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Protocol of HIV TDR and Subtype test in Beijing V.2

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1 Works for me dx.doi.org/10.17504/protocols.io.8ahhsb6

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ABSTRACT

We analyzed the demographic, clinical, and virological data of residents newly diagnosed with HIV in Beijing. We did population-based sequencing of the pol gene on plasma specimens and identified drug resistance mutations using the World Health Organization (WHO) list for surveillance of drug resistance mutations. HIV-1 subtype analyses utilized the automated subtyping tool COMET.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

1.Jingrong Ye, Hongyan Lu, Weishi Wang, et al. The prevalence of drug resistance mutations among treatment-naive HIV-infected individuals in Beijing, China.AIDS Res Hum Retroviruses 2012;28:418-423. 2.Daniel Struck, Glenn Lawyer, Anne-Marie Ternes,et al.COMET: adaptive context-based modeling for ultrafast HIV-1 subtype identification.Nucleic Acids Res. 2014;42:e144. 3.Diane E Bennett, Ricardo J Camacho, Dan Otelea,et al.Drug resistance mutations for surveillance of transmitted HIV-1 drug-resistance: 2009 update.PLoS One 2009;4:e4724.

MATERIALS TEXT

1.TaKaRa PrimeScript™ One Step RT-PCR Kit Ver 2.0;
2.Bio-Rad T100™ Thermal Cycler;
3.Takara TaKaRa Ex Taq DNA Polymerase

SAFETY WARNINGS

Biosafety 2 class laboratory for all this test.

1 Ethics: Beijing CDC Ethics Committee approved the study. By law, consent was not required because these data were collected and analyzed in the course of routine public health surveillance.

2 Study patients:The Beijing HIV laboratory network (BHLN), established in 1986, is a collaboration engaged in HIV diagnosis in Beijing authorized by the Beijing Municipal Commission of Health, which now includes one central HIV confirmed laboratory in the Beijing Center for Disease Prevention and Control (CDC),4 HIV confirmed laboratories (DiTan, YouAn, Peking Union Medical College, and PLA General Hospital), and more than 280 HIV screening laboratories. The collaboration boasts a biobank, which includes more than 50,000 samples from 21,886individuals from across China ever diagnosed in Beijing since 1986, and an HIV epidemiology database, which tracks everyone who receives a diagnosis of HIV infection in Beijing and records a baseline CD4 cell count for all newly identified individuals. We used BD FACSCalibur, BD FACSCanto II, and Beckman Coulter FC500 for CD4 cell counting.Each year we will conduct a survey of HIV TDR among individuals newly diagnosed with HIV. To ensure representative sampling, we designed a standardized sampling strategy. For simplicity, however, we just randomly selected half of the samples from all newly identified individuals. In addition, we retrospectively included half of samples that were stored in the biobank before routine genotyping was introduced in Beijing. We included study participants if they were aged 18 years or older, were newly diagnosed with HIV infection, and not pregnant. We excluded individuals who reported previous use of antiretroviral drugs for treatment or prophylaxis.

3 HIV subtyping: We inferred HIV subtype by automated subtyping in Context-Based Modeling for Expeditious Typing (COMET)-HIV.Sequences classified as “unassigned” by COMET were also analyzed by neighbor-joining phylogenetic analysis. The trees were estimated based on Kimura 2-parameter Model with 1000 bootstrap replicates, using Mega 6.0.

4 HIV TDR analyses: We did population-based sequencing of HIV protease and codons 1–300 of reverse transcriptase on all specimens, using in-house methods. We did all virological testing at two reference laboratories: the Division of Research on Virology and Immunology, China CDC (for the 2011 and 2013 survey) and the Beijing Central HIV confirmed laboratory, Beijing CDC (for the survey of the other years). Both laboratories participated in external quality assessment schemes for genotypic DR testing from the National AIDS Reference Laboratory of the National Center for AIDS/STD Control and Prevention. We defined TDR in two steps. First, we estimated the prevalence of TDR with the Stanford Calibrated Population Resistance (CPR) method, based on the WHO list of surveillance drug-resistant mutations 2009 (SDRM 2009) update. [12]Second, for patients harboring a virus with at least one drug-resistant mutation, we used the Stanford drug-susceptibility algorithm (version 8.5) to classify sequences as susceptible (Stanford level 1 or 2), low-level resistance (Stanford level 3), intermediate-level resistance (Stanford level 4), or high-level resistance (Stanford level 5) to the drug classes (NRTIs, NNRTIs, and PIs) and specific drugs.
We entrust three commercially available sequencing companies(Beijing Sino Geno Max Co., Ltd , Beijing Tsingke Biological technology Co., Ltd, Beijing Tianyi Huiyuan Biological technology Co., Ltd) to do the sequencing with an ABI 3500Analyzer. All these companies participated in external quality assessment schemes for sequencing test from us.

5 Analysis: We captured the baseline data from these individuals, including demographic characteristics, transmission risk group, and CD4 cell count in the Beijing HIV epidemiology database. We anonymized and de-identified the patients’ information prior to analysis. We categorized those with Beijing Hukou as Beijing residents. Hukou is a basic system of household registration in China. It officially identifies a person as a resident of an area and includes identifying information such as name, parents, spouse, and date of birth. We established four sampling phases for convenient sake:2001–2008, 2009–2011, 2012–2014, 2015–2016. We compared categorical data with the χ^2 test and continuous data with one-way ANOVA where appropriate. We calculated the prevalence of sequences containing at least one drug-resistant mutation and further specified for transcriptase inhibitors (NRTIs), non-NRTIs (NNRTIs), and protease inhibitors (PIs). We analyzed the potential risk factors for acquiring any drug-resistant mutations by using logistic regression. We assessed biologically plausible interactions in the multivariable model. Variables investigated were sex, age (18-24, 25-44, 45-64, and ≥65 years), ethnicity, HIV subtype, CD4 cell count (<200, 200-349, 350-499,and ≥500 cells per μL), transmission risk group, and sampling phase. In the model, we included a binary response, indicating detection of any drug-resistant mutation from each patient as an outcome. We analyzed variables independently and included those that were associated ($p<0.1$) with the outcome in the multivariable model. We expressed our results as odds ratios (ORs) with 95% confidence intervals (CIs) and two-sided P values, with a P value of <0.05 considered statistically significant. We did all analyses with R (version 3.5.0). We used listwise deletion to handle missing data.