

Strains of *Staphylococcus aureus* Obtained from Drug-Use Networks Are Closely Linked

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Drug users have a higher incidence of colonization with *Staphylococcus aureus* than does the general population, and, as a result, they are at increased risk of infection with their colonizing strain. The purpose of this study was to examine the role of drug-use risk networks in *S. aureus* transmission, the similarity of *S. aureus* isolates within these networks, and the possible role of inhalation drug use paraphernalia in the transmission of *S. aureus*. Strains of *S. aureus* are shared among colonized inhalation drug users within drug-use risk networks. It suggests that patterns of drug use and the geographic location where drug sharing occurs are major contributors to the transfer of staphylococci and, as a result, to the high prevalence of staphylococcal colonization and perhaps disease in this population.

Staphylococcus aureus is the single most common cause of bacterial infection among drug users. These infections vary in severity from minor skin and soft-tissue infections to life-threatening systemic infections that include endocarditis and septic shock [1–5]. The frequency, spectrum, and severity of these infections have been extended by the HIV epidemic, the increased use of inhalation drugs in the past 25 years, and the spread of methicillin-resistant *S. aureus* (MRSA) within drug-using communities [3, 6, 7]. Numerous studies have catalogued the diversity of staphylococcal infections among drug users; however, insight into the basis for the high prevalence of *S. aureus* disease in this population is limited.

Drug users, along with people with diabetes, patients undergoing hemodialysis, and HIV-infected individuals, have an increased incidence of nasal colonization with staphylococci, and, as a result, they are at increased risk of developing infections with their colonizing strains [8–10]. Tuazon and Sheagren [4] and Tuazon et al. [11] showed that the staphylococcal strains responsible for nasal carriage in drug users, rather than strains recovered from heroin or injection drug paraphernalia, were responsible for the patients' subsequent infections. In a later study, HIV-infected inhalation drug users were found to have a higher incidence of colonization with *S. aureus* than were other drug users, raising the possibility that shared inhalation drug paraphernalia served as a vehicle for bacterial transmission [12]. Although these earlier studies identified the role of nasal colonization in subsequent infection, little information exists as to whether strain transmission among drug users who share drugs or paraphernalia contributes to the high prevalence of staphylococcal disease within this population.

The purpose of the present investigation was to examine the prevalence and patterns of *S. aureus* carriage among drug users from a geographically defined area,

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to examine the ability of molecular epidemiologic techniques to help define drug-use risk networks, and to examine the potential of inhalation drug paraphernalia to transmit *S. aureus*.

PATIENTS AND METHODS

Data Collection

During an 8-month period (June 2000–January 2001), inhalation drug users participating in a community outreach program were asked to participate in the study. Subjects were included in the study if they were ≥ 18 years of age and admitted inhalation drug use (inhalation drug use is defined as snorting ≥ 1 drug [12]). After receiving informed consent, the interviewer requested the following from study participants: (1) the completion of a brief questionnaire concerning demographic information, drug-use behavior, medical history, antibiotic use, and basic social network information; (2) the donation of used inhalation paraphernalia (e.g., a straw or matchbook); and (3) the permission to obtain a culture sample from the subject's anterior nares with a sterile cotton swab (Culturette; Becton Dickinson) to screen for *S. aureus*.

Data were obtained in 1 of 5 locations where subjects actively use drugs. Three sites were within a 4-block radius and included a crack house, and 2 sites were 10 blocks away from the crack house, adjacent to the outreach program headquarters. As part of the recruiting strategy, participants were asked to invite other individuals with whom they used drugs to participate in the study. This study was reviewed and approved by the Columbia University Institutional Review Board (New York).

Laboratory Analysis

Screening for nasal carriage of *S. aureus*. For culture, nasal samples were plated directly onto mannitol salt agar for 48 h at 37°C, followed by isolation on blood agar plates for 24 h at 37°C. Identification was confirmed by use of StaphAurex (Murex Biotech). Samples from inhalation drug paraphernalia were vortexed in 5.0 mL Todd Hewitt broth and plated directly onto mannitol salt agar. To maximize the recovery of *S. aureus*, an aliquot of this broth (0.5 mL) was incubated overnight in 5.0 mL of Todd Hewitt broth at 37°C and then processed as described above. Aliquots from positive samples were frozen at -80°C in 20% glycerol for future typing.

Antibiotic susceptibility testing was performed at 35°C on Mueller-Hinton agar (Becton Dickinson) by means of the disk diffusion technique, as outlined by the National Committee for Clinical Laboratory Standards [13]. The antibiotic susceptibility panel included vancomycin, trimethoprim-sulfamethoxazole, rifampin, penicillin, oxacillin, levofloxacin, gentamicin, erythromycin, clindamycin, cephalothin, ampicillin, and amikacin (Becton Dickinson).

PFGE was performed on *S. aureus* isolates as described else-

where [14]. An overnight culture of the test strain (37°C) was pelleted and washed, and the bacterial density was adjusted to an optical density (620 nm) of 0.025. The bacterial suspension was incorporated into agarose plugs (1.5% agarose; 20- μL plugs). The plugs were then placed in lysis buffer (6.0 mM Tris, 1.0 M NaCl, 100 mM EDTA, 0.2% sodium deoxycholate acid, 0.5% sarkosyl) containing lysostaphin (50 $\mu\text{g}/\text{mL}$; Ambi), lysozyme (100 $\mu\text{g}/\text{mL}$; Sigma), and RNase (5 U/mL) and incubated for 3 h at 37°C, followed by overnight incubation with proteinase K (1 mg/mL in 500 mM EDTA, 1.0% sarkosyl) at 50°C. After washing with Tris (10 mM)–EDTA (1 mM) buffer (pH, 7.6), the DNA-containing plugs were digested with *Sma*I (500 U/mL) for 3 h at 37°C, then loaded into the gel and separated by size with the CHEF-DR III System (Bio-Rad). The conditions for PFGE were as follows: 50 mM Tris–50 mM Borate–1mM EDTA running buffer, 6.0 V/cm, 14°C, 120° angle, and 1.0–30.0-s switch time for 23 h.

The PFGE results were entered into an archival, analytic program (Diversity Database Software, version 2.0; Bio-Rad). All DNA fragments were identified, and their sizes were determined by comparison with molecular size standards from each gel. The strain profiles were then compared using the analytical software. Analysis of strain relatedness was performed with the Dice coefficient (DC). Strain similarity was determined by means of a tolerance of $\pm 1\%$ for band size variations from different strains [15]. Strains with differences of $\leq 25\%$ were considered to be closely related [16–18]. On the basis of this analysis, a phylogenetic tree was constructed to illustrate the degree of similarity of the strains. The relatedness of the study strains was further evaluated by merging data for these isolates with a preexisting database of strains collected from an ongoing study of *S. aureus* carriage among attendees of a methadone maintenance program from an adjacent geographical area. This database contains 350 unique isolates obtained from different subjects (including former and current drug users). The PFGE profile from every tenth isolate up to a total of 28 strains from this cohort was used for comparison.

Assay for the presence of heroin or cocaine in the paraphernalia. A section of each piece of drug paraphernalia was sent for toxicology testing (performed by Bendiner & Schlesinger, New York) to verify that the apparatus was used for snorting drugs (cocaine, heroin, or both). Drug or drugs were eluted from the paraphernalia and concentrated through evaporation. This was followed by gas chromatography and selected ion monitoring. The sensitivity of the assay was 37 ng/mL for heroin and 22 ng/mL for cocaine.

Network Analysis

A “biologically linked network” was defined as isolates from either nasal cultures or paraphernalia with a PFGE profile that

was $\leq 25\%$ different when compared via the DC for pairwise comparisons.

Social network data were derived from 3 sources: the questionnaire, the recruitment strategy, and ethnographic observation of the social networks by the outreach manager from the community outreach program, who also conducted the interviews. A “social network” refers to a group of persons connected by social relationships [19]. In our study, the “social risk networks” were defined as drug users who either reported sharing drugs with another study participant or who were known to do so by the outreach manager. On the basis of both historical and current knowledge and of observations of this population, the outreach manager was able to provide specific information regarding drug-sharing partners. These data were collected independently, without the knowledge of the patients being assessed. Survey data were analyzed using SAS software, version 8 (SAS Institute).

RESULTS

Subject characteristics. Fifty-four inhalation drug users participated in this study. Thirteen individuals provided nasal samples or drug paraphernalia for culture on ≥ 2 occasions (11 subjects contributed samples on 2 occasions, and 2 subjects contributed samples on 3 occasions). The subjects’ demographic characteristics and drug-use behavior, as well as their medical information, are summarized in table 1. The subjects were predominantly of Hispanic ethnicity. Drug users inhaled heroin (60.8% of subjects), cocaine (3.9%), or both (35.3%). Most subjects (81.5%) reported using inhalation drugs for >1 year. Sixty percent stated that they shared drugs with others. Fifty-six percent stated that they also injected drugs.

Microbiologic findings for nasal swabs and drug paraphernalia. There were 55 nasal swabs and 48 pieces of paraphernalia (46 straws and 2 matchbooks) cultured from 54 drug users. Seventeen (32%) of the 54 drug users’ nasal or paraphernalia cultures were positive for *S. aureus*. These 17 drug users contributed 18 positive cultures (14 nasal cultures and 4 cultures of pieces of inhalation drug paraphernalia; the latter consisted of 3 straws and 1 matchbook). Twenty-eight of 43 paraphernalia samples assayed for heroin or cocaine were positive. Three of the 28 assay-positive straws were culture positive; 1 of the 15 assay-negative straws was culture positive. For only 1 subject were both nasal sample and paraphernalia cultures positive. It is of note that this subject’s paraphernalia was colonized with 2 closely related *S. aureus* strains. Both paraphernalia strains were analyzed by PFGE, resulting in a total of 19 samples obtained from 17 different individuals.

All *S. aureus* isolates were susceptible to oxacillin, cephalothin, gentamicin, rifampin, trimethoprim-sulfamethoxazole, and vancomycin. They were uniformly resistant to penicillin.

Table 1. Characteristics of 54 drug users assessed for *Staphylococcus aureus* carriage.

Characteristic	Value
Age, years	
Mean \pm SD	37.2 \pm 7.4
Range	24–52
Sex	
Male	42 (77.8)
Female	12 (22.2)
Race/ethnicity	
Hispanic	33 (61.1)
African American	14 (25.9)
White	5 (9.3)
Other	2 (3.7)
Drug use ^a	
Heroin only	31 (60.8)
Cocaine only	2 (3.9)
Both cocaine and heroin	18 (35.3)
HIV positive	10 (18.5)
Use of inhalation drugs for >1 year	44 (81.5)
History of injection drug use ^b	25 (55.6)
History of staphylococcal infection	2 (3.7)
Antibiotic use in 2 weeks before study	5 (9.3)
Medical conditions	
Skin infections	7 (13.0)
Complications of inhaling drugs	6 (11.1)
Nose bleeds	4 (7.4)
Sinusitis	4 (7.4)
Hepatitis	4 (7.4)
Shared drugs ^c	
Yes	29 (60.4)
No	19 (39.6)

NOTE. Data are no. (%) of subjects, unless otherwise indicated.

^a Data missing for 3 patients.

^b Data missing for 9 patients.

^c Data missing for 6 patients.

A single isolate was resistant to clindamycin, erythromycin, and levofloxacin.

Results of PFGE. The 19 isolates were analyzed using PFGE (figure 1). Four biologically linked groups of strains were identified (group A, 5 isolates, which contained 2 phenotypically distinct paraphernalia isolates from 1 subject; group B, 4 isolates; group C, 2 isolates; and group D, 2 isolates). There were 2 sets of identical isolates in group A (one set with 3 isolates and the other with 2 isolates, with a DC of 96.6 relatedness between the 2 sets). The 4 isolates in group B had varying DCs in comparison with one another, but all were considered to be very closely related, with DC values of 82.8–93.3. The isolates from groups C and D were closely related to each other within each group, with DCs of 90.3 and 86.7, respectively.

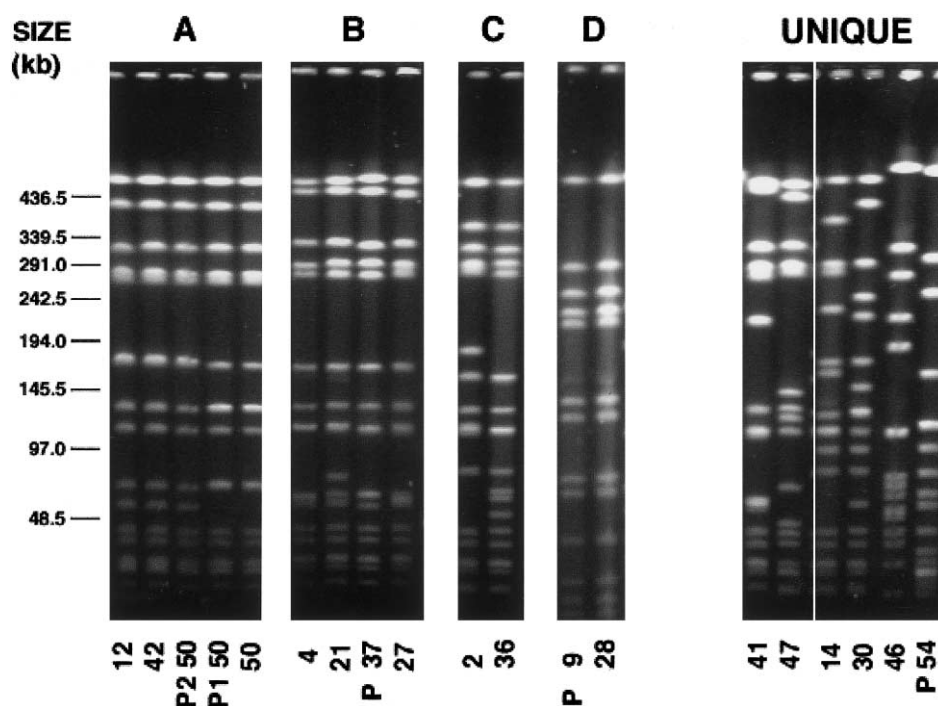


Figure 1. PFGE of all isolates found to be *Staphylococcus aureus* on culture. The 4 groups of related strains are grouped separately and identified by letter. Unique strains are on the right. Numbers on the bottom refer to subjects. P, paraphernalia.

To demonstrate the extent of similarity of the 4 biological networks, *S. aureus* isolates recovered from attendees at a methadone maintenance program were combined with the isolates from this study to see whether the former isolates randomly distributed within the latter database (figure 2). The resulting dendrogram illustrates that the biological linkages were preserved without the other strains intervening, and, in fact, the majority of the strains from this study remained closely nested within the entire sample of isolates.

Drug-use network analysis. There were 14 social networks identified, accounting for 36 of the 54 drug users interviewed. The size of the networks varied from 2 to 4 people. Eighteen individuals remained unlinked to other drug users (i.e., they were social isolates). The 5 locations where subjects were approached and where drug use occurred are summarized in figure 3. The crack house used by most of the subjects (30 [56%] of 54) was the epicenter of the biological networks, with ≥ 1 member of each biological network frequenting the site. Seven (41%) of 17 drug users with cultures positive for *S. aureus* reported that they shared paraphernalia with others. Two of the 4 positive paraphernalia samples came from subjects who shared paraphernalia. Of the culture-negative users, 22 (60%) of 37 claimed to share their drugs or paraphernalia in general.

The results of the biological and social network analyses are summarized in figure 3. The data for subjects with negative culture results are only displayed when these subjects are socially linked to individuals with a positive nasal or paraphernalia

culture. Ten of the 17 positive subjects were contained within 7 social networks. Seven positive subjects were social isolates (i.e., they were not linked to any other drug users). Only 2 of the biologically linked (PFGE) networks were also identified in the social drug-use networks. Thus, the PFGE profiles suggested 2 additional linkages that were not otherwise identifiable. For example, subjects 12 and 50 (group A) were first linked by PFGE, and the outreach manager, on the basis of her knowledge of this drug-use network, independently linked these drug users. Two other biological linkages (B and C) were linked by use of the crack house.

DISCUSSION

To our knowledge, our study is the first to have investigated staphylococcal carriage and transmission among drug users outside a clinical or hospital environment in a nonepidemic setting. Three intriguing observations result from this study. First, the striking similarity of strains recovered from these drug users suggests that drug-use behavior, combined with the social organization of drug use, is critical to our understanding of staphylococcal transmission within this population. Second, molecular epidemiologic analysis complemented and expanded the understanding of strain transmission within social networks. Third, inhalation drug-use paraphernalia was identified as a potential vehicle of transmission of staphylococci.

PFGE identified 4 networks of drug users with related strains.

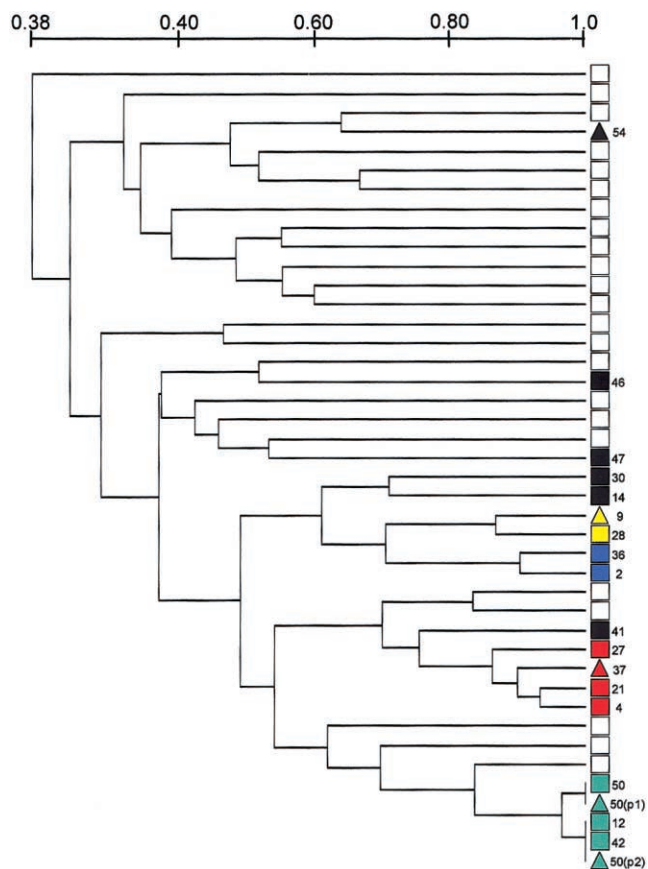


Figure 2. Dendrogram illustrating strain similarity of *Staphylococcus aureus* isolates analyzed by PFGE. Squares, nasal cultures; triangles, paraphernalia cultures; green, group A isolates; red, group B isolates; blue, group C isolates; yellow, group D isolates; black, unique isolates; white, isolates from the methadone clinic.

The similarity of the study strains was illustrated when these networks remained closely linked after integration with a sample of isolates selected from a cohort of current and former drug users attending a methadone clinic in an adjacent neighborhood (figure 2). The biological networks identified by PFGE were further strengthened by the susceptibility of the isolates to methicillin. Unlike MRSA, which evolved from a small number of genetic events and therefore has a more limited number of PFGE patterns, methicillin-susceptible strains of *S. aureus* are genetically diverse and are therefore less likely to appear related [20]. In contrast with the biological networks, the social linkages were more diffuse, with 7 networks and 7 independent isolates. Only 2 of the biologically identified networks were also identified by means of social network methodology, illustrating the complementary role of molecular epidemiologic techniques in this type of investigation.

Earlier reports documenting outbreaks of bacterial infections among drug users also suggest that drug-use behavior is a major risk factor for the transmission of infectious diseases. The first

community outbreak of infection with MRSA in the United States occurred among drug users in Detroit [7, 21]. Person-to-person spread, possibly via contaminated needles, was a suspected mode of transmission during the Detroit outbreak of infection [7]. Craven et al. [22] reported a clonal MRSA outbreak among injection drug users that was linked to a “shooting gallery” (i.e., a place where injection drug users gather to inject drugs). These earlier outbreak investigations established the link between colonization and infection in drug users, as well as the potential for dissemination of strains among drug users who share drugs or paraphernalia or who use drugs communally in settings that are specifically for drug use (e.g., shooting galleries).

Studies in which molecular epidemiologic methodology is integrated with social network analysis to investigate the basis for transmission of infectious diseases are uncommon. The

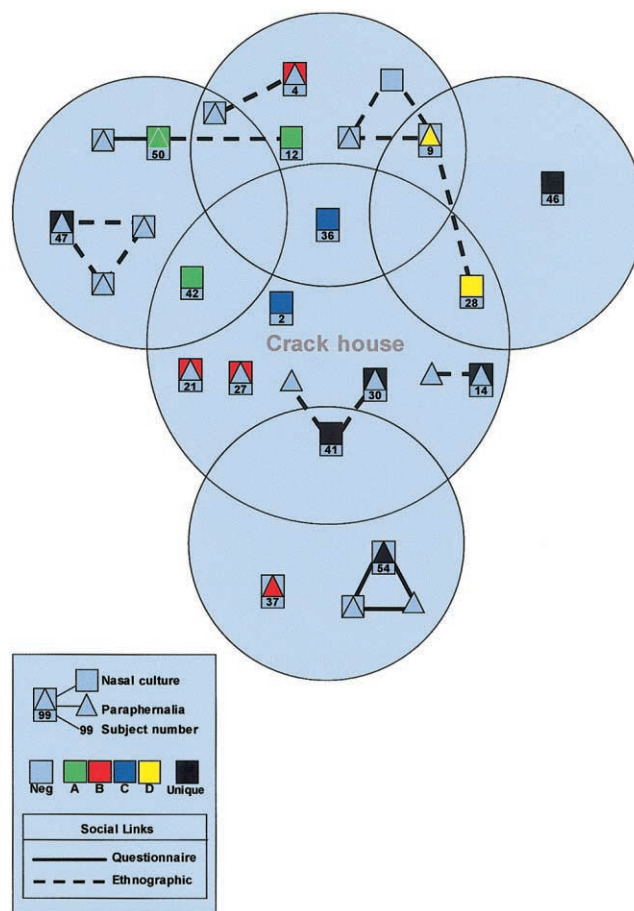


Figure 3. Linkage of culture-positive study participants, based on ethnographic, interview, and molecular epidemiologic information. Placement of subjects was based on where the subject was interviewed and where the outreach manager indicated that a person used drugs or lived. Data from subjects with negative culture results are only displayed when the subjects are socially linked to individuals with positive nasal or paraphernalia culture.

biological linkages established by PFGE defined drug-use networks, some of which were identified by social network data. Social network studies describe relationships among individuals in a population. The integrity of such information has been limited when it has relied on personal reports, particularly with regard to information on topics of a sensitive nature, such as sexual activity [23] or drug-use practices [22, 24], that is derived solely from interviews. Ward et al. [25] recently used *opa* typing of gonococcal strains to identify transmission links that could not be identified by means of social network analysis alone. Similarly, Klovdahl et al. [19] combined methods from molecular biology, epidemiology, and network analysis to document an outbreak of tuberculosis.

Our study illustrates the importance of this approach, demonstrating the similarity of strains among drug users who were interviewed in a limited geographic area. Furthermore, the integrated approach identified a likely epicenter of transmission. Representatives from all 4 biologically linked networks were found in the crack house. Our study differs from the others in that it traces carriage, not infection, in a nonoutbreak situation. It shows the potential for strains of *S. aureus* to become resident in a population, serving as potential reservoirs for future infections and further transmission.

We are unaware of other studies that have examined the potential contribution of inhalation drug use to infection, let alone the transmission of *S. aureus* as a basis for subsequent colonization. Holbrook et al. [12] found an increased rate of nasal colonization with *S. aureus* among HIV-infected inhalation drug users, which suggests that inhalation drug paraphernalia play a potential role in transmission. Unlike earlier studies of injection drug paraphernalia [11, 26, 27], 4 pieces of inhalation paraphernalia were culture positive in the present study. This is likely related to the high density of staphylococci in the nares of carriers and the ability of staphylococci to survive for a prolonged period on environmental surfaces. These data support the notion that paraphernalia are a potential means of strain transmission. Possible important variables that were not assessed in the present study and that warrant further investigation include the frequency and nature of drug use, skin cleaning behavior, the presence or absence of open skin lesions, and HIV infection status.

Although the *S. aureus* strains isolated from this network of drug users were susceptible to most antimicrobials, other drug-use networks are likely to serve as reservoirs for antimicrobial-resistant strains of *S. aureus* (such as MRSA) in the community [21]. Exposure of drug-using network members to individuals with AIDS, recent hospitalization, or use of antibiotics (either prescribed or self-administered) will increase the likelihood of resistance emerging in the drug-use network and beyond. Among a San Francisco population of urban poor persons with

a history of injection drug use, 26.1% were colonized with *S. aureus*, 20.2% of whom were colonized with MRSA [28].

Data from the current study should be interpreted with caution, because detailed social network data were not collected, and it is possible that more of the biological linkages could have been supported by additional data. Furthermore, the toxicology assays performed on the paraphernalia samples (43 of the 48 samples were tested) did not confirm that all of the donated samples were used. One negative sample was positive for *S. aureus*, perhaps suggesting a role for hands in the transfer of staphylococci. Subjects were often high, in a hurry, unwilling to provide information regarding their networking and behavior practices, or suspicious of our intentions. Others have noted these limitations when working with this population [22, 24]. Although the ability to define the drug-use networks in this study was limited as a result of the constraints of data collection in an open environment, sufficient information was available to demonstrate the defining role of a specific crack house in strain acquisition and transmission and the power of molecular analysis as a complement to social network methodology in identifying the extent and spread of similar staphylococcal isolates.

The present investigation demonstrates that drug users remain a potential urban reservoir of *S. aureus*. Transmission of strains within this group appears to be linked to drug-use behavior, the social organization of drug-use networks, and the environment in which communal drug use occurs. This report illustrates the value of combining molecular epidemiologic tools with social network methodology. The use of a multifaceted approach to identify social relationships resulted in the demonstration of far more extensive linkages than was suggested by ethnographic data or than was anticipated at the outset of the investigation. Ultimately, the transmission of strains within this high-risk group increases the chance of subsequent infection [4, 8, 9], particularly in HIV-infected subjects [6]. Further studies incorporating molecular epidemiologic tools to pursue the issues of *S. aureus* transmission, colonization, and disease within drug-use networks are likely to yield important information on the pathogenesis and prevention of staphylococcal disease in this population. This study adds to the growing body of research that integrates molecular biology, epidemiology, and social network methodologies in an effort to understand the transmission dynamics of infectious diseases in drug-using populations.

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