Emerging HIV-1 drug resistance after roll-out of antiretroviral therapy in sub-Saharan Africa

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Purpose of review
This review provides an update of recent data on the development of HIV-1 drug resistance during treatment and its transmission in sub-Saharan Africa after the scale-up of antiretroviral therapy (ART).

Recent findings
Evidence is accumulating of a rising prevalence of transmitted HIV drug resistance (TDR), predominantly associated with nonnucleoside reverse transcriptase inhibitors (NNRTIs), in east and southern Africa. Pretherapy resistance is associated with first-line therapy failure. Accumulation of resistance mutations during first-line failure can be prevented by early detection and timely switching to second-line ART. Important gaps in service delivery and programme performance, associated with resistance development, affect a considerable proportion of ART programmes, particularly with respect to inadequate supply systems and patient retention. The reduction in new HIV infections associated with earlier use of ART is predicted to outweigh the risk of increasing TDR. Future levels of TDR are estimated to be diminished by improving switching practices to second-line regimens.

Summary
TDR is on the rise after the recent scale-up of ART in Africa. To prevent the development and spread of drug resistance and sustain the effectiveness of ART programmes, there is a need to improve drug supply systems, patient retention and access to routine viral load monitoring. Enhanced resistance monitoring is warranted in Africa.

Keywords
antiretroviral therapy, drug resistance, HIV-1, review, sub-Saharan Africa

INTRODUCTION

Sub-Saharan Africa continues to have the highest burden of HIV/AIDS worldwide [1]. In response, the WHO-recommended public health approach to antiretroviral therapy (ART) has been widely implemented [2]. This includes standard ART regimens based on first-line nonnucleoside reverse-transcriptase inhibitors (NNRTIs) and ritonavir-boosted protease inhibitors (bPIs) as second-line [3]. Obstacles to full ART access in Africa include weak ART programme functioning, lack of long-term funding and inadequate human resources. A consequence of ART scale-up is treatment failure that selects for drug-resistant HIV-1 (acquired drug resistance). Such virus has the potential to limit the response to subsequent treatment and can be transmitted to newly infected individuals (transmitted drug resistance, TDR). Factors contributing to acquired drug resistance in Africa include the lack of plasma viral load monitoring [4▪], treatment interruptions due to drug stockouts [5], drug interactions [6] and the use of substandard antiretroviral regimens [7▪]. These are leading to the concern of drug-resistance and becoming a potential threat to the worldwide control of HIV/AIDS [8]. This review provides an update of the latest data on acquired and transmitted HIV-1

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KEY POINTS

- Cumulated data indicate a rise in transmitted HIV drug resistance, predominantly associated with NNRTIs, in east and southern Africa, after ART scale-up.
- Pretherapy resistance to standard first-line therapy in Africa is associated with higher risk of virological failure and the further acquisition of drug resistance mutations.
- Routine viral load monitoring prevents accumulation of mutations, preserves HIV susceptibility and prevents unnecessary switching to second-line therapy.
- Early antiretroviral therapy initiation (at CD4 cell count <350 or <500 cells/μl), including TasP, according to scientific modelling leads to a rise in TDR. However, this risk is outweighed by a reduction in the number of new HIV infections. Future levels of TDR can be diminished by improving strategies for early failure detection and switching practices.
- HIV treatment programmes should optimize their functioning and sustainability, including robust supply chains, access to viral load monitoring and alternative drug regimens, strategies to maximize patient retention and enhanced resistance monitoring and surveillance in Africa.

drug resistance in sub-Saharan Africa after the ART scale-up and discusses public health implications.

TRANSMITTED DRUG RESISTANCE

To date, the WHO has reported 44 surveys in recently infected populations from 18 African countries, which suggest that the prevalence of TDR has increased between 2004 and 2010, driven primarily by NNRTI resistance [9▪▪]. Recent local surveys document moderate (5–15%) levels of TDR in Ouagadougou, Burkina Faso [10], KwaZulu-Natal, South Africa [11], Kampala, Uganda [12] and Mombasa, Kenya [13].

A challenge of TDR surveys is to identify persons who have been recently infected with HIV. A practical alternative is to study HIV-infected persons at the time of ART initiation. The PharmAccess African Studies to Evaluate Resistance Monitoring (PASER-M) cohort study among 2436 antiretroviral-naive individuals in 11 geographic areas in Kenya, Nigeria, South Africa, Uganda, Zambia and Zimbabwe reported overall baseline prevalence of drug resistance mutations (DRMs) of 5.6% [95% confidence interval (CI) 4.6–6.7] in 2007–2009 [14▪▪]. Drug class-specific resistance prevalence was 2.5% for NRTIs, 3.3% for NNRTIs, 1.3% for bPIs and 1.2% for dual-class resistance to NRTIs and NNRTIs. The most common DRMs were K103N (1.8%), thymidine analogue mutations (TAMs) (1.6%) and M184V (1.2%). Particularly high resistance levels were observed in Uganda (pooled estimate 11.6%), where antiretrovirals were introduced well ahead of nearby countries. PASER-M estimated a 38% increase of the average rate of resistance per year since ART roll-out [14▪▪]. Another study [15▪] in Yaoundé, Cameroon, demonstrated an increasing prevalence of DRMs in antiretroviral-naive persons over time, that is 0.0% in 1996–1999, 1.9% in 2001, 4.1% in 2002 and 12.3% in 2007.

Recently, a comprehensive global assessment of published studies and WHO surveys was performed on HIV-1 drug resistance in untreated patients, including 26 102 persons in 42 countries [16▪▪]. East Africa had the highest estimated rate of increase at 29% per year (95% CI 15–45; $P = 0.0001$) since roll-out, with an estimated resistance prevalence at 8 years after roll-out of 7.4% (4.3–12.7). An annual increase of 14% (95% CI 0–29; $P = 0.054$) was estimated in southern Africa, and a nonsignificant increase of 3% (95% CI −0.9 to 16; $P = 0.618$) in west and central Africa. NNRTI resistance increased substantially in east Africa [36% per year (95% CI 21–52); $P < 0.0001$] and southern Africa [23% per year (95% CI 7–42); $P = 0.0049$]. The prevalence of TAMs also seemed to increase in East Africa [31% per year (95% CI 4–66); $P = 0.021$], but not in other regions. The meta-analysis was limited by the fact that most studies included urban populations, and that the available data were not collected systematically, with standardized methods or definitions, or with representative sampling.

In summary, cumulated data suggest a rise in TDR in east and southern Africa after the ART scale-up. The rise is mostly driven by NNRTI resistance, which is of particular concern, as this drug class constitutes the foundation of current first-line ART regimens and prophylaxis for prevention of mother–child transmission (PMTCT) [3,17].

Because of clinical benefits, international guidelines recommend earlier initiation of ART at CD4 cell counts less than 350 or even less than 500 cells/μl [3,18,19]. Early treatment has attracted significant attention as a promising tool to reduce the number of people acquiring HIV infection [20]. Little is known on what will be the population effects of widespread implementation of early treatment in Africa, in terms of survival, HIV prevention and drug resistance development. A mathematical model on the basis of combined data on transmitted and acquired drug resistance from Kampala, Uganda, and Mombasa, Kenya, predicted that earlier initiation of ART, that is at CD4 cell count less than 350 or less than 500 cells/μl, will lead to increased prevalence of TDR in the coming 10 years, up to

11.6–13.4% and 17.8–18.7%, respectively (B.E. Nichols, K.C. Sigaloff, C. Kityo, K. Mandaliya, in preparation; this is a mathematical model based on data on transmitted and acquired drug resistance from Kampala, Uganda, and Mombasa, Kenya. Earlier initiation of ART is predicted to lead to increased prevalence of TDR, but this risk is outweighed by a reduction in the absolute number of new HIV infections). However, this risk of increased TDR would be outweighed by the reduction in the number of new HIV infections. Moreover, future levels of NNRTI resistance would be diminished because of improved switching practices to second-line bPI regimens.

EFFECT OF PRETHERAPY DRUG RESISTANCE ON THE RESPONSE TO FIRST-LINE ANTIRETROVIRAL THERAPY

The PASER-M cohort study demonstrated that the presence of pretherapy resistance to components of standard first-line treatment more than doubled the risk of virological failure and the further acquisition of DRMs after the first year of first-line ART [21▪]. These findings are in agreement with results from a collaborative analysis in Europe [22]. Pretherapy NNRTI mutations, either as major [21▪,23] or minority variants [24▪], have been associated with an increased risk of virological failure with first-line ART. In addition, previous use of ART or PMTCT has been shown to increase the risk of virological failure and the acquisition of drug-resistant virus during first-line ART [21▪]. These findings support the consideration of bPI-based first-line regimens in individuals who report previous antiretroviral exposure as well as in populations in which TDR levels exceed a yet to be defined threshold.

Pretherapy drug resistance in children is primarily associated with perinatal nevirapine exposure and may be present in up to 60% of infants below 6 months of age when PMTCT fails [25]. Infant prophylaxis with two-drug or three-drug ART regimens can reduce the rate of HIV drug resistance in HIV-infected children to less than 12% [26]. New evidence indicates the superiority of ritonavir-boosted lopinavir-based regimens over nevirapine-based regimens in terms of both efficacy and safety in all infants below the age of 3 years, regardless of whether they were previously exposed to nevirapine [27▪,28]. This poses a dilemma to policymakers because the use of ritonavir-boosted lopinavir implicates higher cost, poor palatability, inconvenient formulation and cold chain requirements.

ACQUIRED DRUG RESISTANCE FOLLOWING FIRST-LINE ANTIRETROVIRAL THERAPY

The WHO-defined early warning indicators (EWI) for HIV drug resistance aim to identify specific programmatic deficiencies that are associated with resistance development during ART [29]. Bennett *et al*. [30▪▪] assessed data from 907 ART programmes in the region between 2004 and 2009, documenting drug stock-outs in about 40% of sites, more than 20% of loss to follow-up in 40% of sites and ART prescription congruent with national guidelines to 100% of patients in 74% of sites. Important gaps in service delivery and programme performance affect a considerable proportion of ART programmes, particularly with respect to the fragility of procurement and supply systems and inadequate patient retention.

WHO surveys of acquired resistance indicate that after 12 months on ART, 91% of patients achieved full viral load suppression [9▪▪,31]. Among those failing therapy, 72% had drug resistance, implying that 28% of patients potentially switch to costlier second-line regimens unnecessarily. The predicted level of viral susceptibility observed among patients with drug resistance at 12 months suggests that the NRTI component of currently recommended second-line regimens, in combination with a bPI, is likely to be effective for the majority [9▪▪].

Recent observational studies from the region corroborate the notion that routine viral load monitoring prevents accumulation of DRMs and preserves HIV susceptibility. In patients who experienced prolonged first-line failure in the absence of viral load monitoring, 88% had at least one DRM, including high frequencies of accumulated NRTI and NNRTI mutations, for example TAMs, K65R and Q151M, all conferring broad cross-resistance [4▪]. In patients who experienced virological failure after the first year of ART, 70% had at least one DRM [32▪]. Frequent (3-monthly) viral load monitoring in a South African cohort resulted in even less extensive DRM patterns [33▪] (Fig. 1a and b).

There is limited knowledge of DRMs in the African non-B HIV-1 subtypes and their clinical relevance [34]. Studies [35,36] have suggested increased rates of the K65R mutational pattern in subtype C, compared with subtype B, which may be due to the nature of the subtype C RNA template of the viral reverse transcriptase. In a multicountry study [32▪] of mostly subtype A, C or D infections, K65R was frequently selected after failure on stavudine (15%) or tenofovir (28%) containing first-line regimens. After stavudine failure, zidovudine, rather than tenofovir (as recommended by the current

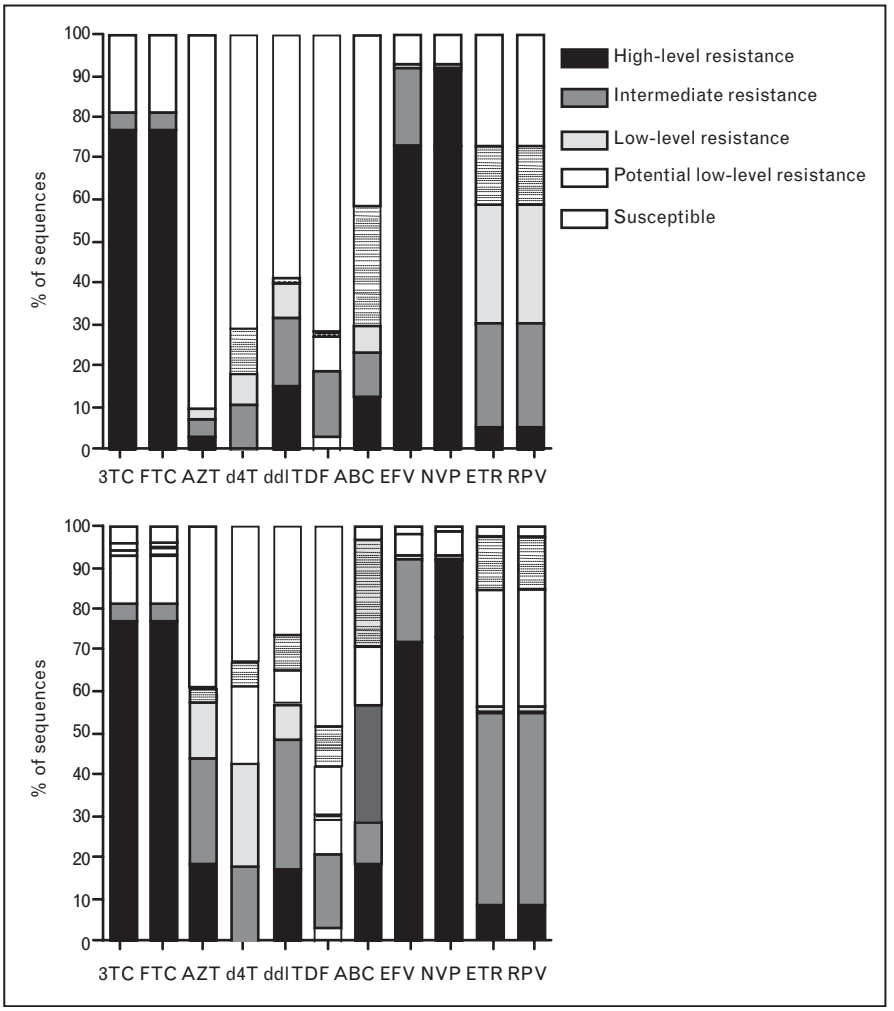

FIGURE 1. Predicted viral susceptibility to reverse transcriptase inhibitors. (a) Percentage of HIV sequences with at least one drug resistance mutation in patients with virological failure after 12 months of ART (standard viral load testing performed at month 12) ($n = 100$) [33▪]. (b) Percentage of HIV sequences with at least one drug resistance mutation in patients with clinico-immunological failure after a median of 26 months of ART (no viral load testing) ($n = 161$) [4▪]. Genotypic drug susceptibility was predicted using the Stanford HIVdb algorithm (version 6.1.0). 3TC, lamivudine; ABC, abacavir; AZT, zidovudine; d4T, stavudine; ddI, didanosine; EFV, efavirenz; ETR, etravirine; FTC, emtricitabine; NVP, nevirapine; RPV, rilpivirine; TDF, tenofovir.

WHO guidelines [3]), might be the preferred second-line NRTI in non-B infected populations in whom stringent viral load monitoring is lacking. Reports of K65R transmission are rare [16▪▪].

Several recent studies [4▪,32▪,37,38] have reported high frequencies of Y181C and G190A mutations, which means that the second-generation NNRTIs etravirine or rilpivirine are unlikely to be effective as part of second-line ART if combined with two NRTIs. Particularly, patients treated with nevirapine seem more likely to have resistance associated with the second-generation NNRTIs than those treated with efavirenz.

The patterns of reverse transcriptase mutations and rates of drug resistance at the time of virological failure seem to be similar for children and adults. However, the rates of virological failure are higher in children, resulting in an overall higher emergence of drug resistance among children [39]. A systematic review on HIV-1 drug resistance after failure of first-line regimens in children in resource-limited settings reported high prevalence of DRMs, 80% for NRTIs, 88% for NNRTIs and 54% for bPIs [40]. Although protease inhibitors are more commonly prescribed in paediatric first-line ART, resistance to this drug class is rare among children [41] except when ritonavir is used as single protease inhibitor [42,43]. Misclassification of treatment failure in children in the absence of virological monitoring is common [44,45,46▪]. A modelling study [47]

informed by a Thai cohort estimated that even infrequent viral load monitoring is likely to substantially reduce the duration of virological failure.

ACQUIRED DRUG RESISTANCE FOLLOWING SECOND-LINE ANTIRETROVIRAL THERAPY

Two observational studies [48,49▪] have suggested that empiric bPI-based regimens can successfully resuppress HIV-1 replication (in 85–86% of patients), despite the presence of extensive NRTI resistance. By contrast, the ACTG5230 trial, evaluating bPI monotherapy after first-line failure, found that about one-third of patients who achieved less than 400 HIV-RNA copies/ml after 24 weeks appeared to have incomplete suppression at between 40 and 200 HIV-RNA copies/ml [50▪]. In addition, the randomized HIV Star Study [51▪] in Thailand found that second-line bPI monotherapy was virologically inferior to triple therapy in patients failing NNRTI-based first-line therapy (61 versus 83% HIV-RNA <50 copies/ml). Thus, bPI monotherapy should not currently be recommended, and large international trials are underway to further assess this issue. A recent systematic review of patient outcomes on second-line ART in resource-limited settings showed that rates of virological failure are high (23, 27 and 38% at 12, 24 and 36 months, respectively) and associated with poor adherence, rather than resistance development to bPIs, which is likely attributable to their high genetic barrier [52▪]. Therefore, a major concern in Africa is maintaining long-term adherence, especially given that therapeutic options beyond second-line are very expensive and largely nonexistent.

Although data on second-line ART outcomes in children are scarce, most studies [40,41,44,46▪] of children failing first-line ART predict preserved HIV susceptibility to bPIs. A South African study [53] evaluating 82 children after the use of ritonavir as a single protease inhibitor demonstrated compromised effectiveness of lopinavir/ritonavir-based second-line therapy due to the accumulation of major protease inhibitor mutations, mostly I54 V and V82A/M/S. Among 40 children from Cameroon failing NNRTI-based regimens, response to second-line bPI-based therapy was favourable, with 70% achieving undetectable viral load after 48 weeks [54].

ACQUIRED DRUG RESISTANCE FOLLOWING PRE-EXPOSURE PROPHYLAXIS

The use of antiretroviral agents by HIV-uninfected persons before sexual exposure to HIV-infected partners, known as preexposure prophylaxis (PrEP), has been evaluated in various trials, with disparate results [55▪,56▪,57▪▪,58,59]. Most recently, the Botswana TDF/FTC Oral HIV Prophylaxis Trial (TDF2) [55▪] and Partners PrEP [56▪] study found that PrEP reduced the risk of HIV acquisition by 62–75%. By contrast, the FEM-PrEP study among HIV-negative women in Kenya, South Africa, and Tanzania was discontinued early because of a lack of protection, due to poor drug adherence [57▪▪]. HIV-1 infection acquired during PrEP has the potential to develop resistance to the agents used [55▪,56▪,58]. Mathematical modelling has suggested that, although TDR may increase, the potential benefits of PrEP outweigh the risks associated with resistance [60–62] and that drug resistance resulting from ART is expected to far exceed that from PrEP [63]. Further studies are needed to determine how best to optimize adherence before widely implementing PrEP in African populations [57▪▪].

ACQUIRED DRUG RESISTANCE IN WOMEN FOLLOWING PREVENTION OF MOTHER-TO-CHILD TRANSMISSION

Given that single-dose nevirapine (sdNVP) for PMTCT has been shown to diminish the effectiveness of subsequent NNRTI-based ART [64] because of frequent resistance selection [65], the use of combined antiretroviral regimens is being advocated [17]. In women in Mozambique, sdNVP along with zidovudine reduced postpartum nevirapine resistance [66]. In the Kesho Bora randomized study, HIV-1 drug resistance was present in 17.1% of mothers who received sdNVP along with zidovudine versus 1.4% who received triple ART [67,68]. A study [69] in Botswana found no DRMs 1 month after discontinuing triple ART for PMTCT. A new WHO strategy (Option B+) proposes to continue triple ART for all women for life [70]. However, no studies have yet investigated the effects on resistance development.

VIROLOGICAL MONITORING

The benefits of routine monitoring of viral load in preventing unnecessary switches and accumulation of drug resistance are increasingly being acknowledged [4▪,71]. A cost–effectiveness analysis estimated that 'VL-only' (without additive CD4 cell counts) holds promise as a diagnostic strategy to save costs for long-term ART management by avoiding the incremental costs associated with unnecessary switching [72▪]. A mathematical model of HIV transmission predicted that some form of routine viral load monitoring in patients on ART can reduce TDR [73▪▪].

Very few molecular diagnostic laboratories in the region have the capacity to conduct HIV genotypic resistance testing, of which only six have been WHO-accredited [74]. Because of technical and resource constraints, the use of drug resistance testing in the region is expected to remain restricted to research and population-based surveillance. Of note, a Markov model from South Africa's public sector programme estimated that adding drug resistance testing for patients with virological failure (and retaining patients without resistance on first-line therapy, rather than switching all failures to second-line) is potentially cost neutral and can conserve treatment options [75].

A comprehensive evaluation of dried blood spots (DBS) for genotyping assay validation and proficiency testing in the WHO laboratory network found that an amplification sensitivity of 1000 HIV-RNA copies/ml can be achieved, that reproducibility and accuracy of nucleotide sequence determination and DRM identification is similar from DBS to that previously determined for plasma and that international shipping at an ambient temperature had no significant effect on amplification success [76]. However, extreme storage conditions may negatively impact on HIV-1 amplification from DBS [77,78]. Current evidence suggests that DBS-based genotyping is probably reliable for population-based applications, but that further optimization of assay conditions and sensitivity is still warranted for individual patient management.

CONCLUSION

Cumulated data suggest a rising TDR prevalence in east and southern Africa in the context of growing coverage and use of antiretrovirals. The rise in NNRTI resistance is of particular concern, as this drug class is the cornerstone of first-line ART and PMTCT regimens. Important gaps in service delivery and programme performance affect a considerable proportion of ART programmes, particularly with respect to the fragility of procurement and supply systems and inadequate patient retention. To address this issue, national HIV treatment programmes should focus on optimizing their functioning and sustainability, including robust supply chains, access to routine viral load monitoring and alternative regimens, strategies to maximize patient retention and enhanced surveillance of drug resistance. Earlier ART initiation, including TasP, is anticipated to lead to a further rise in TDR in the coming 10 years. However, this risk is likely to be outweighed by a reduction in the number of new HIV infections. Future levels of NNRTI-associated TDR can be diminished by improving strategies for early failure detection and switching practices. Mathematic modelling and economic assessments remain needed to establish the cost–effectiveness of different drug regimens and diagnostic monitoring strategies. Concerted action by international agencies, national governments and ART programmes will be critical to preserve the long-term effectiveness of available ART regimens in Africa.

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R.L.H. and K.C.E.S. performed the literature search and drafted the report. All authors contributed to intellectual content, helped to revise the report and approved the final version.

Conflicts of interest

The authors are members of the Affordable Resistance Test for Africa (ART-A) Consortium, a public–private partnership that develops a simplified, affordable HIV drug resistance testing protocol for Africa (http://www.arta-africa.org).

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