Access to highly active antiretroviral therapy (HAART) 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 HAART, 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.

Methods

To identify eligible studies, a systematic search of the English language literature published before 2008 was conducted.
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 quasispecies, 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 population, 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 HAART 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 HAART 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 HAART is largely limited to this subtype. Preliminary data suggests that short-term immunological and virological outcomes on HAART 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 intersubtype 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 intersubtype 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 intersubtype 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 intersubtype 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 intersubtype 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 HAART in Africa. The studies were conducted in Uganda, Senegal, Zimbabwe, Rwanda, Cameroon, Botswana, Côte d’Ivoire and Tanzania.
Observational data from patients on a first-line HAART regimen show large variations in the rate of resistance, reported at 3.7%–49% after 24–163 weeks on HAART [40,48–58]. Earlier studies showed that the use of non-suppressive regimens (mono or bitherapy) 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 HAART, 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
Table 1. Summary of studies on acquired HIV drug resistance in patients receiving HAART in sub-Saharan Africa

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>n</th>
<th>Study design</th>
<th>Study population</th>
<th>Median baseline CD4+ T-cell count, cells/mm³</th>
<th>Main subtypes</th>
<th>Main HAART regimen</th>
<th>Median HAART duration, weeks</th>
<th>Comment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1998–2000</td>
<td>Uganda</td>
<td>94</td>
<td>CSA</td>
<td>Failing patients on HAART for &gt;90 days, subset of DAI cohort</td>
<td>73</td>
<td>A, D</td>
<td>2 NRTIs versus HAART</td>
<td>36</td>
<td>VF=400 copies/ml: 2 NRTIs 20% versus HAART 50%, overall HIVDR: 2 NRTIs 29/37 (78%) versus HAART 22/45 (49%)</td>
<td>[56]</td>
</tr>
<tr>
<td>1998–2000</td>
<td>Senegal</td>
<td>58</td>
<td>PC</td>
<td>ARV-naïve, advanced disease, subset of SAARV cohort</td>
<td>109</td>
<td>CRF02_AG</td>
<td>d4T+d4T+DDV</td>
<td>78</td>
<td>VF=500 copies/ml: 41%, HMVR 2/54 (3%)</td>
<td>[50]</td>
</tr>
<tr>
<td>2001</td>
<td>Zimbabwe</td>
<td>25</td>
<td>CSA</td>
<td>Failing patients on HAART for &gt;2 months</td>
<td>95*</td>
<td>C</td>
<td>Triple therapy, mostly PI-based, first- and second-line regimens</td>
<td>48</td>
<td>VF=400 copies/ml: 2/125 (8%), Genotyping 21/25, HMVR 17/21: NRTI 18%, NNRTI 18%, PI 41% ≥1 drug class 59%, 50% treatment interruption</td>
<td>[58]</td>
</tr>
<tr>
<td>2002</td>
<td>Rwanda</td>
<td>60</td>
<td>CSA</td>
<td>Failing patients on HAART for &gt;3 months</td>
<td>NA</td>
<td>A, C</td>
<td>91% triple therapy, mostly NNRTI-based</td>
<td>NA</td>
<td>VF=1,000 copies/ml: 26/60 (43%), Genotyping 22/24, HMVR 11/22: NRTI 6.3%, NNRTI 59%, PI 27%</td>
<td>[49]</td>
</tr>
<tr>
<td>2001–2003</td>
<td>Cameroon</td>
<td>109</td>
<td>PC</td>
<td>ARV-naïve, ≥18 years, CD4+ T-cell count &lt;350 cells/mm³ or AIDS, Karnofsky score ≥50%</td>
<td>150</td>
<td>NA</td>
<td>3TC+NVP+ZDV or d4T</td>
<td>70</td>
<td>VF=400 copies/ml: 18% at 24 months, overall HIVDR: 4/109 (3.7%), incidence 3.2/100 persons</td>
<td>[48]</td>
</tr>
<tr>
<td>2003</td>
<td>Uganda</td>
<td>137</td>
<td>CSA</td>
<td>Failing patients on HAART for &gt;12 weeks, mostly ARV-naïve at baseline</td>
<td>163*</td>
<td>A, D</td>
<td>Triple therapy, mostly NNRTI-based</td>
<td>38</td>
<td>VF=400 copies/ml: 46/137 (3%), genotyping 36/46, HMVR 30/36: (mostly K103N)</td>
<td>[53]</td>
</tr>
<tr>
<td>2003</td>
<td>Uganda,</td>
<td>377</td>
<td>CT</td>
<td>ARV-naïve, advanced disease, DART trial</td>
<td>101</td>
<td>A, C, D</td>
<td>3TC+ZDV+TDF</td>
<td>24</td>
<td>VF=1,000 copies/ml: 53/377 (14%), genotyping 20/53, HMVR 18/20 (mostly M184V, K65R less common)</td>
<td>[54]</td>
</tr>
<tr>
<td>1998–2004</td>
<td>Senegal</td>
<td>176</td>
<td>CSA</td>
<td>Failing patients, mostly AIDS and ARV-naïve</td>
<td>144</td>
<td>NA</td>
<td>2 NRTIs+NNRTI or PI</td>
<td>131</td>
<td>VF=500 copies/ml: 22.5% at 30 months, overall HIVDR 22/1(76): NRTI 10%, NNRTI 9%, PI 8%</td>
<td>[51]</td>
</tr>
<tr>
<td>2002–2004</td>
<td>Cameroon</td>
<td>128</td>
<td>CSA</td>
<td>Failing patients on HAART for ≥3 months</td>
<td>NA</td>
<td>CRF02_AG</td>
<td>2 NRTIs+NNRTI or PI</td>
<td>44</td>
<td>Genotyping 35/128, HMVR 21/35: NRTI 13% (mostly M184V, NNRTI 10%, mostly K103N), PI 2%</td>
<td>[52]</td>
</tr>
<tr>
<td>2002–2005</td>
<td>Botswana</td>
<td>155</td>
<td>CSA</td>
<td>Patients failing NFV-based second-line HAART</td>
<td>96</td>
<td>C</td>
<td>ddI+d4T+NFV, NNRTI-based second-line regimen</td>
<td>57</td>
<td>VF: 16/155 (10%), suggested subtype C specific</td>
<td>[40]</td>
</tr>
<tr>
<td>2005</td>
<td>Tanzania</td>
<td>150</td>
<td>CSA</td>
<td>Failing patients on HAART for median ≥6 months</td>
<td>114</td>
<td>A, C, D</td>
<td>3TC+d4T+NVP</td>
<td>52</td>
<td>VF=1,000 copies/ml: 35/150 (23%), genotyping 22/35, HMVR 13/27: NRTI 9%, NNRTI 10%</td>
<td>[57]</td>
</tr>
<tr>
<td>2004–2006</td>
<td>Côte d'Ivoire</td>
<td>106</td>
<td>CSA</td>
<td>Failing patients in ACONDA/ISPED cohort</td>
<td>122</td>
<td>NA</td>
<td>2 NRTIs+NNRTI or PI</td>
<td>163</td>
<td>VF=300 copies/ml: 44/106 (42%), overall HIVDR 23/106 (22%), 30% ≥1 drug class</td>
<td>[55]</td>
</tr>
</tbody>
</table>

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; HAART, highly active antiretroviral therapy; HMVR, 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.
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 months on HAART, 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 HAART 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 HAART 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 counsellors 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, subtherapeutic 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 coinfection 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 HAART. Therapeutic monitoring based on clinical and immunological parameters alone might result in unnecessary switches to second-line HAART 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,
Table 2. Summary of studies on rates of transmitted HIV drug resistance in sub-Saharan Africa

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>n</th>
<th>Study population</th>
<th>Median (CD4^+) T-cell count*</th>
<th>Main HIV-1 subtypes</th>
<th>Reported resistance rate, %†</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000 NA</td>
<td>Abidjan, Côte d'Ivoire</td>
<td>20</td>
<td>ARV-naïve, DAI cohort</td>
<td>84 CRF02_AG</td>
<td>0 0 0 0 0 0 0</td>
<td>–</td>
<td>[80]</td>
</tr>
<tr>
<td>2000 Soweto</td>
<td>Antenatal clinic attendees, ARV-naïve</td>
<td>37</td>
<td>C</td>
<td>479</td>
<td>0 0 0 0 0 0 0</td>
<td>–</td>
<td>[89]</td>
</tr>
<tr>
<td>2000 Lusaka, Zambia</td>
<td>Antenatal clinic attendees in first pregnancy</td>
<td>28</td>
<td>NA C</td>
<td>NA</td>
<td>0 0 0 0 0 0 0</td>
<td>–</td>
<td>[84]</td>
</tr>
<tr>
<td>1997–2000</td>
<td>Abidjan, Côte d'Ivoire</td>
<td>99</td>
<td>Regulars, volunteers and blood donors, estimated time since seroconversion</td>
<td>9.4 months</td>
<td>NA CRF02_AG</td>
<td>0 0 0 0 0 0 0</td>
<td>[91]</td>
</tr>
<tr>
<td>1996–2001</td>
<td>Llonyewe and Blantyre, Malawi</td>
<td>21</td>
<td>ARV-naïve, STD clinic and hospital attendees</td>
<td>NA C</td>
<td>0 0 0 0 0 0 0</td>
<td>–</td>
<td>[88]</td>
</tr>
<tr>
<td>1998–2001</td>
<td>Dakar, Senegal</td>
<td>41</td>
<td>ARV-naïve, subset of SIAARV cohort ARV-naïve</td>
<td>112 CRF02_AG</td>
<td>0 0 0 0 0 0 0</td>
<td>–</td>
<td>[93]</td>
</tr>
<tr>
<td>2001 South Africa</td>
<td>Antenatal clinic attendees</td>
<td>56</td>
<td>Multiple C</td>
<td>376</td>
<td>5.4 0 5.4 0 0</td>
<td>RT: G190A, K103N, A98G</td>
<td>[84]</td>
</tr>
<tr>
<td>2001</td>
<td>11 health districts, Botswana</td>
<td>71</td>
<td>Sentinel survey among antenatal and STD clinic attendees</td>
<td>NA C</td>
<td>0 0 0 0 0 0 0</td>
<td>–</td>
<td>[82]</td>
</tr>
<tr>
<td>2001–2002</td>
<td>Abidjan, Côte d'Ivoire</td>
<td>107</td>
<td>Blood donors (PRIMO-CI) and ARV-naïve women (DITRAME Plus)</td>
<td>395 CRF02_AB</td>
<td>5.6 0.9 3.7 0.9 0</td>
<td>RT: K101E, K103N, P236L, K219Q, PR: N88D</td>
<td>[92]</td>
</tr>
<tr>
<td>2000–2002</td>
<td>Six rural villages, Cameroon</td>
<td>128</td>
<td>Random subset of HIV diagnostic samples</td>
<td>NA CRF02_AB</td>
<td>0 0 0 0 0 0 0</td>
<td>–</td>
<td>[86]</td>
</tr>
<tr>
<td>2002</td>
<td>Dijklouali</td>
<td>47</td>
<td>Subset of general population survey</td>
<td>NA C</td>
<td>10.6 2.1 2.1 6.4 0</td>
<td>RT: L100I, K65R, PR: N88D</td>
<td>[87]</td>
</tr>
<tr>
<td>2002</td>
<td>Four major cities, DRC</td>
<td>70</td>
<td>Subset of sentinel survey population representing various subtypes</td>
<td>NA Multiple</td>
<td>4.3 0 1.4 2.9 0</td>
<td>RT: K103N, V179D, PR: M46L, L90M</td>
<td>[96]</td>
</tr>
<tr>
<td>2002</td>
<td>Burundi</td>
<td>101</td>
<td>Selected subset of sentinel serosurvey</td>
<td>NA C</td>
<td>1.0 1.0 0 0 0</td>
<td>RT: G190E</td>
<td>[97]</td>
</tr>
<tr>
<td>2003</td>
<td>Maputo, Mozambique</td>
<td>58</td>
<td>ARV-naïve, subset of DREAM cohort</td>
<td>361 C</td>
<td>0 0 0 0 0 0 0</td>
<td>–</td>
<td>[81]</td>
</tr>
<tr>
<td>2003</td>
<td>Ouagadougou and Bobo Dioulasso, Burkina Faso</td>
<td>97</td>
<td>Recently diagnosed hospital and treatment centre attendees, median age 33 years</td>
<td>166 CRF02_AB</td>
<td>8.3 2.1 4.1 2.1 0</td>
<td>RT: M41L, T695, V106A, V108I, PR: L13F</td>
<td>[94]</td>
</tr>
<tr>
<td>2001–2004</td>
<td>Yaoundé, Cameroon</td>
<td>102</td>
<td>Recently diagnosed blood donors and hospital attendees, median age 36 years</td>
<td>400 CRF06_cpx CRF_AG</td>
<td>7.8 2.9 2.0 2.9 0</td>
<td>RT: A62V, T69N, V108I, M184V, P236L; PR: L13F, M46/L, V82A</td>
<td>[94]</td>
</tr>
<tr>
<td>2001–2004</td>
<td>Yaoundé, Cameroon</td>
<td>96</td>
<td>Pregnant women attending antenatal care, diagnosed &lt;12 months, ARV-naïve</td>
<td>365 CRF02_AB</td>
<td>2.1 1.0 0 1.0 0</td>
<td>RT: L210W, T215S, PR: N88S</td>
<td>[95]</td>
</tr>
<tr>
<td>2004</td>
<td>Western Cameroon</td>
<td>54</td>
<td>Antenatal/STD clinic attendees, median age 33 years</td>
<td>NA CRF02_AB</td>
<td>13.0 3.7 5.6 7.4 3.7</td>
<td>RT: V75L, L100I, M184V, Y188C, PR: M46/L, V82A</td>
<td>[85]</td>
</tr>
<tr>
<td>2005–2006</td>
<td>Dar es Salaam, Tanzania</td>
<td>39</td>
<td>Sentinel serosurvey (WHO Threshold Survey)</td>
<td>NA A, C</td>
<td>0 0 0 0 0 0 0</td>
<td>–</td>
<td>[90]</td>
</tr>
</tbody>
</table>

Table includes studies published between 2002 and 2007, with a minimum of 20 study participants. Table sorted by year of study. *cells/mm^3. †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.
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 HAART became available in 1996. By contrast, in Africa, where widespread treatment was only introduced when HAART 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 changing sexual behaviour, HAART 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 HAART 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 HAART 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 SD-NVP 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 44.4% in women receiving SD-NVP only, 20.4% in women receiving SD-NVP plus other ante- or intrapartum antiretrovirals and 4% 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 post-partum [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 HAART 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 HAART 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 HAART 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 HAART following SD-NVP, WHO guidelines recommend that HIV-infected mothers and infants who require HAART and have previously been exposed to SD-NVP should still be considered eligible for NNRTI-based regimens [119].

Priorities for public health action and research

As the number of individuals on HAART 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

Table 3. Summary of studies on rates of NVP resistance in women and infants after exposure to single-dose NVP for pMTCT of HIV-1 in sub-Saharan Africa

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>n</th>
<th>Time, weeks*</th>
<th>Main subtypes</th>
<th>Main subtypes</th>
<th>NVPR women, %</th>
<th>NVPR infants, %</th>
<th>Comments</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA</td>
<td>Côte d'Ivoire</td>
<td>29</td>
<td>4</td>
<td>CRF02_AG</td>
<td>21</td>
<td>NA</td>
<td>–</td>
<td>Two-dose NVP</td>
<td>[117]</td>
</tr>
<tr>
<td>NA</td>
<td>South Africa</td>
<td>111+40</td>
<td>4–6</td>
<td>C</td>
<td>67</td>
<td>53</td>
<td>–</td>
<td>–</td>
<td>[116]</td>
</tr>
<tr>
<td>NA</td>
<td>South Africa</td>
<td>456</td>
<td>7</td>
<td>C</td>
<td>39</td>
<td>42</td>
<td>–</td>
<td>–</td>
<td>[113]</td>
</tr>
<tr>
<td>NA</td>
<td>South Africa</td>
<td>155+20</td>
<td>26</td>
<td>C</td>
<td>35</td>
<td>65</td>
<td>–</td>
<td>–</td>
<td>[115]</td>
</tr>
<tr>
<td>NA</td>
<td>South Africa</td>
<td>30+30</td>
<td>6</td>
<td>C</td>
<td>40</td>
<td>40</td>
<td>–</td>
<td>–</td>
<td>[111]</td>
</tr>
<tr>
<td>NA</td>
<td>South Africa</td>
<td>68+9</td>
<td>6</td>
<td>NA</td>
<td>57</td>
<td>78</td>
<td>–</td>
<td>–</td>
<td>[114]</td>
</tr>
<tr>
<td>1997–2001</td>
<td>Uganda</td>
<td>140</td>
<td>1+6</td>
<td>A, D</td>
<td>22</td>
<td>NA</td>
<td>–</td>
<td>–</td>
<td>[41]</td>
</tr>
<tr>
<td>2000–2001</td>
<td>Zimbabwe</td>
<td>32</td>
<td>8</td>
<td>C</td>
<td>34</td>
<td>NA</td>
<td>–</td>
<td>–</td>
<td>[112]</td>
</tr>
</tbody>
</table>

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.
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 HAART 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 HAART 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.

Acknowledgements

This work resulted from the PASER programme which is part of the Linking African and Asian Societies for an Enhanced Response to HIV/AIDS (LAASER) programme. LAASER is a collaboration of the Dutch Aids Fonds, The Foundation for AIDS Research/TREAT Asia, PharmAccess Foundation and the International Civil Society Support group, and is supported in part by a grant from the Ministry of Foreign Affairs of The Netherlands (12454).

Disclosure statement

The authors declare no competing interests.

References


