The World Health Organization's global strategy for prevention and assessment of HIV drug resistance

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Antiretroviral treatment (ART) for HIV is being scaled up rapidly in resource-limited countries. Treatment options are simplified and standardized, generally with one potent first-line regimen and one potent alternate first-line regimen recommended. Widespread HIV drug resistance (HIVDR) was initially feared, but reports from resource-limited countries suggest that initial ART programmes are as effective as in resource-rich countries, which should limit HIV drug resistance if programme effectiveness continues during scale-up. ART interruptions must be minimized to maintain viral suppression on the first-line regimen for as long as possible. Lack of availability of appropriate second-line drugs is a concern, as is the additional accumulation of resistance mutations in the absence of viral load testing to determine failure. The World Health Organization (WHO) recommends a minimum-resource strategy for prevention and assessment of HIVDR in resource-limited countries. The WHO's Global Network HIVResNet provides standardized tools, training, technical assistance, laboratory quality assurance, analysis of results and recommendations for guidelines and public health action. National strategies focus on assessments to guide immediate public health action to improve ART programme effectiveness in minimizing HIVDR and to guide regimen selection. Globally, WHO HIVResNet collects and analyses data to support evidence-based international policies and guidelines. Financial support is provided by major international organizations and technical support from HIVDR experts worldwide. As of December 2007, 25 countries were planning or implementing the strategy; seven countries report results in this supplement.

Background to the WHO strategy

Antiretroviral treatment scale-up in resource-limited countries

The rapid scale-up of antiretroviral therapy (ART) for human immunodeficiency virus (HIV) in resource-limited countries is an international priority. The Group of Eight countries and the United Nations member states have endorsed the global goal of universal access to ART by 2010. The World Health Organisation (WHO), the Joint United Nations Program on AIDS, the US President's Emergency Plan for AIDS Relief, the Global Fund to Fight AIDS, Tuberculosis and Malaria along with numerous partner organizations are heavily committed to supporting ART expansion. Numerous countries have targeted coverage for 80% of individuals in need of ART as their 2010 goal [1]. Meeting this need rapidly and maintaining patients on appropriate ART is challenging, given the minimal health infrastructure, lack of trained personnel and facilities, complexities of drug ordering, delivery and storage and inadequate laboratory capacity.

By December 2006 it was estimated that 2,015,000 people living with HIV/AIDS were receiving treatment in low- and middle-income countries, representing coverage of 28% of the estimated 7.1 million people in need of ART [2]. In Sub-Saharan Africa and in South and Southeast Asia, the two areas where >90% of individuals in need of ART reside, expansion has been rapid, but the need remains great. In December 2006, an estimated 1.3 million people were receiving ART in Sub-Saharan Africa, with coverage of 28% of those in need, whereas 3 years earlier there were 100,000 on treatment and coverage was only 2%. In East, South and Southeast Asia, 280,000 people (19% of those in need) were receiving ART in December 2006, a four-fold increase compared with the 70,000 receiving ART at the end of 2003.

The context for ART scale-up makes impractical the approach used in resource-rich countries, which consists of specialized patient management based on complex laboratory monitoring to guide selection among extensive antiretroviral (ARV) options. In Sub-Saharan Africa and Southeast Asia, there are only 2–4 healthcare workers per 1,000 population in contrast to the 18–23 per 1,000 in Europe and the Americas [3]. Numerous other medical problems also require urgent

attention. The public health approach to extend ART rapidly to the maximum number of individuals in need is therefore based on standardized simplified treatment protocols, standardized management approaches and decentralized service delivery [4]. The approach uses the coordinating principle of the 'Three Ones' (one comprehensive HIV/AIDS Action Framework agreed by key stakeholders, one National AIDS Coordinating Authority and one agreed country-level Monitoring and Evaluation System) [5]. Under the public health approach, clinicians do not make individualized regimen decisions; regimen selection is a matter of national policy guided by WHO recommendations [6]. This approach enables healthcare workers with minimum training to deliver care to large numbers of patients in facilities without sophisticated resources. ART scale-up is based on one first-line regimen consisting of one non-nucleoside reverse transcriptase inhibitor (NNRTI) supported by two nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs). An alternate regimen, involving a different NNRTI and/or a different NRTI, can be substituted if required because of toxicity, drug interactions or other health conditions. One second-line regimen, consisting of a protease inhibitor (PI), ideally boosted by ritonavir, and two NRTIs, should be used when the first regimen fails. National selection of the first-line regimen for the population takes into account the possible efficacy, durability and tolerability (the criteria used in resource-rich countries), but also whether the ARVs are registered and marketed in the country, especially in fixed-dose combinations [7], their cost [6,8], and whether drugs can be transported and stored unrefrigerated [9]. Second-line regimens used in resource-limited countries generally follow WHO recommendations, but selection of second-line drugs is based primarily on availability and affordability. To the extent possible, the aim is to minimize cross-resistance resulting from first-line regimen [10]. Given limited laboratory facilities, decisions to start, substitute one first-line ARV for another or switch to second-line therapy are largely made on the basis of clinical observation and WHO clinical staging or, if available, $CD4^+$ T-cell count [11], haemotology and biochemistry. Viral load measurements are not recommended or routinely performed, due to cost, complexity and lack of laboratory facilities. National ART policy is based on WHO guidelines in almost all countries where ART scale-up is taking place [2,12].

HIV drug resistance

Given the limited number of regimens available in resource-limited countries, minimizing HIV drug resistance (HIVDR) is especially important. The evolution of drug-resistant subpopulations of HIV can significantly limit the ability of ARVs to suppress replication. HIV evolves rapidly within the human body; it has a high replication rate (up to ten billion new virions are produced daily in an untreated person [13]) and a high mutation rate. The enzymes HIV produces to support its own life cycle, which include protease and reverse transciptase (the targets of the drugs on which ART scale-up is based) can function even with numerous mutations [14]. During ART, strains containing mutations associated with resistance can emerge within days if drug pressure is insufficient to suppress replication. Once resistant strains emerge and replicate, they persist indefinitely. After treatment stops or the regimen changes, the strains resistant to the previous regimen will quickly cease to constitute the majority circulating quasi-species and may become undetectable by conventional genotyping methods. However, they are 'archived' within memory cells and can re-emerge if drugs to which they are resistant are restarted. Mutations conferring resistance to one drug frequently confer cross-resistance to others within the same ARV class [15,16]. Treatment with fewer than three drugs, or with a non-potent three-drug regimen, can lead to HIVDR quickly, as can the addition of one drug to an already failing regimen. Interruption of treatment, even for a few days, can also lead to suboptimal ARV levels, treatment failure and drug resistance. ART should ideally maintain plasma HIV-1 RNA levels below the limits of detection of the commercially available assays (that is, <50–400 copies/ml), which will also minimize the development and replication of additional resistance mutations [17]. Transient 'blips' to 1,000 copies/ml may not lead to development and replication of new resistance mutations [18], but prolonging a failing regimen when the viral load is higher can cause the addition of more resistance mutations and also compensatory mutations that may increase the fitness of the resistant strains [19,20]. However, continuing ART with a regimen to which resistance has developed is associated with substantially better clinical outcomes than stopping ART [20–22].

Factors potentially associated with HIV drug resistance in resource-limited countries

Some aspects of the public health ART strategy, where it is successfully implemented, support limitation of HIVDR. All ART patients are treated with potent three-drug regimens that can reliably suppress HIV replication to levels of <50 copies/ml and also suppress the emergence and replication of drug-resistant strains [23]. The availability of a limited number of regimens and the use of fixed-dose combinations supports adherence [24] and can also limit unnecessary regimen switching and selective drug taking [25]. Widespread

prescribing of inappropriate ART is seen more frequently in countries where many different ARV drugs are available, such as Mexico [26], than in countries following the public health approach with a limited number of regimens used in the public sector.

Despite earlier doubts, evidence is available that even in countries with very limited resources, ART programmes based on the public health approach have shown effectiveness equal to that seen in clinical cohorts in the USA and Europe using similar regimens [27–31]. A major challenge is to replicate these optimal outcomes in new ART sites as ART is expanded to approach universal access. Further challenges include training additional personnel, retaining currently trained staff (when more lucrative jobs may be offered in resource-rich countries or when they themselves may be HIV-infected [32]), and expansion of supervision, monitoring, laboratory services and drug delivery systems. In this context there are many potential sources of interruptions to treatment or suboptimal treatment, which can lead to insufficient drug pressure and drug resistance.

Patient factors facilitating non-adherence and resulting treatment interruptions are similar to those in rich countries [33]. However, programmatic barriers to continuous ART access play a greater role in non-continuous drug taking and preventable HIVDR emergence in resource-limited countries. Barriers include charges for treatment or drugs [27,34–37], as well as long distances to be travelled to ART sites and lack of affordable transport [33,38–41]. Interruption of ARV supplies at both site and country level also leads to ART interruptions [42,43]. Failure to pick up drugs on time, which may result from transport difficulties, illness, other obligations or lack of funds in programmes where payment is required, may result in NNRTI resistance [44,45], even if drugs are picked up as few as 48 h after previously dispensed drugs run out, because an NNRTI will persist at subtherapeutic levels in the human body longer than NRTIs leading to the equivalent of monotherapy. Temporary 'losses to follow-up' from ART programmes or ART stops may also be a source of resistance [36,46,47]; particularly if adherence was less than perfect beforehand, a lapse in clinic attendance of days to months may lead to NNRTI resistance for the same reason. In most resource-limited countries, when patients with interrupted treatment eventually return for ART, they are likely to be treated with the same first-line regimen to which they may have archived NNRTI resistance. These sources of drug resistance are to some extent preventable by the application of targeted resources within the existing public health approach in current programmes [48,49]. Maintaining continuity of treatment during human conflict and natural disasters is especially challenging, although careful planning and targeted resources have minimized interruptions in some settings [50].

In resource-limited countries, successfully treating patients for as long as possible on uninterrupted first-line ART, which is simpler to administer [51], associated with higher levels of adherence and less costly than second-line ART, is crucial to both prevention of resistance and supporting good health for individuals living with HIV [49]. Adverse events during a first-line regimen may sometimes trigger unnecessary switches to more complex second-line regimens where appropriate alternate first-line ARVs for substitution are unavailable because of cost [52].

Some aspects of the public health approach as currently implemented are associated with the emergence of additional resistance mutations during failing ART regimens. The most common first-line regimens in countries following the public health approach currently consist of stavudine or zidovudine plus lamivudine and a NNRTI [2]. Clinical or immunological determinations of ART failure in the absence of viral load monitoring are associated with unnecessary switches to second-line ART in the absence of virological failure, but also with the prolongation of a failing regimen and a 'late' switch to second-line [53–55]. Late switches in a first-line regimen that includes NRTIs are associated with the accumulation of thymidine analogue and other resistance mutations [20,54], which can cause cross-resistance to many, and possibly to all, NRTIs that might be used in a second-line regimen. This phenomenon may be less likely when tenofovir (TDF) is included in the first-line regimen [56], but TDF is still commonly unavailable or unaffordable [52]. Even where viral load testing is available, the patterns of resistance developed may severely limit NRTI options for second-line regimens [10]. Key drugs in WHO-recommended second-line regimens, including TDF, abacavir (ABC), and ritonavir-boosted PIs are still unavailable or unaffordable in many countries [9,57]. Many ART sites still have no second-line regimens for patients whose first-line regimens fail; the lack of second-line availability is a major source of preventable emergence of resistance.

There is little chance that salvage ART regimens (to be used after a second-line regimen has failed) including new drug classes will be widely available in resource-limited countries during the next 5 years, because of their cost and complexity and because there is little economic incentive for pharmaceutical companies to register them in resource-limited countries [1]. In March/April 2006, only 4% of individuals on ART in resource-limited countries were receiving second-line regimens [2], so lack of salvage regimens is currently unlikely to contribute substantially to resistance. However, eventually the necessity of maintaining

patients on a failing second-line regimen may also contribute to the emergence of resistance.

HIV drug resistance associated with the prevention of mother-to-child HIV transmission

NNRTI resistance mutations have been reported in 20–69% of women [58,59] who have received single-dose nevirapine (sd-NVP). Although suboptimal, for programmatic reasons sd-NVP is the most common ARV prophylaxis intervention used for prevention of mother-to-child transmission (PMTCT) of HIV in resource-limited countries because of its safety, efficacy, simplicity and low cost [60]. NNRTI mutations associated with sd-NVP, unlike those that appear with inadequate ART-associated drug pressure lasting weeks to months, are reported not to affect the outcome of ART provided it commences >6 months after PMTCT [59,61]. The short duration of sd-NVP exposure may limit the number of archived mutations in viral reservoirs [62]. Previous administration of sd-NVP is reported not to reduce its PMTCT efficacy in subsequent pregnancies [61]. Because relatively few eligible women are receiving PMTCT (11% in resource-limited countries as of December 2006 [2]), and because fewer than 20% of women who receive sd-NVP are immediately eligible for ART [60], PMTCT is currently unlikely to have an important effect on HIVDR at a population level. Increasingly, women eligible for ART during pregnancy are receiving potent three-drug ART [47]. For those not ART-eligible, PMTCT utilizing ARV combinations to minimize resistance is recommended [47], although greater complexity of administration and cost may slow expansion.

Cases of NNRTI resistance have been reported in infants whose mothers have received sd-NVP for PMTCT and has been associated with failure of NNRTI-based regimens subsequently used for paediatric ART [59,63]. However, because 85–95% of infants whose mothers receive sd-NVP do not become HIV-infected [64], and because only 11% of eligible pregnant women currently receive PMTCT in resource-limited countries, this is currently an infrequent cause of resistance in children on a population basis. Combination regimens recommended for PMTCT and to prevent post-partum transmission to breastfeeding infants are being scaled-up and should minimize resistance [47], but it is important to treat HIV-infected infants whose mothers received sd-NVP with appropriate regimens.

In resource-limited countries, ART failure and HIVDR emergence in children are also associated with inappropriate use of adult formulations where paediatric formulations are unavailable, with inappropriate dosing or poor absorption and with adherence problems [65]. New formulations have been prioritized and are beginning to be made available with more guidence on appropriate dosage for weight [7,47,66], but better strategies to reduce barriers to access and support adherence are still needed.

Transmission of HIV drug resistance

The prevalence of transmitted HIVDR depends on many factors, but one of the most important is ART use: that is, the extent to which ART is used in an area, how long it has been widely used and the numbers and percentages of those who are currently on a failing regimen [67–69]. In most resource-limited countries, 15–20% of HIV-infected individuals are estimated to be in need of ART [2] and only 28% of these were receiving ART as of December 2006; in 2003, only 2% of those in need were receiving ART in resource-limited countries [2]. On the basis of these figures, models predict transmitted HIVDR at a level of ≥5% is unlikely for many years in most of these countries [67–69]. As the majority of ART patients are starting on highly potent regimens [4], the rise of drug resistance transmission is likely to be delayed compared with resource-rich countries, where ART scale-up was initially implemented with resistance-associated monotherapy and dual therapy. The effect of maintaining individuals on a failing regimen in the absence of viral load testing may contribute to increased transmission of resistant HIV strains as ART becomes more widespread, although quantifying the extent of the contribution will require research and mathematical modelling. Residual activity and partial viral suppression is generally seen with a regimen to which resistance has developed [22,70,71], which could lower the risk of transmission. Also, resistant viruses may be less fit and thus less transmissible [22,51,72]. Success in limiting risk behaviour among ART patients will also decrease transmission of resistant strains. 'Prevention for positives' programmes that focus on reducing risky behaviour among patients in care, as well as supporting adherence, have demonstrated beneficial reduction in risk behaviours among ART patients in resource-limited countries, lowering the risk for HIV transmission including transmission of resistant strains [73–77].

Estimating transmitted resistance on the basis of baseline genotyping of patients eligible for ART is common in resource-rich countries. Currently, in resource-limited countries most individuals starting ART are likely to have been infected 10–12 years earlier when there was little or no ART in the country, or even longer. In resource-limited countries, age-related HIV disease progression is similar to that observed in resource-rich countries [78], so most individuals starting ART are likely to have been

infected at least 10–12 years earlier. Because ART starts at a significantly lower CD4+ T-cell count [79,80], time from infection to ART may be even longer. When individuals currently starting ART were infected with HIV, it is unlikely that transmission of resistant strains was occurring at a level detectable in surveys, because ART use was very limited before 2004 [2]. It is more likely that baseline resistance in reportedly drug-naive patients starting ART in resource-limited countries is due to undisclosed ARV experience than to transmitted resistance [54,81].

WHO strategy for prevention and assessment of HIV drug resistance

The WHO has brought together WHO HIVResNet, a global network of over 50 institutions, laboratories, clinicians, epidemiologists and other HIVDR experts to support HIVDR prevention and assessment, capacity building and data analysis. Standardized assessment tools have been developed and implemented in resource-limited settings.

At the global level, WHO and HIVResNet will perform analyses to inform evidence-based recommendations on ART regimens and implentation of programmes to limit HIVDR. Data from the implementation of national surveys and indicators will be analysed centrally as more results accumulate. Results from clinical trials, ART cohorts, molecular epidemiology research and other studies are being collated and used in relevant mathematical models. HIVResNet experts also contribute to a variety of WHO and other international organizations' working groups to develop guidelines for ART regimens and regimen cycling, the determination of time to switch regimens, monitoring and evaluation, HIV transmission prophylaxis and prevention, and prioritizing ARV drug formulations to be made available to developing countries.

Specific methods are described in the following sections. Some elements of the methodology are more fully described in other articles in this supplement [82–85].

National HIVDR prevention and assessment strategies for countries scaling up ART

A national HIVDR prevention and assessment strategy should ideally be integrated into national HIV care, treatment and prevention planning. Standardized routine minimum-resource assessments and surveys provide information to support optimal use of available ARVs to minimize ART interruptions and to guide population-based selection of ART regimens. The emphasis is on strengthening aspects of existing ART programmes that will minimize HIVDR, including support for adherence and follow-up, removal of barriers to ART access, and drug supply continuity at the individual and the ART site level. The WHO supports local capacity building to perform these assessments and then to act on them. The strategy does not seek to duplicate already existing programmes to support adherence, drug supply continuity and other HIVDR-related issues or ongoing research projects, but to strengthen them and to emphasize their importance in limiting HIVDR.

Formation of a national HIV drug resistance working group

WHO recommends that the Ministry of Health/National AIDS Council in each country establish an HIVDR working group to develop a country-specific HIVDR prevention, surveillance and monitoring plan, and make evidence-based recommendations for HIVDR prevention. The group generally includes epidemiologists, clinicians and laboratorians with expertise in HIV surveillance, HIV prevention, ART and related laboratory issues. Inclusion of national and international research scientists performing HIVDR-related research in the country, to increase their awareness of and potential contributions to the strategy, is important.

Among the main tasks of the working group is the collection of indicators and the implementation of surveys described below, and the collation of any additional HIVDR-related information from projects or research being performed in the country. The working group plans further evaluations to clarify issues raised by survey results and to address other country-specific issues.

HIV drug resistance 'early warning indicators'

The WHO recommends use of information collected routinely in medical and pharmacy records to monitor the functioning of ART sites for factors potentially associated with HIVRDR prevention or emergence (Box 1). Laboratory assessments are not needed to predict the emergence of preventable resistance where inappropriate prescribing practices, treatment interruptions and ARV supply shortages are occurring at unacceptable levels. ART early warning indicators (EWI) are monitored either at all ART sites in the country or at representative sites. The most important indicators monitor the extent to which prescribing practices meet national and international guidelines; the percentages of patients still on first-line and lost to follow-up, respectively, 12 months after ART start; the percentages of patients picking up ARVs before their previous prescriptions run out and keeping appointments regularly, respectively; and ARV drug supply continuity at the site. The WHO recommends targets for the indicators; more stringent targets may be set by individual countries.

Plans for these assessments should be integrated with the country-level monitoring and evaluation system, in collaboration with major institutions working within the country to support and monitor ART. Implementation is facilitated in countries where records at the majority of ART sites reflect the international agreement on standard minimum dataset for ART records [86,87], but the WHO HIVDR team has collaborated with governments and other organizations providing ART to develop methods of extracting EWI from a variety of electronic and paper-based systems.

Recommendations for quick action either at the site level or, if many sites do not meet targets, at the national ART programme level, are based on results. These may include increased training and resources for specific aspects of care and provision of targeted support for adherence and reduction of barriers to continuous ARV access [49,88,89]. Additional research to clarify the source of problems and the support required to address them may also be recommended.

Surveys to monitor HIV drug resistance prevention and associated factors in sentinel antiretroviral treatment sites

Details on the HIVDR prevention at sentinel ART site can be found in a separate article in this supplement

Box 1. WHO HIV drug resistance early warning indicators*

Prescribing practices: target 100% of regimens prescribed are congruent with national or international guidelines

- Percentage of patients starting first-line ART over a selected time period who are prescribed or pick up initial ARV regimens that meet national or WHO guidelines.
- Percentage of all ART patients who during a specified time period are prescribed or pick up an ARV regimen meeting national or WHO guidelines.

Percentage of patients lost to follow-up in first year of ART: target <20%

- Percentage of patients starting ART during a specified time period who, 90 days from their last scheduled appointment within the first 12 months of care, have not returned to the clinic or pharmacy and who are not known to have transferred out or died.

Percentage of persons starting first-line antiretroviral treatment who are still on a recommended first-line ART regimen 12 months after ART start: target ≥70%

- Percentage of patients initiating ART at site during a selected time period who are taking a first-line ART regimen that meets national or WHO guidelines 12 months after ART start.

On-time antiretroviral drug pickup: target ≥90%

- Percentage of ART patients who pick up all their prescribed ARV drugs on time (that is, before previously dispensed drugs run out) for two consecutive drug pick-ups following a pick-up in a selected month.
- Percentage of patients starting ART during a specified time period who, over the course of a year, pick up all ARV drugs before previously dispensed ARVs run out.

On-time appointment keeping: target >80%

- Percentage of ART patients who, following an appointment attended in a selected month, attend their next two appointments within 7 days of the expected or schedule dates.
- Percentage of patients starting ART during a selected time period who attend all their appointments within 7 days of the expected or scheduled dates during the first year.

ARV stock-outs and shortages: target 0%

- Percentage of patients on first-line ART, whose regimen was stopped, modified, or incompletely dispensed at the pharmacy due to ARV stock-outs or shortages during a designated year.
- Percentage of patients initiating ART at the site during a selected time period, whose regimen was stopped, modified or incompletely dispensed at the pharmacy during the first 12 months of ART due to ARV stock-outs or shortages.
- Number of months with any ARV supply stock-outs or shortages during one year.

Standardized adherence measures: target >90% (monitored only in countries where pill counts or standardized adherence measurements are routinely performed and recorded)†

- Percentage of ART patients who demonstrate >90% adherence by pill count of each drug in their regimen at each appointment or drug pick-up during the course of a selected time period.
- Percentage of ART patients who demonstrate >90% adherence using a routinely administered standard assessment such as the visual analogue scale at each appointment during the course of a selected time period.

Viral load at 12 months: target >70% undetectable (monitored only in countries where viral load testing is performed routinely at 12 months)†

- Percentage of patients starting ART during a specified time period who, at 12 months, had an HIV RNA level of less than the detection limit of the test used.

*HIV drug resistance early warning indicators (EWI) are collected either at all antiretroviral treatment sites in the country, or in a representative sample of sites, and are reported separately for each site. Countries collect only the EWIs that can be abstracted from current medical records. Sites unable to meet multiple targets may require increased resources, training or targeted support. If many sites fail to meet a target, action at the national antiretroviral therapy (ART) programme level may be indicated. Additional investigations may be required to identify sources of problems and the actions required. †Optional indicators. ARV, antiretroviral.

[84]. The surveys estimate the proportion of patients starting ART at each site who achieve HIVDR prevention (defined by viral load suppression) at 12 months after ART start. The surveys also identify specific HIVDR mutations and mutation patterns if resistance emerges and evaluates patient and site factors potentially associated with the prevention or emergence of HIVDR. WHO recommends sentinel ART sites be selected to represent the main clinic types in the country in terms of factors such as geography, resources and staff available, populations treated and size of the population in treatment and regimens used. The cohort-based assessment methodology is designed to be incorporated into the routine functioning of ART sites, with minimum data collection and genotyping of remnant specimens collected for routine clinical purposes. The main outcome measure, prevention of HIVDR, is supplemented by HIVDR genotyping and patient information. A separate protocol has been developed for paediatric ART programmes.

The assessment focuses on a cohort of 99–129 patients consecutively beginning first-line ART during a selected time period in each site. It includes a baseline sequence of the relevant regions of the HIV *pol* gene to assess HIV-1 subtype and HIVDR, collection of demographic and clinical data and a brief history of ARV use. Viral load (VL) testing is performed at 12 months for individuals still on first-line ART (including substitutions) or prior to a switch to a second-line regimen before 12 months; if the VL is detectable, HIVDR genotyping is performed. Surveys are performed for a minimum of 12 months after the enrolment of the last participant; where resources permit, the cohort may be followed up to 72 months, with annual VL and genotyping. At each site, an analysis will be performed on the associations between HIVDR prevention or emergence and key factors such as ARV history, baseline drug resistance mutations, ARV drugs prescribed, timeliness of ARV drug pick ups and clinic appointment-keeping, adherence and clinical variables.

ART site factors are recorded at survey initiation, anually and at survey end; these include the site's standard ARV regimens, support for adherence and follow-up at the site, provider-to-patient ratio, ARV continuity and barriers to continous ARV access for patients (for example, costs, transport and waiting times). The site factors, and other issues such as the association among patterns of resistance mutations arising and HIV-1 subtype, will be analysed in multisite analyses. Results are expected to support recommendations for specific actions to improve outcomes at sentinel clinics and potentially at other sites. Patterns of baseline HIVDR and HIVDR accompanying virological failure may contribute to recommendations for first- and second-line regimens nationally and internationally. The surveys might also provide information on accumulation of mutations where clinical and immunological determinants are used to define time to switch. Results will be used to guide planning of more detailed studies within each country.

Countries plan a rolling cycle of surveys at 9–30 representative sites, performing surveys in 3–10 sites annually for 3 years; in the fourth year, the cycle starts again in the year 1 sites.

Surveillance of transmitted HIVDR in recently infected individuals

Details on the WHO methods and the statistical background for the methods for surveillance of transmitted HIVDR can be found in two articles in this supplement [83,85]. WHO recommends a minimum-resource strategy to assess transmitted HIVDR in specific geographic areas of resource-limited countries where transmitted HIVDR is likely to be seen first (that is, in specific geographic areas where ART has been widely available for ≥3 years). If HIVDR transmission is low in such areas, it is unlikely to be higher in other areas. Modelling suggests that in the initial years of ART scale-up, HIVDR transmission is likely to be minimal [69,90]. Using a variation on methods designed to survey other health conditions using small sample sizes in limited geographic areas, specimens are collected and sequenced from individuals consecutively diagnosed with HIV. The surveys are called HIVDR threshold surveys (HIVDR-TS), because results categorize transmitted resistance to relevant ARV drugs and drug classes as above or below two thresholds (5% and 15%).

Site selection and individual eligibility criteria are designed to minimize the likelihood that individuals included will have been infected before ART was widely available in the country or will be ARV-experienced. Where possible, the surveys use remnant specimens and data from regularly performed serosurveys to estimate HIV prevalence, which are already in place in most resource-limited countries [91].

Results will contribute to ART policy decisions, including ART regimen and HIV prophylaxis guidelines. Specific public health actions are recommended on the basis of the prevalence category for resistance to the drugs and drug classes.

Designation of one or more genotyping laboratories for HIVDR surveillance and monitoring

Each national HIVDR working group is asked to designate one or more genotyping laboratories accredited by the WHO Global HIVDR Laboratory Network, described in more detail elsewhere in this supplement [82], to provide quality-assured sequencing for the surveys described above.

Genotyping for countries without genotyping facilities or where designated in-country laboratories are moving towards accreditation is performed in regional and specialist laboratories accredited by the Network. Designated national laboratories that do not meet all accreditation criteria are provided technical support and training by accredited laboratories within the Network.

The national HIV drug resistance database

The WHO recommends maintaining a national database to hold the HIV sequences collected through surveillance, monitoring, research projects and other sources for HIVDR-related analyses. A simple database application that can be used to collect data at the country-level and to transfer a subset of data to the regional- and central-levels is provided by the WHO in collaboration with HIVResNet colleagues at the US Centers for Disease Control and Prevention (CDC) and Brown University. Standardized clinical and demographic information should accompany each sequence. As well as supporting specific analyses planned for the surveys, the data collected will support additional analyses on HIVDR and HIV genetic diversity within the country.

Annual reports and recommendations

Each national HIVDR working group should produce an annual report summarizing results from HIVDR EWI, HIVDR monitoring in sentinel HIVDR sites and HIVDR-TS. The report should also summarize other relevant HIVDR-related research performed in the country. Lastly, the report should include information on programmes that are not the working group's direct responsibility, but that are likely to affect HIVDR within the country, including adherence support and follow-up programmes, 'prevention for positives' programmes in ART sites, monitoring of and support for continuity of ARV supplies, success in acquiring cheaper and more effective ARVs for country use and other crucial issues.

Recommendations should be made based on the implications of the data collected for continued support of current well-functioning strategies, and for strengthening of elements of prevention and care programmes that are not functioning optimally to minimize drug resistance.

The WHO global HIV drug resistance strategy

The implementation of the strategy at the global level focuses on the development of standardized tools and the collection and analysis of information to support actions and policies that minimize the emergence and transmission of HIVDR. More detail on operation of the strategy at the global level can be found in Box 2.

Resources are provided for the global HIVDR strategy by WHO and other major international institutions and through major grants from the William and Melinda Gates Foundation, and from the Spanish, Canadian, Italian and US governments. Positions are supported both at WHO headquarters and in WHO regions for epidemiologists, clinicians and virologists to develop and support the strategy. Additional guidance and technical assistance is provided by HIVResNet. In addition to members at large, HIVResNet includes two implementation networks: the Global HIVDR Surveillance and Monitoring Network and the WHO Global HIVDR Laboratory Network. The Global HIVDR Surveillance and Monitoring Network is made up of countries and WHO regions implementing the WHO HIVDR strategy and reporting their data to WHO, together with affiliated institutions and individuals actively supporting implementation in at least one country. The WHO Global HIVDR Laboratory Network provides quality-assured genotyping results for HIVDR surveillance and monitoring performed under national HIVDR strategies. The latter network is described fully in an article in this supplement [82]; its functions and the three categories of membership (national, regional and specialist) are summarized in Box 2. To enter or remain in the network, all laboratories must meet accreditation criteria and be re-assessed annually. Requirements include review of standard operating procedures, experience and staff qualifications, on-site laboratory assessments, successful genotyping of an annual proficiency panel and submission of sequences for quality checks within the database. Additionally, national laboratories are required to be designated by their national governments to provide genotyping for HIVDR surveys, regional laboratories are assessed for training capacity, and specialist laboratories are assessed for their particular specialist function.

Current status of the WHO HIVDR strategy

Support for implementation of the strategy in African, Asian, Caribbean, Eastern European and Latin American countries has been provided directly to national HIVDR working groups by the WHO, the Global Fund to Fight AIDS, tuberculosis and malaria, the US CDC, the President's Emergency Plan for AIDS Relief, the Atomic Energy Commission, UNAIDS, national governments and Treat Asia, Pharmaccess, ANRS and other academic and activist non-governmental organizations (NGOs). Requests for technical assistance implementing the HIVDR strategy have been received by WHO and its HIV ResNet partners from >40 countries. Regional training and planning meetings have been held in Asia, Africa, the Caribbean, Latin America and the Middle East for 35 countries. National HIVDR working groups have been set up in 13 African countries (Botswana, Burkina Faso, Burundi, Ethiopia, Kenya, Malawi, Mozambique, Nigeria, Rwanda,

Box 2. Global implementation of the WHO HIVDR strategy

Objectives

- To develop standardized tools that support a public health approach to HIVDR surveillance and monitoring.
- To provide training and technical assistance to countries and regions in implementing HIVDR prevention and assessment plans, including quality-assured sequencing for HIVDR surveillance and monitoring, interpretation of results and planning actions on the basis of the results.
- To collate information from WHO-supported studies and other researches to inform HIV care and prevention guideline policies globally.
- To support research and initiatives that will limit HIVDR.
  - Development and promulgation of cheaper, more feasible HIV-associated laboratory tests.
  - Recommendations to guide the public health approach to HIV care and prevention, to prioritize development of needed ARV drug formulations and to support wider availability and lower prices of ARV drugs.
  - Standardization of a basic minimum dataset for HIV care and harmonization of international indicators.
  - Strengthening ART programmes to support adherence and remove barriers to ART continuity.
  - Implementation of pharmacovigilance and strategies to minimize adverse ART-associated events.

Activities and working groups of the global HIVDR surveillance and monitoring network

- Activities of the global HIVDR surveillance and monitoring network.
  - Development and regular updating of normative guidance, database applications and protocols.
  - Provision of technical assistance and training to countries and regions.
  - Collection and analysis of data.
  - Mathematical modelling to address key questions in HIVDR emergence and transmission.
  - Provision of evidence-based recommendations for guidelines and policy development.
- Working groups
  - Epidemiology/clinical working group (protocols and tools, data analyses and identification of important clinical and research issues).
  - Surveillance mutations working group.
  - Mathematical modelling working group (HIVDR transmission models, re-examination of the thresholds for transmitted resistance, models to inform optimal determination of ART failure/switch to second-line in resource-limited settings, optimal cycling of regimens [ARV classes] given currently available drugs).
  - Regional and subregional working groups (facilitate communication, multisite analyses, regional training and sharing of lessons learned within countries).

Activities of and laboratory categories within the WHO global HIVDR laboratory network

- Provision of quality-assured sequences for HIVDR surveillance and monitoring.
  - Development of proficiency panels, normative guidance and training materials.
  - Training and capacity-building for genotyping.
  - Assessment of laboratories for accreditation within the network.
  - Operational research on simple, low-cost specimen collection methods and genotyping procedures to support the WHO HIVDR strategy – current focus on dried blood spots for genotyping and proficiency panels.
- Laboratory categories
  - National laboratories – designated by the national government and accredited by WHO to provide quality-assured sequences for HIVDR surveys.
  - Regional laboratories – provide training and quality-assured sequences for HIVDR surveys for countries in the region.
  - Specialized laboratories – provide support to regional and country laboratories and serve additional specialist functions including assessments, development of proficiency panels, normative guidance, training materials and operational research.

ART, antiretroviral therapy; ARV, antiretroviral; HIVDR, HIV drug resistance; WHO, World Health Organisation.

Swaziland, Tanzania, Uganda, Zambia and Zimbabwe), in six Asian countries (Cambodia, China, India, Indonesia, Thailand and Vietnam) and in Haiti and the Ukraine. Twenty-two countries have planned national strategies; 16 of these have implemented at least one element of the strategy.

Additional countries will be prioritized for 2008 and 2009; some of these, including South Africa [90], have already implemented aspects of the strategy.

A report of Malawi's HIVDR EWI pilot is included in this supplement [86]; countries that have piloted or are in the process of piloting their EWI include Botswana, Cambodia, Ethiopia, Haiti, Mozambique, Swaziland, Vietnam, Uganda, Zambia and Zimbabwe. HIVDR prevention surveys in sentinel ART sites are being implemented in Burundi, Cambodia, Ethiopia, Haiti, Indonesia, Malawi, Mozambique, Nigeria, Swaziland, Vietnam and Zambia. The Dutch-supported NGO AIDS Fonds is also supporting cohort surveys in which the WHO methodology is embedded, and for which results will be shared with national HIVDR working groups, in additional sites in Ethiopia, Uganda, Zambia, South Africa and Zimbabwe through the NGO Pharmacess, and in Cambodia, China, India, Malaysia, Singapore and Thailand through Treat Asia.

As of December 2007, HIVDR threshold surveys have been implemented in 16 African, Asian and Eastern European countries, seven of which (Ethiopia

[93], Malawi [94], South Africa [89], Swaziland [95], Tanzania [96], Thailand [97] and Vietnam [98]) report results in this supplement. These articles report that transmitted resistance to all relevant drugs and drug classes is <5% in the areas of these countries where ART has been available for the longest time period. Results also confirm that use of remnant HIV serosurvey and diagnostic specimens for HIVDR serosurveys is feasible, although specimen handling procedures to optimize amplification for genotyping must be improved. Six countries (five of the above countries and Botswana) also presented their findings at international conferences in 2007 (the *14th Conference on Retroviruses and Opportunistic Infections* [99–101] the *1st HIV/AIDS Implementers' Conference* [102–104] and the *4th International AIDS Society Conference on HIV Pathogenesis, Treatment and Prevention* [105]).

At the global level, protocols and other standardized tools have been developed both by the HIVDR Surveillance and Monitoring Network and the HIVDR Laboratory Network. Implementation of the surveillance and monitoring protocols at a national level has been described above; the mutations group has also produced and performed the first annual reassessment of a list of mutations for HIVDR transmission surveillance [106,107]. The laboratory network has produced checklists for laboratory assessments and guidance for laboratories, and is actively investigating the use of dried blood spots for HIVDR surveillance and monitoring. As of September 2007, assessments had been conducted in 32 laboratories in Africa, Asia, Europe, North America and the Caribbean; additional assessments are planned for 2008. The first proficiency panel was distributed in 2007 and results returned; the second was distributed in early 2008.

WHO HIVResNet experts have contributed to deliberations on formulations and ARV drugs to be prioritized for expanded availability, updates to ART guidelines and a standardized approach to pharmacovigilence. The mathematical modelling working group is currently focusing on the determination of ART failure and when to switch to second-line ART in resource-limited settings, optimum cycling of regimens for countries on the basis of ARV drugs currently available, predicting the extent of transmitted HIVDR in countries using ART coverage data and other parameters, and evaluation of the current drug resistance thresholds for surveillance of transmitted HIVDR.

The WHO HIVDR strategy will continue to be adapted as more experience is gained, as gaps in the international research agenda are identified and as data are collected and utilized for evidence-based recommendations and data collection methods are evaluated. Global reports of HIVDR data collected using WHO-recommended methods will be produced starting in 2008; national and regional reports from the network will also be available on the website. The WHO will work to create opportunities for countries to analyse and publish their data in peer-reviewed journals, of which this supplement is the first example. HIV drug resistance prevention and assessment strategies have been integrated into the HIV care and treament plans of many countries; the WHO HIV drug resistance team and our HIVResNet partners will continue to support the development and implementation of these strategies.

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The authors declare that they have no competing interests.

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