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Chapter 2

The 2008 status of HIV-1 resistance to antiretroviral drugs in sub-Saharan Africa

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ABSTRACT

Access to combination antiretroviral therapy (ART) for persons infected with HIV in sub-Saharan Africa has greatly improved over the past few years. However, data on long-term clinical outcomes of Africans receiving ART, patterns of HIV resistance to antiretroviral drugs and implications of HIV type-1 (HIV-1) subtype diversity in Africa for resistance, are limited. In resource-limited settings, concerns have been raised that deficiencies in health systems could create the conditions for accelerated development of resistance. Coordinated surveillance systems are being established to assess the emergence of resistance and the factors associated with resistance development, and to create the possibility for adjusting treatment guidelines as necessary. The purpose of this report is to review the literature on HIV-1 resistance to antiretroviral drugs in sub-Saharan Africa, in relation to the drug regimens used in Africa, HIV-1 subtype diversity and overall prevalence of resistance. The report focuses on resistance associated with treatment, prevention of mother-to-child transmission and transmitted resistance. It also outlines priorities for public health action and research.

INTRODUCTION

The use of combination antiretroviral therapy (ART) in individuals infected with HIV type-1 (HIV-1) has effectively reduced morbidity and mortality in the industrialized world [1]. The implementation of ART in developing countries with a high HIV prevalence, including the hardest-hit area of sub-Saharan Africa (herein referred to as Africa), is a global public health priority [2]. The number of HIV-infected persons in Africa who have access to ART is estimated to have increased 10-fold over the past 3 years. By December 2006, it was estimated that more than 1.3 million Africans had received ART, reaching 28% of those in need, yet leaving over 70% without access [3].

Deficiencies in health systems and resources, such as unreliable supply systems, shortage of staff and lack of virological monitoring, could create the conditions for accelerated development of HIV-1 resistance to antiretroviral drugs. Patients receiving antiretroviral drugs could acquire resistance, and subsequently transmit resistant viruses to newly infected persons. High rates of resistance could eventually compromise the effectiveness of antiretrovirals in the general population [4–6]. Moreover, the high diversity of HIV-1 non-B subtypes prevalent in Africa [7] might have implications for the patterns of resistance development.

The purpose of this report is to review the literature that describes HIV-1 resistance to antiretroviral drugs in Africa, in relation to the drug regimens used in Africa, HIV-1 subtype diversity and overall prevalence of resistance. The report focuses on resistance associated with treatment, prevention of mother-to-child transmission (pMTCT) and transmitted resistance, and outlines priorities for public health action and research.

METHODS

To identify eligible studies, a systematic search of the English language literature published before 2008 was conducted. The search included the Medline database, relevant treatment guidelines, the World Health Organization (WHO) website and abstracts presented at international conferences. The search strategy combined the terms 'antiretroviral therapy', 'public health', 'drug resistance', 'surveillance', 'HIV-1 subtype diversity' and 'sub-Saharan Africa'.

RESULTS

Principles of resistance and WHO treatment guidelines

Principles of resistance

The viral replication process of HIV-1 is exceedingly error-prone, leading to a high mutation frequency [8, 9]. In combination with a rapid viral turnover [10, 11], this results in a pool of genetically related but distinct viruses, called quasi-species, within each infected individual. The most frequently used antiretroviral drugs target the replication enzymes reverse transcriptase (RT) and protease (PR), which are encoded by the HIV-1 polymerase (*pol*) gene. Virus variants that have mutations at specific positions of nucleic acid in *pol* could be selected by drug selective pressure, leading to reduced susceptibility, or resistance, to that particular drug. Selection of resistant viruses occurs in the context of incomplete suppression of viral replication when optimal drug levels are not maintained, either through poor adherence, treatment interruptions or the use of suboptimal drug combinations (acquired resistance). For instance, single-dose nevirapine (SD-NVP), which is commonly used for pMTCT in HIV-infected pregnant women in Africa, is a non-suppressive regimen. A second method of acquiring resistance is via transmission of a resistant strain to a newly infected person (primary resistance). Virus variants harbouring resistance might replicate less efficiently than wild-type virus strains. In the absence of drug selective pressure, resistant viruses might be rapidly outgrown by wild-type virus strains which are fitter. As such, the mutant virus becomes undetectable in the plasma virus populations, but will still be archived in the proviral DNA population of HIV-1-infected cells, re-emerging only if drugs to which they are resistant are restarted [12]. Each antiretroviral drug or drug combination has its own resistance profile, which could be specific to the drug or could express cross-resistance to other drugs within the same class [13]. Drugs with a high genetic barrier, such as zidovudine (ZDV) and most protease inhibitors (PIs), require the accumulation of multiple mutations to overcome antiviral drug activity. On the other hand, drugs with a low genetic barrier, including lamivudine (3TC) and non-nucleoside reverse transcriptase inhibitors (NNRTIs), only require a single point mutation to confer high-level resistance.

WHO treatment guidelines

In view of the public health benefits of accelerating access to ART in resource-limited countries, the WHO has developed a public health approach to treatment based on standardized, simplified guidelines and a decentralized service delivery [14, 15]. In the absence of specialist physicians and extensive virological patient monitoring, which is the standard care model in industrialized countries, the public health model enables healthcare workers with minimum training to deliver care to large numbers of patients.

Clinical decision-making is guided by clinical observation, WHO clinical staging and, if available, haematology, biochemistry and CD4+ T-cell counts.

The standard ART regimens used in Africa are based on relatively inexpensive drugs, which are produced generically in large quantities and are often available in a fixed-dose combination. WHO guidelines include a standard first-line regimen consisting of either two nucleos(t)ide reverse transcriptase inhibitors (NRTIs) plus an NNRTI or a triple NRTI regimen, and a second-line regimen consisting of a boosted PI with at least one NRTI [14]. The most frequently used first-line regimen consists of the dual NRTI backbone (3TC and either ZDV or stavudine [d4T]) plus an NNRTI (either NVP or efavirenz [EFV]). ZDV and d4T are thymidine analogue drugs and both select for a common set of mutations called thymidine analogue mutations (TAMs). Accumulated TAMs induce cross-resistance to other NRTIs. Both 3TC and NNRTIs have a low genetic barrier, presenting a potential vulnerability of the current standard first-line therapy. Because of high costs, the availability of PIs in Africa has been limited, reserving them for second-line therapy only. Given that fewer regimens are available in resource-limited countries, it is of particular importance to minimize resistance.

HIV-1 subtype diversity and resistance

HIV-1 subtypes

HIV-1 has been divided into three distinct genetic groups: M, N and O [16]. Whereas groups N and O represent a small minority of HIV-1 infections in central Africa [17, 18], group M is responsible for over 90% of HIV-1 infections globally, comprising nine subtypes (A–D, F–H, J and K) [19] and a number of circulating recombinant forms (CRFs) [20, 21]. Subtype B is still the predominant subtype in Europe, North America and Australia, but is hardly found on the African continent, where all other (non-B) subtypes are represented with a distinct geographic distribution [7]. In Africa, subtype C is responsible for 56% of infections, mainly in the south and east, whereas smaller proportions of infections are caused by subtypes A (14%), G (10%), CRF02_AG (7%) and other recombinants (9%) [7].

Antiretroviral drugs that are currently available were developed on the basis of their activity to primarily inhibit the replication of subtype B viruses. As a result, scientific data on patterns of resistance and clinical outcome of ART is largely limited to this subtype. Preliminary data suggests that short-term immunological and virological outcomes on ART are similar for Africans compared with their Western counterparts [22, 23]. However, these results have been obtained with a limited set of first-line regimens and long-term outcome data is not yet available.

Nucleotide differences between subtypes could have an effect on the spectrum of amino acid substitutions resulting from point mutations, which in turn might influence the biochemical and biophysical microenvironment in the PR and RT *pol* gene regions [24–26]. As a result, intersubtype differences in the genes targeted by antiretroviral drugs could influence their primary drug susceptibility, the propensity to develop resistance and the spectrum of mutations that emerge during drug selective pressure, either as a consequence of the nucleotide composition at baseline or by the emergence of specific mutations during therapy.

Natural polymorphisms

Certain naturally occurring genetic variations, called polymorphisms, are frequently found in untreated populations infected with a non-B subtype of HIV-1. Analyses of drug-naive virus isolates of various non-B subtypes have shown that 53% and 48% of PR and RT positions, respectively, are naturally polymorphic, as compared to subtype B [27, 28]. In subtype B, some polymorphisms at specific amino acid residues (including PR positions 10, 20, 36, 63, 71, 77 and 93 and RT positions 69, 75, 98, 106, 118, 179 and 214) are known to be associated with resistance [27, 28]. The extent to which the abundance of polymorphisms in the non-B subtypes alters PR and RT function, drug susceptibility or clinical response to therapy is still unclear. For instance, the naturally occurring Y181C and Y181I genotypes in HIV-1 group O and HIV-2 render these viruses resistant to all NNRTIs [29, 30]. Some polymorphisms, such as the frequently occurring M36I in PR, could restore or support the replication capacity of resistant virus thereby facilitating the emergence of resistance under drug pressure [31]. Other data on the possible clinical consequences of inter-subtype differences in polymorphisms are inconclusive [32–37].

Mutational pathways

The most common resistance mutations reported in studies conducted in Africa are M184V and K103N, and are a consequence of the widespread use of 3TC and NNRTIs, respectively, as part of the standard first-line therapy. Both mutations also occur frequently in subtype B viruses. Indeed, there is currently no evidence that non-B viruses develop resistance by 'new' mutations, that is, at positions that have not been associated with resistance in subtype B viruses [26]. Although limited, the available data provides reassurance that, for the most part, the various subtypes share common mutational pathways of resistance. Moreover, a recent analysis concluded that the overall genetic barrier to resistance was similar for the various HIV-1 subtypes [38]. However, some subtype-related mutational pathways have been reported that might have implications for the African context. For instance, tenofovir (TDF) might select the K65R mutation more rapidly in subtype C compared with subtype B [39, 40]. In light of increasing and recommended use of TDF as part of first-line therapy in Africa, this finding could have

implications for therapy effectiveness. Also, several studies have demonstrated inter-subtype differences in frequency and long-term persistence of resistance mutations in women and infants after the use of SD-NVP for pMTCT [41–44]. Finally, *in vitro* EFV rapidly selects the V106M mutation in subtype C, as opposed to the Y181C mutation in subtype B [45]. This is explained by an inter-subtype difference in the genetic barrier to resistance: the wild-type V106A needs two nucleotide changes in subtype B, as opposed to only one in subtype C. Further investigations are warranted to identify additional inter-subtype differences in mutational pathways, to ascertain whether these are caused by the genetic differences between subtypes or are a result of other variations, such as differences in patient monitoring and therapy-switching policies, and to evaluate their effect on clinical outcome.

Genotypic algorithm interpretation

For the clinical interpretation of genotypic resistance data algorithms, such as those from Stanford, REGA and ANRS, are used which apply certain rules to determine the presence of mutations, and subsequently predict their effect on drug activity. However, algorithms used at present are mainly based on subtype B data. As a result, in non-B subtypes their reliability might be limited, as they do not take into account any possible inter-subtype differences in drug susceptibility and resistance evolution outcomes of known and new mutations [46, 47].

Treatment-associated resistance

Available data

Twelve studies reported rates of resistance among patients receiving ART in Africa. The studies were conducted in Uganda, Senegal, Zimbabwe, Rwanda, Cameroon, Botswana, Côte d'Ivoire and Tanzania [40, 48–58] (figure 1, table 1). Observational data from patients on a first-line ART regimen show large variations in the rate of resistance, reported at 3.7%–49% after 24–163 weeks on ART [40, 48–58]. Earlier studies showed that the use of non-suppressive regimens (mono or bi-therapy) with inappropriate therapeutic monitoring rapidly led to high levels of resistance [56, 59, 60]. However, comparison of study results is difficult because of dissimilarities in drug regimens used, previous use of antiretroviral drugs, duration of follow-up and HIV-1 subtypes. Overall, the reported resistance rates do not appear to exceed rates reported in industrialized countries, where the prevalence of resistance mutations has been estimated at 9% in patients after 2 years on ART, rising to 27% by 6 years [61]. Resistance outcome data on second-line regimens in Africa is virtually non-existent [40, 58]. A cohort study in Côte d'Ivoire has been the first to report data on clinical and immunological outcomes in African patients who are resistant. In patients who had a major resistance mutation by a median of 37

Figure 1. Map of sub-Saharan Africa showing the geographical location of studies that were included in the literature review.
Each symbol represents one study. Studies are subdivided by acquired resistance in patients receiving ART (stars), transmitted resistance (circles) and nevirapine resistance after single-dose nevirapine (squares).

months on ART, subsequent 20-month clinical and immunological outcomes were compromised when compared with patients who had no resistance [55]. Even less data is available on the prevalence of resistance in African children on ART and their clinical outcome [62–64]. Due to the success of pMTCT in industrialized countries, the bulk of this data will have to be generated in developing countries.

Contributing factors: inadequate health systems

After years of inadequate administration, insufficient funding and brain-draining, health systems in many African countries feature poorly functioning medical facilities and unreliable supply systems. Breakdowns in health systems create the conditions for accelerated resistance. Factors that most directly affect resistance arise from weak regulation, poor supply chain management (for example, for drugs and laboratory reagents), inadequate equipment maintenance arrangements, a lack of knowledge and training among providers and inadequate monitoring and control systems in hospitals and other care facilities [65, 66]. Moreover, Africa is facing a human resource crisis with serious

shortages of nurses and doctors, a problem that has been aggravated by the high rate of HIV infection among healthcare providers [67]. Ultimately, these weaknesses affect adherence to treatment regimens and quality of care, which are key factors in the prevention and containment of drug resistance.

Contributing factors: patient adherence to therapy

Meticulous adherence to therapy is considered the most important factor in the prevention of resistance [68–70]. Although the widespread introduction of fixed-dose combination drugs in developing countries has greatly simplified ART regimens, there are important sociocultural and environmental factors that pose barriers to the ability of patients to adhere to treatment. These also include the cost of regular transportation to the clinic and the challenge to afford the food needed to take with medicines. Several studies have reported poorer rates of patient retention and viral suppression, and higher mortality for fee-paying patients compared with patients who received their medication free of charge [22, 23, 71]. The risk of resistance development could be reduced by enhancing treatment adherence through uninterrupted drug supply and the provision of medical services, including medication and laboratory tests, at no or low cost. To eliminate barriers to adherence, adherence support and patient education by dedicated counselors should be emphasized [72]. There is a need for novel affordable methods to promote adherence specifically tailored to the sociocultural context of African adult and paediatric patients.

Contributing factors: prescribing patterns

Additional challenges to minimizing resistance include misdiagnosis, poor prescribing practices resulting from lack of training, sub-therapeutic dosage and the distribution of substandard drugs [66]. The availability of adequate second-line drug combinations is limited, leaving patients dependent on suboptimal drug combinations after failing the first-line therapy. The strong long-term side effects of some of the frequently used drugs, such as d4T, could negatively affect adherence, thus promoting resistance. Concomitant use of particular tuberculostatic agents (such as rifampin) could affect the blood levels of antiretroviral drugs such as PIs and EFV [73]. This is particularly relevant in view of the high rates of tuberculosis co-infection in Africa. Moreover, there is insufficient knowledge on potential interactions with other drugs.

Contributing factors: access to virological monitoring

There is insufficient laboratory capacity and financial resources in Africa to perform regular virological monitoring in patients on ART. Therapeutic monitoring based on clinical and immunological parameters alone might result in unnecessary switches to second-line ART in the absence of virological failure, but could also increase the risk that

patients will stay on a virologically failing regimen for longer periods [71,74]. This could result in accumulation of resistance mutations, which might compromise the efficacy of subsequent second-line therapy [75]. Once clinical failure arises, the ability to select an optimal treatment regimen will be further limited by the inability to test for resistance.

Transmitted resistance

Available data

The prevalence of transmitted resistance is highest in industrialized countries, estimated between 9% and 20% [5, 6, 76–78]. The WATCH study found that the rate of resistance (to any drug) among treatment-naive individuals was 5.5% in Africa [79]. Between 2002 and 2007, 19 studies reported rates of resistance among treatment-naive populations in Africa. Studies were conducted in South Africa, Zambia, Côte d'Ivoire, Malawi, Senegal, Botswana, Cameroon, Djibouti, Democratic Republic of Congo, Burundi, Mozambique, Burkina Faso and Tanzania [80–97] (figure 1, table 2). NNRTI resistance rates ranged from 0% to 5.6%, NRTI resistance ranged from 0% to 3.7% and primary PI mutations were rare. To date, most reports from Africa have described low rates of transmitted resistance to NRTIs and NNRTIs, which might reflect the restricted availability of antiretroviral drugs until recently. Most studies conducted in Africa have small samples and substantial dissimilarities in assay methodology, the time period in which data were collected, the population under study and HIV-1 subtypes, which limit generalizability and the possibilities for comparison.

Contributing factors

The most important risk factor for transmitted resistance seems to be widespread access to antiretroviral drugs in the area where infection occurred, particularly where drugs were used as part of non-suppressive regimens, such as industrialized countries before ART became available in 1996. By contrast, in Africa, where widespread treatment was only introduced when ART was available, it has been hypothesized that less resistant viruses are expected to circulate [76].

Mathematical modelling has shown that at currently planned levels of treatment coverage and unchanging sexual behaviour, ART rollout in Africa will not initially drive an epidemic of drug-resistant HIV [98]. However, if the assumptions made in the model (for example, those regarding ART coverage, level of transmission, rate of persistence of resistant viruses and replicative capacity of resistant viruses) are modified, it appears equally plausible that resistance transmission will have a substantial effect on disease epidemiology [99, 100]. Notably, recent studies have suggested that resistance acquired during HIV infection could persist over time. This might be due to the fact that the new

infection is caused by a relatively homogeneous virus population derived from the actively replicating virus population in the donor [101, 102]. This could not only impair the individual's response to treatment, but could also have an effect on the risk of becoming infected with resistant viruses that persist over time. Therefore, more sophisticated models are urgently needed to effectively inform policy.

pMTCT-associated resistance

In industrialized countries, the rate of mother-to-child transmission of HIV-1 has been reduced to <2% by the use of ART during pregnancy, elective caesarean delivery and avoidance of breastfeeding [103–105]. However, in the developing world, access to antenatal care is limited, leaving mother-to-child transmission the second major route of HIV infection and rendering the use of shorter and more practical regimens of NRTIs and/or NNRTIs for pMTCT widespread. Peripartum administration of SD-NVP to the mother at the onset of labour and to the infant at 48–72 h of life has been shown to be an easy and low-cost intervention, reducing HIV-1 transmission by 41%–47% [106, 107].

Data on resistance in women and infants following SD-NVP

SD-NVP, which has a low genetic barrier and a long half-life, does not provide maximum viral suppression, inducing the selection of resistance mutations in mothers and infants. Thirteen studies evaluated NVP resistance following SD-NVP. Studies were conducted in Côte d'Ivoire, South Africa, Uganda, Malawi and Zimbabwe. The most common resistance mutations were K103N and Y181C. Resistance rates ranged from 19% to 69% in women and from 40% to 87% in infants, with possible variations between subtypes [41, 42, 44, 108–117] (figure 1, table 3).

Data on resistance in women following other pMTCT regimens

Several studies have examined the emergence of resistance following other pMTCT regimens. A randomized trial comparing women receiving SD-NVP alone with women who received SD-NVP followed by either 3 or 7 days of ZDV and 3TC post-partum found that the prevalence of NVP resistance in these three groups was 57%, 13% and 9%, respectively [114]. Similarly, a non-controlled study found that the rates of NVP resistance in women were reduced when SDNVP was followed by the administration of ZDV plus 3TC for 3 days post-partum [118]. Accordingly, revised WHO pMTCT guidelines for resource-limited settings recommend the use of a combination of ZDV and 3TC post-partum, in addition to SD-NVP, in order to reduce the risk of NVP resistance [119]. A recent study from Zambia showed that a single dose of TDF and emtricitabine at delivery, in addition to SD-NVP and a short course ZDV, reduced NVP resistance in women by half at 6 weeks after delivery [120]. A recent meta-analysis reported NVP resistance rates at 4–8 weeks post-partum of 35.7% in women receiving SD-NVP with or without other ante

or intrapartum antiretrovirals, and 4.5% in women receiving SD-NVP plus post-partum antiretrovirals [121].

Data on resistance in infants following other pMTCT regimens

A number of studies evaluated resistance following other pMTCT regimens in infants. Mother-infant pairs who were treated with ZDV or SD-NVP showed NVP resistance in half of the pairs receiving SD-NVP and no ZDV mutations in those receiving ZDV at 6 weeks post-partum [122]. Infants who received SD-NVP plus 7 days of ZDV and 3TC showed no NVP resistance at 6 weeks compared with 78% of those who received SD-NVP only [114]. NVP resistance in infants could be reduced by adding a short-course of ZDV postpartum [123]. A recent meta-analysis reported NVP resistance rates at 4–8 weeks post-partum of 52.6% in infants receiving SD-NVP only and 16.5% in infants with additional post-partum antiretrovirals [121].

Clinical consequences of previous pMTCT

The clinical consequences of NVP exposure on effectiveness of NNRTI-based ART and/or pMTCT in later pregnancies are still unclear. Studies have reported that SD-NVP decreased the virological response of women to subsequent NVP-containing ART at 6 months [124, 125]. Others have suggested that effectiveness was not compromised at 18 months of follow-up [126] and that initial virological response was also not compromised if ART was started more than 6 months after delivery [125]. Furthermore, preliminary data suggest that there is no increase in NVP resistance when SD-NVP is taken for a second time in a subsequent pregnancy [127], and that effectiveness of SD-NVP for pMTCT used in successive pregnancies is probably not impaired [128,129]. Additional randomized trials are needed to definitively answer these questions. Meanwhile, because relatively few women (11% of those eligible [3]) are currently receiving SD-NVP and because most women will not immediately initiate ART following SD-NVP, WHO guidelines recommend that HIV-infected mothers and infants who require ART and have previously been exposed to SDNVP should still be considered eligible for NNRTI based regimens [119].

Priorities for public health action and research

As the number of individuals on ART across the African continent grows, the main challenge is to maintain the momentum in the rollout of treatment and prevention programmes achieved so far and to sustain those already in care. The next challenge will be to develop more effective and sustainable health systems, which include the appropriate infrastructure for logistics, administration, information management, laboratories and other facilities [130], and to take specific measures to prevent and contain resistance and to improve the quality of HIV care and treatment.

Preserving first-line regimens

Due to limited availability of virological monitoring, detection of resistance mutations and second-line therapy, prolonging the clinical efficacy of first-line therapy will be crucial [131]. Meticulous adherence to therapy must therefore be emphasized [68–70, 72]. Clinical trials evaluating which therapeutic monitoring strategies are essential to ensure long-term effectiveness of ART in resource-limited countries are ongoing. In addition, data are needed to determine the optimal time to switch from first-line to second-line therapy in the absence of resistance testing and salvage regimens.

Coordinated surveillance of resistance

Currently, in developing countries, the emergence of acquired and transmitted resistance is not routinely evaluated as part of treatment programmes. The coordinated assessment of the proportion of HIV-infected individuals who have developed resistance, patterns of resistance and the factors associated with resistance emergence and spread, will provide crucial information for adjusting treatment guidelines as necessary. To this end, the WHO launched a global public health strategy through the Global HIV Drug Resistance Surveillance Network (HIVResNet) and national governments [132]. Although the validity of the proposed study methodologies, which include early warning indicators, sentinel monitoring and threshold surveillance, needs to be confirmed, an important first step has been taken towards standardization and coordination of resistance surveillance efforts. The PharmAccess African Studies to Evaluate Resistance (PASER) programme is a major contributor to the global public health strategy in Africa. Together with its counterpart programme in Asia, TREAT Asia Studies to Evaluate Resistance (TASER), PASER aims to build capacity for coordinated resistance surveillance by establishing a network of HIV clinics, reference laboratories and research centres that collaborate in an observational resistance database [133]. Results are expected to support recommendations to policy makers for optimal ART practices.

Improved laboratory capacity

Over time, laboratory capacity in Africa should be improved to expand access to laboratory-dependent patient monitoring strategies, such as haematology, biochemistry, CD4+ T-cell counts and viral load testing, as feasible technologies become available [131]. Currently, the use of conventional resistance detection methods, mainly genotypic and phenotypic assays [134], are limited by prohibitively high costs, high capital outlay and significant technical skill required to conduct the assays. At present, WHO does not recommend resistance testing for individual patient management in resource-limited settings. The development of affordable and more practical alternatives for laboratory monitoring tools, including resistance assays, simple specimen carrier devices, in-house genotyping protocols and point mutation assays, should be pursued actively. As part

of the coordinated surveillance efforts, there is a need to build the laboratory capacity for quality-assured genotypic resistance testing. To this end, it seems most feasible to adopt a centralized approach with a limited number of regional reference laboratories in strategic African countries. Both HIVResNet and PASER are currently supporting the set-up of the appropriate infrastructure, including quality assurance schemes.

DISCUSSION

Breakdowns in health systems might create the conditions for accelerated emergence of antiretroviral resistance in resource-limited countries. The main contributing factors include interrupted drug supply, poor adherence to therapy, suboptimal prescribing patterns and limited access to virological monitoring. Studies conducted in Africa to date reported low rates of transmitted resistance, but predictions for the future are difficult to make. The use of non-suppressive drug regimens in HIV prevention strategies, such as in pMTCT, and the possible future use of microbicides and pre-exposure prophylaxis, warrants careful investigation of their consequences for resistance development.

This literature review was limited by the quality and quantity of the available studies. Small and selected samples in many studies meant data could not be easily extrapolated to the general population. Also, because of heterogeneity in study design, populations under study, HIV-1 subtypes and time of data collection, the possibilities of study comparison are limited.

In view of the numerous risk factors, the public health community should anticipate the realistic possibility of exacerbated emergence of resistance among African HIV-infected populations, as treatment and prevention programmes are scaled up. The containment of resistance in Africa is particularly important given the limited number of drug regimens that are available. Many important questions concerning patterns and prevalence of resistance, therapeutic monitoring strategies and implications of subtype diversity and pMTCT, remain to be definitively answered. The next main challenge is to vitalize the health systems and to take specific measures to minimize resistance. To this end, coordinated resistance surveillance systems are being established throughout the developing world.

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Table 1. Summary of studies on drug resistance in patients receiving ART in sub-Saharan Africa.

Year	Location	n	Study design	Study group	Median baseline CD4 count	Main HIV-1 subtypes	Main ART regimen	Median ART duration (weeks)	Comment	Ref
1998-2000	Uganda	94	CSA	Failing patients on ART for >90 days, subset of DAI cohort	73	A, D	2NRTIs vs. triple ART	36	Virologic failure (>400cps): 2NRTIs 20% vs ART 50% Overall HIVDR: 2NRTIs 29/37 (78%) vs. ART 22/45 (49%)	[56]
1998-2000	Senegal	58	PC	ARV naïve, advanced disease, subset of ISAARV cohort	109	CRF02_AG	d4T+ddI+IDV	78	Virologic failure (>500cps): 41% Overall HIVDR 2/58 (3%)	[50]
2001	Zimbabwe	25	CSA	Failing patients on ART for >2 months	95 *	C	Triple therapy, mostly PI-based; 1st and 2nd line	48	Virologic failure (>400cps): 21/25 (84%) Genotyping 21/25, HIVDR 17/21 (NRTI 82%, NNRTI 18%, PI 41%, ≥1 drug class 59%)	[58]
2002	Rwanda	60	CSA	Failing patients on ART for >3 months	na	A, C	91% triple therapy, mostly NNRTI-based	na	50% treatment interruption Virologic failure (>1000cps) 26/60 (43%) Genotyping 22/26, HIVDR 11/22 (NRTI 63%, NNRTI 55%, PI 27%)	[49]
2001-2003	Cameroon	109	PC	ARV naïve, ≥18yrs, CD4 <350/mm3 or AIDS, Karnofsky >50%	150	NA	3TC+NVP+ZDV or d4T	70	Virologic failure (>400cps): 18% at 24 months Overall HIVDR 4/109 (3.7%) Incidence: 3.2 per 100 person yrs	[48]
2003	Uganda	137	CSA	Failing patients on ART for >12 weeks, mostly ARV naive at baseline	163 *	A, D	Triple therapy, mostly NNRTI-based	38	Virologic failure (>400cps) 46/137 (34%) Genotyping 36/46, HIVDR 30/36 (mostly K103N)	[53]

Table 1 (continued)

Year	Location	n	Study design	Study group	Median baseline CD4 count	Main HIV-1 subtypes	Main ART regimen	Median ART duration (weeks)	Comment	Ref
2003	Uganda, Zimbabwe	377	CT	DART: ARV naïve, advanced disease	101	A, C, D	3TC+ZDV+TDF	24	Virologic failure (>1000cps) 53/377 (14%) Genotyping 20/53, HIVDR 18/20 (mostly M184V, K65R less common)	[54]
1998-2004	Senegal	176	CSA	Failing patients, mostly AIDS and ARV naïve	144	NA	2NRTIs+NNRTI or PI	131	Virologic failure (>500cps) 22.5% at 30 months Overall HIVDR 22/176 (13%) (NRTI 10%, NNRTI 9%, PI 8%)	[51]
2002-2004	Cameroon	128	CSA	Failing patients on ART for >3 months	NA	CRF02_AG	2NRTIs+NNRTI or PI	44	Genotyping 35/128, HIVDR 21/35 (NRTI 13% (mostly M184V), NNRTI 10% (mostly K103N), PI 2%)	[52]
2002-2005	Botswana	155	CSA	Failing patients	96	C	ddI+d4T+NFV, NFV-based 2nd line regimen	57	Virologic failure 16/155 (10%) Suggest subtype-C specific NFV resistance pathways (D30N 54%, L90M 31%)	[40]
2004-2006	Ivory Coast	106	CSA	Failing patients in ACONDA/ISPED cohort	122	NA	2NRTIs+NNRTI or PI	163	Virologic failure (>300cps/ml) 44/106 (42%) Overall HIVDR 23/106 (22%) (≥1 drug class 30%)	[55]
2005	Tanzania	150	CSA	Failing patients on ART for median ≥6 months	114	A, C, D	3TC +d4T+NVP	52	Virologic failure (>1000 cps/ml) 35/150 (23%) Genotyping 27/35, HIVDR 15/27 (NRTI 9%, NNRTI 10%)	[57]

Table sorted by year of study. *CD4+ T-cell count at time of cross-sectional analysis (CSA), not baseline. ARV, antiretroviral; CT, clinical trial; ddI, didanosine; d4T, stavudine; ART, combination antiretroviral therapy; HIVDR, HIV drug resistance; IDV, indinavir; NA, not available; NFV, nelfinavir; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NVP, nevirapine; PC, prospective cohort; PI, protease inhibitor; PR, protease; RT, reverse transcriptase; TDF, tenofovir; VF, virological failure; ZDV, zidovudine; 3TC, lamivudine.

Table 2. Summary of studies on rates of transmitted drug resistance in sub-Saharan Africa [a].

Year	Location	n	Study group	Median CD4 count	Main HIV-1 subtypes	Reported resistance rate (%)*					Mutations	Ref
						Any	NRTI	NNRTI	PI	MDR		
NA	Abidjan, Ivory Coast	20	ARV naïve (DAI cohort)	84	CRF02_AG	0	0	0	0	0		[80]
2000	Soweto, South Africa	37	Antenatal clinic attendees, ARV naive	479	C	0	0	0	0	0		[89]
2000	Lusaka, Zambia	28	Antenatal clinic attendees in first pregnancy	NA	C	0	0	0	0	0		[84]
1997-2000	Abidjan, Ivory Coast	99	Regulars, volunteers and blood donors, estimated time since seroconversion 9.4 months	NA	CRF02_AG	0	0	0	0	0		[91]
1996-2001	Lilongwe and Blantyre, Malawi	21	ARV naive, STD clinic and hospital attendees	NA	C	0	0	0	0	0		[88]
1998-2001	Dakar, Senegal	41	ARV naive subset (SIAARV cohort)	112	CRF02_AG	0	0	0	0	0		[93]
2001	KwaZuluNatal, South Africa	56	ARV naive, heterogenous	366	C	5.4	0	5.4	0	0	RT: G190A, K103N, A98G	[83]
2001	11 health districts, Botswana	71	Sentinel survey among antenatal and STD clinic attendees	NA	C	0	0	0	0	0		[82]
2001-2002	Abidjan, Ivory Coast	107	Blood donors (PRIMO-CI) and ARV naïve women (DITRAME Plus)	395	CRF02_AG	5.6	0.9	3.7	0.9	0	RT: K101E, K103N, P236L. K219Q PR: N88D	[92]
2000-2002	6 rural villages, Cameroon	128	Random subset of HIV diagnostic samples	NA	CRF02_AG	0	0	0	0	0		[86]
2002	Djibouti	47	Subset of general population survey	NA	C	10.6	2.1	2.1	6.4	0	RT: L100I, K65R PR: N88D	[87]
2002	4 major cities, DR Congo	70	Subset of sentinel survey population representing various subtypes	NA	Multiple	4.3	0	1.4	2.9	0	RT: K103N, V179D PR: M46L, L90M	[96]

Table 2 (continued)

Year	Location	n	Study group	Median CD4 count	Main HIV-1 subtypes	Reported resistance rate (%)*					Mutations	Ref
						Any	NRTI	NNRTI	PI	MDR		
2002	Burundi	101	Selected subset of sentinel serosurvey	NA	C	1.0	0	1.0	0	0	RT: G190E	[97]
2003	Maputo, Mozambique	58	ARV naive subset (DREAM cohort)	361	C	0	0	0	0	0		[81]
2003	Ouagadougou and Bobo Dioulasso, Burkina Faso	97	Recently diagnosed hospital and treatment center attendees (median 33 yrs)	166	CRF02_AG, CRF06_cpx	8.3	2.1	4.1	2.1	0	RT: M41L, T69S, V106A,V108I PR: L33F	[94]
2001-2004	Yaounde, Cameroon	102	Recently diagnosed blood donors and hospital attendees (median 36 yrs)	400	CRF02_AG	7.8	2.9	2.0	2.9	0	RT: A62V, T69N, V108I, M184V, P236L PR: L33F, M46I/L	[94]
2001-2004	Yaounde, Cameroon	96	Pregnant women attending antenatal care, diagnosed <12 months and ARV naive	365	CRF02_AG	2.1	1.0	0	1.0	0	RT: L210W, T215S PR: N88S	[95]
2004	Western Cameroun	54	Antenatal/STD clinic attendees (median 33 yrs) †	NA	CRF02_AG	13.0	3.7	5.6	7.4	3.7	RT: V75I, L100I, M184V, Y188C PR: M46I/L, V82A	[85]
2005-2006	Dar Es Salaam, Tanzania	39	Sentinel serosurvey (WHO Treshold Survey)	NA	A, C	0	0	0	0	0		[90]

Table includes studies published between 2002 and 2007, with a minimum of 20 study participants. Table sorted by year of study. *Resistance subdivided per drug class. †Clonal analysis of proviral analysis, excluding minor populations of drug-resistant mutants from analysis reduces prevalence of any resistance from 13.0% to 1.9%. ARV, antiretroviral; DRC, Democratic Republic of Congo; HIV-1, HIV type-1; MDR, multidrug resistance or resistance to ≥2 classes; NA, not available; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; PR, protease; RT, reverse transcriptase; STD, sexually transmitted disease; WHO, World Health Organization.

Table 3. Summary of studies on rates of NVP resistance in women and infants after exposure to single-dose NVP for pMTCT in sub-Saharan Africa

Year	Location	n	Time (wks) *	Main HIV-1 subtypes	% NVPR women	% NVPR infants	Comments	Ref
NA	Ivory Coast	29	4	CRF02_AG	21%	NA		[118]
NA	South Africa	111+40	4-6	C	67%	53%	2-dose NVP	[117]
NA	South Africa	456	7	C	39%	42%		[114]
NA	South Africa	155+20	26	C	35%	65%	NVPR rate at 6 months of individuals with NVPR at 7 wks. Resistance associated with higher VL and lower CD4 counts	[116]
NA	South Africa	30+30	6	C	40%	40%		[112]
NA	South Africa	68+9	6	NA	57%	78%	Addition of ZDV+3TC to SD-NVP significantly decreased NVPR rates	[115]
1997-2001	Uganda	111+24	6-8	NA	19%	46%	Mutations faded from detection within 12-24 months	[109]
1997-2001	Uganda	279	6-8	A, D	25%	NA	Higher NVPR rates in subtype A (19%) than D (36%)	[111]
1997-2001	Uganda	65	1+6	A, D	28%	NA	Y181C often fades from detection by 6–8 weeks. K103N emerges more slowly, but remains detectable longer	[110]
1997-2001	Uganda	140	1+6	A, D	22%	NA	Y181C fades from detection faster in subtype A and K103N accumulates faster in subtype D	[41]
1997-2003	Uganda, Malawi	306	6-8	A, C, D	69%	NA	Higher NVPR rates in subtype C (69%) than A (19%) or D (36%).	[42]
1997-2003	Uganda, Malawi	41	6-8	A, C, D	na	87%	NVPR more frequent in Malawian subtype C infants (87%) than Ugandan subtype A or D infants (50%)	[44]
2000-2001	Zimbabwe	32	8	C	34%	NA	20 paired breastmilk/plasma samples; NNRTI-resistance in 65% of breastmilk and 50% of plasma RT sequences, with divergent mutation patterns	[113]

Table includes studies using standard genotypic sequencing methods only. Table sorted by country. *Time in weeks after delivery. HIV-1, HIV type-1; NA, not available; NNRTI, non-nucleoside reverse transcriptase inhibitor; NVP, nevirapine; NVPR, nevirapine resistance; pMTCT, prevention of mother-to-child transmission; RT, reverse transcriptase; SD-NVP, single-dose nevirapine; ZDV, zidovudine; 3TC, lamivudine.

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