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▶ **To cite this version:**

Anna Zhukova, Teresa Cutino-Moguel, Olivier Gascuel, Deenan K Pillay. The Role of Phylogenetics as a Tool to Predict the Spread of Resistance. Journal of Infectious Diseases, 2017, 216 (suppl_9), pp.S820-S823. 10.1093/infdis/jix411 . lirmm-02078446

HAL Id: lirmm-02078446

https://hal-lirmm.ccsd.cnrs.fr/lirmm-02078446

Submitted on 25 Mar 2019

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The Journal of Infectious Diseases
SUPPLEMENT ARTICLE

The Role of Phylogenetics as a Tool to Predict the Spread of Resistance

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Drug resistance mutations emerge in genetic sequences of HIV through drug-selective pressure. Drug resistance can be transmitted. In this review we discuss phylogenetic methods used to study the emergence of drug resistance and the spread of resistant viruses.

Keywords. HIV; drug resistance; phylogenetics.

The clear majority of publications on human immunodeficiency virus (HIV) drug resistance emanate from the resource-rich world, including those pertaining to the clinical epidemiology of resistance. Although there remain important lessons to be drawn to understand the spread of drug resistance in resource-limited settings, it is worth comparing these settings in considering drivers of drug resistance. We witnessed high levels of resistance in treated and untreated individuals in the 1990s and early 2000s in those settings with access to therapy. To a large extent, this rise was associated with what is now recognized to be suboptimal therapy—limited drug classes, pill burden, toxic effects, late initiation of therapy—together with continuing transmission in high-risk communities. Since that time, the availability of >25 antiretroviral (ARV) drugs across 5 classes, individualized therapy including the use of resistance testing, and simplified regimens have led to a dramatic reduction in resistance in these settings [1]. Indeed, some predictions at the time of ever increasing levels of resistance [2] have not been borne out [3]. By contrast, we are observing the opposite phenomenon in resource-limited settings, where the burden of infection is greatest [4]. Table 1 identifies some of the drivers of such high levels of resistance.

CLINICAL EPIDEMIOLOGY OF HIV DRUG RESISTANCE

How can we better understand this phenomenon, and develop tools for predicting future trends? The overall population burden of resistance is contributed to both by the emergence of resistance in treated individuals and by the transmission of resistance. It is self-evident that the dynamics of the epidemic itself must be considered in modeling future spread of resistance—in other words, the proportion of infected individuals diagnosed and receiving treatment, as well as the ongoing incidence of infection must be considered. From an overall health burden and policy perspective, there is a big difference between a transmitted drug resistance (TDR) rate of 15% with a population HIV incidence of 2% and a TDR rate of 5% with a population incidence of 6%.

This contrast is exemplified in a modeling approach undertaken by [5], which addresses the likely impact of a widespread HIV testing and treatment strategy within the South African epidemic. Based on a 2012 TDR prevalence of <10%, their model suggests that over a 20-year period of such a test-and-treat strategy, the overall incidence of infection would be reduced by 50%. Nevertheless, by that time, up to 30% of new infections would be caused by drug-resistant viruses [5]. For this reason, drug resistance surveillance programs need to be placed in the wider clinical epidemiological context of the epidemic in question.

It is also important to consider the developing use of ARV drugs for preexposure prophylaxis (PrEP). Following the PROUD and IPERGAY study results [6, 7], there is a strong push for rollout of PrEP within high-risk populations in resource-poor settings. The first case of PrEP failure due to resistance has now been reported [8]. Abbas et al [9] modeled the potential impact of PrEP on HIV transmission and drug resistance in South Africa. They predicted that ARV therapy (ART) combined with PrEP over 10 years would reduce the number of infections. Supervie et al performed 2 modeling studies on rolling out of PrEP: in San Francisco (ie, in a resource-rich country) [10] and in Botswana (resource limited) [11]. They showed that if PrEP is widely used in a "high-risk" community in San Francisco, the number of infections and the rate of transmitted ART resistance are both likely to decrease (if risk behavior does not increase significantly). In contrast, the introduction of PrEP interventions in Botswana is likely to lead to an increase of transmitted ART resistance (while decreasing the overall number of infections). This occurs because the level of ambient

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The Journal of Infectious Diseases® 2017;216(S9):S820–3
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DOI:10.1093/infdis/jix411

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Table 1. Drivers of High Levels of Resistance

Characteristics	Resource-Rich Countries	Resource-Limited Countries	Effect on Population Drug Resistance in Resource-Limited Countries
Calendar time of ARV drug availability	1980s	2000s	Decreased resistance
Treatment paradigm from time of ARV drug availability	Monotherapy to dual therapy to triple therapy	Triple therapy	Decreased resistance
Availability of 2nd- and 3rd-line regimens	Yes	No	Increased resistance
Single-dose nevirapine[a] for PMTCT	No	Yes	Increased resistance
Viral load monitoring availability	Extensive	Limited	Increased resistance
Incidence and prevalence	Low	High	Increased resistance

Abbreviations: ARV, antiretroviral; PMTCTC, prevention of mother-to-child transmission.

[a]Viramune (Boehringer Ingelheim, 800-243-0127).

resistance is higher in San Francisco than in Botswana, owing to a longer treatment history. The differences in the results obtained by Abbas et al [9] and by Supervie et al [10, 11] highlight the importance of accounting for the assumptions that are made, for example, the initial levels of resistance when the rollout begins.

Several studies have used phylogenetics together with detailed clinical and epidemiological data to explore the origin of incident infections. Fisher et al [12] demonstrated that up to 30% of new infections were from individuals in the highly infectious primary stage of infection. Brenner et al [13] used phylogenetic clustering analysis of a Quebec HIV-infected population to show that early infections may account for a major proportion of onward transmissions. This approach was expanded to the ATHENA cohort in the Netherlands [14] to show that both primary and undiagnosed infections together accounted for the bulk of new infections. By contrast, few transmissions came from patients in care and receiving ART. However, the incidence of transmissions from treated patients with resistance still undetected owing due to poor monitoring (a typical situation in developing countries) remains to be estimated.

Against this background, what is the potential role of phylogenetics in enhancing our understanding of emergence and spread of drug resistance? First, who are the main transmitters of drug resistance, and are they receiving ART? Second, what is the contribution of transmission during acute infection to the spread of drug resistance? Third, what is the persistence of drug-resistant virus strains within the population? Finally, as PrEP becomes widespread, can we identify the emergence and transmission of resistant strains from patients who are infected while receiving PrEP?

PHYLOGENETICS AND DRUG RESISTANCE

HIV viruses rapidly accumulate genetic variation because of short generation times and high mutation rates. Phylogenetic inference methods use these variations for reconstruction of *phylogenies* (phylogenetic trees) from contemporary sequencing data. The root of the tree represents the ancestral lineage, and the tips correspond to the virus sequences at the moment of sampling. Going from the root to the tips corresponds to moving forward in time. When a lineage splits (speciation), it is represented as a branching node of the phylogeny. When the sampling is dense, such a split can be interpreted as a virus transmission infecting a new individual, and the whole tree is an approximation of the transmission tree [15].

To access the robustness of the reconstructed tree, the *support* values on its branches can be calculated using statistical methods, such as bootstrap [16]. These values tend to decrease when going back in history, from tips to the root. To remove the uncertain data from the study, *genetic clusters* are often used instead of the whole tree. Such clusters correspond to the well-supported subtrees that contain sequences closely related to each other and distant from the rest of the tree (see [17] for an overview of genetic clustering methods). A cluster of sequences that also share a common trait values (eg, geographic location, risk group, presence of a given drug resistance mutation [DRM]) is called a *phylotype* [18]. The branch lengths in genetic clusters are typically short, and therefore a cluster can be interpreted as representing a recent outbreak, as, for example, when a virus acquires a DRM under drug-selective pressure and the patient starts transmitting the resistant virus. The subtree including this patient, individuals infected by him/her, and those infected by them would form a resistance cluster if these persons are sampled before their virus strains diverge significantly. The root of the cluster would correspond to the first transmission event.

Viral phylodynamics is defined as the study of how epidemiological, immunological, and evolutionary processes act and potentially interact to shape viral phylogenies [19, 20]. Phylodynamics methods have been used to estimate the parameters shaping the emergence of drug resistance and spread of resistant viruses, such as, for example, the persistence time of DRMs in the untreated population.

Wensing et al [21] used phylogenetic reconstruction and genetic clustering to study the persistence of DRMs in HIV infected treatment-naive patients from 19 countries across Europe. They found a significant difference in the level of baseline resistance between recently infected patients (13.5%) and patients infected for >1 year (8.7%).

The origin of TDR has been addressed by several groups. Yerly et al [22] reconstructed HIV transmission clusters in Geneva using phylogenetic analysis, showing that newly diagnosed HIV infections are a significant source of onward transmission, notably of resistant strains. Audelin et al [23] studied

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TDR among individuals with newly diagnosed HIV-1 infection in Denmark, and they concluded that TDR isolates mostly originate from patients failing therapy. The same conclusion was reached by Lewis et al [24] in a study including approximately 2000 patients from London, predominantly men who have sex with men, using a similar transmission-cluster-based approach.

Hué et al [25], and later Mourad et al [26], obtained different results while studying HIV-1 transmission in the United Kingdom. Hué et al studied treatment-independent viral clusters with DRMs and demonstrated that sustainable reservoirs of resistance persist in the HIV-1–infected population through continuous transmission of resistant viruses among treatment-naive individuals. Mourad et al used a parsimony-based approach [27] to extract phylotypes of sequences, the most recent common ancestor of which was bearing a DRM that is still shared by the majority of the sequences in the phylotype.

Once dated and combined with the treatment-naive/treatment-experienced status of those represented by the sequences, these phylotypes were used to zoom on the most readable parts of the phylogeny and compute simple statistics which are immediately accessible from the annotated tree; for example, the number of naive-to-naive transmissions of DRMs or the fraction of extant sequences having lost the ancestral resistance. The simplicity of the method makes it computationally very efficient. It was applied to a large set of approximately 25 000 HIV-1 subtype B sequences from the United Kingdom, where it showed that about 70% of TDR had a treatment-naive source. In this population, the most commonly transmitted mutations were L90M in the protease gene and K103N, T215D, and T215S in reverse-transcriptase. Moreover, reversion to wild type occurred at a low frequency, and drug-independent reservoirs of resistance have persisted for up to 13 years.

These conclusions are very close to those of Drescher et al [28], who studied the transmission of resistances among men who have sex with men in the Swiss HIV Cohort. Their method was different, because they did not reconstruct the ancestral resistance status of the sequences, but they also extracted well -supported transmission clusters from a large sequence phylogeny and searched for the potential sources of the resistances observed in these clusters. The discrepancy between the results obtained by Mourad et al [26] and Drescher et al [28] and those obtained by Audelin et al [23] and Lewis et al [24] is most likely attributable to the size of the data sets, ranging from approximately 2000 ([24]; published in 2008) to approximately 25 000 ([26]; published in 2015). Moreover, the sampling density is of prime importance (>50% in [26] and [28]), because relatively large resistance clusters with no or little missing data are needed to demonstrate naive-to-naive TDR. When the proportion of missing data is high, it is not possible to determine the origin of the transmission for isolated drug-naive patients harboring DRMs.

CONCLUSIONS

In summary, we argue for building phylogenetics into a more detailed epidemiological surveillance of HIV drug resistance. With an ever-reducing cost of genetic sequencing, there is a move to generate full-length HIV sequences [29]. This has the capacity to increase the phylogenetic resolution, owing to longer sequences. Through a large simulated data set, we have shown that the accuracy of trees was nearly proportional to the length of sequences, with gag-pol-env data sets showing best performance compared with the partial pol sequences commonly created through drug resistance testing [30]. An added advantage of extended sequencing is the ability to capture integrase inhibitor resistance. Care must be taken in the sampling frame in the context of HIV prevalence, to produce realistic estimates. This will facilitate a better understanding of the drivers of resistance spread, the source of transmitted resistance, and how this is changing over time in the face of ARV drug rollout.

Notes

Funding. D. P. was supported by Wellcome Trust and the Bill & Melinda Gates Foundation.

Supplement sponsorship. This work is part of a supplement sponsored by the National Institute of Allergy and Infectious Disease, NIH, and the Centers for Disease Control and Prevention.

Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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