Molecular analysis of methicillin-resistant
_Staphylococcus aureus_ dissemination among healthcare professionals and/or HIV patients from a tertiary hospital


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**ABSTRACT**

**Introduction:** Methicillin-resistant _Staphylococcus aureus_ (MRSA) is a nosocomial pathogen in community settings. MRSA colonized individuals may contribute to its dissemination; the risk of MRSA infection is increased in human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) patients, although the prevalence of colonization in this group is not well established. The present study addressed this issue by characterizing MRSA isolates from HIV/AIDS patients and their healthcare providers (HCPs) to determine whether transmission occurred between these two populations.

**Methods:** A total of 24 MRSA isolates from HIV-infected patients and five from HCPs were collected between August 2011 and May 2013. Susceptibility to currently available antimicrobials was determined. Epidemiological typing was carried out by pulsed-field gel electrophoresis, multilocus sequence typing, and _Staphylococcus_ cassette chromosome (SCCmec) typing. The presence of heterogeneous vancomycin-intermediate _Staphylococcus aureus_ (hVISA) and heterogeneous daptomycin-resistant _Staphylococcus aureus_ (hDRSA) was confirmed by population analysis profile. Isolates characterized in this study were also compared to isolates from 2009 obtained from patients at the same hospital.

**Results:** A variety of lineages were found among patients, including ST5-SCCmecII and ST30-SCCmecIV. Two isolates were Panton-Valentine leukocidin-positive, and hVISA and hDRSA were detected. MRSA isolates from two HCPs were not related to those from HIV/AIDS patients, but clustered with archived MRSA from 2009 with no known relationship to the current study population.

**Conclusions:** ST105-SCCmecII clones that colonized professionals in 2011 and 2012 were already circulating among patients in 2009, but there is no evidence that these clones spread to or between HIV/AIDS patients up to the 7th day of their hospitalization.

**Keywords:** Methicillin-resistant _Staphylococcus aureus_. HIV patients. Teicoplanin resistance. h-VISA. Daptomycin.
**METHODS**

**Sample collection**

MRSA samples from two colonization sites [nares (N) and saliva (S)] were obtained from HIV/AIDS patients on days 1 and 7 of hospitalization and from HCPs with whom they had contact, in two specific units of a large Brazilian public hospital with 600 beds, of which 24 are occupied almost exclusively by HIV patients. From August 2011 to May 2013, 317 individuals with HIV/AIDS were hospitalized, and 266 agreed to participate in the study along with 73 HCPs; staffs that were on leave were not included. Samples were collected using swabs and stored in Stuart agar until bacterial isolation and identification. *S. aureus* was isolated on Mueller Hinton agar supplemented with 3% sheep blood and phenotypic identification was carried out using the Vitek system (BioMérieux, Marcy l’Etoile, France). Methicillin resistance was detected using the AST-P855 card (BioMérieux) and broth dilution. Once an HCP was identified as being colonized with MRSA, a decolonization protocol was carried out that included a chlorhexidine bath and application of 1ml silver sulfadiazine to the nares, which was repeated daily for 5 days. Additionally, 22 MRSA isolates from other patients from different wards at the same hospital collected between June and September 2009 that were archived by our group, and *S. aureus* strain N315 were included for determination of clonality. Isolates representing each pulsotype detected by pulsed-field gel electrophoresis (PFGE) were randomly selected for typing by multilocus sequence typing (MLST) and *Staphylococcus* Cassette Chromosome (SCCmec) typing. The study protocol was approved by the Research Ethics Committee of the Ribeirão Preto School of Nursing (no. 1304/2011).

**Susceptibility profiling**

The minimum inhibitory concentration (MIC) was determined for oxacillin, vancomycin, teicoplanin, daptomycin, tigecycline, linezolid, and quinupristin-dalfopristin by broth dilution, according to Clinical and Laboratory Standards Institute (CLSI) guidelines. MIC₅₀ and MIC₉₀ were calculated and CLSI breakpoints were adopted for classification except in the case of tigecycline, for which the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendation was followed.

**Heterogeneous vancomycin-intermediate *Staphylococcus aureus* screening**

Heterogeneous vancomycin-intermediate *Staphylococcus aureus* screening (hVISA) screening was performed as previously described². If a countable number (one to 30) of colonies was observed within 48h of incubation at 37°C on brain-heart infusion agar containing 4µg/ml vancomycin, the strain was designated as a possible hVISA. *S. aureus* Mu3 and Mu50 were used as hVISA and VISA control strains, respectively, and were kindly provided by Keiichi Hiramatsu and Teruyo Ito (Juntendo University, Tokyo, Japan).

**Population analysis profile**

The population analysis profile (PAP) (6) to vancomycin or daptomycin was determined for samples that were positive for the hVISA screening or exhibited resistance to daptomycin after 48h of incubation(6)(7)(8). PAP to daptomycin was used to identify heterogeneous daptomycin-resistant *Staphylococcus aureus* (hDRSA) strains⁸.

**Panton-Valentine leukocidin gene and hemolysis analysis**

The PVL gene lukSF was amplified by PCR as previously described⁹. Mueller Hinton agar supplemented with 5% sheep blood was used to assess hemolytic activity.

**Molecular characterization**

SCCmec type was determined by multiplex-PCR as previously described¹⁰ using the following reference strains: *S. aureus* RN4220 (SCCmec negative), 10442 (SCCmecI), N315 (SCCmecII), 85/2082 (SCCmecIII), 4744 (SCCmecIVa), and WIS (SCCmecV).

PFGE was carried out after DNA digestion with Smal¹¹. Data were analyzed with BioNumerics v.7.1 software (Applied Maths NV, Belgium)¹² using the unweighted pair-group method with arithmetic mean based on Dice coefficients, where optimization and tolerance were set to 0.5% and 1.25%, respectively. A similarity coefficient of 80% was selected to describe patterns representative of each pulsotype, which were further characterized by MLST¹³. Sequence types were identified using the MLST database¹⁴. To limit redundancy, duplicate isolates from the same patient with identical SCCmec and pulsotype were considered as the same strain and included only once in the analysis.

**RESULTS**

*Staphylococcus aureus* was cultured from 101 (38%) tested individuals, and resistance to oxacillin was observed in 15 participants (5.6% of all participants or 14.8% of those colonized by *S. aureus*). A total of 13/15 HIV patients were found to be colonized by MRSA on the day of hospital admission, and only five of these remained colonized on day 7. Additionally, two patients were found to be colonized only on day 7 of hospitalization. Only 3/73 (4.1%) of HCPs (P1, P2, and P3) were colonized by MRSA during the study. P1 was colonized on three different dates, despite having undergone decolonization procedures¹⁵. Therefore, a total of 29 MRSA isolates were characterized: 24 from patients and five from HCPs (Figure 1).

Susceptibility analysis confirmed β-lactam resistance in all putative MRSA isolates. Daptomycin resistance in isolate 80N was observed after a 48h incubation; this was unexpected, since daptomycin is considered bactericidal. A population analysis of the isolate revealed a heterogeneous phenotype that included some daptomycin-resistant cells (Figure 2A).
MIC<sub>50</sub> and MIC<sub>90</sub> (μg/ml) of all antimicrobials were determined for each strain, and were as follows: 128/>256 for oxacillin, 4/4 for linezolid, 0.5/1 for teicoplanin, 0.125/0.5 for tigecycline, 0.5/0.5 for quinupristin/dalfopristin and daptomycin, and 1/2 for vancomycin. MICS for each isolate are shown in Figure 1.

One isolate (176N) was identified as hVISA, which was confirmed by PAP (Figure 2B). According to CLSI breakpoints<sup>(3)</sup>, this isolate exhibited an intermediate level of resistance to teicoplanin after 24h of incubation (MIC = 16μg/ml) and full resistance after 48h. However, it would be classified as resistant after 24h based on EUCAST breakpoints<sup>(4)</sup>, as resistant after 24h based on EUCAST breakpoints<sup>(4)</sup>, and 1.25% tolerance.

FIGURE 1 - Similarity dendrogram. Similarity was calculated with BioNumerics v.6.5 software using the Dice coefficient with 0.5% optimization and 1.25% tolerance. ST: sequence typing; SCCmec: Staphylococcus cassette chromosome; DPC: daptomycin; LNZ: linezolid; TCP: teicoplanin; TGC: tigecycline; OXA: oxacillin; VAN: vancomycin; N: nares; S: saliva; HCPs: healthcare providers. Isolates collected in this study are indicated in bold to differentiate them from those obtained in 2009, which were used only for clonality analysis.
Only 2/29 isolates (6.9%) were PVL-positive (strains 199N and 273N), while 25 (86.2%) were fully hemolytic. Isolates from HIV patients showed considerable variation in terms of PFGE band patterns, which included eight pulsotypes (A-E, G, H, and J) in contrast to three pulsotypes (F, I, and K) among isolates from HCPs.

ST5, ST105, ST148, ST239, and ST30 were detected among isolates. Two of three HCPs were colonized by ST105-SCCmecII, a single locus variant (SLV) of the New York/Japan Clone, whereas one was colonized by ST5-SCCmecIV, the Pediatric Clone. The following lineages were observed in HIV patients: ST5-SCCmecIV, ST239-SCCmecIII, ST30-SCCmecIV (Southern Pacific clone), ST5-SCCmecV, ST105-SCCmecII, and ST148-SCCmecIV, all of which have been previously described. Different SCCmec types were observed in isolates collected from Patient #7 on days 1 and 7 of hospitalization, although they were indistinguishable by PFGE (Figure 1).

FIGURE 2 - Population analysis profile to: A) daptomycin and B) vancomycin. CFU: colony forming units.
Only Patients #1 and #12 were readmitted to this hospital during the period of study. Isolates from these patients from days 1 and 7 of hospitalization were indistinguishable. Although both isolates from Patient #1 collected at readmission were of the same lineage as that obtained at the first hospitalization (ST239-SCCmecIII), they were of a different pulsotype (G instead of H), and the isolates from days 1 and 7 of readmission were of different subtypes (G3 and G2). Isolates obtained from Patient #12 over a two-month period were indistinguishable and MRSA was detected only on day 1 of both hospitalizations.

The first P1 isolate (PAS 4N) was characterized by PFGE as pulsotype F. After decolonization, this HCP remained colonized by the same MRSA clone (PAS 63N). A second decolonization procedure was carried out, and P1 was again found to be colonized, but this time by pulsotype K strain PAS 65N. Although the pulsotype had changed, all three isolates from P1 were of ST105-SCCmecII. There were no HIV patients colonized by the same type of MRSA as determined by PFGE of HCP samples examined during the course of this study.

When MRSA isolated in 2009 from different infection sites from patients in different wards of the same hospital was compared to those of HIV patients and HCPs in the current study, we observed that with the exception of pulsotype H associated with Brazilian Epidemic Clone (BEC), all other HIV patient isolates were unrelated to those obtained from patients in 2009 (< 80% similarity) (Figure 1). However, HCPs P1 (isolates PAS 4N and PAS 63N) and P3 (isolate PAS 31N) were colonized in 2011 by clones that were indistinguishable from those isolated from infections at this hospital in 2009.

ST105 and ST148 are SLVs of ST5 and account for the majority of isolates typed in this study. ST30 is a prototype of CC30 and is associated with community-acquired infection. The isolate ST30-SCCmecIV was positive for PVL, which is consistent with MRSA strains found in the community.

Although many subtypes isolated from patients can be considered as closely related according to Tenover’s criteria, only four pairs of patient strains yielded indistinguishable band patterns by PFGE. Interestingly, the isolate from P3 and the first two isolates from P1 clustered with MRSA from different patients isolated 2 years prior–i.e., a clone related to ST5-SCCmecII that was circulating among patients in 2009. There was no evidence that these clones spread to the HIV patients studied in 2011. This clone is common in hospitals around the world, and has replaced BEC as the predominant MRSA clone in Brazil.

Although it would be prudent, there is currently no rule in Brazil requiring removal from the work environment of a MRSA-positive HCP. British guidelines for the control of MRSA in healthcare facilities include screening of staff for this bacterium. HCPs with colonized or infected hand lesions are required to be off work while receiving clearance therapy since they are a source of dissemination to non-colonized patients. Moreover, HCPs work in different wards of a hospital, which increases opportunities for dissemination. Security and hygienic practices should also be verified more frequently, since HCPs can be colonized on different dates even after undergoing decolonization, as we observed here.

In conclusion, HCPs were found to be colonized by ST105-SCCmecII in 2011 and 2012 and this strain had infected other patients in 2009. Although the same pulsotype was detected, we cannot conclude that these HCPs were in direct contact with patients and that dissemination occurred. Finally, there was no broad dissemination of a specific MRSA clone among HIV patients up to the 7th day of hospitalization.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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