Other (358)	OR (95%CI)	P	OR (95%CI)	P
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		

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.

Predictors	MRSA (10)	Other (358)	OR (95%CI)	P	OR (95%CI)	P
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		

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.

Predictors	MRSA (10)	Other (358)	OR (95%CI)	P	OR (95%CI)	P
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.						

Serial surveys and household contactants

Only 67 subjects (59.8% of those living in Botucatu) could be followed in both second and third surveys. The proportions of subjects colonized with overall S. aureus in one, two or three surveys were 20.9%, 13.4% and 7.5% - and 41.8% had at least one positive sample. A total of 7 subjects (10.4%) were positive for MRSA in a single sample, and none among them had this agent recovered more than once.

At the time of the second survey, we tested 76 household contactants of research subjects for nasal or oropharyngeal colonization. The prevalence of S. aureus in this group was 30.6% (23.6% for nasal colonization, 16.7% for oropharyngeal colonization). We found MRSA in three people (4.2%), of whom 2 were colonized in the nares and 2 in the throat. We found association between colonization in the contactant and in the index subject for both overall S. aureus (OR, 2.95; 95%CI, 1.03–8.52; P = 0.04) and MRSA (OR, 17.71; 95%CI, 1.42-221.16; P = 0.03).

Molecular Epidemiology

Regardless of the history of exposure to healthcare, all but one of the 19 MRSA isolates from subjects and contactants harbored SCCmec 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 Fig. 1, and Table 3 describes the clusters identified. Interestingly, the only isolate for which we could not assign a SCCmec type (345) did not belong to any cluster and was the least similar to other isolates from our study.

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.

Spatial 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 Fig. 3. The autocorrelation test did not find geographical clusters for whole subjects or for MRSA. However, we found that subjects harboring overall S. aureus were more geographically dispersed than expected at random chance (Moran's Index, -0.19; Expected Index, -0.04; P = 0.03). 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).

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 SCCmec 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 more 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. 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. 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 acknowledge 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.

Declarations Section

Ethics approval

The study was approved by the reference Committee for Ethics in Human Research (Faculdade de Medicina de Botucatu, São Paulo State, Brazil). Informed consent was obtained from all study subjects.

Availability of data and materials

Study database (in Portuguese) is available as supplementary file.

Funding

This study was funded by São Paulo Foundation for Research Support (FAPESP, grant # 2011/06988-2).

Author's contributions

LCL, MLRSC and CMCBF conceived the study. LCL and LRS recruited subjects and conducted surveys. MLRSC and CSMS performed molecular typing. CV conducted spatial epidemiology analysis. LCL and CMCBF wrote the manuscript.

Acknowledgements

Partial results were presented as Master of Science dissertation of LCL, with CMBF as advisor.

References

1. Onorato M, Borucki MJ, Baillargeon G, Paar DP, Freeman DH, et al. Risk factors for colonization or infection due to methicillin-resistant *Staphylococcus aureus* in HIV-positive patients: a retrospective case-control study. Infect Control Hosp Epidemiol. 1999; 20:26-30.
2. Tumbarello M , de Gaetano Donati K, Tacconelli E, Citton R, et al. Risk factors and predictors of mortality of methicillin-resistant *Staphylococcus aureus* (MRSA) bacteraemia in HIV-infected patients. J Antimicrob Chemother 2002; 50:375-82.
3. Smith NP, Nelson MR, Azadian B, Gazzard BG. An outbreak of methicillin-resistant *Staphylococcus aureus* (MRSA) infection in HIV-seropositive persons. Int J STD AIDS 1998; 9:726-30.
4. Padoveze MC, Tresoldi AT, von Nowakonski A, Aoki FH, Branchini ML Nasal MRSA colonization of AIDS Patients cared for in a Brazilian university hospital. Infect Control Hosp Epidemiol 2001; 22:783-5.
5. Crum-Cianflone NF, Grandits G, Echols S, Ganesan A, Landrum M, et al. Trends and causes of hospitalizations among HIV-infected persons during the late HAART era: what is the impact of CD4 counts and HAART use? J Acquir Immune Defic Syndr 2010; 54:248-57.
6. Bartlett JG. Methicillin-resistant *Staphylococcus aureus* Top HIV Med 2008; 16:151-5.
7. do Lago RF, Costa N do R. Antiretroviral manufacturers and the challenge of universal access to drugs through the Brazilian National STD/AIDS Program. Cad Saude Publica 2009;25:2273-84.
8. Grinsztejn B, Luz PM, Pacheco AG, Santos DV, Velasque L, et al. Changing mortality profile among HIV-infected patients in Rio de Janeiro, Brazil: shifting from AIDS to non-AIDS related conditions in the HAART era. PLoS One 2013; 8:e59768.

9. Domingues CS, Waldman EA. Causes of death among people living with AIDS in the pre- and post-HAART Eras in the city of São Paulo, Brazil. PLoS One 2014;9:e114661.
10. Grangeiro A, Escuder MM, Castilho EA. Magnitude and trend of the AIDS epidemic in Brazilian cities, from 2002 to 2006. Rev Saude Publica. 2010; 44:430-40.
11. Nemes MI, Melchior R, Basso CR, Castanheira ER, de Britto e Alves MT, et al. The variability and predictors of quality of AIDS care services in Brazil. BMC Health Serv Res. 2009;9:51.
12. Nemes MI, Alencar TM, Basso CR, Castanheira ER, Melchior R, et al. Assessment of outpatient services for AIDS patients, Brazil: comparative study 2001/2007. Rev Saude Publica 2013;47:137-46
13. Skov R, Christiansen K, Dancer SJ, Daum RS, Dryden M, et al. Update on the prevention and control of community-acquired meticillin-resistant *Staphylococcus aureus* (CA-MRSA). Int J Antimicrob Agents. 2012;39:193-200.
14. Casey JA, Cosgrove SE, Stewart WF, Pollak J, Schwartz BS. A population-based study of the epidemiology and clinical features of methicillin-resistant Staphylococcus aureus infection in Pennsylvania, 2001–2010. Epidemiol Infect 2013; 141: 1166–1179.
15. Gorwitz RJ, Kruszon-Moran D, McAllister SK, McQuillan G, McDougal LK, et al. Changes in the prevalence of nasal colonization with *Staphylococcus aureus* in the United States, 2001–2004. J Infect Dis 2008; 197: 1226–1234.
16. Scerri J, Monecke S, Borg MA. Prevalence and characteristics of community carriage of methicillin-resistant *Staphylococcus aureus* in Malta. J Epidemiol Glob Health 2013; 3: 165–173.
17. Vlack S, Cox L, Peleg AY, Canuto C, Stewart C, et al. Carriage of methicillin-resistant *Staphylococcus aureus* in a Queensland Indigenous community. Med J Aust 2006;184:556-9.
18. Schaumburg F, Köck R, Friedrich AW, Soulanoudjingar S, Ngoa UA, et al. Population structure of *Staphylococcus aureus* from remote African Babongo Pygmies. PLoS Negl Trop Dis 2011;5: e1150.
19. Crum-Cianflone NF, Shadyab AH, Weintrob A, Hospenthal DR, Lalani T, et al;Association of methicillin-resistant *Staphylococcus aureus* (MRSA) colonization with high-risk sexual behaviors in persons infected with human immunodeficiency virus (HIV). Medicine (Baltimore) 2011;90:379-89.
20. Popovich KJ, Hota B, Aroutcheva A, Kurien L, Patel J, et al. Community-associated methicillin-resistant Staphylococcus aureus colonization burden in HIV-infected patients. Clin Infect Dis. 2013; 56:1067-74
21. Popovich KJ, Zawitz C, Weinstein RA, Grasso AE, Hotton AL, et al. The Intersecting Epidemics of Human Immunodeficiency Virus, Community-Associated Methicillin-Resistant Staphylococcus aureus, and Incarceration. Open Forum Infect Dis 2015;2:ofv148
22. Imaz A, Camoez M, Di Yacovo S, Gasch O, Dominguez MA, et al. Prevalence of methicillin-resistant *Staphylococcus aureus* colonization in HIV-infected patients in Barcelona, Spain: a cross-sectional study. BMC Infect Dis 2015; 15:243.
23. Lemma MT, Zenebe Y, Tulu B, Mekonnen D, Mekonnen Z. Methicillin Resistant *Staphylococcus aureus* among HIV Infected Pediatric Patients in Northwest Ethiopia: Carriage Rates and Antibiotic Co-Resistance Profiles. PLoS One 2015; 10:e0137254.

24. Camargo CH, da Cunha M de L, Bonesso MF, da Cunha FP, Barbosa AN, et al. Systemic CA-MRSA infection following trauma during soccer match in inner Brazil: clinical and molecular characterization. Diagn Microbiol Infect Dis 2013; 76: 372–374.
25. Pires FV, da Cunha MdLRdS, Abraa˜o LM, Martins PYF, Camargo CH, et al. Nasal Carriage of *Staphylococcus aureus* in Botucatu, Brazil: A PopulationBased Survey. PLoS One 2014; 9: e92537.
26. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility tests; approved standard, tenth edition (M02-A10). Wayne, PA: CLSI. 76 p, 2009.
27. Murakami K, Minamide K, Wada K, Nakamura E, Teraoka H, et al. Identification of methicillin-resistant strains of staphylococci by polymerase chain reaction. J Clin Microbiol 1991; 29: 2240–2244.
28. Milheiriço C, Oliveira DC, de Lencastre H. Update to the multiplex PCR strategy for assignment of mec element types in *Staphylococcus aureus*. Antimicrob. Agents Chemother 2007; 51: 3374–3377.
29. McDougal LK, Steward CD, Killgore GE, Chaitram JM, McAllister SK, et al. Pulsed-field gel electrophoresis typing of oxacillin-resistant *Staphylococcus aureus* isolates from the United States: establishing a national database. J Clin Microbiol. 2003; 41: 5113–5120.
30. Greenland S. Modeling and variable selection in epidemiologic analysis. Am J Public Health 1989; 79: 340–349.
31. Hosmer DW, Hosmer T, Le Cessie S, Lemeshow S. A comparison of goodness-of-fit tests for the logistic regression model. Stat Med 1997; 16:965-80.
32. Bailey TC, Gatrell AC. Interactive spacial analysis. Essex, UK: Prentice Hall.413p, 1995.
33. Zervou FN, Zacharioudakis IM, Ziakas PD, Rich JD, Mylonakis E. Prevalence of and risk factors for methicillin-resistant *Staphylococcus aureus* colonization in HIV infection: a meta-analysis. Clin Infect Dis 2014; 59:1302-11.
34. Padoveze MC, de Jesus Pedro R, Blum-Menezes D, Bratfich OJ, Moretti ML *Staphylococcus aureus* nasal colonization in HIV outpatients: persistent or transient? Am J Infect Control 2008;36:187-91
35. DeLeo FR, Otto M, Kreiswirth BN, Chambers HF. Community-associated meticillin-resistant *Staphylococcus aureus*. Lancet 2010; 375:1557-68.
36. Mediavilla JR, Chen L, Mathema B, Kreiswirth BN. Global epidemiology of community-associated methicillin resistant *Staphylococcus aureus* (CA-MRSA). Curr Opin Microbiol 2012; 15:588-95.
37. El-Sharif A, Ashour HM. Community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) colonization and infection in intravenous and inhalational opiate drug abusers. Exp Biol Med (Maywood) 2008;233:874-80.
38. Otter JA, French GL. Community-associated meticillin-resistant *Staphylococcus aureus*: the case for a genotypic definition. J Hosp Infect 2012; 81:143-8.
39. Nunes CL, Andrade T, Galvão-Castro B, Bastos FI, Reingold A. Assessing risk behaviors and prevalence of sexually transmitted and blood-borne infections among female crack cocaine users inSalvador–Bahia, Brazil. Braz J Infect Dis. 2007; 11:561-6.

40. Ferri CP, Gossop M. Route of cocaine administration: patterns of use and problems among a Brazilian sample. Addict Behav 1999;24:815-21.
41. Stefani S, Chung DR, Lindsay JA, Friedrich AW, Kearns AM, et al. Meticillin-resistant *Staphylococcus aureus* (MRSA): global epidemiology and harmonisation of typing methods. Int J Antimicrob Agents 2012; 39:273-82.
42. Monecke S, Coombs G, Shore AC, Coleman DC, Akpaka P, et al. A field guide to pandemic, epidemic and sporadic clones of methicillin-resistant *Staphylococcus aureus.* PLoS One. 2011;6:e17936.
43. Knox J, Van Rijen M, Uhlemann AC, Miller M, Hafer C, et al. Community-associated methicillin-resistant *Staphylococcus aureus* transmission in households of infected cases: a pooled analysis of primary data from three studies across international settings. Epidemiol Infect 2015; 143:354-65.
44. Farr AM, Marx MA, Weiss D, Nash D. Association of neighborhood-level factors with hospitalization for community-associated methicillin-resistant *Staphylococcus aureus*, New York City, 2006: a multilevel observational study. BMC Infect Dis; 2013; 13:84.
45. Miller M, Cook HA, Furuya EY, Bhat M, Lee MH, et al. *Staphylococcus aureus* in the community: colonization versus infection. PLoS One 2009; 4:e6708.
46. Cook HA, Furuya EY, Larson E, Vasquez G, Lowy FD. Heterosexual transmission of community-associated methicillin-resistant *Staphylococcus aureus.* Clin Infect Dis. 2007;44:410-3.
47. Cocchi P, Taccetti G, Montagnani C, Campana S, Galli L, et al. Evidence of transmission of a Panton-Valentine leukocidin-positive community-acquired methicillin-resistant *Staphylococcus aureus* clone: a family affair. Clin Microbiol Infect 2013; 19:1158-62.
48. Miller LG, Diep BA (2008) Clinical practice: colonization, fomites, and virulence: rethinking the pathogenesis of community-associated methicillin-resistant Staphylococcus aureus infection. Clin Infect Dis 2008; 46: 752–760.

Figures

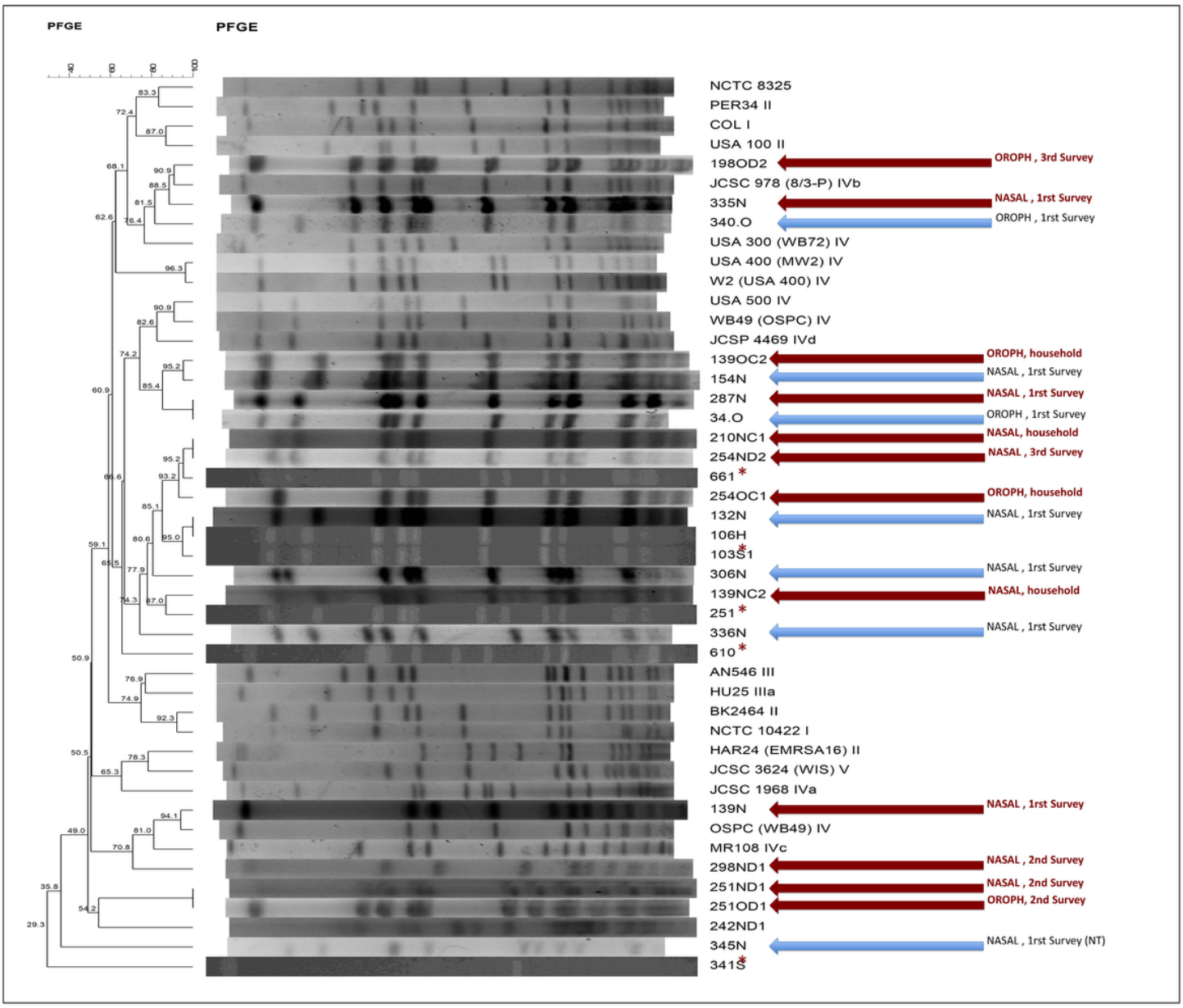

Figure 1

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 SCCmec type IV, except number 345 (NT=not typable).

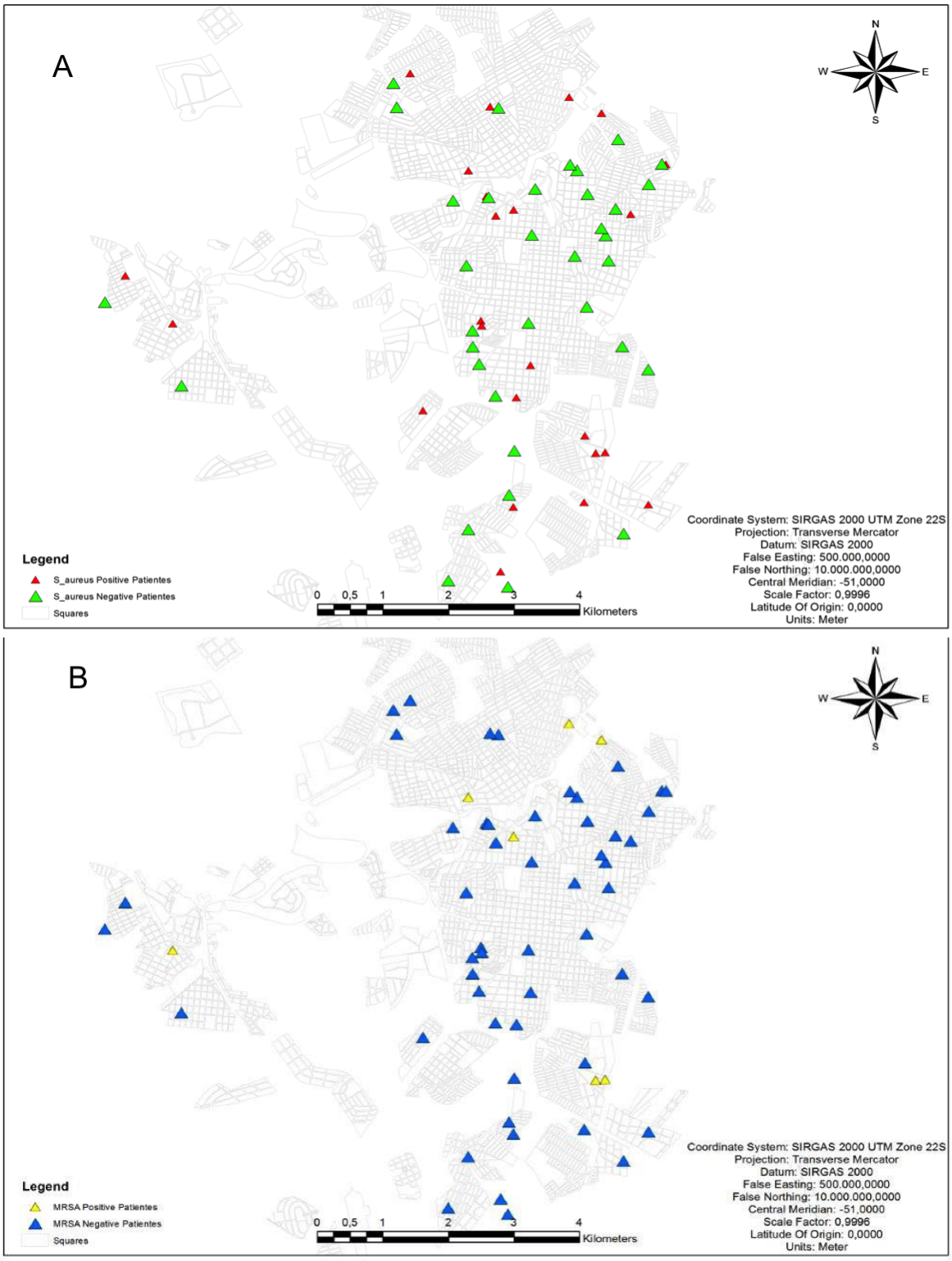

Figure 4

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).

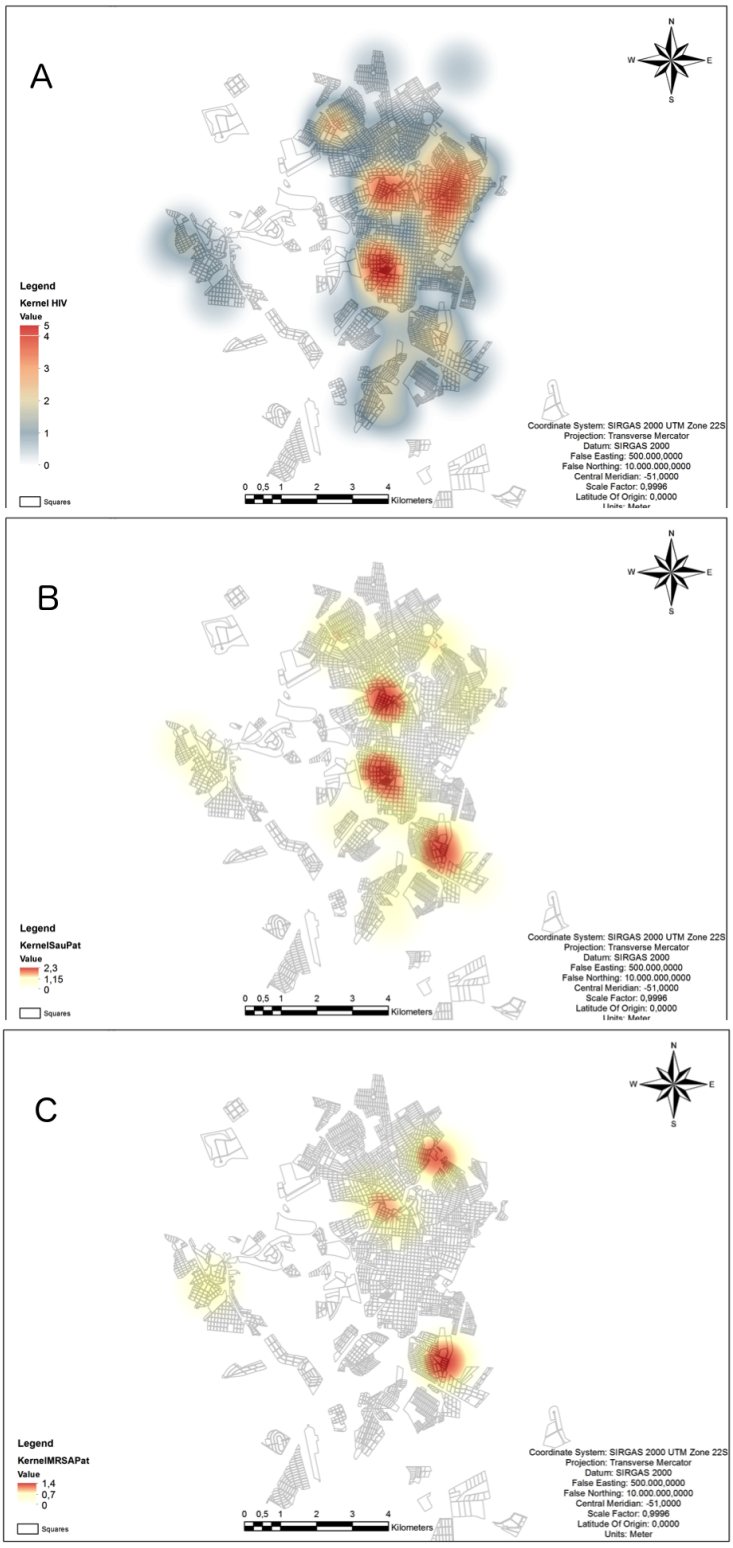

Figure 6

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

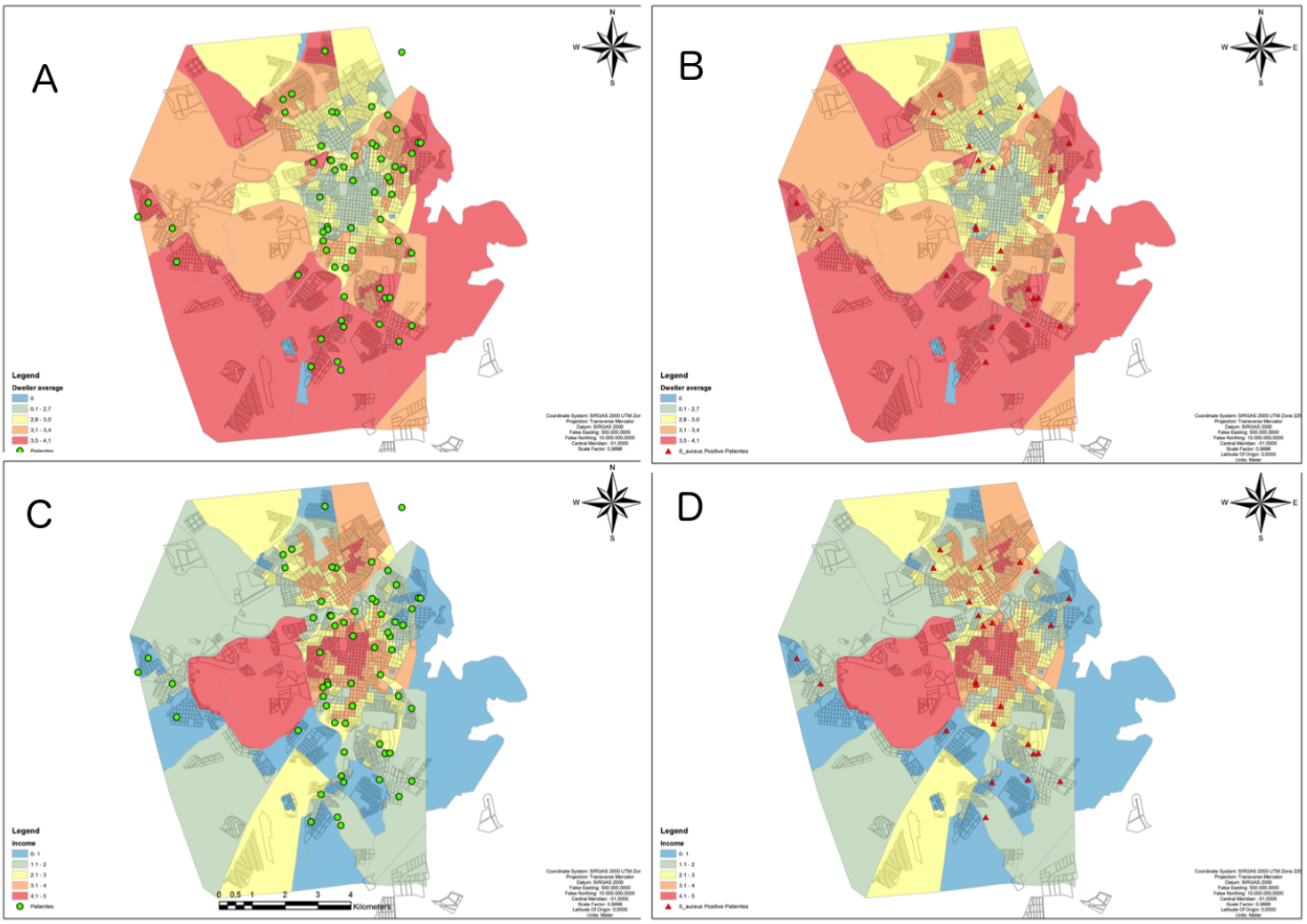

Figure 8

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.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- hivsaureus.xlsx
- hivsaureus.xlsx