

Inferences about HIV Transmission: Phylogenetics in Forensic Science

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Abstract: Connecting people with infectious diseases is not only a matter of epidemiological interest but also has legal implications when the disease is transmitted during a criminal act or when there is a civil dispute for compensation made by an infected person who acquired the disease as a result of lifestyle or work-related accidents (such as needle injuries). When the disease is a consequence of crimes like rape, the characteristics can be useful in linking the criminal and the victim, and the transmission itself can be inferred as the criminal's responsibility. Using phylogenetics as an inference method, it is possible to analyze the viral population genomes of people infected with HIV. Brazil already has ongoing judicial cases and can indeed benefit from the aid of complementary evidence, especially molecular-level proof that can have high reliability.

Keywords: Forensics; Biotechnology; Genetics; Criminalization of HIV; Transmission Chains.

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1. Introduction

The World Health Organization reveals that 35.3 million people are living with HIV, with 2.3 million new infections and 1.6 million people who died due to AIDS in 2012 worldwide. In Brazil, during the same year, between 530,000 to 600,000 people were living with HIV, and 11,000 to 19,000 deaths occurred due to AIDS [1]. HIV-1 and HIV-2 are the agents of AIDS and are classified as lentiviruses (retroviruses). In their genomic map, genes that code for their structure (gag and env), their metabolism (pol), and resulting regulatory proteins can be observed [1]. One of the fundamental characteristics of HIV-1 is its high genetic variability. Currently, HIV-1 can be divided into groups M, N, and O. Group M is responsible for the majority of HIV-1 infections worldwide. It can be further subdivided into 9 phylogenetic subtypes (A, B, C, D, F, G, H, J, and K) and circulating recombinant forms (CRF). In Brazil, the subtypes are equidistant among themselves, with subtype B being the most prevalent, accounting for 69.1% of the sequences deposited in the HIV-LANL database [3].

HIV-1 is transmitted in three ways: blood (transfusion of contaminated blood products, needle sticks or sharing of needles previously used by an infected person, artificial insemination, skin grafts, organ transplants); vertical transmission (mother-to-child transmission during pregnancy, childbirth, and breastfeeding); and sexual transmission (unprotected sexual intercourse with an infected partner) [4]. Regarding HIV-1 transmission, various quasispecies are present in the semen, vaginal secretion, or blood of chronically infected individuals. However, frequently only a single virion or infected cell is transmitted and generates the clinical condition (with the exception of transmissions among intravenous drug users) [5]. This single virion results from mutations over time and can be traced when compared with samples from local populations.

Genetic factors that generate mutation markers include the CCR5 delta 32 variation, which reduces the frequency of the CCR5 receptor on CD4+ T cells since HIV uses this receptor as an entry point [6]. Another important factor for the diversity of HIV-1 is treatment. Before the introduction of highly active antiretroviral therapy, the long-term mortality rate of the infection was close to 100%. There are five classes of antiretroviral drugs in clinical use, each acting at different stages of the virus's life cycle. These are: protease inhibitors, integrase inhibitors, reverse transcriptase inhibitors, fusion inhibitors, and CCR5 antagonists. Drug resistance becomes a reality after about seven years of common treatment or is associated with poor adherence to treatment. In South America, the prevalence of resistance can reach up to 22% of patients [7].

Individuals infected with HIV-1 contain a population that is dynamic in terms of evolution. Factors contributing to the expansion of multiple viral lineages are high mutation [8] and recombination rates [9], together with an estimated replication of 108 to 1010 viruses per day [10]. This expansion is compensated by the extinction of lineages of defective non-replicating virions [11], the effectiveness of the host's immune system and the effectiveness of active antiretroviral therapy [12]. However, viral dynamics limit a researcher from using the usual methods of comparing DNA profiles, thus, phylogenetic methods are ideal for determining the pattern of HIV inheritance in suspected cases of criminal transmission [13].

The use of phylogenetics in forensic sciences focuses on proving evidence of transmission and, together with circumstantial evidence, is a complementary tool of interest for legal cases in Brazil. The objective of this review is to show the scenario of the criminalization of conscious transmission of HIV, demonstrating the capacity of phylogenetic analysis to expose a chain and origin of transmission. The intention is to address questions such as: *what is the application of this technique, along with modes of interpretation? How can Brazil benefit from clarifying a transmission chain? What are the advantages of scientific evidence for cases within Brazilian criminal law?*

2. Forensic Phylogenetics

When applied to cases of infections by highly evolving pathogens, phylogenetics is an essential tool for revealing the possibilities of transmission and its direction (who infected and who is infected), the origins of episodes, and estimating the time of infection. In its application to judicial cases, a basic question to resolve is determining if the samples obtained from distinct patients share a more recent common ancestor with that of the possible source of infection; this is compared to infected individuals from the general population (the latter used as external controls of the method) [14].

Various methods are available to construct trees, but maximum likelihood tends to be used more frequently by those working with HIV transmission chains. These use statistical techniques to determine the probability that a proposed phylogenetic tree and the hypothetical evolutionary history could explain the observed virus population profile. Generating different phylogenetic trees using computer simulations allows the one with the highest likelihood to be identified (using algorithms estimating tree reliability, such as the bootstrap). Bayesian inference is also used to generate phylogenetic trees. Although it has been found that, in two criminal cases, the phylogenetic trees generated by Bayesian and maximum likelihood analyses obtain the same results. Whatever approach is adopted, it is essential that the tree includes appropriate control samples from people with the same HIV subtype as the donor and recipient, and who live in the same locality [15].

The interpretation of viral phylogenetic trees can be complicated when the infected person has several different HIV lineages, as is quite possible in those who are sexually promiscuous, while similarities in viral genomes (e.g., drug resistance mutations) can also arise by chance because of convergent or parallel evolution [16]. It is suggested that instead of making categorical judgments, statements such as "the viral sequences of

person A and person B demonstrate a high level of similarity" or "the viral sequences are compatible with the possibility that person A infected person B" should be used [17].

Providing molecular evidence to define the direction of transmission would increase the strength of a hypothesis under investigation. This is possible if there is a so-called paraphyletic relationship in the phylogenetic tree (e.g., a group of source viral sequences is more related to all the sequences obtained from the infected individual than to other sequences). Despite the large population of related HIV genomes in infected individuals, paraphyly is the result of a significant reduction in the genetic population when establishing an infection in a recipient. Several studies support the high probability of this pattern by demonstrating that most (>75%) productive infections are derived from a single virus [18].

Following the initial infection, the rapid evolution rate of HIV generates increased diversity of viral sequences within a newly infected individual. If the sequences are collected as samples from the source of infection and the recipient shortly after transmission, then the sequences of the source will be paraphyletic to the sequences of the recipient. Paraphyly then supports the direction of transmission but depends on the alignment of homologous sequences, the choice of target genes, the choice of local controls or database sequences that well represent the virion population of the donor (suspected source) and recipient, and how the tree rooting is done [13].

3. And Brazil?

Phylogenetic studies worldwide have elucidated transmission chains in cities, even to rule out the possibility of iatrogenic transmission [19]; one of the first and most famous cases being the Florida dentist suspected of infecting patients [20]. It is noteworthy that AIDS has ceased to be just a public health issue, as more countries are punishing individuals who, knowing they are HIV-positive, do not warn their partners of the risk of infection and transmit the virus. Brazil is already part of this list. The methods of AIDS prevention are so widespread that some judges, despite the lack of specific legislation on the subject, interpret this situation as a crime.

There are several cases in our courts that should be remembered:

CASE 1: This happened in the case of José Luis Correa de Moura from São Paulo, who infected his lover. First tried in 2004, Moura was sentenced to eight years in prison for attempted murder. The legal reasoning was that by having unprotected sex with his partner, he knew he could kill her. After a new trial last month, the crime was changed to grievous bodily harm, with the possibility of the defendant serving the sentence under an open regime. The prevailing argument this time was that, since AIDS is an incurable but not always fatal disease, the infection should be considered a health damage, not a life risk [21].

CASE 2: The defense of Jayr Galhardo Júnior from Cosmópolis, in the interior of São Paulo, imprisoned for contaminating two girlfriends, wants him to be tried for the milder crime of danger of serious illness contagion. The Brazilian justice system follows a global trend. Thirty-five countries have already convicted citizens for transmitting the AIDS virus [22].

CASE 3: An incident occurred at a Basic Health Unit (UBS) in the city of Londrina, PR. The case involved a pregnant woman with HIV who was instructed by the health team to use antiretroviral drugs (commonly known as the "AIDS cocktail") during her pregnancy. According to the Ministry of Health's guidelines, regular use of these drugs—along with cesarean delivery and avoidance of breastfeeding—reduces the possibility of newborn infection to less than 1%. However, despite being instructed by the health team, the patient did not take the medication correctly, failing to adhere to the prescribed dosage and routine. Thus, the professionals involved posed the following

question: is it possible to hold this patient criminally responsible who, knowing the risks of her actions, still failed to take the "anti-HIV cocktail"? [23].

4. Final Considerations

Examining the doctrine and jurisprudence, one can see that the debate regarding the classification of those who engage in behaviors capable of transmitting HIV is intense and ambiguous. This is because, in cases of virus transmission through sexual intercourse—the only ones that have gained judicial repercussions—there is still no definitive position. Sometimes this conduct has been classified as attempted murder, grievous bodily harm, the crime of danger of serious illness contagion, endangerment of the life or health of others, among others [24]. Among all the judicial decisions on the subject, two recent ones from our main courts should be highlighted: the first from the Supreme Federal Court (STF), which on that occasion rejected the categorization of HIV transmission as an intentional crime against life, a position previously favored by most Brazilian judges. The Superior Court of Justice (STJ) faced a similar case, classifying the conduct as grievous bodily harm [22].

In Table 1, Guimarães [24] outlines the possible classifications based on Brazilian court decisions regarding the generic conduct of having unprotected sexual intercourse with a person living with HIV/AIDS, where the sexual relations are consensual.

Table 1. Tipos de crimes aplicados a casos de transmissão do HIV no Brasil [24].

CP Article	Crime	Penalty
Art. 130, caput: "exposing someone, through sexual relations or any libidinous act, to the contagion of a venereal disease, which they know or should know they are contaminated with."	Danger of Venereal Contagion.	Detention from 3 months to 1 year or a fine.
Art. 131: "practicing, with the intention of transmitting to another person a serious illness from which they are contaminated, an act capable of producing the contagion."	Danger of Serious Illness Contagion. This is a preferred classification by the Judiciary as an alternative to attempted murder.	Imprisonment from 1 to 4 years and a fine.
Art. 132, caput: "exposing the life or health of another to direct and imminent danger."	Danger to the Life or Health of Others. The TJ/RJ has judged sexual exposures of HIV as this crime.	Detention from 3 months to 1 year if the fact does not constitute a more serious crime.
Art. 129, § 2, clause II: "offending the bodily integrity or health of another, if it results in: (see adjacent table)."	Grievous Bodily Harm (Extremely Severe) by Transmission of Incurable Disease. This has been a preferred classification by the Brazilian Judiciary as an alternative to attempted murder.	Imprisonment from 2 to 8 years for the consummated crime.
Art. 129, § 3: "if it results in death and the circumstances show that the agent did not intend the result nor assumed the risk of producing it."	Bodily Injury Followed by Death (intent to injure + negligence causing death). It is a preterintentional crime; it goes beyond intent: there is negligence.	Imprisonment from 4 to 12 years for the consummated crime.
Art. 121, caput: "killing someone" combined with article 14, clause II (attempt).	Attempted Simple Homicide: frequent incrimination.	Imprisonment from 6 to 20 years, reducing the penalty by one-third to two-thirds.
Art. 121, § 2, clause III combined with article 14, clause II.	Attempted Qualified Homicide by insidious means (means used without the victim perceiving or being aware). Frequent incrimination in Brazil.	Imprisonment from 12 to 30 years, reducing the penalty by one-third to two-thirds.

It is difficult to obtain validation of epidemiological links in judicial cases involving HIV transmission, especially because, depending on the circumstances in which a doctor-patient interview occurs, the information may not always be accurate or clear. It is therefore important to have an option to obtain accuracy in the process of identifying host and transmission; this is the objective of molecular characterization. Phylogenetics

should not be used to prove a criminal act but rather as complementary evidence, where there may be an epidemiological link, but the possibility of intermediaries cannot be excluded (it does not prove direct transmission).

Phylogenetics ultimately supports the notion that those involved in criminal HIV transmission cases are related when compared to other individuals with a similar epidemiological profile; and it can be used in court to exonerate an accused person, when the hypothesis of HIV transmission with unrelated viral populations among individuals is rejected. Brazil has the possibility of using this inference method in ongoing cases, thereby increasing the potential for discussing the application of forensic phylogenetics not only in developed countries but also bringing this scope to our criminal expertise.

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Supplementary Materials: None.

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