Social network based recruitment successfully reveals HIV-1 transmission networks among high risk individuals in El Salvador

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Abstract

Objective—HIV in Central America is concentrated among certain groups such as men who have sex with men (MSM) and female sex workers (FSW). We compared social recruitment chains and HIV transmission clusters from 699 MSM and 757 FSW to better understand factors contributing to ongoing HIV transmission in El Salvador.

Methods—Phylogenies were reconstructed using pol sequences from 119 HIV-positive individuals recruited by respondent driven sampling (RDS) and compared to RDS chains in three cities in El Salvador. Transmission clusters with a mean pairwise genetic distance ≤0.015 and Bayesian posterior probabilities=1 were identified. Factors associated with cluster membership were evaluated among MSM.

Results—Sequences from 34 (43%) MSM and 4 (10%) FSW grouped in 14 transmission clusters. Clusters were defined by risk group (12 MSM clusters) and geographic residence (only one spanned separate cities). In 4 MSM clusters (all n=2), individuals were also members of the same RDS chain but only 2 had members directly linked through recruitment. All large clusters (n ≥3) spanned more than one RDS chain. Among MSM, factors independently associated with
cluster membership included recent infection by BED assay ($P=0.02$), sex with stable male partners ($P=0.02$), and sex with $\geq 3$ male partners in past year ($P=0.04$).

**Conclusions**—We found few HIV transmissions corresponding directly with the social recruitment. However, we identified clustering in nearly one half of MSM suggesting RDS recruitment was indirectly but successfully uncovering transmission networks, particularly among recent infections. Interrogating RDS chains with phylogenetic analyses may help refine methods for identifying transmission clusters.

**Keywords**

HIV-1; molecular epidemiology; phylogenetic; respondent-driven sampling; transmission networks; El Salvador

**INTRODUCTION**

Despite considerable improvements in access to testing and treatment for HIV/AIDS throughout Central America over the past decade, targeted prevention efforts among high-transmission settings remain at low-levels.\(^1\) Prevention in these settings, particularly among men who have sex with men (MSM) and female sex workers (FSW) is hampered by the continued stigma and discrimination surrounding HIV among the general population. In El Salvador, although the HIV prevalence is low overall ($<$1% in the general population), a significant concentrated epidemic was documented in 2002 among MSM (15.5%) and FSW (3.2%).\(^2\) Repeat surveillance in 2008 revealed little change in HIV prevalence among these high risk groups.\(^3\)

Recently, more innovative recruitment methods have been employed in El Salvador to reach high risk groups. Respondent driven sampling (RDS), a chain referral method often used to recruit hard-to-reach or “hidden” populations,\(^4\) has been successfully implemented for HIV biological and behavioral surveillance in international settings.\(^5\) In 2008, RDS was implemented in El Salvador to estimate the size of the MSM and FSW population,\(^5\) assess HIV and sexual transmitted infection (STI) prevalence rates, and to gain information on high-risk behaviors in these groups.\(^3\) Although many studies have utilized RDS for HIV surveillance, few have integrated molecular data from HIV-positive participants to better understand relationships between social recruitment chains and HIV transmission networks.\(^7,8\) Typically in RDS, recruitment is performed from social rather than sexual networks. However, the RDS social recruitment chain takes advantage of potential networks where HIV and other STIs are transmitted. HIV-1 sequence data can be exploited to reconstruct transmission networks based on sequence similarity and inference of common ancestors using phylogenetic methods.\(^9\)

A better understanding of the relationship between social recruitment and groups of individuals who share related HIV strains (transmission clusters) may assist in the design of intervention programs including the assessment or refinement of recruitment methods. The objective of this study was to reconstruct transmission networks among HIV-positive MSM and FSW in El Salvador who were recruited by RDS and compare the relationship between these networks to their RDS recruitment chains. Clinical and demographic data were
evaluated to assess factors associated with membership in HIV transmission clusters among MSM.

**METHODS**

**Study Design and Population**

We conducted a cross-sectional analysis of HIV-positive individuals who were recruited through RDS in El Salvador during 2008 and had a specimen available for genotypic sequencing. From March to September 2008, MSM and FSW residing in three cities in El Salvador were recruited during an integrated biological and behavioral survey implemented to assess HIV/STI prevalence rates and to collect behavioral data. Recruitment and data collection methods for this survey, the Encuesta Centroamericano de Vigilancia de VIH y Comportamiento en Poblaciones Vulnerables (ECVC), have been previously described in detail.³ MSM were men who had anal sex with another man in the past 12 months; they were recruited in San Salvador and San Miguel. FSW were women who reported exchanging sex for money in the past 30 days; they were recruited in San Salvador and Sonsonate. Individuals in both groups were ≥18 years old, signed informed consent, and also lived and worked in the city in which they were recruited.

To initiate RDS recruitment of peers, various ‘seeds’ diversified with respect to age, self-identification of sexual orientation and income levels were located through local organizations working with the populations. Eleven seeds were used in San Salvador and five in San Miguel for MSM. Ten seeds were selected in San Salvador and five in Sonsonate for FSW. Participants were asked to recruit three other individuals from their social network or peers, and each new recruit was asked to continue this process, or “waves” of recruitment. Recruits who presented a valid coupon to a study site were screened for eligibility and provided information about the study. They were then asked to provide written informed consent. They responded to questions in a computer-based as well as a face-to-face interview and were asked to submit blood, urine and anal or vaginal swab samples. Participants were not directly asked about sexual contact with the individuals they recruited. All participants received promotional materials, condoms and a choice of other incentives (T-shirt, towel, make-up kit, lubricants, and additional condoms) for each recruited peer they referred (up to three). Recruitment was tracked using RDS coupon numbers; names were not collected.

HIV testing was conducted with two rapid HIV tests (Determine HIV-1/2 and OraQuick Rapid HIV-1 Antibody Test); positive results were confirmed with HIV ELISA and/or Western blot. All reactive sera were analyzed with the BED test HIV-1 EIA capture assay (BED-CEIA; Calypte Biomedical Corp., Lake Oswego, OR, USA) to estimate probable duration of infection. Recent infections were defined as a confirmatory OD-n of <0.8 on the BED-CEIA, indicative of a mean seroconversion period of 162 days (95% CL, 146-179 days). Patients were also screened for STIs using polymerase chain reaction (PCR) for *Trichomonas vaginalis*, *Mycoplasma genitalium*, *Neisseria gonorrhoeae*, and *Chlamydia trachomatis*. Active syphilis was defined as a reactive rapid plasma reagin (RPR) >1:8 and positive *Treponema pallidum* hemagglutination assay (TPHA).
HIV-1 Sequences and Phylogenetic Inference

Population based sequencing of the full protease and partial reverse transcriptase gene was performed by previously described in-house methods.\textsuperscript{13,14} Sequences were aligned using Clustal X and edited manually with removal of gapped positions.\textsuperscript{15} Subtypes and recombination were assessed using Subtype Classification Using Evolutionary Algorithms (SCUEAL).\textsuperscript{16} Subtyping was confirmed with phylogenetic analysis using Mega v4 and the neighbor-joining method with GTR-$\Gamma$ estimated distances and 1000 bootstrap replicates.\textsuperscript{17} The sequences most closely related to each one of our sequences were downloaded from the Los Alamos National Laboratory (LANL) HIV database (http://www.hiv.lanl.gov) using BLAST. At the time of our search in 2011, no sequences from El Salvador were deposited in LANL. The BLAST search generated 30 unique sequences, and these were incorporated in the phylogenetic analyses. Additionally, 4 more HIV-1 B-subtype references were included, accession numbers: IT_AF251949, FR_L31963, FR_D86069, and B.FR.83.HX.

Potential transmission clusters involving highly related sequences were first identified as clades with $\geq 2$ sequences on the neighbor joining tree with bootstrap support $\geq 90\%$ and mean intra-cluster pairwise genetic distance $\leq 0.015$ nucleotide substitutions per site. Neighbor joining trees were reconstructed in MEGA 4.0 with the Tamura-Nei model.\textsuperscript{17} Clusters were then confirmed using Bayesian methods in MrBayes\textsuperscript{18} using the GTR-$\Gamma$ model with a proportion of invariant sites. The model was run to $40 \times 10^6$ generations, sampling every 1000th generation, and with a 10% burn-in. Convergence of the estimates was evaluated with generation vs. log probability plots in Tracer v.1.5\textsuperscript{19} using an Effective Sample Size $>200$. A maximum clade credibility tree was generated using TreeAnnotator in BEAST.\textsuperscript{20} Clusters with posterior probabilities=1 were considered robust. Analyses were also repeated after removing sites associated with major drug resistance mutations to avoid theoretic convergent evolution, which showed similar topology (data not shown). Mutations associated with transmitted drug resistance (TDR) were identified using the 2009 standardized surveillance list from the World Health organization.\textsuperscript{21}

Social Network and Statistical Analyses

Social network data were visualized using NetDraw (Analytic Technologies, Lexington, KY) to visualize recruitment waves among participants originating from each seed. The outcome of interest was membership in phylogenetic clusters. Descriptive statistics were used to assess the relationship between the RDS recruitment chains and membership in phylogenetic clusters. Bivariate associations between characteristics of interest including demographics, prevalence of STIs and selected behaviors among MSM in clusters compared to those not in clusters were obtained using chi-squared for categorical variables and Kruskal-Wallis for continuous variables. Independent predictors of membership in MSM clusters were assessed using log-linear binomial regression. Prevalence ratios (PRs) were calculated instead of odds ratios to avoid overestimation of the relative risk because our outcome was common ($>10\%$).\textsuperscript{22} Data were analyzed with Stata version 11.0 (StataCorp, College Station, TX). All analyses were done unweighted without adjusting for RDS recruitment and therefore findings are not generalizable beyond the sample.

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RESULTS

Characteristics Study Population

In total, 824 MSM and 848 FSW were recruited during the RDS in 2008. Of those participants consenting to HIV testing, 95/699 (13.6%) of MSM and 46/787 (5.8%) of FSW tested HIV-1 positive. Among these 141 HIV-positive participants, 119 (84%) had specimens available for genotypic sequencing. The participants who did not have sequences were similar to those with sequences by risk group (66% vs. 67% MSM) and residence (83% vs. 81% from San Salvador). Individuals without sequences appeared randomly distributed in the recruitment chains; approximately one-half of the chains included at least one individual without a sequence.

Of the 119 participants with sequences, 90 (67%) were MSM and 39 (33%) were FSW. All sequences were subtype B and have been deposited in GenBank (Accession # KC412724-KC412842). The majority of both risk groups were recruited in San Salvador, however the groups differed by several characteristics (Table 1). MSM were more likely to be younger (median 24 years [IQR 21-29] vs. 32 [IQR 26-38] for FSW) and have recent infection (24% vs. 0%) than the FSW. Rates of STIs at the time of recruitment were higher among FSW (59% vs. 23% MSM), likely due to high rates of *Mycoplasma genitalium* and Trichomonas. However, only MSM had active syphilis (11%). Only two participants reported use of ART. The prevalence of transmitted drug resistance was similar (9% MSM vs. 10% FSW) with K103N being the most frequent mutation (6 of the 11 sequences with drug resistance).

Identification and Characteristics of Phylogenetic Transmission Clusters

We identified 14 transmission clusters involving 38 (32%) sequences on the neighbor-joining tree with >90% bootstrap and ≤.5% mean intra-cluster genetic distance. All clusters remained robust with high support of the nodes in the Bayesian analysis (Figure 1). The mean cluster size was 3 members (range 2-6) with 72% in dyads. Median intra-cluster genetic distance was low at 0.005 substitutions per site (IQR 0.003-0.012), indicating that sequences were highly related. Clusters were defined by risk group and recruitment city (Table 2). Not surprisingly, MSM were more likely to be in clusters (43% vs. 10% FSW; \(P\textless0.001\)). However, four sequences from FSW formed two separate cluster dyads and one dyad spanned two cities. Among MSM, similar rates of cluster membership were seen between men recruited in San Salvador (44%) and San Miguel (35%) though no clusters spanned both cities. While no sequences from both MSM and FSW clustered together at the 1.5% genetic distance cutoff, more distantly related clusters are noted where both risk groups share a robust common ancestor (Figure 1).

Comparison to Social Recruitment Chains

In the RDS recruitment chains, 12/16 (75%) MSM and 13/15 (87%) of the FSW seeds recruited HIV-positive individuals. Of these, all 12 MSM and three of the FSW recruitment chains included at least one participant who was also a member of a transmission cluster. Recruitment in an earlier wave of the chain may indicate more network ties compared to recruitment in later waves, which may be expected for members of transmission clusters. However, the median number of waves until recruitment into the sample was similar for
HIV-positive individuals who were members of the transmission clusters compared to non-members (5 waves [IQR 3-9] vs. 6 [IQR 4-9]; \( P = 0.73 \)). Among the 14 transmission clusters (labeled A-N), half included at least two members recruited from the same social chain, and four MSM dyads were composed of members of the same chain (Table 2). However, the two members were directly linked through the RDS recruitment in only two of these dyads. The most successful RDS chain at identifying clusters had 65% (11 of 17) HIV-positive MSM who were members of clusters; however, these spanned 6 discrete clusters (Figure 2). Overall, participants with recent infection tended to be in clusters that included members of their same RDS chain compared to those with chronic infection (67% vs. 35%, respectively; \( P = 0.07 \)). Finally, to assess whether RDS tended to enrich the network sample for MSM clusters, we evaluated the association between clustering and the participants’ reported personal network size (number of MSM the participant estimates to know). Men who were cluster members were significantly more likely to report larger networks than non-members (median 13 persons [IQR 7-20] vs. 5 [IQR 3-10]; \( P = 0.006 \)).

**Characteristics Associated with Cluster Membership among MSM**

Among MSM, 43% were members of phylogenetic clusters. On bivariable analysis, these patients were significantly more likely to be younger, have recent infection, and report any sex (protected or unprotected) with a stable male partner over the past year (Table 3). In the multivariable regression analysis (adjusted for variables \( P < 0.1 \) in the bivariable analysis), recent infection (PR 1.59 [95% CI 1.07-2.34], \( P = 0.02 \)), sex with stable male partner (PR 1.69 [95% CI 1.08-2.54], \( P = 0.02 \)), and sex with \( \geq 3 \) male partners in past year (PR 1.70 [95% CI 1.02-2.80; \( P = 0.04 \)]) were independently associated with cluster membership. No associations were found with illicit drug use and, of note, injection drug use was uncommon with only one MSM reporting injection of cocaine or heroin in the past year.

**DISCUSSION**

Our study demonstrates that respondent-driven sampling not only identified many HIV-positive individuals but by using phylogenetic analysis, also provides important insight into local HIV transmission in El Salvador that would not have been apparent from participant history alone. HIV sequences from one-third of the study population grouped into 14 highly related transmission clusters using stringent criteria including short genetic distance and Bayesian posterior probabilities. For MSM, 44% were found in clusters, indicating that RDS recruitment was successfully identifying transmission networks. Cluster members were more likely to be recently infected and report both stable partners and a higher number of partners than men who were not in clusters. Altogether, this combination of social and molecular data could help target prevention interventions for these high risk groups as well as assist in the refinement of social recruitment methods to better enable detection of transmission networks.

To our knowledge, compared to previous studies, we have analyzed phylogenetically the largest number of HIV sequences with RDS chains to date. We found very few individuals who were members of the same HIV transmission cluster and who were directly linked in the social recruitment chains. These results are similar to the few other studies which
compared RDS chains to HIV\textsuperscript{7,8} and/or hepatitis C viral phylogenies.\textsuperscript{8,23} Among 18 HIV-positive MSM recruited in Croatia, no direct linkage with RDS recruitment and genetic relatedness was found.\textsuperscript{7} Similarly, among injection drug users recruited in Canada, very few of the 18 HIV-positive individuals with clustered sequences were directly linked in the RDS chains.\textsuperscript{8} The discordance between direct social recruitment and genetic relatedness studies that use HIV-1 phylogenetics to interrogate RDS chains is likely due to imperfect alignment of social and sexual/transmission networks. Social networks are dynamic - the RDS represents a snapshot of the current social network while phylogenetic clusters represent historical transmission networks. RDS studies also limit the number of recruits for each participant, and usually recruits are not restricted to sexual partners, but to any social contact such as in our study. These limitations may result in a partial description of the social or sexual network. Unlike contact networks, the reported social or sexual connections between all the participants in the RDS recruitment are not collected; another limitation of our study which prevents us from evaluating the overlap between sexual and social networks. Thus, many participants in our study who were not directly connected in the RDS chain may have indeed been direct sexual contacts, as is indicated by the phylogenetic analysis. Limiting RDS recruitment to sexual partners rather than social contacts may enhance the ability to find recruitment chains more closely aligned to phylogenetic clusters.

Nonetheless, the high degree of phylogenetic clustering seen among MSM in our study supports the view that RDS may be a choice method for penetrating the networks HIV-positive MSM. This is suggested by the finding that larger personal networks were associated with increased cluster membership among MSM. However, due to lack of a comparison group in our study, it is unclear whether RDS would be superior to other recruitment methods at finding phylogenetic clusters. In our study, this method seems particularly beneficial in the case of identifying incident HIV infections as cluster members were more likely to be recently infected compared to non-members. The phylogenetic reconstruction of HIV sequences offers a unique view on the movement of the virus within the population which provides an estimate of the transmission network structure. The fact that several highly related clusters were found shows that the population sampled is sexually networked and that ongoing transmission is occurring locally. However, there are several limitations to be noted when interpreting phylogenetic trees. The direction of transmission cannot be determined in these types of analyses and there may also be unrecognized third parties involved in the transmission chain. Transmission cluster members may go unrecognized through incomplete sampling because of unavailability of HIV sequence or if individuals are undiagnosed, refuse HIV testing, or are not linked to care. For example, the FSW in our study were very unlikely to have sequences which clustered. This is expected because partners of FSW were not included in the study, so half of each FSW transmission event is missing from the phylogenetic analyses. However, identifying clusters is possible where FSW share similar transmission sources. We describe two such cases in our study, of which one cluster spanned cities of Sonsonate and San Salvador.

The HIV and STI prevalence among high risk groups such as MSM in El Salvador, as in other Central American countries, remains unacceptably high. Nearly 14% of MSM recruited in the RDS were HIV-infected and many were likely recently infected. Most of these recent infections were among young men, a group of high concern for an expanding
epidemic. Similar HIV prevalence rates have been reported among MSM in the surrounding countries of Guatemala (12.1%) and Honduras (12.4%). The high level of stigma surrounding both homosexuality and HIV in Latin America presents a significant barrier to reaching this group for prevention. Fortunately, ART coverage for eligible patients is high in El Salvador with 96% reported coverage in 2008. The prevalence of transmitted drug resistance in our sample is strikingly similar to developed countries, reflecting this high ART coverage. Altogether, this information further emphasizes the need to diagnose and link patients to clinical care (especially young men) to take advantage of the morbidity and mortality benefits of ART, decrease onward HIV transmission, and limit onward spread of transmitted drug resistance.

Better methods are needed to effectively control HIV transmission at the population level. For these methods to be successful, at-risk persons must to be identified either before or early in the course of infection to take advantage of interventions known to be successful at the individual level such as early ART, circumcision, and condom use. Social recruitment methods offer ways at finding those persons who may not only be at highest risk of infection, but may also be the hardest to reach for prevention campaigns. Integrating molecular tools, such as the phylogenetic reconstruction of HIV sequences, with social recruitment data can be a complimentary method to detect the transmission networks driving local epidemics. This integrated approach has the potential to be a powerful tool in assessing the success of different types of recruitment methods, including time-location or venue-based recruitment, to detect HIV transmission networks.

**ACKNOWLEDGMENTS**

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Figure 1. Bayesian phylogenetic tree based on pol sequences from MSM (n=80) and FSW (n=39, shown with black dots) who were identified with HIV-1 infection during respondent-driven sampling (RDS) recruitment in 2008.

Transmission clusters were identified with a mean intra-cluster pairwise genetic distance ≤0.015 nucleotide substitutions per site and were confirmed with Bayesian posterior probability=1 (shaded circles labeled A-N). Colored circles correspond to individuals involved in the example RDS chain shown in Figure 2. Nodes with posterior probabilities >0.90 are indicated. Control sequences from the Los Alamos HIV database are labeled by accession number.
Figure 2.
Respondent-driven sampling (RDS) chain demonstrating the most successful chain at identifying phylogenetic transmission clusters. The RDS chain started from one MSM seed in San Salvador and resulted in the recruitment of 134 men. Arrows indicate the direction of recruitment. Eleven men belonged to six discrete clusters (noted by colored circles which also correspond to the phylogenetic clusters indicated in Figure 1. However, none of these men were directly linked in the RDS chain. *Indicates three HIV-positive participants without sequences.
### Table 1
Characteristics of 119 HIV-positive individuals recruited by respondent driven sampling in El Salvador.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>MSM % (n/N)*</th>
<th>FSW % (n/N)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Patients</td>
<td>80</td>
<td>39</td>
</tr>
<tr>
<td>Age ≤25 years</td>
<td>66 (53/80)</td>
<td>21 (8/39)</td>
</tr>
<tr>
<td>Recruitment City</td>
<td></td>
<td></td>
</tr>
<tr>
<td>San Salvador</td>
<td>79 (63/80)</td>
<td>85 (33/39)</td>
</tr>
<tr>
<td>Other†</td>
<td>21 (17/80)</td>
<td>15 (6/39)</td>
</tr>
<tr>
<td>Recent Infection</td>
<td>24 (19/80)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>ART naive</td>
<td>98 (78/80)</td>
<td>100 (39/39)</td>
</tr>
<tr>
<td>Drug Resistance Mutation</td>
<td>9 (7/80)</td>
<td>10 (4/39)</td>
</tr>
<tr>
<td>Any STI</td>
<td>23 (23/79)</td>
<td>59 (22/37)</td>
</tr>
<tr>
<td>Chlamydia</td>
<td>5 (3/57)</td>
<td>18 (6/33)</td>
</tr>
<tr>
<td>Gonorrhea</td>
<td>12 (7/57)</td>
<td>15 (5/33)</td>
</tr>
<tr>
<td>Trichomonas</td>
<td>3 (2/71)</td>
<td>36 (12/33)</td>
</tr>
<tr>
<td>Mycoplasma genitalium</td>
<td>6 (4/71)</td>
<td>48 (16/33)</td>
</tr>
<tr>
<td>Active Syphilis</td>
<td>11 (9/79)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Seropositive HSV-2</td>
<td>76 (60/79)</td>
<td>95 (25/37)</td>
</tr>
</tbody>
</table>

MSM, men who have sex with men; FSW, female sex worker; ART, antiretroviral; STI, sexually transmitted infection; HSV-2, herpes simplex virus type 2

* Varying denominators indicate missing data

† Other = San Miguel for MSM and Sonsonate for FSW
Table 2

Description of the HIV transmission clusters involving 38 participants and relation to social recruitment chains among 119 HIV-positive individuals. Cluster letter (A-N) corresponds to the phylogenetic tree shown in Figure 1.

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Transmission Risk</th>
<th>Number of Sequences</th>
<th>City</th>
<th>Intra-cluster Genetic Distance *</th>
<th>% in Same Social Recruitment Chain</th>
<th>Directly Linked†</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>FSW</td>
<td>2</td>
<td>San Salvador</td>
<td>0.011</td>
<td>0</td>
<td>---</td>
</tr>
<tr>
<td>H</td>
<td>FSW</td>
<td>2</td>
<td>San Salvador/Sonsonate</td>
<td>0.001</td>
<td>0</td>
<td>---</td>
</tr>
<tr>
<td>C</td>
<td>MSM</td>
<td>2</td>
<td>San Miguel</td>
<td>0.015</td>
<td>100</td>
<td>No</td>
</tr>
<tr>
<td>D</td>
<td>MSM</td>
<td>2</td>
<td>San Miguel</td>
<td>0.003</td>
<td>100</td>
<td>Yes</td>
</tr>
<tr>
<td>F</td>
<td>MSM</td>
<td>2</td>
<td>San Miguel</td>
<td>0.012</td>
<td>0</td>
<td>---</td>
</tr>
<tr>
<td>B</td>
<td>MSM</td>
<td>2</td>
<td>San Salvador</td>
<td>0.001</td>
<td>100</td>
<td>No</td>
</tr>
<tr>
<td>G</td>
<td>MSM</td>
<td>2</td>
<td>San Salvador</td>
<td>0.003</td>
<td>0</td>
<td>---</td>
</tr>
<tr>
<td>I</td>
<td>MSM</td>
<td>2</td>
<td>San Salvador</td>
<td>0.005</td>
<td>100</td>
<td>Yes</td>
</tr>
<tr>
<td>M</td>
<td>MSM</td>
<td>2</td>
<td>San Salvador</td>
<td>0.003</td>
<td>0</td>
<td>---</td>
</tr>
<tr>
<td>N</td>
<td>MSM</td>
<td>2</td>
<td>San Salvador</td>
<td>0.005</td>
<td>0</td>
<td>---</td>
</tr>
<tr>
<td>L</td>
<td>MSM</td>
<td>3</td>
<td>San Salvador</td>
<td>0.006</td>
<td>67</td>
<td>No</td>
</tr>
<tr>
<td>E</td>
<td>MSM</td>
<td>4</td>
<td>San Salvador</td>
<td>0.014</td>
<td>0</td>
<td>---</td>
</tr>
<tr>
<td>K</td>
<td>MSM</td>
<td>5</td>
<td>San Salvador</td>
<td>0.003</td>
<td>80</td>
<td>No</td>
</tr>
<tr>
<td>J</td>
<td>MSM</td>
<td>6</td>
<td>San Salvador</td>
<td>0.013</td>
<td>50</td>
<td>No</td>
</tr>
</tbody>
</table>

FSW, female sex worker; MSM, men who have sex with men.

* Mean intra-cluster pairwise genetic distance (nucleotide substitutions/site) based on the Tamura-Nei model

† Indicates individuals with related sequences who were directly linked in the recruitment chain
Table 3
Comparison of factors associated with membership in phylogenetic transmission clusters among the 80 MSM recruited during respondent-driven sampling

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>In cluster % (n/N)</th>
<th>Not in cluster % (n/N)</th>
<th>( p^\dagger ) unadjusted</th>
<th>( p^\ddagger ) adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>34</td>
<td>46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age ≤25 years</td>
<td>82 (28/34)</td>
<td>54 (25/46)</td>
<td>0.009</td>
<td>0.13</td>
</tr>
<tr>
<td>Recruited in San Salvador</td>
<td>82 (28/34)</td>
<td>76 (35/46)</td>
<td>0.50</td>
<td>---</td>
</tr>
<tr>
<td>Recruited in ≤5 waves in RDS chain</td>
<td>55 (18/33)</td>
<td>37 (17/46)</td>
<td>0.12</td>
<td>---</td>
</tr>
<tr>
<td>Recent Infection</td>
<td>35 (12/34)</td>
<td>15 (7/46)</td>
<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>Drug resistance mutation</td>
<td>3 (1/34)</td>
<td>13 (6/46)</td>
<td>0.23</td>
<td>---</td>
</tr>
<tr>
<td>Any STI</td>
<td>30 (10/33)</td>
<td>28 (13/46)</td>
<td>0.84</td>
<td>---</td>
</tr>
<tr>
<td>Sex with stable male partner( ^8 )</td>
<td>63 (21/33)</td>
<td>32 (15/45)</td>
<td>0.008</td>
<td>0.02</td>
</tr>
<tr>
<td>Sex with ≥3 male partners( ^8 )</td>
<td>54 (18/33)</td>
<td>33 (13/40)</td>
<td>0.06</td>
<td>0.04</td>
</tr>
<tr>
<td>Exchanged sex for money( ^8 )</td>
<td>41 (14/33)</td>
<td>41 (18/44)</td>
<td>0.98</td>
<td>---</td>
</tr>
</tbody>
</table>

MSM, men who have sex with men; RDS, respondent-driven sampling; STI, sexually transmitted infection

\( ^* \) Varying denominators indicate missing data

\( ^\dagger \) Based on Pearson's \( \chi^2 \) or Fishers exact if cell values <5 for bivariable comparison

\( ^\ddagger \) Based on multivariable model adjusted for age, recent infection, sex with a stable male partner, and sex with ≥3 male partners

\( ^8 \) In the past 12 months