Prevalence of transmitted drug resistance mutations among patients infected with human immunodeficiency virus type 1 (HIV-1) in Henan Province, China

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To the Editor: Even with the emergence of highly active antiretroviral therapy, human immunodeficiency virus type 1 (HIV-1) remains a serious public health problem worldwide. The widespread use of antiretroviral therapy (ART) has, on the one hand, significantly improved life expectancy in patients infected with HIV-1; on the other hand, it has inevitably brought along with it a difficult problem—viral resistance. Viral resistance is a common phenomenon evolutionarily, particularly in patients with poor adherence and suboptimal drug levels. Failed viral suppression due to viral resistance emerging in treatment-experienced patients can be avoided by adopting viral-susceptible ART regimens. However, viral resistance obtained during transmission, known as "transmitted drug resistance," can be a major hurdle against the elimination of HIV-1 infection. As genotypic drug resistance testing in treatment-naive or newly diagnosed HIV-1 patients is not as widely implemented as in treatment-experienced patients. Transmitted drug resistance mutations (TDRMs) in HIV-1 pose a serious threat to the efficacy of current ART regimens and pre-exposure prophylaxis (PrEP). A better understanding of the prevalence of local TDRMs can provide valuable data for clinical- and government-level decision-making.

This study was approved by the Institutional Ethics Committee of Henan Infectious Diseases Hospital, China (No. IEC-2021-006). Written informed consent was provided by each recruited participant. All participants enrolled were HIV-1-infected inpatients and outpatients visiting our hospital from June 2018 to December 2021, with an interval between the visit and HIV confirmed within 3 months and without evidence of HIV ART use before the date of sample collection. Demographic data and medical records, including HIV ribonucleic acid (RNA) viral load (VL), $CD4^+$ T-cell count, and transmission route, were collected. Furthermore, whole blood samples were collected and HIV-1 partial *pol* gene and complete integrase gene sequences were separately amplified, sequenced, and analyzed for drug resistance mutations (DRMs), as described previously.[1,2] DRMs and antiretroviral susceptibility were analyzed by submitting the determined sequences to the regularly updated Stanford HIV-1 drug resistance database (http://hivdb.stanford.edu/). Subtyping of HIV isolates was performed using the REGA HIV-1 Subtyping Tool (http://dbpartners.stanford.edu:8080/RegaSubtyping/stanford-hiv/typingtool/) based on the partial pol region, which was further confirmed by phylogenetic analysis. A phylogenetic tree was constructed using Molecular Evolutionary Genetic Analysis (MEGA) software (version X) based on the Maximum Likelihood method and General Time Reversible model with 1000 bootstrap replicates. To facilitate visualization, only portions of sequences from major subtypes (a subtype with a number of sequences >100) were used while all the sequences of the minor subtypes (a subtype with a number of sequences <100) were included in the construction of the phylogenetic tree. Statistical analysis was carried out using the SPSS statistics program (version 20) for Windows (SPSS, Inc., Chicago, IL, USA). Continuous variables were expressed as mean ± standard deviation if normally distributed or a median with the first to third quartile. Categorical variables were expressed as numbers with percentages. The differences between or among groups were analyzed by Student's *t*-test or chi-squared test; a $P < 0.05$ was considered to be statistically significant.

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Of 1262 patients, we obtained partial *pol* sequences from 1228 and integrase sequences from 827. So, 1228 HIV-1 patients, with a median age of 35 years (Q1–Q3: 7–89 years), were included in this analysis. Male patients accounted for 89.33% (1097/1228); Han ethnicity accounted for 84.53% (1038/1228) of the total patients. Furthermore, 83.06% (1020/1228) of patients were from Henan Province, and the remaining (16.94%) were from adjacent provinces or with unknown residence; 42.51% (522/1228) of the patients were married, 42.10% (517/1228) were single, and 189 had unknown marriage status. Non-workers accounted for 42.51% (522/1228) of the total patients, followed by, in descending order, workers (34.77%), unknown (17.51%), and students (5.21%). Transmission route data were available for 755 patients, of which men who have sex with men (MSM) dominated, accounting for 33.06% (406/1228), followed by, in descending order, heterosexual orientation (27.12%, 333/1228), plasmapheresis (1.30%, 16/1228), and injection drug use (0.08%, 1/1228). Baseline $CD4^+$ T-cell count data were available for 923 patients; the median $CD4^+$ T-cell count was 127 cells/μL (Q1–Q3: 1–1102 cells/mL). Baseline HIV-1 RNA copy (VL) data were available for 895 patients; the median baseline VL was 64,235 (182–67,946,648) copies/μL. Detailed demographic data of the patients are listed in Supplementary Table 1, http://links.lww.com/CM9/B370.

Subtyping analysis indicated that the prevalence of each subtype was as follows: CRF07_BC (34.77%, 427/1228), CRF01_AE (31.51%, 387/1228), B (25.65%, 315/1228), CRF55_01B (3.34%, 41/1228), A (1.14%, 14/1228), C (0.81%, 10/1228), CRF08_BC (0.73%, 9/1228), CRF52_01B (0.41%, 5/1228), CRF59_01B (0.008%, 1/1228), CRF67_01B (0.008%, 1/1228), CRF68_01B (0.008%, 1/1228), CRF45_cpx (0.008%, 1/1228), and other/recombinant (1.30%, 16/1228) [Supplementary Table 2, http://links.lww.com/CM9/B370]. Phylogenetic analysis with reference strains, including all confirmed subtypes, was performed along with these determined sequences [Supplementary Figure 1, http://links.lww.com/CM9/B370]. Based on the inferred phylogenetic tree, all determined subtypes clustered with reference strains, indicating consistent results and correct subtyping. When analyzed by individual drug class, the prevalence of TDRM was 2.20% (27/1228), 17.26% (212/1228), 2.77% (34/1228), and 1.81% (15/1228) for nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), and integrase strand transfer inhibitors (INSTIs), respectively [Supplementary Table 3, http://links.lww.com/CM9/B370]. The most common TDRMs were M184VI (17, 1.38%) for NRTIs, V179DEIT (125, 10.18%) for NNRTIs, Q58E (21, 1.71%) for PIs, and E157Q (8, 0.97%) for INSTIs. Two hundred and thirty-five patients showed TDRM to one drug class, 22-to-2 drug classes, and 3-to-3 drug classes. For individual drugs, the most commonly observed high-level, medium-level, low-level, and potential low-level resistant drugs were nevirapine (NVP), NVP, rilpivirine (RPV), and etravirine.

As more than 90% of newly diagnosed HIV-1 patients chose available free drug regimens for treatment, we further analyzed the prevalence of TDRM against those drugs. Based on our results, the prevalence of TDRM that led to low- or higher-level resistance to abacavir (ABC), zidovudine (AZT), lamivudine (3TC), and tenofovir (TDF) of the NRTIs was 1.63% (20), 0.57% (7), 1.63% (20), and 0.98% (12), respectively. The prevalence of TDRM that led to low- or higher-level resistance to NVP and efavirenz (EFV) of the NNRTIs was 6.03% (74) and 5.21% (64), respectively. The prevalence of TDRM that led to low- or higher-level resistance to lopinavir/ritonavir (LPV/r) was 0.24% (3). The overall prevalence of TDRM in the above eight drugs was 21.17% (260/1228). Detailed information about the TDRM-related drug resistance to first- and second-line regimens is presented in Supplementary Figure 2, http://links.lww.com/CM9/B370.

When analyzed by year, the overall prevalence of TDRM in 2019 and 2020 was similar to the overall prevalence, while the prevalence was lower in 2018 and 2021, possibly due to the small sample sizes. Due to the low prevalence of TDRMs to INSTIs, the prevalence trend of INSTIs could not be determined. Distribution of transmission routes stratified by age among patients newly diagnosed with HIV-1 showed that the incidence of MSM was higher in age groups younger than 20 years and 30 to 39 years [Supplementary Table 4, http://links.lww.com/CM9/B370]

Of the study population, 89.33% of patients were males, 75.98% were within the age range of 20 to 49 years, and heterosexual (HSX) and MSM accounted for >50%, which are consistent with data in the latest government report. The proportion of HSX and MSM transmission can be as high as >90% if the proportion of patients with unknown data is removed.

INSTIs were not freely available to patients and not covered by medical insurance in most provinces in China before 2022, including Henan Province, so only a limited number of patients were treated with this class of drugs, and resistance testing against INSTIs is thus not highly demanded in clinical settings. Phylogenetic analysis based on the determined partial *pol* gene sequences indicated that the most common subtype was CRF07_BC, accounting for 34.77%, followed by CRF01_AE, B, CRF55_01B, and A, which is consistent with subtype patterns in other regions.[3] The changes in subtype distribution from primary subtype B before the 2000s to more recent newly emerging recombinants in Henan Province, are not only a reflection of the transmission route from illegal blood donation to MSM and HEX but also increased population mobility.

In our previous study, DRMs were most commonly observed in B, the dominant subtype in treatment-experienced patients.[2] The discrepancy between TDRMs in treatment-naive and DRMs in treatment-experienced patients might be due to historical reasons. However, along with social and economic improvements, the number of patients infected with subtype B is expected to continue to decrease. The prevalence of TDRMs in subtypes showed a similar trend with the distribution of subtypes, which can be easily explained by the dominant

transmission routes (HEX and MSM) and is also consistent with other reports.[4] The most common TDRMs were M184VI for NRTIs, V179DEIT for NNRTIs, Q58E for PIs, and E157Q for INSTIs. M184V/I caused high-level *in vitro* resistance to 3TC and emtricitabine (FTC) and low-level resistance to ABC. V179DEIT only caused potential low-level resistance to EFV and NVP. Q58E, the most commonly observed PI-associated mutation, is a non-polymorphic accessory PI-selected mutation associated with reduced susceptibility to tipranavir (TPV) and possibly other PIs. E157Q is a polymorphic mutation selected in patients receiving raltegravir (RAL) and elvitegravir (EVG) and appears to have little effect on INSTI susceptibility.

The prevalence of TDRMs for NRTIs, NNRTIs, PIs, and INSTIs was consistent with the prevalence noted in other reports.[4-6] The higher prevalence of TDRMs against NNRTIs when compared with that of NRTIs might be due to their lower-resistance barrier and most frequent uses as a component of first-line therapy. The prevalence of TDRMs against the most commonly used NRTIs 3TC, ABC, AZT, and TDF and the most commonly used NNRTIs EFV and NVP were 1.30%, 0.65%, 0.08%, 0.49%, 3.58%, and 3.91%, respectively. 3TC is a component of the dual-drug compound, Dovato (3TC/dolutegravir [DTG]), which is gaining traction in China following its inclusion as one of the national basic medical insurance-covered drugs. The high prevalence of TDRMs against 3TC indicates that pretreatment genotypic resistance testing for patients receiving Dovato or 3TC containing regimens might be necessary. However, recent studies showed that the presence of pretreatment resistance mutations against 3TC did not affect the efficacy of the dual-drug compound (3TC/DTG) or triple-drug compound (ABC/3TC/DTG), possibly due to the high efficacy of DTG.[5] The other commonly used regimen is the triple-drug compound, Biktarvy, which is composed of bictegravir/tenofovir alafenamide/emtricitabine (BIC/TAF/FTC) and has also been included in the national basic medical insurance program. The high prevalence of TDRMs against FTC and TAF also warrants the implementation of genotypic drug resistance testing in treatment-naive patients.

LPV/r, the most commonly used PI in China, is associated with low resistance rate and high efficacy. LPV/r also has high resistance barriers, and the rate of DRMs is low even in treatment-experienced patients.[6] However, LPV/r is associated with potentially severe long-term effects such as fat redistribution and risks of cardiovascular events, and thus, is only used in second-line therapy. For INSTIs, only low levels of TDRMs against obsolete EVG and RAL were observed, and the result is consistent with that found in other reports.[6]

In summary, the prevalence of TDRMs is high for commonly used NRTIs and NNRTIs and low for commonly prescribed PIs and INSTIs in Henan Province. Standard genotypic drug resistance testing is currently recommended for all patients at entry into care to assist in the selection of effective and available drug regimens; furthermore, based on our data, more than 90% of newly diagnosed patients receive drug resistance testing before the initiation of ART. Since the use of INSTIs is expected to increase significantly in the near future, close monitoring of INSTI-related DRMs is thus necessary.

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Conflicts of interest

None.

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