Original Article

# Understanding drug resistance patterns across different classes of antiretrovirals used in HIV-1-infected treatment-Naïve and experienced patients in Mumbai, India

Raj Gurubuxrai Harjani[1,2], Asha Krishnaraj Iyer[1], Ankita Chaurasia[1]

[1]Centre for Excellence in HIV/AIDS, Ashirwad Hospital, [2]AIDS Research and Control Centre (ARCON-VCTC) Rajiv Gandhi Medical College and CSM Hospital Kalwa (Collaborative Program of Thane Municipal Corporation TMC, Government of Maharashtra, Maharashtra, India and the University of Texas, Houston, USA), Thane, Maharashtra, India

**Address for correspondence:**
Dr. Raj Gurubuxrai Harjani, Centre for Excellence in HIV/AIDS, Ashirwad Hospital, Thane - 400 603, Maharashtra, India.
E-mail: drrajharjani@gmail.com

## Abstract

**Background:** The aim of this study is to find out the proportion of treatment-naïve (Tn) and treatment-experienced (Te) patients experiencing HIV drug resistance (DR) to different classes of antiretrovirals (ARVs) being used for HIV treatment and their in class DR correlation. **Methods:** A cross-sectional study was done on 109 HIV patients enrolled at a private hospital in Thane, India, from 2014 to 2019. All patients were tested for CD4 count, viral load, and resistance to ARVs. **Results:** Sixty-six patients were Tn and 43 patients were Te. Among Tn and Te patients, the percentage of high-level resistance (HLR) for nonnucleoside reverse transcriptase inhibitors (NNRTI) was 4.55% and 37.8%, respectively, for nucleoside reverse transcriptase inhibitors (NRTI) was 0.43% and 36.4%, respectively. No HLR was observed for protease inhibitors (PIs) among Tn patients, while Te patients showed 2.62% HLR. Tn and Te patients showed high susceptibility for Darunavir (98.48% and 95.34%, respectively) followed by Atazanavir and Lopinavir (96.96%, each and 90.69%, each). Tn patients showed HLR for Lamivudine and Emtricitabine (1.52%, each). Integrase Strand Transfer Inhibitors were susceptible (100%) in both Tn and Te patients. A positive correlation was observed for within class across ARVs. **Conclusion:** An increased incidence of HLR was observed for NNRTI as compared to NRTI while PIs and integrase strand transfer inhibitors (INSTIs) demonstrated no HLR in either group of patients. When selecting a regimen for Tn patients consisting of NRTIs + NNRTIs genotypic DR test is essential. While with PIs or INSTIs its optional. Among Te patients, DR testing is recommended for all classes of drugs.

**Key words:** Antiretrovirals drug resistance, human immunodeficiency virus, India, Mumbai, treatment-experienced, treatment-naïve

## Introduction

India HIV Estimation fact sheet 2017 reported that, there are 2.1 million people living with HIV with thousands of people getting newly infected each year.[1] In 2004, the National AIDS Control Organization Department of AIDS Control initiated free antiretroviral treatment (ART) which broadened the access to ART.[2] In India, nearly 1.17 million people are receiving ART.[1] To suppress HIV replication a combination of ARVs drugs has been recommended, thus, preventing HIV-linked mortality and morbidity apart from improving the quality of life of HIV/AIDS infected people.[3] All newly diagnosed HIV patients in Indian ART centers are offered ARVs consisting of two nucleoside reverse transcriptase inhibitors (NRTIs) and 1 nonnucleoside reverse transcriptase inhibitor (NNRTI). Regimens with protease inhibitors (PIs) are reserved as second-line treatment options for patients who fail the first-line ART.[4] In recent years, however, there has been a growing concern of the emergence of pretreatment drug resistance mutations (DRMs).[5] pretreatment drug resistance (DR) has the ability to contribute to the increasing rates of virological failure at a population level, thus, compromising the long-term effectiveness of recommended first-line regimens.[6] Emerging data have revealed an increased prevalence of DR HIV strains ranging from 10% to 20% among ART-naïve patients.[7-9]

Access this article online

Quick Response Code:

Website: www.ijstd.org

DOI: 10.4103/ijstd.ijstd_101_20



**How to cite this article:** Harjani RG, Iyer AK, Chaurasia A. Understanding drug resistance patterns across different classes of antiretrovirals used in HIV-1-infected treatment-Naïve and experienced patients in Mumbai, India. Indian J Sex Transm Dis 2022;43:150-5.

**Submitted:** 25-Sep-2020 **Revised:** 26-Jun-2022
**Accepted:** 12-Jul-2022 **Published:** 17-Nov-2022

Thus, there is a concern about the onward transmission of DR strains after ART scale-up. A study done in Western India in Mumbai showed a prevalence of DR strains among ART-naïve patients to be 9.6%.[10] Another study done in Mumbai demonstrated that in treatment-naïve (Tn) patients, the proportion of high-level resistance (HLR) was 2% for NRTIs, 5% for PIs, and 11% for NNRTIs.[2] This is an unique study done in Mumbai to further our understanding of DR and its patterns both in Tn and treatment-experienced (Te) patients. The aim of this study is to find out the proportion of Tn and Te patients experiencing HIV DR to different classes of drugs being used for HIV treatment and their in class DR correlation pattern.

## Methods

For the purpose of this cross-sectional study, 121 HIV-positive patients were enrolled from a private hospital in Thane, Maharashtra, India, between 2014 and 2019. Out of 121 patients, 12 patients dropped out from the study due to low viral load (VL) which could not be amplified, thus, a total of 109 patients were included in the study for the analysis. Of these 109 HIV-positive patients, 66 were newly diagnosed Tn patients and 43 were Te patients. All patients were enrolled in the study after obtaining written informed consent. Resistance testing was done at a private diagnostic center and was chargeable to the patients. Given the cost associated with HIV DR testing in India, patient consent was obtained for the same across different classes of ARVs. All patients included in the study were tested for CD4 count, VL, and resistance to antiretroviral (ARVs). Tests were conducted on following drugs: (1) PIs: Atazanavir (ATV), Darunavir (DRV), Fosamprenavir (FPV), Indinavir (IDV), Lopinavir (LPV), Nelfinavir (NFV), Saquinavir (SQV), and Tipranavir (TPV); (2) NRTIs: Lamivudine (3TC), Abacavir (ABC), Azidothymidine (AZT), Stavudine (D4T), Didanosine (DDI), Emtricitabine (FTC), and Tenofovir (TDF); and (3) NNRTIs: Efavirenz (EFV), Etravirine (ETR), Nevirapine (NVP), and Rilpivirine (RPV); (4) Integrase strand transfer inhibitors (INSTI): Elvitegravir, Dolutegravir (DTG), Raltegravir. The HIV RNA polymerase chain reaction (PCR) was done using QIAGEN One Step Reverse transcription PCR Kit (QIAGEN, Hilden, Germany), and HIV RNA real-time PCR was done using RoboGene HIV-1 Quantification Kit (ROBOSCREEN, Germany). HIV Stanford Database was used for genotypic DR analysis. The resistance patterns were classified as: susceptible (resistance mutation score [RMS] 0 and 5), potential low-level resistance (RMS 10), low-level resistance (RMS 15–25), intermediate resistance (RMS 30–55), and HLR (RMS 60 and above).

Analyses for all recorded variables were performed using IBM SPSS software version 21.0.(IBM Corp., Armonl, N.Y., USA) For qualitative data, Chi-square/Fisher's exact test was applied for low cell counts. We also calculated the overall prevalence of HLR in all the resistance tests conducted among PIs, NRTIs, and NNRTIs. *P* values were considered statistically significant at a level of $P < 0.05$.

## Results

Table 1 shows the levels of resistance in each class of drug of Tn and Te patients. The mean age of 66 Tn patients was 37.59 (±11.49) years (age range = 14–67 years; 45 males, 20 females, 1 transgender) and Te patients was 44.47 (±10.41) years (age-range = 13–

**Table 1: Levels of resistance among nonnucleoside reverse transcriptase inhibitors, nucleoside reverse transcriptase inhibitors and protease inhibitors in 66 treatment-naïve (no; not exposed to treatment) and 43 treatment-experienced patients (yes; exposed to treatment)**

| Drugs | Susceptible, *n* (%) | PLLR, *n* (%) | LLR, *n* (%) | IR, *n* (%) | HLR, *n* (%) | *P* |
|---|---|---|---|---|---|---|
| NNRTI | | | | | | |
| EFV | | | | | | |
| Yes | 13 (30.23) | 0 | 3 (6.97) | 0 | 27 (62.80) | <0.05* |
| No | 58 (87.88) | 0 | 0 | 2 (3.03) | 6 (9.09) | |
| ETR | | | | | | |
| Yes | 24 (55.81) | 3 (6.98) | 7 (16.28) | 7 (16.28) | 2 (4.65) | <0.05* |
| No | 61 (92.42) | 4 (6.06) | 1 (1.52) | 0 | 0 | |
| NVP | | | | | | |
| Yes | 13 (30.23) | 0 | 0 | 1 (2.33) | 29 (67.44) | <0.05* |
| No | 58 (87.88) | 0 | 1 (1.52) | 1 (1.52) | 6 (9.09) | |
| RPV | | | | | | |
| Yes | 24 (55.81) | 0 | 6 (13.95) | 6 (13.95) | 7 (16.25) | <0.05* |
| No | 61 (92.42) | 0 | 3 (4.54) | 2 (3.03) | 0 | |
| NRTI | | | | | | |
| 3TC | | | | | | |
| Yes | 15 (34.88) | 0 | 0 | 1 (2.33) | 27 (62.79) | <0.05* |
| No | 65 (98.48) | 0 | 0 | 0 | 1 (1.52) | |
| ABC | | | | | | |
| Yes | 15 (34.88) | 1 (2.33) | 9 (20.93) | 3 (6.97) | 15 (34.88) | <0.05* |
| No | 65 (98.48) | 0 | 0 | 1 (1.52) | 0 | |
| AZT | | | | | | |
| Yes | 31 (72.10) | 0 | 0 | 3 (6.97) | 9 (20.93) | <0.05* |
| No | 66 (100.0) | 0 | 0 | 0 | 0 | |
| D4T | | | | | | |
| Yes | 25 (58.1) | 0 | 0 | 8 (18.60) | 10 (23.26) | <0.05* |
| No | 65 (98.48) | 0 | 0 | 1 (1.52) | 0 | |
| DDI | | | | | | |
| Yes | 15 (34.88) | 10 (23.26) | 0 | 3 (7.0) | 15 (34.88) | <0.05* |
| No | 65 (98.48) | 0 | 0 | 1 (1.52) | 0 | |
| FTC | | | | | | |
| Yes | 15 (34.88) | 0 | 0 | 1 (2.33) | 27 (62.79) | <0.05* |
| No | 65 (98.48) | 0 | 0 | 0 | 1 (1.52) | |
| TDF | | | | | | |
| Yes | 24 (55.81) | 1 (2.33) | 4 (9.30) | 7 (16.28) | 7 (16.28) | <0.05* |
| No | 65 (98.48) | 0 | 1 (1.52) | 0 | 0 | |
| PI | | | | | | |
| ATV | | | | | | |
| Yes | 39 (90.69) | 1 (2.33) | 1 (2.33) | 1 (2.33) | 1 (2.33) | 0.46 |
| No | 64 (96.96) | 0 | 1 (1.52) | 1 (1.52) | 0 | |
| DRV | | | | | | |
| Yes | 41 (95.34) | 0 | 1 (2.33) | 1 (2.33) | 0 | 0.48 |
| No | 65 (98.48) | 0 | 1 (1.52) | 0 | 0 | |
| FPV | | | | | | |
| Yes | 39 (90.69) | 0 | 1 (2.33) | 2 (4.65) | 1 (2.33) | 0.25 |
| No | 64 (96.96) | 1 (1.52) | 0 | 1 (1.52) | 0 | |
| IDV | | | | | | |
| Yes | 39 (90.69) | 1 (2.33) | 0 | 1 (2.33) | 2 (4.65) | 0.04* |
| No | 64 (96.96) | 0 | 2 (3.03) | 0 | 0 | |
| LPV | | | | | | |
| Yes | 39 (90.69) | 1 (2.33) | 0 | 1 (2.33) | 2 (4.65) | 0.04* |
| No | 64 (96.96) | 0 | 2 (3.03) | 0 | 0 | |
| NFV | | | | | | |
| Yes | 39 (90.69) | 1 (2.33) | 0 | 1 (2.33) | 2 (4.65) | 0.1 |

*Contd...*

**Table 1: Contd...**

| Drugs | Susceptible, *n* (%) | PLLR, *n* (%) | LLR, *n* (%) | IR, *n* (%) | HLR, *n* (%) | *P* |
|---|---|---|---|---|---|---|
| No | 62 (93.94) | 0 | 3 (4.54) | 1 (1.52) | 0 | |
| SQV | | | | | | |
| Yes | 39 (90.69) | 2 (4.65) | 0 | 1 (2.33) | 1 (2.33) | 0.18 |
| No | 64 (96.96) | 0 | 1 (1.52) | 1 (1.52) | 0 | |
| TPV | | | | | | |
| Yes | 39 (90.69) | 1 (2.33) | 0 | 3 (6.98) | 0 | 0.02* |
| No | 64 (96.96) | 0 | 2 (3.03) | 0 | 0 | |

*Significant at *P*<0.05, #INSTIs were 100% susceptible in both Tn and Te patients. NRTI=Nucleoside reverse transcriptase inhibitors; NNRTI=Non-NRTI; PIs=Protease inhibitors; Tn=Treatment-naïve; Te=Treatment-experienced; EFV=Efavirenz; ETR=Etravirine; NVP=Nevirapine; RPV=Rilpivirine; 3TC=Lamivudine; ABC=Abacavir; AZT=Azidothymidine; D4T=Stavudine; DDI=Didanosine; FTC=Emtricitabine; TDF=Tenofovir disoproxil fumarate; ATV=Atazanavir; DRV=Darunavir; FPV=Fosamprenavir; IDV=Indinavir; LPV=Lopinavir; NFV=Nelfinavir; SQV=Saquinavir; TPV=Tipranavir; INSTIs=Integrase strand transfer inhibitors, PLLR=Potential low-level resistance; LLR=Low-level resistance; IR=Intermediate resistance; HLR=High-level resistance

64 years; 11 females, 32 males). All 109 patients were tested for PIs, NRTIs, and NNRTIs. Out of 66 Tn patients, 48 patients agreed for INSTIs resistance test. Among PIs, high susceptibility was observed for DRV (98.48%), followed by ATV and LPV (both 96.96%, respectively). No HLR was observed in PI mutation. Susceptibility was high for all NRTIs. The susceptibility was similar for all NNRTIs tested. For ETR, RPV it was 92.42%, respectively and for EFV and NVP it was 87.88%, respectively. We recorded HLR (9.09%) for both EFV and NVP, respectively. Resistance patterns were significantly different in Tn and Te patients for IDV, LPV, and TPV only ($P < 0.05$). Similarly, it was significantly different for NRTIs and NNRTIs ($P < 0.05$). All 43 Te patients were tested for PIs, NRTIs, and NNRTIs. Out of 43 Te patients, INSTIs resistance test was conducted on 22 patients. In PIs, high susceptibility was found in DRV (95.34%), followed by ATV, FPV, IDV, LPV, NFV, SQV, and TPV (each 90.69%, respectively). HLR was found in IDV, LPV, and NFV (each 4.65%, respectively). Among NRTIs, HLR was observed for 3TC, FTC (both 62.79%), ABC, DDI (both 34.88%), D4T (23.26%), AZT (20.93%), and TDF (16.28%). Among NNRTIs, HLR was observed for NVP (67.44%), EFV (62.80%), RPV (16.25%), and ETR (4.65%). In INSTIs, all 22 patients were found to be susceptible. Resistance patterns were also found to be significant for all NRTIs and NNRTIs in both groups of patients, respectively ($P < 0.05$).

Table 2 shows the prevalence of resistance levels and RMS s among PIs, NRTIs, NNRTIs, and INSTIs in both Tn and Te patients. Among Tn patients, a total of 528 resistance tests were conducted for PIs. Of these, no cases of HLR were observed. Similarly, out of a total of 462 NRTI resistance tests, the prevalence of HLR among them was 0.43% and out of 264 NNRTI resistance tests, the occurrence of HLR was 4.54%. However, no HLR was observed in INSTI. Among Te patients, the highest percentage of HLR cases was observed in NNRTIs (37.79%), followed by NRTIs (36.54%), respectively. A total of 344 resistance tests were conducted for PIs of which 2.62% of patients reported to have HLR. Out of 301 NRTI resistance tests, the prevalence of HLR was 36.54%. Similarly, out of 172 NNRTI resistance tests, the occurrence of HLR was 37.80%. However, no HLR was observed in the INSTI tests.

Table 3 represents the major mutations associated with resistance to ARVs. Among Tn patients, major mutations associated with resistance NNRTIs was K103N/S (10.60%), followed by E138A/Q (6.06%) and Y188D/F (4.54%). Similarly, the frequency of mutation for M184V, K70E, and D67N in the NRTI class was 1.51% each. In the NNRTI group, the most frequent mutation was F53 L and T74S at (3.03% each). In Te patients, the most frequent mutation in PIs was V82C/VA/VG at 6.97%, while the frequency of M184V (62.79%) mutation was found to be highest in NRTI resistance, followed by D67N (27.90%), K219E (25.58%), K70N (23.25%), and T215E (23.25%), respectively. Similarly, in NNRTI resistance, the most common mutations detected was K103N (32.55%), followed by G190A/S (27.90%) and V106A/M (25.58%). Among the 109 patients included, Subtype C was the most prevalent (92.66%), followed by subtype B, CRF02-AG (each 2.75%), respectively.

The correlation of the resistance pattern of drugs among Tn patients is shown in Table 4. In PI mutations, the resistance of ATV is found to be highly significantly correlated with the resistance of FPV, IDV, LPV, SQV, and TPV ($P < 0.05$ and $r = 0.99$ each), respectively. In NRTIs, the resistance of 3TC is found to be significantly correlated with the resistance of ABC, D4T, DDI, FTC, and TDF ($r = 0.99$ each; $P < 0.05$). We also found a significant correlation between the resistance patterns of D4T and DDI, D4T and FTC, and D4T and TDF, respectively ($P < 0.05$). In NNRTIs, a highly significant correlation was observed in the resistance pattern of EFV and NVP ($r = 0.99$) and ETR and RPV ($r = 0.99$) ($P < 0.05$).

The correlation of resistance pattern of drugs among Te patients is shown in Table 5. Among Te patients, resistance of ATV is highly correlated with resistance of FPV, IDV, LPV, NFV, SQV and TPV ($r = 0.99$). FPV is found to be highly correlated with IDV, LPV NFV and SQV, respectively ($r = 0.99$). Similarly, significant correlation is observed between IDV and LPV ($r = 0.99$), IDV and NFV ($r = 0.99$), IDV and SQV ($r = 0.99$) and IDV and TPV ($r = 0.99$), respectively. Among NRTIs, significant correlation was observed between 3TC and FTC ($r = 0.99$), 3TC and ABC ($r = 0.85$), 3TC and DDI ($r = 0.83$). As the resistance of ABC increases, resistance of FTC, DDI, and TDF also increases. AZT is highly correlated with D4T ($r = 0.82$). Significant correlation was observed in resistance level of D4T and TDF ($r = 0.90$) and DDI and TDF ($r = 0.90$) ($P < 0.05$). Among NNRTIs, we found a highly significant correlation between ETR and RPV ($r = 0.97$), EFV and NVP ($r = 0.95$) ($P < 0.05$). In general, the resistance patterns were similar within same classes of drugs.

## Discussion

Though recent advances in HIV treatment and effective adherence to treatment allow for successful management of HIV, DR still remains a major challenge in achieving viral suppression. Studies done in India about 4–5 years ago, reported that the prevalence of primary DR ranged from 0% and 6.7%.[3] However, during the last few years, ART usage has increased, and with this increase, there have been higher levels of NRTIs and NNRTIs DR among Te patients. Similarly, adherence to medication too has increased in the last few years which explains lower levels of DR among Tn patients as reported in this study. Better understanding among HIV patients and enhanced adherence to medication have enabled patients to achieve sustained virological

**Table 2: Levels of resistance and resistance mutation scores among protease inhibitors, nucleoside reverse transcriptase inhibitors, nonnucleoside reverse transcriptase inhibitors and integrase strand transfer inhibitors in all tests conducted among 66 treatment-naïve patients and 43 treatment experienced patients**

| Patients group | Anti-retroviral tests | Total | Susceptible, *n* (%) | PLLR, *n* (%) | LLR, *n* (%) | IR, *n* (%) | HLR, *n* (%) |
|---|---|---|---|---|---|---|---|
| Treatment naive | PI | 528 | 511 (96.78) | 1 (0.19) | 12 (2.27) | 4 (0.76) | 0 |
| | NRTI | 462 | 456 (98.70) | 0 | 1 (0.22) | 3 (0.65) | 2 (0.43) |
| | NNRTI | 264 | 238 (90.15) | 4 (1.52) | 5 (1.89) | 5 (1.89) | 12 (4.55) |
| | INSTI | 144 | 144 (100.00) | 0 | 0 | 0 | 0 |
| Experienced patients | PI | 344 | 314 (91.28) | 7 (2.03) | 3 (0.87) | 11 (3.2) | 9 (2.62) |
| | NRTI | 301 | 140 (46.51) | 12 (3.99) | 13 (4.32) | 26 (8.64) | 110 (36.54) |
| | NNRTI | 172 | 74 (43.02) | 3 (1.74) | 16 (9.30) | 14 (8.14) | 65 (37.8) |
| | INSTI | 66 | 66 (100.00) | 0 | 0 | 0 | 0 |

NRTI=Nucleoside reverse transcriptase inhibitor; NNRTI=Non-NRTI; PI=Protease inhibitor; INSTI=Integrase strand transfer inhibitors, PLLR=Potential low-level resistance; LLR=Low-level resistance; IR=Intermediate resistance; HLR=High-level resistance

**Table 3: Antiretroviral drug resistance mutations detected in treatment-naïve and treatment-experienced patients**

| Mutations detected in naïve patients (*n*=66) | | | Mutations detected in treatment-experienced patients (*n*=44) | | |
|---|---|---|---|---|---|
| PI (*n*) | NRTI (*n*) | NNRTI (*n*) | PI (*n*) | NRTI (*n*) | NNRTI (*n*) |
| V32I (1) | D67N (1) | K103K, N, S (7) | L23I (1) | M41L (8) | V90I, V (2) |
| M46L (1) | K70E (1) | V106I, M (2) | V32I (2) | E44D (1) | A98G (8) |
| F53L (2) | M184V (1) | E138A, Q (4) | M46I, L (2) | K65R (6) | L100I (1) |
| I54S (1) | | Y188D, F (3) | G48I (1) | D67N (12) | K101E, EK, H (8) |
| T74S (2) | | G190A (1) | I54V (2) | T69D (4) | K103S, N, KN (14) |
| | | F227FL (1) | L76V (1) | K70E, R, KN, KR (10) | V106A, M (11) |
| | | | V82C, VA, VG (3) | L74I (1) | V108I (5) |
| | | | L89T (1) | V75M (3) | E138Q, E (2) |
| | | | | F77FL (1) | V179D, T (4) |
| | | | | Y115F (3) | Y181C (4) |
| | | | | M184V, MV (27) | V181C (1) |
| | | | | T215E, F, I, N, Y (10) | Y188L, C (2) |
| | | | | K219E, Q, M, N, R, Rm (11) | G190A, S (12) |
| | | | | | H221Y (2) |
| | | | | | P225H (3) |
| | | | | | F227L (5) |
| | | | | | K238T (1) |
| | | | | | Y318F (2) |

NRTI=Nucleoside reverse transcriptase inhibitor, NNRTI=Non-NRTI, PI=Protease inhibitor

suppression, thereby lowering the transmission rates (wild or mutated virus) in the community.

The WHO recommends the use of 2 NRTIs + EFV or 2NRTIs + DTG as the first line for HIV treatment.[11] The primary DR mutations observed in this study are recognized as per the Stanford DR database. This study found that among NRTIs, M184V is the most frequent mutation conferring resistance to NRTIs in both Tn and Te HIV patients, followed by D67N and K70E. Previous studies have shown that M184V could result in HLR to 3TC and FTC[12,13] and low-level resistance to DDI and ABC *in vitro*.[14-17] Remarkably, M184V could reduce the viral fitness of HIV, and increase susceptibility to AZT, d4T, and TDF and slow the emergence of AZT, d4T, and TDF resistance.[14,15,17,18] D67N is a TAM associated with low-level resistance to AZT and d4T. When present with other TAMs, it adds to reducing susceptibility to ABC, DDI, and TDF. Accessory TAMs like K219Q/E are associated with decreased susceptibility to AZT and possibly d4T when present with other TAMs.[17] T215Y/F confers intermediate-level resistance to AZT and d4T and low-level resistance to ABC, DDI, and TDF. K70E/G produces low-level resistance to TDF, ABC, DDI and possibly 3TC and FTC while increasing susceptibility to AZT.[19] K219N/R mutations are also selected by AZT and d4T and seem to contribute to reduced NRTI susceptibility in combination with other TAMs.[17]

Similarly, among NNRTIs, K103N/K/S was the most common mutation seen in both Tn and Te patients. K103N is a mutation selected in patients receiving NVP and EFV reducing susceptibility by about 50- and 20-fold, respectively.[20] K103S, on the other hand, usually occurred in patients who earlier had K103N mutations. This mutation is linked with intermediate-level reductions in susceptibility to EFV. We also found a noteworthy difference in mutation patterns among Tn and Te patients on NNRTIs. Tn patients showed E138A and Y188D/F mutations, while Te patients showed V106A/m, A98G, and K101 E/EK/H mutations. V106A is a mutation selected by NVP and Doravirine (DOR) and causes about a 50-fold reduction in NVP susceptibility and about a 5-fold reduction in EFV susceptibility. Unaccompanied, it causes intermediate declines in DOR susceptibility but in combination with other DOR-associated DRMs, it is associated with high-level DOR resistance. On the other hand, V106M is a mutation selected mainly by EFV and NVP. It is most common in subtype C viruses and causes more than 30-fold reduced susceptibility to NVP and EFV.

**Table 4: Correlation matrix of resistance pattern of protease inhibitors, nucleoside reverse transcriptase inhibitors and nonnucleoside reverse transcriptase inhibitors among 66 treatment-naïve patients**

| Drugs | ATV | DRV | FPV | IDV | LPV | NFV | SQV | TPV | 3TC | ABC | D4T | DDI | FTC | TDF | EFV | ETR | NVP | RPV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PIs | | | | | | | | | | | | | | | | | | |
| ATV | 1.00 | | | | | | | | | | | | | | | | | |
| DRV | **0.71** | 1.00 | | | | | | | | | | | | | | | | |
| FPV | **0.99** | **0.71** | 1.00 | | | | | | | | | | | | | | | |
| IDV | **0.99** | **0.70** | **0.99** | 1.00 | | | | | | | | | | | | | | |
| LPV | **0.99** | **0.70** | **0.99** | **0.99** | 1.00 | | | | | | | | | | | | | |
| NFV | **0.71** | **0.51** | **0.70** | **0.71** | **0.71** | 1.00 | | | | | | | | | | | | |
| SQV | **0.99** | **0.69** | **0.99** | **0.99** | **0.99** | **0.71** | 1.00 | | | | | | | | | | | |
| TPV | **0.99** | **0.70** | **0.99** | **0.99** | **0.99** | **0.71** | **0.99** | 1.00 | | | | | | | | | | |
| NRTIs | | | | | | | | | | | | | | | | | | |
| 3TC | | | | | | | | | 1.00 | | | | | | | | | |
| ABC | | | | | | | | | **0.99** | 1.00 | | | | | | | | |
| D4T | | | | | | | | | **0.99** | **0.99** | 1.00 | | | | | | | |
| DDI | | | | | | | | | **0.99** | **0.99** | **0.99** | 1.00 | | | | | | |
| FTC | | | | | | | | | **0.99** | **0.99** | **0.99** | **0.99** | 1.00 | | | | | |
| TDF | | | | | | | | | **0.99** | **0.99** | **0.99** | **0.99** | **0.99** | 1.00 | | | | |
| NNRTIs | | | | | | | | | | | | | | | | | | |
| EFV | | | | | | | | | | | | | | | 1.00 | | | |
| ETR | | | | | | | | | | | | | | | **0.59** | 1.00 | | |
| NVP | | | | | | | | | | | | | | | **0.99** | **0.59** | 1.00 | |
| RPV | | | | | | | | | | | | | | | **0.60** | **0.99** | **0.59** | 1.00 |

Values in bold indicate *P*<0.05. NRTI=Nucleoside reverse transcriptase inhibitor; NNRTI=Non-NRTI; PIs=Protease inhibitors; EFV=Efavirenz; ETR=Etravirine; NVP=Nevirapine; RPV=Rilpivirine; 3TC=Lamivudine; ABC=Abacavir; D4T=Stavudine; DDI=Didanosine; FTC=Emtricitabine; TDF=Tenofovir disoproxil fumarate; ATV=Atazanavir; DRV=Darunavir; FPV=Fosamprenavir; IDV=Indinavir; LPV=Lopinavir; NFV=Nelfinavir; SQV=Saquinavir; TPV=Tipranavir

**Table 5: Correlation matrix of resistance pattern of protease inhibitors, nucleoside reverse transcriptase inhibitors, and nonnucleoside reverse transcriptase inhibitors among 43 treatment-experienced patients**

| Drug | ATV | DRV | FPV | IDV | LPV | NFV | SQV | TPV | 3TC | ABC | AZT | D4T | DDI | FTC | TDF | EFV | ETR | NVP | RPV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PIs | | | | | | | | | | | | | | | | | | | |
| ATV | 1.00 | | | | | | | | | | | | | | | | | | |
| DRV | **0.68** | 1.00 | | | | | | | | | | | | | | | | | |
| FPV | **0.99** | **0.71** | 1.00 | | | | | | | | | | | | | | | | |
| IDV | **0.99** | **0.69** | **0.99** | 1.00 | | | | | | | | | | | | | | | |
| LPV | **0.99** | **0.69** | **0.99** | **0.99** | 1.00 | | | | | | | | | | | | | | |
| NFV | **0.99** | **0.69** | **0.99** | **0.99** | **0.99** | 1.00 | | | | | | | | | | | | | |
| SQV | **0.99** | **0.68** | **0.99** | **0.99** | **0.99** | **0.99** | 1.00 | | | | | | | | | | | | |
| TPV | **0.99** | **0.67** | **0.99** | **0.99** | **0.99** | **0.99** | **0.99** | 1.00 | | | | | | | | | | | |
| NRTIs | | | | | | | | | | | | | | | | | | | |
| 3TC | | | | | | | | | 1.00 | | | | | | | | | | |
| ABC | | | | | | | | | **0.85** | 1.00 | | | | | | | | | |
| AZT | | | | | | | | | **0.47** | **0.66** | 1.00 | | | | | | | | |
| D4T | | | | | | | | | **0.57** | **0.89** | **0.82** | 1.00 | | | | | | | |
| DDI | | | | | | | | | **0.83** | **0.98** | **0.63** | **0.89** | 1.00 | | | | | | |
| FTC | | | | | | | | | **0.99** | **0.99** | **0.47** | **0.57** | **0.83** | 1.00 | | | | | |
| TDF | | | | | | | | | **0.57** | **0.88** | **0.55** | **0.90** | **0.90** | **0.57** | 1.00 | | | | |
| NNRTIs | | | | | | | | | | | | | | | | | | | |
| EFV | | | | | | | | | | | | | | | | 1.00 | | | |
| ETR | | | | | | | | | | | | | | | | **0.49** | 1.00 | | |
| NVP | | | | | | | | | | | | | | | | **0.95** | **0.54** | 1.00 | |
| RPV | | | | | | | | | | | | | | | | **0.47** | **0.97** | **0.53** | 1.00 |

Values in bold indicate *P*<0.05. NRTI=Nucleoside reverse transcriptase inhibitor; NNRTI=Non-NRTI; EFV=Efavirenz; ETR=Etravirine; NVP=Nevirapine; RPV=Rilpivirine; 3TC=Lamivudine; ABC=Abacavir; D4T=Stavudine; AZT=Azidothymidine; DDI=Didanosine; FTC=Emtricitabine; TDF=Tenofovir disoproxil fumarate; ATV=Atazanavir; DRV=Darunavir; FPV=Fosamprenavir; IDV=Indinavir; LPV=Lopinavir; NFV=Nelfinavir; SQV=Saquinavir; TPV=Tipranavir

It is also chosen *in vitro* and *in vivo* by DOR and initial data suggests it is linked with low/intermediate decreases in DOR susceptibility. A98G is an accessory mutation selected in patients receiving NVP and EFV. It reduces NVP, EFV, RPV, and DOR susceptibility by about 2- to 3-fold. K101E generally occurs in combination with other NNRTI-resistance mutations. Alone it reduces susceptibility to NVP by 3–10-fold, to EFV by 1–5-fold, and to ETR and RPV by about 2-fold.[21] Results from this study also reported that patients who developed resistance to EFV would also develop resistance to NPV likewise those resistant to ETR would develop resistance to RPV.

Given the DR mutations observed among NRTIs and NNRTIs in both Tn and Te patients and the high level of in-class cross-resistance among NRTIs, NNRTIs, it may be beneficial to assess the resistance before starting 2NRTIs + NNRTI-based regimen in patients.

In India, PI-based regimens are often reserved as second-line treatment. This study found that PIs were susceptible in both, Tn and Te patients. In this study, V32I and M46 L mutations were reported among PIs, in both groups. V32I is a mutation related to reduced susceptibility to each of the PIs except SQV. In combination with other PI-resistance mutations, they are linked with reduced susceptibility to each of the PIs except DRV.[22]

Limitations of this study are a relatively small population size in each group with a still smaller proportion of patients consenting for INSTI resistance testing. Thus, the generalization of this data is limited; however, a detailed analysis of all four groups of ARVs is presented. In addition, correlation matrix has also been included for better understating of in-class resistance patterns.

Despite these limitations, this study significantly contributes to the literature especially, in the Indian context, and has important clinical implications. Understanding these mutation patterns among Tn and Te patients will allow clinicians to better choose an appropriate treatment regimen that would reduce the development of ART resistance.

## Conclusion

A combination of 2 NRTIs + 1 NNRTIs is the most frequently used regimen for HIV treatment. This study reported that among Tn patients, the prevalence of HIV DRMs across NRTIs is 0.43% and NNRTIs is 4.55%, respectively, while among Te patients, it is 36.54% for NRTIs, 37.8% for NNRTIs, and 2.62% for PIs. INSTIs were susceptible in both groups of patients.

Thus, in resource-limited settings when selecting a regimen for Tn patients consisting of NRTIs + NNRTIs genotypic resisting testing is essential while a combination of NRTIs with PIs or INSTIs can be safely used as no HLR is observed with PIs and INSTIs. Among Te patients, DR testing is recommended for all classes of drugs when the VL is high.

### Financial support and sponsorship

Nil.

### Conflicts of interest

There are no conflicts of interest.

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