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***Staphylococcus aureus* Nasal Colonization in HIV-Seropositive and HIV-Seronegative Drug Users** [Epidemiology]

Holbrook, Karen A.*; Klein, Robert S.*; Hartel, Diana*; Elliott, David A.; Barsky, Todd B.; Rothschild, Liza H.; Lowy, Franklin D.

*Department of Medicine, Division of Infectious Diseases, and Department of Epidemiology and Social Medicine, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, New York; George Washington University, Washington, D.C.; University of Stony Brook, Stony Brook, New York, U.S.A.

Address correspondence and reprint requests to Karen A. Holbrook, Department of Medicine, Division of Infectious Diseases, Montefiore Medical Center, 111 E. 210th Street, Bronx, NY 10467, U.S.A.

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Summary

Nasal colonization plays an important role in the pathogenesis of *Staphylococcus aureus* infections. To identify characteristics associated with colonization, we studied a cross-section of a well-described cohort of HIV-seropositive and-seronegative active and former drug users considered at risk for staphylococcal infections. Sixty percent of the 217 subjects were Hispanic, 36% were women, 25% actively used injection drugs, 23% actively used inhalational drugs, 23% received antibiotics, and 35% were HIV-seropositive. Forty-one percent of subjects had positive nasal cultures for *S. aureus*. The antibiotic susceptibility patterns were similar to the local hospital's outpatient isolates and no dominant strain was identified by arbitrarily primed polymerase chain reaction (AB-PCR). Variables significantly and independently associated with colonization included antibiotic use (odds ratio [OR] = 0.37; confidence interval [CI] = 0.18-0.77), active inhalational drug use within the HIV-seropositive population (OR = 2.36; CI = 1.10-5.10) and female gender (OR = 1.97; CI = 1.09-3.57). Characteristics not independently associated included injection drug use, HIV status, and CD4 count. The association with active inhalational drug use, a novel finding, may reflect alterations in the integrity of the nasal mucosa. The lack of association between HIV infection and *S. aureus* colonization, which is contrary to most previous studies, could be explained by our rigorous control for confounding variables or by a limited statistical power due to the sample sizes.

Nasal colonization with *Staphylococcus aureus* is epidemiologically linked to an increased risk of subsequent infection (1-9). In parenteral drug users, insulin-injecting diabetic patients, and dialysis patients, nasal colonization appears to predispose to infection by providing a reservoir from which the skin is seeded. Frequent needle injections then provide a portal of bacterial entry. Although

Links

[Abstract](#)
[Complete Reference](#)



Outline

- Summary
- METHODS
 - Population
 - Microbiologic Evaluation
 - Strain Typing With Arbitrarily Primed Polymerase Chain Reaction
 - Statistical Analyses
- RESULTS
- DISCUSSION
- REFERENCES

Graphics

- Table 1
- Table 2
- Table 3
- Fig. 1

Recent History

Staphylococcus aureus Nas...  

HIV-seropositive populations have been studied, the role HIV infection plays in predisposing patients to *S. aureus* infection is not clear. Several studies describe an increased nasal carriage of *S. aureus* in HIV infected groups (10-14). Neutrophils, monocytes, and immunoglobulins from HIV-seropositive patients have impaired function against *S. aureus*(15-17), and several reports document an increased incidence of *S. aureus* infection in people with severe HIV infection (18-22). Thus, some evidence and a rationale for HIV predisposing patients to increased infection rates exist. However, many of the studies of infection and colonization contained cases with risk factors for *S. aureus* infection other than HIV disease (i.e., injecting drug use [IDU], presence of long-term intravenous catheters, lymphedema from Kaposi's sarcoma). Not controlling for these confounding variables could have led an overstatement of the role that HIV seropositivity plays in predisposing patients to *S. aureus* infection.

Another potential patient characteristic that could predispose to *S. aureus* infection is noninjecting drug use. This type of drug use, in particular inhalational drug use, which could alter the integrity of the nasal mucosa, has not been studied.

Because nasal carriage appears to be an important precondition to infection, identification of additional characteristics associated with increased carriage would improve our assessment of the risk of future disease (9). Such studies not only further our understanding of the disease process but also could allow for targeted preventive intervention. With the exception of the use of topical antimicrobial agents, limited work has been directed at prevention. The high frequency of *S. aureus* infections, their severity, and the specter of multidrug-resistant isolates underscores the importance of such work.

To further elucidate factors associated with nasal colonization by *S. aureus*, we studied a well-characterized group of active and former drug users, both HIV-seropositive and -seronegative, who were participating in a prospective study of the natural history of HIV infection. This paper describes nasal colonization data on a cross-section of this cohort. Because this study uses an epidemiologically well-described cohort of patients and multivariate analyses, it is unique by comparison with past studies in its ability to identify patient characteristics independently associated with *S. aureus* colonization. In addition to assessing colonization status and correlating this to patient characteristics, we determined the antibiotic susceptibility pattern on all strains and performed DNA strain analysis through arbitrarily primed polymerase chain reaction (AP-PCR) to determine if a dominant colonizing strain or pattern existed within the cohort.

METHODS

Population

The study sample was drawn from a cohort of current and former drug addicts recruited for an ongoing longitudinal study of the natural history of HIV disease from a hospital-affiliated methadone maintenance program in the Bronx, New York (23). After providing informed consent, patients undergo a confidential standardized interview that includes questions about their demographics, drug use and medical history. Detailed information about the type of drug, routes of administration, and frequency of use is obtained. Drugs used intravenously included heroin, cocaine, and speed (methamphetamines). Inhalation is defined as snorting but not smoking drugs. Inhaled drugs included heroin, cocaine, and speedballs, a mixture of heroin and cocaine. Recent drug use is defined as occurring within 6 months of the interview. Physical examinations are performed, and blood specimens are obtained for HIV antibody and CD4 lymphocyte counts. Reevaluations with interviews and physical exams are scheduled every 6 months.

From March 1994 to June 1995, additional information regarding recent (within 2 weeks) antibiotic use was obtained. Swabs of the anterior nares for isolation of *S. aureus* were collected from consenting patients available during periods when laboratory personnel were able to process specimens.

Microbiologic Evaluation

Nasal cultures were obtained by rotating a sterile rayon-tipped swab(Becton Dickinson, Cockeysville,

MD, U.S.A.) in one anterior nares and placing it in transport media. Subsequent processing of specimens was done by personnel blinded to clinical information including HIV status. Specimens were plated onto mannitol-salt agar within 24 hours. Positive plates were numerically graded according to the number of colonies: 0, no growth; 1, rare positive colonies; 2, many discrete positive colonies; and 3, a lawn of positive colonies. All positive cultures were confirmed by catalase and the Staphaurex test (Murex Diagnostics Limited, Dartford, Kent, U.K.), which detects clumping factor and protein A. Antibiotic susceptibilities were determined via the Kirby-Bauer disk diffusion method. Antibiotics tested included penicillin, oxacillin, cephalothin, trimethoprim-sulfamethoxazole, erythromycin, clindamycin, rifampin, ciprofloxacin, vancomycin, gentamicin, and mupirocin. Isolated colonies were grown in Todd-Hewitt broth (THB) overnight and aliquots frozen in 20% glycerol for future strain typing.

Strain Typing With Arbitrarily Primed Polymerase Chain Reaction

Bacteria from stored aliquots were streaked onto blood agar plates. Single colonies were inoculated into 5 mL THB and incubated overnight at 37°C in a gyrosaker. DNA was extracted from 500 Åµl using Qiagen tips (Qiagen Inc., Chatsworth, CA, U.S.A.). The manufacturer's protocol was modified for lysis of *S. aureus* and included lysostaphin (Applied Microbiology, New York, NY, U.S.A.) at a final concentration 15 Åµg/ml. M13 reverse sequencing primer (GGAAA-CAGCTATGACCATG) was chosen for amplification reactions based on its previous use with *S. aureus* (24). The AP-PCR was performed in 50-Åµl volumes that contained 0.25 U Taq polymerase, single strength Taq buffer (Gibco BRL, Life Technologies, Gaithersburg, MD, U.S.A.), 2.5 mmol/L of magnesium chloride, 0.2 mmol/L of each deoxynucleoside triphosphate, 5 ÅµM of primer, and 5 Åµl of template genomic DNA (total template DNA ~100 ng). The amplification procedure was modified from previously published protocols (24). After a 4-minute period at 95°C for complete denaturation, 45 low stringency cycles were performed: 94°C for 1 minute for denaturation, 34°C for 1 minute for annealing, and 72°C for 2 minutes for extension. Ten microliters of reaction mix was run on an agarose gel electrophoresis at 100 V for 2 hours and stained with ethidium bromide. Negative controls of reaction mix without template DNA and a positive control of a laboratory strain (25) were included in all experiments. Isolates were considered distinct if they consistently differed by one or more bands (24).

Statistical Analyses

After examining the distributional properties of variables, the statistical relation of each variable to colonization (binary outcome) was studied. For categorical variables, $[\chi]^2$ and Fisher's exact tests were performed. *t*-Tests for continuous variables were calculated for normally distributed data or for variables normally distributed after transformation. Multivariate analyses (logistic regression) were carried out to examine the independent association of the primary study variables, controlling for potential confounders. Variables were retained in models if they had a *p* value of 0.20 or less. Variables were considered statistically significant at an alpha level of 0.05 (two-sided). OR and their 95% CI were calculated from regression coefficients and their standard errors in logistic regression models.

RESULTS

As of February 28, 1994, 881 current and former drug users were actively participating in the longitudinal study of the natural history of HIV disease. From March 1994 to May 1995, 230 patients were enrolled into the present study. Four specimens had questionable labeling and nine visits were duplicates; thus, 217 patients were included in the analysis. The demographic characteristics of the study subjects, listed in Table 1, were not statistically different from the larger longitudinal cohort. The majority of patients were Hispanic. Thirty-six percent of those studied were women, 35% HIV-seropositive, 25% were recent injecting drug users, 23% inhalational drug users, and 23% had taken antibiotics within the past 2 weeks. Of the HIV-seropositive patients, the median CD4 count was 375/Åµl (range, 7-1557/Åµl). Of the 50 inhalational drug users, 33 snorted heroin, 30 snorted cocaine, and 4 snorted speedballs (a mixture of heroin and cocaine). Antibiotics used included trimethoprim-sulfamethoxazole (18 patients), penicillin (10 patients), ampicillin (3 patients), amoxicillin

(2 patients), dicloxacillin(3 patients), cefuroxime (2 patients), amoxicillin-clavulanic acid (1 patient), erythromycin (3 patient), ciprofloxacin (2 patients), doxycycline (1 patient), and rifabutin (1 patient).

	n (%)
Gender (female)	58 (36)
Race	
White	39 (25)
Black	26 (17)
Hispanic	103 (65)
Drug use	
Injecting	41 (25)
Inhalational	44 (23)
Antibiotic	44 (23)
HIV seropositivity	48 (33)
CD4 count <20%	14 (42)

Total number of subjects, 217; mean age of subjects, 41 years (range, 22-62 years).

TABLE 1. *Characteristics of subjects cultured for nasal colonization with Staphylococcus aureus*

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Of the 217 patients, 89 (41%) had positive nasal cultures for *S. aureus*. Of these, 73 (82%) were heavily colonized (plates graded ≥ 2). Single variable analyses revealed an association of colonization with female gender and antibiotic use. Antibiotic use provided a protective effect (OR = 0.38; CI = 0.17, 0.83). Logistic regression (Table 2) confirmed this finding and also demonstrated the association with female gender. Inhalational drug use was noted to be significantly and independently associated with colonization in the HIV-seropositive group. Within the total group, inhalational drug use demonstrated a trend toward increased colonization. Characteristics not independently associated with colonization included injection drug use, HIV status, and CD4 count and percentage.

Variable	Odds ratio (CI)	p Value
Total sample (model 1)		
Antibiotic use	0.37 (0.18-0.77)	.01
Inhalational drug use	1.63 (0.89-2.97)	.10
Female gender	1.97 (1.09-3.57)	.02
HIV-seropositive sample (model 2)		
Antibiotic use	0.34 (0.13-0.91)	.03
Inhalational drug use	2.36 (1.10-5.10)	.05

* Each model controlled for: age, race/ethnicity, gender, number of injections/month, types of drugs used, HIV status (Model 1), CD4 percent (Model 2), in addition to drug use route.
CI, confidence interval.

TABLE 2. *Logistic regression analyses: variables associated with positive nasal colonization with Staphylococcus aureus*

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The antibiotic susceptibility patterns of the *S. aureus* isolates were similar to patterns found with the local hospital's outpatient *S. aureus* strains (Table 3). Forty-one randomly selected isolates were compared by AP-PCR. Although several isolates were similar, most were unique and no dominant strain was identified. Figure 1 displays a representative sample of isolates.

	Our study	Montefiore outpatients, 1994
Total number	89	170
Penicillin G	3.3%	7%
Erythromycin	54.8%	63%
Clindamycin	97.7%	90%
Genamycin	100%	92%
Methicillin	97.7%	83%
Vancomycin	100%	100%
Ciprofloxacin	97.7%	86%

TABLE 3. *Susceptibility of isolates to antibiotics*

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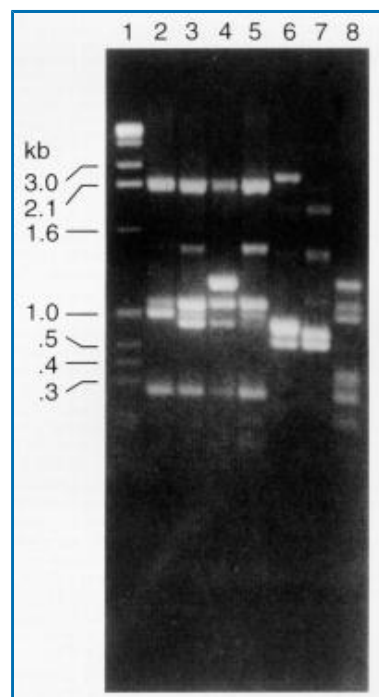


FIG. 1. Arbitrarily primed polymerase chain reaction of representative strains from the longitudinal study cohort. Lane 1, molecular size standards; lanes 2 through 8, strains from subjects in the cohort. Strains in lanes 3 and 5 are similar.

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DISCUSSION

Because nasal colonization with *S. aureus* is an important precondition to infection, investigations of the factors associated with colonization may shed light on the pathogenesis of *S. aureus* disease (2-9). In this cross-sectional study, *S. aureus* nasal colonization was independently correlated with use of antibiotics, inhalational drug use in HIV-seropositive persons, and female gender. Injection drug use, HIV status, and CD4 count were not independently associated.

The protective effect of antibiotic use is not surprising and is consistent with prior studies (12). The association of inhalational drug use with nasal colonization has not been previously described and is intriguing. It confirms the prominent role the nasal mucosa plays in *S. aureus* pathogenesis. This effect was independent of antibiotic use, implying that inhalation of drugs may defeat the protective effect of antibiotics for some individuals. The exact mechanisms by which staphylococci bind to the nasal mucosa are not known, although studies describe bacteria binding to both epithelial cells and mucin (26-28). Recent studies of the staphylococcal-mucin binding interaction suggest the presence of specific bacterial protein receptors for a carbohydrate moiety in mucin (28). Perhaps inhalational drug use alters this interaction and increases bacterial adherence. Although more work needs to be done to confirm if colonized inhalational drug users have more infections with *S. aureus*, this observation provides an opportunity to further study the pathogenesis of staphylococcal infections.

The increased colonization in women may relate to estrogen levels as suggested by a study of women that linked high estrogen levels with increased *S. aureus* nasal colonization (29). A previous survey described increased carriage in black women (30). Alternatively, unknown and uncontrolled confounding variables may explain this finding.

The lack of association with injection drug use in our study requires further study. Previous studies showing an association with injecting drug use did not examine frequency of drug injection or other routes of drug use (3). In the study by Tuazon and Sheagren (3), recent drug use (<2 weeks) was correlated with colonization. We defined drug use as occurring within the past 6 months and collected data on frequency of injection over the entire period.

Surprisingly and contrary to most other studies, we did not demonstrate an association between HIV

infection and *S. aureus* colonization. One other study also found no association of bacterial (21% was staphylococcal) nasopharyngeal colonization and HIV infection (31). However, HIV infection has been purported to be a risk factor for infection based on several retrospective studies of staphylococcal infection, one case control study, several cross-sectional colonization investigations, a prospective study of carriage, and studies documenting impaired immune function against *S. aureus*(10-22). Similar to research on other high-risk groups, these previous studies in HIV-seropositive patients demonstrate the presence of a *S. aureus* reservoir and subsequent breaks in the body's defense system. However, whether HIV infection is an independent predictor of *S. aureus* colonization and infection remains unclear. It is possible that other risk factors might explain the increased colonization and infection rates in these studies. Perhaps because our study rigorously controlled for confounding variables, the association with HIV infection was not identified. Alternatively, the lack of statistical association in our study could be due to the limited sample sizes for HIV-seropositive persons with low CD4 counts and no antibiotic use.

Although we were unable to demonstrate an association between HIV infection and *S. aureus* colonization, this issue is clinically relevant. If there is such an association, identifying a subpopulation of patients to offer prophylaxis might prevent substantial morbidity and mortality from *S. aureus* infections. Moreover, a theoretic risk of accelerated HIV replication caused by *S. aureus* membrane constituents activating HIV-1 NF- κ B sites exists (32). Prophylactic intervention has been successful in dialysis patients (5) and may already be inadvertently occurring in AIDS patients, given the standard use of trimethoprim-sulfamethoxazole for *Pneumocystis carinii* pneumonia prophylaxis and increased use of rifabutin and the azolides, azithromycin and clarithromycin, for prophylaxis of *Mycobacterium avium* intercellulare. However, development of bacterial resistance remains a potential negative effect of long-term antibiotic use (33). At present, sufficient data on appropriate antibiotic prophylaxis do not exist. Further studies should be done to address the utility and best approach to prevent staphylococcal infections in this setting.

The AP-PCR fingerprinting analyses demonstrated a heterogeneous group of isolates at the time of this study. Previous workers have described clonal dissemination resulting in outbreaks of virulent *S. aureus* in the hospital setting (34,35). The potential for such dissemination in a clinic setting clearly exists. As our HIV population lives longer, they will require more long-term antibiotic use. The risk of a dominant strain arising under this selective pressure may therefore increase.

Nasal colonization is a fundamental step in the pathogenesis of *S. aureus* infections. Thus, rigorous investigations of colonization can provide a better understanding of infection, guide basic science investigations, and ultimately lead to better prophylaxis and better treatment regimens. In the present investigation, the ability to study staphylococcal colonization in a demographically well-defined cohort using sophisticated statistical techniques resulted in several unexpected observations: an association between colonization and inhalational drug use and a lack of association between colonization and HIV seropositivity even when stratified by CD4 count. Prospective follow-up to define the long-term effects of drug use, antibiotics, or HIV infection are clearly warranted.

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