

Implementing HIV-1 Genotypic Resistance Testing in Antiretroviral Therapy Programs in Africa: Needs, Opportunities, and Challenges

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Abstract

Tremendous progress has been made with the scale-up of antiretroviral therapy in Africa, with an estimated seven million people now receiving antiretroviral therapy in the region. The long-term success of antiretroviral therapy programs depends on appropriate strategies to deal with potential threats, one of which is the emergence and spread of antiretroviral drug resistance. Whilst public health surveillance forms the mainstay of the World Health Organization approach to antiretroviral drug resistance, there is likely to be increasing demand for access to drug resistance testing as programs mature and as HIV clinical management becomes more complex. African-owned research initiatives have helped to develop affordable resistance testing appropriate for use in the region, and have developed delivery models for resistance testing at different levels of the public health system. Some upper-middle-income countries such as Botswana and South Africa have introduced drug resistance testing for selected patient groups to guide clinical management. The scale-up of resistance testing will require substantial expansion of clinical and laboratory capacity in the region, but the expertise and resources exist in Africa to support this. The long-term population health impact and cost-effectiveness of resistance testing in the region will also require further investigation. (AIDS Rev. 2013;15:221-9)

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Introduction

The scale-up of antiretroviral therapy (ART) for the treatment of HIV infection has been one of the largest and most successful public health initiatives in recent years. In sub-Saharan Africa, which accounts for approximately 70% of all people living with HIV (an estimated 25 million), improved access to treatment in the last decade has had profound benefits and has transformed HIV from a death sentence to a manageable

chronic disease¹. The long-term success of HIV treatment programs is still threatened by certain issues, one of which is antiretroviral drug resistance². The emergence and transmission of drug-resistant HIV has the potential not only to impair individual health outcomes, but also to limit the population-level benefits of ART³.

In 2012, an estimated seven million people were receiving ART in sub-Saharan Africa, largely through the public health approach recommended by the World Health Organization (WHO), with standardized drug regimens and simplified monitoring systems¹. The WHO global strategy for the prevention and assessment of drug resistance has three main components: monitoring of HIV drug resistance early warning indicators; surveys of acquired drug resistance in populations receiving ART and surveys of transmitted drug resistance in recently infected populations⁴⁻⁶. This strategy focuses on population-based methods assuming

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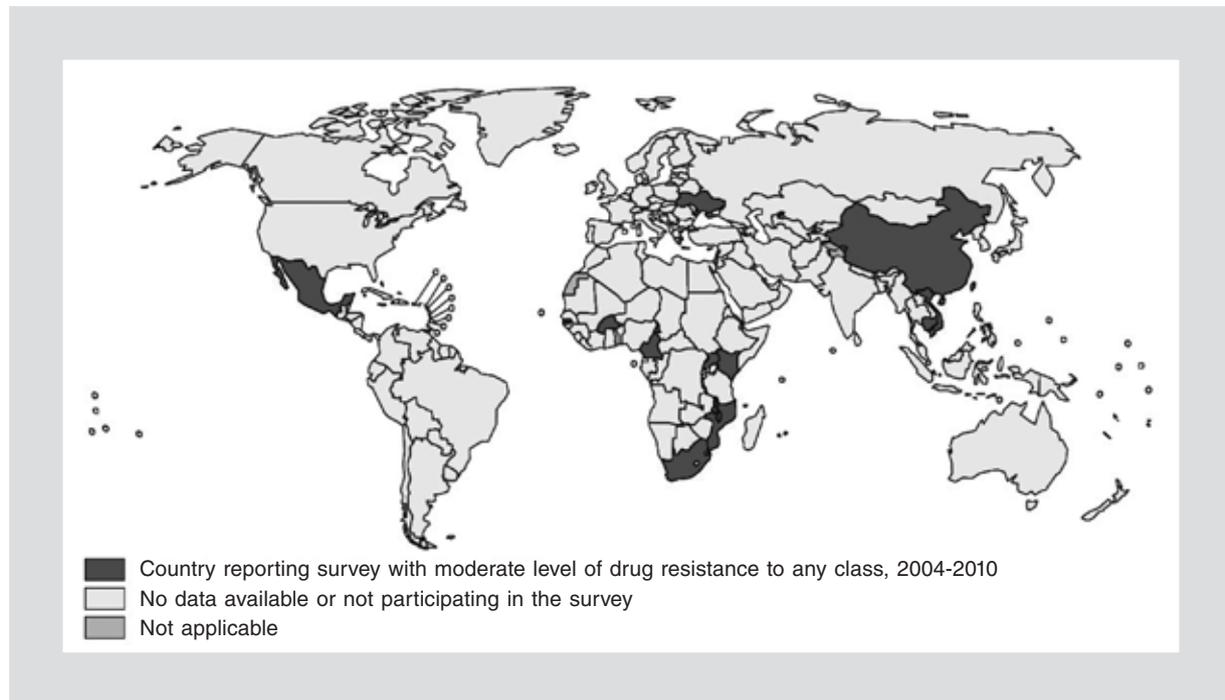


Figure 1. Location of World Health Organization surveys with moderate levels (5-15%) of drug resistance to any drug class (reproduced with permission from World Health Organization HIV drug resistance report⁹). The seven African countries are Burkina Faso, Cameroon, Kenya, Malawi, Mozambique, South Africa, and Uganda.

individualized assessment of drug resistance is not available or affordable in the majority of low- and middle-income countries. However, as HIV treatment programs mature and clinical management becomes more complex, there is likely to be a need for more advanced diagnostic tools, such as drug resistance testing, to assist treatment monitoring and to guide clinical decision-making^{7,8}. In this article, we provide an update on the current knowledge about HIV drug resistance levels in Africa and recent advances in genotypic resistance testing and consider the potential role of drug resistance testing in routine clinical care.

Current status of drug resistance

Adult transmitted resistance

The WHO HIV Drug Resistance Report 2012 summarized the results from 72 surveys of transmitted drug resistance in 26 countries between 2004 and 2010 (60% of the surveys were from the African region)⁹. While the majority of surveys ($n = 52$; 72.2%) found a low prevalence (< 5%) of drug resistance-associated mutations (DRAM) in all three drug classes, there was an increase over time in surveys reporting moderate prevalence (5-15%) of DRAM for at least one class. In

Africa, seven countries have had at least one survey reporting a moderate prevalence of DRAM (Fig. 1), and the proportion of surveys reporting moderate prevalence of DRAM increased from 17.6% in 2004-2006 to 40.7% in 2007-2010⁹.

In a systematic review of 218 datasets from 2001-2011 containing data on 26,102 untreated adults (15 years or older), a similar increase in transmitted drug resistance over time was revealed¹⁰. The increase was most pronounced in East Africa, where the prevalence of any DRAM increased from 0.9% (95% CI: 0.5-1.6) to 7.4% (95% CI: 4.2-12.9) after 8-9 years of program roll-out. The increase was less pronounced in Southern Africa, from 2.1% (95% CI: 1.6-2.6) to 3.7% (95% CI: 2.5-5.4) after 5-7 years. A separate review of studies specifically from South Africa, home to the largest ART program in the world, found no evidence of an increase in transmitted drug resistance between 2002 and 2010, and apart from 2002, the level was below 5%¹¹. However, one study in the KwaZulu-Natal region of South Africa has reported approximately 6% of naive patients with DRAM¹².

Adult acquired resistance

The majority of adults treated with ART achieve virological suppression. Systematic reviews have shown

that around three-quarters of adults initiated on ART in low- and middle-income countries are still on therapy with virological suppression ($< 1,000$ copies/ml) at 12 months¹³, although there seems to be substantial variation in program performance¹⁴. Surveillance for acquired resistance usually involves sampling those individuals with viremia $> 1,000$ copies/ml at around 12 months of ART, which corresponds to 1-2 in 20 of all adults who initiated ART.

In the WHO African region, an estimated 70% of adults with viremia $> 1,000$ copies/ml at 12 months had at least one DRAM⁹. As most individuals surveyed (87%) were receiving regimens which included thymidine analogues (stavudine or zidovudine), the prevalence of thymidine analogue mutations (TAM) was of particular interest as these mutations can confer cross-resistance and impair susceptibility to standard second-line regimens. A total of 13.4% of adults in the WHO African region had one or more TAM⁹. Similar results have been reported from other multicentre studies. The PharmAccess African Studies to Evaluate Resistance (PASER), which incorporated data from 13 sites in six African countries, estimated 70.4% of those with viral load $> 1,000$ copies/ml after 11-15 months of first-line ART to have at least one DRAM and 8.5% to have one or more TAM¹⁵. Another study involving countries in West Africa and in south-east Asia, reported 71.0% of those with viral load $> 1,000$ copies/ml at 12 months to have at least one DRAM and 13.8% to have one or more TAM¹⁴. A recently published study in rural KwaZulu-Natal identified a much higher level of resistance in patients with longer duration of ART including thymidine analogues (medium duration on ART 42 months. In this study, 40% of patients had one or more TAM and 15% of patients had a genotypic susceptibility score for the standard second-line regimen of less than two, suggesting a significantly compromised regimen¹⁶.

Recently, tenofovir disoproxil fumarate (TDF) was added as part of first-line ART regimens in Africa. Drug resistance studies are emerging about the use of TDF. At present, mixed reports have been presented in South Africa, where HIV-1 subtype C dominates the epidemic. One study pointed to a failure rate of 6% (35/585) at six months of ART, but with very high levels (69.7%; 23/33) of K65R, which is one of the main mutations causing resistance to TDF¹⁷. However, a more recent study did not see this effect, with 16% (270/1,682) of patients failing ART at six months but only 12% (5/40) of the genotyped patients with K65R¹⁸. It is important to note that the two studies were done

on subtype C viruses and used low sample numbers. Subtype C has been reported to develop K65R with greater propensity than other subtypes¹⁹ and more research is needed to determine the patterns and impact of drug resistance with TDF-based regimens.

Pediatric acquired resistance

Studies of pediatric drug resistance are often limited by difficulties in combining data for children on non-nucleoside reverse transcriptase inhibitor (NNRTI)-based and protease inhibitor (PI)-based regimens, and by the lack of data on the presence of drug resistance prior to ART. In a systematic review of 30 studies, including children on both NNRTI-based and PI-based regimens, the pooled proportion of children with at least one DRAM was 90% (95% CI: 88-93). The proportion was considerably higher in NNRTI-exposed children than in PI-exposed children (88 vs. 54%) and the high proportion with at least one TAM (56%) was also noteworthy²⁰.

The relatively low proportion of children failing ritonavir-boosted PI-based regimens with resistance has also been reported from individual programs²¹. This not only highlights the challenges of HIV treatment and care in the population of young children, but also underlines the potential importance of drug resistance testing for this group, especially as there is little evidence to inform standardized second-line options in this group.

Importance and impact of drug resistance testing

The most common form of resistance testing is genotypic resistance testing, where HIV-specific mutations known to be associated with reduced susceptibility to certain antiretroviral drugs are detected. Individualized resistance testing is routinely used in high-income countries prior to ART and at the time of virological failure in order to guide clinical management²²⁻²⁵. Within developing countries, where a sequence of standardized ART regimens is carefully chosen, taking into account potential drug resistance, the role of resistance testing is, at present, less clear²⁶⁻²⁸. However, as clinical case management becomes more complex and as more drugs and regimens are used over time, the demand for individualized resistance testing to guide clinical management of virological failure is likely to increase.

The potential benefits of resistance testing are not restricted to selecting an appropriate drug regimen in

the presence of DRAM. The absence of drug resistance can point to particularly poor adherence, the undisclosed interruption of therapy, or problems with drug dosing or absorption. Accurately identifying these issues allows for targeted interventions to be made in order to conserve first-line regimens. Studies from South Africa using standard population sequencing have reported 5-33% of adults with virological failure on first-line ART to have wild-type genotypes²⁹. The PASER study in six sub-Saharan countries also documented a relatively high proportion (30%) with wild-type genotype¹⁵. In the absence of resistance testing, these individuals will be switched to second-line ART regimens and the root cause of their virological failure may not be addressed. This may partially explain the poorer outcomes on second-line ART for individuals with wild-type genotype^{30,31} and also the high levels of wild-type genotype in studies reporting early virological failure on second-line regimens in Africa (38-85% had no DRAM at the time of second-line ART failure)³²⁻³⁵.

The association between virological failure on second-line regimens and subtherapeutic drug concentrations before and after regimen switch, further strengthens the interpretation that unresolved adherence issues remain a significant driver of second-line ART failure^{30,35}. Even in settings with routine viral load monitoring, the identification of treatment failure can be difficult and decisions around whether or not to switch can be complex^{30,36}. At the root of this are the challenges in accurately assessing antiretroviral adherence³⁷ as well as the relative paucity of evidence-based adherence interventions³⁸. Whether outcomes could be improved, through the use of genotypic resistance testing to identify those with poor adherence and to target adherence interventions, requires further investigation.

In high-income countries, the use of genotypic resistance testing to guide regimen selection in adults with virological failure has been shown to have a positive, albeit modest, impact on subsequent virological outcomes³⁹⁻⁴¹. There is as yet no published evidence about the effectiveness of resistance testing in Africa, although there is at least one clinical trial investigating the impact of pre-ART resistance testing on virological outcomes of first-line ART⁴². Estimates from mathematical modeling studies have suggested that, in the context of the South African ART program, genotypic resistance testing would have a modest beneficial effect on clinical outcomes⁴³ and would be cost-effective at failure of first- and second-line ART^{43,44}.

The cost-effectiveness of genotyping at failure of first-line ART was shown to be particularly sensitive to

the prevalence of wild-type genotype: resistance testing was estimated to be very cost-effective (i.e. the incremental cost-effectiveness ratio was less than the per capita gross domestic product) when the prevalence of wild-type genotype was $\geq 12\%$ and the cost of drug resistance testing was US\$ 250. Given the recent decrease in costs of genotyping, resistance testing is expected to become more affordable and cost-effective. For example, in Botswana, the cost of second-line ART is three-times the costs of first-line ART and third-line ART is ten-times the cost of first-line. A resistance genotype in Botswana would cost approximately the same as one month on third-line therapy.

If higher levels of transmitted drug resistance ($> 15\%$) are documented during public health surveillance, resistance testing prior to treatment to guide first-line regimen choice might be required⁶. Whether or not the use of resistance testing would be a more cost-effective strategy than modification of the standard regimens for all individuals (for example from a NNRTI-based to a PI-based first-line regimen) will require further research, should the situation arise.

Opportunities for resistance testing within the public health approach to antiretroviral therapy

In most low- and middle-income countries, ART is delivered through the public health approach, with standardized drug regimens and simplified laboratory monitoring. Drug regimens are selected on the basis of predicted drug resistance patterns, and regimen sequencing aims to achieve optimal long-term ART efficacy^{26-29,45}. The use of routine viral load monitoring is intended to monitor adherence, limit the emergence of drug resistance, and enable regimen switching before the onset of immunological and clinical failure⁴⁶.

High coverage of routine virological monitoring has so far been achieved only in South Africa and Botswana, but other countries are scaling up the implementation of viral load testing⁴⁷. In South Africa, the implementation of routine virological monitoring has required rapid growth in laboratory capacity, with 17 laboratories now performing almost two million viral load tests annually^{48,49}. In the past three years, the South African National Health Laboratory Service (NHLS) has also scaled up laboratory capacity to deliver Xpert MTB/RIF testing for the diagnosis of tuberculosis and antituberculosis drug resistance⁵⁰. Whilst these laboratory systems are still largely centralized, there

are plans to decentralize services as appropriate technologies and systems are developed⁴⁹. In Botswana, where viral load monitoring was offered from the beginning of the national ART program and laboratory services are largely decentralized, there are now 24 laboratories performing routine viral load monitoring. The rapid scale-up of Xpert MTB/RIF testing is also underway.

In both South Africa and Botswana, genotypic resistance testing is now recommended for adults when virological failure occurs on second-line ART^{51,52}. This is in order to preserve second-line regimens due to the limited availability and the high cost of third-line regimens. Additional priority groups in both countries include children with virological failure on PI-based regimens (first- or second-line)^{51,52}. Specialist treatment failure management teams to coordinate the management of complex cases have already been established in Botswana⁵² and are planned in South Africa.

Recent advances with genotypic resistance testing

The main barriers to implementation of genotypic resistance testing have been related to the cost, the need for complex laboratory infrastructure, and issues with specimen transport to centralized laboratories. In the past decade, there has been a rapid evolution of genome sequencing technologies and this has driven huge reductions in genome sequencing costs⁵³. Whilst these cost reductions can be slow to translate into more affordable technologies for use in clinical practice, there is already evidence that this can happen. The Southern African Treatment and Resistance Network (SATuRN) has developed laboratory methods to reduce the cost of resistance testing and has, through collaboration with a major biotechnology company, enabled access to cheaper sequencing reagents⁵⁴. The Affordable Resistance Test for Africa (ART-A) initiative has similarly developed and validated simple, robust genotyping methods specifically designed for subtype C viruses⁵⁵⁻⁵⁷. In both of these initiatives, cost reductions were achieved through a reduction in the number of sequencing primers, a reduction in reagent volumes, simplification of polymerase chain reaction methods, and targeted amplification of reverse transcriptase and protease fragments or reverse transcriptase alone^{16,55-58}. The availability of open-source bioinformatics software and publicly accessible databases specific to the region also help to reduce costs associated with the analysis

and interpretation of genotypic data and to maximize the value of the data generated through drug resistance testing⁵⁹.

Standard genotypic resistance testing incorporates population (Sanger) sequencing methods. One limitation of this method is the inability to detect minority variants present at frequencies below 20%²⁵. The use of next-generation sequencing technologies allows detection of these minority variants⁶⁰. Further research is necessary to determine the true significance of minority variants in relation to clinical outcomes, and to compare the cost-effectiveness and impact of different sequencing technologies.

The issue of specimen collection, handling, and transport to centralized laboratories is an important one in low- and middle-income countries, where the laboratory infrastructure often exists remotely from where patients access healthcare. Dried blood spots (DBS) are easy to collect in the field, easily transported, and HIV-1 nucleic acids remain stable on DBS for long periods at ambient temperatures⁶¹. Several studies have reported successful genotyping from DBS^{61,62}. However, the sensitivity is lower than from whole plasma specimens and depends largely on the HIV-1 viral load. In a study involving ten WHO/HIVResNet-accredited laboratories, HIV-1 was successfully amplified for more than half the replicates with viral load 1,000 copies/ml in only two of ten laboratories. At a threshold of 10,000 copies/ml, seven of ten laboratories were able to successfully amplify more than half the replicates⁶³.

Scaling up capacity for public health implementation

At present, drug resistance surveillance activities in low- and middle-income countries are coordinated through a network of national and regional WHO-accredited genotyping laboratories. As of 2011, this included laboratories in seven African countries (Fig. 2). Both the PASER and SATuRN networks are working to develop laboratory capacity in order to provide quality assured genotypic resistance testing in the region^{58,64,65}. Implementation of resistance testing in the clinical care context would require substantial expansion of existing laboratory capacity. Given the laboratory infrastructure required and the relative complexity of the techniques, it is unlikely that genotypic resistance testing could be decentralized below the level of provincial or national laboratories.

In addition to the expansion of laboratory capacity, introducing resistance testing into clinical care will

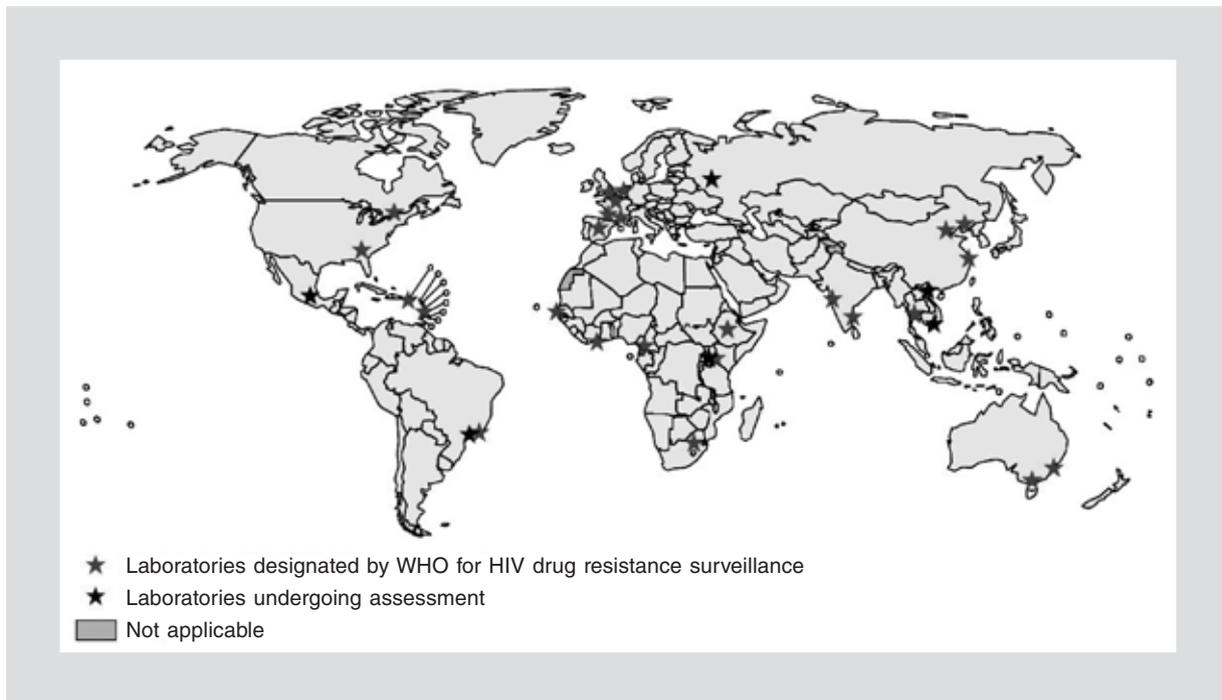


Figure 2. WHO-accredited laboratories performing HIV drug resistance testing for public health surveillance (reproduced with permission from World Health Organization HIV drug resistance report⁹). This includes laboratories in Cameroon, Cote d'Ivoire, Ethiopia, Kenya, Senegal, South Africa, and Uganda.

require the parallel development of clinical capacity. Genotypic resistance testing provides most value when the results are interpreted by a specialist clinician in parallel with relevant clinical information. In sub-Saharan Africa, clinical virologists and specialist HIV clinicians are a scarce resource. There is, therefore, a need to expand teaching on HIV drug resistance in existing training programs and to develop educational methods around drug resistance for frontline health-care workers. It is likely that initially services would be centralized around existing clinical and laboratory expertise, but systems could be decentralized over time as demand grows. This is the model that has been adopted in Botswana⁵² and it is also similar to how programs for the management of drug-resistant tuberculosis (TB) have evolved in South Africa⁶⁶.

The Botswana national ART program currently incorporates the use of genotypic resistance testing, primarily at the time of second-line ART failure in adults⁵². Capacity for resistance testing has been available since the beginning of the national program in 2002 and resistance testing is carried out at one reference laboratory, located in Gaborone. With the maturation of the ART program, the demand for resistance testing has continued to increase and with it the need for a more decentralized HIV specialist care model. Therefore, plans are now underway to develop HIV/TB specialty centers

in six districts across Botswana in order to meet the need for advanced clinical management of both HIV and TB. These centers will build upon existing HIV/TB specialty care and also allow for more decentralized HIV/TB clinical training and HIV/TB resistance surveillance. This model of care is illustrated in figure 3. Under this model, identification of second-line virological failure at primary healthcare clinics and district hospitals prompts referral of the patient to one of six HIV/TB specialty centers. After assessment at these centers, a specimen is submitted to the laboratory for HIV genotyping with a form containing clinical information (including a detailed treatment history), which has been approved by an HIV specialist clinician on the basis of the clinical information provided. Once the resistance report is generated, it is interpreted by the same specialist clinician, who then sends the report with interpretation and management recommendations directly back to the referring doctor.

A similar model has been adapted for the primary healthcare context by SATuRN and has been implemented in Hlabisa sub-district in northern KwaZulu-Natal, South Africa¹⁶. The difference in this model is that patients receive care throughout at the primary healthcare clinic, while specimens are transported to a central laboratory and the specialist clinician can function remotely through electronic communication (Fig. 3).

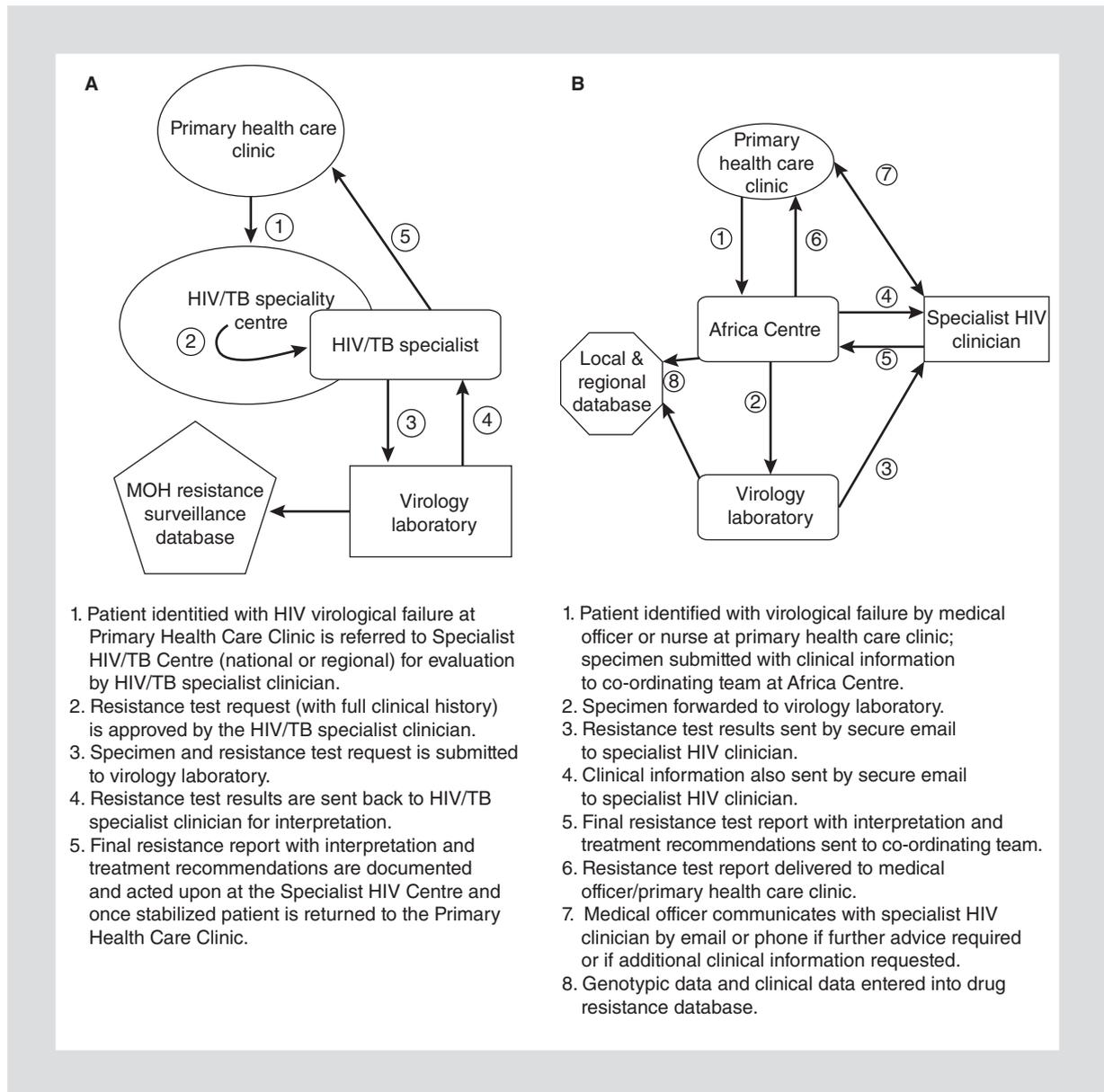


Figure 3. Models for the implementation of HIV-1 genotypic resistance testing for routine clinical management: Botswana national model (A) and a primary healthcare HIV program in Hlabisa sub-district, KwaZulu-Natal, South Africa (B).

A feature of both these models is the integration of data systems where the sequence data is accumulated along with clinical information⁶⁷. These databases can then be used by frontline healthcare workers for patient management, but can also be resources for real-time surveillance, research, and training^{59,67}.

Other than these examples, there is a paucity of published evidence around the use of genotypic resistance testing for clinical care in Africa and there is certainly the need to develop and evaluate different systems for the programmatic implementation of genotypic resistance testing.

Challenges for implementation

There are substantial challenges around the expansion of laboratory monitoring strategies in low- and middle-income countries, as previously highlighted in the context of viral load testing⁶⁸. However, the scale-up of laboratory capacity to deliver CD4⁺ T-cell count and viral load testing also provides a framework through which genotypic resistance testing could be introduced. However, CD4⁺ T-cell count and viral load technologies are starting to move out of the laboratory to more peripheral levels of the health system where patients

access care^{69,70}. From the perspective of frontline healthcare workers, technologies integrating viral load measurement and identification of DRAM might have the most value in low- and middle-income countries and it is important that such tools are developed and evaluated.

The interpretation of genotypic resistance data is complex. A number of algorithms exist to assist with interpretation, such as the Stanford HIVdB⁷¹, Rega algorithm⁷² and the ANRS algorithm⁷³. Whilst the algorithms do differ in their interpretation of mutational patterns, particularly for non-B HIV-1 subtypes⁷⁴, the evidence suggests that they perform equally well in terms of predicting virological response to subsequent antiretroviral regimens⁷⁵. SATuRN hosts a mirror of RegaDB and of Stanford HIVdB in order to gather and analyze subtype C HIV-1 sequences. This data is then used to guide regional public health policies⁵⁹.

Lastly, while genotypic resistance testing might have a role in routine HIV care in Africa, we should be cautious about the extent to which health technologies can contribute to improved programmatic and population outcomes. Resistance testing should not be seen as a technological solution to the problem of drug resistance. Long-term reductions in the emergence and spread of drug resistance will best be achieved by improving the quality of care in HIV programs in low- and middle-income countries⁷⁶.

Conclusions

Genotypic resistance testing may become an increasingly important component of routine care in Africa as HIV programs mature. Local research and development, coupled with advancements in genomic sequencing technologies, have made resistance testing affordable for upper-middle-income countries such as South Africa and Botswana and models for implementation in these countries are continuing to be developed. The introduction of resistance testing to support clinical care will require substantial expansion of laboratory and clinical capacity. To guide the future scale-up of resistance testing, research is needed to demonstrate its impact on individual clinical outcomes and population health outcomes.

Conflicts of interest and source of funding

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