Abstract

**Background:** The introduction of the highly active antiretroviral therapy in the mid-1990s has significantly reduced morbidities and prolonged the lifespan of people living with HIV. However, the emergence of resistance to the antiretroviral drugs is becoming a major cause of treatment failure. While the problem of drug resistance is being tackled in developed countries, not much seems to be done in this regard in developing countries of Africa, Asia and Latin America. This review looked at the regional distribution of HIV groups and subtypes and how this has affected the pattern of antiretroviral resistance.

**Methods:** The review was sourced from papers presented at international conferences on HIV/AIDS and rational drug use, relevant journals and Medline search using the keywords- Antiretroviral drugs, drug resistance, HIV subtypes and resistance testing.

**Results:** The types, groups, subtypes, sub-subtypes and recombinant forms of HIV-1 have been identified according to their geographical distributions. The evolution of HIV viral mutations, process(es) involved in development of primary and secondary antiretroviral drug resistance, including the role of HIV genetic polymorphisms, and transmitted resistance have been discussed.

**Conclusion:** The pitfalls in the current resistance testing based on HIV-1 subtype B have been highlighted. The design of resistance testing algorithm based on HIV-1 subtype non-B has been suggested for the developing world.

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Introduction

Twenty seven years after the Acquired Immunodeficiency Syndrome (AIDS) was first described in a cohort of homosexuals in the United States of America (USA), this deadly disease has killed over 40 million people and currently affects another 33 million worldwide, over 70% of these in sub-Saharan Africa. The human immunodeficiency virus (HIV), the causative agent of AIDS, is classified into types, groups, subtypes and sub-subtypes according to its genetic diversity. Two major types of HIV are currently recognized, HIV type 1 (HIV-1) and HIV type 2 (HIV-2). While HIV-2 is restricted to West Africa, where it represents about 3% of total HIV infections, and was reported to be decreasing in prevalence, HIV-1 group M is globally disseminated, accounting for the AIDS pandemic. Nine pure subtypes of HIV-1 group M (A-D, F-H, J and K) are currently known. The other HIV-1 groups, O (outlier) and N (new or non-M, non-O) are restricted to countries of central Africa, notably Cameroun and Congo.

In 2004 a study on molecular epidemiology of HIV-1 subtypes from 23,874 HIV-1 samples in 70 countries (which accounted for 89% of all people living with AIDS [PLHWA] worldwide) showed that HIV variants are heterogeneously distributed with subtype B prevalent in developed countries of America, Western Europe, Japan and Australia, while non-B subtypes predominate in developing countries. The non-B subtypes are distributed as followed:- subtype A typically found in Eastern Europe and countries of former Soviet Union, Democratic Republic of Congo (DRC) and Tanzania; subtype C in most countries of sub-Saharan Africa, Ethiopia, Zambia and India; subtype D in Libya, DRC and Tanzania; subtype F in West Africa and DRC. Subtypes and sub-subtypes can form additional mosaic forms through recombination of different strains inside dually-or multiply-infected individuals giving rise to circulating recombinant forms (CRFs). Currently over 40 CRFs are recognized in different parts of the world giving rise to 18% of infections globally. Some of these CRFs have achieved epidemic relevance in certain geographic regions, such as CRF01_AE in Southeast Asian countries, CRF02_AG in West African countries, and CRF07_BC and CRF08_BC in China.

Because these HIV-1 M subtypes and CRFs are the result of founder effects and localized evolution in different geographic locales, they are heterogeneously
distributed worldwide. This genetic variability of HIV-1 will surely impact on current efforts at virus eradication through development of newer antiretroviral drugs and vaccines. Since different subtypes display distinct biological and clinical properties, such as tropism, transmission propensities, disease progression and effectiveness profile in diagnostic and monitoring assays.

Evolution of HIV viral mutations

HIV replication is a highly dynamic process in which large numbers of virions are created and destroyed by the immune system each day. Recent studies have calculated that the half-life of an HIV virion is approximately 30 minutes, and the production of virus can amount to \(10^4\) to \(10^5\) virions per day. The plasma viral load (pVL) thus reflects the balance between the production and clearance of viral particles. Most HIV-infected cells are short-lived T cells, which have a half-life of about 2 days; therefore, the pool of virus-producing cells is maintained through the constant infection of new cells. Individual cells can be infected by more than 1 virion, which may represent different members of a pool of quasispecies. In these multiply infected cells, the reverse transcriptase (RT) enzyme can randomly jump from one ribonucleic acid (RNA) template to the other during viral replication, exchanging segments of genetic information between viral genomes. This template switching can facilitate the accumulation of mutations in a viral genome that may otherwise take a long time to develop.

Genetic recombination may play an important role in the development of multidrug-resistant HIV strains, whereby a viral strain can accumulate several drug resistance mutations in a short period of time. Mutations in the HIV genome are primarily generated during the initial steps of the HIV replication cycle. Genomic RNA carried by HIV is copied into deoxyribonucleic acid (DNA) early in the replication cycle; this process is mediated by the HIV RT enzyme. Spontaneous errors have been shown to occur during the RT process, which result in the placement of an incorrect nucleotide in the growing DNA strand, either as point mutation (e.g., the replacement of guanine by adenine, GAC to AAC) or insertion of an extra nucleotide (e.g., AAA-GAC-AGT to AAA-GAC-AGT-AGT). This happens approximately once in every 10,000 to 30,000 nucleotides.

Because the HIV genome is about 10,000 bases long, an average of 1 error (mutation) occurs each time a viral genome is replicated. These aberrant nucleotides may result in changes in the amino acid coding of the HIV proteins from HIV DNA, potentially altering the structure and/or function of these proteins and affecting the replication competence of the viral strain. Many examples abound; point mutation from GAC to AAC leads to substitution of glutamate for aspartate; insertion of an extra AGT leads to the production of a mutant serine. The first phenomenon describes the viral mutation as D30N, in which the initial letter represents the wild-type amino acid of the protein [D: aspartate]; the number represents the codon or position of the affected amino acid within the protein [30]; and the end letter represents the mutant amino acid that is present [N: glutamate]. Thus, D30N describes the substitution of glutamate for aspartate at the 30 codon in HIV RT, representing a shift from nucleotide codon GAC [aspartate] to AAC [glutamate]. Since mutations in these proteins are the determining factor in drug susceptibility, the nomenclature for drug resistance mutations describes amino acid sequence substitutions in the proteins.

Selection of mutations by HIV-1 genome during antiretroviral therapy

i. Development of primary antiretroviral drug resistance.

Factors such as viral tropism, replication kinetics and fitness, and host immune responses, are likely to influence virus transmission (both horizontal and vertical) and disease progression in people infected with different subtypes. In uncontrolled HIV infection, the high HIV replication rate coupled with the RT-induced mutation rate generates every possible mutation in the HIV genome each day. These factors generate a large pool of genetically related but distinct HIV strains called quasispecies, each of which has the potential to develop into the dominant strain. Most of these quasispecies have either deleterious mutations or mutations that make their growth rate inferior to that of other quasispecies. However, even a small proportion of functional mutations will generate a significant population of modified genomes because of the high overall replication rate. Strains with a mutation that provides a growth advantage in a particular environment, e.g., in the presence of antiretroviral (ARV) drugs will out compete the other quasispecies and become the dominant viral strain in the population. Even strains with mutations that cause impaired replication rate (compared with a non-mutated strain) can still accumulate additional mutations during replication, some of which can repair the strain’s replicative defect. The character of the quasispecies population is constantly shaped by both viral and host factors such as viral tropism, replication kinetics and fitness, and host immune responses, are likely to influence virus transmission (both horizontal and vertical) and disease progression in people infected with different subtypes.
factors. Virus strains with replication rates that are severely impaired by genetic features will be less able to compete for target cells than strains with a higher replication rate, and cannot become the dominant strain of the viral population. Also, the immune system of the host may recognize and attack quasispecies that have specific immunologic epitopes, limiting the ability of those variants to compete with other quasispecies.

Antiretroviral therapy (ART) is an additional source of pressure on the viral population. Initial therapy with a potent, combination antiretroviral regimen will suppress the replication of most quasispecies and reduce the plasma viral load to a level below the limit of detection of sensitive viral load assays. But some variants will possess mutations that enable replication to occur at a rate determined by the inherent fitness and degree of resistance of the quasispecies to all drugs in the regimen. Studies have shown that mutations can be generated and viral evolution can occur even in patients undergoing highly active antiretroviral therapy (HAART)14-16. Viral mutations are accelerated in the presence of preexisting drug resistance, impotent drugs, inadequate adherence and therefore inadequate drug levels17-19. As drug-resistant quasispecies continue to replicate, continued reverse transcription events generate further mutations in the surviving viral population, resulting in an increased plasma viral load composed of quasispecies that have acquired sufficient resistance (and resistance mutations) to become the dominant viral strains20.

Inherent replication rate of the resistant variant also influences the rate of its emergence. Certain resistance mutations such as Y181C and K103N, which are associated with broad cross-resistance to the first generation non-nucleoside reverse transcriptase inhibitors (NNRTIs), do not appear to substantially affect the viral replication rate; and as such, virus containing these mutations can appear as the dominant quasispecies in a matter of weeks in patients receiving failing nevirapine or efavirenz-containing regimens21,22. On the other hand, some mutations may confer resistance and permit replication in the presence of the drug, but compromise the viral replication rate compared with that of wild-type virus. Strains with these types of mutations may take longer to emerge as a major quasispecies, or may acquire (through selection) additional compensatory mutations that help to restore the viral replication rate.

High levels of resistance to some drugs, such as zidovudine and most protease inhibitors (PIs) may require the accumulation of several mutations, which may involve single, double or multiple nucleotide base changes. And, depending on the viral replication rate, these mutations may take several months or years to occur. Examples abound. The M184V mutation on RT confers high level resistance to lamivudine and emtricitabine, but increases susceptibility to zidovudine, stavudine and efavirenz23. Secondary protease (PR) mutations may confer reduced susceptibility to PI in vitro24,25; mutations D123N and I135T in RT of some CRF02_AG isolates have reduced susceptibility to abacavir26; mutations at positions 181I, 188L and 190A of HIV-2 virus and HIV-1 group O isolates from drug-naïve Cameroonian have been linked to resistance to NNRTIs, the fusion inhibitors (enfuvirtide and T-12249) and some PIs27-29.

Although preliminary in vitro inter-subtype differences in resistance to the CCR5 co-receptor antagonist maraviroc have not been found, reported differences in co-receptor tropism among subtypes may influence the in vivo response to that drug. For instance, subtype D isolates have been reported to develop CXCR4 tropism more frequently than subtype C20. It is also anticipated that amino acid differences in proteins from non-B HIV-1 subtypes may impair the efficacy of investigational drugs that are not yet even approved for clinical use. For example, a total of 13 amino acid differences between the integrase (IN) proteins of subtypes B and CRF02_AG have been pinpointed, which according to the predicted 3D model of the pre-integration complex, may impact on IN function. Particularly, the amino acid residue T125, whose variation has been implicated in resistance to the IN inhibitor L870, 810, differs between both subtypes21.

ii. Development of secondary antiretroviral drug resistance

A similar process of selection for resistance mutations occurs when a patient changes to a new treatment regimen. In this case, the rate of viral replication and selection of mutations is influenced by the extent to which the mutations selected by the previous regimen also confer resistance to the new drugs. If cross-resistance exists between the previous drugs and the new drugs, mutation-generating replication events continue to occur at a rate determined by the inherent fitness of the viral strain and the overall effectiveness of the new regimen. This continued replication and selection is the cause of the more rapid virologic failure typically seen with second-line and third-line treatment regimens as compared with initial regimens. If the mutations selected during therapy with the initial
regimen do not confer cross-resistance to drugs in the new regimen, the mutant strains will be inhibited by the new drugs. No longer having a selective advantage, these variants will become minority members of the quasispecies population as they are out competed by newly selected resistant strains. The disappearance of mutations in the predominant population can occur in a matter of weeks or may take several months.

A puzzling issue in the development of drug resistance among different HIV-1 subtypes lies in the context of prophylactic ART for prevention of mother-to-child transmission of HIV (PMTCT). Most of the studies addressing this came from the use of single-dose of nevirapine for PMTCT in African countries. Several studies derived from the HIVNET 012 program in Uganda showed a higher occurrence of nevirapine (NVP) - related drug resistance, namely the mutation K103N in RT, in subtypes C- and D-infected women and babies compared with subtype A counterparts. Mutations K103N and Y181C have also been seen in subtype C-infected women exposed to single-dose NVP in South Africa, Zambia and Zimbabwe, as well as in CRF02_AG-infected women in the Ivory Coast. In 2008, Hosseinipour et al. studied the development of drug resistance among patients in Malawi who had mostly received a first-line ART consisting of stavudine, lamivudine, and nevirapine. Patients who failed were usually switched from this regime to one containing zidovudine, lamivudine, tenofovir, and lopinavir/ritonavir. Evaluation of drug resistance in 101 of this cohort of patients who had failed first-line therapy, with viral loads > 1000 copies/mL revealed that the most common mutation was the M184V followed by a number of NNRTI mutations associated with reduced responsiveness against nevirapine and/or efavirenz. 16% of the patients had either the K65R or K70E mutations that are associated with stavudine failure. These findings confirm earlier data from Botswana that suggested that the K65R mutation was far likely to occur among patients failing a stavudine-containing regimen in the context of subtype C viruses than would be expected with viruses of subtype B origin. This finding may be of considerable significance in view of the fact that both K65R and K70E are able to confer broad cross-resistance against a wide array of nucleoside compounds, thereby potentially compromising the therapeutic usefulness of this family of drugs, which are the backbone of ART in resource poor countries.

**HIV genetic polymorphisms and development of antiretroviral drug resistance**

### i. Alterations in genetic barrier to antiretroviral drug resistance

The polymorphic nature of HIV genes encoding proteins that are targeted by ARVs can influence the genetic barrier for acquiring drug-resistance mutations. The genetic barrier a particular HIV variant faces in reaching resistance is also influenced by the relative fitness and/or the replication capacity of the virus carrying particular mutations compared with its wild-type counterpart. Genetic differences among HIV-1 subtypes may explain the lower occurrence of certain resistance mutations in particular strains. Thus, different drug-resistance mutations impact differentially on viral fitness, more in some subtypes than others. Mutations L210W and Q151M, both providing resistance to nucleoside reverse transcriptase inhibitors (NRTIs), can only emerge in subtype F1 after two nucleotide changes from a consensus baseline sequence, compared with other subtypes. Of note, such lower predicted prevalence was found in NRTI-treated Brazilian subjects infected with subtype F1, compared with subtypes B and C. Among European drug naïve subjects, additional higher genetic barrier differences were found for I82A in subtypes C and G and V108I in subtype G respectively. Conversely, a lower barrier was found for I82T in subtypes C and G and V196M in subtype C. D30N mutation in PR produces a lower PI (nelfinavir) resistance in non-B subtypes than in subtype B, and also impairs the replication capacity or fitness of both viruses, affecting the former more. On the other hand, mutations K20I and M36I in PR of subtype G and CRF02_AG viruses, in the absence of PIs, increases the replication of these isolates faster than their wild-type counterparts. A drug’s genetic barrier to viral resistance is a measure of the number of mutations that is required for resistance to the drug to develop as well as the frequency with which such mutations emerge. For example, it is well established that ritonavir-boosted PI-containing regimens have a higher genetic barrier to resistance than do non-boosted regimens, the NRTIs and NNRTIs. Also, the very low genetic barrier of first generation NNRTIs (nevirapine, efavirenz, and delavirdine) to mutations such as Y181C and K103N is associated with broad cross-resistance to them.

### ii. Developing alternative pathways to antiretroviral drug resistance

HIV variants can also display preferential selection of drug resistance mutations and acquisition of alternative pathways to drug resistance. This phenomenon is most prevalent among PIs, although it is seen with the other ARV drugs. For instance, D30N and L90M are PR mutations occurring in equal frequency in subtype B,
but while D30N is selected more frequently and confer resistance exclusively to nefavirvir; L90M lowers susceptibility of this subtype to most PIs currently in clinical use. L90M also reduces susceptibility of non-B subtypes to most PIs currently in clinical use, but confers resistance to nefavirvir in these subtypes only. This scenario may have direct implications for nefavirvir usage in countries where HIV-1 non-B subtypes are found. Other examples are: - the acquisition of a threonine (82T) or a methionine (82M) mutation, at position 82 of PR of subtype G reduces its susceptibility to tiprinavir and indinavir respectively. CRF01_AE, on the other hand, appears to have a lower genetic barrier to V82F than subtype B, and may develop faster resistance to indinavir through this pathway.

Preferential selection of drug- resistance mutations to NRTIs and NNRTIs has also been described. Subtype C may acquire the tenofovir related mutation K65R more rapidly than other subtypes, perhaps influenced by nucleotide polymorphisms at codons 64-66. Different changes at the same amino acid residue may also be selected in distinct subtypes as a result of the same drug-selective pressure. This is the case for codon 106V, which changes to an alanine (106A) in subtype B, but to a methionine (106M) in subtype C. The 106M mutation has been shown to confer resistance not only to efavirenz, which selects it, but to all NNRTIs.

HIV-2 exhibits more complex genetic pathways towards drug resistance than HIV-1, because of several differences in the PR and RT backbones. As a result, amino acid changes selected by ARVs are different and more difficult to interpret. For instance, PR mutation for tiprinavir in HIV-2 is I82L as distinct from V82A in HIV-1. Several other changes associated with drug resistance in HIV-2 PR, such as K7R, V62A/T and L99F are uncommon to detectable transmitted NNRTI resistance substantially decreases the likelihood of having a virologic response to a first-generation NNRTI-based regimen. Surveillance of transmitted HIV drug resistance in some African countries revealed that about 5% of Zambian adults beginning first-line therapy possessed at least 1 resistance-associated mutation (RAM). In HIV-2 infected, ART-experienced Senegalese adults, multiclass drug resistance that includes mutational profiles revealing high levels of M184V/I was commonly found, while the Q151M mutation that can cause broad class resistance to all nucleosides, and the tenofovir related mutation K65R was found in 9% of them. In contrast, thymidine analog-associated mutations (TAMs) were very rare, with the exception of K70R, which was found in 1 person.

**Assessment of Antiretroviral Drug Resistance**

The prevalence of drug resistance and the role of drug resistance testing as an adjunct to the management of patients who are initiating or changing an antiretroviral treatment regimen has been endorsed in the recent update of the guidelines for resistance testing.

There are 2 types of assays available for measuring viral resistance. Genotypic assays identify specific nucleotide changes within the HIV-1 genome that correlate with drug resistance, whereas phenotypic tests more directly measure antiretroviral susceptibility in vitro by the use of a resistance test vector derived from viruses present in a patient plasma sample. Drug resistance testing can only be reliably performed if plasma HIV-1 RNA levels are > 500 copies/mL and should ideally be performed while the patient remains on the failing regimen so that the test results reflect the drug resistant viral species present under pressure of the treatment regimen, because some drug-resistant strains may not replicate as rapidly as more fit wild-type virus once antiretroviral treatment is modified or stopped. Even in some patients who have been off therapy, it is reasonable to perform drug resistance testing to determine the pattern of resistance of the fit viral population, although one must realize that without active drug selection pressure, resistant mutations will not be evident. Therefore, all previous ARV drug exposure must be considered before choosing a second-line regime.
Results of genotypic drug resistance tests are reported as a list of predefined drug resistance mutations, often with interpretations that classify individual drugs as “susceptible”, “possibly resistant”, or “resistant”, as determined by rules-based algorithms 
21, 59. A better understanding of resistance mutations and larger clinical trial results has allowed such algorithms to more accurately predict clinical response to specific drug regimens. However, the interpretation of resistance tests must take in cognizance the full drug history, including the current failing regimen. In cases in which resistance test results do not indicate selection of resistant virus at failure and in which the clinician believes the patient maintained appropriate adherence, but nonetheless failed therapy, there is another approach that may have value in selected settings.

The US Department of Health and Human Services (DHHS) treatment guidelines suggest that in certain situations, therapeutic drug monitoring may be useful in patient management 60. However, this is currently possible only for the PIs and NNRTIs for which concentration-response data exist 61-63. It can not be recommended for NRTIs which are probably more dependent on intracellular drug concentrations for their effect. This has therapeutic implications in developing countries where the NRTIs are the backbone of first-line regimen.

**Antiretroviral Drug Resistance and Implications for Clinical Management of HIV Infection in Developing Countries**

Current ARV drugs of all classes have been developed exclusively in high income countries, where a predominance of subtype B of HIV-1 group M is found. These drugs were developed through molecular dynamics and rational drug use, using template target proteins of subtype B origin. Moreover, the vast majority of data regarding toxicity, pharmacokinetics and the development of drug resistance interpretation algorithms and rules are being conducted in a context of subtypes B-infected subjects from the resource rich Western Europe and United States of America (USA) 64-66. Presently there is no centre for antiretroviral drug resistance testing in Africa, although plans are ongoing to establish one each in Zambia, South Africa and Nigeria. Thus, the effectiveness of ARV drugs and their impact on drug-resistance development is poorly known or understood for HIV-1 non-B subtypes which predominate in low income developing countries. Algorithms designed to interpret HIV genotyping resistance are ultimately aimed at predicting ART response and therefore guidance. Interpretation is based on the sequence of HIV genes that are targeted by ARVs such as PR, RT, gp41 and IN enzymes, determined from a circulating virus infecting an individual. Sequencing or mutation lists are subjected to the rules composing the algorithm, and a prediction of drug resistance to individual ARV drugs is provided 59.

Historical models have been developed to predict that over the next decade, the rate of transmission of drug resistant virus in Africa would remain below 5% and that most resistant strains would result from acquired, not transmitted, resistance 64. But these models failed to recognize the peculiarities of Africa in terms of diversity of populations, races and ethnicities.

**Antiretroviral Drug Resistance and the Future of HAART in Sub-Saharan Africa**

As antiretroviral therapy is becoming widespread in sub-Saharan Africa, it is expected that drug resistance, both primary and secondary, may become a major problem in future. Some studies have already shown that among treatment-naïve subjects initiating HAART, 25% developed drug-resistance mutations during a 30-month follow-up, while multi-class resistance was noted in about 10% 65. Epidemiological studies in Europe and USA 66 found the prevalence of primary resistance to at least one drug to be 10.9% and 11.5% respectively in patients infected for less than 1 year, and 7.5% in patients infected for more than 1 year. In both studies, the most common resistance was to NRTIs. However, the emerging trend in these developed countries with a long history of ART is that resistance is low if HAART is comprehensive and widely available and adherence is high. In sub-Saharan Africa, the current recommended first line regimen consisting of 2 NRTI ( stavudine or zidovudine and lamivudine) and NNRTI ( nevirapine or efavirenz) are not optimal enough to prevent development of resistance for many reasons. The high toxicity profiles of these drugs promote non-adherence and their low barrier to resistance considering reports of high level resistance from the use of single-dose nevirapine and the poor response of these patients to subsequent nevirapine containing HAART combinations 67. Africa will definitely not be unaffected by the high prevalence of NRTI resistance already reported in developed countries 68 and other parts of the world 69,70. Most of all, factors which are associated with poor adherence are prevalent in sub Saharan Africa. These include irregular availability and supply of ARV drugs, ignorance, stigmatization and poor motivations, poverty, malnutrition, young age and therefore illicit drug and alcohol abuse, multiple drug dosing for HIV,
opportunistic infections (since many present at stage of AIDS), malaria and other co-morbidities.

Conclusions
The use of ART for treating HIV-infected people in developing countries has increased significantly in the past few years and has already witnessed the gains of reduced mortality and morbidity seen in the developed world in the mid-1990s. But this gain may be curtailed by the emergence of drug resistant HIV strains and consequently virologic failure. Therefore understanding the response of different HIV variants, (especially HIV-1 non-B subtypes, and HIV-2 which are prevalent in developing countries), to ART is of paramount importance as the effectiveness of ARVs to infected patients from these areas is currently largely unknown. Establishment of drug resistance testing centres in Africa is the key to understanding drug resistance patterns to ARVs on this continent. This is fundamental to the treatment of patients who have not responded to or have failed a prior treatment regimen. Increasing international initiatives to disseminate the use of ART in developing countries and the design of more controlled and extended clinical trials and observational studies are needed, as these will ultimately lead to a better understanding of the actual impact of HIV variability on treatment in these areas.

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