A Molecular Epidemiological Analysis of 2 Staphylococcus aureus Clonal Types Colonizing and Infecting Patients with AIDS

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Background. Persons with acquired immune deficiency syndrome (AIDS) who use drugs appear to be at increased risk for colonization and infection with Staphylococcus aureus. Little is known about the nature of and risk factors responsible for this association. This study is among the first to prospectively follow carriage and infection in this uniquely high-risk population.

Methods. We prospectively followed the cases of 75 patients with AIDS in a residential drug treatment facility and screened for S. aureus nasal colonization and infection.

Results. Thirty-seven baseline cultures (49%) were positive for S. aureus, and 81% of subjects were colonized at least once during the study. Thirteen subjects experienced 17 infections. Pulsed-field gel electrophoresis and sequence-based typing methods revealed that 244 (92%) of the isolates belonged to either clonal type A or B. Clonal type A was methicillin-susceptible. Clonal type B consisted of 3 main subtypes (B1, B2, and B3), all with the same allelic profile (ST8) and staphylococcal protein A gene (spa) type (7). Of note, subtype B1 was methicillin-susceptible (ST8 and spa type 7), lacking mecA, whereas the other B clones were methicillin-resistant. Both clones were resistant to trimethoprim-sulfamethoxazole. Clonal type B isolates were relatively resistant, suggesting prior exposure to the health care setting.

Conclusions. This study demonstrates a sustained high rate of S. aureus carriage and infection. It demonstrates the capacity of unique methicillin-resistant S. aureus clones with an established linkage to earlier outbreaks of methicillin-resistant S. aureus, as well as to human immunodeficiency virus—infected subjects, to persist in this residential setting. It also illustrates the apparent genetic instability or transmissibility of the staphylococcal chromosomal cassette mec type IV element.

People with HIV infection and AIDS who use drugs appear to be at increased risk for nasal colonization with Staphylococcus aureus [1–5], which, in turn, increases the risk of S. aureus infection [1, 6, 7]. The basis for this increased risk is still not clearly defined. Immunocompromised status, hospital contact, and anti-biotic selective pressure, among other factors, may all contribute. Among past and present drug users, HIV-positive status remains a major risk factor for S. aureus nasal colonization [5]. Drug use may be an independent risk factor for colonization with S. aureus [8, 9].

To better understand the nature of and risk factors for S. aureus colonization and infection in this high-risk population, we prospectively followed the cases of subjects at a residential treatment facility for drug users with AIDS. We identified a high proportion of subjects colonized with only 2 clonal types. Here we provide a comprehensive genetic and epidemiological analysis of 2 clones that accounted for the majority of S. aureus colonization and infection in a population of HIV-infected patients with a history of drug use.
MATERIALS AND METHODS

Study population. Seventy-five patients at Project Samaritan (PSI) participated in the study between January and December 2001. PSI, located in the Bronx, New York, is a long-term inpatient drug rehabilitation facility for patients with AIDS. Both newly admitted patients and those already residing at PSI were eligible to enroll in the study. Subjects at PSI slept on 1 of 3 floors. Two floors had 12 double rooms with 4 shared bathrooms. The remaining floor had 2 single and 2 double rooms. There were also meeting rooms, common dining and outdoor areas, and a clinic.

Data were collected from the medical record. An interviewer, unaffiliated with PSI, conducted an entrance interview and collected an initial nasal sample for culture from each subject. Nasal samples for culture were collected, when possible, on a monthly basis thereafter for the remainder of the study. The study was reviewed and approved by the PSI and Columbia University institutional review boards.

Specimens for culture. Samples were collected from the anterior nares of each subject with a cotton swab (Becton Dickinson Culturette Systems). Isolates from infection samples with culture results positive for S. aureus were forwarded to our laboratory from Bendiner and Schlesinger Laboratories. Nasal samples for culture were also obtained from PSI personnel. Environmental samples were collected from the lobby, common rooms, and bathrooms by use of premoistened culturette swabs. One colony from each sample with positive culture results was collected and stored for further analysis, as described elsewhere [10].

PFGE. All available colonization (264), infection (13), environmental (4), and PSI personnel (4) isolates were typed by PFGE with use of SmaI digestion, as described elsewhere [10]. PFGE images were captured, archived, and analyzed with Diversity Database software, version 1.0, coupled with the GelDoc 1000 system (Bio-Rad). Dendrograms were constructed by the Dice coefficient method, as described elsewhere [10, 11].

Multilocus sequence typing (MLST). MLST was performed as described elsewhere [12, 13]. Sequences were analyzed with DNAStar software, and MLST sequence types (STs) were assigned through the MLST database (http://www.mlst.net).

Staphylococcal protein A (spa) gene typing. spa typing was done as described elsewhere [14, 15]; spa types were assigned with GeneSearch.

Antibiotic susceptibility testing. Susceptibility to antibiotics (amikacin, clindamycin, erythromycin, gentamicin, levofloxacin, oxacillin, penicillin, rifampin, trimethoprim-sulfamethoxazole, and vancomycin; Becton Dickinson) was determined for all isolates according to the Kirby-Bauer disk diffusion method [16]. Meticillin susceptibility was established by Etest (AB Biodisk), SCCmec typing, or Southern blot hybridization (described below) of representative isolates from the different clones and the unique isolates.

Southern blot hybridization. Southern blotting for mecA was done on a PFGE gel of 26 isolates, including 15 representative type B isolates. Southern blot hybridization was done as described elsewhere [17, 18].

Population analysis profiles. Population analysis profiles for oxacillin were constructed for 16 study samples, including type A isolates (3), type B isolates (11), and unrelated isolates (2), by means of previously published methods [19]. The type B isolates investigated included B1 (3), B2 (5), B3 (1), and BO (2). Five reference strains—BM79, CDC-1, COL, NCTC8325, and NYHB3—were used as controls. Oxacillin MICs generated from population analysis profiles were arbitrarily defined as the lowest concentration of oxacillin that caused a minimum 100-fold reduction in the viable titer of the culture (data not shown).

mecA typing. Multiplex PCR for mecA typing was performed as described elsewhere [20] on 32 isolates, including 14 type B isolates, 1 negative control isolate, and 6 positive control isolates.

Statistical analyses. A cross-sectional analysis was used to assess potential risk factors for S. aureus colonization. These included the relationship between S. aureus colonization and demographics, CD4+ cell count, HIV load, antibiotic use, hospitalization, medical conditions, drug use practices, and past incarceration. In addition to baseline colonization status, we evaluated the same risk factors for subsequent S. aureus infection. In univariate analyses, the χ² test was used to assess associations between colonization status and categorical dependent variables. Student’s t test or the Wilcoxon signed rank test was used to compare means for continuous dependent variables. A logistic model was created to examine the association between infection status and the proportion of positive nasal cultures (as a proxy for persistent colonization), controlling for the number of nasal specimens collected. For this analysis, data were categorized as follows: 0, <50%, and ≥50% of nasal specimens that were culture positive. Two-tailed P values were significant at P ≤ .05. Fisher’s exact test was used when expected cell counts were <5. All statistical analyses were performed with use of SAS software, version 9.0 (SAS Institute).

RESULTS

Study population. PSI subjects had a mean age (±SD) of 42 ± 6 years (range, 26–57 years) and were predominantly male (65%) and black (49.3%) or Latino (41.3%). Although 45 (60%) were admitted from an apartment, 14 (18.7%) were admitted to PSI from a correctional facility. Sixty-seven (89%) were previously imprisoned. Subjects had been residing at PSI for a median of 11.3 months before study enrollment. At study baseline, 63 (84%) of 75 subjects were receiving antiretroviral therapy. Forty-
eight subjects (64%) had CD4+ cell counts of <200 cells/mL, and 31 (41%) had HIV loads of <400 copies/mL.

**Analysis of colonization and infection isolates.** Thirty-seven subjects (49.3%) were colonized with *S. aureus* on the first nasal culture, and 61 (81.3%) had at least 1 positive culture result for *S. aureus* during the study period. Each participant had samples obtained for culture a mean (± SD) of 4.3 ± 2.9 times. On average, 51.6% (SD, ± 36%) of a subject’s nasal swabs were positive for *S. aureus*. Fourteen subjects (19%) did not have any nasal cultures positive for *S. aureus*. In total, 497 nasal samples were obtained and cultured, and 267 were positive for *S. aureus*. Three isolates were lost, resulting in a total of 264 nasal isolates available for study.

Seventeen *S. aureus* infections occurred in 13 (17.3%) of the subjects (table 1). This is a high incidence of infection in a residential patient population. Thirteen of these isolates were available for further analyses. Most of the infections were skin or soft-tissue infections. Two subjects were bacteremic. Nine subjects had documented nasal colonization before developing an infection. For 8 (88.9%) of 9 infections in which both the infection isolate and most recent nasal isolate were available, the 2 isolates were the same subtype.

**Isolate relatedness.** The PFGE profiles of the nasal isolates obtained from each subject’s first nasal culture sample could be grouped into 2 major clonal types (A and B) with various subtypes (figure 1). A dendrogram was constructed from the PFGE results of these initial nasal isolates (figure 2) that revealed that clonal type A (methicillin-susceptible *S. aureus* [MSSA]) consisted of 2 subtypes, A1 and A2, with 77% similarity. Type B, a cluster of closely related subtypes with similarities of ≥65%, consisted of 3 main subtypes: B1 (MSSA) and B2 and B3 (both methicillin-resistant *S. aureus* [MRSA]). Within these subtypes, isolates differed by 0–2 bands. Various minor B subtypes were classified as subtype B “other” (BO). Unrelated isolates were categorized as “U” isolates.

MLST and spa typing were performed on representative subtypes to provide further evidence of their relatedness (table 2). Type A isolates had the same allelic profile (ST25) and had similar spa types. Type B isolates had the same allelic profile (ST8) and were spa type 7. Subtype B1 was methicillin susceptible but was also ST8 and spa type 7, like the other MRSA B subtypes.

**Characterization of isolates.** On the first nasal culture, 11 (29.7%) of 37 colonized subjects were found to be colonized with type A isolates, and 22 (59.5%) were found to be colonized with type B isolates. Only 4 (10.8%) colonized subjects were colonized with unrelated isolates. Of the 264 total available nasal isolates, 244 (92%) belonged to either clonal type A or B. The distribution of clonal types was similar to the baseline colonization results: 36% were type A, and 57% were type B. Over the entire study period, 14 subjects (19%) never had a positive culture result, 34 (45%) were consistently colonized with the same clonal subtype, and 27 (36%) were colonized with ≥2 different clonal subtypes (table 3). Of the 13 infection

<table>
<thead>
<tr>
<th>Identification no.</th>
<th>Type of infection</th>
<th>Infection isolate subtype</th>
<th>Nasal culture result before infection</th>
<th>Nasal culture result after infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>101</td>
<td>Lip</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>121</td>
<td>Catheter sepsis—blood culture</td>
<td>NA</td>
<td>B2</td>
<td>NA</td>
</tr>
<tr>
<td>127</td>
<td>Buttock abscess</td>
<td>B2</td>
<td>B2</td>
<td>Negative</td>
</tr>
<tr>
<td>129</td>
<td>Left cheek abscess</td>
<td>B2</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>130</td>
<td>Nasal pustule</td>
<td>A1</td>
<td>A1</td>
<td>B2</td>
</tr>
<tr>
<td>131</td>
<td>Right leg abscess</td>
<td>B2</td>
<td>B2</td>
<td>A1</td>
</tr>
<tr>
<td>134</td>
<td>Ear</td>
<td>NA</td>
<td>NA</td>
<td>B2</td>
</tr>
<tr>
<td>134</td>
<td>Sepsis—blood culture</td>
<td>NA</td>
<td>Negative</td>
<td>NA</td>
</tr>
<tr>
<td>152</td>
<td>Scalp pustule</td>
<td>A1</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>152</td>
<td>Scalp pustule</td>
<td>B2</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>165</td>
<td>Leg wound</td>
<td>A1</td>
<td>A1</td>
<td>A1</td>
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<tr>
<td>165</td>
<td>Right first finger</td>
<td>A1</td>
<td>A1</td>
<td>A1</td>
</tr>
<tr>
<td>165</td>
<td>Left leg wound</td>
<td>A1</td>
<td>B2</td>
<td>A1</td>
</tr>
<tr>
<td>174</td>
<td>Wound</td>
<td>U</td>
<td>NA</td>
<td>Negative</td>
</tr>
</tbody>
</table>

**NOTE.** NA, not available.
isolates, 7 were subtype B2, and 5 were subtype A1 (1 isolate was unrelated).

**Antibiotic susceptibility.** Antibiotic susceptibility data for all isolates collected are displayed in figure 3. Subtypes A1 and A2 were oxacillin-susceptible, and none were multidrug resistant. Subtype B1 was also oxacillin susceptible and was not particularly multidrug resistant—66% of isolates were resistant only to penicillin, erythromycin, and trimethoprim-sulfamethoxazole. In contrast, subtypes B2 and B3 were both oxacillin and multidrug resistant. Of note, 93% of all isolates were resistant to trimethoprim-sulfamethoxazole. Clonal types A and B were resistant, whereas almost all trimethoprim-sulfamethoxazole—susceptible isolates were from the group of unrelated (type U) isolates.

Antibiotic susceptibility was stable among subtypes obtained by culture from any 1 patient. To demonstrate this, for each subject, the mean and range of the number of antibiotics to which a particular subtype was resistant were determined. The means of these intrasubject means and ranges were determined for each subtype as follows: A1 (2.9 ± 0.3; 0.2 [0–2]), A2 (2.8 ± 0.4; 0.2 [0–1]), B1 (3.3 ± 0.6; 0.6 [0–2]), and B2 (5.8 ± 0.7; 0.6 [0–3]).

**Analysis of mecA.** Multiplex PCR for SCCmec typing of representative B2 and B3 isolates (table 2) revealed the presence of SCCmecIV. B1 and type A isolates did not contain SCCmec.

Population analysis profiles of cultures of types B2, B3, and BO showed that they expressed resistance in a heterogeneous manner (population analysis profile expression class 2) [19]. Although the majority of bacteria were inhibited by low concentrations of oxacillin, starting at an MIC of 0.75 μg/mL, subpopulations of cells with MICs of >200 μg/mL were also present in ranges of 10^{-1}–10^{-3} cfu/mL.

Southern blot analysis confirmed the presence of mecA on the 209-kbp band in the B2 and B3 subtypes and the absence of mecA in B1 and type A isolates (data not shown). Thus, the 3-band difference between B1 and B2 was, in part, due to the absence of mecA.

By PFGE, a single MSSA isolate (isolate 5007) differed from the MRSA B2 subtype by 3 bands. On PFGE, it contained a 196-kbp band instead of the 209-kbp band that contains the mecA element. Southern hybridization indicated the absence of mecA. Multiplex PCR mecA typing of isolate 5007 amplified the 342-bp dcs region of the SCCmecA cassette but did not amplify the 162-bp mecA internal control, suggesting an imperfect excision of the mecA chromosomal cassette.

**Environmental sampling and nasal cultures from PSI personnel.** Thirty-eight environmental samples for culture were collected on 3 separate occasions. Four had positive results (iron [B2], faucet [B2], and telephones [A1 and A2]).

Thirty-five PSI employees had nasal samples obtained for

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**Figure 1.** PFGE profiles of initial nasal *Staphylococcus aureus* isolates demonstrating different clonal types. Lanes 1–5, subtype B2; lane 6, BO strain 5007 with partial SCCmecA deletion; lanes 7–12, subtype B1; lanes 13 and 17, BO isolates; lanes 14–16, subtype B3; lanes 18–21, U (unrelated) isolates; lanes 22–24, subtype A1; lanes 25–26, subtype A2; lane 27, strain Newman (methicillin-susceptible); lane 28, strain COL (contains staphylococcal chromosomal cassette mec type 1 [SCCmec]). Arrow, location of mecA.
culture 1 time. There were 4 positive results; 2 were type B clones (B1 and B2), and 2 were unrelated (1 SCCmecII MRSA, 1 MSSA) as shown by PFGE. These data likely reflect the overall prevalence of these clonal types at PSI rather than a major environmental or personnel reservoir for these clones.

**Potential risk indicators.** There were no statistically significant associations between either *S. aureus* colonization or infection and any of the potential demographic, medical, or behavioral risk factors that were examined. Change in CD4+ cell count (≤100 to >100, ≥100 to <100, <200 to ≥200, ≥200 to <200) had no effect on colonization status. The effect of relapse of illicit drug use was examined. Sixteen people expe-
rienced possible relapses (2 as determined by physician report and 14 as determined by toxicology data), although in 8 cases, the toxicology results were positive for metabolites also found in drugs used for therapeutic purposes (e.g., opiates). Relapse of drug use was not associated with the risk of colonization or infection. Subjects’ living arrangements were also examined and were not found to be associated with colonization status.

**DISCUSSION**

This study is among the first to prospectively investigate *S. aureus* nasal colonization and infection among patients with AIDS in an inpatient drug rehabilitation setting. Major findings include a very high level of both nasal colonization and infection, occurring overwhelmingly with the same 2 clonal types not only at baseline but also over the year-long study period. One clonal type was MSSA, whereas the other consisted of MRSA and MSSA subtypes, suggesting either instability or transmissibility of SCCmec IV. The MRSA subtypes are related to SCCmecIV-containing MRSA previously implicated in numerous outbreaks, especially among immunocompromised individuals [22–26].

In this study, 49.3% of subjects were found to be colonized with *S. aureus* according to the first nasal culture, and 81.3% were colonized at least once during the 1-year study. The high level of colonization in our study population relative to that in the general population (21%–37%) [27] provides further evidence that patients with AIDS with histories of illicit drug use are at high risk for nasal colonization with *S. aureus*.

In addition to the consistently high colonization rate, PSI subjects were colonized almost entirely with 2 clonal types. Although 45% were always colonized with the same subtype, 36% were colonized with ≥2 different subtypes. These clonal types were important because they were responsible for 12 of 13 infections. The 2 clonal types could simply represent common clones found in New York City (the community or jails/prisons), or they could be the result of importation and subsequent clonal dissemination in the closed PSI environment.

Clonal type A was ST25 by MLST and had similar spa types. It is likely that this is a highly transmissible MSSA, because it was identified in 29.7% of colonized subjects at baseline and continued to colonize and infect subjects over the course of the study. Clonal type B consisted of 1 MSSA subtype (B1) and 2 MRSA subtypes (B2 and B3), all of which were ST8 and spa type 7. ST8 was a successful MSSA that gave rise to another closely related clone, ST250 [13, 21]. ST250 later acquired the mecA gene to become the first known MRSA. In fact, ST8 is believed to have acquired the mecA gene at a later date [21]. Because subtype B1 MSSA is ST8 by MLST, it appears to be related to the early MSSA described by Enright et al. [21]. In addition, it is possible that the B1 MSSA subtype acquired SCCmec IV and gave rise to the B2 and B3 MRSA subtypes. Only ST8 and 1 other ST (ST5) have been found to harbor all 4 SCCmec types [21]. Therefore, ST8 may be particularly receptive to acquiring mecA.

SCCmec may also be unstable and subject to deletion from the MRSA genome. Donnio et al. [28] described an epidemic MRSA and the emergence of a virtually identical MSSA with a highly resistant antibiogram, suggesting that the MSSA resulted from the loss of SCCmec from the epidemic MRSA. Isolate 5007, described above, provides evidence for the partial excision of mecA by subtype B2 because it contained only dcs, a downstream gene in the SCCmecA cassette. These data provide further support for the instability of the mecA element.

The same SCCmecIV-containing MRSA found at PSI were identical by PFGE to those previously identified in hospitalized patients with AIDS in New York City in 1997 [23, 29]. An MRSA with the same MLST and spa type was responsible for outbreaks of infections among HIV-positive men who have sex with men in Los Angeles [22, 25] and in a Mississippi prison in 1999–2000 [24, 26]. Although these strains carry SCCmecIV

**Table 3.** Characterization of colonization isolates over study period among 75 patients with AIDS in a residential drug treatment facility prospectively followed up for *S. aureus* nasal colonization and infection.

<table>
<thead>
<tr>
<th>Colonization results over the study perioda</th>
<th>No. (%) of subjects (n = 75)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All culture results negative</td>
<td>14 (19)</td>
</tr>
<tr>
<td>Colonized with 1 clonal subtype</td>
<td>34 (45)</td>
</tr>
<tr>
<td>All</td>
<td>5</td>
</tr>
<tr>
<td>A1</td>
<td>5</td>
</tr>
<tr>
<td>A2</td>
<td>0</td>
</tr>
<tr>
<td>B type</td>
<td>25</td>
</tr>
<tr>
<td>All</td>
<td>10</td>
</tr>
<tr>
<td>B1</td>
<td>11</td>
</tr>
<tr>
<td>B2</td>
<td>1</td>
</tr>
<tr>
<td>B3</td>
<td>3</td>
</tr>
<tr>
<td>B type (B1 and B2)</td>
<td>1</td>
</tr>
<tr>
<td>A and B types</td>
<td>14</td>
</tr>
<tr>
<td>B and unrelated types</td>
<td>5</td>
</tr>
<tr>
<td>A, B, and unrelated types</td>
<td>3</td>
</tr>
</tbody>
</table>

NOTE. A1 and A2 are methicillin-susceptible *S. aureus* (MSSA) clonal subtypes. B1 (MSSA), B2 and B3 (methicillin-resistant *S. aureus*), and BO are related clonal subtypes.

a Represents subjects’ culture results over time, January–December 2001 (not co-colonization).
and exhibit heterogeneous expression of methicillin resistance, they were probably derived from a hospital-based strain of *S. aureus* that acquired SCCmecIV. The ST8 MRSA in this study is multidrug-resistant and was isolated from people with a high burden of disease and frequent contact with the health care system.

One interesting feature of both clonal types identified at PSI was that they were uniformly resistant to trimethoprim-sulfamethoxazole. It is likely that the frequent use of trimethoprim-sulfamethoxazole for prophylaxis against *Pneumocystis carinii* among patients with AIDS is responsible for the acquisition of resistance in these clonal types. Either the resistance developed in patients or the frequent use of trimethoprim-sulfamethoxazole among these patients predisposed them to colonization with these trimethoprim-sulfamethoxazole-resistant types [30–32].

Although we attempted to identify individual-level risk factors associated with *S. aureus* nasal colonization, this study was limited by a combination of several factors, including the small sample size, the high frequency of colonization, and the fact...
that all of the subjects had 1 of the strongest risk factors for colonization (AIDS) [5]. Finally, because patients at PSI were entering the institution for drug rehabilitation, PSI staff did not ask them to participate in the study on admission. This limited the ability to determine whether subjects were entering with the clonal types or became colonized with them at PSI.

In conclusion, this cohort of patients with AIDS in drug rehabilitation were consistently colonized and infected primarily with 2 clonal types. This study clearly illustrates the capacity of these 2 clones to persist over time and to cause significant disease. Once discharged, these colonized patients will constitute a reservoir of antibiotic-resistant strains in the community. Given that these clones have repeated past associations with outbreaks as well as their remarkable persistence and spread within the PSI environment, the data suggest that these clones have a unique survival advantage.

Acknowledgments


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References

