HIV-1 drug resistance before initiation or re-initiation of first-line antiretroviral therapy in low-income and middle-income countries: a systematic review and meta-regression analysis

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Summary

Background Pretreatment drug resistance in people initiating or re-initiating antiretroviral therapy (ART) containing non-nucleoside reverse transcriptase inhibitors (NNRTIs) might compromise HIV control in low-income and middle-income countries (LMICs). We aimed to assess the scale of this problem and whether it is associated with the initiation or re-initiation of ART in people who have had previous exposure to antiretroviral drugs.

Methods This study was a systematic review and meta-regression analysis. We assessed regional prevalence of pretreatment drug resistance and risk of pretreatment drug resistance in people initiating ART who reported previous ART exposure. We systematically screened publications and unpublished datasets for pretreatment drug-resistance data in individuals in LMICs initiating or re-initiating first-line ART from LMICs. We searched for studies in PubMed and Embase and conference abstracts and presentations from the Conference on Retroviruses and Opportunistic Infections, the International AIDS Society Conference, and the International Drug Resistance Workshop for the period Jan 1, 2001, to Dec 31, 2016. To assess the prevalence of drug resistance within a specified region at any specific timepoint, we extracted study level data and pooled prevalence estimates within the region using an empty logistic regression model with a random effect at the study level. We used random effects meta-regression to relate sampling year to prevalence of pretreatment drug resistance within geographical regions.

Findings We identified 358 datasets that contributed data to our analyses, representing 56,044 adults in 63 countries. Prevalence estimates of pretreatment NNRTI resistance in 2016 were 11·0% (7·5–15·9) in southern Africa, 10·1% (5·1–19·4) in eastern Africa, 7·2% (2·9–16·5) in western and central Africa, and 9·4% (6·3–13·2) in Latin America and the Caribbean. There were substantial increases in pretreatment NNRTI resistance per year in all regions. The yearly increases in the odds of pretreatment drug resistance were 23% (95% CI 16–29) in southern Africa, 17% (5–30) in eastern Africa, 17% (6–29) in western and central Africa, 11% (5–18) in Latin America and the Caribbean, and 11% (2–20) in Asia. Estimated increases in the absolute prevalence of pretreatment drug resistance between 2015 and 2016 ranged from 0·3% in Asia to 1·8% in southern Africa.

Interpretation Pretreatment drug resistance is increasing at substantial rate in LMICs, especially in sub-Saharan Africa. In 2016, the prevalence of pretreatment NNRTI resistance was near WHO’s 10% threshold for changing first-line ART in southern and eastern Africa and Latin America, underscoring the need for routine national HIV drug-resistance surveillance and review of national policies for first-line ART regimen composition.

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Introduction The scale-up of antiretroviral therapy (ART) for the treatment of HIV has reached 19·5 million individuals globally and is an unprecedented public health achievement.1 Despite this accomplishment, millions more people with HIV need to initiate and be maintained on ART for life. WHO and UNAIDS have set ambitious targets to end the AIDS epidemic as a public health threat by 2030. These widely adopted targets reflect the global community’s commitment to expanding access to ART and are aiming, by 2020, to diagnose 90% of all people with HIV infection, provide treatment to 90% of those diagnosed, and ensure that 90% of people on treatment achieve virological suppression.2 As ART scale-up proceeds, some degree of HIV drug resistance is anticipated and will have to be managed. Should the prevalence of HIV drug resistance in people starting treatment rise to substantial levels, global efforts to
Evidence before this study

We searched PubMed for meta-analyses of pretreatment HIV-1 drug resistance over time in adults starting antiretroviral therapy (ART) in low-income and middle-income countries (LMICs), published in English, Spanish, or Portuguese. We limited our search to studies published between Jan 1, 2012, and Aug 31, 2017, because we were interested in contemporary trends and prevalence estimates for drug resistance. We used the search terms “HIV” AND “transmitted drug resistance” AND “systematic review”; “HIV” AND “pretreatment drug resistance” AND “systematic review”; “HIV” AND “transmitted drug resistance” AND “meta-analysis”; “HIV” AND “pretreatment drug resistance” AND “meta-analysis”. We did not identify any such studies in adults.

Added value of this study

Our findings provide up-to-date estimates of the prevalence of HIV drug resistance in people initiating or re-initiating first-line ART and we found worrying increases in prevalence in all regions of sub-Saharan Africa, Asia, and Latin America and the Caribbean. The prevalence of HIV drug resistance seems to be 10% or higher in several regions and was much higher in studies in which individuals reported previous antiretroviral exposure. We also noted an increase in virological failure after first-line ART in individuals who reported previous antiviral exposure.

Implications of all the available evidence

Our results show that some LMICs might be reaching WHO’s 10% threshold for changing first-line non-nucleoside reverse transcriptase inhibitor (NNRTI)-based ART to integrate inhibitor-based ART. Individuals with previous ART exposure should be identified and NNRTI-based regimens should be avoided in this group.
pretreatment drug resistance in LMICs is therefore crucial in the global response to HIV/AIDS. We did this study to help fill this knowledge gap.

**Methods**

**Search strategy and selection criteria**

This study was a systematic review and meta-regression analysis of the regional prevalence of pretreatment drug resistance and risk of pretreatment istance among ART initiators reporting previous ART exposure. We searched for studies in PubMed and Embase and conference abstracts and presentations from the Conference on Retroviruses and Opportunistic Infections, the International AIDS Society Conference, and the International Drug Resistance Workshop for the period Jan 1, 2001, to Dec 31, 2016. We supplemented the systematic review with additional unpublished datasets from WHO-supported surveys of drug resistance.

We searched for studies in adults (aged >15 years) infected with HIV who were eligible to initiate first-line NNRTI-based ART in LMICs in the WHO-defined regions western Pacific, southeast Asia, Africa, eastern Mediterranean, and Latin America and the Caribbean. For the purpose of this analysis, countries in the southeast Asia, western Pacific region, eastern Mediterranean regions, as well as Turkey (Europe region) are grouped under the regional heading of “Asia”. We analysed data within subregions of sub-Saharan Africa: eastern Africa, southern Africa, and west and central Africa. Because we included studies from only resource-limited settings, we used the search term “Latin America and the Caribbean” instead of the “Americas”. We excluded studies with fewer than ten HIV-1 genotypes. We used the search terms “antiretroviral therapy” AND “transmitted drug resistance”; “antiretroviral therapy” AND “pretreatment drug resistance”; “antiretroviral therapy” AND “( stavudine OR zidovudine OR nevirapine OR efavirenz)”; “HIV” AND “transmitted drug resistance”; “antiretroviral therapy” AND “( stavudine OR zidovudine OR nevirapine OR efavirenz)”; “HIV” AND “pretreatment drug resistance”; “HIV” AND “(antiretroviral therapy)”; “HIV” AND “( genotyp* OR “HIV” AND “naive”)”; “HIV” AND “( genotyp* OR “HIV” AND “resistance”)”; and “HIV” AND “( genotyp* OR “HIV” AND “resistance”)” and “primary”, with the search restricted to records in English, Spanish, or Portuguese. We did not contact study authors for unavailable data.

The following study-level data were extracted from each study: country, year of sample collection, sex, risk groups, setting, pretreatment CD4 cell count, number of pretreatment genotypes reported in the study, and exposure to antiretroviral drugs prior to treatment initiation (yes, no, or unknown). Additionally, the number of people with more than one drug-resistance mutation, one or more NRTI mutations, one or more thymidine analogue mutations, one or more NNRTI mutations, and one or more protease inhibitor mutations were extracted. When individual sequences were made available for analysis, drug resistance-mutations were defined as those appearing on the 2009 WHO surveillance drug-resistance-mutations list.

In all other cases, study authors’ interpretations of HIV drug resistance based on the Stanford HIVdb algorithm, International Antiviral Society-USA mutations list, and the Agence autonome de l’Inserm algorithms mutations list were used. RKG JGr RK and TC did the searches and data extraction. Conflicts over inclusion were decided by RKG.

**Data analysis**

The two major aims of our study were to characterise changes in drug resistance over time and to compare the prevalence of drug resistance in people restarting ART after reported previous antiretroviral exposure versus treatment-naïve patients. We also assessed whether factors including sex, CD4 cell count, rural or urban setting, or risk groups were predictors of HIV drug resistance in any geographical region. We therefore extracted information on drug resistance separately for each calendar year, treating each year as a separate datapoint in our analyses. We extracted information separately for patients before treatment initiation with and without prior exposure (reported or unreported) to antiretroviral drugs in studies where this information was available. The database was manually scanned for duplicates by JGr, NP and RKG. Where there were duplicate publications, the publication with information on the largest number of genotypes was used.

Statistical analysis was done in Stata version 14.1. To assess the prevalence of drug resistance within a specified region at any specific timepoint, we pooled prevalence estimates within the region using an empty logistic regression model with a random effect at the study level (appendix pp 20, 21). We did not formally assess study quality.

To assess associations between study-level characteristics (eg, calendar year at the midpoint of the study year) and drug resistance, we used univariate meta-regression analyses within each region (appendix pp 20, 21). We also explored the prevalence of specific drug-resistance mutations among all individuals with any WHO surveillance drug-resistance mutations. We calculated the proportion with specific mutations after crudely pooling the numbers of individuals with any mutation and the number with specific mutations. Heterogeneity was assessed using the I² statistic.

**Role of the funding source**

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. RKG had full access to all the data in the study and had final responsibility for the decision to submit for publication.

**Results**

We initially identified 19458 potential studies or reports of 697 full-length papers assessed. 339 records were
excluded on the basis of our eligibility criteria. We included 358 datasets in our analysis, which represented 56,044 adults with data on HIV drug resistance across 63 countries (appendix pp 1–13). 277 (93%) of 299 dataset with unambiguous location information were derived from urban settings (table 1). The identified studies included 23,948 genotypes from sub-Saharan Africa, (42.7% of all genotypes), 16,008 (28.6%) from Latin America and the Caribbean, and 16,088 (28.7%) from Asia. The median number of genotypes per study was 95 (table 1). There was a large amount of variation in the prevalence of drug-resistant mutations reported by studies, even within geographical regions (appendix p 14). We did not find study level summaries of sex or CD4 cell count to be associated with the prevalence of HIV drug resistance in any region (appendix pp 15–17). We detected an association between men who have sex with men and overall HIV drug resistance in Asia (p=0.047), but not for NNRTI or NRTI resistance specifically or in other geographical regions. We found insufficient data to assess other risk groups or the impact of rural versus urban or peri-urban setting on HIV drug resistance.

NNRTI resistance was more prevalent in more recent studies than in older studies across all regions (p<0.05 for all regions; figure 1; appendix p 18). Annual increases in the odds of pretreatment NNRTI resistance per year were 23% (95% CI 16–29) in southern Africa, 17% (6–29) in western and central Africa, and 11% (2–20) in Asia. In eastern Africa and Latin America and the Caribbean, there was evidence that the annual increase in the odds of pretreatment drug resistance, but not necessarily absolute changes in pretreatment drug resistance, have declined in more recent years (p for change in odds over time 0-027 for eastern Africa and 0-033 for Latin America and the Caribbean). Estimated annual increases in the odds of pretreatment NNRTI resistance since 2007 were 17% (5–30) in eastern Africa and 11% (5–18) in Latin America and the Caribbean. The model-predicted year-on-year increases in prevalence levels of NNRTI resistance between 2015 and 2016 were 11.0% (7.5–15.9) in southern Africa, 10.1% (5.1–19.4) in eastern Africa, 7.2% (2.9–16.5) in western and central Africa, 9.4% (6.6–13.2) in Latin America and the Caribbean, and 3.2% (1.8–5.6) in Asia (figure 1). As expected, the prevalence of NNRTI resistance in studies done between 2014 and 2016 agreed well with model-predicted prevalence levels for 2016 in all regions (appendix p 18).

By contrast with the increasing prevalence of NNRTI resistance over time, we saw little change in the prevalence of NRTI resistance, with model predicted prevalence in 2016 below 5% in all regions (figure 2). For Latin America and the Caribbean, western and central Africa, and Asia, there was no discernible trend in NRTI resistance over time (p=0.05 for all), and although increases in NRTI resistance were significant over time in southern Africa (p=0.015) and eastern Africa (p=0.017), changes in the prevalence of resistance were small (figure 2).

The most common NNRTI mutations (present in 10% or more of individuals with pretreatment drug resistance) from the WHO surveillance drug-resistance mutations list were Lys103Asn (present in 793 [34%] of 2349 people with pretreatment drug resistance), Tyr181Cys (215 [9%] patients), and Gly190Ala (200 [9%] patients; figure 3). The most common NRTI mutation was Met184Ile/Val (292 [12%] patients). Tenofovir resistance (Lys65Arg/Asn or Leu74Val/Ile) was relatively uncommon (3% of patients; patient-level data not available), although the thymidine analogue mutations Asp67Asn (134 [6%] patients) and Met41Leu (267 [11%] patients), which confer resistance to zidovudine, were more common. The prevalence of drug resistance to protease inhibitors was universally very low (<1%). The total number of drug-resistance mutations was available from 249 studies involving 29,898 patients in total and 1452 patients with any drug-resistant mutation. In these 1452 patients, the mean number of mutations per patient was 1.53 (SD not available from study-level data).

In a subset of 27 studies with 6534 patients, information was available on the presence of previous antiretroviral exposure. Previous antiretroviral exposure occurred in

| Region                     | Number of studies | Number of genotypes | Genotypes per study | Sampling year | Studies in urban populations |
|----------------------------|-------------------|---------------------|---------------------|---------------|------------------------------|
| Eastern Africa             | 53                | 7169                | 92 (57–187)         | 2008 (2005–09)| 32/44 (73%)                  |
| Southern Africa            | 61                | 11,852              | 102 (53–208)        | 2007 (2004–09)| 41/47 (87%)                  |
| Western and central Africa | 56                | 4924                | 79 (49–104)         | 2007 (2004–09)| 48/50 (96%)                  |
| Latin America and the Caribbean | 90              | 16,008              | 98 (52–221)         | 2008 (2003–10)| 67/69 (97%)                  |
| Asia                       | 98                | 16,088              | 97 (47–223)         | 2009 (2006–10)| 89/89 (100%)                 |
| Overall                    | 358               | 56,044              | 95 (50–194)         | 2008 (2005–10)| 277/299 (93%)                |

Data are n, median (IQR), or n/N (%). *Denominators restricted to studies with unambiguous information on location available. †For the purpose of this analysis, countries in the southeast Asia, the western Pacific, and eastern Mediterranean regions and Turkey (Europe region) are grouped under the regional heading of Asia.

Table 1: Characteristics of included studies by region
388 (8%) patients and was associated with substantially higher NNRTI resistance. Despite scarce data for many geographical regions, we were able to detect significant differences in the prevalence of pretreatment NNRTI resistance between patients with and without previous antiretroviral drug exposure in Asia, Latin America and the Caribbean, and southern Africa (table 2). Differences were not significant in eastern Africa, or western and central Africa; although statistical power was limited, particularly for western and central Africa where few patients had prior exposure (table 2; appendix p 19). NNRTI resistance was also more common among individuals with previous antiretroviral drug exposure compared with antiretroviral drug naive in all regions, with significant differences in all regions except for east Africa (table 2; appendix p 19).

We identified a subset of 13 studies with prospective data on treatment outcomes in adults in Africa who reported previous antiretroviral drug exposure. People who initiated NNRTI-based ART and self-reported previous antiretroviral drug exposure (n=83) had significantly greater risk of virological failure (defined as a viral load of 1000 copies per mL) at 12 months than antiretroviral-naive people (n=1944; odds ratio 2.91, 95% CI 1.48–5.72 after adjustment for age, baseline viral load, baseline CD4 cell count, initial ART adherence, WHO clinical stage, year of ART initiation, sex, and pretreatment drug resistance, p=0.002).

Discussion

Pretreatment HIV drug resistance can be detected in people naive to antiretroviral drugs who are initiating ART or people who are initiating or re-initiating first-line ART who have had previous exposure. Pretreatment drug resistance can be either transmitted or acquired drug resistance, or both. This resistance could have been transmitted at the time of infection (i.e., transmitted drug resistance), or it might be acquired after antiretroviral drug exposure: e.g., in women exposed to antiretroviral drugs for the prevention of mother-to-child transmission of HIV, people who have received pre-exposure prophylaxis, people re-initiating first-line ART after a period of treatment interruption without documented virological failure, or off-prescription use of ART through sharing within families or friends or black market availability.

Our analysis shows that the prevalence of pretreatment drug resistance is rising in many LMICs. This situation contrasts with that in high-income regions where the prevalence of pretreatment drug resistance has been in decline over the past decade and
has stabilised at around 10%. WHO 2016 antiretroviral guidelines recommend the use of NNRTI as a component of first-line ART regimens, which is widely implemented in LMICs, irrespective of previous exposure to antiretroviral drugs. A single aminoacid mutation can confer resistance to efavirenz or nevirapine, and the presence of NNRTI resistance is particularly crucial to predict the efficacy of recommended first-line regimens. Our analysis shows that, in several regions, pretreatment NNRTI resistance in populations initiating ART has reached levels exceeding the recently established 10% prevalence threshold above which countries should urgently consider responding. This response could be by either introducing a non-NNRTI-based first-line regimen or, in circumstances where use of a non-NNRTI-containing first-line regimen is not feasible because of cost or other considerations, using pretreatment drug-resistance testing to guide first-line ART regimen selection, if laboratory infrastructure and costs allow.

To our knowledge, our analysis provides the first robust evidence that people with disclosed previous antiretroviral drug exposure at the time of treatment initiation (ie, women exposed to treatment for the prevention of mother-to-child transmission or defaulters re-initiating first-line...
ART after a period of treatment interruption) are substantially more likely to have viruses resistant to both NNRTIs and NRTIs compared with people who report being antiretroviral-drug naive, in whom we infer resistance as being due to transmitted drug resistance. Data from several LMICs suggest that this pre-exposed population represents around 10–30% of people initiating or re-initiating first-line NNRTI-containing ART, a proportion that is likely to increase substantially following the rapid expansion in HIV treatment coverage.1

The present study also provides the first estimates of the risk of virological failure in individuals with previous exposure to antiretroviral drugs who initiate or re-initiate first-line NNRTI-based regimens in LMICs. The risk of virological failure was almost three times higher in the previously exposed group than in the treatment-naive group, which is reminiscent of data showing that presence of NNRTI resistance mutations before first-line treatment is associated with two to three times higher prevalence of virological failure after 12 months of treatment.10,12 These results suggest that self-reported previous exposure might be useful in clinic to identify people at increased risk of treatment failure and resistance. These findings underpin WHO guidelines on pretreatment drug resistance, which recommend the identification of individuals starting ART who are at increased risk of pretreatment drug resistance due to previous antiretroviral exposure (or other risk), with prioritisation of treatment with non-NNRTI based ART in this subpopulation. An alternative approach would be to identify patients with previous exposure and improve monitoring for early virological failure, although the problems of feedback of viral load results to patients and clinicians are likely to be significant in sub-Saharan Africa.

Notably, NRTI resistance also increased significantly over time in eastern and southern Africa. The prevalence of NRTI resistance was substantially lower than that of NNRTI resistance. This finding is not surprising for several reasons. First, in patients with virological failure, NNRTI mutations are among the earliest to emerge20 and therefore greater transmission of these resistant variants retaining almost wild-type replication fitness could be expected.21 The NRTI mutation Met184Val/Ile also emerges early, but is not commonly transmitted because of its significant fitness cost to the virus. In our analysis, about 10% of patients with pretreatment drug resistance

| Any resistance | Studies with data on previous treatment | Treatment naive | Any resistance | Studies with data on previous treatment | Treatment naive |
|----------------|----------------------------------------|----------------|----------------|----------------------------------------|----------------|
|                 | Number of patients | Proportion (95% CI) |                  | Number of patients | Proportion (95% CI) |
| Asia            | 4 | 1030 | 3·8% (2·8–5·1) | 73 | 24·0% (7·0–56·9) | 6·35 (2·15–18·76) | <0·0001 |
| Eastern Africa  | 7 | 1790 | 8·7% (6·4–11·8) | 91 | 17·6% (11·1–26·8) | 2·31 (1·36–3·92) | 0·023 |
| Latin America   | 4 | 797  | 11·7% (9·6–14·1) | 138 | 43·6% (21·6–68·5) | 3·46 (2·07–5·81) | <0·0001 |
| Southern Africa | 9 | 1810 | 4·9% (3·2–7·3) | 203 | 34·3% (27·5–41·9) | 7·42 (3·86–14·26) | <0·0001 |
| Western and central Africa | 2 | 328 | 2·4% (0·9–4·4) | 4 | 25·0% (3·4–76·2) | 15·68 (not estimable) | 0·085 |
| NNRTI resistance | Asia | 4 | 1030 | 2·6% (1·6–4·0) | 73 | 19·8% (7·6–42·8) | 8·05 (4·25–15·26) | <0·0001 |
| Eastern Africa  | 7 | 1790 | 6·0% (3·9–9·1) | 91 | 9·9% (5·2–17·9) | 2·04 (1·07–3·89) | 0·30 |
| Latin America   | 4 | 797  | 9·2% (7·3–11·4) | 138 | 32·8% (15·1–57·2) | 3·40 (1·79–6·48) | <0·0001 |
| Southern Africa | 9 | 1810 | 3·8% (2·3–6·0) | 203 | 31·5% (23·3–40·9) | 8·31 (4·23–16·24) | <0·0001 |
| Western and central Africa | 3 | 569 | 2·9% (1·5–5·8) | 33 | 12·1% (4·6–28·2) | 6·89 (0·41–117·23) | 0·061 |
| NRTI resistance  | Asia | 4 | 1030 | 1·6% (1·0–2·5) | 73 | 9·9% (0·6–65·7) | 13·29 (2·29–77·03) | 0·00034 |
| Eastern Africa  | 6 | 1790 | 3·5% (1·8–6·7) | 58 | 6·9% (2·6–17·0) | 2·76 (0·56–13·53) | 0·17 |
| Latin America   | 4 | 797  | 3·4% (1·9–6·0) | 138 | 15·5% (6·9–31·4) | 4·58 (1·96–10·71) | <0·0001 |
| Southern Africa | 9 | 1810 | 0·7% (0·4–1·4) | 203 | 7·9% (4·1–12·5) | 9·52 (4·17–21·76) | <0·0001 |
| Western and central Africa | 4 | 569 | 0·7% (0·3–1·9) | 81 | 6·2% (0·3–61·5) | 40·89 (not estimable) | <0·0001 |

p values are for the difference between treatment naive and previously treated people using random effects meta-regression. Where feasible, drug-resistance mutations were defined as those appearing on the 2009 WHO surveillance drug-resistance mutations list. Otherwise, the study authors’ interpretation was used. *Odds ratios use a random effects meta-analyses of within-study odds ratios. †Uses Freeman–Tukey arcsin transformation because mixed models did not converge. NNRTI = non-nucleoside reverse-transcriptase inhibitor. NRTI = nucleoside reverse-transcriptase inhibitor.

Table 2: Prevalence of HIV-1 drug resistance among antiretroviral-naive individuals compared with those starting first-line ART reporting prior antiretroviral drug exposure.
had Met184Val/Ile mutations. Although a sub-analysis showed a significant doubling of the prevalence of Met184Val/Ile in individuals with previous exposure to antiretroviral drugs, this could nevertheless suggest previous exposure even in patients who did not report it.

Thymidine analogue mutations were common, consistent with rapid accumulation of such mutations following the failure of first-line thymidine analogue-containing ART.27-29 By contrast, despite the scale-up of tenofovir-based ART and reports of the emergence of resistance to tenofovir in patients with treatment failure on tenofovir across sub-Saharan Africa,4 resistance to tenofovir was infrequent in our analysis. This finding is somewhat reassuring given concerns about the use of tenofovir for both the treatment and prevention of HIV infection.30 Nonetheless, continued surveillance for Lys65Arg/Asn and associated mutations such as Ala62Val remains important as tenofovir use is relatively new in many LMICs.25

Our study reports the prevalence of pretreatment drug resistance among 56000 people initiating ART over two decades, which we identified via a systematic review that included information from nine nationally representative unpublished pretreatment drug-resistance surveys (n=7138) and five additional unpublished studies (n=2757), making ours the largest such study to date. In addition to adding contemporary data to our previous report,9 this analysis includes 2-4 times more data from Latin America and the Caribbean and 2-9 times more data from Asia than in our previous work, allowing us to do statistically robust analyses in these regions, where it was previously unclear whether NNRTI resistance was increasing.

Our study has important limitations including the large amount of between-study heterogeneity and the inclusion of studies that are not nationally representative (appendix). The large degree of unexplained heterogeneity reduces the statistical power to detect region-specific trends over time. The inclusion of non-nationally representative studies has the potential to yield estimates of resistance that are too high or low. However, we were reassured by noting that the results from nationally representative surveys included in our analysis showed estimates of the prevalence of pretreatment drug resistance that were broadly similar to those from studies using a convenience sample in the same countries. In addition to these limitations, there were few studies from rural settings, so the analysis reflects the prevalence of pretreatment drug resistance primarily in urban and peri-urban areas. However, as access to ART has substantially increased in sub-Saharan Africa, similar trends might well be expected in rural areas with similar ART programmes. The inclusion of results using different genotyping methods and interpretation systems is a potential drawback of this analysis. Finally, we did not account for mutations in the connection domain of reverse transcriptase, which is known to confer NRTI and NNRTI resistance with mutations such as Asn348Ile.26-27 Therefore, the prevalence of pretreatment drug resistance might have been underestimated.

With increasing global use of ART for both the treatment and prevention of HIV infection and increasing global trends in the prevalence of HIV drug resistance, efforts to improve HIV programme quality and prevent the emergence and transmission of drug-resistant HIV should be strengthened. Although, HIV drug-resistance testing is not routinely available for individual patient management in many LMICs, the monitoring of patient and clinical factors associated with the emergence of preventable HIV drug resistance and successful population-level viral load suppression are relatively inexpensive, and can be used to identify gaps in service delivery and programme quality that can be corrected. These factors include retention on ART 12 months after treatment initiation, on-time pill pick-up, drug stock outs, viral load suppression, viral load completion, and timely switching to second-line ART. Monitoring of these factors, or early warning indicators of HIV drug resistance, is recommended by WHO on an annual basis at all ART clinics through the implementation of the consolidated strategic information guidelines for HIV in the health sector.30

Our findings reinforce the need for routine, robust nationally representative surveillance of pretreatment drug resistance and the need for each country to assess the prevalence of pretreatment drug resistance in people starting ART, irrespective of reported previous exposure to antiretroviral drugs. WHO guidelines provide recommendations on the appropriate public health response in the face of rising prevalence of pretreatment NNRTI resistance. The data presented in this report will help to inform policy makers and donors on the urgent need to respond to pretreatment drug resistance in settings where a high prevalence of resistance has already been reached.

At present, point-of-care drug resistance testing is not available, although substantial efforts and resources have been directed to this field and candidate signature mutations for surveillance have been identified.30 Earlier treatment initiation combined with routine viral load monitoring in people on treatment should lead to more viral load suppression and less pretreatment drug resistance, providing that the people who are identified as having confirmed virological failure are promptly switched to active second-line and or third-line regimens. One important caveat, however, is the fact that individuals on first line treatment sometimes experience virological rebound with subsequent re-suppression even in the absence of viral load monitoring,32 reinforcing the continual need for adherence support. Particular attention should be paid to people initiating ART who have had previous exposure to antiretroviral drugs, given that our data show increased prevalence of not only NNRTI but also NRTI mutations in this group compared with antiretroviral drug-naive individuals as well as an independently higher risk of virological failure. Finally, routine surveillance of population-level HIV drug
resistance coupled with strengthened approaches to prevent the development of resistance and sound evidence-based responses will be essential to the attainment of the global goal to eliminate AIDS as a public health threat by 2030.

Contributors
SB, JG, MRJ, and RKG conceived and designed the study, RKG, JG, RR, and TC did the literature search. RH, SI, LAF, CW, PK, LMF, MHC, NM, AT, SH, ZZH, AA, JD, Gr, ZG, PM, DP, TAO, KN, AK, EB, and MRJ generated and analysed data. RKG wrote the first draft of the manuscript. JG, NP, HH-S, SB, MRJ, AG, MD, JGa, SAR, and RKG analysed data and contributed to writing the manuscript.

Declaration of interests
We declare no competing interests.

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References
1. UNAIDS. UNAIDS data 2017. http://www.unaids.org/en/resources/documents/2017/20170720_Data_book_2017 (accessed July 21, 2017).
2. UNAIDS. 90-90-90—an ambitious treatment target to help end the AIDS epidemic. 2014. http://www.unaids.org/sites/default/files/media_asset/90-90-90_en_0.pdf (accessed May 14, 2017).
3. WHO. WHO HIV drug resistance report 2012. 2012. http://www.who.int/hiv/pub/drugresistance/report2012/en/ (accessed June 15, 2017).
4. WHO. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection Recommendations for a public health approach—second edition. 2016. http://www.who.int/hiv/pub/arv/arv-2016/en (accessed April 14, 2017).
5. Boender TS, Sigaloff KC, McMahon JH, et al. Long-term virological outcomes of first-line antiretroviral therapy for HIV-1 in low- and middle-income countries: a systematic review and meta-analysis. Clin Infect Dis 2015; 61: 1453–61.
6. Gupta RK, Hill A, Sawyer AW, et al. Virological monitoring and resistance to first-line highly active antiretroviral therapy in adults infected with HIV-1 treated under WHO guidelines: a systematic review and meta-analysis. Lancet Infect Dis 2009; 9: 409–17.
7. Gregson J, Kaleebu P, Marconi VC, et al. Occult HIV-1 drug resistance to thymidine analogues following failure of first-line tenofovir combined with a cytosine analogue and nevirapine or efavirenz in sub Saharan Africa: a retrospective multi-centre cohort study. Lancet Infect Dis 2016; 17: 296–304.
8. TenoRes Study Group. Global epidemiology of drug resistance after failure of WHO recommended first-line regimens for adult HIV-1 infection: a multicentre retrospective cohort study. Lancet Infect Dis 2016; 16: 565–75.
9. Gupta RK, Jordan MR, Sultan BJ, et al. Global trends in antiretroviral resistance in treatment-naive individuals with HIV after rollout of antiretroviral treatment in resource-limited settings: a global collaborative study and meta-regression analysis. Lancet 2012; 380: 1250–58.
10. Ávila-Ríos S, García-Morales C, Matías-Florentino M, et al. Pretreatment HIV-drug resistance in Mexico and its impact on the effectiveness of first-line antiretroviral therapy: a nationally representative 2015 WHO survey. Lancet HIV 2016; 3: e579–91.
11. Kanter R, Smeaton L, Vardhanbhatti S, et al. Pretreatment HIV drug resistance and HIV-1 subtype C are independently associated with virological failure: results from the multinational PEARLS (ACTG A5175) clinical trial. Clin Infect Dis 2015; 60: 1541–49.
12. Hamers RL, Schuurman R, Sigaloff KC, et al. Effect of pretreatment HIV-1 drug resistance on immunological, virological, and drug resistance outcomes of first-line antiretroviral treatment in sub-Saharan Africa: a multicentre cohort study. Lancet Infect Dis 2012; 12: 307–17.
13. WHO. HIV drug resistance report 2017. http://www.who.int/hiv/pub/drugresistance/hiv-dr-res-report-2017/en/ (accessed July 25, 2017).
14. NICD. Prospective sentinel surveillance of human immunodeficiency virus-related drug resistance. Communicable Dis Communiqué. 2016. http://nicd.ac.za/assets/files/NICD%20Communicable%20Diseases%20Communique_Mar2016_final.pdf (accessed May 22, 2017).
15. Hamers RL, Siwale M, Wallis CL, et al. HIV-1 drug resistance mutations are present in six percent of persons initiating antiretroviral therapy in Lusaka, Zambia. J Acquir Immune Defic Syndr 2010; 55: 95–101.
16. Phillips AN, Stover J, Cambiano V, et al. Impact of HIV drug resistance on HIV/AIDS associated mortality, new infections and antiretroviral therapy program costs in sub-Saharan Africa. J Infect Dis 2017; 215: 1362–65.
17. WHO. Guidelines on the public health response to pretreatment HIV drug resistance. 2017. http://who.int/hiv/pub/guidelines/hivdr-guidelines-2017/ (accessed July 28, 2017).
18. Bennett DE, Camacho RJ, Otelea D, et al. Drug resistance mutations for surveillance of transmitted HIV-1 drug-resistance: 2009 update. PLoS One 2009; 4: e6724.
19. Rhee SY, Blanco JL, Jordan MR, et al. Geographic and temporal trends in the molecular epidemiology and genetic mechanisms of transmitted HIV-1 drug resistance: an individual-patient- and sequence-level meta-analysis. PLoS Med 2015; 12: e1001810.
20. Hoffmann CJ, Charalambous S, Sim J, et al. Viremia re-suppression, and time to resistance in human immunodeficiency virus (HIV) subtype C during first-line antiretroviral therapy in South Africa. Clin Infect Dis 2009; 49: 1928–35.
21. Wang J, Bambara RA, Demeter LM, Dykes C. Reduced fitness in cell culture of HIV-1 with nonnucleoside reverse transcriptase inhibitor-resistant mutations correlates with relative levels of reverse transcriptase content and RNase H activity in virions. J Virol 2010; 84: 9377–89.
22. Goodall RL, Dunn DT, Nkurunziza P, et al. Rapid accumulation of HIV-1 thymidine analogue mutations and phenotypic impact following prolonged viral failure on zidovudine-based first-line ART in sub-Saharan Africa. J Antimicrob Chemother 2017; 72: 1450–55.
23. Boender TS, Kiyoi CM, Boerma RS, et al. Accumulation of HIV-1 drug resistance after continued virological failure on first-line ART in adults and children in sub-Saharan Africa. J Antimicrob Chemother 2016; 71: 2918–27.
24. Gupta RK, Wainberg MA, Brun-Verzit F, et al. Oral antiretroviral drugs as public health tools for HIV prevention: global implications for adherence, drug resistance, and the success of HIV treatment programs. J Infect Dis 2013; 207 (suppl 2): S101–06.
25. Rhee SY, Varghese V, Holmes SP, et al. Mutational correlates of virological failure in individuals receiving a who-recommended tenofovir-containing first-line regimen: an international collaboration. EbioMedicine 2017; 18: 225–35.
26. McCormick AL, Parry CM, Crombe A, et al. Impact of the N348I mutation in HIV-1 reverse transcriptase on nonnucleoside reverse transcriptase inhibitor resistance in non-subtype B HIV-1. Antimicrob Agents Chemother 2011; 55: 1806–9.
27. Yap SH, Sheen CW, Fahey J, et al. N348I in the connection domain of HIV-1 reverse transcriptase confers zidovudine and nevirapine resistance. PLoS Med 2007; 4: e335.
28. WHO. Global report on early warning indicators of HIV drug resistance. 2016. http://www.who.int/hiv/pub/drugresistance/ewi-hivr-2016/en/ (accessed May 5, 2017).
29. WHO. Consolidated strategic information guidelines for HIV in the health sector. 2015. http://www.who.int/hiv/pub/guidelines/strategic-information-guidelines/en/ (accessed May 5, 2017).
30. WHO. Consolidated guidelines on person-centred HIV patient monitoring and case surveillance 2012. http://www.who.int/hiv/pub/guidelines/person-centred-hiv-monitoring-guidelines/en/ (accessed July 28, 2017).
31. Rhee SY, Jordan MR, Raiser E, et al. HIV-1 drug resistance mutations: potential applications for point-of-care genotypic resistance testing. PLoS One 2015; 10: e0145777.
32. Gupta RK, Goodall RL, Ranopa M, et al. High rate of HIV re-suppression after viral failure on first line antiretroviral therapy in the absence of switch to second line. Clin Infect Dis 2014; 58: 1023–26.