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Regional Variations of Rates and Determinants of Drug Resistance Mutations in People Failing First-line Therapy for HIV-1: A Substudy from the D^{2}EFT Phase 3b/4 Clinical Trial

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Background. D^{2}EFT (Dolutegravir and Darunavir Evaluation in Adults Failing Therapy) was a phase 3b/4 randomized clinical trial designed to assess second-line treatment options systematically. This substudy evaluated the distribution of drug resistance mutations (DRMs) before treatment randomization in individuals failing first-line therapy.

Methods. From a total of 826 participants across 14 countries, 727 sequences that covered the *PR-RT-INT* (700), *PR-RT* (24), and *RT* (3) regions were analyzed for drug resistance. Sequences were submitted to the Stanford HIV drug resistance database to detect DRMs and assign subtypes. By adjusting for country and antiretroviral therapy regimen, we assessed the association between DRMs and country and reported DRMs.

Results. Subtype C of human immunodeficiency virus type 1 (HIV-1) accounted for most (59.3%) infections. There were extraordinarily high rates of high-level resistance to lamivudine and emtricitabine (both at 88.3%) and for efavirenz and nevirapine (92.8% and 96.8%, respectively). On average, nucleoside reverse transcriptase inhibitor mutations had the highest occurrence across countries with M184V/I detected in 86.2% of the samples, while the highest proportion of nonnucleoside reverse transcriptase inhibitor mutations was K103N at 57.8%. K103N had an increased likelihood of occurrence in African and South American countries ($P < .05$). Participants with prior exposure to a zidovudine-containing regimen had an increased likelihood of T215F/Y mutations (6.80 [2.59–17.86]), while those with nevirapine/rilpivirine exposure had a decreased likelihood of K103N mutations (0.29 [0.15–0.56]).

Conclusions. The regional specificity of mutations underscores the dynamic nature of HIV-1 drug resistance patterns and the importance of monitoring and understanding local mutation profiles.

Keywords. HIV-1; drug resistance mutations; HIV-1 subtypes; low- and middle-income countries.

Human immunodeficiency virus type 1 (HIV-1) infection remains a significant global health challenge, with an estimated 38 million people with the virus worldwide [1]. Antiretroviral therapy (ART) has revolutionized the management of HIV-1 infection, greatly improving disease outcomes. However, ART is not without limitations, and some individuals may experience drug resistance or adverse effects that limit their treatment options.

As ART scales up, the World Health Organization (WHO) recommends the use of sentinel surveys with opportunistic sampling for monitoring HIV-1 drug resistance mutations (DRMs) in populations receiving ART, which involves standardized, minimum-resource prospective survey methodologies implemented at sentinel sites [2]. The data generated

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from these surveys inform evidence-based decision-making for national and global ART regimen selection, ultimately aiming to minimize the emergence of DRMs at a population level [3, 4]. Nucleoside reverse transcriptase inhibitor (NRTI)–associated DRMs are more frequent in persons who were on first-line nonnucleoside reverse transcriptase inhibitor (NNRTIs)–containing regimens and had experienced virological failure [5]. However, resistance testing at first-line failure is uncommonly performed in resource-limited settings. This makes it difficult to assess resistance for clinical care of patients and compare patterns for and between countries within regional areas. Essentially, considering second-line regimens with potent, tolerant, and high-genetic-barrier drugs could improve outcomes such as viral suppression and immunological rebound.

Studies assessing DRMs in low- and middle-income countries (LMICs) have revealed concerning trends in recent years. A systematic review and meta-analysis by Gupta et al [6] reported a substantial annual increase in NNRTI resistance across LMICs. This trend is corroborated by the WHO's 2019 HIV drug resistance report, which found that in 12 of 18 surveyed countries, primarily in African regions, the level of NNRTI-associated DRMs among adults initiating first-line NNRTI–based ART exceeded 10% [7]. In LMICs, where NNRTI-based regimens were until recently common for first-line treatment, NNRTI mutations such as K103N, Y181C, and G190A/S are frequently observed in persons experiencing virological failure [8]. HIV-1 subtype has also been identified as a factor influencing DRM patterns. For instance, the V106M mutation, which confers resistance to most NNRTIs, is more prevalent in subtype C and CRF01_AE compared to subtype B [9]. These findings underscore the importance of considering geographical, treatment, and viral genetic factors when assessing and addressing HIV-1 drug resistance in LMICs.

Generally, comparative data on DRMs among persons failing first-line NNRTI-based ART across LMICs is lacking and hence the ability to assess trends across countries is limited [10]. Here, data from the Dolutegravir and Darunavir Evaluation in Adults Failing Therapy (D^2EFT) trial were used. For this trial, HIV-1 sequences were generated for individuals failing a NNRTI-based first-line ART regimen, and before starting second-line ART [11]. It is important to have an assessment of the frequency of DRMs, allowing a snapshot of resistance profiles in those failing therapy in a study population predominantly of non-B subtypes across LMICs. With its large sample size and standardized methodology, the data allow for a reliable comparison between countries. Focusing on individuals failing first-line NNRTI-based therapy provides crucial insights into the types and frequencies of DRMs that emerge under these conditions in different geographical contexts [12]. Moreover, including multiple treatment strategies allows for examining how various ART regimens might influence the development or persistence of DRMs across different populations [6]. Leveraging the rich dataset provided by the D^2EFT clinical trial, the aim was to quantify and compare DRM rates across participating countries, identify potential geographical patterns, and analyze the relationship between specific first-line ART regimens and the emergence of acquired DRMs.

METHODS

Ethical Statement

As detailed in the primary trial report [11], the study protocol was reviewed by the University of New South Wales Human Research Ethics Committee (primary study committee), as well as through each of the local ethics committees and regulatory authorities of the participating countries using local approval guidelines and each country holds those approval documents. Protocol amendments were reviewed and approved by each local ethics committee and regulatory authorities. Each of the participants in the study provided informed consent before enrollment. The study was approved with the protocol number NTC03017872.

Study Design and Participants

This was an analysis of HIV-1 resistance data within the parent open-label phase 3b/4 randomized controlled trial of D^2EFT. Details of this trial were previously published [11]. The D^2EFT study enrolled patients into 3 arms with the participants randomized to 1 of the following treatment regimens for 96 weeks: arm 1, WHO standard of care (ritonavir-boosted darunavir [DRV/r] and 2 recycled NRTIs [13]); arm 2, dolutegravir (DTG) and DRV/r; and arm 3, DTG and tenofovir disoproxil fumarate (TDF) and either lamivudine (3TC) or emtricitabine (FTC). Enrolled participants in D^2EFT were integrase and protease inhibitor naive. Patients were randomized without access to resistance genotyping; the choice of NRTIs in arm 1 was reliant on recycling. Persons aged ≥18 years of age whose first-line NNRTI-based therapy failed, as defined by D^2EFT eligibility criteria (2 HIV RNA loads >500 copies/mL at least 7 days apart) and who provided written informed consent for D^2EFT had genotyping performed on stored samples at baseline (defined as timepoint before starting 1 of the randomized treatment arms). A total of 826 participants were enrolled from 14 D^2EFT sites in Mexico, Argentina, Brazil, Chile, Columbia, Thailand, Malaysia, Indonesia, India, Zimbabwe, South Africa, Nigeria, Mali, and Guinea.

Sample and Data Collection

All samples collected for the study were sent from participating countries to the central laboratory at the Applied Centre for Medical Research (Sydney, Australia) where drug resistance testing was done on the Vela automated sequencing platform

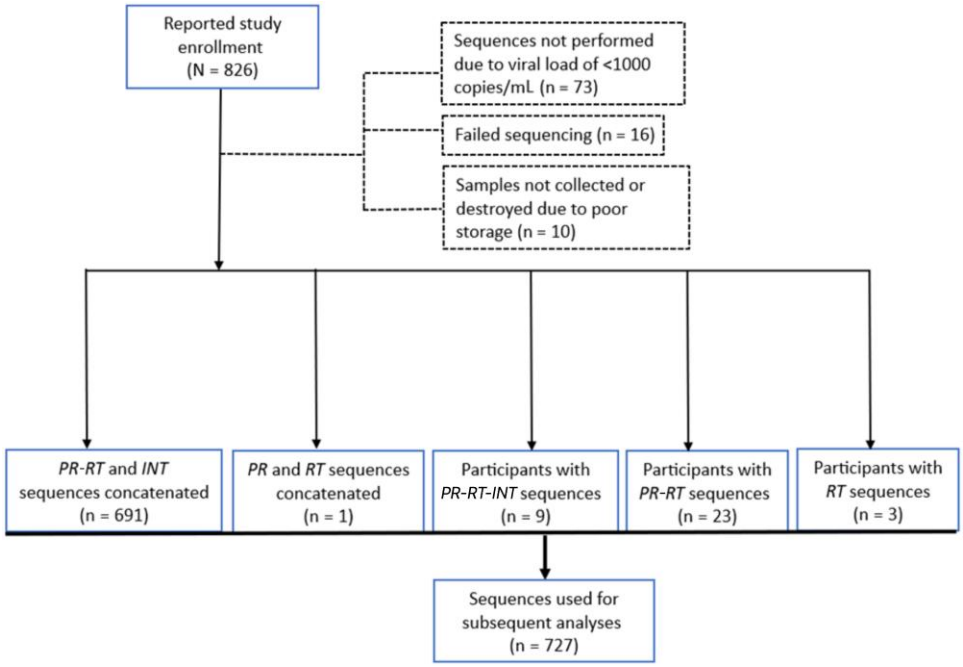

Figure 1. Flowchart of participant enrollment and sequencing outcomes. Successful sequence outputs included participants who had both *PR-RT* and *INT* sequenced to form *PR-RT-INT* combined (9), *PR-RT* and *INT* separately (691), only *PR-RT* (23), *PR* and *RT* sequenced separately (1) and only *RT* (3). A final dataset of 727 sequences that covered *PR-RT-INT* (700), *PR-RT* (24), and *RT* (3) was used for subsequent analyses.

(Sentosa SQ HIV Genotyping Assay) [14] with *PR-RT* and *INT* sequencing kits and interpreted on the standard algorithm (version 9.5.0; 22 August 2023) as previously described [11]. This excluded the 39 Indonesian samples processed on-site using an in-house assay as previously reported [15]. The detailed methods of both these assays can be found in the Supplementary Materials.

The sequences from 99 participants were excluded due to either poor sample quality or repeated sequencing failures, including those with <1000 copies/mL and a documented lower sequencing success rate [14]. Successful sequence outputs included participants who had both *PR-RT* and *INT* sequenced to form *PR-RT-INT* combined (9), *PR-RT* and *INT* separately (691), only *PR-RT* (23), *PR* and *RT* sequenced independently (1), and only *RT* (3). For the 691 participants with separate sequences covering the *PR-RT* and *INT* regions, we concatenated both sequences (joined together) to obtain a combined *PR-RT-INT* sequence. All sequence concatenations, alignment, and editing were done in Geneious Prime software [16]. A final dataset of 727 sequences that covered *PR-RT-INT* (700), *PR-RT* (24), and *RT* (3) was used for subsequent analyses (Figure 1). Sequences were submitted to the Stanford HIV drug resistance database version 9.4 (https://hivdb.stanford.edu/hivdb/by-patterns/) to detect DRMs as per the WHO list of primary and accessory drug resistance [17] and assign subtypes. All data were stored on the secure D^{2}EFT database at The Kirby Institute for further analysis. A phylogenetic analysis was performed in IQTree (http://www.iqtree.org) [18] to allocate the subtypes across participating countries.

Data and Statistical Analysis

All data were presented as percentage occurrence for each of the 14 countries and statistical associations between covariates and individual DRMs were assessed using logistic regression and presented as adjusted odds ratios. Because of the adverse effect on power, *P* values and confidence intervals were not adjusted for multiple comparisons and the findings should be interpreted accordingly. Analyses were performed in SAS version 16 software. Graphical representation of those associations that were statistically significant ($P < .05$) was performed using forest plots [19].

RESULTS

Baseline Characteristics of Study Participants

The study included sequence data from 727 participants recruited from 14 countries. The data were categorized by gender, country, prior ART regimen, and HIV-1 subtype (Table 1). More than half of participants (55.3%) were female, and Zimbabwe had the largest proportion of participants (33.8%), followed by South Africa (20.1%) and Nigeria (13.5%), while enrollment from each of the other countries did not exceed 10% of the total (Table 1). Most viral sequences were subtype C (59.3%), with the next most prevalent being CRF01_AE (15.8%), then CRF02_AG (9.6%), subtype B (6.9%), and other

Table 1. Number of Study Participants by Gender, HIV-1 Subtype, Prior Antiretroviral Therapy Regimen, and Country of Enrollment

Variable	Frequency (%), N = 727
Gender	
Male	325 (44.7)
Female	402 (55.3)
Country	
Mexico	6 (0.8)
Argentina	13 (1.8)
Brazil	19 (2.6)
Chile	7 (0.9)
Colombia	2 (0.3)
Thailand	67 (9.2)
Malaysia	25 (3.4)
Indonesia	39 (5.4)
India	39 (5.4)
Zimbabwe	246 (33.8)
South Africa	146 (20.1)
Nigeria	98 (13.5)
Mali	13 (1.8)
Guinea	7 (0.9)
HIV-1 subtype	
B	50 (6.9)
C	431(59.3)
CRF01_AE	115 (15.8)
CRF02_AG	70 (9.6)
Other subtypes[a]	61 (8.4)
Prior ART regimen	
d4T + EFV + 3TC	2 (0.3)
d4T + NVP + 3TC	4 (0.6)
EFV + ABC + 3TC	14 (1.9)
EFV + TDF + 3TC	334 (45.9)
EFV + TDF + FTC	271 (37.3)
EFV + ZDV + 3TC	20 (2.8)
ETR + TDF + FTC	1 (0.1)
NVP + ABC + 3TC	1 (0.1)
NVP + TDF + 3TC	13 (1.8)
NVP + TDF + FTC	13 (1.8)
NVP + ZDV + 3TC	47 (6.5)
RPV + TDF + 3TC	1 (0.1)
RPV + TDF + FTC	6 (0.8)

Abbreviations: 3TC, lamivudine; ABC, abacavir; ART, antiretroviral therapy; d4T, stavudine; EFV, efavirenz; ETR, etravirine; FTC, emtricitabine; HIV-1, human immunodeficiency virus type 1; NVP, nevirapine; RPV, rilpivirine; TDF, tenofovir disoproxil fumarate; ZDV, zidovudine.

[a]Other subtypes here include pure subtypes and recombinants that occurred at very low frequencies: A, D, F, G, B + F, CRF06_cpx, CRF12_BF, CRF45_cpx.

subtypes and recombinants (8.4%). Of the 13 different reported NRTI and NNRTI regimen combinations used as first-line therapy, nearly half (45.9%) of study participants had received efavirenz (EFV), TDF, and 3TC in combination, followed by EFV, TDF, and FTC in combination (37.3%). No other first-line regimen exceeded 7% of the total enrolled (Table 1).

Gender and HIV-1 Subtype Distribution by Country

The gender distribution was balanced overall, but males dominated in Mexico, Argentina, Brazil, Chile, Colombia, Thailand, Malaysia, Indonesia, and India whereas females dominated in South Africa, Zimbabwe, Nigeria, Mali, and Guinea (Figure 2*A*). For the distribution of subtypes, the predominant subtype was B in the Americas (Mexico, Argentina, Brazil, Chile, Colombia), CRF01_AE in Asia (Thailand, Malaysia, Indonesia), and CRF02_AG in West Africa (Nigeria, Mali, and Guinea). In India, unlike in the other Asian countries participating in the study, the most predominant subtype was subtype C as seen in South African countries (South Africa and Zimbabwe) (Figure 2*B*). Confirmation of subtypes was performed with a maximum-likelihood phylogenetic analysis (Figure 2*C*).

ART Regimen Prior to Enrollment by Country

Thirteen different drug regimen combinations were reported to have been prescribed as first-line therapy for participants across the countries with Thailand recording 11 different regimens, while all participants in Mexico reported the same ART combination (Figure 3). Among all of the regimens, 3TC was included in 9 of 13 regimens and FTC in 4 of 13 regimens. TDF with either 3TC or FTC was used in 7 of 13 regimens. For the NNRTIs, EFV and NVP were used in 5 of 13 regimens each. Other drugs—abacavir (ABC), stavudine (d4T), and rilpivirine (RPV)—were used in 2 regimens each and etravirine in only 1 regimen (Figure 3). Even though most countries used EFV, TDF, and FTC/3TC, the combination regimens greatly differed by country (Figure 3). For example, the proportion of participants in Argentina who received NVP + zidovudine (ZDV) + 3TC was 0.31, and 0.38 for EFV + ABC + 3TC, and 0.57 in Chile received EFV + ABC + 3TC.

Prevalence of DRMs Identified Across the Countries

A very high prevalence of mutations was found in this population failing a first-line NNRTI-based regimen. In 11 of 14 countries, the prevalence of mutations was >90%. Notably, NRTI- and NNRTI-associated mutations are ubiquitous, present in a significant number of samples across all countries. Protease inhibitor–associated mutations were rare, reported only in Zimbabwe and Nigeria, while integrase strand transfer inhibitor–associated DRMs were the least common, with a single case in Thailand (Table 2).

NRTI and Associated DRMs Across Countries, Subtypes, and Prior ART Exposure

Overall, 17 codon sites were detected and classified as major NRTI DRM by the Stanford algorithm. M184V/I amino acid changes were the most predominant with a proportion of >0.8 across all countries (Figure 4*A*). Samples from India and Brazil had the lowest proportion of M184V/I mutations at 0.6, while Mexico, Chile, Colombia, and Guinea had the highest proportion at 1 (Figure 4*A*). The K65R mutation was the second most predominant with a proportion of 0.38 and much more diverse mutation patterns. K65R was highest in Zimbabwe at 0.6 and lowest in Argentina at 0.1, though this mutation was not

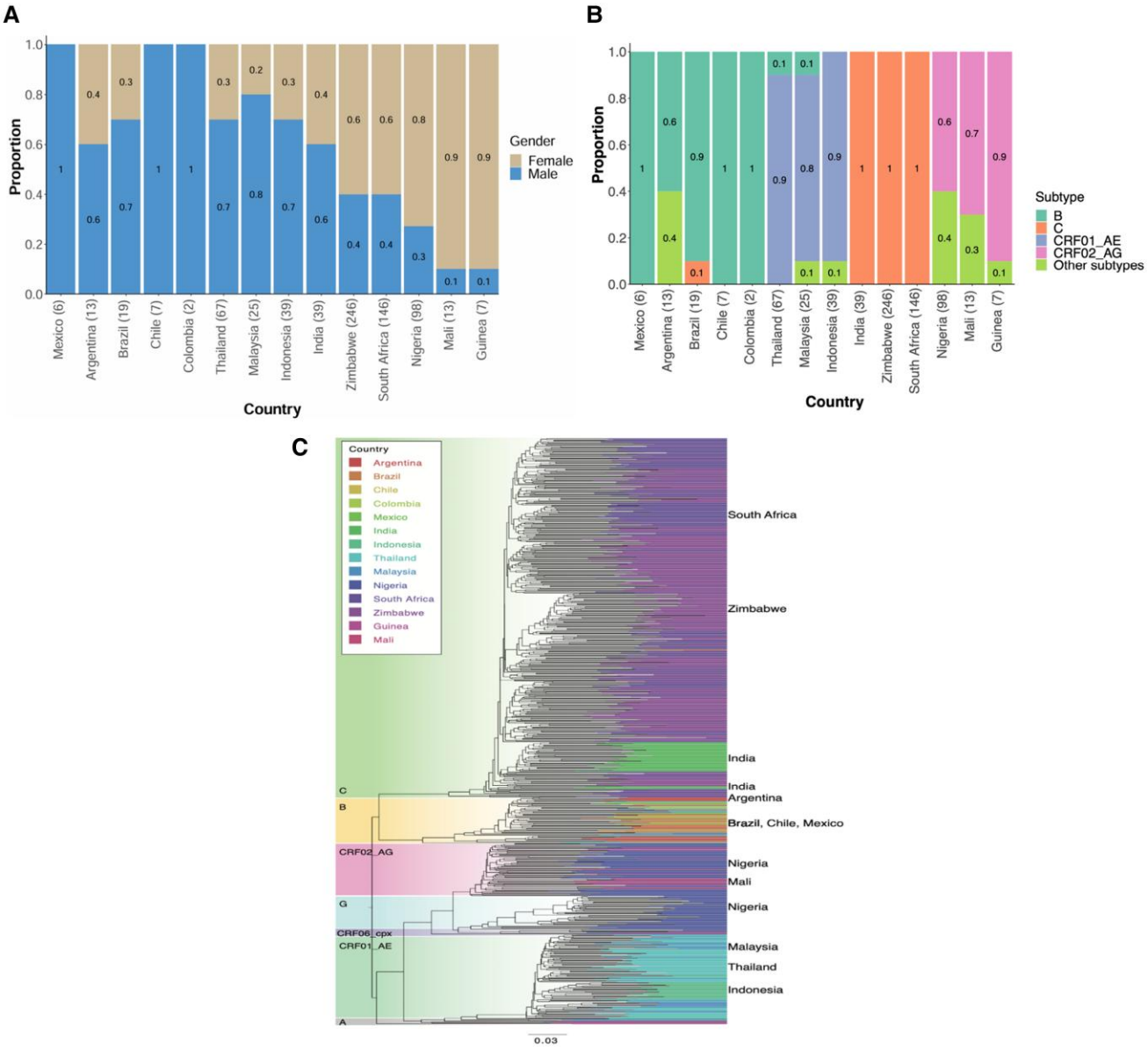

Figure 2. Number of participants across gender and human immunodeficiency virus (HIV) subtypes. Shown is gender distribution of enrolled participants across the 14 countries (*A*) and the HIV-1 subtypes detected (*B*). Phylogenetic analysis with maximum-likelihood algorithm was used to confirm the subtypes across the countries (*C*). The branches are colored by country and the subtypes are indicated to the left. Branch length indicates nucleotide substitutions per site.

detected at all in Mexico consistent with the predominant use of a d4T-containing regimen. Data from Guinea had the highest proportion for K70E/R (0.7), K219Q/E (0.7), and D67N (0.6). Data from Mali also had the highest proportion for S68G (0.6), and Chile for L74V (0.6). M184V/I and K70E/R mutations were most common for CRF02_AG (1.0 and 0.5, respectively) and K65R was most common for subtype C (0.5) (Figure 4*B*).

Genome sequences from participants using d4T + NVP + 3TC had overall the highest occurrence for D67N (0.8) and K219Q/E (0.8), while participants on NVP + TDF + FTC had the highest proportion for K65R (0.6), followed by participants on EFV + TDF + 3TC (0.5) (Figure 4*C*). Importantly, the multidrug NRTI resistance mutation Q151M was only detected in Thailand and only in 1 individual with CRF01_AE infection who reported d4T + EFV + 3TC exposure (Figure 4). Figure 4*D* illustrates the levels of resistance to various NRTIs, revealing concerning patterns of drug resistance in LMICs. Notably, it shows extraordinarily high rates of high-level resistance for certain NRTIs, particularly 3TC and FTC, both at 0.88. Also, there exist varying levels of resistance across different NRTIs, with drugs like ABC and d4T showing lower rates of high-level resistance (0.54 and 0.31, respectively) but significant intermediate-level resistance.

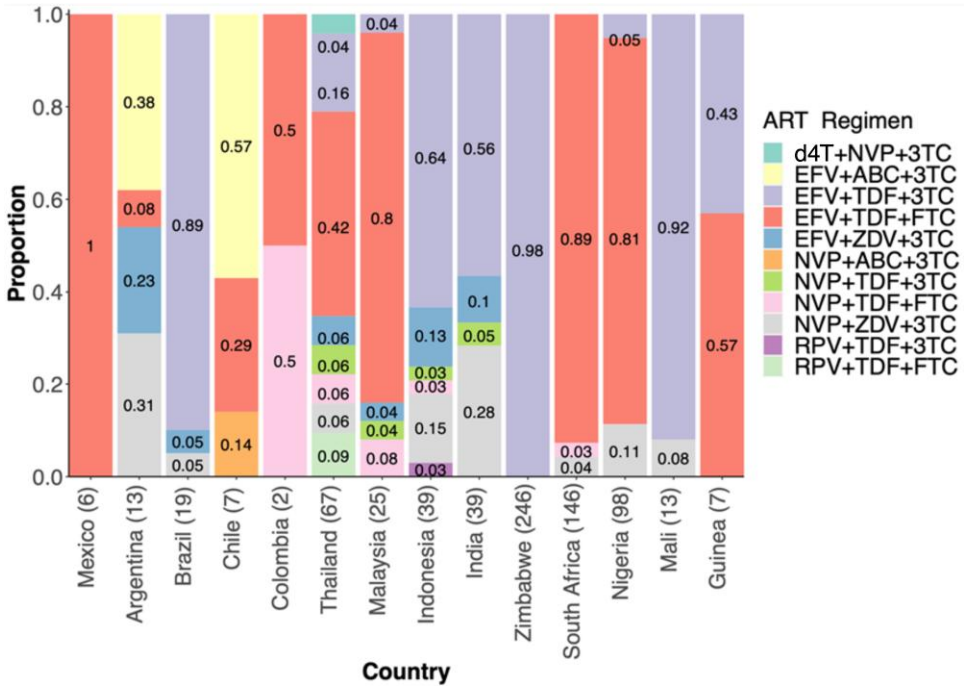

Figure 3. Reported ART regimen of participants prior to study enrollment. Shown are the 13 different first-line combination ART regimen participants used before enrollment. The proportion of the different ART combinations is shown for each country. Abbreviations: ART, antiretroviral therapy; 3TC, lamivudine; ABC, abacavir; d4T, stavudine; EFV, efavirenz; FTC, emtricitabine; NVP, nevirapine; RPV, rilpivirine; TDF, tenofovir disoproxil fumarate; ZDV, zidovudine.

Table 2. Prevalence of Drug Resistance Mutations Identified Across the 14 Countries (N = 727 Individuals)

	No. (%) of Individuals With DRMs			
Country (No.)	NRTI-Associated DRMs	NNRTI-Associated DRMs	PI-Associated DRMs	INSTI-Associated DRMs
Mexico (6)	6 (100.0)	6 (100.0)	…	…
Argentina (13)	12 (92.3)	13 (100.0)	…	…
Brazil (19)	13 (68.4)	17 (89.5)	…	…
Chile (7)	7 (100.0)	7 (100.0)	…	…
Colombia (2)	2 (100.0)	2(100.0)	…	…
Thailand (67)	62 (92.5)	62 (92.5)	…	1 (1.5)
Malaysia (25)	25 (100.0)	24 (96.0)	…	…
Indonesia (39)	36 (92.3)	37 (94.9)	…	…
India (39)	27 (69.2)	37 (94.9)	…	…
Zimbabwe (246)	237 (96.3)	243 (98.8)	4 (1.6)	…
South Africa (146)	137 (93.8)	145 (99.3)	2 (1.4)	…
Nigeria (98)	96 (98.0)	98 (100.0)	2 (2.0)	…
Mali (13)	11 (84.6)	11 (84.6)	…	…
Guinea (7)	7 (100.0)	7 (100.0)	…	…

Abbreviations: DRM, drug resistance mutation; INSTI, integrase strand transfer inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

NNRTI and Associated DRMs Across Countries, Subtypes, and Prior ART Exposure

A total of 15 major codon site NNRTI DRMs were detected, with amino acid change at position 103 (K103 codon site mutation) being the most predominant (>0.57) across all countries (Figure 5*A*). The proportion of K103N codon site mutations ranged from 0.33 in Indonesia to 0.83 in Mexico. Surprisingly, K103N was found in each of the 50 subtype B samples and the proportion recorded for CRF02_AG was 0.71 (Figure 5*B*). K103N was most

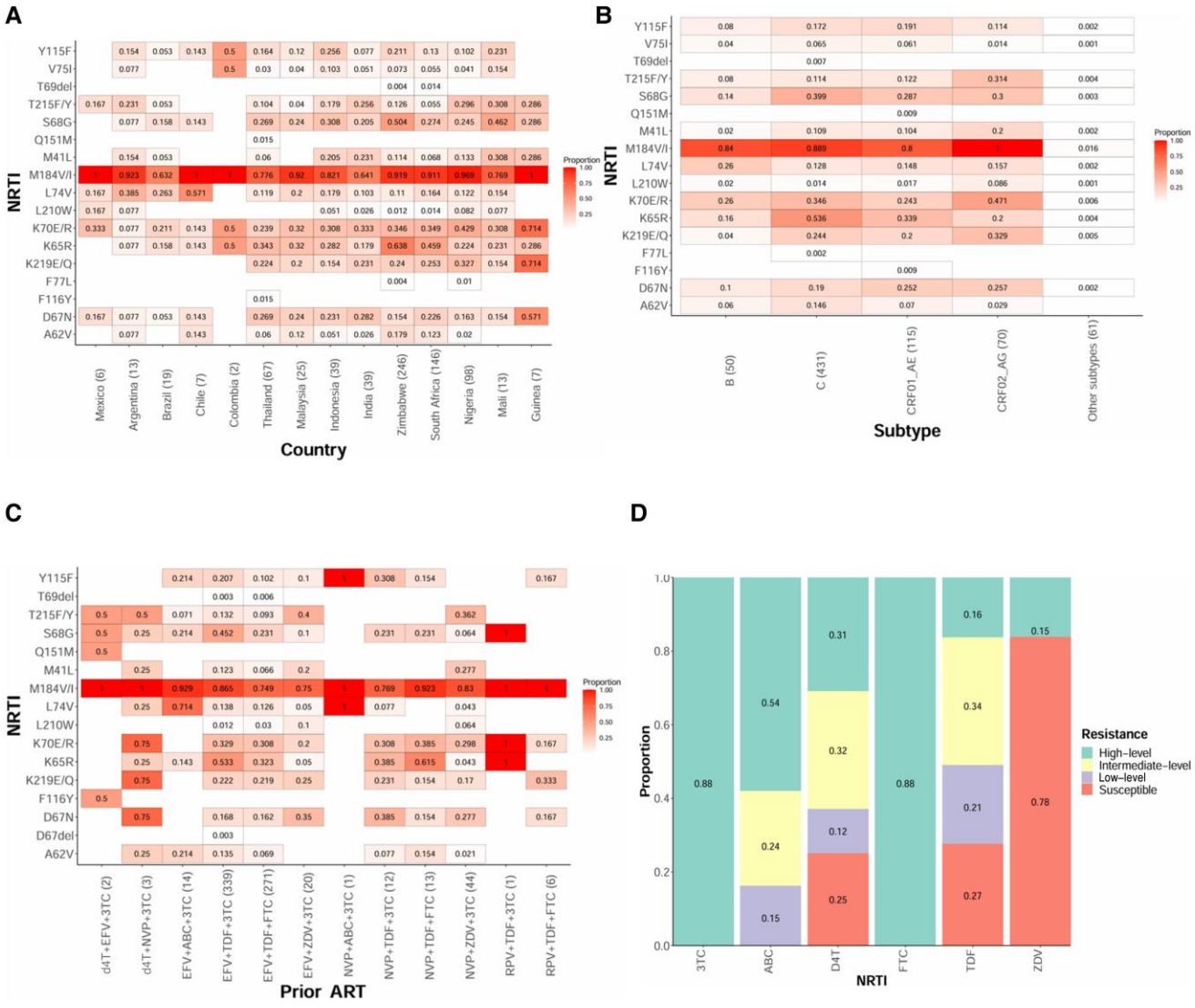

Figure 4. NRTI and associated drug resistance mutations across countries, subtypes, and prior ART exposure. The proportion of 17 major NRTI-codon site mutations detected across the different countries (*A*) and HIV-1 subtypes (*B*), and prior ART exposure (except for etravirine + tenofovir disoproxil fumarate + emtricitabine with no observation) (*C*) and the level of resistance for each NRTI drug as reported in the Stanford HIV database (*D*). Proportions <0.1 are not shown. Abbreviations: ART, antiretroviral therapy; 3TC, lamivudine; ABC, abacavir; d4T, stavudine; EFV, efavirenz; FTC, emtricitabine; NRTI, nucleoside reverse transcriptase inhibitor; NVP, nevirapine; RPV, rilpivirine; TDF, tenofovir disoproxil fumarate; ZDV, zidovudine.

prevalent among participants with first-line EFV + ABC + 3TC (0.71) and EFV + TDF + FTC (0.69) but was not found in those with RPV + TDF + FTC (Figure 5*C*). Finally, extraordinarily high proportions of high-level resistance to EFV and NVP were observed at 0.93 and 0.97, respectively (Figure 5*D*).

Correlation Analysis

Some countries were grouped by region due to small sequence numbers (Supplementary Table 1). Associations were found between NRTI mutations, prior ART exposure, and country/region, influencing HIV treatment outcomes. K65R and M184V/I mutations were less likely in South Africa, West Africa, Asia, and the Americas (Figure 6*A*, Supplementary Table 2). Prior ZDV exposure increased likelihood of T215F/Y, but decreased K65R, Y115F, and S68G (Figure 6*B*, Supplementary Table 2). FTC exposure correlated with the presence of the M184V/I mutations (Supplementary Table 2). Regional associations were found for NNRTI mutations (eg, K103N, L234I, Y181C/L) (Figure 7*A*, Supplementary Table 3). Prior NVP/RPV exposure influenced the presence of K103N and V108I mutations (Figure 7*B*, Supplementary Table 3). For detailed results on these correlations, see the Supplementary Results.

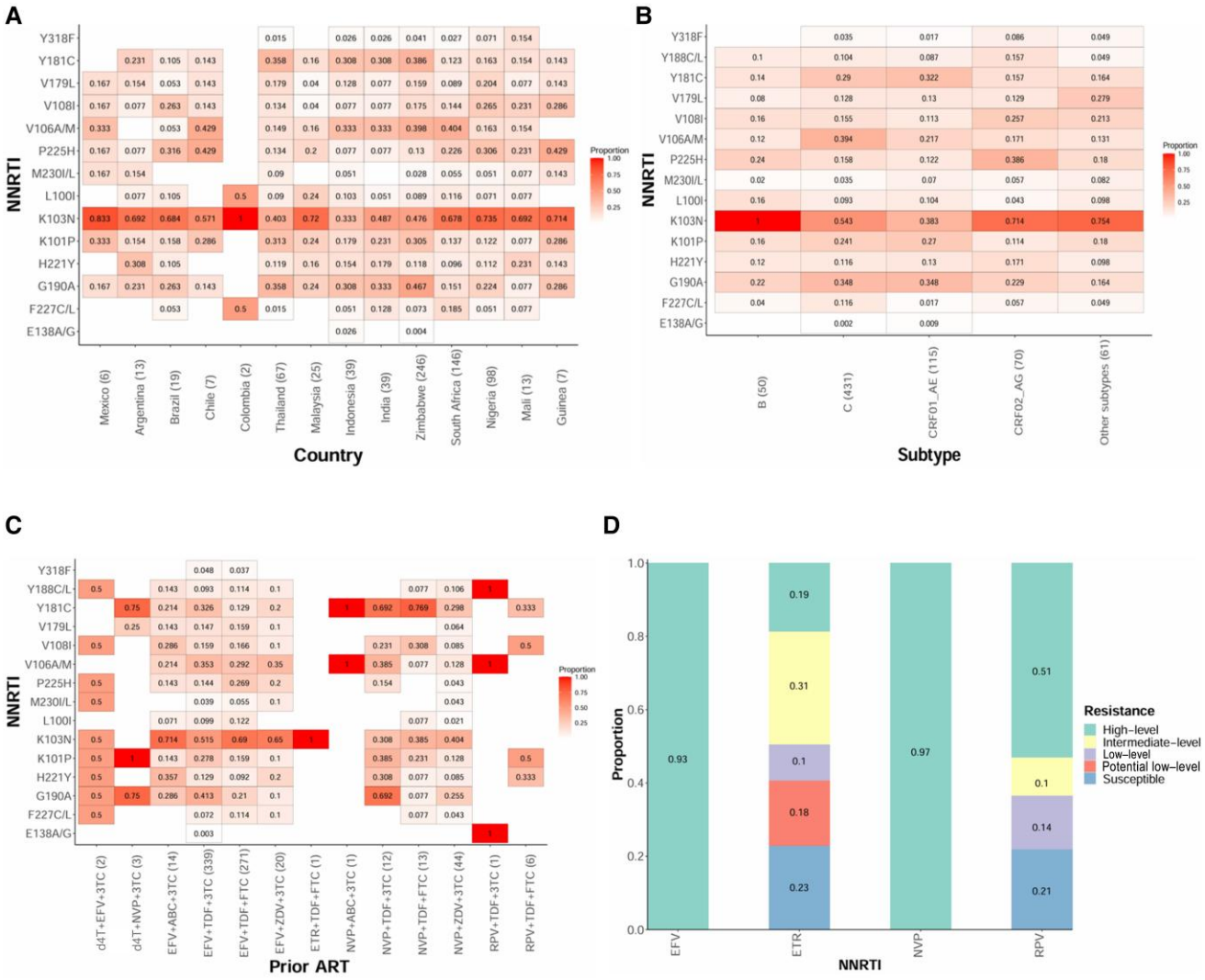

Figure 5. NNRTI-associated drug resistance mutations across countries, subtype, and prior ART exposure. The proportion of 15 major NNRTI-codon site mutations detected are shown for the different countries (*A*), first-line regimen (*B*), HIV-1 subtypes (*C*), and the level of resistance for each NNRTI drug as reported in the Stanford HIV database (*D*). Proportions <0.1 are not shown. Abbreviations: ART, antiretroviral therapy; 3TC, lamivudine; ABC, abacavir; d4T, stavudine; EFV, efavirenz; ETR, etravirine; FTC, emtricitabine; NNRTI, nonnucleoside reverse transcriptase inhibitor; NVP, nevirapine; RPV, rilpivirine; TDF, tenofovir disoproxil fumarate; ZDV, zidovudine.

DISCUSSION

This study examines the prevalence and patterns of DRMs across different countries, focusing on individuals failing first-line NNRTI-based ART. The study used a systematically collected data set, not dominated by B subtype, with sequences from both male and female participants with HIV-1. This study expands on existing knowledge by detailing regional variations in DRM prevalence and associating these with prior ART exposure. Importantly, we record high rates of resistance mutations across the participating countries, suggesting that the failure may not just be driven by noncompliance or lack of tolerability first-line ART alone, but may be as a result of preexistence of resistance mutations [20]; however, this study did not assess participants' drug resistance status when they were first diagnosed and started on ART. One important issue was the low enrollment across certain sites such as Colombia, Chile, and Mexico where participants did not exceed 10; the occurrence of DRMs per country was unable to be assessed with any certainty, so the data were aggregated by regional area to increase statistical power.

The study provides insight into the prevalence and significance of NRTI-associated DRMs and their association with different treatment regimens. Consistent with another study [21], the prominence of M184V/I in the presence of failed therapy with 3TC and FTC was found. Generally, M184V/I mutations were detected across all countries and were the most predominant among all NRTI DRMs, consistent with the widespread use of 3TC/FTC. Statistical correlation between this codon site mutation and country, and 3TC/FTC use was also found. The

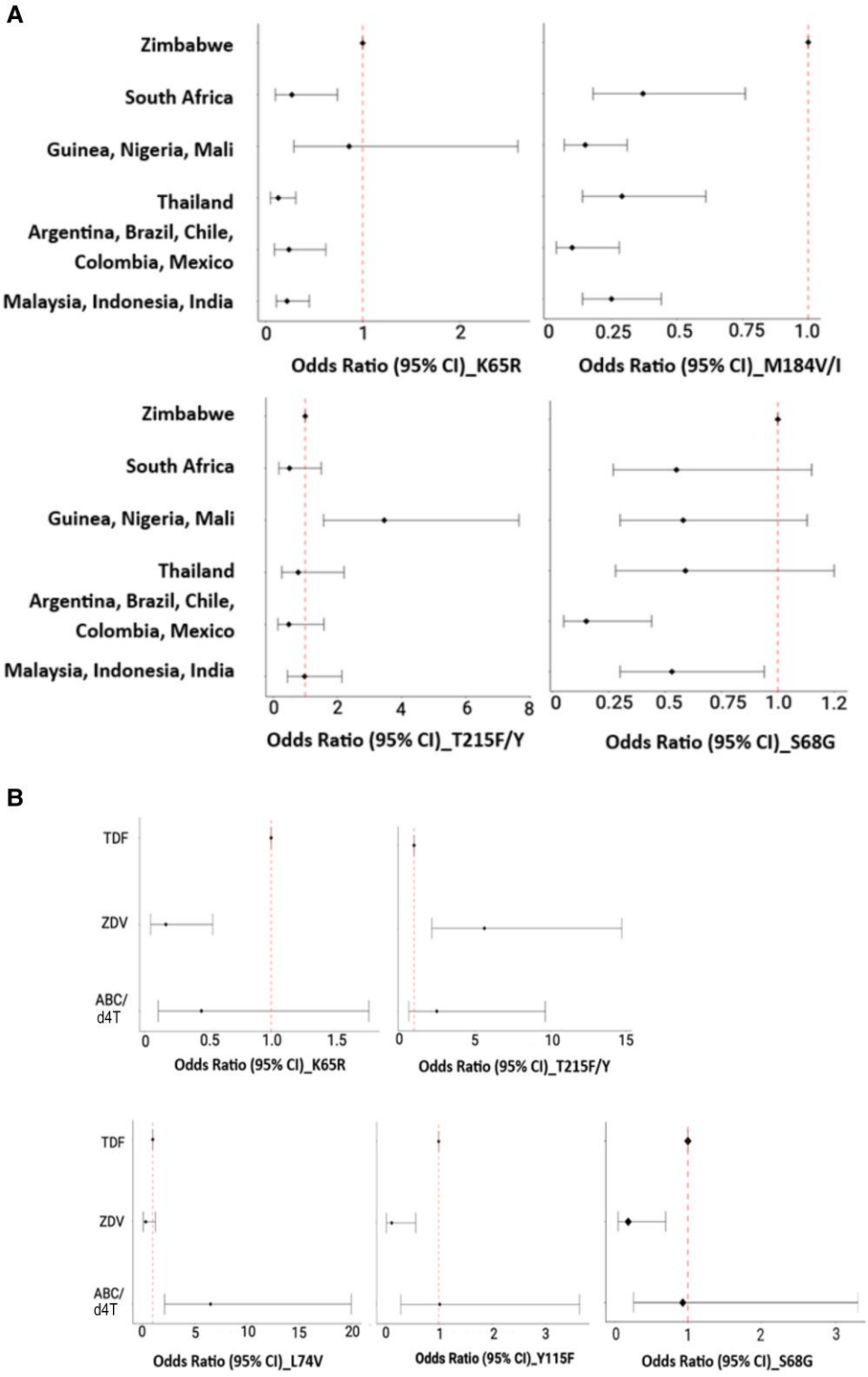

Figure 6. Statistical associations between nucleoside reverse transcriptase inhibitor (NRTI)–associated drug resistance mutations (DRMs) and countries and antiretroviral therapy drug categories. The adjusted odds ratios are illustrated as forest plots for those mutations with significant associations ($P < .05$) under a multivariate logistic model to assess the association between DRMs and countries (*A*) and NRTI drug categories (*B*). Abbreviations: ABC, abacavir; CI, confidence interval; d4T, stavudine; TDF, tenofovir disoproxil fumarate; ZDV, zidovudine.

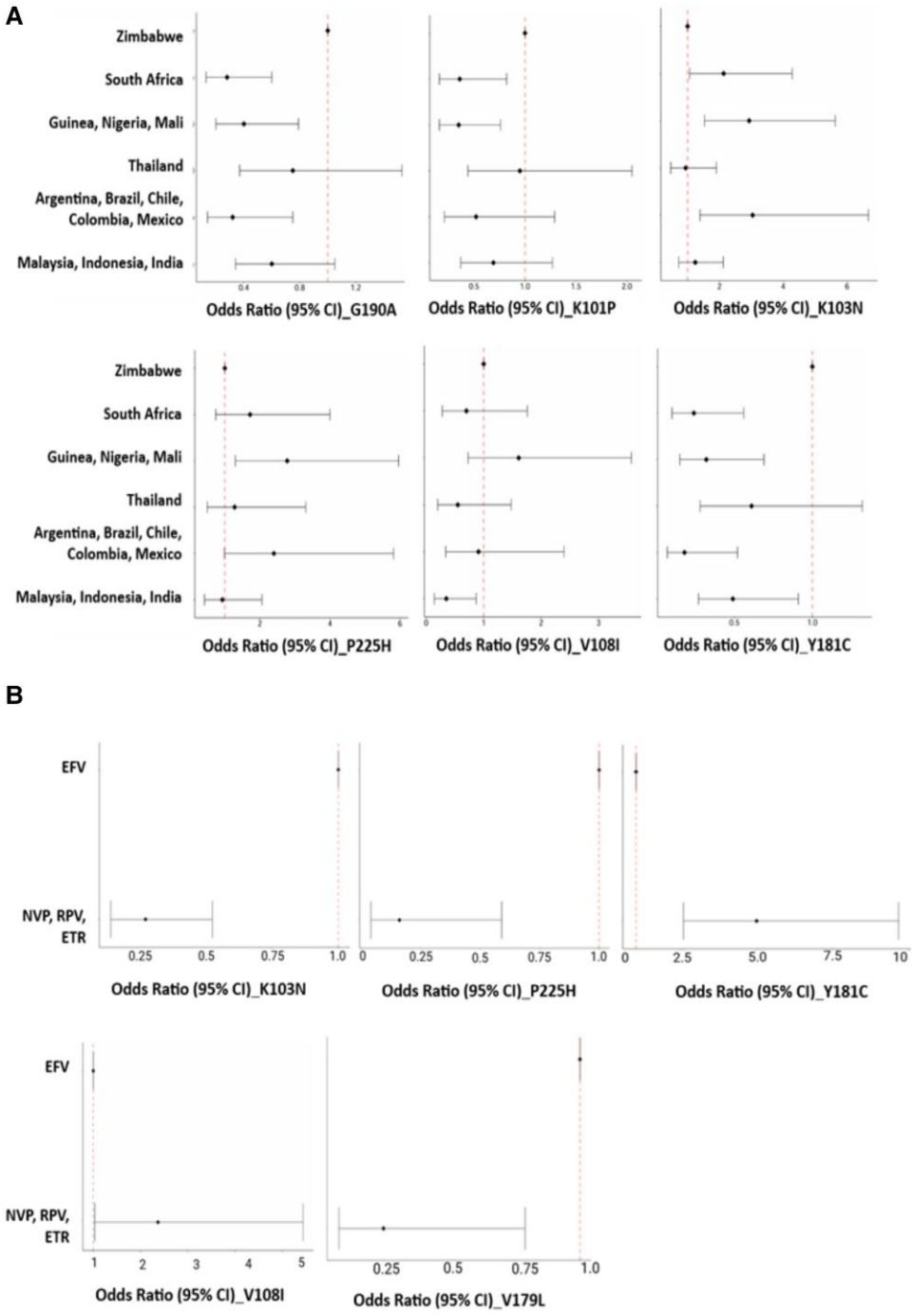

Figure 7. Statistical associations between nonnucleoside reverse transcriptase inhibitor (NNRTI)–associated drug resistance mutations (DRMs) and country and antiretroviral therapy drug categories. The forest plots show the adjusted odds ratios for mutations that showed significant association ($P < .05$) under a multivariate logistic model to assess the association between DRMs and country (*A*) and NNRTI drug categories (*B*). Abbreviations: CI, confidence interval; EFV, efavirenz; ETR, etravirine; NVP, nevirapine; RPV, rilpivirine.

M184V/I mutations have been shown to cause high-level resistance to 3TC and FTC (mostly used interchangeably), and low/intermediate resistance to ABC [22]. One study discriminated between the potency of FTC as against 3TC and found that M184V/I occurs more frequently in individuals with a 3TC-containing regimen compared to FTC [23]. However, this is not a contraindication to the continued use of these drugs, as M184V/I also increases susceptibility to ZDV and TDF while significantly reducing viral fitness [24] and is identified to prevent the emergence of resistance to DTG [25].

The regional specificity of mutations underscores the dynamic nature of HIV-1 drug resistance patterns and the

importance of monitoring and understanding local mutation profiles [26]. The increased likelihood of selecting K103N across African and American countries, but not for Asian countries, suggests a distinct regional pattern in the prevalence of this mutation. The K103N mutation is nonpolymorphic and confers high-level resistance to EFV and NVP. It causes at least a 50-fold decreased susceptibility to EFV and NVP [27].

The alarmingly high resistance rates seen in this study are still of relevance to LMICs despite the scaled-up use of DTG. First, it potentially informs which ART drugs to use with DTG; second, it has implications for the choice of second-line regimens, especially in the context of increasing rates of DTG resistance reported in LMICs [28]. This situation is particularly concerning given that in many LMICs, options for second-line and salvage therapies may be limited due to cost and availability constraints [29]. Acquired resistance increased steadily with time on ART, from 7.2% at 6–11 months to 20.7% at ≥36 months. These findings highlight the need for virological monitoring, drug resistance testing, and alternative drug regimens [30]. The results highlight the need to implement robust HIV drug resistance surveillance programs, optimizing adherence support, and ensuring the quality of service delivery in LMICs. Efforts should be made to develop cost-effective point of care resistance testing to identify DRM following the current integrase-based ART regimen to optimize future regimens. There is an urgent need for continued research and development of new antiretroviral drugs and treatment strategies to combat the growing challenge of HIV-1 drug resistance in resource-limited settings. In light of recent destabilization in ART programs, these results highlight the need for enhanced monitoring of treatment outcomes and resistance patterns, improved adherence support and retention strategies, and timely switching to effective second-line regimens, while strengthening surveillance to track the spread of resistant strains.

CONCLUSIONS

The high prevalence of DRMs, particularly to commonly used first-line ART regimens, suggests that individuals failing treatment may have limited effective treatment options. The emergence of resistance to multiple drugs could compromise the efficacy of second-line regimens, potentially leading to increased morbidity and transmission of drug-resistant strains.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. B. M. O., F. D. G., and A. D. K. conceptualized and designed the project. F. D. G., A. D. K., M. La., G. Ma., R. K., and I. A. provided study oversight and leadership. A. D. K., G. Mu., M. Lo., R. K., N. K., and I. A. contributed to funding acquisition. B. M. O., F. D. G., M. La., J. H., A. D. K., and A. S. provided formal data analysis. P. C., I. A., W. N. W., N. K., M. W., R. K., M. S. D., N. E., G. Mu., J. K., A. B., M. F., J. B., and D. P. M. contributed patient data, oversaw project implementation at the study sites, and contributed to data interpretation. B. M. O., F. D. G., A. D. K., G. Ma., R. K., and I. A. contributed to interpretation. B. M. O., F. D. G., M. La., and J. H. directly accessed and verified the data reported in the manuscript. B. M. O. was responsible for writing the original draft. All authors provided review and editing of final draft. All authors had access to the study data and had final responsibility for the decision to submit for publication.

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Data availability. The data in this study cannot be made publicly available due to ethical restrictions in the study's informed consent documents and in countries in which D²EFT was conducted; public data availability could compromise participant confidentiality. However, de-identified study data might be available upon request and submission and approval from the D²EFT protocol steering committee of a concept sheet summarizing the analyses to be done. The data archive will be held at The Kirby Institute and kept on a secure network drive accessible to authorized personnel only. Data generated from the study results might be used in future reports, or used in scientific presentations at both national and international conferences. Further inquiries can be directed to the study administration (D2EFT@kirby.unsw.edu.au).

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