Major Article

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 (SCC*mec*) 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-SCC*mec*II and ST30-SCC*mec*IV. 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-SCC*mec*II 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: Methicilin-resistant *Staphylococcus aureus*. HIV patients. Teicoplanin resistance. h-VISA. Daptomycin.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a leading cause of nosocomial and community infections[1]. It is usually present in the environment as well as in the microbiota of the superior respiratory tract and skin. MRSA is transmitted by direct contact, and patient-to-patient transmission is largely via the hands of health care providers (HCPs). Undetected MRSA-colonized HCPs represent a major source of the bacterium in hospitals, since it can be transmitted from these individuals to high-risk patients, which can limit the success of other control measures. Indeed, a case in which MRSA transmission was reduced after carrier HCPs were identified and successfully decolonized has been described[2].

Individuals with human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) are at increased risk for MRSA colonization and infection mainly due to their high antibiotic use[1] and high rates of hospital readmission. These patients can also harbor community-acquired MRSA, which often produces Panton-Valentine leukocidin (PVL)[1]. However, the prevalence of MRSA colonization in this group is not well established.

We addressed this in the present study by investigating the rate of MRSA colonization in hospitalized AIDS patients and their HCPs. MRSA isolates were characterized genetically and phenotypically and compared to determine whether any transmission occurred between patients and/or HCPs. The isolates were also compared to isolate from 2009 that were collected from different patients at the same hospital.

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 5% sheep blood and phenotypic identification was carried out using the Vitek system (BioMérieux, Marcy l'Etoile, France). Methicillin resistance was detected using the AST-P585 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 (SCC*mec*) 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 oxacilin, vancomycin, teicoplanin, daptomycin, tigecyclin, linezolid, and quinupristin-dalfopristin by broth dilution, according to Clinical and Laboratory Standards Institute (CLSI) guidelines[3]. MIC_{50} and MIC_{90} 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[4].

Heterogeneous vancomycin-intermediate *Staphylococcus aureus* screening

Heterogeneous vancomycin-intermediate *Staphylococcus aureus* screening (hVISA) screening was performed as previously described[5]. 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[5]. *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)[5] [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) strains[8].

Panton-Valentine leukocidin gene and hemolysis analysis

The PVL gene *luk*SF was amplified by PCR as previously described[9]. Mueller Hinton agar supplemented with 5% sheep blood was used to assess hemolytic activity.

Molecular characterization

SCC*mec* type was determined by multiplex-PCR as previously described[10] using the following reference strains: *S. aureus* RN4220 (SCC*mec* negative), 10442 (SCC*mec*I), N315 (SCC*mec*II), 85/2082 (SCC*mec*III), 4744 (SCC*mec*IVa), and WIS (SCC*mec*V).

PFGE was carried out after DNA digestion with *Sma*I[11]. 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 MSLT[13]. Sequence types were identified using the MLST database[14]. To limit redundancy, duplicate isolates from the same patient with identical SCC*mec* 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 procedures[15]. 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 48-h 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**).

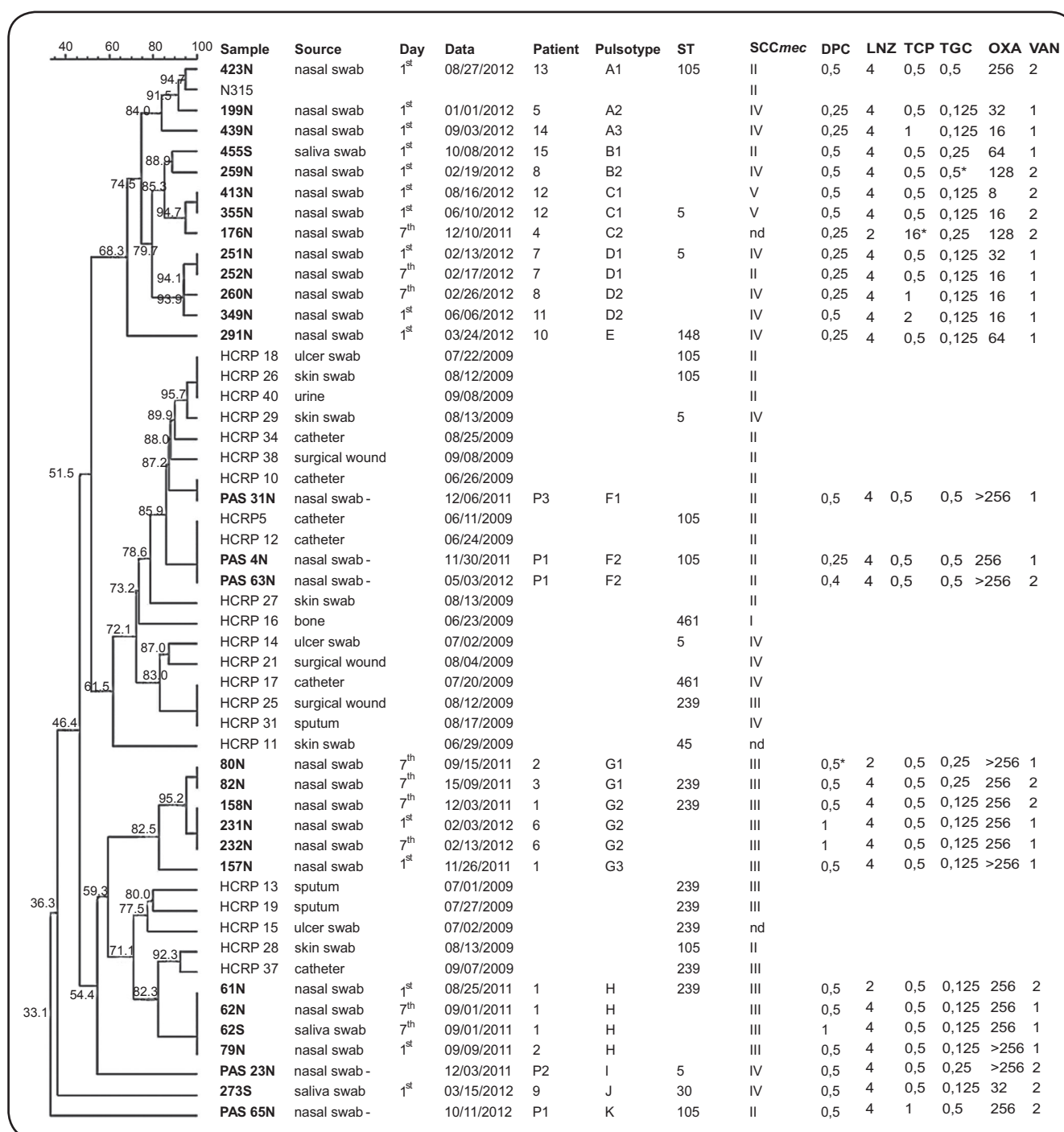| Sample	Source	Day	Data	Patient	Pulsotype	ST	SCCmec	DPC	LNZ	TCP	TGC	OXA	VAN
423N	nasal swab	1st	08/27/2012	13	A1	105	II	0,5	4	0,5	0,5	256	2
N315							II						
199N	nasal swab	1st	01/01/2012	5	A2		IV	0,25	4	0,5	0,125	32	1
439N	nasal swab	1st	09/03/2012	14	A3		IV	0,25	4	1	0,125	16	1
455S	saliva swab	1st	10/08/2012	15	B1		II	0,5	4	0,5	0,25	64	1
259N	nasal swab	1st	02/19/2012	8	B2		IV	0,5	4	0,5	0,5*	128	2
413N	nasal swab	1st	08/16/2012	12	C1		V	0,5	4	0,5	0,125	8	2
355N	nasal swab	1st	06/10/2012	12	C1	5	V	0,5	4	0,5	0,125	16	2
176N	nasal swab	7th	12/10/2011	4	C2		nd	0,25	2	16*	0,25	128	2
251N	nasal swab	1st	02/13/2012	7	D1	5	IV	0,25	4	0,5	0,125	32	1
252N	nasal swab	7th	02/17/2012	7	D1		II	0,25	4	0,5	0,125	16	1
260N	nasal swab	7th	02/26/2012	8	D2		IV	0,25	4	1	0,125	16	1
349N	nasal swab	1st	06/06/2012	11	D2		IV	0,5	4	2	0,125	16	1
291N	nasal swab	1st	03/24/2012	10	E	148	IV	0,25	4	0,5	0,125	64	1
HCRP 18	ulcer swab		07/22/2009			105	II						
HCRP 26	skin swab		08/12/2009			105	II						
HCRP 40	urine		09/08/2009				II						
HCRP 29	skin swab		08/13/2009			5	IV						
HCRP 34	catheter		08/25/2009				II						
HCRP 38	surgical wound		09/08/2009				II						
HCRP 10	catheter		06/26/2009				II						
PAS 31N	nasal swab -		12/06/2011	P3	F1		II	0,5	4	0,5	0,5	>256	1
HCRP5	catheter		06/11/2009			105	II						
HCRP 12	catheter		06/24/2009				II						
PAS 4N	nasal swab -		11/30/2011	P1	F2	105	II	0,25	4	0,5	0,5	256	1
PAS 63N	nasal swab -		05/03/2012	P1	F2		II	0,4	4	0,5	0,5	>256	2
HCRP 27	skin swab		08/13/2009				II						
HCRP 16	bone		06/23/2009				I						
HCRP 14	ulcer swab		07/02/2009			5	IV						
HCRP 21	surgical wound		08/04/2009				IV						
HCRP 17	catheter		07/20/2009			461	IV						
HCRP 25	surgical wound		08/12/2009			239	III						
HCRP 31	sputum		08/17/2009				IV						
HCRP 11	skin swab		06/29/2009			45	nd						
80N	nasal swab	7th	09/15/2011	2	G1		III	0,5*	2	0,5	0,25	>256	1
82N	nasal swab	7th	15/09/2011	3	G1	239	III	0,5	4	0,5	0,25	256	2
158N	nasal swab	7th	12/03/2011	1	G2	239	III	0,5	4	0,5	0,125	256	2
231N	nasal swab	1st	02/03/2012	6	G2		III	1	4	0,5	0,125	256	1
232N	nasal swab	7th	02/13/2012	6	G2		III	1	4	0,5	0,125	256	1
157N	nasal swab	1st	11/26/2011	1	G3		III	0,5	4	0,5	0,125	>256	1
HCRP 13	sputum		07/01/2009			239	III						
HCRP 19	sputum		07/27/2009			239	III						
HCRP 15	ulcer swab		07/02/2009			239	nd						
HCRP 28	skin swab		08/13/2009			105	II						
HCRP 37	catheter		09/07/2009			239	III						
61N	nasal swab	1st	08/25/2011	1	H	239	III	0,5	2	0,5	0,125	256	2
62N	nasal swab	7th	09/01/2011	1	H		III	0,5	4	0,5	0,125	256	1
62S	saliva swab	7th	09/01/2011	1	H		III	1	4	0,5	0,125	256	1
79N	nasal swab	1st	09/09/2011	2	H		III	0,5	4	0,5	0,125	>256	1
PAS 23N	nasal swab -		12/03/2011	P2	I	5	IV	0,5	4	0,5	0,25	>256	2
273S	saliva swab	1st	03/15/2012	9	J	30	IV	0,5	4	0,5	0,125	32	2
PAS 65N	nasal swab -		10/11/2012	P1	K	105	II	0,5	4	1	0,5	256	2

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; **SCC*mec: Staphylococcus* cassette chromosome; **DPC:** daptomycin; **LNZ:** linezolide; **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.***resistance after 48h.**

One isolate (176N) was identified as hVISA, which was confirmed by PAP (**Figure 2B**). According to CLSI breakpoints[3], 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[4].

MIC_{50} and MIC_{90} (µg/ml) of all antimicrobials were determined for each strain, and were as follows: 128/>256 for oxacilin, 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**.
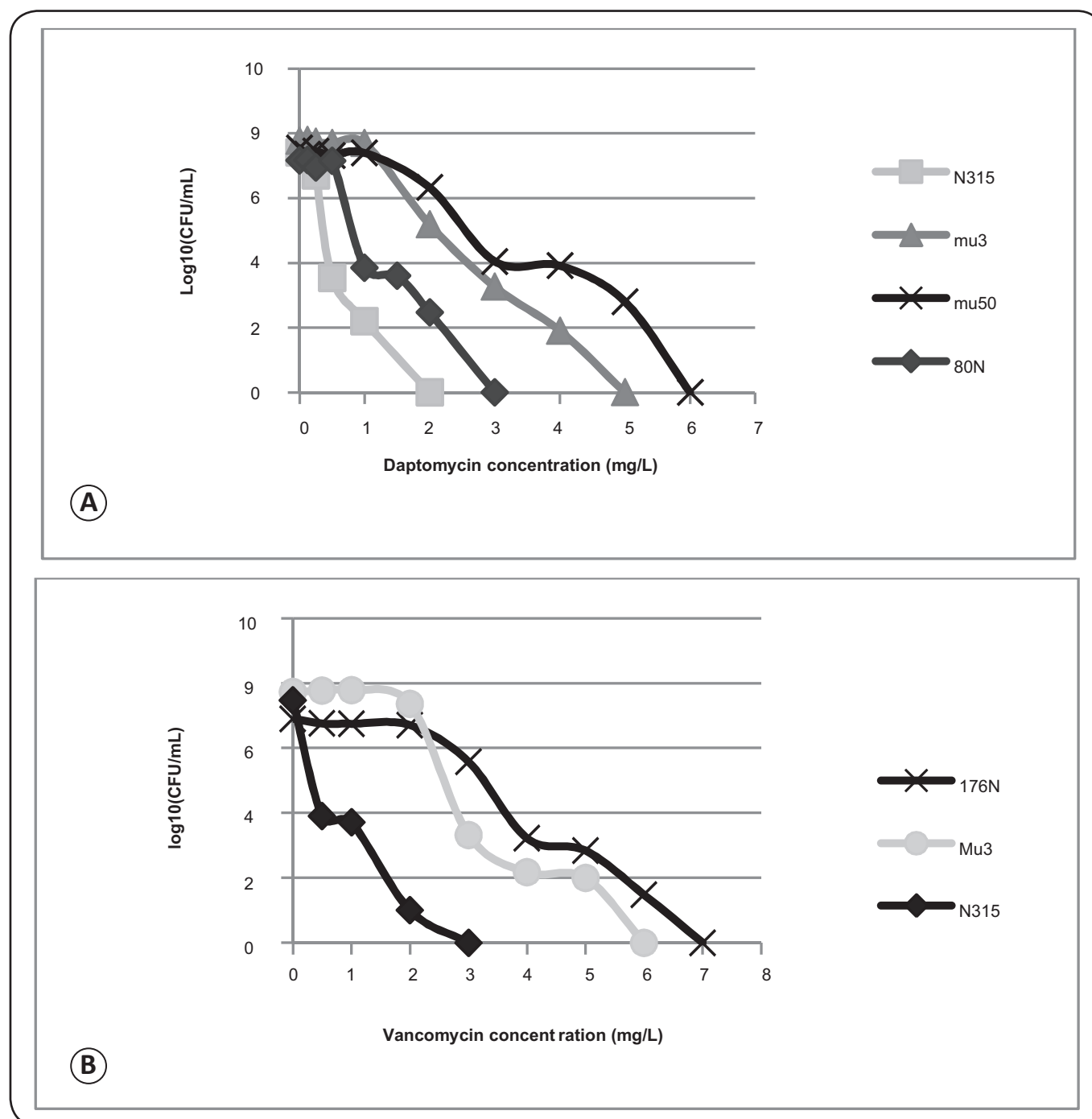
FIGURE 2 - Population analysis profile to: A) daptomycin and B) vancomycin. CFU: colony forming units.

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-SCC*mec*II, a single locus variant (SLV) of the New York/

Japan Clone, whereas one was colonized by ST5-SCC*mec*IV, the Pediatric Clone. The following lineages were observed in HIV patients: ST5-SCC*mec*IV, ST239-SCC*mec*III, ST30-SCC*mec*IV (Southern Pacific clone), ST5-SCC*mec*V, ST105-SCC*mec*II, and ST148-SCC*mec*IV, all of which have been previously described[16] [17]. Different SCC*mec* types were observed in isolates collected from Patient #7 on days 1 and 7 of hospitalization, although they were indistinguishable by PFGE (**Figure 1**).

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-SCC*mec*III), 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[15], 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-SCC*mec*II. 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.

DISCUSSION

In this study, a variety of MRSA pulsotypes with few subtypes were isolated from HIV patients and HCPs. We observed no correlation between pulsotype and reduced susceptibility to antimicrobials. We found one hVISA isolate that also exhibited intermediate resistance to teicoplanin. The *vanA* gene responsible for complete glycopeptide resistance in *S. aureus* was not detected in any samples (data not shown). Teicoplanin resistance after vancomycin use has been reported[18]; however, none of the patients in our study were treated with vancomycin or teicoplanin.

The mechanisms underlying daptomycin resistance are not well understood[19][20], but it is known that decreased susceptibility to daptomycin and vancomycin can co-occur both in vivo[20] and in vitro[21] after selection with either antimicrobial[21]. Although there is evidence for cross-resistance[21], in this study, the hDRSA isolate remained susceptible to vancomycin. Since none of the patients had been treated with daptomycin, the basis for resistance is unclear, but is currently under study. Heterogeneous type resistance to daptomycin has been previously observed, and is in part attributed to *dltA* overexpression. The detection of teicoplanin-intermediate *S. aureus*, hVISA, and hDRSA is of great concern since these represent last-line antimicrobials for treating MRSA infection.

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-SCC*mec*IV was positive for PVL, which is consistent with MRSA strains found in the community[22].

Although many subtypes isolated from patients can be considered as closely related according to Tenover's criteria[12], 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-SCC*mec*II 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[23].

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[24][25] 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[24][25]. 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-SCC*mec*II 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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