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Challenges in evaluating the use of viral sequence data to identify HIV transmission networks for public health

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Abstract: Great efforts are devoted to end the HIV epidemic as it continues to have profound public health consequences in the United States and throughout the world, and new interventions and strategies are continuously needed. The use of HIV sequence data to infer transmission networks holds much promise to direct public heath interventions where they are most needed. As these new methods are being implemented, evaluating their benefits is essential. In this paper, we recognize challenges associated with such evaluation, and make the case that overcoming these challenges is key to the use of HIV sequence data in routine public health actions to disrupt HIV transmission networks.

Keywords: contact tracing; HIV; phylogeny; public health.

Introduction

Significant accomplishments have been made in the fight against HIV in the United States and globally (Jones et al. 2019). To address remaining challenges in this battle, prevention and treatment strategies are being rolled out with ambitious goals to end the pandemic (United Nations General Assembly 2016). The use of HIV sequence data to infer transmission networks is an emerging tool that has been used to halt HIV transmission outbreaks (Wertheim et al. 2019). This tool is now recommended by the United States Center for Disease Control and Prevention (CDC) and is being used by public health services and departments of health to identify new cases and to better understand HIV transmission dynamics (Centers for Disease Control and Prevention 2019a). The precise nature of the information carried in these sequence data, and how they should be used optimally to inform public health practice, is not entirely clear. This challenge motivates the need for careful evaluation of the use of such data with rigorous research methods. Recognizing this challenge is particularly important today, as motivation grows to apply these tools to real-life public health interventions

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fielded to eliminate HIV in the United States and around the world. This paper describes some of the challenges in doing so, both from a technical aspect, and from the standpoint of how best to use these new methods in practice, towards incorporating them into routine public health actions.

Defining the problem: eliminating HIV transmission in the U.S.

According to the latest (2018) estimates of the World Health Organization, between 33 and 44 million people live with HIV throughout the world (WHO 2018). About 75 million people have contracted HIV since its widespread emergence in the human population, and about 32 million have died of the disease. The United Nations has declared an ambitious target, to end the AIDS pandemic by 2030, a goal which the U.S. has adopted (Jones et al. 2019; United Nations General Assembly 2016; United States Health Resources and Services Administration). To do so, it must overcome three factors which facilitate continued person-to-person transmission: (1) 15% of individuals with HIV do not know they are infected; (2) 23% of individuals with HIV know they are infected but are not in care; and (3) 11% of individuals with HIV are in care, but are not virally suppressed (Centers for Disease Control and Prevention 2019b). Of these three pools of individuals who may transmit HIV to others, the first two account for the majority of new transmissions (38 and 43%, respectively) (DHHS 2015). Getting these two groups into effective treatment programs, to render them virally-undetectable, and therefore untransmittable, is a major U.S. goal, towards which many resources have been targeted (Eisinger et al. 2019). A major challenge is to identify these individuals, and to do so with any practical success, given that they represent less than one percent of the U.S. population, we must know where to look. In this regard, gaining information about the genomic proximity of HIV sequence data may provide insights regarding HIV transmission networks, which in turn may be associated with social phenomena by means of which public health interventions may be targeted.

A current solution: contact tracing and partner services

The latency period of HIV infection can be up to 8–10 years, during which individuals who carry the virus but are unaware of it can transmit it to others for this extended period of time, before symptoms present themselves (DHHS 2015). Current antiretroviral therapy is highly effective in inhibiting viral replication, arresting disease progression, lessening mortality and dramatically reducing transmission (Antiretroviral Therapy Cohort Collaboration 2008; Cohen et al. 2011; Gueler et al. 2017). Of course, detection must precede treatment, and regular and universal HIV testing is essential.

Owing, at least in part, to concerns about privacy and the potential societal consequences of a positive HIV diagnosis, regular and universal HIV testing is not common practice in many countries. This motivates other strategies to prevent HIV transmission, including health education to avoid high risk behaviors and to adopt harm reduction strategies, and promotion of free HIV testing (Joint United Nations Programme on HIV/AIDS 2019). In addition, U.S. public health agencies typically perform statewide surveillance and reporting of new diagnoses, as well as contact tracing and partner services (termed ‘contact tracing’ from here on) (Centers for Disease Control and Prevention 2008; Workowski and Bolan 2015). Contact tracing is the process in which newly identified people with HIV are interviewed by disease intervention specialists to identify sexual partners and other at-risk contacts, who, in turn, are contacted, counseled, and offered HIV testing. This public health approach is also implemented to prevent the transmission of many other serious diseases, with variations related to the nature of the diseases in question, e.g., vaccination or prophylactic treatment in place of testing (Armbruster and Brandeau 2007).

Unfortunately, though this strategy is essential and beneficial, it also has its limitations: Newly diagnosed persons may have been diagnosed years after being infected, and therefore may have had numerous contacts over time and in different places, thus taxing even the best of memories; and they and their contacts are often reluctant or are unable to identify contacts (who may have chosen to remain anonymous to the newly

diagnosed persons), or simply may have become inaccessible to public health before information, counseling and testing could be offered. Additionally, public health resources for contact tracing are limited (sometimes, quite limited), which makes it impossible in most jurisdictions to exhaust all opportunities for contact tracing, thus limiting an already limited public health strategy (Armbruster and Brandeau 2007; Magaziner et al. 2018; Tributino et al. 2018). The effect of all these challenges is to obscure key sources (persons) and facilitators (venues, etc.) of disease transmission, and thus, to complicate the targeting of public health interventions.

Using analytic methods from molecular epidemiology to improve contact tracing

Molecular epidemiology refers to the study of pathogen evolution by examination of pathogen genetic sequences (Grabowski and Redd 2014). One of the primary products of such studies is a pathogen phylogeny, which indicates the inferred evolutionary relationships of the pathogens. Though they are often conflated, transmission networks and pathogen phylogenies are distinct entities. The transmission network describes transmission events between humans, and the pathogen phylogeny describes evolutionary relationships between pathogens. Because the human transmission network constrains the evolution of the pathogen, the pathogen phylogeny contains extensive information about the transmission network. Adjacent patients in the transmission network, for example, may have viruses that are closely related in the pathogen phylogeny. The topology of transmission networks and pathogen phylogenies can be incongruent due to multiple factors, including incomplete lineage sorting, saturation of phylogenetic signal, and homoplasy that results in phylogenetic error (Maddison 1997; Som 2015) Using molecular epidemiology methods to enhance human health is therefore a three step process: (1) collecting genetic sequence data from pathogens and inferring a pathogen phylogeny; (2) examining the pathogen phylogeny to infer features of the human transmission network; and (3) making decisions based on features of the transmission network thus revealed to optimize patient care and disrupt transmission. There are many types of insights that molecular epidemiology can provide, both to improve our understanding of an epidemic and to impact it. In addition to providing a critical perspective on the structure of the transmission network, it can provide a dynamic perspective on an epidemic. For example, it can identify longitudinal changes in transmission rates through time among particular clusters of individuals.

These methods have been used to characterize local HIV epidemics and to identify risks for forward transmission (Brenner et al. 2011; Little et al. 2014); characterize epidemics longitudinally (Chan et al. 2015); and assist in the development of targeted prevention strategies and interventions (Peters et al. 2016). Using sequence data to infer transmission networks may therefore augment contact tracing by actions such as identifying sources and facilitators of disease transmission which may otherwise be obscured by the limitations of contact tracing; focusing contact tracing on rapidly-expanding or other existing transmission networks (possibly indicative of active disease transmission and/or at-risk contacts); and mapping the migration of strains of HIV into (and with regional public health cooperation, out of) defined geographic areas, providing clues to target public health interventions. Observing phenomena such as these appears to hold promise for the targeting of public health resources where they will have the greatest impact, i.e., where HIV transmission appears to be most rapid or otherwise problematic, but also perhaps in routine care.

To date, the potential benefits of HIV molecular epidemiology methods for HIV sequences have been demonstrated in a growing number of independent studies. For example: (1) in 2015, 11 new HIV diagnoses in a small community in Indiana led to an outbreak investigation conducted by the CDC. Of 181 cases newly diagnosed between November 2014 and November 2015, 88% were found to have used IV oxymorphone. Among 159 cases with sequence data, 99% were in a close transmission network. Contact tracing guided by the sequence data identified 536 contacts; 87% were located, tested for HIV, and, if infected, linked to care. As a result, a syringe-service program in Indiana was established for the first time (Peters et al. 2016). (2) in 2014, real time phylogenetic inference conducted in British Columbia identified an HIV transmission network that had

expanded by 11 cases in three months, including eight cases with transmitted drug resistance. The ensuing investigation assured access to care (and actual initiation of therapy) for the affected subpopulation. During the next 12 months, 12 new cases had a reduction in onward resistance transmission (Poon et al., 2016). (3) in evaluating the yield of contact tracing in new diagnoses and acute/early HIV between 1996 and 2014 in San Diego, California, 107/574 persons identified 119 sex partners (57% infected; 33% newly diagnosed). Of 62 sero-concordant partners with sequences, 61% were members of phylogenetic networks. Partners enrolled within 30 days of their naming partners were more likely to be newly diagnosed and in networks (Green et al. 2017). The data from these and a growing number of other studies suggest that combining contact tracing with phylogenetic analysis, the optimal specifics of which is to be determined, may be an effective tool in identifying and targeting real time outbreaks, and thus, to enhance investigation of transmission networks.

Given the results of these and similar studies, the CDC has recommended the use of viral sequence data for HIV-prevention (Centers for Disease Control and Prevention 2019a). To assist in this process, in addition to existing methods from the molecular epidemiology toolbox, the CDC has adopted and disseminated molecular surveillance tools to state and local public health entities throughout the U.S., and is prepared to provide them with additional technical support, as necessary, when active HIV transmission networks are identified (Centers for Disease Control and Prevention 2019c). The CDC also collects de-identified sequence data from all jurisdictions, which it uses to identify networks that are concerning for recent and rapid transmission of HIV (Centers for Disease Control and Prevention 2019c). An advantage of doing so at the national level is that the CDC can identify cross-jurisdictional networks, getting a handle, for example, on the effects of tourism and migration from one jurisdiction to another.

Challenges in evaluating the utility of using viral sequence data to identify transmission networks for public health

The use of HIV sequence data analysis as a routine tool in the public health armamentarium is promising. However, despite substantial strides made thus far, its practical application faces specific challenges in each of three steps: (1) accurately inferring pathogen phylogenies; (2) analyzing these phylogenies to gain insight about the structure and dynamics of the actual but unobservable transmission networks; and (3) translating these insights into actions that disrupt transmission.

The challenges encountered in the first two steps relate to the difficulty of describing and validating the structure of phylogenies and transmission networks from data which are imperfect (i.e. with unknown epidemiologic linkages) and incomplete (i.e. with not all individuals with HIV represented) data.

As to the first issue of imperfect data, even if we had a perfect and complete virus phylogeny, we would still have an incomplete understanding of the transmission network. That is because not every feature of the transmission network is expected to be retained in the phylogeny. The transmission network has nodes (people) and edges (transmission events). The transmission events occur at specific times but do not have a time duration. The virus phylogeny is a directed acyclic graph with nodes corresponding to virus populations and edges that are not instantaneous but instead have time duration that indicate evolutionary distance between nodes. The transmission network imposes demographic structure on the virus phylogeny, so the virus phylogeny contains information about the transmission network, but they are not equivalent entities.

The second reason for incompleteness of information is a sampling issue: not all individuals with HIV in the transmission network will be sampled; hence their HIV sequences are not included in the phylogeny. This type of incompleteness leads to false negatives in the sense that the inferred phylogeny may not recognize a sampled individual as a member of a cluster because we do not have HIV samples from other members of that cluster. Similarly, we cannot claim that the largest cluster in the phylogeny is the largest cluster in the transmission network because we may have missed some clusters altogether, or missed some members of other, larger clusters.

These two types of incomplete information highlight the difficulties of using standard statistical techniques for assessing both bias and uncertainty as they relate to inferences about the transmission network. One line of work that could provide insight about the relationship between transmission network structure and virus phylogeny is development of generative models that explain what we expect the virus phylogeny to look like given different, real transmission networks, and the conduct of simulation studies to assess which features of the transmission network can be reliably recovered from a phylogenetic analysis of a partial sample of sequence data taken from individuals in the network. In the absence of a generative model underlying a transmission network of interest, it is difficult to assess whether one transmission network is better supported than another by a given virus phylogeny.

As to incomplete sampling, standard methods for handling missing data may be difficult to apply here because we do not typically have any information about individuals who are missing. Standard approaches to missing data such as inverse probability weighting and imputation typically rely on knowing the size of the target sample and the fraction of individuals who do not respond. Partial information about the non-respondents usually is available as well. Here the 'non-respondents' are those people with HIV in the transmission network for whom we do not have sequences. In most instances we do not even know who these individuals are.

Despite these limitations we can still assess empirically the potential impact of missing information. Approaches include analyses of simulated data and assessment of concordance with independent data, such as those obtained from contact tracing. We also can assess sensitivity of the clustering patterns to missing data by refitting the phylogeny after removing individual sequences. From a design point of view, improved sampling density of individuals, virus genomes sampled within individuals, and number of genomic regions sequenced, all have the potential to reduce uncertainty about the clustering structure.

The challenges related to step 3 are distinct from those encountered in steps 1 and 2. Assessing whether application of molecular epidemiology methods disrupts disease transmission, and optimizing the impact of these methods on human health, require a framework for quantifying the improvement on specific metrics and indicators that is attributable to using sequence data to infer transmission networks. Ideally, hypotheses about the effect of using sequence data could be tested using a randomized trial; absent that, methods for causal inference from observational data can be applied.

The application and evaluation of molecular epidemiology methods in the field is therefore challenging, primarily because they cannot be used to infer *actual* disease transmission networks. Instead, analyses of these sequence data can identify groups of individuals whose viruses have some genomic similarity. While this does not and cannot identify direct epidemiologic linkage between individuals, it can suggest that there is a latent larger network of individuals between whom transmission may have taken place, and/or those who may be at high risk of being infected.

Because there is unlikely to be any source of data that could illuminate an actual HIV transmission network, it follows that using sequence analysis methods, such as phylogenetics, to discover or validate the *real* transmission network may not be a public health priority. To support the routine incorporation of information gained from analyses of sequence data into public health surveillance and outreach, including contact tracing, we must find ways to identify those methods and quantitative summaries that yield measurable improvements in important public health outcomes. Improvements can be quantified in terms of identifying more individuals with HIV who are not yet aware of it, those aware of their positive HIV status but who are not linked to care, or those who are at high risk of contracting HIV. Specific questions that can be used to frame the evaluation include: Can summaries of molecular-level data be reliably evaluated *in vivo*, with so many moving parts and uncontrolled variables associated with implementation of public health monitoring and intervention? Does using these data improve the effectiveness of traditional public health methods, as quantified by specific metrics such as those outlined above?

Several factors complicate our ability to answer such questions: First, the process of contact tracing can vary by jurisdiction and can vary over time. As discussed above, 'contact tracing' refers to an approach that is used to identify, locate and interview those who may be undiagnosed cases, or who may be at risk for contracting or transmitting HIV. The approach may depend on the person obtaining the information, and may

vary from one contact to another, and can be dictated by psychological and social considerations. Contact tracing interviews are necessarily open-ended (therefore highly variable); interviews are completely voluntary and individuals may help or hinder investigations; and scale of implementation can be limited by the temporal bunching of newly diagnosed cases. Second, contact tracing does not exist in a vacuum. It usually takes place in parallel with other public health interventions such as the promotion of condom use, HIV testing, pre-exposure prophylaxis, and other prevention activities. This makes isolating the effect of contact tracing per se difficult. Third, the inference of transmission networks is limited by the representation of the available sequence dataset (Mehta et al. 2019). Finally, other factors have an impact, such as migration patterns, social norms, the economy, the availability of intimate partners, health promotion efforts, access to prophylaxis and treatment, new treatments, the availability of addictive drugs and access to drug treatment programs. None of these factors are under the control of public health agencies, except partially or tangentially. Importantly, ethical factors must be incorporated into these considerations (Mehta et al. 2019).

Addressing these challenges in Rhode Island

In Rhode Island, universal contact tracing of the partners of persons newly diagnosed with HIV has been a routine strategy for many years. With the emerging exploration of the use of sequence data into this process, our group is currently conducting an NIH-funded study in Rhode Island. We are leveraging availability of sequence data from drug resistance testing as part of clinical care for a high (~80) percentage of HIV cases in the state, which will enable evaluation of benefits of integrating information on transmission networks derived from HIV sequence data into public health activities, focusing on routine contact tracing. One of our objectives is to lay out a roadmap for evaluating the efficacy of these methods and comparing and contrasting different ways to use and evaluate them.

At least three important research questions emerge in quantifying the role of molecular epidemiology methods in public health interventions designed to reduce and eventually halt HIV transmission. First, are there substantive differences in the output of existing methods used in transmission networks inference? (Kosakovsky Pond et al. 2018; Novitsky et al. 2019). Second, does the targeted use of viral sequence data to infer transmission networks improve public health interventions to disrupt HIV transmission? And third, what are the relative benefits of different analytic methods for summarizing sequence data analyses, and is there an optimal way to use these methods in a public health context? We are in the process of designing and implementing methods to address these questions. Relevant considerations include how and when sequences are sampled from the population, how many and what percentage of the population are needed and why, which analytic method or methods to employ, how to properly carry out statistical comparisons between methods in the presence of multiple sources of confounding, and how to identify a strategy that optimizes impact while keeping cost and labor within certain boundaries.

Conclusions

To achieve set goals and end the HIV epidemic in the United States and globally, existing interventions to prevent transmission must be improved, and new ones developed. Enhancing routine public health actions with the use of HIV sequence data to infer transmission networks holds great promise for disease control efforts, and ultimately, for the disruption of HIV transmission. Importantly, public health direction for using these tools should be focused not on whether they represent actual transmission networks (they do not), but whether they improve public health outcomes. Recognizing challenges in integration of such methods into routine public health actions and enhancement of contact tracing, and evaluating their benefits, are significant steps towards optimizing the use of these strong tools in disrupting HIV transmission in the U.S. and beyond.

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