THE EPIDEMIOLOGY OF HIV-1 TRANSMITTED DRUG RESISTANCE

DINEKE FRENTZ

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General introduction

1 The epidemiology of HIV

The human immunodeficiency virus (HIV) was first discovered in 1983. In 2009, it was estimated that 33.3 (31.4 -35.3) million individuals are infected with HIV worldwide. In that year 1.8 (1.6-2.1) million people died from HIV. Although the virus continues to spread, the number of new infections has fallen from an estimated 3.2 (3.0 -3.5) million in 1997 to 2.6 (2.3 – 2.8) million in 2009. [1] There are several explanations for this decrease. First, the use of antiretrovirals has slowed down the epidemic by suppressing viral replication and thereby the HIV RNA load [2]. This RNA load is a key factor in determining transmissibility of HIV [3]. Second, sexual risk behaviour has decreased in most countries [1]. Third, HIV prevalence follows an 'S' curve, like any infectious disease where it start slowly and gradually. In the final phase of the epidemic, people are either no longer infectious (due to effective treatment) or deaths outnumber new cases, so that the total number alive and infected passes its peak and begin to decline or reach a plateau. [4]

The majority of new HIV infections continue to occur in sub-Saharan Africa. Here, an estimated 1.8 (1.6 -2.0) million people were newly infected in 2009 and 22.5 million (20.9 -24.2) people were living with HIV in this region (figure 1).

In Europe, the HIV-1 epidemic is much smaller, with an estimated 130,000 (110,000-160,000) newly infected in Western and Central Europe in 2009. This results in 820,000 (720,000-910,000) individuals living with HIV-1 and a prevalence of 0.2% in this region. [1]

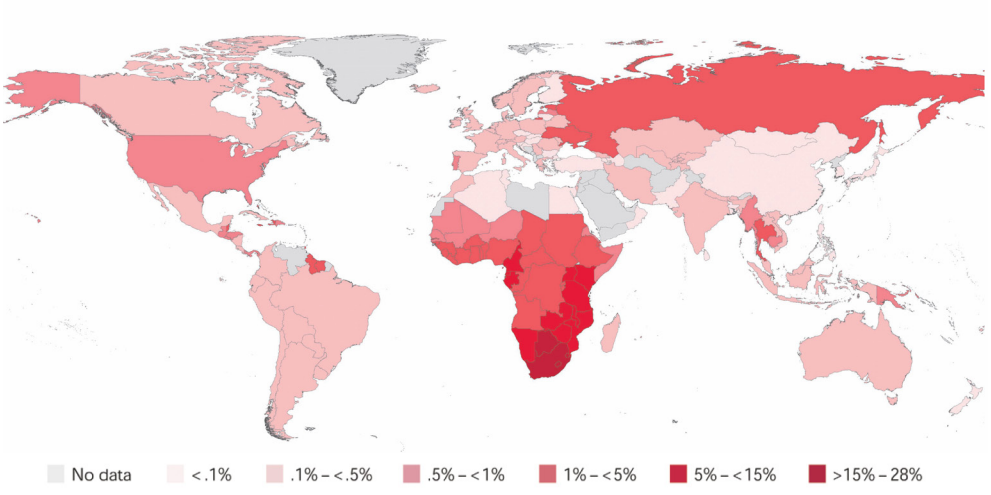

Figure 1. Adapted from www.unaids.org [1].

2 Phases of HIV-1 disease progression

The HIV infection can be broken into three distinct stages: primary infection, chronic infection, and acquired immunodeficiency syndrome (AIDS) [5].

The primary (or acute) infection starts after HIV enters the body. The high replication of the virus initiates an immune response by producing HIV antibodies and cytotoxic lymphocytes. This stage of infection lasts for 10-16 weeks and is often accompanied by a flu-like illness. It is characterized by high viral loads [6] which is the key determinant in explaining transmission of HIV [7]. Many studies observed that recently infected patients account for a disproportionally high number of new infections [8-10]. After the acute stage of HIV infection the patient progresses to the chronic stage which lasts for an average of eight years [11]. The initial immune response leads to a large down-regulation of amount of HIV plasma RNA in the blood. However, the virus is not completely eliminated from the body and viral replication and CD4 decrease is continued at a low level. Because of the low level of plasma HIV-RNA in this stage, the infectiousness of persons in this stage is much lower than of those in the acute stage [12]. If patients remain untreated and CD4 cell numbers have declined below a critical level, the infection leads to the phase of AIDS, where fatal opportunistic infections and cancers can develop. In the AIDS stage, the viral load increases to high levels which again coincide with a high infectiousness [12].

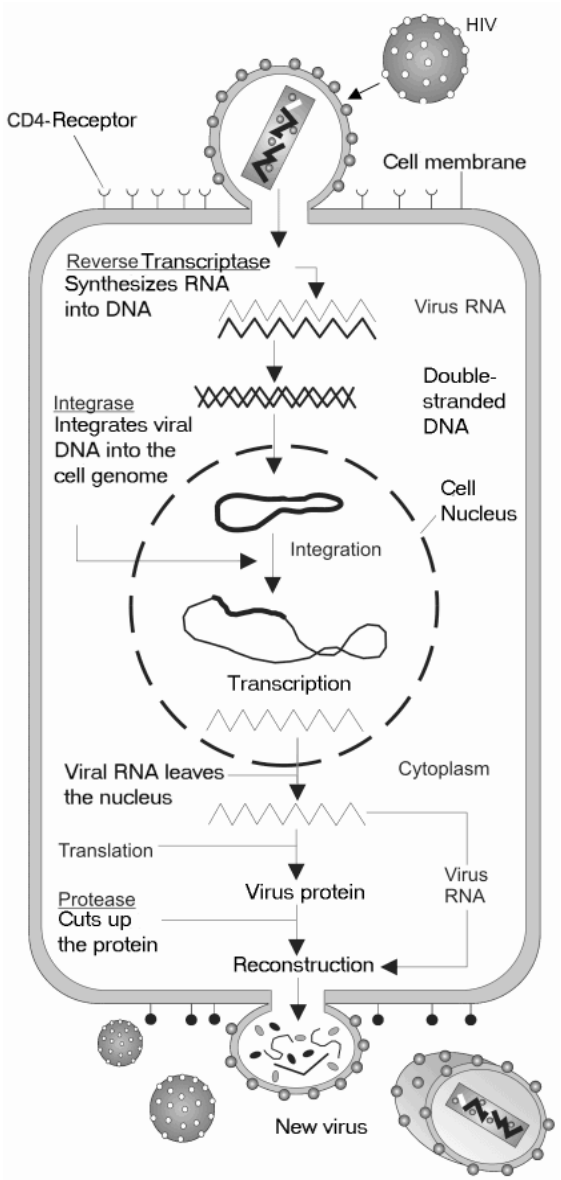

Figure 2. Schematic overview of the HIV replication cycle (adapted from Wikipedia).

3 Antiretrovirals

Currently, six classes of antiretrovirals have been developed and approved for clinical use: nucleos(t)ide reverse transcriptase inhibitor (NRTI), non-nucleoside reverse transcriptase inhibitor (NNRTI), protease inhibitors (PI), fusion inhibitors, entry inhibitors, and integrase inhibitors. Each class inhibits HIV-1 at a different stage in its replication cycle (figure 2).

The NRTIs compose the first class of antiretroviral drugs developed. This class contains 7 drugs that have been Food and Drug Administration (FDA) approved (lamivudine, abacavir, zidovudine, stavudine, didanosine, emtricitabine, tenofovir, and zalcitabine) [13]. The chemical structure of this class of drugs resembles the natural nucleoside [14]. For example, zidovudine contains an azido group in place of the hydroxyl group at the 3' position of the deoxyribose ring (figure 3). Presence of this azido group prevents formation of phosphodiester linkages needed for DNA replication, causing chain termination and thereby suppressing replication of the virus.

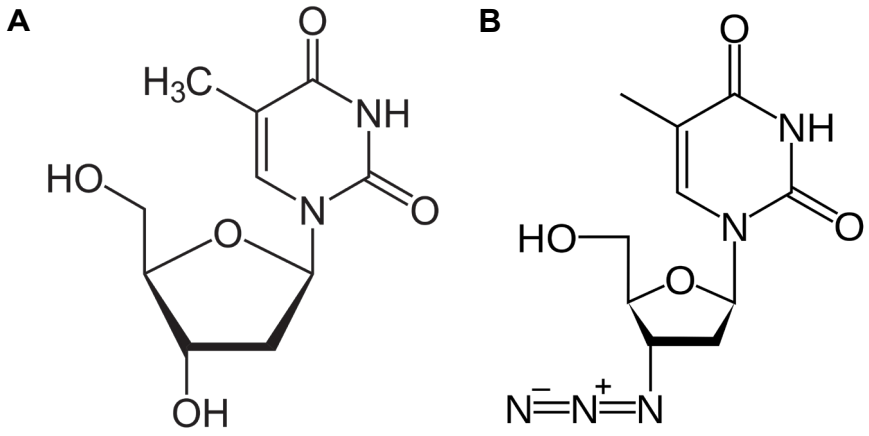

Figure 3. Structural formula of the natural nucleoside thymidine and the therapeutic analogue of a) thymidine and b) zidovudine (adapted from Wikipedia).

The PI drug class was the second class of antiretroviral drugs developed. It consists of 10 drugs approved for use in the treatment of HIV-1 (saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, lopinavir, atazanavir, fosamprenavir, tipranavir, and darunavir) [13]. PI drugs prevent viral replication by inhibiting the activity of the protease enzyme, which is required for cleavage of the HIV precursor polyproteins into proteins that are essential for viral assembly and subsequent activity [14]. The majority of currently available PIs are coadministered with low-dosed ritonavir as a pharmaco-enhancer that significantly increases PI level in plasma (saquinavir, inidnavir, amprenavir, and lopinavir) [15]. Boosting the PI results in a reduced pill burden and the higher plasma levels make it difficult for the virus to develop resistance as more mutations are required to escape the higher plasma levels [16-

19].

In the NNRTI drug class, 5 drugs have been FDA approved (delavirdine, efavirenz, etravirine, nevirapine, and rilpivirine) [13]. These compounds bind to the reverse transcriptase enzyme. This binding results in an enzyme which is incapable of interacting properly with the viral RNA to produce viral DNA [14].

The most recently developed drug classes are the fusion-, entry- and integrase inhibitors. As fusion inhibitor, the drug enfuvirtide has been developed. This binds to the HIV gp41 molecule and thereby inhibits fusion of the viral and the cell membranes [14]. The entry inhibitor maraviroc is a CCR5 coreceptor antagonist. It binds to the CCR5 receptor on the host cell membrane, and thereby prevents the interaction and binding of the HIV-1 gp120 and CCR5. This binding is necessary for the membrane fusion of the viral and the cell membranes [20]. The HIV integrase inhibitor raltegravir was approved by the FDA in 2007 [13]. By blocking the action of the integrase enzyme, the viral genome cannot be inserted into the DNA of the host cell [21].

Currently, HIV-1 treatment consists of a combination of 3 or more antiretrovirals, so-called highly active anti-retroviral therapy (HAART). The use of HAART has strongly reduced morbidity and mortality among patients infected with HIV [22]. Treatment has further improved by the introduction of regimens that are less toxic, have better efficacy (e.g. boosted PIs), and reduced pill burden. Treatment guidelines now recommend to start therapy with 2 NRTIs (tenofovir/emtricitabine or abacavir/lamivudine) and a third agent from another drug class (efavirenz, boosted atazanavir, boosted darunavir, or raltegravir). Furthermore, patients should start treatment when having a CD4 cell counts of <350 cells/mm^3 or <500 cells/mm^3, according to the European [23] and American treatment guideline [24], respectively. The importance of early treatment has been shown by its reduction in mortality [25-27] and the reduction of transmission of HIV [28-33].

For patients living in North America and Western Europe, treatment was highly accessible from the moment the first antiretroviral drugs became available. However, very few people living in the developing world had access to HIV treatment from the introduction of antiretrovirals until the beginning of the 21st century. In 2001, generic drugs were produced resulting in a large reduction in price. The next step was the initiation of the 3 by 5 target (3 million people in low- and middle-income countries on antiretrovirals by 2005) of the WHO. However the largest impact on expanding treatment on global scale was initiated by the President's Emergency Plan For AIDS Relief (PEPFAR) which was a commitment of $15 billion over five years (2003-2008) aimed to provide antiretroviral treatment to 2 million HIV-infected people in resource-limited settings. In 2008, PEPFAR was renewed, revised and expanded to $48 billion through 2013.

Due to this large effort to improve the access to treatment, the number of patients on treatment increased from 400,000 in 2003 to a 1.3 million in 2005. This number even further increased to an estimated 5.2 million people in 2009. However, though now more people are receiving antiretroviral therapy in all regions of the world than at any previous time in the epidemic, many people eligible for treatment still do not have access to treatment. This lack of access is highest in Eastern Europe and Central Asia, sub-Saharan Africa, and Central and South America, with 82%, 63%, and 58%, respectively. [1]

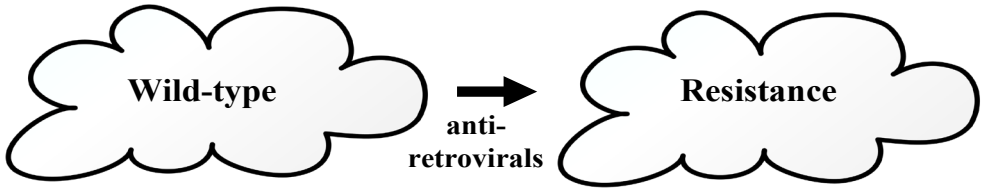

Figure 4. Cloud of quasispecies.

4 HIV drug resistance

4.1 Mechanism of HIV drug resistance

The HIV virus is characterized by its high genetic diversity. First, this high diversity is a result of the high levels of virus production and turnover. Second, HIV has a very high rate of nucleotide sequence evolution which in turn is due to the high error rate of the viral reverse transcriptase. This leads to the generation of many variants of HIV in a single infected patient. The swarm of genetic viral variants is called 'quasispecies'. [34] Viruses with mutations that result in a fitness advantage will outgrow other variants and become the dominant viral population among the quasispecies.

4.2 Acquired HIV drug resistance

In treated patients, drug resistance associated mutations can be acquired when virus suppression is not completely achieved and replication of the virus can continue at low levels (figure 4). The genetic barrier, defined as the number of viral mutations required to escape from the selective pressure of the drug, is an important factor for the development of drug resistance [35-37]. Boosted PIs have a high genetic barrier as they require multiple (3-5) mutations to overcome the drug pressure [19, 35, 37]. Conversely, all other drugs have a low genetic barrier as a single mutation is sufficient for viral breakthrough [35, 37-38]. Many mutations selected by the use of one drug also cause cross-resistance to other drugs of the same drug class, complicating further treatment options. Often, viruses with major resistance mutations have reduced replication rates. This can be compensated by compensatory mutations that emerge after the major mutations. They do not reduce drug susceptibility, but improve the replication of the virus.

4.3 Transmitted HIV drug resistance

Viruses with resistance mutations can be transmitted to other individuals. Because a wild-type is rarely co-transmitted together with the drug-resistant HIV, the quasispecies have no 'memory' of the wild-type [39]. There are 3 possible evolutionary pathways for this transmitted drug-resistant variants described. First, when there is a profound effect on the replication rate of the virus, the resistant variant may revert back to wild-type. Second, atypical variants (a novel amino acid that is neither the wild-type amino acid nor an intermediate towards wild-type) may be observed when it results in higher replication rate than the original transmitted resistant variant. Finally, the resistant variant can persist. Mutations that induce only a limited decrease in the

replication rate tend to persist. Furthermore, in the treated failing patients, multiple compensatory mutations may appear after the initial selection of resistance mutations that lower the replicative capacity. After transmission to a new host, evolution may be expected to occur in a stepwise manner. However, if all possible nucleotide changes would initially decrease the replicative capacity, reversion to wild-type will be blocked. [40]

4.4 Detection of HIV drug resistance

Resistance can be detected both with phenotypic and genotypic assays. The phenotypic assay measures the ability of an HIV-1 variant to grow *in vitro* in the presence of an antiretroviral drug in comparison with the wild-type variant. Genotypic assays identify drug-resistant mutations by sequencing the virus. The cost of a genotypic assay is 50% or less of the price of the phenotypic test. Furthermore, the genotypic assay is performed in only one to two weeks, while for a phenotypic assay up to four weeks may be needed. Therefore, the genotypic test is the preferred test in clinical practice and is recommended by the European HIV Drug Resistance Guidelines Panel [41] and the International AIDS Society-USA Panel [24]. However, genotypic testing is challenging due to the complexity of interpreting the many different drug-resistant mutations and translating these mutations into treatment response. Several interpretation systems have been developed, which provide rules to help physicians interpret genotypic HIV drug resistance results.

When a resistant virus reverts back to wild-type, the initial resistant variant may persist in resting memory cell cells, which can have a very long half life. When such an individual starts treatment, the replication of the wild-type virus will be blocked and the resistance-variants will re-emerge quickly. The current clinically-used genotypic test is population sequencing. This technique fails to identify drug-resistant minority variants that are present in <20% of the virus population infecting a patient [42-43]. These minority variants have been detected in almost 14% of antiretroviral naïve HIV-infected individuals [44]. The presence of minorities, particularly involving NNRTI resistance, is associated with an increased risk of virological failure to first-line therapy [44]. Therefore, the level of resistance is underestimated using the population sequencing assay.

4.5 Patterns of transmitted drug resistance over time

Due to the increased risk for virologic failure when patients start therapy [45], transmitted drug resistance is an important public health concern. Therefore, surveillance of transmitted drug resistance is necessary. Transmitted drug resistance was first detected in patients resistant to the NRTIs zidovudine or stavudine [46-47]. These drugs were initially prescribed to HIV patients in North America and Western Europe as mono- or dual-therapy. This mono- and dual-therapy of zidovudine and stavudine led to a rapid development of thymidine analogue mutations (TAMs) [48-49]. Subsequently a rapid increase was observed in the prevalence of NRTI transmitted drug resistance mutations (TDRM), and specifically the TAMs in North America and Western Europe [50-52]. After 1996 HAART was introduced, which is virologically more active [53-54] and is associated with a substantially lower risk of resistance. Among the most common mutations in treated patients is the M184V [55]. The M184V mutation can be selected by the drugs emtricitabine and lamivudine,

which are both currently popular in first-line regimens [24, 56-59]. This mutation has a strong effect on replication capacity and if transmitted, reverts back to wild-type rapidly (68% after 6 months of HIV infection [60]).

5 Objective of this thesis

This thesis focuses on three topics. First, we investigated the epidemiology of HIV-1 TDRM. Second, we studied different sides of the interpretation of HIV drug resistant mutations. Finally, we performed cost-effectiveness analyses on baseline genotypic testing. The next paragraphs discuss the topics for the different chapters.

Epidemiology of HIV transmitted drug resistance

Substantial differences in TDRM to particular drug classes can be expected over time in different parts of the world due to the differences in drug use as described before. To our knowledge no review has been published summarizing the published articles on TDRM. Therefore, we conducted a review describing available data on HIV-1 transmitted drug resistance mutations, with a major emphasis on the time trends of drug resistance prevalence in the different regions across the world (chapter 2). We identified relevant literature by searching in PubMed through September 2009.

A limitation of this review was the use of many different algorithms in the included studies to interpret, which makes it more difficult to compare the studies. In the WATCH study, we collected and analyzed data of currently available studies on TDRM from across the world using a single algorithm to score drug resistance (chapter 3). Using this approach, we were able to give insights in different profiles of TDRM over time between continents. However, in this study we could not rule out the occurrence of convenience sampling (i.e. an over-representation of patients suspected to carry a drug-resistant virus), due to the different sampling strategies used among the included studies.

In the European SPREAD study, we were able to uniformly sample newly diagnosed patients in a representative way. The SPREAD programme combines the efforts of virologists, clinicians, and public health institutes to study the epidemiology of transmission of drug resistant HIV. The programme started in September 2002 and now includes data until December 2007, enrolling 4,317 patients from 27 countries. In chapter 4, we present the analyses performed on the data including the newest data collected in this programme. In Europe, we expect differences in TDRM to the particular drug classes over time also due to changes in use of treatment over time in Europe, as described before. Therefore, the objective in this study was to determine the trends in transmitted drug resistance in newly diagnosed HIV-1 infected patients over time in Europe.

The SPREAD study also resulted in the study described in chapter 5. Here, we further explored the prevalence of TDRM in the three main HIV transmission groups: men who have sex with men, heterosexual patients, and injection drug users. The prevalence of HIV resistance-associated mutations are expected to be different among different routes of transmission. Men having sex with men (MSM) mostly originate from western countries where antiretroviral drugs have been available for many years. In contrast, heterosexual patients mostly originate from Sub-Saharan

Africa where large scale antiretrovirals have only been available recently. Injection drug users infected with HIV are mostly found in Central and East European countries where the coverage of antiretrovirals in HIV patients in need of treatment has been low in many countries [61]. These differences in drug use between the transmission groups are reflected in several studies showing a higher likelihood in MSM patients to be infected with a resistant virus compared to other patients [62-64]. Furthermore, due to differences in the use of these drug classes over time, TDRM to specific drug classes are expected to have evolved differently over time. Yet, there are no European-wide studies performed analysing time trend of the prevalence of TDRM in the different transmission groups. In chapter 5, we present the time trends in the prevalence of TDRM in the different transmission groups for the main drug classes in Europe.

Although travel and migration played a key role in the early spread of HIV, it is not known to what extent travel currently explains transmission of HIV. We therefore performed phylogenetic analyses on the patient data of the SPREAD programme to estimate the proportion of individuals newly diagnosed with HIV that was infected within their own country (chapter 6).

The interpretations of HIV-1 drug resistant mutations

The estimation of the prevalence of transmitted drug resistance using genotypic testing has some limitations. Two of these limitations are discussed in chapters 7 and 8. In chapter 7, we focus on the difficulties of the interpretation of mutations towards therapy response. Several algorithms for the interpretation of HIV-1 genotypic drug resistance information have been designed [65]. These interpretation systems provide rules to help physicians interpret the drug mutations. The purpose of our study was to compare the different interpretation systems that have been developed. The three most commonly used interpretations systems, ANRS, Stanford HIVdb, and Rega have been validated in different studies [66-68]. To compare the systems, it is important to include virological response data in correlation with the prediction of interpretation systems. We performed a comparison between the systems in patients with virological failure (transmitted and acquired resistance) using three different virological outcome time points.

Another limitation of genotypic testing is the possibility of overestimating the prevalence of TDRM due to the presence of low-level polymorphisms. These polymorphisms are naturally occurring amino acid substitutions at positions associated with antiretroviral drug resistance. We examined how these polymorphisms influence the classification method developed by the WHO used for global surveillance of TDRM in resource-limited countries. This is presented in chapter 8.

Cost-effectiveness of baseline genotypic testing

The use of genotypic testing has been proven. However, the cost-effectiveness analyses that have been published were all performed before the year 2001 [69-71]. Because of changes in TDRM, decrease in rates of opportunistic infections and mortality, and changes in health care costs, we investigated the cost-effectiveness of baseline genotypic testing (chapter 9).

The results of this thesis are summarized and discussed in chapter 10.

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2

Temporal changes in the epidemiology of transmission of drug resistant HIV-1 across the world

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ABSTRACT

Background: A substantial number of studies have been performed across the world to determine transmitted drug resistance. Large variations between different parts of the world can be expected because of differences in availability over time of treatment. Time trend analyses are often not possible, because of small numbers of included patients. In this review, we present the available data on the transmission of drug resistant HIV with a major emphasis on the time trends of drug resistance prevalences.

Methods: We identified relevant literature by searching in PubMed through Sept. 2009. Studies were grouped, according to the year of data-collection, into the following time periods: <2001, 2001-2003, >2003.

Results: We selected a total of 215 studies which included 43,170 patients. The following prevalences of transmission of drug resistant HIV were found in rank order: North America (12.9%), Europe (10.9%), Latin America (6.3%), Africa (4.7%) and Asia (4.2%). Changes over time in particular drug classes were found in all parts of the world. Nucleoside reverse transcriptase inhibitor (NRTI) resistance was declining over time in North America (p-value: 0.03), Europe (p-value: <0.001) and Latin America (p-value: <0.001). The decline in NRTI resistance reflects the improvement of treatment regimens in resource-rich settings. In contrast NRTI resistance prevalence was increasing in Asia (p-value: 0.047) and Africa (p-value: <0.001). This can be explained by the antiretrovirals becoming more available during recent years in these continents.

Conclusions: Non-nucleoside reverse transcriptase inhibitor (NNRTI) resistance was rising over time in North America (p-value: <0.001), Europe (p-value: <0.001), Latin America (p-value: <0.001), and Asia (p-value: 0.01). This paper gives a complete overview of the epidemiology of resistance of antiretroviral drug in drug-naïve patients worldwide. The time trends that were observed seem to reflect changes in describing prescriptions over time. Changes include the more wide-spread of anti-retroviral drugs in developing countries and the development of therapies from low-active mono-therapies to highly active anti-retroviral regimens in the industrialized countries.

INTRODUCTION

The use of highly active antiretroviral therapy has substantially improved survival among patients infected with HIV-1. But the success of antiretroviral treatment can be limited by the emergence of HIV drug resistance which in turn can be transmitted to newly infected individuals. Transmission of drug resistance is associated with an increased risk for virological failure 12 months after start of treatment [1].

A large number of studies reported on transmitted drug resistance across the world. These studies report a prevalence of transmitted drug resistance that ranges between 0 to 25% [2-4]. The prevalence is lowest in resource-limited settings [5]. But the prevalence in resource-limited countries may have increased in recent years as access to antiretroviral drugs has been expanding.

Substantial differences in resistance to particular classes of antiretroviral drugs may exist over time between different parts of the world. For example, use of nevirapine in Africa to prevent mother-to-child transmission could have increased the prevalence of transmitted resistance to non-nucleoside reverse transcriptase inhibitors (NNRTI) in Africa [6]. Similarly, in resource-rich settings zidovudine was given as mono-therapy before 1996 resulting in transmitted nucleoside reverse transcriptase inhibitor (NRTI) drug resistance [7-8]. In recent years, other classes of antiretrovirals have become popular which could have changed the epidemic of transmission of drug resistance.

We conducted a systematic review of literature to compare temporal changes in the prevalence of transmission of drug resistant HIV-1 across different continents.

Selection of studies on transmitted drug resistance

PubMed was used to identify studies written in English on the epidemiology of transmission of drug resistant HIV-1, until Sept 1st 2009 (key words “HIV” and “resistance” or “HIV” and “transmission”). Primary research studies that investigated the prevalence of HIV drug resistance in antiretroviral naïve HIV-1 infected persons were eligible for inclusion.

Transmitted drug resistance was reported in 215 papers including 43,170 patients (table 1). Most studies came from Europe (82 studies/ 25,446 patients), followed by Africa (47/ 3,096), North America (36/ 8,718), Latin America (26/ 3,218), Asia (23/ 2,507), and Australia (1/ 185). The characteristics of included patients varied among the continents. The proportion of risk groups per continent in the included studies followed the regional mode of HIV-1 transmission across the world. For example, in North America and Europe, patients were predominantly infected through men having sex with men (MSM) (41% and 47%, respectively), whereas in other continents this did not exceed 20%, as described in literature [9].

Definition of transmission of drug resistance

We compiled transmitted drug-resistance as reported in the studies. Resistance to NRTI, NNRTI, and protease inhibitors (PI) was defined as the presence of at least one drug resistance associated mutation to that particular drugs class. Multiclass resistance was defined as the presence of resistance-associated mutations to at least two different classes of antiretroviral drugs. The list used to define transmitted drug resistance was extracted from the studies.

Statistical analysis

Time trends were analyzed by grouping the studies according to the year of data-collection: before 2001, 2001-2003, and 2004 or later. We used these cut-offs so that we could include time periods with comparable numbers of patients. Taking different time periods did not result in different trends over time (data not shown). Studies reporting the epidemiology of transmission of resistance over a range of years were grouped according to the average of the years.

Sixteen studies did not report the year of data collection. The average difference between year of data collection and year of publication was 4 years. We therefore calculated the missing data-collection years, by subtracting 4 years from the year of publication. Exclusion of these studies or subtraction of 0, 2, or 6 years from the year of publication did not change the results (data not shown).

Prevalence estimates are presented with 95% confidence intervals calculated according to the Wilson score interval. Poisson regression analysis was used to calculate the time trends analyses for each continent.

Epidemiology of transmission of drug resistance

Europe

The studies were predominantly performed in Western-Europe (n=75). A smaller number of studies (n=7) came from Central Europe and the former Soviet-Union. Studies from the former Soviet-Union are of particular interest as this part of the world has the strongest growing epidemic world-wide due to an explosive outbreak of HIV-1 infections among intravenous drug users [10-12].

The prevalence of transmission of drug resistance across Europe was 10.9% (95% confidence interval 10.6-11.3%) (figure 1). Transmission of drug resistance most frequently involved NRTIs with a prevalence of 7.4% (7.1-7.7%). The prevalence of resistance to NNRTIs was with a prevalence of 3.4% (3.2-3.6%) slightly higher than the prevalence of 2.9% (2.7-3.2%) found for protease inhibitors.

Transmission of drug resistance declined over time in Europe (figure 2). The prevalence was around 11.5% before 2003 and reduced to 7.7% after that year ($p<0.001$). A closer examination of the classes showed that this decrease was ascribed to the decline in resistance to NRTI (from 8.0 to 4.3%) and protease inhibitors (from 3.3 to 1.4%) (both p-values: <0.001). Resistance to NNRTIs increased from 2.9% to a small peak in 2001-2002 of 4.4%, after which it decreased again to 3.2% (p-value: 0.004).

Two European studies that reported on the epidemiology of transmitted drug resistance over time confirm our results. First, the pan-European SPREAD programme also reported a decrease in the prevalence of transmitted NRTI resistance and an increase in the prevalence of transmitted NNRTI resistance over time (2002-2006). These changes were however not statistically significant which could be ascribed to a smaller sample size in the SPREAD programme [13]. The second study confirming the decline in transmitted drug resistance over time was performed in the United Kingdom. This study reported a small increase in NRTI resistance, with some evidence of a levelling off from 1996 to 2003. This British study also reported an increase in transmission of NNRTI resistance [14].

North America

Europe and North America have the longest access to antiretrovirals across the world. There were, however, several differences between the two continents. In North America, the prevalence of transmission of drug resistance was higher with a proportion of 12.9% (12.2-13.7%). Similar to Europe, transmission of drug resistance was for the largest part ascribed to NRTIs; prevalence 7.4% (6.8-8.0%). But transmission of NNRTI resistance was in North America with a proportion of 5.7% (5.2-6.2%) higher than the prevalence of 3.4% found in Europe. Similar to Europe, resistance to protease inhibitors was also uncommon in North America with a prevalence of 3.2% (2.8-3.6%) as compared to 2.9% in Europe.

Contrary to Europe, the prevalence of resistance showed an increase over time from 11.6% (10.7-12.7%) in studies performed before 2001 to 14.3% (12.8-16.1%) in studies performed after 2003 (p-value: 0.003) (Fig. 2). This increase in overall transmitted resistance was ascribed to the increase in NNRTI resistance (from 4.1% to 8.3%, p-value: <0.001), whereas the NRTI resistance was decreasing from 8.0% to 6.4% (p-value: 0.032).

Studies that included longitudinal data confirm the time trends we observed. A study performed in San Francisco showed a decrease in transmitted NRTI resistance from 21% in 1996-1997 to 3.3% in 1998-1999 and a subsequent increase to 6.2% in 2000-2001 [8].

The decline in NRTI resistance in resource-rich settings reflects the improvement of treatment regimens. Before 1996, antiretroviral therapy consisted of mono-therapy or dual-therapy of NRTI's, which lead to the appearance of drug-resistant HIV-1 in many patients [15-16]. After 1996, HAART was introduced, which is virologically more active and is associated with a substantially lower risk of resistance. As a consequence, NRTI resistance was initially high and then decreased in recent years.

The increase of NNRTI resistance in Europe and North America coincides with the more frequent use of this drugs class in the developed world in the previous years. NNRTIs were approved in 1996 and clinical trials in 1999 indicated that virologic outcomes during treatment with this drugs class were better compared with those of PI-based treatment [17].

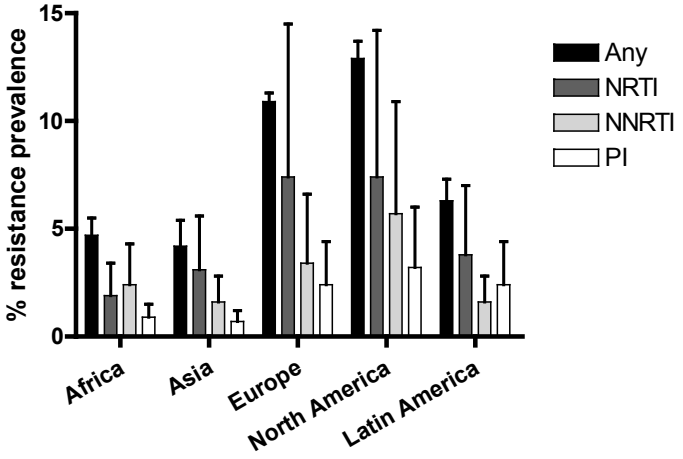

Figure 1. Prevalence of transmitted drug resistance to any of the drug classes (Any), NRTI (nucleoside reverse transcriptase inhibitor), NNRTI (non-nucleoside reverse transcriptase inhibitor) and PI (protease inhibitor) in Africa, Asia, Europe, North America, and Latin America.

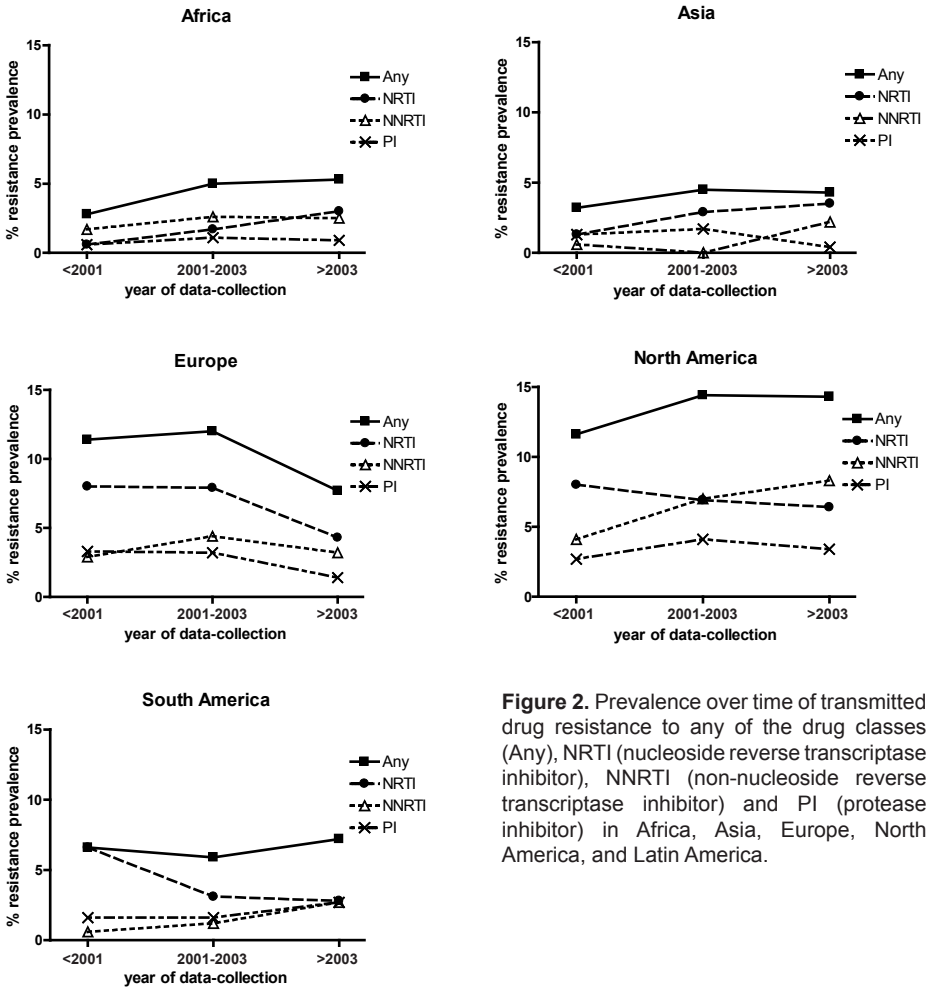

Figure 2. Prevalence over time of transmitted drug resistance to any of the drug classes (Any), NRTI (nucleoside reverse transcriptase inhibitor), NNRTI (non-nucleoside reverse transcriptase inhibitor) and PI (protease inhibitor) in Africa, Asia, Europe, North America, and Latin America.

Latin America

Large Latin American countries as Argentina and Brazil have sponsored a policy of universal access to antiretroviral drugs since the 1990s. Interestingly, transmission of drug resistance was reported in 6.3% (5.5-7.3%) of HIV-1 patients from Latin American studies suggesting that universal access did not result in high levels of resistance.

Studies from Latin America reported a low prevalence of transmission of drug resistance to the different drug classes, with 3.8% (3.2-4.6%) for NRTI, 1.6% (1.2-2.1%) for NNRTI and 2.4% (2.0-2.8%) for protease inhibitors. The time trends for resistance to particular classes followed the same trend as in Europe and North America. Resistance to NRTIs decreased over time (6.6% to 2.8%, p-value: <0.001). The prevalence of transmission of NNRTIs increased from 0.6% to 2.7% (p<0.001).

Resistance to protease inhibitors increased but remained limited (from 1.6% to 2.7%, p-value: 0.01).

Transmitted drug resistance to protease inhibitors was uncommon in all parts of the world (less than 3.2%). This may be explained by the high genetic threshold for resistance to boosted protease inhibitors. Moreover, protease inhibitors are not used in treatment of all patients as they are frequently reserved for second line therapy.

Africa

Transmission of drug resistance was variable in Africa and 30 out of 47 studies reported a prevalence <5%. The combined prevalence of transmission of drug resistance in studies from Africa was low with a proportion of 4.7% (4.0-5.5%). However, many parts of Africa do still not have access to antiretrovirals. Epidemiological studies on transmitted resistance will not be performed in these areas as resistance is unlikely. Therefore, the transmitted resistance prevalence that we calculated from the available studies performed in Africa is an overestimation of the real prevalence in this continent.

Importantly, transmission of drug resistant HIV increased over time. The prevalence was 2.8% (1.7-4.5%) before 2001 and almost doubled to 5.3% (4.0-6.9%) after 2003. This increase was, however, not statistically significant (p=0.06). The increase can be explained by the increase in NRTI drug resistance over time from 0.6% before 2001 to 3.0% after 2003 (p-value: <0.001). The prevalence of PI resistance was low (0.9%; CI: 0.6-1.3%) and NNRTI prevalence showed a non-significant increase from 1.7% to 2.5%.

In Africa, different patterns of resistance to particular antiretroviral drug classes were seen as in other parts of the world. Contrary to the Americas and Europe, the prevalence of NRTI resistance was increasing over time. This increase can be explained by the antiretrovirals becoming more widely available during recent years (e.g. due to the efforts of the Global Fund and PEPFAR -President's Emergency Plan for AIDS Relief). Due to the increased use of HAART (which includes NRTIs as the backbone), resistant mutations have developed, and as a consequence transmitted NRTI resistance in Africa has been rising.

A high proportion of NNRTI-resistance was initially observed and is decreasing over time. This high contribution reflects the prophylactic use of a single dose of NNRTI-monotherapy for prevention of mother-to-child-transmission [6, 18]. Due to the low genetic threshold for resistance to NNRTIs, viral resistance could be induced [19]. Currently, the WHO recommends combinations of different antiretroviral drugs (including NRTIs) to prevent vertical transmission, instead of using the simplest regimen of single-dose nevirapine [20]. Furthermore, universal access of HAART has been scaled up in developing countries [21-22]. As a consequence, transmitted NRTI resistance has increased and the contribution of NNRTI resistance to the total resistance has decreased.

Asia

We found a lack of data on transmission of drug resistant in Asia. Data from Asia should therefore be interpreted with caution. Only a low number of studies (and patients) could be extracted from literature. Consequently, time trend analyses showed less significant results. For example, the overall resistance prevalence of

4.2% (3.4-5.4%) was stable over time (p-values: 0.496). However, NRTI and NNRTI resistance were slightly increasing from 1.3% to 3.5% (p-value: 0.047) and 0.6% to 2.2% (p-value: 0.01), respectively. Transmitted resistance to protease inhibitors declined over time from 1.3% to 0.4% (p-value: 0.02).

Oceania

Only one study was included from Australia in this review. This study reported a high prevalence of 23.2% (17.7-29.8%). No further analyses were performed with this data.

DISCUSSION

In this review, we examined all literature available on HIV-1 transmitted drug resistance epidemiology. Reviewing all literature on this subject allowed us to calculate the change over time in the prevalence of transmission of drug resistant HIV-1 for the different drug classes in each continent.

The prevalence of transmitted resistance ranged between 0% e.g. [23-26] and 27% [8]. This means that most HIV infections are with a virus that is susceptible to antiretrovirals. There were, however, clear differences across the world. The highest prevalence of transmitted resistance was found in North America (12.9%) and Europe (10.9%) in which antiretroviral drugs are available for prolonged periods of time. Lower proportions of transmitted resistance were found in Latin America (6.3%), Africa (4.7%), and in Asia (4.2%).

Time trends observed in this study may be caused by true differences in temporal changes in treatment regimens between continents, or by others sources of variability. An important factor may be the inclusion of recent or chronic infected patients, a distinction sometimes made in studies performed in resource-rich countries. Resistance in recently infected patients has been reported to be higher than resistance in patients infected >1 year [27]. This can be explained by several factors. First, the difference partly reflects the variation of resistance prevalence among different HIV risk groups. The majority of the recently infected patients are MSM [28]. Transmitted drug resistance is often much higher in MSM HIV infected patients compared to the heterosexual risk group, because most HIV patients who acquired HIV through heterosexual contact are more likely to come from regions with limited access to antiretroviral drugs [13, 29]. In addition, the lower prevalence of transmitted drug resistance in chronic patients can be explained by the outgrowth of the wild type or the reversion of the transmitted drug resistance mutations. Remarkably, some resistance viruses remain present in patients, despite the negative effect on replication capacity, due to the appearance of compensatory mutations and the reduced replication capacity of the required intermediate viruses [30]. In this review, the effect of differences between studies in including recently or chronically infected patients on the time trends is probably limited, as most differences in studies were seen between continents and not over time.

Another source of variation in resistance prevalence between studies may be the use of different methods to define drug resistance. The majority of the studies we included have defined resistance either with the IAS-USA or the Stanford genotypic

resistance interpretation algorithm. However, the use of different algorithms to score resistance may not have a large impact. This is supported by a previous study reporting that scoring resistance using the IAS-USA mutation list of 2006 [31], or the Stanford HIVdb (version 4.3.0, 2007) or the Shafer list of 2007 [32] was associated with comparable levels of transmitted drug resistance in 8272 genotypic resistance tests of drug-naïve patients conducted during 1997-2005 [33].

This review is limited by the data that could be extracted from published reports. Convenience sampling (i.e. an over-representation of patients suspected to carry a drug resistant virus) may have an impact on the prevalence estimates. Although we cannot rule out that convenience sampling occurred, the vast majority of included studies used well-defined sampling strategies to identify relevant patients.

Heterogeneity is another bias that can occur within reviews. Heterogeneity applies to differences in the strategy used to sample patients and in research methodology. We reduced the heterogeneity by taking into account the year of data-collection and performing analyses per continent.

The studies that were collected used population sequence analysis. This method fails to detect minor populations of drug-resistant quasi-species [34]. As resistance variants in the absence of drug-selection pressure in the antiretroviral naïve host may be present in minority viral variants population-sequence analysis will underestimate the prevalence of drug resistant HIV-1.

Despite these shortcomings, this review is the first, to our knowledge, to summarize all the published articles on transmitted drug resistance.

CONCLUSION

In this paper, we gave an overview of the epidemiology of resistance to antiretroviral drug in drug-naïve patients worldwide. The resistance profiles of the three antiretroviral drug classes seem to be different among continents and reflect changes in prescribing behaviour of antiretroviral drugs. Although the prevalence of resistance to antiretroviral drugs decreases, resistance can become a larger problem in third world continents, where antiretroviral drug therapy is becoming more widespread. Continuous global surveillance is needed to monitor the circulating HIV-strains and ensure that the development of treatment is adjusted to the drug resistance evolution.

SUPPLEMENTARY DATA

Supplementary data is available in the APPENDIX.

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APPENDIX

Table 1. Summary of studies on transmitted drug resistance per continent

Ref.	Region	Methodology	Years of sampling	(n)	Africa										
					HIV risk factor (%)			Resistance prevalence (%)					Population		Sampling of patients
					MSM	IVDU	HSX	Any	NRTI	NNRTI	PI	MDR	Recent (time to infection)	Newly diagnosed	
[35]	Africa	Stanford HIVdb	1995-1999	142				3.5	0	3.5	0	0			From hospital serosurveillance study
[36]	Gabon	Stanford HIVdb 2001	2000	13				0	0	0	0	0			
[37]	Cote d'Ivoire	Not reported	1997-2000	99				0	0	0	0	0	99 (<3 years)		Blood donors
[38]	Cameroon	Not reported	1998	110							1.8				Individuals attending a (military) hospital
[39]	South Africa	Not reported	2000	37				0	0	0	0	0			Drug-naive pregnant woman enrolled in study to prevent MTCT
[40]	Cote d'Ivoire	IAS and ANRS, version not reported	2001-2002	107			100	5.6	0.9	3.7	0.9	0			Blood donors and pregnant from a MTCT prevention study
[41]	South-Africa	Stanford HIVdb	2001	72				5.4	0	5.4	0	0			Representing patients attending clinics
[42]	Zambia	Stanford HIVdb, IAS 1998[43]	2000	28			100	0	0	0	0.0	0			Pregnant woman
[44]	Senegal	Stanford HIVdb	1998-2001	41				0	0	0	0	0			Patients enrolled for ARV therapy
[45]	Cote d'Ivoire	IAS 1998[43]	Unreported	20				0	0	0	0	0			Patients enrolled for ARV therapy
[46]	Cameroon	Birk 1998[47]	Unreported	128							0				HIV-1 infected patients from 6 provinces
[48]	South Africa	Stanford HIVdb	2001	14	0	0	100	0	0	0	0	0			HIV infected outpatients at a clinic
[49]	Botswana	Shafer[50]	2001	70				0	0	0	0	0			HIV-1 infected patients from 11 health districts
[51]	Mozambique	Stanford HIVdb	2003	58				0	0	0	0	0			HIV patients visiting hospitals and enrolled for a therapy program
[52]	Malawi	Stanford HIVdb	1996-2001	21				0	0	0	0	0			Patients attending to hospitals and woman participating in a breast-milk study
[53]	Djibouti	IAS 2005[54]	2002	47				21.3	2.1	14.9	6.4	2.1			Patients randomly selected for HIV test
[55]	Cameroon	IAS 2005[54]	2004	54				13.0	3.9	5.9	7.4	3.7			Patients attending clinics
[23]	Nigeria	Not reported	2005	18				0	0	0	0	0			Patients enrolled for a therapy program
[56]	From 7 countries in Africa	ANRS Sept. 2004, Stanford 2005, Rega version 6.2	1996-2003	35				11.4	0	11.4	0	0			Patients attending hospitals

(continue)

Table 1. Summary of studies on transmitted drug resistance per continent (*continued*)

Africa														
Ref.	Region	Methodology	Years of sampling	(n)	HIV risk factor (%)			Resistance prevalence (%)					Population		Sampling of patients
					MSM	IVDU	HSX	Any	NRTI	NNRTI	PI	MDR	Recent (time to infection)	Newly diagnosed	
[57]	Democratic Republic of Congo	Stanford HIVdb	2002	70				4.3	0	1.4	2.9	0			Patients from a sentinel population groups
[58]	Cameroon	ANRS Sept. 2005	2001-2004	96				2.1	1	0	1	0			Pregnant woman
[59]	Burkina Faso	Stanford HIVdb	2003-2004	38				5,3	0	5,3	0	0			Pregnant woman and other HIV positive patients attending the hospital
[5]	Cameroon	IAS, version not reported	2001-2002	102				7.8	2.9	2.0	2.9	0			Patients attending hospitals and treatment centers
[60]	Nigeria	Stanford HIVdb	Unreported	RT: 35 PR: 43				17.1	8.6	11.4	0	2.8			Patients enrolled for a treatment program
[61]	Burkina Faso	Stanford HIVdb	2003-2004	17				0	0	0	0	0			Patients from a HIV centre
[5]	Burkina Faso	IAS, version not reported	2003	97				8.3	2.1	4.1	2.1	0			Patients attending hospitals and treatment centers
[62]	Madagascar	ANRS Sept. 2005	2005	28				3.6	0	0	3.6	0			HIV surveillance in sex workers, STD patients and pregnant women
[63]	Central African Republic (CAR)	IAS fall 2005[64], ANRS Sept. 2005	2005	53				0	0	0	0	0			PMTCT program
[65]	South Africa	Stanford HIVdb Algorithm	2001-2004	40				5.0	0	0	5.0	0			HIV patients attending a clinic
[66]	Mozambique	IAS 2005[54]	2003	43				14.0	4.7	7.0	2.3	0			Patients attending clinics
[67]	Burundi	IAS 2005[54], ANRS 2005. 7, Stanford db 4.1.7, Rega 6.4.1	2002	101				1.0	0	1.0	0	0			HIV sero-surveillance study with samples from sentinel population groups in four major cities
[68]	Mali	IAS fall 2005[64]	2005	98			100	2	1	1	0	0			Patients attending a clinic
[69]	Cote d'Ivoire	IAS fall 2006[31]	2002-2006	100				6.0	3.0	2.0	1.0	0			Blood donors
[70]	Uganda and Rwanda	Not reported	Unreported	97				7.2	4.1	3.1	0	0			Plasma samples from pregnant women at week 36 of gestational age
[71]	Cameroon	IAS fall 2006[31]	2004	79				13.9	7.6	8.8	2.6	5.1			Patients attending clinics
[72]	Swaziland	IAS fall 2006[31]	2002-2003	47				2.1	0	2.1	0	0			Randomly chosen plasma samples from HIV patients
[73]	Uganda	IAS 2007[74]	2006-2007	46				0	0	0	0	0		46	Patients attending a clinic
[75]	Tanzania	Shafer 2007[32]	2005-2006	39				0	0	0	0	0			Surveillance among pregnant women
[76]	South Africa	Shafer 2007[32]	2002	65				0	0	0	0	0			Young women with first pregnancy
			2004	48				4.2	4.2	0	0	0			

(continue)

Table 1. Summary of studies on transmitted drug resistance per continent (*continued*)

Africa

Ref.	Region	Methodology	Years of sampling	(n)	HIV risk factor (%)			Resistance prevalence (%)					Population		Sampling of patients
					MSM	IVDU	HSX	Any	NRTI	NNRTI	PI	MDR	Recent (time to infection)	Newly diagnosed	
[77]	Malawi	Shafer 2007[32]	2006	34				0	0	0	0	0			PMTCT patients program
[78]	Ethiopia	Shafer 2007[32]	2005	39				0	0	0	0	0			Pregnant women in surveillance study
[79]	Swaziland	WHO list	2006	34				0	0	0	0	0			Young women with first pregnancy
[80]	Mozambique	Shafer 2007[32] and Shafer 2008[81]	2002-2004	104				3.8	3.8	1.0	0	1.0			HIV-1 patients attending hospitals and clinics
[82]	Angola	Shafer 2008[81] and 2007[32]	2001	122	4		70	1.6	1.6	0.8	0	0.8			HIV patients naive for ARV therapy
[83]	Uganda	Shafer 2007[32]	1998-2003	104				5.8	2.9	0	2.9	0			Annual testing of individuals in a longitudinal study
[84]	Burkina Faso	Stanford HIVdb and IAS 2008[85]	2004-2006	104				12.5	10.6	6.1	0	3.8			Patients attending a HIV hospital
[86]	Central Africa	Shafer 2007[32]	2006-2007	102											HIV infected woman with first
				34				0	0	0	0	0			pregnancy < 25 years
				34				5.9	2.9	2.9	0	0			
				34				8.8	5.9	2.9	0	0			

North America

Ref.	Region	Methodology	Years of sampling	(n)	HIV risk factor (%)			Resistance prevalence (%)					Population			Sampling of patients
					MSM	IVDU	HSX	Any	NRTI	NNRTI	PI	MDR	Recent (time to seroconversion)	Recent (time between neg. and pos. test)	Chronic (time to diagnosis)	
[87]	USA	IAS 1998[43]	1997-1998	31	50		50	25.8	6.5	12.9	16.1	6.5	31 (< 3 years)			HIV patients assigned to a military bases
[88]	Canada	IAS 1998[43]	1996-1998	57		100		0	0	0	0	0	57 (< 15 months)			Injection drug users infected with HIV
[89]	USA	VircoGEN interpretation software	1997-1998	95				22.1	4.2	14.7	10.5	3.2	54 (< 1 year), 37 (< 2 years), 6 (2-3 years)			Military personnel with HIV infection
[90]	USA	IAS 1998[43]	1993-1998	99	26	3	71	7.1	6.1	2.0	1.0	2.0		99 (< 2 years)		HIV patients attending public STD clinics
[91]	USA	IAS 1998[43]	1997-1998	458				6.3			3.8					Naive HIV patients attending the HIV/AIDS Centre
[92]	USA	Not reported	1998-1999	199	74	3	15		14	16	3		(< 3 years)	(< 3 years)		Prospective study with ARV-naive patients

(continue)

Table 1. Summary of studies on transmitted drug resistance per continent (*continued*)

North America															
Ref.	Region	Methodology	Years of sampling	(n)	HIV risk factor (%)			Resistance prevalence (%)					Population			Sampling of patients
					MSM	IVDU	HSX	Any	NRTI	NNRTI	PI	MDR	Recent (time to seroconversion)	Recent (time between neg. and pos. test)	Chronic (time to diagnosis)	
[93]	USA	Visible Genetic software	1999	44	34	7	55	11	4.5	4.5	2.3	0			44	HIV patients who planned to initiate ARV therapy in a HIV clinic
[94]	USA	Hirsch 2000[95]	1996-2001	62	41.9	4.8	53.2	12.9	8.1	3.2	3.2	1.6			62 (> 6 months and < 5 years)	Drug-naive patients attending a outpatients clinic
[8]	USA	IAS 2001[96]	1996-1997	40	80			25.0	25.0	0	2.5	2.5	(0)	(< 12 months)		Patients recruited through physicians, HIV-1 testing and counseling sites, community health centers, and self-referral
			1998-1999	94	86			18.1	7.4	6.4	5.3	1.1				
			2000-2001	91	89			27.4	20.9	13.2	7.7	14.3				
[97]	USA	IAS 2000[95]	1995-1998	213				8	8.5	1.7	0.9	3.8	(< 12 months)	(< 12 months)		Subjects with signs or symptoms of an acute HIV infection
			1999-2000	88				22.7	15.9	7.3	9.1	10.2				
[98]	USA	IAS 2000[95]	1997-1999	69	84	3	6	4.3	1.4	2.9	0	0		58 (< 2 years)		Recently infected recruited from different clinics, private practitioners and by advertisement
[99]	USA	Hanna 1999[100]	2000-2001	20				5.0	5.0	0	5.0	5.0			20	Voluntary testing in a prison
[101]	USA	IAS 2002[96]	2000-2001	18				17	0	17	0	0			18	HIV-positive pregnant women at a university hospital
[102]	USA	Hanna 1999[100] and IAS 2000[95]	1999	88	20	51	31	18.2	13.6	4.6	3.4	2.3	1 (< 6 months)		87 (> 6 months)	HIV-infected patients newly presenting for HIV care in a hospital
[103]	USA	IAS 2002[96]	1997-2001	1082	44.5	10.2	45.3	8.3	6.4	1.7	1.9	1.3	182 (< 170 days)		767 (< 12 months)	Patients consecutively enrolled from HIV care clinics, HIV counseling and testing sites, and other clinical settings
[104]	USA	IAS 2000[95]	1996-2000	152	64.6	33.1	35.5	13.8	2.6	7.1	4.9					Homeless and marginally housed persons from major lunch lines and shelters and low-rent residence hotels
[105]	Canada	IAS 2003[106]	1997-2003	305	54	34	12	19.3	14.8	7.2	4.6		305 (< 6 months)			HIV-infected persons from a Montreal cohort
[107]	USA	IAS 2003[108]	1999-2001	491	56	15		11.6	7.8	3.0	0.7	0.7				Demographically diverse treatment-naive subjects from 18 encompassing 25 US communities
[109]	USA	IAS 2005[54]	1991-2001	128				8.6	8.6	1.6	1.6	1.6			128	Pregnant women infected with HIV

(continue)

Table 1. Summary of studies on transmitted drug resistance per continent (*continued*)

Ref.	Region	Methodology	Years of sampling	(n)	HIV risk factor (%)			Resistance prevalence (%)					Population		Sampling of patients
					MSM	IVDU	HSX	Any	NRTI	NNRTI	PI	MDR	Recent (time to seroconversion)	Recent (time between neg. and pos. test)	Chronic (time to diagnosis)	
North America																
[110]	Canada	IAS 2003[111]	2000-2001	715	26	33	17	8.1	4.1	1.4	1.5	1.0	221 (< 170 days) 494 (> 170 days)			Newly diagnosed treatment-naive patients
[112]	USA	IAS 2005[54]	1995-1998	76	93			13.2	11.8	2.6	1.3	2.6		253 (< 6 months)		Newly diagnosed patients
			1999-2000	71	97			19.7	15.5	5.5	5.6	5.6				self-referred or recruited
			2001-2002	102	97			16.7	8.8	7.8	4.9	3.9		72 (6-12 months)		through community referrals
			2003-2004	112	97			24.1	16.1	13.4	7.1	9.8				
[113]	USA	IAS 2005[54]	2003-2005	192	37.0	59.0	4.0	17.7	7.3	7.3	4.2	1.0			192	Patients from a HIV outpatient care clinic
[114]	USA	IAS 2005[54]	2004	129				13.2	7,0	8.5	2.3	3.1	55 (0)		81 long-term infections	Consecutive sample of persons presenting for HIV counseling and testing at a STD clinic
[115]	USA	IAS 2005[54]	2004	55				18.2	3.6	14.6	3.6	1.8	55 (< 180 days)			HIV-infected adolescents aged 12-24
[116]	USA	IAS fail 2005[54], Stanford HIV	2003	317	74	6	28	9.8 12.0	3.5 6.6	6.0 6.3	1.9 0.6	1.6				ARV-naive patients enrolled for a clinical trial
[117]	USA	IAS 2006[31]	2005	103				25.2				6.8				ARV-naive patients attending a public health site
[118]	USA	Shafer 2007[32]	1999-2003	195	100			15.9	8.7	6.7	5.6	3.6		195 (< 6 months)		MSM with documented HIV-1 seroconversion
[119]	USA	IAS 2005[54]	2000-2004	151	28.5	11.3	53	11.3	6.6	5.3	0.7				93 (< 12 months), 57 (> 12 months)	ARV-naive patients presenting for care at clinics
[120]	USA	ViroSeq software	1999-2005	18 44				11.1 4.5	0 2.3	5.6 0	5.6 2.3	0 0	18 (0)		44	Blood donors at American Red Cross centers
[121]	USA	Stanford algorithm	2002-2006	112	100			12.5					112 (< 12 months)			MSM from a cohort of recently HIV-infected patients
[122]	USA	Stanford algorithm 2006	2005-2007	228	55.7		35.1	12.1	4.5	9.8	1.8	2.2				Not recently infected ARV-naive patients attending one of 19 HIV clinics
[123]	Greenland	Stanford HIVdb	1999-2007	60				25	21.7	3.3	10.0	10.0				ARV-naive patients
[124]	USA	IAS 2008[125]	1997-2007	848	78.3	13.0	8.7	14.9	6.8	7.6	5.0	2.1		894 (< 6 months)		Laboratory requisitions completed by prescribing physicians

(continue)

Table 1. Summary of studies on transmitted drug resistance per continent (*continued*)

North America

Ref.	Region	Methodology	Years of sampling	(n)	HIV risk factor (%)			Resistance prevalence (%)					Population			Sampling of patients
					MSM	IVDU	HSX	Any	NRTI	NNRTI	PI	MDR	Recent (time to seroconversion)	Recent (time between neg. and pos. test)	Chronic (time to diagnosis)	
[126]	North America	IAS 2007[74]	2004-2006	913	61			13.6	6.1	7.3	3.6					Naive patients attending a clinic
[127]	USA	IAS 2008[128]	2006-2008	100			54	8	2	6	0	0				ARV-naive patients from a urban HIV clinic
[129]	USA	Shafer 2007[32]	1998-2007	253	53.8			17.8	7.5	9.5	3.2	2.4		120 (< 45 days) 133 (45-180 days)		Patients from a Acute HIV and a Acute Transmission database

Latin America

Ref.	Region	Methodology	Years of sampling	n	HIV risk factor (%)			Resistance prevalence (%)					Population			Sampling of patients
					MSM	IVDU	HSX	Any	NRTI	NNRTI	PI	MDR	Recent (time to seroconversion)	Newly diagnosed	Chronic (time to infection)	
[130]	Brazil	Not reported	1993-1997	81							2.5					HIV-1 infected patients attending several hospitals and clinics
[131]	Martinique	Not reported	1988-1997	69					24.6				35		34	ARV-naive patients attending a hospital
[132]	Cuba	IAS 1998[43]	1999	27				7.4	7.4	0	0	0				Patients recruited for a UNAIDS study to monitor HIV resistance
[133]	Argentina	IAS 2000[95]	1997, 1999, and 2000	Chronic: 86		8		2.3	1.2	0	1.2	0	13		86	HIV patients attending the clinic and seroconversions
		Hammond 1997[134]		Recent: 13				15.4	7.6	0	7.6	0				
[135]	Venezuela	IAS 2000[95]	unreported	RT: 31 PR: 56	63	3	30	3.2	3.2	0	1.8					Patients recruited for a UNAIDS study to monitor HIV resistance
[136]	Brazil	IAS 2000[95]	1998	47				0	0	0	0	0				Blood donors
[137]	Brazil	IAS 1998[43], 2000[95]	2001	339	26.9	5.0	61.7	6.5	2.4	2.1	2.4	0.3				Consecutive HIV patients from different voluntary counseling and testing centers
[138]	Brazil	IAS 1998[43], 2000[95]	2000-2002	51	9.8	0	74.5	14.0	14.0	0.0	0.0	0.0				Patients attending an army hospital
[139]	Mexico	IAS 2003[108]	2002-2003	96				15.6	11.5	6.3	3.1	5.2			96	Consecutive ARV-naive patients with evidence of disease progression in three large public medical centers

(continue)

Table 1. Summary of studies on transmitted drug resistance per continent (*continued*)

Ref.	Region	Methodology	Years of sampling	(n)	HIV risk factor (%)			Resistance prevalence (%)					Population		Sampling of patients
					MSM	IVDU	HSX	Any	NRTI	NNRTI	PI	MDR	Recent (time to seroconversion)	Recent (time between neg. and pos. test)	Chronic (time to diagnosis)	
North America																
[140]	Brazil	Stanford HIVdb[141]	1999-2005	27				0	0	0	0	0				Patients attending a local ambulatory facility in a resource-poor setting region
[142]	Venezuela	Stanford HIVdb	unreported	13				7.7	7.7	0	0	0				ARV-naive patients
[143]	Brazil	Stanford HIVdb	unreported	108	<15	3	>15	3.1	1.0	2.1	0	0		108		Consecutive samples with sequence information
[144]	Brazil	IAS-USA 2003[54]	1998-2002	341				6.2	3.5	0.9	1.2	0.6	55		280	Blood donors, including paid donors and excluding HIV risk group donors
[145]	Peru	IAS-USA 2003[108]	2002-2003	359	100			3.3	2.2	0.8	1.9	1.7	33		326	MSM referred to clinic sites through recruiters and peer educators
[146]	Argentina	IAS 2005[54]	2004-2005	52	45	2	52	7.7	1.9	5.8	0	0	52 (< 9 months)			Recently HIV-infected subjects selected during routine clinical practice
[147]	Brazil	IAS 2002[96]	2002-2003	84	41.8	3.5	58.2	3.6	3.6	0	0	0			84	HIV-1-infected patients
[148]	Brazil	Stanford HIVdb 2000 and IAS 2005[54]	1994-1997 1999-2001	27 38				22.2 15.8	22.2 13.2	3.7 0	0 7.9	3.7 5.3				consecutive attending a hospital Recruited ARV-naive injection venous drug users
[149]	Mexico	IAS fall 2005[84]	2001-2003	39	97			2.8	2.8	0	0	0				HIV-1 patients attending a hospital
[150]	Brazil	Stanford HIVdb	2000, 2001, 2004	74				1.35	1.35	0	0	0	16 (< 153 days)		58 (> 153 days)	Blood samples from the routine of blood banks
[151]	Venezuela	Stanford HIVdb	unreported	20				10.0	5.0	5.0	0	0				-
[152]	Cuba	Stanford HIVdb	2003	250				8.4	4.8	0.4	3.2	0				HIV-1 patients attending the Tropical Medicine Institute
[153]	Chile	Stanford HIVdb	2000-2005	79				2.5	2.5	0	0	0				HIV-1 patients
[154]	Brazil	Shafer 2007[32]	2000-2006	123				6.5	1.6	1.6	0.8	2.4				HIV patients who were followed at a HIV out-clinic
[155]	Venezuela	Stanford HIVdb	2004-2007	63				11.1	6.3	3.2	1.6					Representative group of HIV-1-infected Venezuelan patients (mostly chronically infected)

(continue)

Table 1. Summary of studies on transmitted drug resistance per continent (*continued*)

North America

Ref.	Region	Methodology	Years of sampling	(n)	HIV risk factor (%)			Resistance prevalence (%)					Population			Sampling of patients
					MSM	IVDU	HSX	Any	NRTI	NNRTI	PI	MDR	Recent (time to seroconversion)	Recent (time between neg. and pos. test)	Chronic (time to diagnosis)	
[156]	Brazil	IAS 2008[85]	2007	387	43	2.5	54	5.7	1.3	4.4	1.0	0.8		387		HIV-infected patients from 20 sites in 13 cities
[157]	Brazil	SDRM-2009 IAS, version not reported	2007-2008	97	25.8	1.9	48.5	10.3 8.2	3.1	1.0	2.1	4.1				Naive patients recruited at the main regional reference public hospital for HIV-1 care (mostly chronically infected)

Europe

Ref.	Region	Methodology	Years of sampling	(n)	HIV risk factor (%)			Resistance prevalence (%)					Population			Sampling of patients
					MSM	IVDU	HSX	Any	NRTI	NNRTI	PI	MDR	Recent (time to seroconversion)	Newly diagnosed (time to diagnosis)	Chronic (time to diagnosis)	
[158]	Luxembourg	Stuyver 1997[159] NRTI mutations: 41, 69, 70, 74, 184, 214, 215	1992-1997	135					11.9					135		Newly diagnosed patients from national reference laboratory
[160]	Spain	NRTI mutations: 41, 70, 74, 184, 215	1993 1997	74 75	29.3 13.3	52 50.7	14.7 22.7		13.3 12.0							Patients attending HIV reference centers
[161]	Italy	Schnatz 1997[162] NRTI mutations: 41, 67, 70, 184, 215, 219	1985-1994 1995 1996 1997	44 28 15 11	44	23	33		9.1 7.1 20 36.4				64		34	HIV patients referred to the Division of Infectious Diseases
[163]	United Kingdom	Not reported	Unreported	16				0	0	0	0	0				Patients attending HIV centre
[164]	Greece	Hammond 1997[134]	Unreported	17				29	5.9	23.5						–
[165]	Switzerland	Schinazi 1997[166]	1996-1998	82	40	20	40	11	10	1.4	4.3	1.4	82 (< 3 months)			Patients attending AIDS centre
[167]	Italy	Not reported	1994-1997	38	44.7	26.3	28.9	21.1	21.1	2.6	0	0	38 (< 8 months)			Enrolment of seroconverters in AIDS institute

(continue)

Table 1. Summary of studies on transmitted drug resistance per continent (*continued*)

Ref.	Region	Methodology	Years of sampling	(n)	HIV risk factor (%)			Resistance prevalence (%)				Population			Sampling of patients
					MSM	IVDU	HSX	Any	NRTI	NNRTI	PI	MDR	Recent (time to seroconversion)	Newly diagnosed (time to diagnosis)	Chronic (time to diagnosis)	
Europe																
[168]	Italy	Barber 1990[169]	Unreported	10				0	0	0	0	0	10 (within a few weeks)			Patients with a few weeks primary infection
[170]	Italy	Infrared sequencing[171]	1996-2000	116	52.6	6.9	27.6	12.9	12.9	0.9	0.9	1.8	116 (< 12 months)			Seroconverters at different clinics
[172]	Spain	Line prop assays	1998	59 124					17	NA						Patients attending HIV clinics
[173]	United Kingdom	HRP-ASAPv1.0 program[174]	1995-1999	20				5.0	5.0	0	6 0	0	20 (< 446 days)			Seroconverters from UK register
[175]	Spain	IAS 1998[43]	Unreported	103				4.9	3.9	0	0.9	0				Representative epidemiological sampling
[176]	Belgium	Line prop assays	1995	45	40	2	51	26.6	15.6	17.6	4.4	2.2		45		Patients visiting hospital for the first time
			1997	75	47	0	35	26.6	14.7	13.3	8.0	4.0		75		
			1998	111	27	2	21	31.5	10.8	16.5	9.9	2.7		111		
[177]	France	IAS 1998[43]	1995-1998	48				18.8	17	0	2.1		14 (< 3 months)			Patients attending AIDS centers
[178]	Spain	Line prop assays	1997	Recent: 52 Chronic: 150					13.5 4.7				52 (< 9 months)		150	Well characterized group of individuals
[179]	The Netherlands	IAS 1998[43]	1992-1999	RT:74 PR:28					10.8	0	0		74			Monitoring MSM and IVDU
[180]	Germany	Los Alamos database 1998	1996-1999	64				12.5	6.3	3.1	4.7	1.6	64 (< 3 years)			Seroconversion study
[181]	Italy	Visible Genetics software	Unreported	17				5.9	0	5.9	0	0	13 (< 12 months) 4 (> 12 years)			HIV-1 seropositive persons
[182]	United Kingdom	IAS 2000[95]	1994-2000	69	84.1	2.9	13.0	14.5	11.6	4.3	1.4	2.9	69 (< 18 months)			Patients of UK register of HIV seroconverters
[183]	United Kingdom	Not reported	1996-2000	60	75	23	1.7	8.3	5.0	1.7	1.7	0	39 (< 12 months) 21 (> 12 months)			Patients attending HIV clinics
[184]	France	IAS 2000[95]	1998	391	48.5	12.9	29.8	3.7	3.3	0.8	1.9					Patients from HIV treatment centers

(*continue*)

Table 1. Summary of studies on transmitted drug resistance per continent (*continued*)

Europe															
Ref.	Region	Methodology	Years of sampling	(n)	HIV risk factor (%)			Resistance prevalence (%)					Population			Sampling of patients
					MSM	IVDU	HSX	Any	NRTI	NNRTI	PI	MDR	Recent (time to seroconversion)	Newly diagnosed (time to diagnosis)	Chronic (time to diagnosis)	
[185]	Spain	IAS 2000[95]	2000	351				10.8	3.1	1.5	6.1					First 8 naive consecutive patients at 18 outpatient clinics were selected
[186]	Switzerland	Schinazi 2000[187]	1996-1999	197	42	13	42	8.8					197 (< 12 months)			HIV-1 patients from AIDS centers
[188]	United Kingdom	Not reported	2000	15				0	0	0	0	0	15 (< 6 months)			Acute primary HIV infection study
[189]	France	IAS 1999[43]	1992 1997-1999	41 105	44 36	22 28	34 36			2 2						Patients prior to the initiation of NNRTI therapy
[190]	Italy	IAS 2000[95]	1996-1998	Recent: 68 Chronic: 347	25.0 23.3	19.1 37.2	41.1 31.4	19.1 11.5	14.7 7.8	0 4.9	5.9 1.4	1.5 2.6	68 (< 12 months)		347	Patients enrolled in the Italian Cohort of ARV-Naive patients.
[191]	Greece	Hertogs 2000[192]	1999-2000	24				0	0	0	0	0				Patients recruited before ARV treatment use
[193]	United Kingdom	VircoGENm	1988-1991	37	100			5.0	5.0	0	0	0	37 (< 6 months)			Seroconverted patients attending a HIV clinic
[194]	Spain	IAS 2000[95], and Medscape Guide	1996-2000	91				12.5	2.2	0.0	12.0	0.0				HIV-infected patients attending a center
[195]	France	Delfraissy 2000[196]	1996-1999	204	60	3	34	8.8	6.8	1.0	3.9	0.5	61 (< 3 months) 152 (< 6 months)			HIV-infected patients
[197]	Spain	IAS 2000[95], 2001[198]	1996-1999 2000-2001	31 26	70 67	10 0	20 28	25.8 4.3	23.3 0	3.3 0	6.7 4.3	6.7 0	57 (< 12 months)			HIV-infected patients attending a HIV centre
[199]	United Kingdom	IAS 2000[95]	1999-2001	71			100	5.9	1.5	3.0	4.0	2.2	14 (0), 25 (< 12 months)			Representing new HIV-1 diagnosed in UK
[200]	Spain	IAS 2001[198]	1999 2001	47 47				12.7 15.0	8.5 2.1	4.2 12.7	2.1 2.1	2.1 2.1			94 (>3 years)	Randomly collected ARV-naive individuals
[201]	Sweden	IAS 2000[95]	1998-2001	100	40	2	41	9.0	5.0	3.0	1.0	0				Randomly chosen from newly diagnosed and naive HIV patients
[202]	France	IAS 2002[96]	1999-2000	249	57	2	32	10.4	7.6	4.0	5.6	4.8	249 (< 6 months)			Patients from primary infection therapeutic trial teams

(continue)

Table 1. Summary of studies on transmitted drug resistance per continent (*continued*)

Ref.	Region	Methodology	Years of sampling	(n)	HIV risk factor (%)			Resistance prevalence (%)					Population			Sampling of patients
					MSM	IVDU	HSX	Any	NRTI	NNRTI	PI	MDR	Recent (time to seroconversion)	Newly diagnosed (time to diagnosis)	Chronic (time to diagnosis)	
Europe																
[203]	Denmark	www.hivresistanceweb.com, IAS, version not reported	2000	97				2.1	2.1	0	0	0	12	91		Patients who were seroconverted or newly diagnosed
[204]	Italy	Trugene	Unreported	Recent: 38 Chronic: 24				5.3 8.3	2.6 8.3	0 0	2.6 0	0 0		38 (1-12 months)	24 (> 3 years)	Cohort with naive patients
[205]	Spain	Stanford HIVdb	2000-2002	85	18	32	47	7.1	5.9	0	1.2	0		85		All newly diagnosed HIV-1 patients attending hospitals
[206]	Italy	IAS 2003[111]	1996-2001	112	33.0	20.5	40.2	16.1	14.3	0.9	2.7	1.8	112 (< 12 months)			Cohort with naive patients
[207]	Belgium	IAS 2003[111]	2000	83			58.1	7.2	5.0	2.5	1.2	1.3		83		Recently diagnosed naive HIV patients
[208]	Switzerland	IAS 2000[95]	Unreported	220				10.5	8.6	0.9	2.3	1.4	220 (< 1 year)			Individuals attending a hospital
[209]	The Netherlands	IAS 2003[111]	1994-2003	100	61	27	12	13.0	10.0	2.0	1.0	0.0	100 (< 6 months)			Seroconverters from a group of MSM and IVDU
[210]	Nordrhein-Westfalen, Germany	IAS 2001[198] and 2002[96]	2001-2002	184	46.2	3.5	22.4	14.0	10.5	2.8	2.1	2.1				Patients attending clinics/ hospitals at time of first HAART administration
[211]	Russia Federation, Ukraine, Kazakhstan	Los Alamos national laboratory	1996-2002	27	0	100	0				0					Samples from IVDU
[212]	Spain	Line prop assays	1996-2003	145	10.1	54.1	5.4	20.3	4.1	1.4	16.6	1.4				Untreated HIV patients from different centers
[213]	Italy	Stuyver 1997[159] and Clarke 2000[214]	< 1996 > 2000	21 27	9.5 11.2	42.9 29.6	23.8 29.6	0 11.1	0 7.4	0 0	0 11.1	0 7.4				Naive patients attending clinics

(*continue*)

Table 1. Summary of studies on transmitted drug resistance per continent (*continued*)

Europe																
Ref.	Region	Methodology	Years of sampling	(n)	HIV risk factor (%)			Resistance prevalence (%)					Population			Sampling of patients
					MSM	IVDU	HSX	Any	NRTI	NNRTI	PI	MDR	Recent (time to seroconversion)	Newly diagnosed (time to diagnosis)	Chronic (time to diagnosis)	
[215]	Israel	IAS 2003[108]	1999-2003	176	10	24	58	14.8	2.8	8.5	5.1	0.6				Random samples selected from naive patients
[216]	Former Soviet Union	Stanford HIVdb	1997-2004	278	2.9	84.0	11.5	13.3	5.4	7.5	2.5	0.4				HIV-1-infected patients
[217]	Greece	IAS 2004[218]	2002-2003	101	55	3	23	8.9	5.0	4.0	0	0	18 (< 1 year)	83		Representative sample of newly HIV-patients IV-1 diagnosed patients
[219]	France	IAS 2003[108]	2001-2002	Recent: 303 Chronic: 363	57.7 31.2	0.35 7.8	31.7 51.1	14 9.1	10.3 7.2	3.3 1.7	4.3 1.9	2.0 1.7	303 (< 6 months)	218 (< 1 year), 145 (> 1 year)		National survey
[27]	Europe, Israel	IAS 2003[111]	1996-2002	2208	43	15	41	10.4	7.6	2.9	2.5	2.0	777 (< 1 year) 607 (> 1 year)			Surveillance program to collect samples of newly diagnosed HIV patients. Have been partly published elsewhere
[220]	Spain	IAS 2005[54]	1997-2004	198	70	10	19.5	12.1	9.6	4.0	2.0	3.5	198 (< 12 months)			Consecutive newly diagnosed patients
[14]	United Kingdom	Stanford HIVdb version 2004.04	1996-2003	2357	76.4	2.6	21	14.2	9.9	4.5	4.6	3.3	172 (< 18 months)			Resistance database with test carried out as part of routine clinical care
[221]	Western Europe and Canada (CASCADE)	Stanford 3+ Stanford 4+ IAS 2004 ANRS	1987-2003	438	74.9	10.3	12.3	10.3 15.1 15.5 11.2	5.7 10.3 10.5 7.8	3.4 3.9 3.4 3.0	3.0 4.1 3.9 2.3		438 (< 18 months)			Patients belonging to the CASCADE Collaboration
[222]	Italy	IAS 2005[54]	1997-2004	155	23.9	1.3	16.8	16.8	12.9	4.5	1.9	2.5	155 (≤ 12 months)			HIV-1 patients who received routine testing
[223]	Nordrheim-Westfalen, Germany	IAS 2003[106]	2001-2003	269	48			11.2	8.6	4.1	1.5	1.5			269	HIV-1 patients tested before first application of HAART
[25]	Romania	Stanford HIVdb	Unreported	14				0	0	0	0	0				ARV-naive HIV-1 patients

(continue)

Table 1. Summary of studies on transmitted drug resistance per continent (*continued*)

Ref.	Region	Methodology	Years of sampling	(n)	HIV risk factor (%)			Resistance prevalence (%)					Population			Sampling of patients
					MSM	IVDU	HSX	Any	NRTI	NNRTI	PI	MDR	Recent (time to seroconversion)	Newly diagnosed (time to diagnosis)	Chronic (time to diagnosis)	
										Europe						
[224]	Georgia	Stanford HIVdb 2000	1998-2003	48	2	65	27	4.2	4.2	0	0	0				Naive HIV-1 patients represented different transmission modes
[225]	United Kingdom	IAS 2005[54]	2000-2004	140	91		9	6.4	2.1	4.3	1.4	1.4	140 (< 6 months)			HIV patients recruited into a therapy intervention study
[226]	Sweden	IAS fall 2005[64]	1992-2002	201	100			13.9	13.4	0.5	0	0	87 (< 12 months)			HIV diagnosed patients at a MSM HIV clinic
[227]	Spain	ANRS and IAS 2003[106]	2002	43		90.7		11.6	6.9	6.9	6.9	2.3				Randomly chosen prison inmates infected with HIV
[228]	Slovenia	IAS 2005[54]	2000-2004	77	62.3	2.6	29.9	3.9	3.9	0	0	0		77 (< 3 months)		Patients with HIV confirmed at an AIDS centre
[29]	United Kingdom	IAS fall 2005[64]	2004-2006	239	52.7	2.1	45.2	7.1	4.2	1.7	1.7	0.4	85 (< 164 days)			Patients newly diagnosed at a hospital
[229]	Portugal	IAS 2005[54]	2003	180	19	20	54	7.8	3.9	1.7	0	1.7		180 (< 3 months)		Newly diagnosed patients in representing centre
[230]	Italy	IAS 2006[31]	Unreported	29				17.2	10.3	6.9		6.9			29	ARV-naive patients
[231]	United Kingdom	IAS 2005[54]	1996-1997	310				8.4	7.1	1.3	1.3		19 (< 18	291		Database with samples as part
			1998	340				10.0	8.2	2.1	2.9		months)	312		of routine clinical care
			1999	358				11.2	9.8	5.0	2.8		28	329		nationwide
			2000	458				14.2	9.0	5.2	3.5		29	413		
			2001	517				13.0	9.3	5.2	3.9		45	458		
			2002	519				15.6	11.4	6.0	5.0		59	470		
			2003	767				12.5	7.4	6.0	2.9		49	730		
			2004	1185				9.2	4.6	4.4	2.1		37	1135		
													50			
[232]	Germany	IAS 2004[218]	1997-2004	504	88	7	2.3	16	9	2	2.8	1.4	294 (< 12 months), 178 (< 24 months), 23 (< 36 months, 9 (> 36 months)			Open cohort of HIV persons with known date of seroconversion
[233]	Switzer-land	Shafer 2007[32]	1996-2005	822	42	20	32	7.7	5.5	1.9	2.7	2	822 (< 1 year)			Representative populations of recently HIV patients
[234]	Germany	IAS 2003[106]	2001-2005	831	51.5	5.0	18.5	9.0	5.4	3.0	2.4	1.3			831	HIV patients from the majority of treatment centers of Germany

(continues)

Table 1. Summary of studies on transmitted drug resistance per continent (*continued*)

Europe															
Ref.	Region	Methodology	Years of sampling	(n)	HIV risk factor (%)			Resistance prevalence (%)					Population			Sampling of patients
					MSM	IVDU	HSX	Any	NRTI	NNRTI	PI	MDR	Recent (time to seroconversion)	Newly diagnosed (time to diagnosis)	Chronic (time to diagnosis)	
[235]	Belgium	Shafer 2007[32]	2003-2006	285	55	0.3	38	9.5	7.0	3.5	1.8	4.2		285 (< 6 months)		Patients from all eight Belgian AIDS Reference Centers
[236]	Europe, Israel and Argentina	Shafer 2007[32] and IAS 2007[74] and Stanford version 4.3.4	1994-2007	525 (510 from Europe)				11.4 13.5 16.0	9.3	1.0	3.0	1.7		13 (< 1 year)	512 (> 1 year)	HIV patients in 93 HIV centers
[237]	United Kingdom	IAS fall 2006[31]	2005-2007	392			70.6	3.3	0.5	1.8	1.0	0		392		Newly diagnosed HIV patients from a representative number of centers
[7]	Europe	IAS 2005[54]	2002-2003	1050	44	8	41	9.1	5.4	2.6	3.0	1.3	235 (< 1 year)	815 (< 3 months)		Newly diagnosed HIV patients from 16 European countries with a standardized sampling strategy
[238]	United Kingdom	Stanford HIVdb 2007	2006	99				7.0	5	1	1	0		76		Naive patients with samples in the virology resistance test database
[239]	Cyprus	IAS 2007[74] and Shafer 2006[240]	2003-2006	37	49		46	6.5	4.3	2.2	0	0		37 (< 3 months)		Newly diagnosed patients attending an AIDS clinic
[241]	Spain	Stanford HIVdb	2004-2007	261	37.9	11.1	47.5	11.1	7.6	2.3	0.5	0.9		261		Newly diagnosed patients attending the public hospitals
[242]	Estonia	IAS 2007[74]	2005-2006	115	0	34	65	0	0	0	0	0				HIV patients including from a prison
[243]	Switzerland	Shafer 2007[32]	2000-2008	637				8.5	6.3	3.5	1.9	2.7	131 (< 12 months)	506		Newly diagnosed HIV patients
[126]	Western Europe	IAS 2007[74]	2004-2006	500	54			6.8	3.8	3.2	0.8					Naïve patients attending a clinic
[244]	Italy	Shafer 2007[32]	2004-2007	255	48.6	4.0	47.6	5.9	3.9	3.5	0.4	0	58 (< 6 months)	197		Newly diagnosed HIV-1 patients
[245]	Italy	Bennett 2009[246]	1996-2007	1690	28.4	12.1	41.6	15.1	11	6	4	3.4				Naive HIV patients from ARCA database
[247]	Russia	Not reported	2007-2008	33	5	36	36	9.1	0	9.1	0	0				HIV-infected patients from two Russian regions

(continue)

Table 1. Summary of studies on transmitted drug resistance per continent (*continued*)

Ref.	Region	Methodology	Years of sampling	(n)	HIV risk factor (%)			Resistance prevalence (%)					Population		Sampling of patients
					MSM	IVDU	HSX	Any	NRTI	NNRTI	PI	MDR	Recent (time to seroconversion)	New diagnosed (time to diagnosis)	
Asia															
[248]	Japan	Jennifer 1997[249]	1996-1998	21	17		16	1.0	0		1.0	0			HIV-1 patients from 17 hospitals in various parts of Japan
[250]	Thailand	Los Alamos database	2000-2001	22				0	0	0	0	0			ARV-naive pregnant woman
[251]	Korea	IAS 2000[95]	1998-2002	50	18	0	82	8.0	6.4	0	2.6	2.0			HIV-1-infected persons followed-up at the national hospital randomly included HIV-1 infected attending a HIV Center
[252]	Vietnam	IAS and ANRS, versions not reported	2001-2002	200		42.5		6.5	4.5		2.0				
[253]	Japan	IAS 2000[95]	1999-2002	116	57		28	9.5	6.0	0.0	3.4	0			HIV-1-infected patients at their initial consultation at a hospital
[254]	India	IAS and ANRS, versions not reported	2003	128				1.6	1.6	0	0	0			HIV-1 patients attending the outpatient clinic of a hospital
[26]	India	Software included in VircoSeq	1999-2001	12				0	0	0	0	0	12 (< 4 months)		Patients with recent HIV infection
[255]	Cambodia	ANRS algorithm Update Sept. 2004	2003-2004	144				4.9	2.8	0.7	1.5	0		22 (< 1 year)	Consecutive patients recruited through voluntary counseling and testing center and prenatal clinics
[256]	Japan	IAS 2006[31], Stanford HIVdb[240]	2003-2004	575	66.6	0.19	25.9	4.0	2.8	0.7	0.7	0.2	45 (< 1 year)	530	Newly diagnosed HIV cases through surveillance of 8 AIDS clinical centers
[257]	Malaysia	IAS 2005[54]	2003-2004	100			57	1.0	0	1.0	0	0		60 (< 2 months), 10 (3-23 months), 13 (> 12 months)	HIV-1 patients attending a HIV Clinic
[258]	Hong Kong	Stanford HIVdb	1995-2004	39				0	0	0	0	0			HIV-infected patients
[259]	China	Stanford HIVdb	2004	RT: 71 PI: 174				4.2	4.2	0	0	0			Patients from 4 midland provinces of China
[260]	China	Stanford HIVdb	Unreported	91		8		4.4	1.1	0	3.3	0			Mostly chronically HIV-1-infected patients attending a hospital
[261]	China	Stanford HIVdb	2005	40	100			15	10.0	12.5	0	7.5			patients recruited from the HIV-1 sero-positive MSM management program

(*continue*)

Table 1. Summary of studies on transmitted drug resistance per continent (*continued*)

Asia

Ref.	Region	Methodology	Years of sampling	(n)	HIV risk factor (%)			Resistance prevalence (%)					Population		Sampling of patients
					MSM	IVDU	HSX	Any	NRTI	NNRTI	PI	MDR	Recent (time to seroconversion)	New diagnosed (time to diagnosis)	
[262]	Japan	IAS 2005[54]	1992-2004	54				11.1	1.9	3.7	5.6	0			People visiting STD-related clinics
[263]	Thailand	IAS 2005[54]	2003-2006	305	9	11	75	4.0	4.0	4.0	0	4.0			HIV patients attending HIV-related medical care at a hospital
[264]	Thailand	IAS 2007[74]	Unreported	113	1.3	2.7	90.7	12.4	12.4	0	0	0			ARV-naive patients
[265]	Thailand	Shafer 2007[32]	2005-2006	46				0	0	0	0	0	46 (< 12 months)		Consecutive returning blood donors at the Red Cross National Blood Centre
[266]	Vietnam	Shafer 2007[32] and IAS fall 2006[31]	2006	49				2.0	2.0	2.0	0	2.0			Woman in their first pregnancy attending antenatal clinics or clients at voluntary counseling and testing sites, who were < 25 years old
[267]	Cambodia	WHO HIV Drug resistance database	2006-2007	67			65	1.5	1.5	1.5	0	1.5			Patients of the Medecins du Monde (40), French Red Cross (14) and pregnant woman (13) enrolled at a hospital
[268]	Singapore	IAS 2007[74]	2006-2007	60	73.3	1.7	23.3	1.7	0	1.7	0	0	60 (< 24 months)		Recently infected patients recruited from the outpatient clinic at the CDC hospital
[269]	China	Stanford HIVdb	2007	Subt CRF_08BC 32 CRF_07BC 67		100		 0 1.5	 0 1.5	 0 0	 0 0	 0 0			Patients infected through injection drug use.
[270]	India	Stanford HIVdb	2005-2006	55		100		7.2	3.6	1.8	2.7	0			IDU recruited by field staff

Australia

Ref.	Region	Methodology	Years of sampling	(n)	HIV risk factor (%)			Resistance prevalence (%)					Population		Sampling of patients
					MSM	IVDU	HSX	Any	NRTI	NNRTI	PI	MDR	recent	Newly diagnosed (time to diagnosis)	
[271]	Australia	IAS 1998[43]	1992-2001	185	86.5			23.2	18.4	2.7	1.6	0		185 (< 13 days)	Samples from a reference laboratory of newly acquired HIV

Where several mutation lists were compared (in 3 studies), we used the prevalence numbers of the most recent IAS list.
IAS: International AIDS Society, USA; MSM: men who have sex with men; IVDU: intravenous drug users; HSX: heterosexual; NRTI: nucleoside reverse transcriptase inhibitor; NNRTI: nonnucleoside reverse transcriptase inhibitor; PI: protease inhibitor; ARV: antiretroviral; STD: sexually transmitted disease; PMTCT: preventing mother-to-child transmission; Any: at least one drug resistance mutation; MDR: multidrug resistance to at least two classes.

3

Worldwide analysis of mutational patterns in studies on transmission of drug resistant HIV-1

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ABSTRACT

Background: Transmission of HIV-1 drug resistance occurs in all regions of the world with access to treatment. We collected epidemiological studies on resistance and analyzed their data in a standardized way, which allowed us to determine and compare prevalences across the world.

Methods: Relevant studies were identified in Medline and conference reports. Authors were approached to share protease and reverse transcriptase sequences and clinical and demographic data. The sequences were analyzed for major drug resistance associated mutations included in the IAS-USA mutation figures (Fall 2006).

Results: We included 6244 antiretroviral naïve patients from 44 countries. The prevalence of patients harbouring at least one resistance associated mutation was 10.1% (95% CI: 9.4%-10.9%). This prevalence was higher in the Northern hemisphere (14.2% in Asia, 13.6% in North America, and 10.2% in Europe) than in the Southern hemisphere (approximately 7% in Latin America and Africa). The high prevalence in Asia was ascribed to a monophyletic cluster of A62V in Kazakhstan. The prevalence of resistance to the nucleoside reverse transcriptase inhibitors (NRTI) drug class was the highest (between 3.6% in Latin America and 12.6% in Asia). Multi-class-resistance was limited (<2%). Compared to Europe, the non-nucleoside reverse transcriptase inhibitors (NNRTI) (odds ratio: 2.3; 95% CI: 1.5-3.7) and protease inhibitors (1.8; 1.1-2.9) transmitted resistance were higher in North America. In Asia, resistance prevalence was higher for NRTI (4.5; 2.0-9.8) and lower for NNRTI (0.06; 0.01-0.24) and protease inhibitors (0.3; 0.1-0.6), compared to Europe. No statistical significant results were found in other continents.

Conclusions: The resistance profiles of the antiretroviral drug classes differ slightly between continents. Transmitted resistance was found in all continents stressing the need of continuous global surveillance of transmitted resistance.

INTRODUCTION

In the last decades there has been a substantial progress in the treatment of HIV-infected patients due to the introduction of a large number of antiretroviral drugs. Use of antiretroviral drugs has dramatically reduced mortality among patients living with HIV in Europe and North America [1-2]. In recent years comparable effectiveness of antiretroviral drugs has been reported in resource-poor settings [3].

Resistance, however, may limit the success of antiretroviral drug treatment. Resistance occurs frequently in patients with virological failure and may decrease both the magnitude and the duration of the response to treatment [4]. Transmission of resistant viruses between individuals has been observed [5], which can result in a less favourable response to therapy and limited therapeutic options [6-9].

A substantial number of reports are available on transmitted drug resistance. These studies reported a wide variation in the prevalence of transmitted drug resistance ranging between 0 to 25% [5, 10-11]. Unsurprisingly, a part of this variation in prevalence may be ascribed to differential treatment availability between continents. But the variation between different parts of the world is difficult to characterise due to the use of different algorithms to interpret transmitted resistance profiles that have changed over time [10, 12].

In this paper we will discuss the results of the WATCH study (World-wide Analysis of resistance Transmission over time of Chronically and acute infected HIV patients). WATCH collected and analysed data of currently available studies on transmission of HIV-drug resistance from across the world. Importantly, WATCH used a single algorithm to score drug resistance. Using this approach, we give insight to different profiles of transmitted resistance over time and in differences in prevalence and characteristics of genotypic profiles and viral subtypes between continents.

METHODS

Identification of relevant studies

To identify studies on transmission of drug resistant HIV, literature was searched in PubMed. For this purpose the search terms “HIV OR RESISTANCE” and “HIV OR TRANSMISSION” were used. Also, relevant conference reports were reviewed. The included studies had to include HIV-1 seropositive persons, who were never exposed to antiretroviral drugs, and were at least 18 years. Also, sequences should be available for both the reverse transcriptase (RT) and the protease (PR) gene. For the collection of the data, authors of the compiled articles that met the inclusion criteria were contacted and asked to share HIV-1 pol sequences and additional demographic and clinical data. Part of the data sets has been published elsewhere [13-53].

Patients were considered to be recently infected if they had a negative HIV-1 ELISA test result or a negative, incomplete or indeterminate Western Blot, with subsequent documented HIV-1 seroconversion within 1 year before the drug-resistance analysis was performed. If no reliable information about the duration of infection was available, newly diagnosed cases were classified as having an unknown duration of infection.

Genotypic resistance analysis

Population nucleotide sequence analysis was performed by local laboratories. We used Clustal X (version 1.81) [54] for the alignment of the sequences. Resistance-related mutations were defined according to the International AIDS Society (IAS, Fall 2006) [55] mutation list. The revertants at codon 215, which are listed as footnotes in the IAS figures and are considered to be indicators of transmitted resistance, were included in the analysis as well. The classes of drugs included were nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI) and proteases inhibitors (PI). Other classes of antiretroviral drugs were not considered, as resistance to these drugs in treated patients is not widespread. Multiclass resistance was defined as the presence of resistance mutations to at least two different drug classes.

Sequence quality verification

All sequences encompassed at least codons 30-90 of the protease gene and codons 41-219 of the reverse transcriptase gene. Sequences containing a stop codon (mixtures excluded) at a resistance-related position and individual resistance-related codons with ambiguities consisting of >2 bases per nucleotide position or of >2 ambiguities per codon were excluded from the analysis. Furthermore, codons with <3 nucleotides were recorded as missing.

HIV-subtype classification

HIV-subtypes were assessed by the construction of phylogenetic trees using the neighbour-joining method. We included subtype reference from the Los Alamos Sequence Database (www.hiv.lanl.gov). The Kimura 2-parameter distance estimation method was used to generate pairwise distance matrices with a transition to transversion ratio of 2.0. The consistency of the phylogenetic clustering was tested by bootstrap analysis with 1000 replicates. Bootstrap values above 70 were considered to be sufficient for subtype assignment. Trees were based on *pol* sequences and were constructed for each centre [56].

Statistical analysis

The 95% confidence interval was calculated according to the Wilson interval. Categorical data were compared with the χ^2 test and continuous data by the Mann-Whitney U test. The comparison of the frequencies was analysed by the χ^2 test. Trend analysis was performed using the heterogeneity and trend in proportions tests [57], stratifying the calendar years into 3 intervals: 1996-2000, 2001-2002, and 2003-2007. For the trend analysis, only patients with a recent infection were included, as chronically infected patients could have been infected for many years before diagnosis. Analyses were done on a continent-level, where Israel was considered as being part of Europe. Finally, the comparison for the different drug classes was analysed using logistic regression with Europe as the reference group.

RESULTS

Study population

Data were collected between 1996 and 2007. We collected data from 7482 persons in 41 countries. A total of 1238 individuals were excluded. Of these 1238 patients, 87 patients were aged under 18 years and 12 individuals had a HIV sequence containing a stopcodon at a resistance-related position. The HIV sequence isolated from 1139 individuals did not meet the quality control criteria (predominantly because only the protease region was available). A large part of the excluded individuals came from Africa (37%) and Latin America (23%), where sequence analysis of reverse transcriptase was often not performed.

Most included individuals came from Europe (3362, 54%): Austria (total number of patients: 84), Belgium (125), Cyprus (2), Czech Republic (43), Denmark (130), Finland (8), France (24), Germany (653), Greece (33), Israel (96), Italy (491), Luxembourg (155), Netherlands (23), Norway (21), Poland (35), Portugal (103), Serbia (10), Slovenia (38), Spain (228), Sweden (152), Switzerland (244), United Kingdom (664). But a substantial percentage was derived from other continents, North America: Canada (445), Mexico (44), USA (408); Latin America: Argentina (216), Brazil (548), Chile (37); Asia: China (29), India (118), Japan (76), Kazakhstan (85), South Korea (46), Vietnam (147); Africa: Burkina Faso (101), Cameroon (73), Cote d'Ivoire (135), DR Congo (18), Gabon (13), Mozambique (40), Nigeria (78), Rwanda (97), Senegal (56), South Africa (72).

Table 1 summarizes the characteristics of the 6244 persons that were included. Individuals were predominantly male (except in Africa). The continents had a dissimilar risk group distribution. In Europe and North America, most individuals were men who have sex with men (MSM) (45%). Conversely, in other parts of the world, the majority of patients had reported to be infected through heterosexual contact. Asia had the highest proportion (32%) of individuals who acquired HIV through intravenous drug use.

Subtype B was most common in North America (91%), Latin America (70%) and Europe (68%). In Asia and Africa, HIV subtype A was the most prevalent (52% and 58%, respectively). Subtype C was found in data from all continents (ranging between 4% of the sequences collected in North America to 23% in Asia).

The mean HIV-RNA load was around 4.75 log copies/ml. In North America, however, a somewhat lower RNA load was found, and in Africa a somewhat higher RNA load was found. The median CD4 cell count was quite similar in Europe, North America, and Latin America (around 325 cells/mm^3). In Africa the median CD4 cell count was substantially lower (215 cells/mm^3), which could indicate that patients included from this continent are in an advanced stage of disease.

Resistance analysis

A proportion of 10.1% (95% confidence interval 9.4-10.9%) was infected with HIV containing at least one drug resistance associated mutation (Figure 1A). The highest overall resistance for the different drug classes was observed for the NRTIs (6.8%; 6.5-7.9%), followed by NNRTIs (3.1%; 2.8-3.7%), and PIs (2.6%; 2.3-3.2%). Importantly, multi-drug resistance was relatively rare (1.9%; 1.7-2.4%). In this study population, 1436 patients had evidence of a recent HIV infection enabling calculation

Table 1. Characteristics of patients included in the study grouped according to continent.

	All n = 6244	Africa n = 687	Asia n = 501	Europe n = 3362	North America n = 850	Latin America n = 844
Age, number of patients	5183	372	413	3101	525	769
Age, mean (SD), years	35 (10)	32 (9)	31 (10)	36 (10)	38 (10)	33 (9)
Male, number of patients	6247	115	384	2865	835	489
Male, %	69	44	66	73	72	71
HIV subtype, number of patients	5822	649	498	3237	805	630
HIV subtype, %						
A	18	58	52	13	3	0
B	61	1	24	68	91	70
C	11	21	23	10	4	7
G	2	5	0	3	0	0
12_BF	2	0	0	0	1	19
Other	5	16	2	6	2	4
HIV transmission, number of patients	4909	579	422	2629	785	482
HIV transmission, %						
MSM contact	37	0	14	46	44	42
Heterosexual contact	49	100	51	42	24	52
Injection drug use	13	0	33	11	31	6
Blood transfusion	1	0	2	1	1	0
Baseline values						
CD4 cell count, number of patients	4013	378	422	2452	428	346
$CD4^+$ cell count, median (interquartile range), cells/mm^3	347 (150-526)	215 (93-371)	440 (10-711)	333 (161-540)	313 (66-469)	323 (160-466)
HIV-RNA load, number of patients	3552	193	71	2631	393	252
HIV-RNA load, mean (SD), log copies/mL	4.75 (0.85)	4.97 (0.90)	4.77 (0.95)	4.75 (0.85)	4.50 (0.67)	4.72 (0.86)

MSM, men who have sex with men.

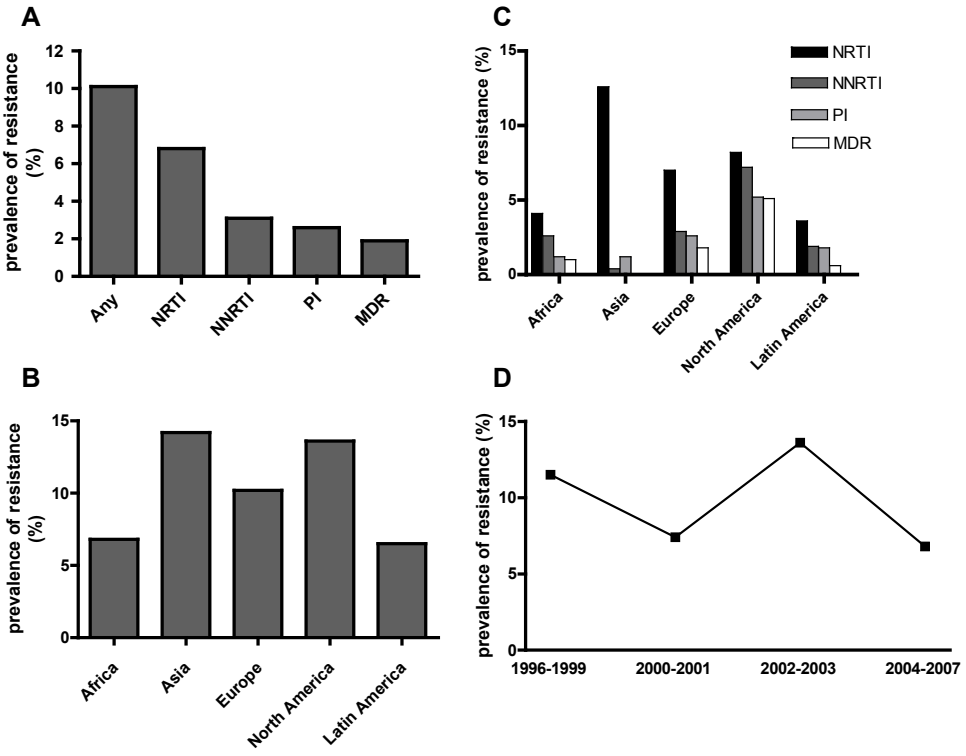

Figure 1. (a) Frequency of HIV resistance within drug classes. **(b)** Frequency of any resistance within continents. **(c)** Frequency of resistance within the drug classes and continents. **(d)** Trend of any HIV resistance from 1996-2007. NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; MDR, multi drug resistance.

of the incidence of transmitted resistance of 9.3% (7.8-10.9%).

Table 2 shows the frequency of transmitted drug resistance mutations (TDRM). The most frequently NRTI-resistance associated mutations were T215 revertants (23.6%), M41L (22.3%), and M184V (12.7%). Mutations associated with the thymidine analogues (TAMs) were present in 48.5% of all sequences with signs of transmitted resistance. For NNRTIs, the most common mutations were K103N (16.6%), Y181C (6.8%), and V108I (4.6%). Finally, the most common PI-related mutations were L90M (11.1%), V82A (6.0%), and M46I (5.7%).

Figure 1 (b and c) show the overall prevalence of HIV TDRM for the different continents. Surprisingly, the highest prevalence of resistance was found in Asia (14.2%; 11.4-17.5%). This substantial prevalence was ascribed to Kazakhstan, where 48 of 85 patients harboured viruses with the NRTI-resistance related A62V amino acid substitution. This A62V mutation was not observed very frequent in other continents. Without this A62V mutation, the TDRM prevalence in Asia was 4.2% (2.4-5.9%).

In Africa, a relatively low prevalence of 6.8% (5.2-8.9%) was found. As in the other continents, the majority of the resistance mutations were at an NRTI-resistance-position. But the distribution of the particular mutations was different in

Africa; TAMs such as M41L and T215 variants were less common. Conversely, M184V was found more frequently. The prevalence of TDRM in Latin America was the same as the estimate for Africa; 6.8% (5.0-9.1%). The highest prevalence estimates for TDRM were found in the industrialized countries. North America had the highest TDRM prevalence at 13.6% (11.5-16.1%). In North America, the highest TDRM prevalence was seen for NNRTI and PI drug classes. The TAMs and M184IV mutations were more present in this population compared to the other continents. Europe showed a resistance prevalence of 10.2% (9.2-11.3%). Here T215 revertants were frequently present compared to the other continents.

Comparison of mutational patterns

Comparing the relative proportion of resistance to the different drug classes showed that Asia differed significantly from Europe in NRTI resistance, with an odds ratio (OR) of 3.7 (95% confidence interval (CI) 1.7-8.0) (figure 2). NNRTI- and protease-inhibitor associated resistance was more common in North America (OR 2.9; 95%

Table 2. Resistance mutations profiles.

	Total	In all patients	In patients with TDRM	Africa n=687	Asia n=501	Europe n=3362	North America n=853	Latin America n=844
Mutation[a]	n	prevalence, %						
NRTI								
M41L	141	2.3	22.3	1.0	0.4	2.3	4.3	1.9
K65R	1	0.0	0.2	0.0	0.0	0.0	0.0	0.0
D67N	67	1.1	10.6	0.9	0.2	1.2	2.1	0.1
K70RE	40	0.6	6.3	0.0	0.2	0.7	1.4	0.1
M184V	80	1.3	12.7	1.5	0.4	1.2	2.6	0.8
L210W	51	0.8	8.1	0.4	0.0	0.8	2.0	0.4
T215FY	48	0.8	7.6	0.4	0.2	0.9	1.4	0.6
T215revertants	149	2.4	23.6	0.4	0.6	3.2	3.3	1.1
K219EQ	57	0.9	9.0	1.0	1.0	1.0	1.3	0.0
TAMs[b]	306	5.0	48.5	2.8	2.0	5.8	7.2	2.4
=> 2 TAMs[b]	147	2.4	23.3	0.7	0.2	2.7	5.0	0.9
TAM +M184V	344	5.6	54.5	3.6	2.4	6.4	8.0	2.8
Multi-NRTI								
A62V	66	1.1	10.5	0.0	9.6	0.3	0.6	0.4
V75I	7	0.1	1.1	0.0	0.0	0.1	0.4	0.0
F77L	11	0.2	1.7	0.1	0.4	0.2	0.1	0.0
Q151M	4	0.1	0.6	0.0	0.0	0.1	0.1	0.1
NNRTI								
K103N	105	1.7	16.6	1.5	0.0	1.6	3.8	1.4
V108I	29	0.5	4.6	0.1	0.4	0.5	0.9	0.2
V181C	43	0.7	6.8	1.3	0.0	0.5	1.5	0.6
PI								
L33F	22	0.4	3.5	0.3	0.4	0.4	0.7	0.0
M46I/L	57	0.9	9.0	0.3	0.4	1.0	1.3	1.1
V82AFSTL	49	0.8	7.8	0.1	0.0	0.9	1.6	0.4
L90M	70	1.1	11.1	0.1	0.6	0.9	3.6	0.4

[a] In addition to these mutations, the following mutations were also studied: Y115F, M184I, F116Y, L100I, V106A/M V181I, Y188L/C/H, G190A/S, P225H, P236L, D30N, V32I, I47AV, G48V, I50LV, I54ML, L76V, I84V, N88S; [b] M41L + D67N + K70R + L210W + 215A/C/D/E/F/N/S/V/Y+ K219E + K219Q; TDRM, transmitted drug resistance mutations; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; and TAM, thymidine analogue mutation.

CI 1.9-4.4; and OR 1.8; 95% CI 1.1-2.7, respectively). Conversely, compared to Europe, lower prevalence of NNRTI and protease resistance associated mutations were found in Asia (OR 0.1; 95% CI 0.02-0.3; OR 0.3; 95% CI 0.1-0.6). In theory, the dissimilar outcomes could have been explained by differences in the calendar years in which the sequences were collected. For example, sequences from the mid-1990s could contain relatively more TAMs due to usage of zidovudine monotherapy. We therefore adjusted our analysis for calendar year. In figure 2, the adjusted odd ratios are shown as white squares and do not differ much from the black (unadjusted) squares. Therefore the calendar year did not have an impact on the statistical significance of our results.

Trend analysis

For patients with a recent infection the incidence varied significantly (p=0.012) among time periods. The incidence of at 'least 1 resistance' mutation, decreased from 1996-1999 to 2000-2001 from 11.5% to 7.4%, increased to 13.6% in 2002-2003 and decreased again to 6.8% in 2004-2007 (figure 1D). Because of the fluctuation in incidences, no overall time trend could be observed (p=0.63).

Genotypic profiles

The most frequently occurring NRTI mutations were the TAM mutations (table 2). The prevalence of these TAMs was higher in North America (7.2%) and Europe (5.8%) than in the other continents (≤2.4%). The most prevalent TAMs found in North America and Europe were the M41L (4.3% and 2.3%, respectively) and the T215 revertants (3.3% and 3.2%, respectively). In Africa, some small differences were seen. Here, the M184V was observed more often (in 1.5% of the patients), whereas the TAMs were not found as frequently (2.8%) compared to the other continents. In Asia, except for A62V, low frequencies were found for all the three classes of antiretroviral drugs. For NNRTIs and PIs, the most prevalent drug resistant mutations across all continents were the K103N (ranging between 0-3.8%) and L90M (0.3%-3.6%) respectively.

The genotypic profiles of the different subtypes are shown in table 3. Frequently found mutations are the A62V in subtype A, and M41L and T215 revertants in subtype B. In subtype C, only mutations with a low frequency were found. These subtype mutation profiles were to a large extent in agreement with the differences that were seen among continents. And although the M46I/L mutation occasionally occurs naturally in untreated individuals with subtypes A, B, and C [58], this mutation was only found in 0.0%, 0.7%, and 0.5% in our study, respectively.

DISCUSSION

The WATCH study is the first large worldwide study on the epidemiology of transmission of drug resistant HIV-1. Using data from 6256 antiretroviral naïve patients from all continents (excluding Australia), we found a worldwide prevalence of 10.1%. Resistance was most frequently found for NRTIs. Importantly, simultaneous resistance to two or more different classes of antiretroviral drugs was limited with a prevalence of approximately 2%. Notably, only little dissimilarity in the type of drug

Table 3. Mutation profiles of different subtypes.

	A n=301 %	B n=3236 %	C n=658 %
NRTI			
M41L	0.0	2.6	0.6
A62V	16.3	0.2	0.3
K65R	0.0	0.0	0.0
D67N	0.0	1.1	0.3
K70RE	0.0	0.6	0.0
M184V	0.3	1.0	0.3
L210W	0.0	0.8	0.5
T215FY	0.0	0.9	0.3
T215revertants	0.0	3.2	0.2
K219EQ	0.0	0.9	0.0
TAMs[a]	0.0	4.4	0.8
=> 2 TAMs[a]	0.0	1.8	0.3
TAM +M184V	0.3	6.4	1.1
NNRTI			
K103N	1.0	1.5	0.9
V108I	0.0	0.5	0.3
V181C	1.3	0.5	0.3
PI			
L33F	1.0	0.2	0.0
M46I/L	0.0	0.7	0.5
V82AFSTL	0.0	0.8	0.0
L90M	0.0	1.0	0.2

[a] M41L + D67N + K70R + L210W + 215A/C/D/E/F/N/S/V/Y+ K219E + K219Q; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; and TAM, thymidine analogue mutation.

resistance was found between continents.

There are several limitations in this study. The first limitation is heterogeneity. Heterogeneity applies to differences in the strategy used to sample patients and in research methodology. We reduced the heterogeneity by re-analysing the HIV-1 sequences and the subsequent application of a single algorithm to score resistance.

The second limitation is publication bias. This bias occurs when studies that report a higher prevalence of resistance are more likely to be published. In recent years, a large number of studies have been published on transmission of drug resistant HIV [5, 10-11]. As a consequence, it may be difficult to publish new resistance results, especially when no resistance is found. The latter will result in an overestimation. Additionally, in African countries without access to antiretroviral drugs, resistance studies will not be performed. The relatively low prevalence estimate (6.8%) we found in Africa is therefore probably an overestimation.

The final limitation relates to the quality of the data. For instance, convenience sampling (i.e. a relative over-representation of patients suspected to carry a drug resistant virus) may have an impact on our prevalence estimates. Although we cannot rule out that convenience sampling occurred, the vast majority of included studies used well-defined sampling strategies to identify relevant patients.

The studies that were collected in WATCH used population sequence

analysis. This method fails to detect minor populations of drug-resistant quasi-species that are present in <20% of the virus population infecting a patient [59-60]. These minority variants have been detected in almost 14% of antiretroviral naïve HIV-infected individuals [61]. The presence of minorities, particularly involving NNRTI resistance, is associated with an increased risk of virological failure to first-line therapy [61]. Due to these minority viral variants, population-sequence analysis will underestimate the prevalence of drug resistant HIV-1.

The prevalence numbers we found for Europe are consistent with the independent European SPREAD study, where a resistance prevalence of 9% was reported [62]. In Asia a higher prevalence was observed and can be ascribed to a monophyletic cluster among intravenous drug users in Kazakhstan which included the NRTI-resistance related amino acid substitution A62V [63].

Prevalence of transmitted resistance varied geographically. A trend was seen of higher resistance prevalences in the Northern hemisphere compared to the Southern hemisphere. This trend could result from the relatively early start of prescribing antiretroviral drugs in North America and Europe, leading to a longer exposure time for viruses that circulate in North America and Europe. In addition, before 1996 antiretroviral drugs were prescribed as part of suboptimal therapy leading to an increased propensity of emergence of drug resistance.

WATCH found a limited differential distribution of resistance for particular classes of antiretrovirals between the continents. This is interesting as drugs were introduced at different moments in time.

The mutations profiles that were seen for the continents were in large agreement with the mutations profiles seen among subtypes. The profile of Asia was closely related with the profile of subtype A, and the profiles of North America and Europe were very similar to the profile of subtype B. This is not surprising since subtype B is the dominant subtype in Europe and North America, whereas in the other continents subtype A and C are more prevalent.

The most commonly observed mutations were associated with resistance to the thymidine analogues, especially the T215 variants and M41L. These mutations might reflect the extensive use of the NRTI zidovudine mono-therapy in the past. T215 variants arise in the absence of antiretroviral drugs due to reversion [5]. At codon 215, the resistance-associated substitutions T215F and T215Y require two nucleotide mutations for reversion to wild type. But in isolates obtained from patients who had not received antiretroviral treatment for their HIV-1 infection, revertant codons are frequently found that are intermediates between wild type and T215F/Y [5, 65-66]. Interestingly, viruses with a reversion at codon 215 have a decreased genetic barrier for the selection of the resistance-associated amino acid substitution T215Y [67]. This may indicate an increased risk for developing resistance when a subsequent treatment is given with zidovudine or stavudine [66].

The high frequency of the M41L mutation may be partly explained by the remarkable persistence of the M41L mutation in plasma over time, shown in several studies [68-71], indicating that reversion of some mutational patterns only occurs to a limited extent. In addition, a recent study proposed compensatory fixation as a possible explanation for the in vivo persistence of some mutational patterns [72]. The study reported the prolonged persistence (up to 4 years) of viruses with multiple protease mutations after treatment with protease inhibitors was stopped (treatment

with RT inhibitors was continued). It was found that these viruses have partially compensated for the initial loss in replication capacity. Reversion of a single mutations therefore causes a further reduction in replication capacity and, as a consequence, the route to wild-type is blocked [67].

In this paper, we gave an overview of the epidemiology of resistance to antiretroviral drug in drug-naïve patients worldwide. The resistance profiles of the three antiretroviral drug classes seem to be different among continents. Although the prevalence of resistance to antiretroviral drugs decreases, resistance can become a larger problem in third world continents, where antiretroviral drug therapy is becoming more widespread. Continuous global surveillance is needed to monitor the circulating HIV-strains and ensure that the development of treatment is adjusted to the drug resistance evolution.

AFFILATIONS

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Increase in transmitted resistance to non-nucleoside reverse transcriptase inhibitors among newly diagnosed HIV-1 infections in Europe

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ABSTRACT

Background: One out of ten newly diagnosed patients in Europe was infected with a virus carrying a drug resistant mutation. We analysed the patterns over time for transmitted drug resistance mutations (TDRM) using data from the European Spread program.

Methods: Clinical, epidemiological and virological data from 4317 patients newly diagnosed with HIV-1 infection between 2002 and 2007 were analysed. Patients were enrolled using a pre-defined sampling strategy.

Results: The overall prevalence of TDRM in this period was 8.9% (95% CI: 8.1-9.8). Interestingly, significant changes over time in TDRM caused by the different drug classes were found. Whereas nucleoside resistance mutations remained constant at 5%, a significant decline in protease inhibitors resistance mutations was observed, from 3.9% in 2002 to 1.6% in 2007 (p=0.001). In contrast, resistance to non-nucleoside reverse transcriptase inhibitors (NNRTIs) doubled from 2.0% in 2002 to 4.1% in 2007 (p=0.004) with 58% of viral strains carrying a K103N mutation. Phylogenetic analysis showed that these temporal changes could not be explained by large clusters of TDRM.

Conclusions: During the last decade transmitted resistance to NNRTI has doubled to 4% in Europe. The frequent use of NNRTI in first-line regimens and the clinical impact of NNRTI mutations warrants continued monitoring.

INTRODUCTION

The use of combination antiretroviral therapy has strongly reduced morbidity and mortality among patients infected with HIV [1]. This use of antiretroviral medication has, however, also led to transmission of drug resistant HIV-1. Approximately 10-15% of antiretroviral naïve patients in Europe [2-5] and North America [6-7] were infected with a virus carrying at least one transmitted drug resistance associated mutation (TDRM). These individuals are at a higher risk for developing virological failure to first-line antiretroviral therapy [8].

The objective of this study is to determine the trends in transmitted drug resistance in newly diagnosed HIV-1 infected patients over time in Europe. For this purpose, we analyzed the data collected by the pan-European SPREAD programme. This programme combines the efforts of virologists, clinicians and public health institutes to study the epidemiology of transmission of drug resistant HIV [2, 9]. SPREAD has used since 2002 the same sampling strategies for inclusion of patients newly diagnosed with HIV-1.

METHODS

Study population

The SPREAD Program includes patients with newly diagnosed HIV-1 infection from September 2002 through December 2007 in 26 European countries (Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Germany, Finland, Greece, Ireland, Italy, Latvia, Lithuania, Luxembourg, the Netherlands, Norway, Poland, Portugal, Romania, Serbia, Slovakia, Slovenia, Spain, and Sweden) and Israel. Although Israel is not officially part of Europe, the WHO includes Israel in the WHO European region definition [10]. Patients were included using a pre-defined sampling strategy based on the geographical and risk group distribution of patients newly diagnosed with HIV in the participating countries. For more details on the sampling strategy, inclusion- and exclusion criteria, and ethical clearance see the previous publications from the SPREAD Programme [2, 9]. Epidemiological, clinical, and behavioral data were collected using a standardized questionnaire within six months of diagnosis. A thorough data verification process preceded the analysis of the data [2, 9].

A blood sample was taken for genotypic resistance testing within six months after diagnosis. Population-based nucleotide sequencing of parts of the reverse transcriptase (RT) and protease (PR) genes of the virus was performed at local laboratories by means of commercially available kits or in-house methods [2, 9]. All countries took part in a blinded quality control program to verify the quality of the genotypic data generated. TDRM was defined according to the mutation list published for surveillance of transmitted drug resistance as recommended by the World Health Organization [11].

Seroconversion was documented in a proportion of the newly diagnosed patients. For some of these patients (n=882) seroconversion could be established because a last negative test was available within 3 years before diagnosis. In these patients, the date of infection was estimated as the midpoint between the date of

Table 1. Characteristics of all included HIV-1 patients and patients carrying a wild-type virus or a virus with transmitted drug resistance mutations to NRTI, NNRTI, or PI drug class.

Characteristics	Categories	Total	Wild-type	NRTI TDRM*	NNRTI TDRM*	PI TDRM*
Patients		4317	3933	218	125	107
Continent of Origin, no (%)	Western Europe	2404 (56)	2164 (55)	135 (62)	85 (68)	66 (62)
	Eastern Europe & Central Asia	919 (21)	848 (22)	48 (22)	16 (13)	17 (16)
	Sub-Saharan Africa	472 (11)	442 (11)	12 (6)	11 (9)	12 (11)
	Other	354 (12)	325 (8)	16 (7)	9 (7)	8 (7)
Gender, no. (%)	Male	3411 (79)	3084 (78)	190 (87)	107 (86)	88 (82)
Risk group, no. (%)	MSM	2084 (48)	1852 (47)	138 (63)	79 (63)	57 (53)
	Hetero	1501 (35)	1402 (36)	50 (23)	28 (22)	37 (35)
	Injecting drug use	355 (8)	337 (9)	7 (3)	10 (8)	5 (5)
Subtype, no. (%)	B	2855 (66)	2553 (65)	183 (84)	94 (75)	78 (73)
	non-B	1381 (32)	1306 (33)	31 (14)	27 (22)	27 (25)
Duration of infection, no (%)	<1 year	1236 (29)	1099 (28)	75 (34)	49 (39)	41 (38)
	1-2 years	144 (3)	130 (3)	8 (4)	6 (5)	2 (2)
	Unknown	2937 (68)	2704 (69)	135 (62)	70 (56)	64 (60)
Plasma HIV-RNA, median (IQR), log copies/ml		4.9 (4.3-5.3)	4.9 (4.3-5.3)	4.9 (4.3-5.5)	4.8 (4.1-5.5)	4.7 (4.3-5.2)
CD4 cell count, median (IQR), cells/mm^3		352 (180-540)	350 (177-534)	400 (186-572)	426 (275-577)	386 (251-593)
Age, median years (IQR)		35 (29-42)	35 (29-42)	35 (28-42)	35 (29-43)	34 (29-39)

TDRM, transmitted drug resistance mutations; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; MSM: men who have sex with men; IQR, interquartile ranges; * TDRM's from multiple classes was found in 49 (22.5%), 39 (31.2%), and 28 (26.2%) patients in the NRTI, NNRTI, and PI drug class, respectively, and are therefore counted in more than one drug resistance column.

the last negative and first positive test. In addition, for 506 patients primary HIV-1 infection was documented based on laboratory data. In these 506 patients, the date of the first positive (and subsequently confirmed) HIV test was used as the estimated date of infection. Patients were defined as recently infected when the duration of infection was <1 year.

For the purpose of analysis, Western Europe was defined to include those countries with a long history of good access to antiretroviral drugs. These countries included: Austria, Belgium, Cyprus, Germany, Denmark, Spain, Finland, Greece, Ireland, Italy, Luxembourg, the Netherlands, Norway, Portugal, Sweden, France, the United Kingdom, Switzerland, and Iceland. In our study, Israel was also included in the Western Europe category.

The HIV-1 subtypes were determined by use of the Rega HIV-1 subtyping tool (version 2.0, available at http://www.bioafrica.net/subtypetool/html/) [12].

Phylogenetic analyses

Phylogenetic analyses were performed to investigate clustering of sequences with TDRM. As controls we included 1) the genetically most closely related sequences in the entire SPREAD dataset (n=46) as identified by neighbour-joining phylogenetic trees constructed using Mega5 [13]; 2) the most closely related sequences (according to the percent of matching bases) in the Los Alamos Sequence Database (www.hiv.lanl.gov) as identified using the HIV BLAST tool (n=55; 3) subtype reference from the Los Alamos Sequence Database.

Sequences were aligned using Clustal W (BioEdit version 7.0.5.3) software [14] followed by manual editing and removal of TDRM-related codons [11]. Maximum likelihood trees were constructed for each relevant subtype using Mega5 and the best fitting nucleotide substitution model estimated by ModelTest v0.1.1 [15] under the Akaike information criterion. Robustness and statistical support of the internal branches of the maximum likelihood tree were evaluated with bootstrap analysis (1000 replicates). Potential non-nucleoside reverse transcriptase inhibitors (NNRTI) transmission clusters were defined as cluster including only sequences with at least one NNRTI TDRM with >70% bootstrap support and a mean genetic distance of <0.03 nucleotide substitutions per site [16-18].

Statistical analyses

The data were analyzed using the statistical software R (version 2.11.1). Categorical data were compared by use of the χ^2 test, Fisher exact test, or logistic regression techniques. Continuous data were investigated by means of the Mann-Whitney *U* test, linear regression, or Poisson regression. Prevalence values were calculated with a 95% Wilson score confidence interval (CI) on the basis of a binomial distribution. Trends in the prevalence of TDRM were calculated by logistic regression. Several factors were investigated as potential risk factors for TDRM: route of infection, recent infection, subtype, sex, age, continent of origin, CDC stage, CD4 cell count (square root transformed), log viral load. All statistically significant (P<0.1) univariate predictors of TDRM were considered as possible confounding factors in the multivariate time trend analysis.

RESULTS

Population characteristics

The SPREAD programme enrolled 4,470 newly diagnosed HIV-1 patients from September 2002 through December 2007. Included here are 4,317 patients for whom genotypic information was available. Data from patients included until 2005 (n=2687) have been reported previously [2, 19]. The current analysis contains 1630 additional patients, included between January 2006 and December 2007.

Table 1 shows the baseline characteristics for all patients. More than half (56%) originated from Western Europe, followed by patients originating from Eastern Europe and Central Asia (21%) and from Sub-Saharan Africa (11%). The most commonly reported transmission risk groups were men who have sex with men (MSM) (48%), followed by heterosexuals (35%) and injection drug users (8%). Most patients were male (80%). Most patients were diagnosed with HIV in their thirties. Nearly one third of patients were defined as recently infected (<1 year). Subtype B was the most frequent viral subtype (66%). At time of diagnosis the median log plasma HIV-RNA was 4.9 copies/ml (IQR: 4.3-5.3) and the median CD4 cell count 352 cells/mm3 (IQR: 180-540).

Prevalence of resistance

The overall prevalence of TDRM in newly diagnosed patients during the period 2002-2007 was 8.9% (95% CI: 8.1-9.8), of those 69% were infected with viruses carrying a single TDRM. Most mutations found were associated with nucleos(t)ide reverse transcriptase inhibitor (NRTI) resistance at 5.0% (95% CI: 4.4-5.7), but NNRTI resistance mutations at 2.9% (95% CI: 2.4-3.4) and protease inhibitor (PI) resistance mutations (2.5%; 95% CI: 2.1-3.0) were also observed. Dual- and multi-class resistance was seen in 0.8% and 0.4% of the patients, respectively. Most NRTI TDRM (184 of 218, 84.4%) were of the thymidine analogue mutations (TAMs) class that are associated with resistance to zidovudine and stavudine. The highest prevalence was found for the revertant mutations at position 215 (S/D/C/E/I/V at 2.7%), followed by M41L (1.7%), and L210W (0.6%). For NNRTI and PI, the most prevalent drug resistant mutations were K103N (1.7%), and L90M (0.6%), respectively.

Factors associated with TDRM

We analyzed which factors were associated with drug resistance for both the total TDRM group (table S1) as well as for the subgroups by drug class (table S2). In a univariate analysis, several factors were significantly associated with an overall increase in TDRM. These factors included a Western European origin (P=0.008), CD4 cell count (square root transformed) (P=0.01), MSM (P<0.0001), subtype B (P<0.0001) and recent infection (P=0.001). The NRTI and NNRTI drug classes showed the same significant predictors for resistance, although the square root CD4 cell count was not being associated with resistance for the NRTI drug class. For the protease inhibitors class, the factors associated with TDRM were log HIV-RNA load, age per 10 years, square root CD4 cell count, and recent infection.

Table 1 shows that most characteristics were similar for patients infected with an NRTI-TDRM, an NNRTI-TDRM, or a PI-TDRM virus. For example, similar

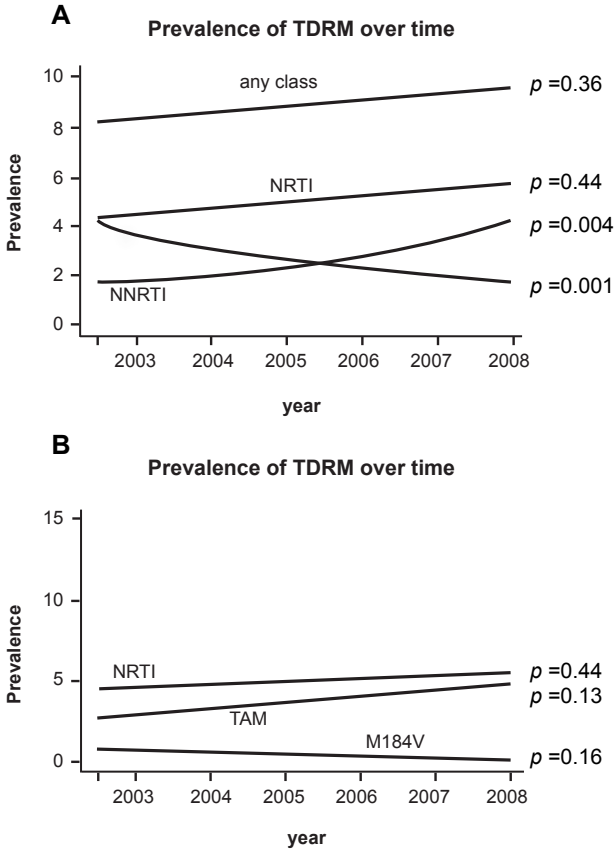

Figure 1. Smoothed line of prevalence of transmitted drug resistant mutations (TDRM) in patients diagnosed from 2002 to 2007 at time of sequence sampling.
(A) Prevalence of TDRM associated with any of the drug classes (any class), nucleoside reverse transcriptase inhibitor (NRTI), non-nucleoside reverse transcriptase inhibitor (NNRTI), and protease inhibitor (PI). (B) Prevalence of mutations associated with nucleoside reverse transcriptase inhibitors (NRTI), thymidine analogue mutations (TAM) and revertants, and the M184V mutation. The p-values of the time trends are shown.

proportions originating from Western Europe were seen in patients infected with a virus with NRTI-TDRM (62%), NNRTI-TDRM (68%), or PI-TDRM (62%). The proportion of males ranged between 82 and 87%, and the proportion of MSM between 53 and 63% in the three resistance groups. The duration of infection was similar in all three groups. The proportion of patients recently infected was 34% in the NRTI, 39% in the NNRTI and 38% in the PI TDRM groups.

TDRM trends over time

Logistic regression showed that the overall prevalence of TDRM (8.8% in 2002 and 9.8% in 2007) was stable over time (odds ratio [OR], 1.03 [95% CI, 0.97-1.10]; p=0.37) (figure 1A). Interestingly, we did observe significant changes in resistance to particular classes of antiretroviral drugs. For the NNRTI TDRM, the prevalence was 2.0% in 2002 and increased to 4.1% in 2007. Logistic regression showed that this increase was significant (OR, 1.18 [95% CI, 1.06-1.32]; p=0.004). In contrast, for PI TDRM, the highest prevalence was found in 2002 at 3.9% and it decreased significantly over time to 1.6% in 2007 (OR, 0.81 [95% CI, 0.72-0.92]; p=0.001). The prevalence of NRTI TDRM was stable, at 5.0% in 2002 and 5.2% in 2007 (OR, 1.03 [95% CI, 0.95-1.13]; p=0.44). Factors associated with TDRM (P<0.1) were included

in the multivariate time trend analyses. Adjusting for these factors did not affect the time trend estimates and significance.

We investigated several hypotheses that could explain the increase in transmission of NNRTI TDRM. The first possible explanation could be that a few patients infected with a strain that contains transmitted NNRTI resistance transmitted their virus to substantial numbers of other individuals. However, this explanation is not plausible as phylogenetic analyses showed only a limited number of clusters containing the K103N amino acid substitution and these clusters were comprised of only a small number of patients (figure 2). Second, an increase in transmitted NNRTI resistance could be explained by migration from Africa, as nevirapine is frequently used for prevention of mother-to-child-transmission in Africa. From Table 1 it can be concluded that this is unlikely given that only eight (9%) patients with a single NNRTI mutation were coming from Sub-Saharan Africa.

We further investigated the time trends for specific TDRM within the NRTI drug class. TAMs were selected in many treated patients before the HAART era by single and dual therapy including zidovudine or stavudine. The M184V mutation can be selected by the drugs emtricitabine, lamivudine, and abacavir. Any of these drugs have been part of the recommended NRTI backbones in treatment that were in use during the time that we collected our data [20-23]. We detected the M184V mutation in 16 patients (0.4%). Figure 1B shows that both the prevalence of the TAMs and corresponding revertants and the M184V mutations were stable over time (OR, 1.07 [95% CI, 0.98-1.18]; p=0.13 and 0.79 [95% CI, 0.56-1.10]; p=0.16, respectively).

DISCUSSION

We studied the prevalence of transmission of drug resistance among patients newly diagnosed with HIV-1 in Europe. The overall prevalence of TDRM remained stable over time in Europe at a level that is just below 10%. But, the underlying prevalence of TDRM associated with particular antiretroviral drug classes showed important changes over time. We found a significant increase in the prevalence of transmitted NNRTI resistance, doubling from 2.1% in 2002 to 4.1% in 2007. In contrast, transmitted PI resistance decreased significantly from 3.9% to 1.6%. Transmitted NRTI resistance mutations remained stable over time (5.7%) and generally involved TAM mutations.

Several studies reported on the changes of TDRM over time in single countries in Europe [24-28]. Recent data from Italy are in agreement with our results. The Italian study reported a similar significant decrease in resistance to PIs and NRTIs and an increase in resistance to NNRTIs in the same time frame [26]. Also, a study in seroconverters in Germany found stable overall resistance and an increase over time for NNRTI resistance (although not significant) between 1996 and 2007. However, transmitted NRTI resistance was decreasing and PI resistance was stable over time [27]. In Sweden, a low overall prevalence of resistance was found (5.8%) and no clear trend over time [28]. In addition, a study from Belgium found no changes over time, which can partly be explained by the smaller sample size in this study and thus the reduced power to detect statistically significant changes [25].

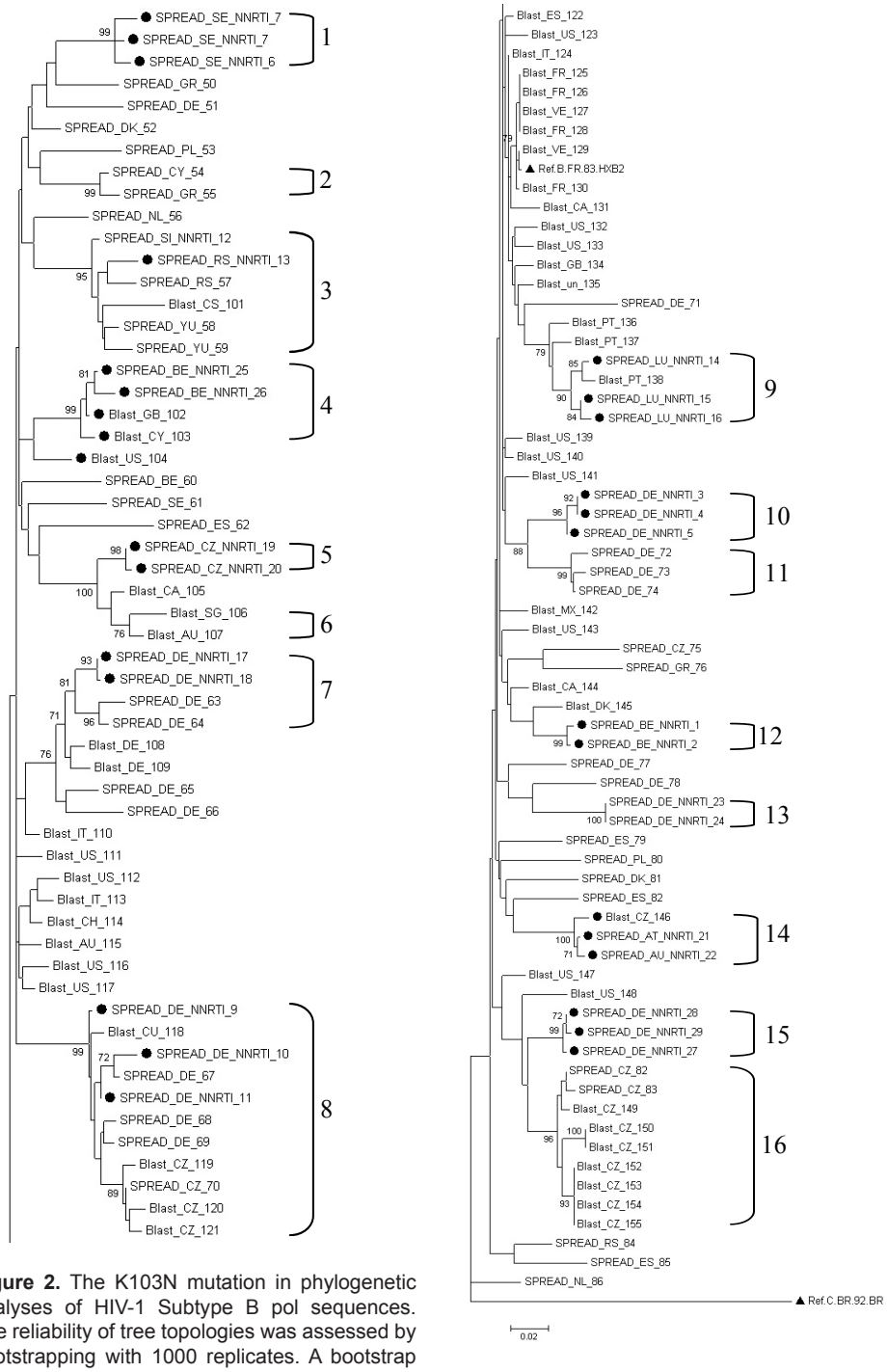

Figure 2. The K103N mutation in phylogenetic analyses of HIV-1 Subtype B pol sequences. The reliability of tree topologies was assessed by bootstrapping with 1000 replicates. A bootstrap support of 70%, or greater, are shown at nodes on the tree. The source of the data, the country of residence, and the presence of a non-nucleoside reverse transcriptase inhibitor (NNRTI) resistance mutation are included in the sequence-label. The 16 square brackets show patients in a phylogenetic cluster with bootstrap support of >70% and a mean genetic distance of <0.03 nucleotide substitutions per site; (●) indicated patients with a K103N mutation; (▲) highlights a reference sequence.

In the previous study published by the SPREAD programme, transmitted NNRTI resistance showed a statistically significant parabolic time trend over the time period of 2002 to 2005 with a peak at the end of 2004 (p=0.02)[2]. The change from a parabolic to a linear increase over time that was found in this study, which includes the years 2006 and 2007, could be explained by the longer time period covered and the increase in power to calculate time trends. Furthermore, the data from 2006 and 2007 showed that the initial increase in NNRTI resistance that was seen in the previous study persisted in these later years.

We investigated several factors that could explain the increase of transmitted NNRTI resistance in Europe. Migration from Africa could have explained the increase as a single-dose of the NNRTI nevirapine has been used extensively for prevention of mother-to-child-transmission, which resulted in increased levels of NNRTI resistance [29-30]. However, this is highly unlikely because only 8 (9%) patients with a single NNRTI mutation came from Sub-Saharan Africa in our dataset. Second, it is important to note that virological studies showed that the K103N, a major NNRTI mutation, can persist in the absence of treatment [31]. However, our phylogenetic analyses indicated that an increase in transmitted NNRTI resistance did not occur within phylogenetic clusters thus suggesting that TDR with K103N originated from different sources.

Changes in prescribing practices most likely explain the increased rates of transmitted NNRTI resistance mutations. NNRTIs have become more popular in first-line treatment as they have good clinical efficacy [32-33] and are convenient to use (low pill burden) which improves adherence [34]. Unfortunately, NNRTIs have a low genetic barrier to drug resistance. A single amino acid change is sufficient for high level drug resistance to the most commonly used NNRTIs in first-line treatment [35]. We believe that with the use of NNRTIs in first line regimens (in combination with emtricitabine/lamivudine plus either tenofovir or abacavir) resistant viruses can become selected in failing patients. Early after failure these viruses carry a single NNRTI mutation often combined with the M184V/I [36]. M184V has a strong effect on replication capacity and if transmitted, reverts back to wild-type rapidly (68% after 6 months of HIV infection [37]). In contrast, the K103N has a limited effect on viral replication capacity and persist for long periods after transmission [31] and strains with this mutation are therefore also transmitted to others (onward transmission) [30, 38].

The decreasing transmission of PI resistant mutations can also be explained by changes in prescribing practices over time. First, PIs have become less popular as randomized clinical trials showed that NNRTIs results in a better virological outcome [32-33]. In addition, over time PIs have increasingly been given with low dose ritonavir (or boosted PIs), which have a high genetic barrier for drug resistance. Therefore the chance of selecting resistant viruses upon treatment failure is very low, likely resulting in a decreased rate of PI versus NNRTI- TDR [39-42].

The persistent high levels of TAMs and revertants over the years are not in line with prescribing practices. TAMs were originally selected by the thymidine analogues stavudine and zidovudine, which have been used extensively in the past but have over time become uncommon in first-line treatment. The persistently high levels of TAMs and revertants can be explained by initial selection in the early 1990s, and subsequently the original selected mutations may have persisted. In addition, revertants or intermediates have evolved in the absence of drug pressure and

persisted since then. This is confirmed by several studies showing that TAMs and revertants tend to persist in the absence of antiretroviral drugs [31, 37]. Given that we find transmitted TAMs also in patients with recent infection despite the limited use of zidovudine during the study period indicates that these viruses are descendants of resistant viruses generated ten to fifteen years ago that still are circulated and being transmitted.

A limitation of our study is that we used population sequencing to identify drug resistance associated mutations. Although population sequencing is standard practice across Europe, this technique fails to identify drug-resistant minority variants that are present in <20% of the virus population infecting a patient [43-44]. These minority variants have been detected in almost 20% of antiretroviral naïve HIV-infected individuals [45]. The presence of minorities, particularly involving NNRTI resistance, is associated with an increased risk of virological failure to first-line therapy [45]. The increasing levels of transmitted NNRTI resistance are therefore worrying, as we most probably underestimate the real prevalence in this study.

Representativeness of the data could also be a limitation in our study. We assessed the representativeness by comparing the distribution of the transmission groups in all countries included in SPREAD with the HIV surveillance data from the European Centre for Disease prevention and Control (ECDC) (data not shown). The proportional distribution of the different transmission groups was very comparable. However, compared to the data from ECDC, MSM were somewhat over-represented in some of the countries participating in SPREAD. This may suggest that the estimated prevalence in our study might be slightly overestimated.

A strength of our study is the data collection that is performed within the SPREAD programme. The SPREAD programme is a large and sufficiently powered pan- European study that has been running since almost ten years. During this time the programme included patients newly diagnosed with HIV using a predefined strategy that is based on the transmission routes and geographical distribution of HIV in the participating countries.

The SPREAD programme studies the prevalence of TDRM in newly diagnosed patients, of which most patients are chronically infected. Several studies showed that resistance levels in recently infected patients are higher compared to those in chronically infected patients [27, 46]. The reason for choosing to investigate newly diagnosed patients is that these patients reflect the patients coming under medical attention. Furthermore, to limit the analyses only to recently infected patients might give a biased result, as MSM (which have higher prevalence of TDRM) are being tested more frequently and are therefore more often recently infected at HIV-diagnosis compared to other risk-groups.

The results from this study have several implications for clinical practice and public health. The single TAMs and revertants found do generally not cause resistance to nucleos(t)ides currently popular in first-line regimens (emtricitabine, tenofovir, lamuvidine, abacavir). Therefore, the high prevalence of resistance to single TAMs that was found in Europe probably will not have a great impact on the efficacy of first-line therapy. The low prevalence of PI mutations and their negligible effect on the efficacy of boosted PIs also implies that they will not have a major public health implication. Conversely, the increasing prevalence of transmitted NNRTI resistance is likely to negatively influence the therapy response to NNRTI-containing

regimens. Since it is it unknown whether the increasing NNRTI resistance levels will increase even more or will level-off, surveillance of TDRM will remain important.

In conclusion, during the last decade, rates of transmitted resistance to certain drug classes have changed considerably. PI resistance declined between 2002 and 2007. In contrast, a significant increase in transmitted NNRTI resistance was observed. This finding underscores the importance of baseline drug-resistance testing prior to the beginning of treatment, given the medical evidence that transmitted NNRTI reduces the efficacy of current first line NNRTI-based regimens [8].

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SUPPORTING INFORMATION

Table S1. Predictors of TDRM: univariable and multivariable models.

	Univariable		Multivariable	
Variable	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***
Continent of Origin				
Western Europe	1.35 (1.08-1.67)	0.008	1.12 (0.87-1.44)	0.38
other				
Baseline values				
HIV-RNA load, log	1.02 (0.90-1.16)	0.77		
Age, per 10 years	1.07 (0.77-1.48)	0.68		
CD4, square root	1.02 (1.00-1.03)	0.01	1.01 (0.99-1.02)	0.45
Risk group				
MSM*	1.80 (1.44-2.27)	<0.0001	1.41 (1.07-1.87)	0.02
other				
CDC stage				
C	0.82 (0.58-1.15)	0.25		
A and B				
Subtype				
B	2.06 (1.59-2.68)	<0.0001	1.49 (1.08-2.06)	0.02
non-B				
Duration of infection				
<1 year	1.43 (1.15-1.78)	0.001	1.13 (0.88-1.46)	0.34
other				

TDRM, transmitted drug resistance mutations; MSM, men who have sex with men. *P*<0.1 was chosen as the cut-off for selecting the predictors into the multivariable analyses; * gender was not included in the model due to multicolinearity with MSM.

Table S2. Predictors of TDRM to individual drug classes: univariable and multivariable models.

	NRTI class				NNRTI class				PI class			
	Univariable		Multivariable		Univariable		Multivariable		Univariable		Multivariable	
Variable	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***
Continent of Origin												
Western Europe	1.33 (1.00-1.76)	0.052	1.04 (0.76-1.43)	0.81	1.69 (1.15-2.48)	0.007	1.40 (0.91-2.16)	0.13	1.27 (0.85-1.89)	0.24		
other												
Baseline values												
HIV-RNA load, log	1.07 (0.90-1.26)	0.454			0.95 (0.76-1.19)	0.67			0.80 (0.63-1.02)	0.07	0.81 (0.62-1.06)	0.13
Age, per 10 years	0.98 (0.63-1.51)	0.92			0.76 (0.41-1.43)	0.40			1.79 (1.09-2.94	0.02	1.51 (0.79-2.88)	0.21
CD4, square root	1.01 (0.99-1.03)	0.20			1.03 (1.00-1.05)	0.03	1.01 (0.99-1.04)	0.34	1.02 (1.00-1.05)	0.10	1.01 (0.98-1.04)	0.64
Risk group												
MSM*	2.13 (1.57-2.90)	<0.0001	1.51 (1.05-2.19)	0.03	1.83 (1.24-2.69)	0.002	1.55 (0.95-2.53)	0.08	1.21 (0.81-1.81)	0.35		
other												
CDC stage												
C	0.92 (0.60-1.41)	0.70			0.61 (0.32-1.18)	0.14			0.59 (0.28-1.22)	0.15		
A and B												
Subtype												
B	2.98 (2.03-4.39)	<0.0001	2.10 (1.33-3.31)	0.001	1.59 (1.04-2.42)	0.03	0.94 (0.56-1.58)	0.81	1.41 (0.90-2.19)	0.13		
non-B												
Duration of infection												
<1 year	1.33 (0.99-1.77)	0.054	1.04 (0.75-1.43)	0.82	1.63 (1.13-2.35)	0.009	1.28 (0.84-1.94)	0.25	1.57 (1.06-2.33)	0.02	1.29 (0.75-2.22)	0.35
other												

TDRM, transmitted drug resistance mutations; MSM, men who have sex with men; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor. *P*<0.1 was chosen as the cut-off for selecting the predictors into the multivariable analyses. * gender was not included in the model due to multicolinearity with MSM.

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Different time trends of transmitted drug resistance among MSM and heterosexual patients in Europe

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ABSTRACT

Background: In Europe 10% of newly diagnosed patients in Europe become yearly infected with drug resistant HIV-1. Little is known about the risk factors for transmission of drug resistant HIV. We analysed data from the SPREAD programme, to gain insight in the prevalence and associated time trends of transmitted drug resistance mutations (TDRM) in different transmission groups.

Methods: The SPREAD programme recruited newly diagnosed HIV-1 patients from September 2002 through December 2007. Sampling was representative for transmission group and geographical distribution in the participating countries. Trends over time were calculated by logistic regression.

Results: From the 4317 patients included, the majority was men-having-sex-with-men -MSM (2084, 48%), followed by heterosexual patients (1501, 35%) and injection drug users (355, 8%). MSM were more often originating from Western Europe, infected with subtype B virus, and recently infected (<1 year) ($p<0.001$). The prevalence of TDRM was highest in MSM (prevalence of 11.1%), followed heterosexuals (6.6%) and injection drug users (5.1%, $p<0.001$). TDRM was predominantly ascribed to nucleoside reverse transcriptase inhibitors (NRTI) with a prevalence of 6.6% in MSM, 3.3% in heterosexuals and 2.0% in injecting drug users ($p=0.001$). The overall prevalence of TDRM was stable for both the MSM ($p=0.19$) and the heterosexual group ($p=0.09$). Interestingly, a significant increase in resistance to non- nucleoside reverse transcriptase inhibitors (NNRTIs) and a decrease in resistance to protease inhibitors was observed in MSM ($p=0.008$ and $p=0.006$, respectively), but not in the heterosexual patients ($p=0.68$ and $p=0.14$, respectively).

Conclusion: MSM showed to have significantly higher TDRM prevalence compared to heterosexual patients and injection drug users. We observed a sharp increase of transmitted NNRTI resistance in MSM requiring further action given that NNRTIs are frequently used in first line regimens.

INTRODUCTION

Antiretroviral therapy has strongly reduced morbidity and mortality in HIV infected individuals [1]. This use of antiretroviral medication, however, also led to transmission of drug resistant HIV-1. Transmission of drug resistance has important clinical ramifications as it is associated with an increased probability for virological failure [2]. Importantly, the problem is large, with prevalence ranging between 10 and 15% of antiretroviral naïve patients infected with a virus carrying at least one transmitted drug resistance associated mutation (TDRM) mutation in Europe [3-6] and North America [6-8].

The prevalence of TDRM is expected to be different among different routes of transmission in Europe. Men having sex with men (MSM) are mostly originating from resource-rich countries where antiretroviral drugs have been available for many years. Until the early 1990s, patients infected with HIV received mono- or dual-therapy with nucleoside reverse transcriptase inhibitors (NRTI). This mono- and dual-therapy led to a rapid development of resistance mutations [9-10]. In contrast, heterosexually infected patients in Europe are mostly immigrants from Sub-Saharan Africa or individuals from Eastern Europe areas where large scale use of antiretrovirals has only recently been initiated. These differences in drug use between the transmission groups are reflected in several studies showing a higher likelihood in MSM patients to be infected with a resistant virus compared to other patients [3, 11].

However, access to antiretrovirals has rapidly been scaled-up during the past decade, leading to prevalence of TDRM already as high as 11.6% in some areas in sub-Saharan Africa [12]. The TDRM epidemic in Africa is often associated with non-nucleoside reverse transcriptase inhibitors (NNRTIs), which is consistent with the use of single-dose nevirapine in Africa to prevent mother-to-child transmission [13]. However, in many areas the size of the problem of TDRM remains limited with a prevalence of <5% [14].

Besides a difference in the overall prevalence of TDRM between HIV transmission groups, variation in particular antiretroviral drug use may therefore also result in differences in the prevalence of TDRM to specific HIV drug classes. However, these differences are difficult to estimate, since the prevalence of resistance to a particular drug class often does not exceed 5% [3-5, 11], leaving a small number of patients with resistant virus in each transmission group. As a result most studies are underpowered to detect statistical significant differences in case there are relevant differences in the prevalences of resistance to specific drug classes between MSM, heterosexuals and injection drug users.

Due to differences in the use of these drug classes over time, TDRM to specific drug classes may have evolved differently over time. Yet, there are no studies performed which analyse these time trends in the different transmission groups European-wide. Therefore, the aim of our study was to examine the prevalence of TDRM for the individual drug classes between various HIV transmission groups in Europe and to study temporal trends of TDRM in these subgroups.

METHODS

Study population

Our analyses included data from the SPREAD Programme. The SPREAD programme recruited individuals newly diagnosed with HIV-1 from September 2002 through December 2007 in 26 European countries. Patients were included using either a i) pre-defined sampling strategy based on the geographical and risk group distribution of patients newly HIV diagnosed or ii) a random sample if there was access to >80% of all patients newly diagnosed within a particular country. These approaches for including patients allowed representative sampling of newly diagnosed patients in the participating countries. For more details on the sampling strategy, see the previous reports of the SPREAD Programme [3, 15]. Epidemiological, clinical, and behavioural data were collected using a standardized questionnaire within six months of diagnosis.

A blood sample was taken for genotypic resistance testing within six months after diagnosis. Population-based nucleotide sequencing of the reverse transcriptase (RT) and protease (PR) genes of the virus was performed at local laboratories by means of commercially available kits or in-house methods. TDRM was defined according to the mutation list published for surveillance of TDRM as recommended by the World Health Organization [16].

Seroconversion was documented in a proportion of the newly diagnosed patients. For some of these patients (n=882) a short term infection could be established because a last negative test was available within 3 years before diagnosis. In these patients, the date of infection was estimated as the midpoint between the date of the last negative and first positive test. In addition, for 506 patients primary HIV-1 infection was documented based on laboratory data. In these 506 patients, the date of the first positive (and subsequently confirmed) HIV test was used as the estimated date of infection. Patients were defined as recently infected when the duration of infection was <1 year.

Statistical analyses

The HIV-1 subtypes were determined by use of the Rega HIV-1 subtyping tool (version 2.0, available at http://www.bioafrica.net/subtypetool/html/) [17]. The data were analyzed using the statistical software R (version 2.11.1). Prevalence values were calculated with a 95% Wilson score confidence interval (CI) on the basis of a binomial distribution. Categorical data were compared using the chi-square test, Fisher's exact test, or logistic regression techniques. Continuous data were investigated by means of a Mann-Whitney *U*-test or the Kruskal Wallis test.

Trends in the prevalence of TDRM were calculated by logistic regression. All statistically significant (P<0.1) univariate predictors of TDRM were considered as possible confounding factors in the multivariate time trend analysis.

RESULTS

Population characteristics

A total of 4317 newly diagnosed HIV-1 patients were included in the SPREAD

Table 1. Characteristics of patients.

Characteristics	Categories	MSM	Hsx	IDU	p-value
Patients		2084	1501	355	
Continent of Origin, no. (%)	Western Europe	1457 (69.9)	589 (39.2)	179 (50.4)	<0.001
	Eastern Europe & Central Asia	378 (18.1)	253 (16.9)	145 (40.8)	
	Sub-Saharan Africa	10 (0.5)	451 (30.0)	4 (1.1)	
	Other	239 (11.5)	208 (13.9)	27 (7.6)	
Baseline values	HIV-RNA load, median (IQR), log copies/ml	4.88 (4.3-5.4)	4.79 (4.2-5.3)	4.76 (4.2-5.3)	<0.001
	CD4 cell count, median (IQR), cells/mm^3	435 (259-585)	280 (110-458)	392 (197-521)	<0.001
	Age, mean years (IQR)	36.1 (29-41)	37.7 (29-45)	33.2 (26-39)	<0.001
Gender, no. (%)	male	2070 (99.3)	765 (51.0)	269 (75.8)	<0.001
CDC stage, no. (%)	A and B	1860 (89.3)	1148 (76.5)	277 (78.0)	<0.001
	C	167 (8.0)	250 (16.7)	37 (10.4)	
Subtype, no. (%)	B	1884 (90.4)	503 (33.5)	218 (61.4)	<0.001
	A	86 (4.1)	275 (18.3)	79 (22.2)	
	C	15 (0.7)	259 (17.3)	6 (1.7)	
	02_AG	22 (1.1)	158 (10.5)	5 (1.4)	
	G	11 (0.5)	96 (6.4)	24 (6.8)	
	F	10 (0.5)	54 (3.6)	2 (0.6)	
	others	30 (1.4)	115 (7.7)	16 (4.5)	
	unassigned	26 (1.2)	41 (2.7)	5 (1.4)	
	non-B	174 (8.3)	957 (63.8)	132 (37.2)	
Duration of infection, no. (%)	<1 year	897 (43.0)	203 (13.5)	83 (23.4)	<0.001
	1-2 years	108 (5.2)	23 (1.5)	12 (3.4)	
	Unknown duration	1079 (51.8)	1275 (84.9)	260 (73.2)	

Data are no. (%) of patients, unless otherwise indicated. Characteristics describe patients from whom a baseline HIV-1 genotypic analysis was available. CDC, Centers for Disease Control and Prevention; IQR, interquartile ranges; MSM, men who have sex with men; Hsx, heterosexual patients; IDU, injection drug users.

programme from September 2002 through December 2007. From these 4317 patients, the majority (2084, 48.3%) was infected through MSM, followed by heterosexuals (1501, 34.8%) and injection drug users (355, 8.2%). The baseline characteristics of these three transmission groups are summarized in table 1. MSMs were more often recently infected (<1 year) (43.0%) than injection drug users (23.4%) and heterosexuals (13.5%) (p<0.001). As a result of the higher proportion of recent infections in MSMs, these patients had a higher median CD4 cell count (435, interquartile range (IQR) 259-585 cells/mm^3) than the corresponding CD4 values found in heterosexually infected patients (median 280, IQR 110-458 cells/mm^3) and in injecting drug users (median 392, IQR 197-521 cells/mm^3) (p<0.0001). Similarly, CDC stage C (advanced stage of the HIV disease) was observed in only 8.0% in MSM compared to 16.7% in heterosexually infected patients and 10.4% in injection drug users (p<0.0001). Furthermore, the proportion of patients originating from Western Europe was highest in MSM (69.9%), followed by injection drug users (50.4%) and heterosexual patients (39.2%) (p<0.0001). Large differences were seen in subtype distribution (p<0.0001). The most reported HIV subtype in viral isolates from MSM was B (90.4%) whereas in injection drug users and heterosexual patients subtype B was only seen in 61.4% and 33.5% of the patients, respectively. In injection drug users, the most commonly found non-B subtype was subtype A, which was observed in 22.2%. In heterosexual infected patients both subtype A (18.3%) and subtype C (17.3%) were the most frequently observed non-B subtype.

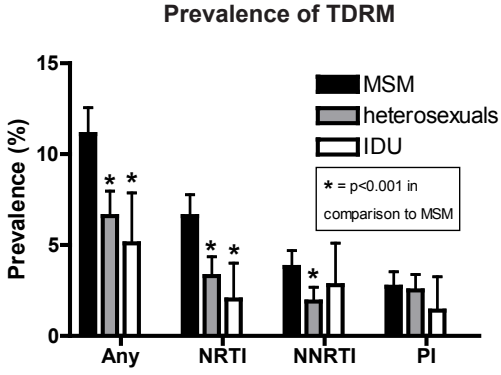

Figure 1. Prevalence of transmitted drug resistance mutations (TDRM) to the specific drug classes in the three main transmission groups.
Prevalences are shown of resistance to at least one of the drug classes (Any), nucleoside reverse transcriptase inhibitor (NRTI), non-nucleoside reverse transcriptase inhibitor (NNRTI) and protease inhibitor (PI) in men who have sex with men (MSM), heterosexual patients, and injection drug users (IDU).

Genotypic resistance analysis

The prevalence of overall TDRM in MSM was 11.1% (95% CI: 9.9-12.6%). This was significantly higher (p <0.001) than in heterosexual patients (6.6%; 95% CI: 5.4-8.0%) and injection drug users (5.1%; 95% CI: 3.2-7.9%) (figure 1). Similarly, for resistance in the NRTI drug class, the prevalence was at least twice as high in MSM (6.6%; 95% CI: 5.6-7.8%) compared to heterosexuals patients (3.3%; 95% CI: 2.5-4.4%; p<0.001) or injection drug users (2.0%; 95% CI: 1.0-4.0%; p=0.001). Most of this NRTI resistance was associated with thymidine analogue resistance mutations in MSM (87.7%), in heterosexuals (70.0%) and in injection drug users (100%).

For the NNRTI drug class, the prevalence in MSM (3.8%; 95% CI: 3.1-4.7%) was significantly higher compared to heterosexual patients (1.9%; 95% CI: 1.3-2.7%;

p<0.001) but not to injection drug users (2.8%; 95% CI: 2.3-1.3%; p=0.44). Notably, the prevalence of NNRTI TDRM in injection drug users was higher than the TDRM prevalence for NRTI in this transmission group. In other risk groups TDRM to NRTI was higher. The most prevalent NNRTI drug resistant mutation was K103N (>57% in all three transmission groups).

In the protease inhibitor (PI) drug class, no statistically significant differences were seen. This could be due to the low prevalence of transmitted PI resistance found in all risk groups; MSM 2.7%, HSX 2.5%; IDU 1.4%). The most prevalent drug resistant mutation was the L90M (>24% in all three transmission groups).

A large proportion (61%) of the heterosexual patients did not originate from Western Europe or North America. We subdivided the heterosexual patients into patients originating in Western Europe or North America and patients originating outside this region. Of patients originating outside Western Europe or North America, 51.8% were from Sub-Saharan Africa. The prevalence of resistance in heterosexual patients originating from Western Europe or North America was 7.8% for overall, 4.1% for NRTI, 2.0% for NNRTI and 2.5% for PI resistance. These prevalences did not differ significantly from the prevalence of resistance in heterosexual patients originating from non-Western countries (5.8% for overall; p=0.14, 2.9% for NRTI; p=0.24, 1.8% for NNRTI; p=0.70, and 2.4% for PI; p=0.87). Also, when excluding the patients originating from outside Western Europe or North America from the analyses, the prevalence of TDRM remained significantly different between MSM and heterosexual patients for overall TDRM (p=0.02), NRTI (p=0.02) and NNRTI (p=0.04). In patients originating from Western Europe or North America, we performed an analysis where we only included patients recently infected (within 1 year). In these patients we found an overall TDRM prevalence of 13.1% in MSM and 6.8% in heterosexual patients.

Notably, transmitted drug resistance to protease inhibitors was largely ascribed to M46I/L. This mutation was found in 8 of the 22 patients infected with a PI-resistant virus and originated from outside Western Europe and North America. In patients originating from Western Europe and North America, this mutation was found in 6 out of 15 patients infected with a PI-resistant virus. These mutations were not associated with a specific subtype. The mutations showed a similar prevalence in patients originating from Western Europe and North America and patients originating from outside this region (1.0 and 0.9%, respectively; p=0.86).

Time trends

Trends over time were only examined in MSM and heterosexuals, as the number of injection drug users was too low for this analysis. The prevalence of overall TDRM slightly increased - but not statistically significant- over time in the MSM group, with 10.1% in 2003 and 12.5% in 2007 (odds ratio [OR], 1.06 [95% CI, 0.97-1.15]; p=0.19) (figure 2A). Conversely, the prevalence declined slightly among the heterosexually infected individuals with a prevalence of 4.4% in 2003 and 2.3% in 2007 (OR, 0.89 [95% CI, 0.78-1.02]; p=0.09) (figure 2B). The NRTI prevalence followed the same time trend as the overall TDRM prevalence, with an OR of 1.07 (95% CI: 0.96-1.19; p=0.22) in MSM and 0.84 (95% CI: 0.69-1.01; p=0.07) in the heterosexual group.

In prevalence of resistance to other drug classes, different trends were observed. Importantly, the NNRTI resistance prevalence increased three fold from

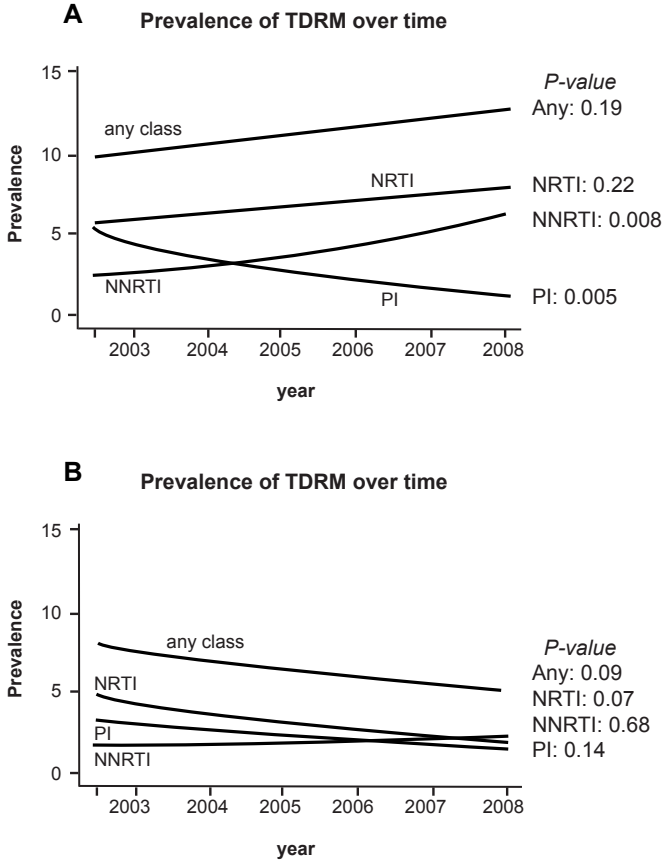

Figure 2. Smoothed line of prevalence of TDRM in patients diagnosed from 2002 through 2008 at time of sequence sampling.
Prevalence of transmitted drug resistance is shown for any of the drug classes (any class), nucleoside reverse-transcriptase inhibitor (NRTI), nonnucleoside reverse-transcriptase inhibitor (NNRTI) and protease inhibitor (PI) in (A) Men having sex with men, and in (B) heterosexual patients. The p-values of the time trends are shown on the right side of the graph.

1.7% in 2003 to 5.0% in 2007 in MSM patients (OR, 1.21 [95% CI, 1.05-1.39] p=0.008). For PI resistance, the prevalence was significantly decreasing over time from 4.6% in 2003 to 2.0% in 2007 (OR, 0.79 [95% CI, 0.66-0.93]; p=0.006). This increase of NNRTI resistance and the decrease of PI resistance was not observed in the heterosexual patients (p=0.68 and p=0.14, respectively). Adjusting for factors significantly associated with TDRM in the univariate analyses did not change any of the time trend effects that were found.

When splitting up heterosexuals into individuals infected in Western Europe or North America and people not infected in this region, no significant time trends were found for any of the drug classes in the heterosexuals infected in Western Europe or North America. In the heterosexuals infected in regions outside Western Europe and North America, the overall TDRM decreased significantly (p=0.03) from 8.1% in 2003 to 3.1% in 2007. This can be explained by the significant (p=0.02) decrease in the NRTI resistance from 4.4% in 2003 to 1.3% in 2007 (data not shown).

DISCUSSION

In this study we examined the prevalence and time trends of TDRM in the three most relevant HIV-transmission groups, MSM, heterosexual patients, and injection drug users. We found significant differences in prevalence of resistance between these three transmission groups. MSM showed to have significantly higher TDRM prevalence of 11.1% compared to 6.6% in heterosexual patients and 5.1% in injection drug users. We were also able to compare the prevalence of resistance to the specific drug classes between transmission groups. The largest difference was found in NRTI resistance, where the prevalence was significantly higher in MSM (6.6%), compared to heterosexual patients (3.3%) and injection drug users (2.0%). But also for the NNRTI drug class, the resistance prevalence were higher in MSM (3.8%) compared to heterosexual patients (2.8%). The prevalence of TDRM to PIs was low in all risk groups (≤2.7%).

Similar observations in overall TDRM were found in the United Kingdom in 2004 to 2006 where MSM showed a significantly higher prevalence of 10.3% compared to other transmission groups (3.5%) [11]. In Germany, the prevalence of resistance in MSM was 10.4% compared to the other transmission groups of 7.6% between 2001 and 2005 [18]. The SPREAD programme also includes data from Germany, so this agreement is not surprising. Our results are in contrast with a time trend study performed in Canada, where a significant decrease in overall TDRM was observed in both MSM (from 12.1% 1997-2000 to 0.0% in 2001-2003) and the injection drug user patients (from 17.0% in 1997-2000 to 7.1% in 2001-2003). The change from 8.3% to 12.5% in the same time ranges in heterosexual patients was not significant (p=0.72) [19]. The differences with the results from our study might be due to the sampling in earlier years (1997-2003), the smaller sample size (180) and the sampling of recent infected patients in the Canadian study.

The prevalence of TDRM in heterosexuals originating from Western Europe and North America was still lower than the TDRM prevalence in MSM originating from the same region. Therefore, the difference in TDRM prevalence between heterosexuals and MSM could not be fully explained by heterosexual migrants originating from countries outside Western Europe or North America. One possible explanation for the low TDRM prevalence in heterosexual patients originating from Western Europe and North America is that heterosexuals in western countries are frequently infected by individuals originating from outside this region. This is supported by a model of Xiridou et al. [20] which showed that a 53% of new HIV infections in the Netherlands was acquired by an African migrant of which most (32%) via sexual contact in the Netherlands . The model was based on data from the Netherlands where migrants reported sexual mixing with Dutch partners and with both Dutch and non-Dutch partners in only 15 and 5%, respectively [21].

Another explanation of the lower TDRM prevalence found in heterosexual patients could be that heterosexual patients are more often chronically infected. In chronically infected patients, virus variants with resistance mutations may revert to wild-type viruses which often have a better replicative capacity. In that case, the resistant virus variants can no longer be detected by population sequencing used in our study, because this method fails to detect minor populations [22-23]. In our study, however, we did not found higher TDRM prevalence in heterosexual patients

originating from Western Europe or North America who where recently infected (6.8%) compared to all patients originating from Western Europe or North America (7.8%). An another explanation for the higher TDRM prevalence in MSM might be that resistance viruses may have spread by onward transmission in HIV clusters of MSM forming a sub-epidemic in these patients [24-26]. Also, MSM may show higher risk behaviour as compared to heterosexual patients by having more often sexual contacts while being on treatment. If they fail treatment and harbour TDRM, they could transmit this resistant virus to other individuals.

In MSM patients, we observed an increase of NNRTI TDRM and decrease in PI TDRM. This could be explained by the change in therapy use in the Western world. NNRTI are described as first line therapy in many patients and have a low genetic barrier as development of resistance can occur after only a single mutation [27]. The use of PI has decreased over time and boosted PI have been recommended in the more recent guidelines minimizes the development of resistant mutations [28-31].

TDRM in injection drug users was found to be very low in our dataset. An explanation for this low resistance prevalence is the high proportion of injection drug users that are originating from East and Central Europe. In many countries from this region, the proportion of HIV-1 patients who receive therapy has been relatively low [32-34]. Additionally, even in countries where the access to antiretrovirals for the general population is good, injection drug users have lower rates of access [35-37].

We observed a similar PI TDRM prevalence between heterosexual patients infected in Europe and North America, heterosexual patients infected outside this region and MSM. This is an interesting finding as PI drugs have often only been used in second-line regimens in regions outside Europe and North America. We found that the PI resistance is often explained by the presence of the M46I/L mutation in patients both infected in Europe and North America and in patients infected outside this region. This mutation was found in a similar proportion of patients originating from Western Europe and North America and patients originating from outside this region. Therefore, this mutation might be present due to natural occurring polymorphisms. This was already reported in Bennett et al. [16] where the M46I/L mutation explains approximately half of the total polymorphisms reported in the PI gene in all subtypes [16]. This mutation could therefore lead to an overestimation of the PI TDRM prevalence [38].

In this study, time trends for TDRM prevalence were analyzed for MSM and heterosexuals using data from countries from different regions in Europe. Regions in Europe can be dissimilar in HIV and TDRM epidemics. For example, Eastern-, Central-, and Western Europe are very different in distribution of transmission groups [39], in (prior) access to antiretrovirals [33] and in the size of the HIV epidemic [39]. Therefore, the difference in resistance prevalences between the transmission groups might also be caused by the patients' region of origin. However, region of origin did not change the time trends, which suggest that the time trend found in MSM and in heterosexuals are not caused by a difference over time in the originating region of patients in Europe.

Alongside the region, other variables also differed between the transmission groups. An example is CD4 count, which was highest in MSM (435 cells/mm^3) and very low in heterosexual patients (280 cells/mm^3). This indicates that heterosexual patients are often diagnosed at a late stage of their disease. This data suggest

focusing HIV testing more to these patients to detect an HIV infection at an earlier stage.

A limitation of our study can lay in the categorizing of transmission group which was done with self-reporting information. This information might be misreported due to the urge to give socially desirable answers. Discrimination and homophobia can lead to fear of disclosure of being MSM [40-41].

A strength of our study is the data collection that is performed within the SPREAD programme. The SPREAD programme is a large and sufficiently powered pan- European study that has been running since almost ten years. During this time the programme included patients newly diagnosed with HIV using a predefined strategy that is based on the transmission routes and geographical distribution of HIV in the participating countries.

In conclusion, TDRM prevalence in MSM is high compared to heterosexuals. Especially a concern is the NNRTI resistance prevalence which increased three times from 1.7% to 5.0% within the study period of five years. This increasing NNRTI resistance is likely to negatively influence the therapy response of first-line therapy, as most include NNRTI drugs. Therefore, special attention is needed to the further development of the prevalence of NNRTI TDRM in MSM patients.

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6

Limited cross-border infections in patients newly diagnosed with HIV in Europe

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ABSTRACT

Background: International travel plays a role in the spread of HIV-1 across Europe. It is, however, not known whether international travel is more important for spread of the epidemic as compared to endogenous infections within single countries. In this study, phylogenetic associations among HIV of newly diagnosed patients were determined across Europe.

Methods: Data came from the SPREAD programme which collects samples of newly diagnosed patients that are representative for national HIV epidemics. 4260 pol sequences from 25 European countries and Israel collected in 2002-2007 were included.

Results: We identified 457 clusters including 1330 persons (31.2% of all patients). The cluster size ranged between 2 and 28. A number of 987 patients (74.2%) were part of a cluster that consisted only of patients originating from the same country. In addition, 135 patients (10.2%) were in a cluster including only individuals from neighbouring countries. Finally, 208 patients (15.6%) clustered with individuals from countries without a common border. Clustering with patients from the same country was less prevalent in patients being infected with B subtype (*P*-value <0.0001), in men who have sex with men (*P*-value <0.0001), and in recently infected patients (*P*-value =0.045).

Conclusions: Our findings indicate that the transmission of HIV-1 in Europe is predominantly occurring within individual countries.

INTRODUCTION

Travel and migration have contributed to the worldwide spread of HIV-1. For instance, HIV was introduced in the America's through travel and migration from Africa and Haiti in the 1960s [1]. Travel has also played a role in the early spread of HIV in East Africa. A phylogenetic study that included geographic information found that the HIV epidemic spread more rapidly in areas in East Africa with a good infrastructure that facilitates travelling [2]. Moreover, we recently showed that within Europe, Mediterranean countries are a source of HIV-1 subtype B infections for other European countries [3].

Although travel and migration played a key role in the early spread of HIV, it is not known to what extent travel currently explains transmission of HIV. On the one hand, the importance of travel may have strongly declined over the years. Travel from sub-Saharan Africa may have decreased due to stricter European immigration laws. But also among native Europeans travel may have become less important for the spread of HIV. In Europe, the HIV prevalence is generally low, and stable at 0.2% over the last decade [4] and is concentrated mainly in specific risk groups (men who have sex with men (MSM) and injection drug users) [5]. Because the HIV epidemic is well spread in all European countries, many transmissions could take place within a country. On the other hand, the role of travel in transmission of HIV-1 may also have increased further in recent years. International travelling has become easier in Europe in the last decade because of low cost airlines and the absence of border control between most countries.

In this study we used data from the pan-European SPREAD project. SPREAD includes individuals newly diagnosed with a HIV-1 infection that are representative for the risk group and geographical distribution of the HIV epidemic in participating countries [6-7]. By performing phylogenetic analyses on this data we estimated the proportion of individuals newly diagnosed with HIV that was infected within their own country.

METHODS

Study population

Data came from the SPREAD programme which included newly diagnosed HIV-1 infected patients of 18 years and older who had never been exposed to antiretroviral drugs from 2002-2007. The sampling strategies were defined in close collaboration with the national public health institutes in the participating countries that had access to the latest information on national HIV epidemics. To obtain representative samples from every country, the investigators selected individuals randomly or according to the national distribution of transmission risk groups and the geographical distribution of patients with new diagnoses of HIV-1 infection. Epidemiological, clinical, and behavioural data were collected using a standardized questionnaire within six months of diagnosis. More details on the sampling strategy are provided in previous publications from the SPREAD Programme [8-9].

Table 1. Characteristics of patients.

Characteristics	Categories	Total patients
Patients		4260
Continent of Origin, no. (%)	Western Europe	2361 (55.4)
	Eastern Europe & Central Asia	915 (21.5)
	Sub-Saharan Africa	467 (11.0)
	Other	517 (12.1)
Baseline values	HIV-RNA load, mean (IQR), log copies/ml	4.8 (4.3-5.3)
	CD4 cell count, median (IQR), cells/mm^3	354 (181-540)
	Age, mean years (IQR)	36.3 (29-42)
Gender, no. (%)	male	3361 (78.9)
Risk group, no. (%)	MSM	2061 (48.4)
	Heterosexual contact	1477 (34.7)
	Injection drug use	347 (8.1)
	Other	39 (0.9)
	unknown	336 (7.9)
CDC stage, no. (%)	A and B	3537 (83.0)
	C	516 (12.1)
Subtype, no. (%)	B	2820 (66.2)
	A	477 (11.2)
	C	291 (6.8)
	02_AG	197 (4.6)
	G	137 (3.2)
	F	92 (2.2)
	others	167 (3.9)
	unassigned	79 (1.9)
	non-B	1361 (31.9)
Duration of infection, no. (%)	<1 year	1228 (28.8)
	1-2 years	141 (3.3)
	Unknown duration	2891 (67.9)
TDRM, no. (%)	present	380 (8.9)

Data are no. (%) of patients, unless otherwise indicated; CDC, Centers for Disease Control and Prevention; IQR, interquartile ranges; MSM, men who have sex with men; TDRM, transmitted drug resistant mutations.

Phylogenetics

HIV-1 subtypes were determined by the Rega subtyping tool (version 2.0) [10]. Isolates suggestive of intersubtype recombination in protease and reverse transcriptase fragments were analyzed by SimPlot 3.5.1 software [11]. All sequences were aligned to consensus sequences from the Los Alamos Sequence Database using Clustal W as implemented in the BioEdit software [12]. Sequences were then trimmed to equal length and the gaps were removed. In order to remove the influence of convergent evolution at antiretroviral drug resistance mutations on the phylogenetic analysis, we excluded all sites associated with major resistance according to the International AIDS Society-USA [13]. In protease these positions are 30, 32, 33, 46, 47, 48, 50, 54, 58, 74, 76, 82, 84, 88, and 90. In reverse transcriptase the following positions were excluded: 41, 62, 65, 67, 69, 70, 74, 75, 77, 100, 101, 103, 106, 108, 115, 116, 151, 181, 184, 188, 190, 210, 215, 219 and 225. This resulted in 920 nucleotides that were used for phylogenetic analysis.

Phylogenetic analyses are computationally intensive. We therefore created two different datasets in order to analyse subtype B sequences (which is the most

common subtype in Europe [6, 9]) separately from non-B subtype sequences. Subtype C was chosen as out-group for analysis of sequences of subtype B. Similarly, subtype B was taken as an out-group for the analysis of non-B subtypes. Phylogenetic trees were constructed using the MEGA5 integrated analysis software [14] by maximum likelihood methods under the general time-reversible model. Bootstrapping was performed on the maximum likelihood trees (1000 replicates) to assess the reliability of the obtained topologies. To identify transmission clusters, the novel methodology for large-scale phylogeny partition was used [15]. This method identifies transmission chains by conjugating the evaluation of node reliability, tree topology and patristic distance analysis and was validated in a large Italian cohort [15].

Clustering was based on high bootstrap values (>98%) and intra-cluster average branch lengths less than 0.03 nucleotide substitutions per site [16]. There is, however, no consensus on the cut-off for bootstrap values and for genetic distances that should be used for defining a cluster. Choosing different cut-off values could, in theory, have a profound impact on clustering. We therefore performed a sensitivity analysis in which clusters were defined using a less strict bootstrap value of 90%. In addition, we also did a sensitivity analysis using stricter cut-off values for the genetic distances of 0.02 and 0.01.

To study the demographics of the transmission clusters, we divided the clusters into clusters containing patients from the same country of residence, clusters with patients from countries of residence with a common border, and clusters with patients from different countries of residence which do not share a common border.

RESULTS

Characteristics

A total of 4,260 patients newly diagnosed with HIV-1 were included. The characteristics of these patients are summarized in table 1. The most commonly reported transmission risk groups were MSM (48%), followed by heterosexuals (35%) and injection drug users (8%). Most patients were male (80%). The most frequently found subtypes were B (66%), A (11%) and C (7%). Other subtypes or circulating recombinant forms were CRF02_AG (5%), G (3%), F (2%), and other (4%). 1.9% of the sequences could not be classified. Nearly one third (29%) of patients were defined as recently infected (<1 year). The median CD4 cell count 354 cells/mm3 (IQR: 181-540), which indicates that approximately half of the included patients were diagnosed at a stage of their infection where they were eligible to receive antiretroviral treatment.

The number of patients per country of residence was for Austria 138, for Belgium 340, for Bulgaria 2, for Croatia 83, for Cyprus 55, for Czech Republic 325, for Denmark 295, for Finland 95, for Germany 685, for Greece 230, for Ireland 93, for Israel 120, for Italia 197, for Latvia 72, for Lithuania 11, for Luxembourg 52, for the Netherlands 97, for Norway 118, for Poland 193, for Portugal 238, for Romania 67, for Serbia 16, for Slovakia 26, for Slovenia 84, for Spain 352, and for Sweden 333. More than half of all patients (55%) originated from Western Europe, followed by patients originating from Eastern Europe and Central Asia (22%) and from Sub-

Saharan Africa (11%). A total of 3322 (77%) patients, patients were originating from a country in Europe. A number of 3035 (70%) patients were living in their country of origin.

We found numerous differences between patients infected with a subtype B virus and patients infected with a non-B subtype virus. Not surprisingly, patients infected with a subtype B virus were less often originating from Sub-Saharan countries (0.7%) as compared to 31.7% in non-B subtype strains (*P*-value <0.0001). From this it follows that individuals harbouring a subtype B strain were more often originating from European countries (89.8%) compared to 50.9% of individuals infected with a non-B strains (*P*-value <0.0001). Furthermore, patients with subtype B strains were more often MSM (71.9%) and recently infected (34.9%), than patients infected with a non-B subtype virus (13.6% and 15.9%, respectively) (both *P*-values <0.0001).

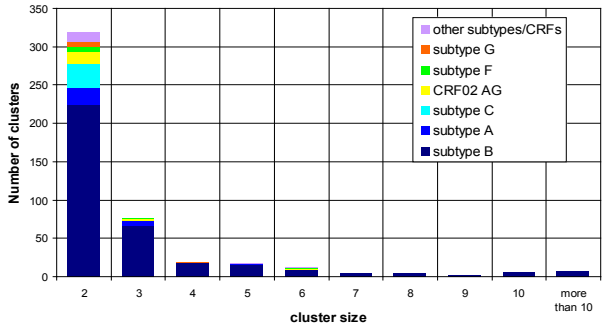

Figure 1. Distribution of cluster size.
Frequency of clusters as defined in the text, of size of 2 or higher, identified by subtype.

Phylogenetic analyses

We identified 457 clusters including 1330 persons (31.2% of all patients). The distribution of the cluster size is shown in figure 1. The cluster size ranged between 2 and 28. Most clusters included two individuals (310 of 457 clusters, 67.8%), 112 clusters contained 3-5 persons (24.5%) and 35 clusters contained >5 persons (7.7%).

Patients that were part of a phylogenetic cluster had different characteristics as compared to patients that were not in a cluster. First, patients included in any cluster were more frequently infected through MSM (63.2% in a cluster vs. 41.3% of individuals that did not cluster, *P*-value <0.0001). Patients that were part of a cluster were more frequently infected with subtype B (82.5%; *P*-value <0.0001), recently infected (39.5%; *P*-value <0.0001) and harbouring a transmitted drug resistance mutation (10.4%, *P*-value =0.03) as compared to non-clustering patients (58.8%, 23.9%, and 8.3%, respectively).

Of the clustering patients infected with a subtype B virus, 1013 (92.1%) patients were originating from a European country (table 2). In patients infected with a non-B subtype that were clustering, a smaller percentage of 63.5% originated from Europe (*P*-value <0.0001). Nonetheless, we found high proportions of patients originating from Europe in clustering patients infected with subtype F (25 out of 26, 96.2%), subtype A (44 out of 61, 72.1%) and subtype G (12 out of 19, 63.2%). Most of these patients infected with subtype F were living in Romania (n=10) and Italy (n=10) and were heterosexually infected (n=17).

Table 2. Proportion of patients originating from Europe per subtype group.

category	Originating from Europe, n (%)
Total	1159 (87.1)
Subtype B	1013 (92.1)
Non-B subtype	146 (63.5)

Table 3. Characteristics of clusters and patients.

Characteristics	category	all clusters	clusters with one country of residence, n (%)	Clusters with neighbouring countries, n (%)
		Characteristics of clusters		
Total		457	380 (83.2)	31 (6.8)
Subtype	Subtype B	357	291 (81.5)	26 (7.3)
	Non-B subtype	100	89 (89.0)	5 (5.0)
		Characteristics of patients in clusters		
Total		1330	987 (74.2)	135 (10.2)
Subtype	Subtype B	1100	787 (71.5)	119 (10.8)
	Non-B subtype	230	200 (87.0)	16 (7.0)
Risk group	MSM	839	578 (68.9)	103 (12.3)
	Heterosexual	278	240 (86.3)	13 (4.7)
	IDU	85	72 (84.7)	10 (11.8)
	other	128	97 (75.8)	9 (7.0)
seroconverters	yes	523	372 (71.1)	61 (11.7)
	no	807	615 (76.2)	74 (9.2)
TDRM	yes	134	100 (74.6)	21 (15.7)
	no	1196	887 (74.2)	114 (9.5)

MSM, men who have sex with men; IDU, injection drug users; TDRM, transmitted drug resistant mutations.

Most of these patients infected with subtype A strains were living in Greece (n=12), Latvia (n=8), Cyprus (n=6) and Austria (n=6). In these patients, transmission through MSM was the most common route of transmission in patients from Greece (11 out of 12) and from Cyprus (3 out of 6), whereas in the other countries subtype A viruses were mostly transmitted among heterosexual patients. The 12 patients that were part of a cluster and were infected with subtype G were living in many different countries and were mainly heterosexual patients (n=10).

Most patients (a number of 987, 74.2%) were part of a cluster that consisted only of patients originating from the same country of residence. The largest clusters were found in Poland (n=15), Germany (n= 12 and 11), and the Czech Republic (n=10). Among the remaining international clusters containing 343 patients, 135 (10.2%) of patients were in a cluster including only individuals from neighbouring countries (the largest had 10 individuals from Denmark and Germany). Finally, 208 patients (15.6%) clustered with individuals from countries without a common border (including the largest cluster of 28 patients). The cluster size of 28 contained patients mostly living in the Czech Republic (n=25) with two patients living in Slovakia and one patient living in Italy. Of these 28 patients, 24 patients reported to be MSM. In the 46 international clusters without a common border, most involved patients living in Spain (n=18) or Germany (n=15).

Table 3 shows the characteristics of the clusters and the patients involved. The proportion of patients in national clusters was different compared to international

clusters for several characteristics. First, clustering with patients from the same residence country was less prevalent in patients infected with a B subtype (71.5% of all clusters) vs. non-B subtypes (87.0% of all clusters; *P*-value <0.0001. Also, MSM (68.9%) and recently infected patients (71.1%) showed less clustering with patients from the same residence country compared to heterosexual (86.3%) or injection drug user (84.7%) (*P*-value <0.0001) and patients with a chronic or unknown duration of infection (76.2%; *P*-value =0.045). The presence or absence of transmitted drug resistance mutations did not influence the proportions of patients clustering in national clusters (74.6 and 74.2%, respectively).

We performed sensitivity analyses using different cut-off values for bootstrap values and for genetic distance (table 4). When we changed the bootstrap value from 98% to 90%, the number of clusters found increased from 457 to 529, including 1643 persons (38.6% of all patients). The smaller bootstrap value did not change the percentage of clusters containing individuals with the same country of residence (from 83.2 to 82.0%; p=0.67). The number of clusters which included persons from neighbouring countries was also highly comparable (7.9 and 6.8%). When we changed the genetic distance of 0.03 to a more stringent value of 0.01, the number of clusters found decreased to 327, including 811 persons (19.0% of all patients). Here, more clusters contained individuals with the same country of residence (90.8%; p=0.002) and a 3.7% of clusters were found with neighbouring-country-patients.

Table 4. Sensitivity analyses on proportion of clusters containing individuals with the same country of residence.

Bootstrap value	Cluster type	Genetic distance		
		0.01	0.02	0.03
90	Within one country	90.6	84.1	82.0
	Neighbouring country	4.5	7.7	7.9
	Without common border	4.9	8.3	10.0
98	Within one country	90.8	84.2	83.2
	Neighbouring country	3.7	6.9	6.8
	Without common border	5.5	9.0	10.1

DISCUSSION

In this representative sample, we found phylogenetic associations between viruses in one third of newly diagnosed individuals. In these clusters, the vast majority of sequences were sampled from persons living in the same country. This suggests that a large part of the spread of HIV-1 in Europe can be explained by transmission of infections taking place between patients within the same country.

A strength of our study is the data collection that is performed within the SPREAD programme. The SPREAD programme is a large and sufficiently powered pan- European study that has been running since 2002. During this time the programme included patients newly diagnosed with HIV using a predefined strategy. This strategy allowed us to include patients that are representative for the national HIV epidemic in participating countries.

The results of this study are in agreement with phylogenetic studies

performed in single European countries [17-18]. First, a phylogenetic transmission study performed in Belgium found that local onward transmission of subtype B virus contributes to an important extent to the epidemic as virtually all patients part of a transmission cluster were of Caucasian origin [17]. Second, a study from Switzerland found that clustering was segregated between different regions in the country, as transmission events occurred preferentially within the same Swiss region [18].

Our study found that patients infected with a non-B subtype virus were less often found in phylogenetic clusters (17.5%) as compared to patients infected with a subtype B virus (39.2%). This finding reflects differences between patients infected with HIV of non-B subtypes and patients infected with a B subtype. First, a much higher proportion of migrants originating from Sub-Saharan countries are infected with a non-B subtype. A Dutch modeling study showed that the migrant groups did not have a large influence on the Dutch HIV epidemic, due to the small number of migrants, their relatively moderate sexual risk behaviour and low mixing with the Dutch heterosexuals [20]. This is in concordance with phylogenetic studies in Switzerland which showed that non-B subtypes are a combined result of both migration and domestic transmission [21] whereas the subtype B epidemic is mainly driven by domestic transmission [18]. Second, patients infected with a non-B subtype are less frequently recently infected (<1 yr) as compared to patients infected with a subtype B virus. Thus, because non-B subtype patients are often chronically infected at time of diagnosis and have originated from many different countries, the chance of phylogenetic clustering in these patients is smaller.

Along these lines, we found that patients involved in non-B clusters had a higher proportion of patients originating from European countries than non-clustering non-B subtype infected patients. Proportions of patients originating from European countries were especially high for clustering patients infected with subtype F, A, and G.

The patients infected with subtype F virus that were phylogenetically related in our study were mainly living in Romania, Italy, and Austria. The spread of subtype F in Europe is explained by nosocomial infections in Romania. In this country, around 10,000 abandoned children have been infected with HIV-1 subtype F due to use of infected hospital equipment or microtransfussions of whole blood around 1990 [22]. Subtype F virus has also been described in Italy, where almost all patients infected with subtype F were originating from Italy [23].

Most clustering patients originating from European countries and infected with subtype A strains were living in Greece, Latvia, Cyprus, and Austria. Subtype A has been described previously in Greece, where most subtype A sequences fell into a single monophyletic cluster, suggesting ongoing transmissions within Greece [24]. In Austria, subtype A infected patients were already reported in 2001, with all patients being immigrants or partners of immigrants [25]. These introductions of subtype A through immigrants could be the start of the circulation of subtype A within Austria. However, no study has yet been published showing the transmission of subtype A within the Austrian country. Furthermore, in Latvia, an HIV-1 subtype A outbreak among injecting drug users was reported which showed shared ancestry with outbreaks among injection drug users in the Ukraine and southern Russia [26].

Transmission of subtype G could, contrary to subtypes F and A, not be ascribed to a few specific countries in Europe. Subtype G clustering patients were

found in many European countries including Portugal and Spain. The presence of subtype G has been described before in these countries (Spain [27] and Portugal [28]). The introduction of subtype G in these countries is probably caused by immigrants coming from West Africa for which the Canary Islands (and therefore also Spain and Portugal) form one of the main gates of entrance of African immigrants into Europe [27].

More studies have been performed showing circulation of particular non-B subtypes within single European countries using phylogenetic analyses [29-31]. Other studies have suggested transmission of non-B subtypes in Europe by observing infections with non-B subtype infections in native or European-originating people [32-34].

In all HIV risk groups, clustering was found mainly between patients with the same country of residence. However, differences were seen between the risk groups. MSM did less often cluster with patients coming from the same country than heterosexuals and injection drug users. This is also reflected in the lower percentage of seroconverters clustering within a country compared to the non-seroconverters, which could be ascribed to the fact that MSM are more often recently infected [35]. The less frequently clustering MSM suggests that MSM more often get infected during travels to other European countries whereas heterosexuals and injection drug users get infected near home. This is supported by studies reporting an association of transmission of HIV-1 in injection users with extensive local epidemics [36-37].

Sensitivity analyses showed that our findings were not distorted by the arbitrary cut-off values that were used for the bootstrap values and for the genetic distance. Using a more stringent genetic distance increased the percentage of patients clustering with patients living in the same country. Therefore, the percentage of patients clustering with patients living in the same country is at least 83.2% or higher, because the initial genetic distance used in the main analyses was taken very wide. Larger bootstrap values did not change the results in our study. Therefore, these results are generally robust and not influenced by the level of bootstrap values used in the cluster definition.

We did not have access to dense samples in which sequences from virtually all newly diagnosed HIV-infected individuals in a particular country are included. We may therefore have underestimated the size of the clusters or missed individuals for whom we currently did not identify a phylogenetically related sequence. Nonetheless, we still found that one out of three individuals was part of a cluster. In addition, dense sampling is expected not to have changed the results to a great extent as the included individuals were representative for the national HIV epidemics.

Our findings indicate that the transmission of HIV-1 in Europe is for a large part occurring between patients coming from the same country. This could have implications for HIV-1 transmission prevention programmes. Because infections attributed to travelling between countries is not frequently observed it is important to have good surveillance of the national HIV-1 epidemics.

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Comparison of HIV-1 genotypic resistance test interpretation systems in predicting virological outcomes over time

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ABSTRACT

Background: Several decision support systems have been developed to interpret HIV-1 drug resistance genotyping results. This study compares the ability of the most commonly used systems (ANRS, Rega, and Stanford's HIVdb) to predict virological outcome at 12, 24, and 48 weeks.

Methods: Included were 3763 treatment-change episodes (TCEs) for which a HIV-1 genotype was available at the time of changing treatment with at least one follow-up viral load measurement. Genotypic susceptibility scores for the active regimens were calculated using scores defined by each interpretation system. Using logistic regression, we determined the association between the genotypic susceptibility score and proportion of TCEs having an undetectable viral load (<50 copies/ml) at 12 (8-16) weeks (2152 TCEs), 24 (16-32) weeks (2570 TCEs), and 48 (44-52) weeks (1083 TCEs). The Area under the ROC curve was calculated using a 10-fold cross-validation to compare the different interpretation systems regarding the sensitivity and specificity for predicting undetectable viral load.

Results: The mean genotypic susceptibility score of the systems was slightly smaller for HIVdb, with 1.92±1.17, compared to Rega and ANRS, with 2.22±1.09 and 2.23±1.05, respectively. However, similar odds ratios were found for the association between each-unit increase in genotypic susceptibility score and undetectable viral load at week 12; 1.6 [95% confidence interval 1.5-1.7] for HIVdb, 1.7 [1.5-1.8] for ANRS, and 1.7 [1.9-1.6] for Rega. Odds ratio's increased over time, but remained comparable (odds ratio's ranging between 1.9-2.1 at 24 weeks and 1.9-2.2 at 48 weeks). The Area under the curve of the ROC did not differ between the systems at all time points; p=0.60 at week 12, p=0.71 at week 24, and p=0.97 at week 48.

Conclusions: Three commonly used HIV drug resistance interpretation systems ANRS, Rega and HIVdb predict virological response at 12, 24, and 48 weeks, after change of treatment to the same extent.

INTRODUCTION

The effectiveness of antiretroviral therapy has been limited by the development of HIV-1 drug resistance. Resistance occurs frequently in patients and may decrease both the magnitude and the duration of the response to treatment [1].

Several prospective studies have shown that the use of genotypic resistance analysis to guide the new treatment choice for patients failing their current HAART improves virologic outcome [2,3,4,5]. The complex mutational patterns are however difficult to interpret, due to the many different drug resistance mutations [6] and the varying levels of decreased susceptibility of these mutations to different drugs. This led to the development of several interpretation systems [7], which provide rules to help physicians interpret HIV-1 drug resistance genotyping results.

ANRS, Stanford HIVdb, and Rega are the three most commonly used and publicly available drug resistance interpretation systems, which are all regularly updated. The systems are rule based algorithms, providing scores for specific (combinations of) mutations. The scores are then translated into different levels of susceptibility. The rules for these scores are based on literature and expert's opinion. The Rega system was the first to be validated in drug experienced patients [8,9], followed by ANRS [5,9] and Stanford [9].

A good way to compare systems is by using virological response data in correlation with the prediction of interpretation systems. However, some systems may be better for short-term virological outcomes, and others may be better for longer-term outcomes. The results of a comparison between systems may therefore depend on the virological outcome time point that is used. In this study, a large data set of HIV-1 patient's sequences was collected together with virological data to compare the three most commonly used interpretation systems in genotypic susceptibility score and in the prediction of virological response. We used 3 different virological outcome time points to analyze the effect of therapy duration on the prediction of systems.

METHODS

Study population

Data was made available through the EU-sponsored ViroLab and EuResist projects [10,11,12]. The ViroLab project comprises data from Belgium (Katholieke Universiteit Leuven), Italy (University of Brescia and Catholic University of the Sacred Heart of Roma), Spain (IrsiCaixa Badalona), and the Netherlands (Erasmus Medical Centre Rotterdam). The EuResist project consists of data from Italy (ARCA database; http://www.hivarca.net/), Germany (AREVIR database); Sweden (Karolinska Infectious Diseases and Clinical Virology Department), and Luxembourg (Retrovirology Laboratory, CRP-Santé). The time-periods of available therapies in the ViroLab and EuResist database ranged between 1996 and 2008. These databases were used to extract treatment change episodes (TCEs). TCEs were defined, in patients aged ≥18, as follows (figure 1): (1) a baseline genotype (reverse transcriptase and protease region) and viral load (detectable being >50 copies/ml) obtained within 90 days before and 8 days after treatment change; (2) at least one follow-up viral load

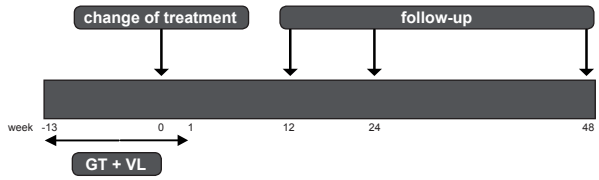

Figure 1. Schematic definition of a treatment change episode.
The treatment change episode requirements are as follows: (1) a baseline genotypic drug-resistance and viral load test (GT + VL) between 90 days before and 8 days after change of therapy (2) at least one follow-up viral load measurement at 12 (8-16), 24 (16-32), or 48 (44-52) weeks.

measurement at 12 (range: 8-16), 24 (16-32), or 48 (44-52) weeks; (3) no changes in therapy between the time of the baseline viral load and the follow-up viral load measurement. In case more genotypic tests or viral load measurements were performed within an analyzed treatment period, the value closest to the start of therapy or the follow-up measurement time was used.

Interpretation systems and genotypic susceptibility scores (GSSs)
The genotypic results were interpreted using three commonly used rule-base interpretation systems: Agence Nationale de recherches sur le SIDA (ANRS) version 17; Stanford HIVdb, version 5.1.2; and Rega Institute version 8.0.1. The ANRS and Rega both report 3 levels of resistance: susceptible, intermediate, and resistant. For ANRS, we translated the definitions 'susceptible', 'intermediate', and 'resistant' into susceptibility scores of 1, 0.5, and 0, respectively. For the Rega scores, we used the weighted score suggested by Rega, which uses the following changes: non-nucleoside reverse transcriptase inhibitors (NNRTI) were scored 0.25 (with the exception of etravirine with a score of 0.5) for intermediate resistance, and ritonavir-boosted protease inhibitors (PI) were scored 0.75 and 1.5 for intermediate resistance and susceptible, respectively. The Stanford algorithm uses 5 levels of resistance. We assigned the following scores to these 5 levels of Stanford: 0, 0.25, 0.50, 0.75, and 1 for respectively the high-level resistance, intermediate resistance, low-level resistance, potential low-level resistance, and susceptible. In a separate analysis we used the unweighted scores for Rega. We assigned the scores 0, 0.5, and 1 to the 'resistant', 'intermediate', and 'susceptible' groups for all drugs, respectively. The three systems did not include a score for ritonavir. We therefore excluded eleven TCEs that used ritonavir as only protease inhibitor, as we could not calculate a GSS of their treatment regimens.

The arithmetic sum of the individual score for the specific drugs provided the total GSS of that treatment. For brevity, we classified the total GSS score in the following categories: 0 to <1, 1 to <2, 2 to <3, 3 to <4, and ≥4. The 0 to <1 group contains viral sequences almost entirely resistant to the drugs in their regimen, and the ≥4 group contains viral sequences susceptible to more than 3 drugs given in their regimen.

To calculate the prevalence of drug resistance we used the mutation list published by the International AIDS Society USA (IAS-USA) [13].

Statistical analysis
Kaplan-Meier curves were estimated to determine the association between GSSs and the proportion of TCEs having an undetectable viral load (<50 copies/ml). The

association between GSS scores and undetectable viral load was analyzed with a logistic regression. In the multivariate analyses we adjusted for real time to viral load measurement (i.e. number of days between the TCEs and the follow-up viral load measurement) and log viral load at start of therapy. Furthermore, we used logistic regression, to calculate odds ratios for each GSS group compared to the GSS group of 0 to <1. The receiver operating characteristic (ROC) curves were calculated to analyze the trade-off between the proportion of true-positive (correct virologic response prediction) and false-positive (incorrect virologic response prediction) results across the range of possible prediction cut-offs. The AUC (Area Under the Curve) is a value between 0 and 1 that corresponds to the probability that a randomly selected virologic success receives a higher score than a randomly selected virologic failure. We used the AUCs to calculate how well the systems separate the GSS groups into those with and without undetectable viral load (<50 copies/ml). Robust extra-sample error estimation was obtained by 10-fold cross-validation [14]. We compared the multiple independent runs of the 10-fold cross validation results with a Kruskal-Wallis test. Analyses were performed with the SPSS software package (version 15.0 for Windows, SPSS).

RESULTS

Baseline characteristics of the study population

The baseline characteristics are shown in table 1. We included 3131 patients in our study, of which most were male (73%), most were infected with subtype B viruses (81.9%), and the median age was 39 years (range 18-78). Of the 3131 patients, 476 (12.7%) had more than one TCE, which leads to a total of 3,763 TCEs included in the study. Of these TCEs, 2,152 had a viral load measurement at week 12, 2,570 at week 24, and 1,083 at week 48. TCEs were retrospectively included between 1996 and 2008. Most TCEs (2085, 55.4%) were included between 2001 and 2004, and fewer TCEs were included between 2005 and 2008 (1029, 27.3%) and between 1996 and 2000 (649, 17.2%). The median HIV RNA level of the TCEs was 4.43 log10 copies/ml [interquartile range (IQR), 3.65-5.08], and the median CD4+ cell count was 233 cells/μL (IQR, 120-371 cells/μL). The most commonly given treatments were lamivudine (59%), tenofovir (37%), and lopinavir (35%). A combination of lamivudine, zidovudine, and lopinavir/r was the most frequently given therapy combination, with a percentage of 8%, followed by 6% for the therapy combination lamivudine, tenofovir, and lopinavir/r.

Prevalence of mutations at baseline

The percentage of sequences having a drug resistance mutation is shown in figure 2. Nucleoside reverse transcriptase inhibitor (NRTI) resistance associated mutations were most frequently found with a prevalence of 62% [13]. The most prevalent NRTI resistance mutations were M41L (27.0%), D67N (23.2%), M184V (35.6%), and T215FY (32.9%). Mutations associated with resistance to NNRTI and PI, were detected less frequently, in 34% and 32% of the cases, respectively. K103N (18.6%), V181C (10.2%), and G190A (8.0%) were the most prevalent NNRTI mutations. The PI mutations with highest prevalences were M46IL (13.2%), V82A (9.6%), and

Table 1. Baseline patient characteristics.

Characteristics	Categories	
Number of patients		3131
Male, number (%)		272 (72.6)
Age, median (IQR*)		39 (18-78)
HIV-1 subtype, number (%)	Subtype B	2563 (81.9)
	Subtype A	158 (5.0)
	Subtype G	118 (3.8)
	Subtype C	90 (2.9)
	Subtype F	62 (1.9)
	CRF 02_AG	28 (0.9)
	CRF 12_BF	24 (0.8)
	other	76 (2.4)
	unclassified	12 (0.4)
Number of treatment-change episodes		3763
Baseline CD4 count (cells/mm^3), median (IQR)		233 (120-371)
Baseline viral load (log_{10})(copies/ml), median (IQR)		4.43 (3.65-5.08)
		Number (%)
Treatment-change episodes	1 treatment-change episode	1555 (41.3)
	>1 treatment-change episodes	476 (12.6)
	>2 treatment-change episodes	108 (2.9)
Year of treatment	1996-2000	649 (17.2)
	2001-2004	2085 (55.4)
	2005-2008	1029 (27.3)
NRTI Drug treatment	lamivudine	2224 (59)
	tenofovir	1400 (37)
	zidovudine	1082 (29)
	didanosine	1007 (27)
	stavudine	932 (25)
	abacavir	590 (16)
	didanosine	653 (18)
	emtricitabine	246 (7)
NNRTI Drug treatment	efavirenz	660 (18)
	nevirapine	447 (12)
	etravirine	1 (0)
	delavirdine	1 (0)
PI Drug treatment	lopinavir	1309 (35)
	nelfinavir	332 (9)
	atazanavir	274 (7)
	indinavir	263 (7)
	saquinavir	221 (6)
	amprenavir	202 (5)
	tipranavir	70 (2)
	darunavir	28 (1)
Other drug treatment	enfuvirtide	135 (4)
therapy combinations	lamivudine + lopinavir + zidovudine	315 (8)
	lamivudine + lopinavir + tenofovir	244 (6)
	lamivudine + zidovudine + abacavir	133 (4)
	lamivudine + tenofovir + efavirenz	133 (4)
	lamivudine + zidovudine + efavirenz	114 (3)
	tenofovir + lopinavir + didanosine	102 (3)

* IQR is interquartile range; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

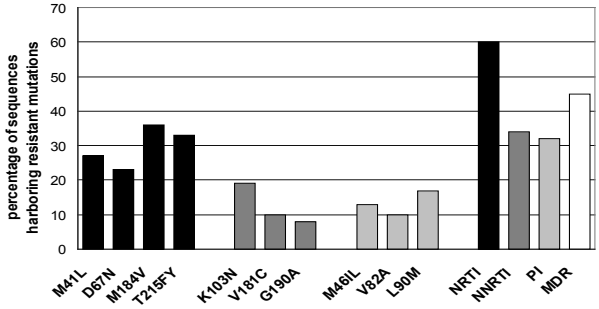

Figure 2. Drug resistance prevalences.
Percentage of sequences having resistance mutations to nucleoside reverse transcriptase inhibitor (NRTI) (black), non-nucleoside reverse transcriptase inhibitor (NNRTI) (dark gray), protease inhibitor (PI) (light gray), and Multi drug resistance (MDR) (white).

L90M (16.9%). The comparisons of the mutation patterns showed no substantial differences between TCEs with a follow-up viral load at 12, 24, and 48 weeks.

Genotypic Susceptibility Score distribution

The genotypic susceptibility scores for a TCE was calculated as the total score of genotypic susceptibility scores for all drugs in one regimen as explained in the 'method' section. Figure 3 displays the proportions of cases in each susceptibility category, according to ANRS, HIVdb, and Rega. All systems show that at least three active drugs were started in a large proportion of TCEs. The mean GSS of the three systems were slightly smaller for HIVdb, with 1.92 ± 1.17, compared to Rega and ANRS, with 2.22 ± 1.09 and 2.23 ± 1.05, respectively. The unweighted Rega scores did not differ much from the other scores with a mean of 2.15 ± 1.09.

The GSS of TCEs with longer follow-up were slightly higher compared to TCEs with a short follow-up time (data not shown), with baseline GSS means ranging between 1.93 and 2.23 at 12 weeks, 1.98 and 2.29 at 24 weeks, and 1.98 and 2.32 for TCEs with viral load measurement available at 48 weeks.

Prediction of virologic outcomes

The virologic responses of all TCEs are described in table 2. The percentage of an undetectable viral load (<50 copies/ml) was higher in week 24 compared to week 12.

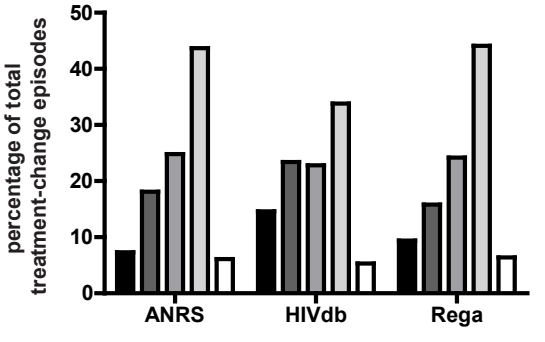

Figure 3. Total Genotypic Susceptibility Scores for ANRS, HIVdb, and Rega.
Total Genotypic Susceptibility Scores were calculated using the arithmetic sum of the individual scores given by the systems for each specific drug given in a regimen. We classified the GSS score for ANRS, HIVdb, and Rega in the following categories: 0 to <1, 1 to <2, 2 to <3, 3 to <4, and ≥4. GSS scores were calculated for 3759 TCEs.

Table 2. The viral load response and GSS groups at different time points.

	ANRS			HIVdb			Rega		
week	12	24	48	12	24	48	12	24	48
GSS 0-<1	7.3	8.3	8.1	12.4	14.8	18.0	10.0	9.3	12.0
GSS 1-<2	23.7	30.0	33.5	28.6	39.4	41.6	19.7	26.4	27.4
GSS 2-<3	36.7	47.6	51.7	44.7	55.9	61.7	39.2	50.2	54.9
GSS 3-<4	46.2	64.4	66.7	47.1	66.4	68.1	46.0	65.0	67.2
GSS ≥4	47.2	69.0	74.6	45.0	68.0	72.0	47.3	65.1	72.1

The percentages of treatment-change episodes with an undetectable viral load (<50 copies/ml) are shown for each GSS group at week 12, week 24, and week 48 for ANRS, HIVdb and Rega.

Week 48 did not show a large increase in percentage compared to week 24. TCEs with higher Genotypic Susceptibility Score had a higher change of reaching an undetectable level of viral load. At 48 weeks, in more than 70% of the TCEs with a Genotypic Susceptibility Score of ≥4, the viral load became undetectable.
Adjusted odds ratios for reaching a viral load below 50 copies/mL for each unit increase in GSS are reported in figure 4. These predictions of the virological response were similar to the odds ratios without adjusting for log viral load at start of therapy and real time to viral load measurement (data not shown). At all time points, the interpretation systems were significantly predictive of the virological response. Odds ratios for each unit increase of the GSSs ranged from 1.77 (95% Confidence Intervals (CI): 1.62-1.94), 1.87 (95%CI: 1.69-2.06), and 1.88 (95%CI: 1.70-2.08) at 12 weeks to around 1.99 (95% CI: 1.84-2.16), 2.20 (95%CI: 2.01-2.41), and 2.16 (95%CI: 1.97-2.37) at 24 weeks for HIVdb, Rega, and ANRS, respectively. Furthermore, the odds ratios for the unweighted Rega scores were similar, ranging between 1.86 (95% CI: 1.69-2.05) at week 12 and 2.16 (95% CI: 1.98-2.36) at week 24. The ROC curves in figure 5 depict different cut-off points, for the three interpretation systems. In the table below the graph, the sensitivity, 1-specificity, and specificity are given for these cut-off points. The sensitivity and specificity of the ROC curves for the systems are all similar. The calculated AUCs were around 0.63 at week 12 and 0.68 at week 24 and 48 (table 3). These AUCs did not significantly differ among the systems (with p-values ranging between 0.60-0.97) at all time points. The AUCs of the unweighted

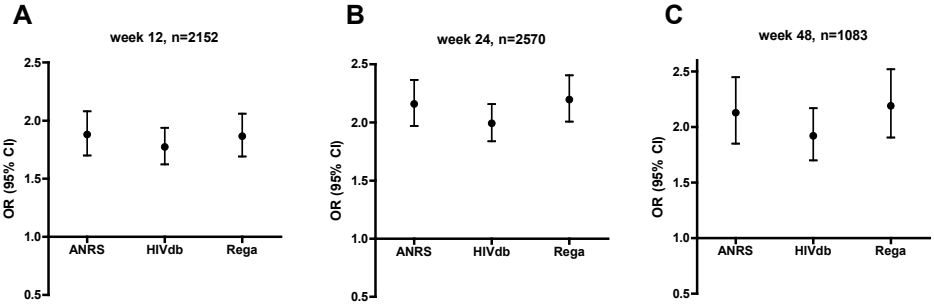

Figure 4. Association between Genotypic Susceptibility Score and undetectable viral load.
The adjusted odds ratios (ORs) with 95% confidence intervals for RNA levels <50 copies/ml at (A) 12 weeks, (B) 24 weeks, and (C) 48 weeks per unit increase of GSS according to ANRS, HIVdb, and Rega. These odds ratios were adjusted for log viral load at start of therapy and real time to viral load measurement, and similar to the unadjusted odds ratios.

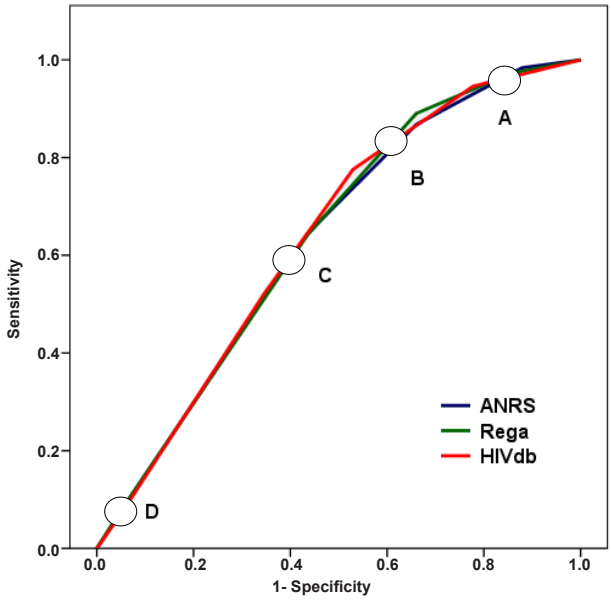

Figure 5. ROC curves for the logistic models for ANRS, HIVdb, and Rega at 12 weeks.
The sensitivity, 1-specificity, and specificity are given in the table for the cut-off points 0.5 (A), 1.5 (B), 2.5 (C), and 3.5 (D) for ANRS, HIVdb, and Rega.

	GSS score	sensitivity			1-specificity			specificity		
		ANRS	HIVdb	Rega	ANRS	HIVdb	Rega	ANRS	HIVdb	Rega
A	0.5	0.98	0.95	0.97	0.88	0.78	0.86	0.12	0.22	0.14
B	1.5	0.87	0.78	0.89	0.66	0.53	0.66	0.34	0.47	0.34
C	2.5	0.64	0.52	0.64	0.43	0.35	0.43	0.57	0.65	0.57
D	3.5	0.08	0.07	0.09	0.06	0.05	0.06	0.94	0.95	0.94

Table 3. Multiple cross-validation for calculating AUC for the different interpretation systems.

week	system	AUC*	sd	Kruskal-Wallis test	
				Chi-square	p-value
Week 12	ANRS	0.629	0.05	0.280	0.597
	HIVdb	0.634	0.05		
	Rega	0.620	0.05		
Week 24	ANRS	0.677	0.04	0.143	0.705
	HIVdb	0.689	0.03		
	Rega	0.689	0.03		
Week 48	ANRS	0.671	0.06	0.001	0.970
	HIVdb	0.680	0.06		
	Rega	0.679	0.06		
All weeks	ANRS	0.671	0.03	0.322	0.570
	HIVdb	0.680	0.03		
	Rega	0.680	0.02		

* AUCs (area under the receiver operating characteristic curve) were obtained from 10-fold cross-validated predictions. AUCs of 0.5 indicate that the interpretation system is not an explanatory factor for the percentage undetectable viral load.

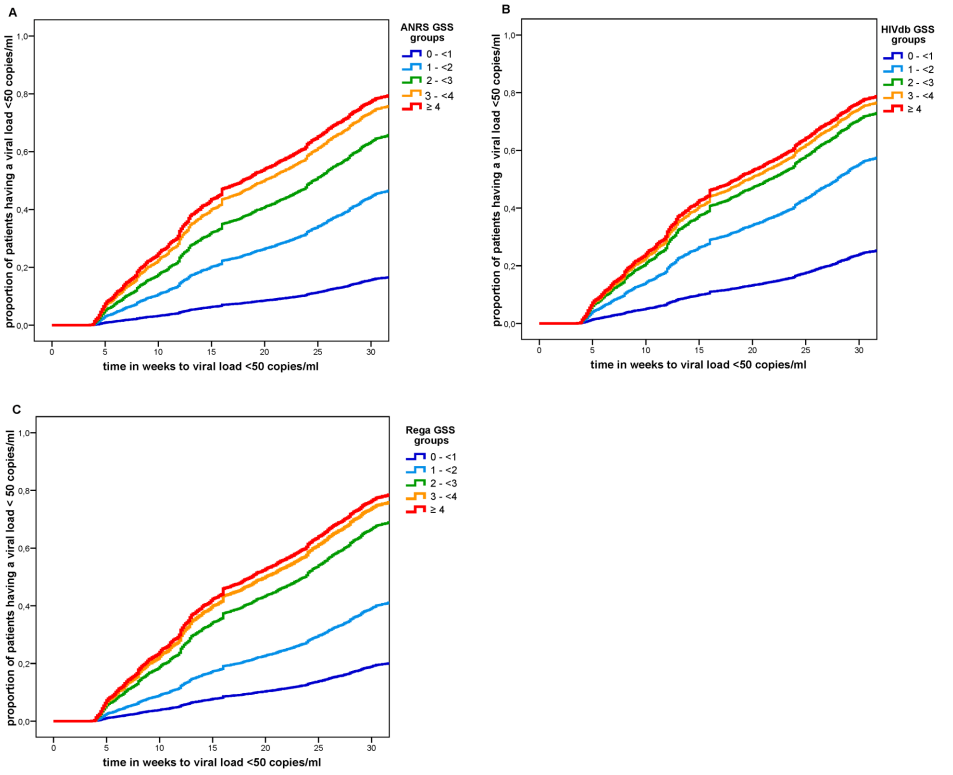

Figure 6. Association of undetectable viral load and Genotypic Susceptibility Score over time.
Kaplan Meier curves showing the association between time to undetectable viral load and the proportion of TCEs having an undetectable viral load for the 5 Genotypic Susceptibility Score groups for (A) ANRS (B) HIVdb and (C) Rega. Due to lost to follow-up at later viral load measurement time points, we limited the follow-up time to 30 weeks.

Rega did not differ from the normal ANRS, HIVdb, and Rega scores, with means of 0.63 at week 12 and 0.68 at week 24 and 48. (data not shown)

In figure 6, Kaplan-Meier curves are given, showing clear associations between the GSS groups and the proportion of TCEs having an undetectable viral load. The GSS group of 4 or higher show the highest proportion of TCEs having an undetectable viral load. The odds ratios of each GSS group are given in table 4 for all time point measurements. In the comparison between the different GSS groups and the GSS group of 0 to <1, increasing odds ratios were found for an increasing GSS. Odds ratios were higher at week 24 compared to week 12 for all GSS groups and in all three interpretation systems, whereas the results at week 48 did not differ much from those at week 24. Due to the low numbers of included TCEs in GSS group ≥4 and at week 48, large confidence intervals were seen in these groups. At week 24, the odds ratios increased from 4.70 (95% CI: 2.57-8.60) to 26.42 (95% CI: 13.49-51.77) for ANRS, from 3.62 (95% CI: 2.56-5.13) to 13.49 (95% CI: 8.25-22.06) for HIVdb, and from 3.46 (95% CI: 2.03-5.91) to 19.34 (10.70-34.94) for Rega.

Table 4. Logistic regression for calculating association between undetectable viral load and the GSS groups.

system	GSS group	OR (95% CI)		
		week 12	week 24	week 48
ANRS	0-<1	ref.	ref.	ref.
	1-<2	3.86 (2.07-7.20)	4.70 (2.57-8.60)	5.70 (2.17-14.95)
	2-<3	7.20 (3.94-13,18)	9.89 (5.48-17.83)	11.94 (4.62-30.83)
	3-<4	13.54 (7.50-24.44)	21.24 (11.87-37.99)	23.11 (9.09-58.72)
	≥4	15.01 (7.65-29.45)	26.42 (13.49-51.77)	34.29 (11.56-101.70)
HIVdb	0-<1	ref.	ref.	ref.
	1-<2	2.55 (1.73-3.75)	3.62 (2.56-5.13)	3.19 (1.91-5.33)
	2-<3	5.39 (3.69-7,89)	7.14 (5.06-10.08)	7.21 (4.33-12.00)
	3-<4	8.05 (5.59-11.59)	12.51 (8.9-17.51)	10.06 (6.14-16.46)
	≥4	7.48 (4.51-12.39)	13.49 (8.25-22.06)	12.28 (5.72-26.35)
Rega	0-<1	ref.	ref.	ref.
	1-<2	2.03 (1.20-3.45)	3.46 (2.03-5.91)	2.75 (1.27-5.99)
	2-<3	5.55 (3.41-9.02)	9.72 (5.83-16.22)	8.83 (4.21-18.52)
	3-<4	8.97 (5.61-14.38)	19.15 (11.60-31.61)	15.26 (7.42-31.37)
	≥4	9.73 (5.53-17.11)	19.34 (10.70-34.94)	19.40 (7.93-47.48)

Logistic regression analysis evaluating the association between undetectable viral load and the GSS groups (with GSS group 0 - <1 as reference) at different time points for the three interpretation systems. The number of treatment-change episodes for the GSS group 0-<1, 1-<2, 2-<3, 3-<4, and ≥4 are: 178, 389, 485, 959, and 142 at 12 weeks; 157, 433, 638, 1206, and 142 at 24 weeks; 62, 182, 242, 540, and 59 at 48 weeks. These numbers were similar for the three systems.

DISCUSSION

In this study, data from treated HIV-1 patients were modeled to predict virological outcome comparing genotypic drug resistance with the most commonly used interpretation systems. We used logistic regression and AUC calculations and showed in 3,763 treatment change episodes that ANRS, HIVdb, and Rega, do not differ in predicting virological outcomes.

Comparisons of interpretation systems have been previously reported [9,10,15,16,17]. In this work, due to the large study population, we were able to compare genotypic susceptibility scores between patients using many different drug therapy combinations and control for important possible confounders. The results of our study were in agreement with previous findings [10,16]. In addition to previous work, our study has extensively looked at the differences between the prediction ability of the systems at different time points. We both included short term responses (week 12) and longer term responses (week 24 and 48).

An explanation for the findings in this study is that the systems all make use of the same literature available on correlations between genotypic and phenotypic analyses as well as correlations with treatment history and clinical response.

Several studies showed small changes in genotypic susceptibility scores between different systems. For example Ravela et al. [18], that compared 4 different

interpretation systems (including ANRS, HIVdb, and Rega), reported a 4.4% complete discordance, with at least 1 system assigning susceptible and another system assigning resistant; 29.2% displayed partially discordance; and 66.4% were complete concordant. However, in this study we found that these differences do not have a large influence on the virological outcome of treatment.

A possible limitation of studies comparing different interpretation systems lies in the translation of the indications from the interpretation systems into numeric values, which are taken arbitrarily. However, we have used the same principles used by authors of HIV drug-resistance algorithms for calculating the genotypic susceptibility score. Therefore we were able to compare the three systems in the way they are used in practice. We also used the Rega scores without the suggestions about weighting of scores for boosted PI drugs and NNRTI. Using these unadjusted scores did not change in GSS distributions and virological outcome to a great extent.

Some novel drugs (etravirine, darunavir, tipranavir) were not frequently used in our study population. Similarly, drugs belonging to the newly approved classes, such as raltegravir and maraviroc, were not included. Therefore, the predictive value we found is not a validation for all individual rules in the system and we did not attempt to validate individual rules. Continuous validations in large dataset with recent drug data will therefore remain needed.

No restriction on therapies was performed; therefore suboptimal regimens (fewer than three full-dose drugs) were included. However, the group of patients receiving suboptimal regimens was small and the same for all three interpretation systems. Furthermore, it was previously demonstrated that removal of suboptimal treatment reduces the accuracy of the models [19].

Much discussion has been going on about which follow-up period is most suitable to validate a system. Short term responses might be more directly attributable to the antiviral drug activity whereas longer term outcomes might be more clinically relevant but more easily confounded by other issues such as loss in adherence, drug discontinuations and switches [20]. In our study less than 1/3 of all cases were left at the 48 week time point measurement. This loss to follow up creates selection bias in this group. Therefore, this 48-week-group may not be representative of the whole study population. The patients, who remain on therapy until the 48th week after start of therapy, will do better on therapy and will have better virological responses than patients who switch to another therapy at earlier stages. In accordance, we found stronger associations between interpretation systems and virological outcomes at later time points compared to earlier time points in the logistic regression analyses. However, in the logistic regression that compared the different GSS groups to the GSS group of 0 to <1, the odds ratios were similar between week 24 and week 48. Therefore, week 24 may be a well suitable time point to measure long term responses. However, confidence intervals in week 48 were large, because of low numbers of included TCEs, therefore creating a bias at this time-point.

In conclusion, we found that the three most commonly used interpretation systems do not differ in their ability to predict virological response. Also, when looking into different time points, the prediction abilities between the systems were similar. Since the overall performance is comparable, these systems might evolve towards a more consistent scoring in the future. New breakthroughs might be needed for further improvement in genotypic resistance test interpretation.

AFFILIATIONS

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8

Estimates of HIV transmitted drug resistance can be inflated due to natural sequence polymorphisms

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TO THE EDITORS:

Transmitted drug resistance (TDR) can limit treatment options in patients newly infected with human immunodeficiency virus (HIV) [1]. The increasing global access to antiretroviral drugs and a prevalence of TDR of around 10% in many countries [2-4] are indications that TDR is an important problem. For this reason the WHO has developed a consensus list of mutations for the global surveillance of TDR as well as detailed guidelines for TDR surveillance and prevention [5-6]. The WHO lists have been updated regularly and used extensively [1, 7-13]. The most recent WHO list, which was published by Bennett *et al.*, is based on mutations that are included in three or more of five expert lists of drug resistance mutations. Furthermore, mutations had to be non-polymorphic defined (with a few exceptions) as being present at a frequency <0.5% in all major subtypes in a dataset of more than 6000 antiretroviral therapy-naive individuals. Thus, Bennett *et al.* have provided the best available estimates of polymorphisms levels in treatment-naïve patients. Nonetheless, application of the WHO list may result in overestimation of TDR because the sum of polymorphisms across all surveillance drug-resistance mutation positions may be as high as 4.8% (for CRF01_AE), which is a substantial proportion of TDR reported from many developed and developing countries [2-4, 7-14].

The purpose of this study was to examine how polymorphisms in the WHO consensus list affect the accuracy for surveillance of TDR. Furthermore, the WHO has recommended a threshold survey that classifies TDR prevalence in resource-poor settings into three categories to avoid large-scale expensive genotypic resistance testing. We have evaluated the effect of polymorphisms at amino acid sites associated with drug resistance on this WHO classification

We define polymorphisms as naturally occurring amino acid substitutions seen in low proportions in the absence of selective drug pressure at the drug-resistance-related positions included in the WHO consensus list [5]. Bennett *et al.* calculated the levels of these polymorphisms by excluding sequences with two or more drug-related-mutations for the reason that these sequences would likely have resulted from previous treatment. We calculated the overall polymorphism prevalence as the sum of the prevalence of individual polymorphisms for the three drug classes: nucleoside reverse transcriptase inhibitors; non-nucleoside reverse transcriptase inhibitors; and protease inhibitors. This calculation was justified because no sequence had more than one resistance mutation. We choose to investigate the two most common subtypes B (polymorphism prevalence: 4.0%) and C (2.1%), as well as subtypes G and CRF01_AE which had the lowest and highest prevalence of polymorphisms (1.4% and 4.8% respectively). Positive predictive values were calculated, which indicated the proportion of patients with surveillance drug-resistance mutations that represented real TDR (hereafter called true TDR), rather than the presence of polymorphisms, among all patients with observed TDR mutations.

For the classification of TDR prevalence, the WHO threshold surveys recommend to perform resistance testing on a minimum of 34 samples and assess if between 1 - 5 samples have resistance mutations [15]. If the number is outside this range further sampling is not required and the prevalence of TDR is classified as being <5% or >15%, otherwise additional samples are tested. Sampling is continued until 47 samples have been tested or until the number of samples with resistance

mutations is outside a pre-defined range. Therefore, we set up a simulation where 34 patients where checked for having TDR. If at the 34th patient, no decision could be made according to the WHO threshold, then up to 13 more patients were included in the simulation. The TDR prevalences were categorized into three strata, <5%, 5-15%, and >15%, according to WHO recommendations. Using 1000 different Monte Carlo simulation, we classified resistance based on randomly generated uniformly distributed numbers which created specific resistance levels we were interested in. Different scenarios were set up to investigate the impact of polymorphisms on the WHO classification of TDR prevalence.

Table 1. Proportion of correct WHO TDR prevalence categorizations simulated (n=1000) for different prevalences of true TDR and polymorphisms for subtypes B, C, G and CRF01_AE.

Subtype	True prevalence of TDR (%)	Prevalence of polymorphisms[1] (%)	Distribution of TDR categorizations (%)		
			TDR category <5%	TDR category 5-15%	TDR category >15%
Any	4	0	48	52	0
Any	10	0	7	85	8
B	0	4.0	51	49	0
C	0	2.1	77	23	0
G	0	1.4	87	13	0
CRF 01_AE	0	4.8	40	60	0
B	4	4	15	85	4
C	4	2.1	26	73	1
G	4	1.4	33	67	0
CRF 01_AE	4	4.8	10	86	4
B	10	4.0	1	68	31
C	10	2.1	3	80	17
G	10	1.4	4	82	13
CRF 01_AE	10	4.8	1	64	35

Proportion of correctly categorized simulated TDR prevalences is highlighted in light gray. [1] For subtypes B, C, G and CRF01_AE the prevalence of polymorphisms were obtained from Bennett et al. [5]; TDR, transmitted drug resistance.

We calculated positive predictive values for subtype B, C, G and CRF01_AE for observed TDR levels of 5%, 10%, and 15%. At an observed TDR level of 5%, positive predictive levels were low especially in CRF01_AE (4%) and subtype B (20%), which have high reported levels of polymorphism, and somewhat higher in subtype C (48%) and G (72%). At a higher observed TDR prevalence of 10%, the proportion of correctly identified cases of TDR increased and ranged from 52% in CFR01_AE to 86% in subtype G. At an observed TDR level of 15% the positive predictive value ranged from 68% in CRF01_AE to 91% in subtype G.

Table 1 shows the effect of different levels of polymorphisms and true prevalence of TDR on the accuracy of the WHO approach for TDR sampling and categorization, calculated in the different simulation-scenarios. In the first two scenarios we simulated the WHO approach in populations with no polymorphism (0%) and a true prevalence of TDR of 4% and 10%, respectively. We found that the WHO categorization strategy was correct in less than 50% of the simulations when the true prevalence of TDR was low (4%), but successful in 85% of the simulations when the true TDR prevalence was higher (10%). We next simulated more realistic

scenarios with the specific polymorphism levels for the different subtypes estimated by Bennett *et al.* and a true TDR prevalence of 0%, 4% and 10%. As shown in table 1, the high level of polymorphisms in CRF01_AE had a dramatic impact on the classification of the prevalence of TDR. If we set the true TDR prevalence at 0% in our simulations, we found that there was a 60% probability that TDR was classified as 5-15%. Furthermore, incorrect categorization was even more common when the true TDR prevalence was just below the 5% cut-off, i.e. 4%. In these scenarios the TDR prevalence was typically incorrectly classified into the 5-15% category. Thus, the WHO TDR categorization would be expected to be incorrect 85% of the time for subtype B and 86% of the time for CRF01_AE when the true prevalence of TDR is 4%. As further discussed below these results are based on the assumption that Bennett *et al*. have correctly estimated the prevalence of polymorphisms in treatment naïve patients.

In this paper the effect of naturally occurring sequence polymorphisms on TDR was evaluated using different ranges of polymorphism levels and TDR. With these approaches we showed that polymorphisms can have a large impact on the estimated prevalence of TDR.

There is an inverse relationship between the likelihood of correctly estimating the prevalence of TDR and the presence of polymorphisms: the higher the prevalence of polymorphisms, the lower the likelihood of correctly estimating the prevalence of TDR. Therefore, the presence of polymorphisms affects the accuracy of the classification method recommended by the WHO and may lead to an incorrect categorization of TDR prevalence. This is especially relevant in resource-poor settings, where WHO recommends that TDR prevalences greater than 5% should trigger several actions, such as performing extra research and more frequent surveillance studies [15].

It is important to stress that our findings are based on the assumption that the prevalence of polymorphisms have been correctly estimated by Bennett *et al*. [5]. In fact, they give two possible explanations for the non-zero background level of mutations at drug resistance positions. First, the mutations may be caused by true polymorphisms. This can be caused by nucleotide misincorporations by the error-prone reverse transcriptase enzyme during replication of the HIV-1 genome in combination with cytotoxic T lymphocyte immune selection pressure; an example is the M46I mutation [16]. Secondly, the dataset may have contained patients with unreported prior treatment or TDR. We feel that it would be very valuable to dissect the relative contribution of these two possibilities. Thus, we advocate for studies on sufficiently large numbers of samples for which unreported prior treatment or TDR can be ruled out with 100% certainty, i.e. ideally samples collected before antiretroviral therapy had been introduced. However, the collection of such a dataset is difficult to achieve.

Because the level of polymorphisms levels might be biased by patients with unreported prior treatment and TDR, the impact of these polymorphisms on the estimated prevalence of TDR might be smaller than indicated in our paper. However, even if a perfect estimate of polymorphism levels could be generated, it is very likely that some low-level of polymorphisms will always be present. Therefore, we feel that it always will be necessary to adjust TDR estimates for the level of polymorphisms, especially when the true prevalences of TDR are expected to be

low. In this context, it should be pointed out that the prevalence of TDR may also be under-estimated because routine assays for genotypic resistance testing (i.e. population Sanger sequencing) cannot detect minority resistance mutations [17]. These minority mutations can influence the response to therapy [18].

In conclusion, polymorphisms are likely to have a large impact on the estimates of TDR prevalence and the level of polymorphisms need to be more accurately estimated and adjusted for in TDR surveillance and prevention.

AFFILIATIONS

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9

Cost-effectiveness of baseline genotypic testing in HIV infected patients in the Netherlands

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ABSTRACT

Background: About 10% of all patients newly diagnosed with HIV-1 are infected with a virus carrying a drug resistance mutation. HIV-1 genotyping before start of treatment is therefore recommended in most guidelines. In recent years, however, baseline genotyping mostly identifies mutations conferring resistance to antiretroviral drugs that are not used anymore. The aim of this study is therefore to determine cost-effectiveness of baseline-genotyping in the Netherlands.

Methods: We designed a probabilistic state-transition model to project clinical and cost outcomes in a hypothetical cohort of antiretroviral-naïve patients with a HIV-infection in the Netherlands. The overall prevalence of transmitted drug resistance mutation (TDRM) was taken to be 8.7% from the 2010 Dutch prevalence data. This most frequently involved TDRM for nucleoside reverse transcriptase inhibitors (5.7%), followed by protease inhibitors (2.1%) and non-nucleoside reverse transcriptase inhibitors (1.9%). Rates of efficacy of treatment, virological failure, opportunistic infections, mortality and health-related utilities were derived from published randomized clinical trials, observational cohort studies, and data from a Dutch HIV care centre.

Results: The magnitude of the cost-effectiveness ratio decreased when the reduction in failure rate of first line regimens increased (€1.2 million, €220,000, and €94,000 per QALY gained for absolute failure rate differences of 1, 5, and 10%, respectively). A 10% absolute failure rate difference would be achieved, for example, when TDRM is found in 20% of HIV patients. Subsequently, of these TDRM, half would cause resistance to the prescribed first-line regimen and therefore experience virological failure, while genotypic testing would prevent all these failures. The cost-effectiveness did not decrease to reasonable values unless the absolute reduction rate difference in patients with baseline genotypic testing exceeded 20% (€30,000). Nonetheless, genotypic testing showed to be more cost-effective in patients with a CD4 count below 200 cells/mm^3 compared to patients with a CD4 count of >200 cells/mm^3, with cost-effectiveness ratios of €65,000 and €330,000 per QALY gained, respectively, at an absolute reduction in failure rate of 5%. Additionally, reducing the costs of genotypic testing by half (by limiting sequence testing to reverse transcriptase only) lowered the cost-effectiveness ratio by 50%.

Conclusion: In 2012, routine use of baseline genotypic testing in all newly HIV-1 infected patients is no longer cost-effective. The use of routine baseline genotypic testing should therefore be reconsidered for use in sub-groups.

INTRODUCTION

The use of combination antiretroviral therapy has strongly reduced morbidity and mortality among patients infected with HIV [1]. Unfortunately, transmission of drug resistant mutations, currently at a rate of 10-15% [2-7], can hamper the success of antiretrovirals. This has recently been shown in a pan-European study, which showed that transmission of drug resistance is associated with an increased probability for virological failure [8]. Detection of drug resistance associated mutations through genotypic testing can help to construct a first line antiretroviral regimen that is virologically fully effective against the drug resistant virus [9]. Therefore, current treatment guidelines recommend use of resistance testing in naïve patients [10-11]. Furthermore, the economic impact of baseline genotypic testing has been addressed by several studies in the past [12-14], showing that baseline genotypic testing was cost effective.

To a great extent, the cost-effectiveness of baseline genotypic testing depends on, among other things, the prevalence and nature of transmitted drug resistance mutations (TDRM) in the population. We showed in a recent European TDRM surveillance study that most TDRM do not cause resistance to nucleos(t)ides (tenofovir, emtricitabine, and lamivudine) currently popular in first-line regimens [15]. As a consequence, the three published cost-effectiveness studies (performed in 1998-2001 [12-14], when these data and drugs were not available) do not reflect the current situation in Europe. Mortality rates and opportunistic infections in HIV patients have also decreased substantially over time, irrespective of the CD4 cell count as a reflection of improved clinical care [16-17]. Therefore, data on clinical parameters and resistance prevalences used in previously published studies on cost-effectiveness of baseline genotypic testing are no longer valid.

In light of the issues mentioned above we decided to evaluate the cost-effectiveness of routine baseline genotypic using data from the Netherlands.

METHODS

Model structure

Population

The analyses were performed using a probabilistic state-transition model to project clinical and cost outcomes in HIV-infected individuals. The target population consisted of HIV-infected patients with characteristics similar to HIV-infected individuals that started HAART in the Netherlands in 2010 (table 1) [18]. The prevalence of TDRM among newly diagnosed patients in the Netherlands was 8.7%. This most frequently involved TDRM for nucleoside reverse transcriptase inhibitors (NRTIs) (5.7%), followed by protease inhibitors (PIs) (2.1%) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) (1.9%). We predicted resistance to particular drugs using the Stanford HIV database system (version 6.2.0). It was found that 4% of all patients were infected with a virus with intermediate or high-level resistance [19]. High-level resistance was mostly found in the NNRTI drug class (1.7%), followed by PI (0.5%) and NRTI (0.4%), while intermediate resistance was more often observed

to the NRTI drug class (1.7%), followed by PI (1.4%) and NNRTI (0.2%). Resistance to NRTIs usually involved the thymidine analogues zidovudine and stavudine (97% of all isolates with NRTI resistance) that were popular in past treatment. Resistance to tenofovir, emtricitabine, and lamivudine was less common (34%, 6%, and 6% of isolates with NRTI resistance). Almost all patients carrying NNRTI resistance showed high-level resistance to nevirapine (97%), while this was lower for efavirenz (62%). Resistance to PIs involved nelfinavir in all patients, which is no longer recommended [11, 20]. Resistance to the currently popular boosted PIs atazanavir (19%) and darunavir (5%) was much lower. For more details on the target population see the report of the Dutch HIV monitoring foundation [18].

Treatment

In our cost-effectiveness analyses, we compared a scenario where baseline genotyping was not available and a scenario where baseline genotyping was available at start of first-line therapy. In the approach where baseline genotyping was not available patients received treatment according to current treatment guidelines [11, 20]. This means that a random proportion of 80% of patients received first-line treatment with tenofovir, emtricitabine and efavirenz. To account for heterogeneity in the standard of care for first-line treatment, 10, 5, and 5% received nevirapine, boosted darunavir or boosted atazanavir, respectively, instead of efavirenz. In the approach where baseline genotyping was available, patients infected with a wild-type virus also received treatment according to treatment guidelines as outlined before. In patients that were infected with a drug resistant virus, treatment was modified so that a fully active regimen was prescribed.

Virological failure

The proportion of patients experiencing virological failure for different susceptibility levels in Europe is reported by Wittkop et al. [21]. In summary, a patient without TDRM that received a NNRTI-containing regimen had a probability of virological failure of 2.8%. Similarly, patients receiving a PI-containing regimen had probability of virological failure of 2.7%. They found that patients with TDRM that received a fully active NNRTI-containing regimen had probability of virological failure of 4.3%, while for PI-containing regimens the same probability was seen as was observed in patients without TDRM. The probability of virological failure was 10.6% and 10.9% in patients infected with a virus resistant to the received NNRTI- and PI-containing regimen, respectively [21].

However, the virological failure rates found in Wittkop et al. seem quite low compared to the virological failure rates ranging between 0 to 14% [22] in randomized clinical trials and 4.6 [23], 7 [24], and 8% [25] in recent cohort studies. We therefore increased the virological failure rate to 50% and 100% in all patients infected with a virus being intermediate- or fully resistant to the prescribed regimen to examine its effect on cost-effectiveness.

Impact on CD4-count

In patients experiencing virological failure, drug plasma levels were measured in 20% of the patients and CD4 cell counts and HIV RNA levels were measured three additional times compared to standard HIV care. Furthermore, in patients with

Table 1. Model parameters.

Variable	Baseline value	Source [Ref]
Cohort characteristics		
Age, mean years	40.9	[19]
CD4 cell count, median cells/mm^3 (IQR)	300 (180-360)	[19]
Prevalence of transmitted drug resistance		[19]
- Overall	8.7%	
- NRTI	5.7%	
- PI	2.1%	
- NNRTI	1.9%	
Virological failure at 12 months in NNRTI-containing regimens	10%	[21]
- no transmitted drug resistance	2.8%	
- TDRM and full-active cART	4.3%	
- TDRM and resistance to cART	10.6%	
Virological failure at 12 months in PI-containing regimens		[21]
- no transmitted drug resistance	2.7%	
- TDRM and full-active cART	2.7%	
- TDRM and resistance to cART	10.9%	
Monthly increase in CD4 cell count in patients receiving effective HAART, cells/mm^3		
- first half year	23	[27, 43]
- second half year	10	[27, 43]
Monthly decrease in CD4 cell count in patients experiencing virological failure		
- NNRTI regimen	3.7	[26]
- PI regimen	1.7	[26]
Costs, €		
- therapy (per month)	638-2,230	[32]
- genotypic testing (per test)	340	
- CD4 cell count (per test)	98	
- HIV RNA level (per test)	67	
- plasma level (per test)	80	
- opportunistic infections (per infection)	1,624-34,697	
Health-related quality-of-life score according to CD4 cell count, cells/mm^3		[34]
- 0 to 50	0.79	
- 51 to 100	0.81	
- 101 to 200	0.87	
- >201	0.94	
opportunistic infections	0.56 – 0.65	

NRTI, nucleoside reverse transcriptase inhibitors; NNRTI, non-nucleoside reverse transcriptase inhibitors; PI, protease inhibitor; TDRM, transmitted drug resistance mutations; cART, combination antiretroviral therapy.

virological failure, we assumed that CD4 cell counts began to decrease, following a 1-month lag time after HAART failure [26]. These patients were switched to another regimen 6 months after start of therapy. If treatment was successful, the CD4 cell count increased [27-28] and treatment was continued. The slope for the increase or decrease of CD4 cell count was fixed for 6 months. Every month, the distribution of patients was calculated for the following 6 CD4 groups: 0 to 50, 51 to 100, 101 to 200, 201 to 350, 350 to 500, and >500 cells/mm^3. The CD4 count was used to predict rates of opportunistic infections and HIV-related death [17, 29-30]. We assumed decreased rates of opportunistic infections and HIV-related death for patients successfully on treatment, independent of CD4 cell count [29-30]. Patients could enter and exit temporary health states corresponding to acute

episodes of cytomegalovirus (CMV) retinitis; *Mycobacterium avium* complex (MAC) infection; cerebral toxoplamosis (TOXO) *Pneumocystis jirovecii* pneumonia (PCP); esophageal candidiasis (EC); pulmonary or extrapulmonary tuberculosis (TB); Kaposi's sarcoma; and non-Hodgkin's lymphoma. The model was programmed in Matlab 7.7.0.

Costs

Calculations were performed according to Dutch cost calculation guideline in health-care [31], and medication costs were based on Dutch whole-sale prices [32]. Costs of opportunistic infections were obtained from the Erasmus medical centre, which is a large Dutch HIV care centre. With the large dataset of HIV patients from this hospital we were able to calculate costs based on micro-costing assessment. Resource utilization included physician visits, emergency visits, and the number of hospital days. Use of medical imaging services, medications, laboratory test, and blood transfusions were based on expert opinion. The prices of plasma levels, CD4 cell count levels, and HIV RNA levels were based on hospital integral cost prices (Erasmus Medical Centre, Rotterdam). We used the figure of €340 for genotypic resistance testing. All costs prices were inflated to 2010 using Dutch consumer price index figures [33] (Table 1). All costs are presented in 2010 Euros.

Quality-Adjusted Life-Years (QALYs)

We expressed clinical benefits in quality-adjusted life-years (QALYs) gained, to reflect the potential gains in both longevity and quality of life associated with the use of baseline resistance testing. Data on health-related quality of life were obtained from AIDS Clinical Trials Group protocols 019, 108, 157, and 204 as published in Freedberg et al. [34]. These utilities correspond closely to utilities obtained in more recent studies [35] where utilities were not available per CD4 cell count group and per opportunistic infection. In a 12-month follow-up, this can lead to a value between 0 and a maximum of 1 QALY, where a QALY of 1 is a year lived in perfect health. Given that the analysis was restricted to a 1-year period, discounting was not used.

Scenario analyses

In the baseline scenario, overall prevalence of TDRM was 8.7%. This corresponded with relatively high susceptibility scores in the Dutch antiretroviral naïve population. In table 2, the proportion of patients in the different susceptibility groups are shown. The numbers were multiplied with failure rates for each susceptibility group reported in Wittkop et al.[21]. This resulted in a small difference in failure rate (0.18%) between the group with and without baseline genotypic testing. Wittkop et al. [21] reported low failure rates. We therefore performed worst-case scenario analyses, where virological failure was experienced in 50% and 100% of all patients infected with a virus being intermediate- or fully resistant to the prescribed regimen.

Also, to estimate the effect of higher TDRM levels on the cost-effectiveness of baseline genotypic testing, we increased failure rates to an absolute difference of 1, 5, 10, and 20%. A 10% absolute failure rate difference would be achieved, for example, when TDRM is found in 20% of HIV patients. Subsequently, of these TDRM half would cause resistance to the prescribed first-line regimen and therefore experience virological failure, while genotypic testing would prevent all these

failures. This was considered as the most pessimistic scenario possible. An absolute difference in failure rate of 20% is an extreme value, which could only be seen if TDRM causing resistance to first-line therapy increase strongly.

Finally, we investigated the effect of changing the baseline failure rate (failure rate in patients where genotypic testing was performed) from 3 to 10% for various levels of absolute reduction in failure rate between patients with and without genotypic testing. This analysis was first performed in the baseline scenario. In the second scenario targeted testing was carried out for patients with CD4 counts that were either below or above 200 cells/mm^3 (a CD4 <200 cells/mm^3 is associated with an increased risk for opportunistic infections and mortality). We also examined the effect of physicians prescribing PI-containing regimens (which are more expensive than NNRTI-containing regimens) when genotypic testing results are not available to avoid virological failure due to NNRTI (minority) mutations. In this scenario, we assumed that all patients that did not have a baseline genotypic test result were receiving a PI-containing regimen. Finally, prevalence of PI mutations was low. We therefore investigated the effect of baseline genotypic testing in the reverse transcriptase gene only.

Sensitivity analyses

With the use of sensitivity analyses, we examined the impact of varying key model parameters. We studied sensitivity in the scenario of 1 and 10% absolute failure rate difference. Antiretroviral treatment and NNRTI drug costs were increased and decreased with 50%, as NNRTIs are likely to become generically available in the near future and thus less expensive. To assess the impact of a lower or higher probability of acquiring an opportunistic infection and related treatment costs, we varied the probability and costs by 50%. The costs of genotypic testing and the QALYs were increased and decreased with 10%. The impact of these changes on the cost-effectiveness was presented in a tornado diagram.

The results of the cost-effectiveness analysis are summarized using the cost-effectiveness ratio, in which each strategy with testing is compared incrementally with a strategy that does not employ resistance testing.

RESULTS

Main results

We performed different analyses in which we used various levels of virological failure that could be observed in the presence and absence of baseline genotyping. We first did an analysis in which we calculated expected rate of virological failure based on a previous paper by Wittkop et al. [21]. Only a small proportion of patients in the group where genotypic testing was not performed were infected with a virus that was intermediate- or fully resistant to the drugs that were prescribed (table 2). As a consequence, the expected absolute difference in virological failure rate between the group of patients with or without genotypic testing was only 0.18% using failure rates reported by Wittkop et al. [21] (table 3). This small difference in proportion of patients failing treatment led to a very small difference in CD4 counts and subsequently

Table 2. Proportion of patients in different susceptibility groups.

	without baseline genotypic testing		with baseline genotypic testing	
	NNRTI regimen	PI regimen	NNRTI regimen	PI regimen
No TDRM	82.17	9.13	82.17	9.13
TDRM and fully-active cART	5.23	0.63	7.83	0.87
TDRM and intermediate resistance to cART	1.53	0.22		
TDRM and resistance to cART	1.07	0.02		

NNRTI, non-nucleoside reverse transcriptase inhibitors; PI, protease inhibitor; TDRM, transmitted drug resistance mutation; cART, combination antiretroviral therapy.

Table 3. Effectiveness, cost and cost effectiveness.

Scenarios	Reduction in failure rate, %	QALYs		Total costs, €		Costs per QALY gained, €
		Baseline genotype test	No baseline genotype test	Baseline genotype test	No baseline genotype test	
Absolute failure rate difference						
1%	1	0.920	0.920	11,280	10,933	1.2 million
5%	5	0.920	0.919	11,217	10,970	220,000
10%	10	0.920	0.918	11,217	11,017	94,000
20%	20	0.920	0.915	11,217	11,109	30,000
Wittkop scenario	0.18	0.920	0.921	11,217	10,926	6.6 million
worst-case scenario 1	1.30	0.920	0.920	11,217	10,936	935,000
worst-case scenario 2	2.72	0.920	0.920	11,217	10,949	430,000

QALY, quality adjusted life year; Worst-case scenario 1: the virological failure rate in untested patients with intermediate- or fully resistance to the prescribed drugs was assumed to be 50%. This led to a failure rate difference between the tested and untested patients of 1.30%; Worst-case scenario 2: the virological failure rate in untested patients with intermediate- or fully resistance to the prescribed drugs was assumed to be 100%. This led to a failure rate difference between the tested and untested patients of 2.72%.

opportunistic infections and mortality between patients where a baseline genotype was available or not. This also explains that the QALY estimations in both groups were approximately equal (0.92). On the other hand, baseline genotypic testing increased health-care costs with 347 euro per person, leading to an incremental cost per QALY of €6.6 million for the baseline genotypic testing strategy.

We also determined cost-effectiveness using higher absolute differences in virological failures between patients with and without baseline genotyping. In the worst-case scenario where 50% of all patients experienced virological failure when showing intermediate- or fully resistance to the prescribed drugs, the reduction in failure rate increased slightly to 1.30%. This failure rate reduction resulted again in a substantial cost-effectiveness ratio of €935,000 per QALY gained. When we assumed that all patients showing intermediate- or full resistance to the prescribed drugs were experiencing virological failure, the absolute reduction in failure rate increased to 2.72% and testing cost changed to €430,000 per QALY gained.

The magnitude of this cost-effectiveness ratio decreased in the other scenarios that were analyzed. For example, if a subset of patients showed an absolute reduction in failure rate of 10% in patients with- compared to patients

without baseline genotypic testing, cost-effectiveness decreased to €94,000 per QALY gained. The cost-effectiveness did not decrease to reasonable values unless the reduction rate difference exceeded 20%.

Changing baseline failure did not have any effect on the results, as shown in table 4. Furthermore, this table shows the effect of targeted genotypic testing in patients who were diagnosed with a CD4 <200 cells/mm^3. When performing genotypic testing in patients with a CD4 cell count below 200 cells/mm^3 we observed reasonable cost-effective ratios of €65,000 and €24,000 per QALY gained at an absolute reduction in failure rate of 5% and 10%, respectively. For patients with CD4 counts above 200 cells/mm^3, baseline genotypic testing appeared to be less cost-effective (€150,000 per QALY gained at an absolute reduction in failure rate of 10%). Prescribing PI-containing regimens to patients without baseline genotypic testing lowered the cost-effectiveness ratio minimally (from €220,000 to €210,000 per QALY gained at an absolute reduction in failure rate of 5%). Finally, performing genotypic testing in only reverse transcriptase lowered the cost-effectiveness ratio with approximately 50%, resulting in a cost-effectiveness ratio of €33,000 per QALY gained at an absolute reduction in failure rate of 10%. The increase in failure rate is small in this scenario as only 13% and 2% of patients infected with a virus intermediate- and fully resistant to the prescribed drugs, respectively, is harbouring PI resistance.

Sensitivity analyses

The results of the sensitivity analyses in the scenario where the absolute failure rate difference was 1% are presented in figure 1A. This tornado diagram ranks the parameters based on the magnitude of their impact on the cost-effectiveness per QALY. The diagram clearly shows that QALY scores and genotypic test costs have the highest impact on the model outcome. However, even then, the lowest cost-effectiveness ratio fell far from the cost-effective cut-off, revealing that the

Table 4. Sensitivity analyses: impact of baseline failure rate and absolute difference in failure rate between patients with and without genotypic testing on cost-effectiveness.

	Failure rate baseline	Absolute reduction in failure rate			
		1%	3%	5%	10%
Baseline scenario	3%	1.2 million	390,000	220,000	94,000
	10%	1.2 million	390,000	220,000	94,000
Targeted testing <200 CD4 cells/mm^3	3%	385,000	120,000	65,000	24,000
	10%	390,000	120,000	65,000	24,000
>200 CD4 cells/mm^3	3%	1.8 million	580,000	330,000	150,000
	10%	1.8 million	580,000	330,000	150,000
Change to PI regimen	3%	1.2 million	375,000	210,000	88,000
	10%	1.2 million	370,000	210,000	87,000
Genotypic testing in reverse transcriptase only	3%	575,000	175,000	95,000	33,000
	10%	580,000	175,000	95,000	33,000

PI, protease inhibitor.

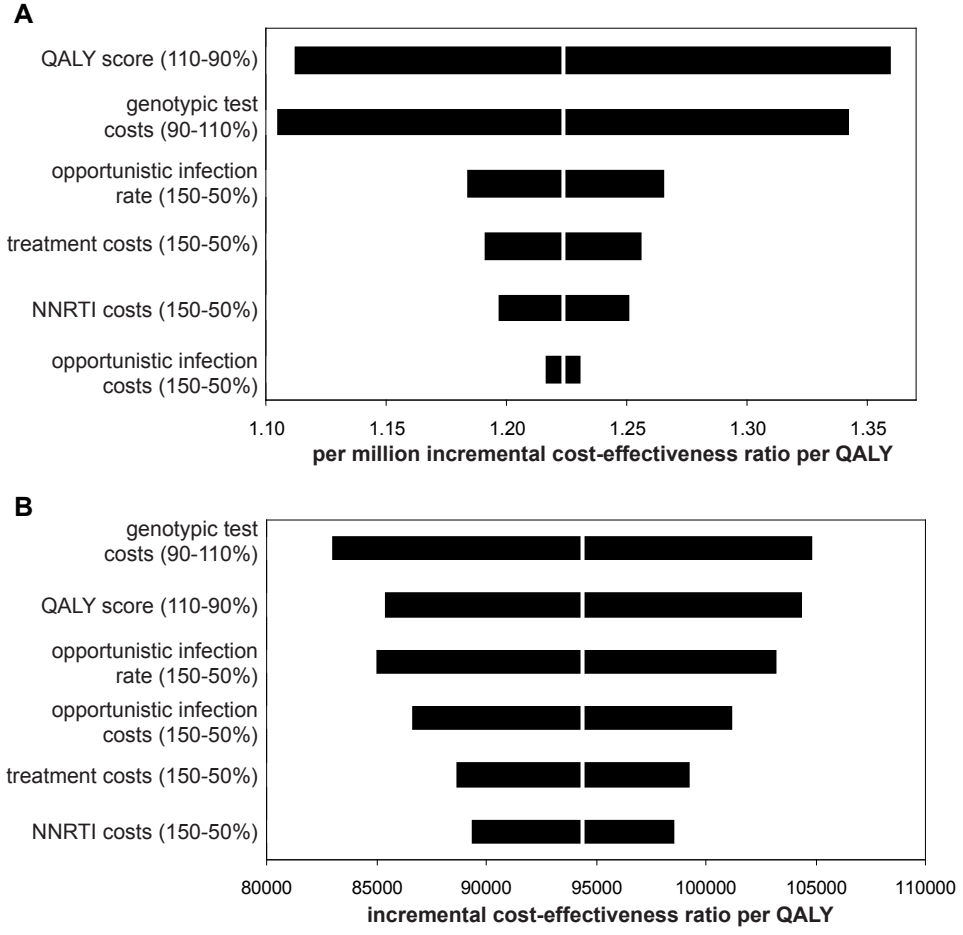

Figure 1. Tornado diagram of the sensitivity analyses.
This diagram summarizes the results of the sensitivity analyses on the incremental cost-effectiveness of baseline genotypic testing in **a**) scenario with an absolute failure rate of 1%, and **b**) scenario with an absolute failure rate of 10%; parameter values of 50% and 150% or 90% and 110% of the baseline scenario value were evaluated and these values are shown on both sides of the bars; QALY, quality adjusted life year; NNRTI, non-nucleoside reverse transcriptase inhibitors.

parameters did not have a very large impact on the results. Opportunistic infection rate and treatment costs had a moderate effect on cost-effectiveness. For the remaining parameters, the model proved to be robust to changes. In the scenario with an absolute failure rate difference of 10%, the tornado diagram showed a very similar structure (figure 1B). However, due to lower cost-effectiveness ratio in this scenario, a larger decrease in costs of genotypic testing than shown here could make baseline genotypic testing cost-effective.

DISCUSSION

Our results show that baseline genotypic testing is not cost-effective. The magnitude of this cost-effectiveness ratio decreased with increasing reduction in failure rate (€1.2 million, €220,000, and €94,000 per QALY gained for absolute failure rate differences of 1, 5, and 10%, respectively). When we applied the expected rates of virological failure as reported by Wittkop et al. [21] we found a cost-effectiveness of more than 6 million euro's per QALY gained. Increasing the virological failure to 50 and 100% in patients infected with a virus intermediate- of fully resistant to the prescribed drugs, gave lower cost-effectiveness ratios of €935,000 and €430,000 per QALY gained, respectively. In the Netherlands, no fixed threshold for cost-effectiveness is used. However, there is a reasonable consensus about the cost-effective threshold lying between 20.000 to 80.000 Euros per QALY depending on the disease [36]. Therefore, even in the most extreme scenario, baseline genotypic testing would not be considered cost-effective. However, in patients with low CD4 cell counts, the cost-effectiveness ratio decreased to €65,000 per QALY gained at an absolute reduction in failure rate of 5%. Furthermore, if resistance testing is performed in the reverse transcriptase gene only, the cost-effectiveness ratio was reduced by approximately 50%.

Our results are very different from the cost-effectiveness studies on baseline genotypic testing that have been performed in the past when different drugs were used in first line therapy [12-14]. Corzillius et al. [14] reported in 2004 a cost-effectiveness ratio of €22,510 per life-year gained in Germany. Sax et al. [13] showed in 2005 that the cost-effectiveness ratio remained less than $50,000 per QALY gained, unless the prevalence of resistance was ≤1%. Weinstein et al. [12] reported in 2001 a cost-effectiveness of $22,300 per QALY gained with a TDRM prevalence of 20%, and $69,000 per QALY gained with 4% prevalence.

This large discrepancy between our study and the reports in literature can predominantly be explained by the difference in calculating the failure rate. Previous studies based virological failure rates on studies among treatment experience patients that failed treatment [37-38]. To calculate failure rates Weinstein et al. [12] used the VIRADAPT trial and the Havana trial, and Corzillius [14] the VIRADAPT trial. The VIRADAPT trial showed a relative risk of 0.79, that is, the probability of primary treatment failure was reduced by 21% [37]. The Havana trial showed a fairly similar value of 0.81% [38]. Sax et al. [13] used several clinic trials where in some of the trials genotypic testing was performed and in others not. All these trials estimated the effect of genotypic testing on virological failure in treatment-experienced patients and not in treatment-naïve patients.

In our study, we used a different approach. We assumed that patients could only fail due to TDRM, when these TDRM were causing intermediate- or full resistance to the prescribed drugs. For the failure rate in these patients we used failure rates reported by Wittkop et al. [21], which gave us a fairly low proportion of patients without TDRM experiencing virological failure (2.91%). Many studies have examined the efficacy of current treatments, showing virological failure rates varying between 0 to 14% [22] in clinical trials and 4.6 [23], 7 [24], and 8% [25] in recent cohort studies. This can be explained by the fact that Wittkop et al. [21] estimated failure rates in patients after being six months on therapy. Also, the virological failure rates found

in Wittkop et al. [21] may be dependent upon underlying mutation profiles, which might be different in our study population. We therefore performed a worst-case scenario analysis, increasing the virological failure by assuming that 50 and 100% of all patients infected with a virus intermediately- or fully resistant to the prescribed drugs experienced virological failure. This increase lowered the cost-effectiveness ratio by approximately 7 and 15 times, respectively. However the cost-effectiveness ratio was still far from being cost-effective. Increasing the baseline virological failure from 3 to 10% did not have any effect on the cost-effectiveness analyses.

The difference between the results in this study and baseline genotypic testing studies previously published could not only be described to difference in failure rate calculations. Even when looking at cost-effectiveness ratios at the same reduction in failure rate as was used in the Weinstein et al. study [12], we found higher cost-effectiveness ratios. Weinstein et al. reported cost-effectiveness ratios of $69,000, $22,300, and $16,100 per QALY gained at a reduction in failure rate of 1, 5, and 10%, respectively. We however, found at the same reduction of failure rate levels, respectively, higher ratios of €1.2 million, €220,000, and €94,000 per QALY gained.

The difference between cost-effectiveness ratios at similar failure rate levels between our study and Weinstein et al. [12] can be explained by several other parameters in our model, for which we used differing estimates. First, other values were taken for CD4 decrease in failing patients. All three previously published studies took estimates from a study reporting a CD4 decrease of 76.5 cells/mm^3 at HIV-1 RNA concentrations of >30,000 per year [39]. This study started when antiretroviral therapy was not yet available and consequently 63% of participants received mono-therapy. In the subgroups that did or did not receive antiretroviral therapy during follow-up, no difference was seen in rate of developing AIDS. Today, the efficacy of antiretroviral therapy has increased greatly [22] and even in patients failing on treatment, a smaller decrease in CD4 is reported [26] than in Mellors et al. [39]. For the definition of the increase in CD4 count in patients successful on treatment, the previous studies used data from clinical trials [40-42] that showed lower efficacies compared to those reported in clinical trials of current regimens [27, 43]. Additionally, rate of opportunistic infections has decreased largely over time. Opportunistic rates from the recent EuroSIDA study [29] are small as compared to opportunistic rates observed in the Multicenter AIDS Cohort Study [44] as used by Weinstein et al. [12]. The mortality rates also came from the Multicenter AIDS Cohort Study [44], but not specifically mentioned. However, use of antiretroviral treatment has reduced mortality independent of CD4 count [30]. Sax et al. [13] also adjusted for the benefits of antiretroviral treatment as they decreased the opportunistic infection rate and death to 54% in treated patients as was shown in Mellors et al. [39].

A large difference was seen in the cost-effectiveness of baseline genotypic testing between patients with a CD4 cell count above or below 200 cells/mm^3. In patients with low CD4 cell counts, the cost-effectiveness ratio decreased to €65,000 per QALY gained at an absolute reduction in failure rate of 5%. This can be explained by the association of low CD4 cell counts and an increased risk for opportunistic infections and mortality [17, 29-30].

A limitation of this study is the short-term follow-up. The viruses in failing patients may develop extra resistance; therefore extra costs may be needed for

second-line and salvage antiretroviral regimens. Also, the decrease in treatment options will lower the potential years of life lost due to premature death [45]. However, the development of extra mutations would not be a major problem as this is only limited to the first half year in our study. Furthermore, we should be careful in extrapolating results from this study to other European countries due to differences in costs. However, our expectation would be that cost-effectiveness studies in other European countries would point in the same direction.

The costs of opportunistic infections were calculated with patient data. When patient data did not follow guidelines, we checked expert opinion to validate this. We noticed that hospital-days are the major contribution to costs of opportunistic infections. Co-morbidity complicates the calculation of hospital days contributed by one opportunistic infection. However, this would not have a major impact on our cost-effectiveness calculations as costs of opportunistic infections was a parameter with only a small impact on the model outcome as shown in the sensitivity analyses.

Our data suggest that especially in patients with high CD4 cell count, baseline genotypic testing is not cost-effective. However, if baseline genotypic testing were no longer standard practice, this could have major implications. First, the data of genotypic testing assays can be used for surveillance purposes. When baseline genotypic testing is not standard practice, we might have a great loss of insight into the epidemiology of TDRM. Also, the cost-effectiveness study was performed on a population level, whereas on an individual basis some patients may benefit significantly from baseline genotypic testing. Furthermore, when physicians would not have access to genotypic information of a patients' virus, they might change their prescribing behaviour. Because NNRTI mutations often cause full resistance to NNRTI drugs, physicians might more often choose to prescribe PI drugs in order to avoid virological failure. PIs are more expensive than NNRTIs and costs may therefore go up once baseline-genotyping is no longer performed. We adjusted for this effect in our analyses, thereby showing that the change to PIs only causes a small decrease in the cost-effectiveness ratio. The cost-effectiveness could, however, decrease more in the near future as NNRTIs are likely to become generically available. Since the costs of these generic NNRTIs are not yet known, we were not able to take this into account in our analyses.

Besides targeting baseline genotypic testing, we could limit population-based nucleotide sequencing to only the reverse transcriptase gene of the virus. Because TDRM levels to PIs are low in our dataset, and other published surveillance studies [2], sequencing the protease gene of the virus seems unnecessary. Without sequencing the protease gene, the population sequencing test cost would be substantially reduced to half of the current sequencing cost. We showed in this study that this also lowers the cost-effectiveness by approximately 50%. Also, performing NNRTI minority assays might be a good alternative. Population sequencing fails to identify drug-resistant minority variants that are present in <20% of the virus population infecting a patient [46-47]. These minority variants have been detected in almost 14% of antiretroviral naïve HIV-infected individuals [48]. The presence of minorities, particularly involving NNRTI resistance, is associated with an increased risk of virological failure to first-line therapy [48]. If minority assays would be implemented as standard practice, costs may lower considerably. With low costs and the provision of valuable information on minority NNRTI mutations, this test could

improve health care in HIV patients.

In conclusion, with different calculations of failure rate reduction and improvement of health-care in HIV-patients cost-effectiveness ratios changed from being cost-effective in previous studies to being far from cost-effective in our study. The use of routine baseline genotypic testing should therefore be reconsidered. Possibilities for reducing costs could be to limit baseline genotypic testing to a targeted population, to perform resistance testing only for the reverse transcriptase gene, or performing NNRTI minority assays only.

AFFILIATIONS

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10

General discussion

1 Introduction

The use of combination antiretroviral therapy has strongly reduced morbidity and mortality among patients infected with HIV [1]. However, the success of antiretroviral drugs has been limited by the emergence of drug-resistant variants which occur frequently in patients with virological failure and may decrease both the magnitude and the duration of the response to treatment [2]. Transmission of resistant viruses between individuals has been observed in approximately 10-15% of antiretroviral naïve patients in Europe [3-6] and North America [7-8]. These individuals are at higher risk for developing virological failure to combination antiretroviral therapy [9].

This PhD thesis deals with transmitted drug resistance mutations (TDRM) and has been divided in three different parts. The first part focuses on the epidemiology of transmission of drug resistance and includes studies on the prevalence and time trends of transmitted drug resistance in, respectively, the world, Europe and among individual transmission groups in Europe. The second part examines the interpretation of acquired- and transmitted- drug resistance mutations. In the last part of the thesis, a study is presented on cost-effectiveness of baseline genotypic testing in the Netherlands.

2 Overall trends in transmitted drug resistance

2.1 Overall trends in resource-rich settings

High prevalence of TAM

As shown in chapter 2, 3, and 4, high TDRM prevalence to nucleos(t)ide reverse transcriptase inhibitors (NRTIs) was found in North America (8.2% -WATCH study in chapter 3 and 7.4% -systematic review in chapter 2) and Europe (>5.0%). Also, in chapter 3 and 4, we reported that most NRTI TDRM in Europe (62% in chapter 3, 84.4% in chapter 4) and in North America (79%) were of the thymidine analogue mutations (TAMs) class that are associated with resistance to zidovudine and stavudine. The highest prevalence on these continents was found for the revertant mutations at position 215 (>45% of NRTI TDRM).

This high prevalence of NRTI TDRM and especially TAMs can be explained by the prolonged use of these drugs in non-suppressive regimens. The NRTI drugs zidovudine and stavudine have been used extensively in the past as mono- and dual therapy in Western countries. The use of the mono- and dual therapy of these drugs has led to the selection of TAMs in many patients [10-11]. The toxicity associated with these drugs and the availability of novel, equally active but less toxic drugs such as tenofovir, lamivudine and emtricitabine, has led to a strong decrease in their use and they have become uncommon in first-line treatment over time. Although the prevalence of NRTI mutations has declined over time due to treatment change, the NRTI-associated TDRM still remain the most frequent. This is due to the long persistence of TAMs in the absence of antiretroviral drugs [12-13]. In the treated failing patients, multiple compensatory mutations may appear after the initial selection of resistance mutations that lower the replicative capacity. After transmission to a new host, evolution may be expected to occur in a stepwise manner. However, if

all possible nucleotide changes would initially decrease the replicative capacity, reversion to wild-type will be blocked [12].

If we carry on along this line of thought, the risk of resistance lowered after HAART was introduced and zidovudine and stavudine are no longer used as mono- and dual therapy. As a consequence, patients starting new on therapy developed TAMs at a much lower frequency. In the patients treated earlier, a part of the viruses containing TAMs did not yet develop compensatory mutations. When this virus was transmitted it could therefore revert to wild-type in the newly infected individual. Other patients were infected with a virus harbouring TAMs together with compensatory mutations. When such viruses were transmitted, the TAMs persisted over time and could be transmitted further. These viruses with compensatory mutations have therefore formed a sub-epidemic spread through North America and Europe. Also in Latin America, this sub-epidemic was observed (although with lower prevalences) due to the universal access to antiretroviral drugs since 1990 in several countries. In Africa, a similar sub-epidemic was described in Uganda [14] which had an early start in the roll-out of antiretroviral treatment [15]. Possible mutations causing fixation have been mentioned before (V60I, K104R and S162A) [16]. The compensative role of these mutations, however, has not yet been confirmed.

As the T215revertant mutation is the most prevalent TAM mutation, the clinical relevance of this high TAM prevalence is limited. T215revertant viruses are only one step away from the resistant variants, compared to the two mutational steps that are needed from wild-type. These revertants do not, by themselves, cause resistance, although an increased risk for developing resistance under treatment with zidovudine or stavudine has been observed [17]. Additionally, single TAMs do not cause resistance to nucleos(t)ides currently popular in first-line regimens (emtricitabine, tenofovir, lamuvidine, and abacavir) [18-20]. As a result, the high prevalence of single TAMs that was mainly observed in Europe and North America will not have a great impact on the efficacy of modern first-line therapy.

Increasing transmitted NNRTI resistance

The increase in non-nucleoside reverse transcriptase inhibitor (NNRTI) TDRM prevalence coincides with the more frequent use of this drug class in the developed world in recent years. NNRTIs have become more popular in first-line treatment as they have good clinical efficacy [21-22] and are convenient to use (low pill burden) which improves adherence [23]. Unfortunately, NNRTIs have a low genetic barrier to drug resistance. A single amino acid change is sufficient for high level drug resistance to the most commonly used NNRTIs in first-line treatment [24]. The most prevalent NNRTI mutation K103N has a limited effect on viral replication capacity and persist for long periods after transmission [12]. Strains with this mutation can therefore also be transmitted to others (onward transmission) [25-26]. This increase in NNRTI resistance is worrying as it is likely to negatively influence the therapy response of first-line therapy, as most include NNRT inhibitors.

Low prevalence of TDRM to PIs

Transmitted drug resistance to protease inhibitors (PI) was uncommon in resource-rich settings (<3.5%). This is be explained by the high genetic threshold for resistance to boosted PIs [27]. Moreover, PIs are not frequently used in first-line

therapy compared to NNRTI-containing regimens as these last regimens showed better clinical efficacy than PI-containing regimens [21].

TDRM in phylogenetic clusters

Although travel and migration played a key role in the early spread of HIV, it is not known to what extent travel currently explains transmission of HIV. We therefore performed phylogenetic analyses on the data of the SPREAD programme to estimate the proportion of individuals newly diagnosed with HIV that was infected within their own country (Chapter 6). We found phylogenetic associations between viruses in one third of newly diagnosed individuals. Patients that were part of a cluster were more frequently harbouring a TDRM (10.4%) as compared to non-clustering patients (8.3%, *P*-value=0.03).

The vast majority of patients were part of a cluster that consisted only of patients originating from the same country. This suggests that a large part of the spread of HIV-1 in Europe can be explained by transmission of infections taking place between patients within the same country. As travel is not of major importance in the transmission of HIV, public health should not make a large effort to focus on this aspect of HIV transmission. The presence or absence of TDRM did not influence the proportions of patients clustering in national clusters (74.6 and 74.2%, respectively).

2.2 Trends in resource-poor countries

NRTI transmitted resistance

In both chapter 2 and 3, we observed different patterns of TDRM to particular antiretroviral drug classes in Africa compared to other parts of the world. Contrary to the Americas and Europe, the prevalence of NRTI TDRM was low and increased over time.

The low prevalence of NRTI TDRM in Africa is due to the limited use of mono-therapy of NRTIs on this continent. The increase of NRTI TDRM can be explained by the antiretrovirals becoming more widely available during recent years (e.g. due to the efforts of the Global Fund and PEPFAR -President's Emergency Plan for AIDS Relief). Due to the increased use of HAART (which includes NRTIs as the backbone), TDRM have developed, and as a consequence NRTI TDRM in Africa has been rising.

In chapter 5, however, we observed a decreasing trend in NRTI resistance in heterosexuals infected in regions outside Western Europe and North America. The difference between the regions could be due to differences in the collection of data. The SPREAD data were collected using representative sampling for the transmission route and geographical distribution of HIV in the participating countries. In SPREAD only 40% of the heterosexual patients who were infected in regions outside Western Europe or North America, originated from Sub-Saharan Africa. Thus SPREAD also represents TDRM time trends also from other continents besides Africa. In Latin America, for example, we observed a decreasing trend in the TDRM prevalence to NRTIs, as reported in chapter 2. Finally, patients migrating to Europe are often chronically infected at time of diagnosis. In chronically infected patients, virus variants with resistance mutations can be outgrown by or revert to wild-type viruses which often have a better replicative capacity. In that case, the resistant virus

variants can no longer be detected by population sequencing as used in our study, because this method fails to detect minority populations [28-29]. The drug class in which reversion to wild-type often occurs is the NRTI drug class. Here, the M184V mutation is replaced by wild-type rapidly (86% within 16 months) [12]. The M184V causes resistance to abacavir, emtricitabine and lamivudine, and the use of these drugs has been increasing over time.

NNRTI transmitted drug resistance

In Africa, we also observed an initially high proportion of NNRTI-resistance, which decreased over time. This high prevalence reflects the prophylactic use of single dose NNRTI-monotherapy for prevention of mother-to-child-transmission [30-31]. Due to the low genetic barrier, resistant viruses were selected [25]. Currently, the WHO recommends combinations of antiretroviral drugs (including NRTIs) to prevent vertical transmission, instead of using the simplest regimen of single-dose nevirapine [32]. Furthermore, access to HAART has been scaled up in developing countries [33-34]. As a consequence, the TDRM prevalence to NRTIs has increased and the contribution of NNRTI TDRM to total resistance has decreased.

Overall prevalence of TDRM

In the review, we reported lower prevalences of TDRM for all individual drug classes in Africa compared to Europe and North America, resulting in a low overall prevalence of TDRM in Africa (6.8%). Similar to Europe, we found limited TDRM to PIs in Africa (1.2%). The review however, only included studies published through 2009. A recent surveillance study in Africa showed an overall TDRM prevalence of 5.6%, ranging from 1.1% in Latin Africa, to 12.3% in Uganda [14]. This higher prevalence found in Uganda is probably related to the earlier start of antiretroviral treatment roll-out in Uganda [15]. So in conclusion, in some areas of Africa with early roll-out of antiretrovirals such as Uganda, TDRM can be high. Nevertheless, the overall TDRM prevalence in Africa still seems to be low (though increasing) compared to Europe and North America. Continuous surveillance is needed to follow the development of this trend on the African continent.

2.3 TDRM in different transmission groups

Supported by other studies [3, 5] we can state that in newly diagnosed patients from Europe higher TDRM prevalence can be found in men who have sex with men (MSM) (11.1%) compared to heterosexual patients (6.6%) (chapter 5). The likely reasons for this are again related to the differences in use of antiretroviral drugs as described before. Heterosexual patients are often migrants infected in countries outside Western Europe or North America. These patients did not have access to antiretroviral treatment until recently. Interestingly however, the prevalence of resistance in heterosexuals originating from Western Europe and North America was still lower than the resistance prevalence we found in MSM patients from these areas. One possible explanation is that heterosexuals in Western Europe are frequently infected by individuals originating from outside Europe. This is supported by a model of Xiridou et al. [35] which showed that a 53% of new HIV infections in the Netherlands was acquired by an African migrant of which most (32%) via sexual contact in the Netherlands . The model was based on data from the Netherlands

where migrants reported sexual mixing with Dutch partners and with both Dutch and non-Dutch partners in only 15 and 5%, respectively [36].

Another explanation for the difference in TDRM between MSM and heterosexual patients can be due to the fact that heterosexual patients are often chronically infected. The resistant variant in heterosexual patients may have reverted to wild-type HIV by that time, and thus undetectable with standard population sequencing. However, we did not observe higher TDRM prevalence in recently infected heterosexual patients originating from Western Europe or North America compared to patients originating from outside these regions. A better explanation might be that resistance viruses may have spread by onward transmission in HIV clusters of MSM, forming a sub-epidemic in these patients.

3. The interpretations of HIV-1 drug resistant mutations

In the second part of the thesis, we discussed the complexity of interpretation of resistance for estimating the prevalence of TDRM in surveillance studies. We started with comparing different interpretation systems and also investigated the influence of low-level polymorphisms in the estimation of TDRM prevalence.

3.1 Comparison of interpretation systems

Interpretation systems have been developed [37] which provide rules devised by experts using information extracted from databases of genotypic and correlated phenotypic or treatment response data. A good way to compare the interpretation systems is by using virological response data in correlation with the prediction of interpretation systems. We performed a comparison between the ANRS, HIVdb, and Rega interpretation systems in patients with virological failure (transmitted and acquired resistance) using three different virological outcome time points (Chapter 7). We were able to show that the ANRS, HIVdb, and Rega interpretation systems do not differ in predicting virological outcomes at all time points at week 12, week 24, and week 48.

3.2 The influence of the presence of low-level polymorphisms

The interpretation of TDRM can be complicated by natural occurring polymorphisms, which could result in an overestimation of TDRM prevalence (chapter 8). In this thesis we showed that these polymorphisms can have a large impact on the estimated prevalence of TDRM (Chapter 8). This is not surprising as the sum of polymorphisms across all surveillance drug-resistance mutation positions varied from 1.4% (for subtype G) to 4.8% (for CRF01_AE) in Bennett et al. [38]. We found an inverse relationship between the likelihood of correctly estimating the prevalence of TDRM and the presence of polymorphisms: the higher the prevalence of polymorphisms, the lower the likelihood of correctly estimating the prevalence of TDRM. Furthermore, a small sample size can decrease the positive predictive value of TDRM even more. Therefore, both the presence of polymorphisms and the number of samples analyzed affects the accuracy of the classification method recommended by the WHO and may lead to an incorrect categorization of TDRM prevalence. This is especially relevant in resource-poor settings, where WHO recommends that TDRM prevalences greater

than 5% should trigger several actions, such as performing extra research and more frequent surveillance studies [39].

4 Cost-effectiveness of baseline genotypic testing

In the last part of this thesis, a cost-effectiveness analysis of baseline genotypic testing was performed (chapter 9). Cost-effectiveness of baseline genotypic testing depends on the prevalence of TDRM in a population. We showed, in chapter 3, 4, and 5 that most TDRM do not cause resistance to the nucleos(t)ides currently popular in first-line regimens (tenofovir/emtricitabine or abacavir/lamivudine with efavirenz or a boosted PI). However, the three previously published cost-effectiveness studies could not incorporate this data as they were performed before these drugs were available (1998-2001) [40-42]. We were able to show that the magnitude of the cost-effectiveness ratio of baseline genotypic testing decreased with increasing levels of an absolute reduction in failure rate to first line regimens (€1.2 million, €220,000, and €94,000 per QALY gained for absolute failure rate differences of 1, 5, and 10%, respectively). A 10% absolute failure rate difference would be achieved, for example, when TDRM is found in 20% of HIV patients. Subsequently, of these TDRM, half would cause resistance to the prescribed first-line regimen and therefore experience virological failure, while genotypic testing would prevent all these failures. The cost-effectiveness did not decrease to reasonable values unless the absolute reduction rate difference in patients with- and without baseline genotypic testing exceeded 20% (€30,000). Nonetheless, genotypic testing showed to be more cost-effective in patients with a CD4 count of below 200 cells/mm^3 compared to patients with a CD4 count of >200 cells/mm^3, with cost-effectiveness ratios of €65,000 and €330,000 per QALY gained, respectively, at an absolute reduction in failure rate of 5%. Furthermore, if resistance testing is performed in reverse transcriptase gene only, the cost-effectiveness ratio was lowered by approximately 50%.

5 Future development of the epidemiology of transmission of drug resistant HIV-1

New developments in HIV prevention and treatment might influence the trends of TDRM in Europe and other continents. Pre- and post-exposure prophylaxis, microbicides, test and treat and the development of new drugs are examples of these new developments. We will discuss their effect on TDRM in the following section.

5.1 Pre-exposure prophylaxis

Pre-exposure prophylaxis (PrEP) is used in high-risk HIV-seronegative populations to prevent HIV infection. The PrEP strategies evaluated for efficacy all consist of the NRTI tenofovir alone or in combination with the NRTI emtricitabine [43]. The use of PrEP in MSM led to a reduction in the incidence of HIV of 73% with high and 21% with low drug adherence [44]. In the patients using PrEP who became with HIV, no resistance was detected to tenofovir or emtricitabine. However, of the 10 subjects who were infected at enrollment, 3 had emtricitabine-resistant infections (2 of 2 in

PrEP group and 1 of 8 in placebo group) [44]. This indicates that testing at the time of initiation of PrEP is very important to limit the development of drug resistance mutations. Fortunately, TDRM to tenofovir and emtricitabine is only found in few patients as shown in this thesis. Circulating TDRM will therefore only have a limited impact on the effectiveness of PrEP. However, since TDRM patterns can change over time, it is important to keep monitoring this in order to change PrEP drugs accordingly.

5.2 Post-exposure prophylaxis

Post-exposure prophylaxis (PEP) is used to prevent HIV infection after sexual or occupational exposure [45]. For PEP, the European guidelines recommend regimens including two NRTIs (tenofovir/emtricitabine or zidovudine/lamivudine) and a ritonavir-boosted PI (lopinavir/r or saquinavir/r). PEP should be started ideally <4 hours after the exposure, and no later than 48 hours for a duration of four weeks [46]. Again, these drugs would not encounter problems related to TDRM, as the prevalence of TDRM to these NRTIs and boosted PIs are not often found in this review. The exception is zidovudine, to which the circulating viruses in approximately half of the patients infected with a transmitted drug resistance virus, are resistant to. Resistance was reported in one patient using PEP who was RNA-positive but antibody-negative. This patient initiated PEP 14 hours after exposure and underwent baseline laboratory evaluation 3 days later [47]. PEP is not likely to cause a rise in TDRM as PEP is prescribed as a combination of antiretrovirals and is not often assessed.

5.3 Microbicides

Microbicides are products that can be applied to vaginal or rectal mucosa and thus prevent or reduce the transmission of sexually transmitted infections including HIV-1. So far, no study reported efficacy of microbicides use for a rectal application. To date only the CAPRISA study found that use of a vaginal tenofovir gel can reduce new infections by approximately 40% [48]. Resistance to tenofovir is rare. TDRM will therefore only have limited impact on the effectiveness of microbicides.

5.4 Test and treat

'Test and treat' is a prevention strategy in which universal testing for HIV is combined with immediate antiretroviral therapy for those individuals being HIV infected [49]. The 'test and treat' strategy has so far mainly been evaluated in modelling studies which predominantly predicted that the strategy can prevent new infections [50]. The most recent HPTN 052 study in serodiscordant couples showed a 96% reduction in HIV transmissions in patients with CD4 count between 350 and 550 cells/mm^3 which started antiretroviral therapy immediately compared to patients who delayed start of therapy to a CD4 cell count of <250 cells/mm^3 [51]. The strategy could lead to an increase in the number of patients being infected with a resistant virus as more patients will receive treatment. If this strategy would be implemented in Europe, the prevalence of TDRM could increase as 50% of the patients newly diagnosed in Europe have a CD4 cell count below 350 copies/mm^3 as indicated by Chapter 4. With the 'test and treat' strategy, patients would be diagnosed and treated in a much earlier stage of their disease than currently is achieved. As more HIV infected patients would

go on treatment, more resistance can be acquired, and the probability of transmitting a resistant virus would increase. This increase could be relatively small as current first-line therapy are more potent, have fewer side effects, and have to be taken less frequently to improve adherence and maintain viral suppression at lower levels of adherence [52] which decreases the risk of drug resistance [53]. Furthermore, new HIV infections can for a large part be explained by the transmission through recently infected patients [54-58]. As recent infections are hard to be identified, the 'test and treat' strategy may be difficult to realize.

5.5 New drugs

Currently, only few HIV drugs are in development. Since these new drugs, after FDA approval will be expensive and often used in highly treated patients only, we do not expect a large change in TDRM prevalences as a consequence of this. In the trials that have been performed recently, resistance to enfuvirtide, maraviroc, elvitegravir/cobicistat, and dolutegravir is seen in only few patients [54, 58-60]. Therefore, we do no expect an increase of resistance prevalences due to these new antiretrovirals.

6 Implications of TDRM

In this thesis we found that half of patients newly diagnosed in Europe have a CD4 cell count below 350 copies/mm^3. Early treatment is important as it has shown to reduce the risk for opportunistic infections and mortality [61-63] and the reduction of transmission of HIV [51, 64-68]. We therefore think substantial effort should be taken to reduce the proportion of patients being diagnosed with HIV in a late stage of their disease.

In this thesis TDRM prevalence remains below 10% in Europe of which most mutations cause resistance to thymidine analogues. These TAMs do not cause resistance to the drugs given in current first-line regimens. Additionally, we demonstrated that baseline genotypic testing would only become cost-effective if it would lead to a 20% of absolute reduction in the probability of failing treatment. Therefore, the use of standard baseline genotypic testing becomes disputable. This is especially true for patients with a CD4 count of >200 cells/mm^3, where failure would not directly lead to a prolonged time of having a high risk of opportunistic infections and mortality.

As a first step in reducing the cost of a baseline genotypic test we would suggest to implement a targeting strategy by performing baseline genotypic testing only in patients with a CD4 cell count <200 cells/mm^3. These patients have an increased risk of developing opportunistic infections and mortality [61-63] and baseline genotypic testing showed to be more cost-effective in these patients compared with patients with a CD4 cell count >200 cells/mm^3. In these late presenters it is more important to prevent ineffective treatment due to TDRM, since virological failure will increase the change of developing opportunistic infections as CD4 cell counts will decline further [69].

Another possibility to reduce costs of baseline genotypic testing would be to limit the population-based nucleotide sequencing to only the reverse transcriptase gene of the virus. Because we have shown low PI TDRM levels on all continents

and no increase over time, the sequencing of the protease gene of the virus seems rather unnecessary. We showed that baseline resistance testing only in reverse transcriptase would lower the cost-effectiveness by 50%. Therefore, without sequencing the protease gene, the population sequencing test cost would become half of the current sequencing cost, which could save sufficient money.

A good alternative for population sequencing is to perform NNRTI minority assays. Population sequencing fails to identify drug-resistant minority variants that are present in <20% of the virus population infecting a patient [28-29]. These minority variants have been detected in almost 14% of antiretroviral naïve HIV-infected individuals [70]. The presence of minorities, particularly involving NNRTI resistance, is associated with an increased risk of virological failure to first-line therapy [70]. If minority assays would be implemented as standard practice, the costs may lower considerably. With low costs and the provision of valuable information on minority NNRTI mutations, this test could improve health care in HIV patients.

However, when recommending different methods of resistance testing, we should take into account the drawback of limiting baseline resistance testing to a targeted population or to reverse transcriptase only. Baseline genotypic testing provides a large pool of data which can be used for surveillance purposes. If baseline genotypic testing is no longer standard practice, we might have a great loss of insight into the epidemiology of TDRM.

7. Overall conclusions

In this thesis TDRM prevalence remains below 10% in Europe of which most mutations cause resistance to thymidine analogues. These TAMs do not cause resistance to the drugs given in current first-line regimens. Importantly, a rise in TDRM to NNRTIs was found in Europe over time. We have also demonstrated that baseline genotypic testing would only become cost-effective if it would lead to a 20% of absolute reduction in the probability of failing treatment. We therefore could consider performing genotypic resistance testing in a targeted population or to reverse transcriptase only.

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Summaries

Summary

In 2009, approximately 33.3 (31.4 -35.3) million individuals were infected with human immunodeficiency virus (HIV) worldwide. Although the virus continues to spread, the number of new infections has fallen from an estimated 3.2 (3.0 -3.5) million in 1997 to 2.6 (2.3 – 2.8) million in 2009. One explanation for the decrease in the number of new HIV infections is the use of antiretrovirals for which the accessibility has increased largely over time.

The HIV virus is characterized by its high genetic diversity. In the swarm of genetic viral variants in a single infected patient, the virus variant showing the highest fitness will outgrow other variants and become the dominant viral population. In treated patients, this mechanism also can lead to the outgrowth of viral variants harbouring drug resistance associated mutations. These resistant viruses can be transmitted to other individuals. Patients infected with a drug resistance virus will have an increased risk for virological failure when starting therapy. This may decrease both the magnitude and the duration of the response to treatment. Therefore surveillance of transmitted drug resistance mutations (TDRM) is necessary.

In the first part of this thesis, we focus on the epidemiology of TDRM. We start with reviewing the literature (chapter 2) on the prevalence of TDRM to determine the prevalence and time trends in the different regions across the world. This review included 215 studies and a total of 43,170 HIV-infected antiretroviral naïve patients. The highest prevalence of TDRM was found in North America (12.9%) and Europe (10.9%). Resistance could, for the largest part, be ascribed to TDRM in the nucleos(t)ide reverse transcriptase inhibitor (NRTI) drug class (in >55% in all continents).

In the WATCH study we collected and analyzed data of available studies on TDRM from across the world to examine the mutational patterns between different continents (chapter 3). The NRTI drug class showed to have the highest TDRM prevalence in all continents. The most frequently occurring NRTI mutations were the thymidine analogue mutations (TAMs) that are associated with resistance to zidovudine and stavudine. The prevalence of these TAMs was higher in North America (7.2%) and Europe (5.8%) than in other continents (≤2.4%).

In chapter 4, 5, and 6 we examined the TDRM prevalence in Europe through the SPREAD programme. This programme included patients using a pre-defined sampling strategy based on the geographical and risk group distributions of patients newly diagnosed with HIV in the participating counties. The SPREAD programme started in September 2002 and now includes data until December 2007 enrolling 4,317 patients from 27 countries. One out of nine patients showed signs of TDRM but in most cases it concerned only a single TDRM. Most mutations found were associated with NRTI resistance at 5.0% of which 84.4% were TAMs. The prevalence of TDRM was stable over time. The underlying prevalence of TDRM associated with particular antiretroviral drug classes, however, showed important changes over time. We found a significant increase in the prevalence of non-nucleoside reverse transcriptase inhibitor (NNRTI) TDRM, doubling from 2.1% in 2002 to 4.1% in 2007. In contrast, transmitted protease inhibitor (PI) resistance decreased significantly from 3.9% to 1.6%. These changes can be explained by the use of different drugs

over time.

Differences in TDRM prevalence were seen between the HIV transmission groups. The largest difference was found in NRTI resistance, where the prevalence was significantly higher in men who have sex with men (MSM) (6.6%) compared to heterosexual patients (3.3%) and injection drug users (2.0%). As heterosexual patients are often originating from Africa, the TDRM prevalence for the different drug classes in these patients follow that of studies performed in Africa. In MSM we see prevalences and time trends that could be explained by the history of drug use of the different antiretroviral drug classes in the western countries.

In chapter 6 we examined the impact of travel on the transmission of HIV. Phylogenetic analyses showed that one third of newly diagnosed individuals were part of a cluster. These patients were more frequently harbouring a TDRM (10.4%) as compared to non-clustering patients (8.3%). The vast majority of patients were part of a cluster that consisted only of patients originating from the same country. This suggests that a large part of the spread of HIV-1 in Europe can be explained by transmission of infections taking place between patients within the same country. As travel is not of major importance in the transmission of HIV, public health should not make a large effort to focus on this aspect of HIV transmissions.

In part two of this thesis we focus on the interpretation of acquired- and transmitted- drug resistance mutations. In chapter 7, we examined the interpretation systems that are developed to guide the new treatment choice for patients failing their current HAART. We have shown that the three most common interpretation systems, ANRS, Stanford HIVdb and Rega did not differ in predicting virological outcomes at all time points (12, 24, and 48 weeks).

The interpretation of TDRM can be complicated by natural occurring polymorphisms, which could result in an overestimation of TDRM prevalence (chapter 8). We found an inverse relationship between the likelihood of correctly estimating the prevalence of TDRM and the presence of polymorphisms: the higher the prevalence of polymorphisms, the lower the likelihood of correctly estimating the prevalence of TDRM. Furthermore, a small sample size can decrease the positive predictive value of TDRM even more. Therefore, both the presence of polymorphisms and the number of samples analyzed affects the accuracy of the classification method recommended by the WHO and may lead to an incorrect categorization of TDRM prevalence. This is especially relevant in resource-poor settings, where WHO recommends that TDRM prevalences greater than 5% should trigger several actions, such as performing extra research and more frequent surveillance studies. These findings suggest excluding some TDRM from the consensus list of mutations in order to prevent the overestimation of TDRM prevalence.

Besides overestimating TDRM prevalence, the prevalence of TDRM can also be underestimated by the presence of minority variants. These minority variants cannot be detected by population sequencing, which was the technique used in all the studies in this thesis. These minority mutations are associated with virological failure, particular involving NNRTI resistance. This finding supports the recommendation of using NNRTI minority assays in routine testing of TDRM.

The high prevalence of TAMs will only have a small clinical relevance as they generally do not cause resistance to nucleos(t)ides currently popular in first-line regimens. We have shown in part 3 of this thesis that with current TDRM prevalence

in the Netherlands baseline genotypic testing is not cost-effective (chapter 9). The magnitude of this cost-effectiveness ratio decreased with increasing the reduction in failure rate (€1.2 million, €220,000, and €94,000 per QALY gained for absolute failure rate differences of 1, 5, and 10%, respectively). The cost-effectiveness did not decrease to reasonable values unless the absolute reduction rate difference in patients with- and without baseline genotypic testing exceeded 20% (€30,000 per QALY gained). Nonetheless, targeting baseline genotyping only to patients with a low CD4 cell count lowered the cost-effectiveness to €65,000 per QALY gained at an absolute reduction in failure rate of 5%. This ratio was much higher in patients with a CD4 cell count of above 200 cells/mm^3 (€330,000 per QALY gained). Therefore, a possibility to reduce costs of baseline genotypic testing would be to implement a targeting strategy by performing baseline genotypic testing only in patients with a CD4 cell count <200 cells/mm^3.

Also the prevalence of PI TDRM was low in all continents. Therefore, this will not have a great impact on the efficacy of first-line therapy. These findings suggest to limit the population-based nucleotide sequencing to only the reverse transcriptase gene of the virus. We have shown that the cost-effectiveness ratio decreased by 50% when baseline genotypic testing was limited to reverse transcriptase only.

In conclusion, we have shown that TDRM prevalence remain relatively low in all continents. Most mutations cause resistance to thymidine analogues. These TAMs do not, however, cause resistance to the drugs given in current first-line regimens. Most importantly, we found a significant doubling in the prevalence of transmitted NNRTI resistance in Europe over time. We also demonstrated that baseline genotypic testing would only become cost-effective if it would lead to a 20% absolute reduction in the probability of failing treatment. We therefore could consider performing genotypic resistance testing in a targeted population or only genotyping the reverse transcriptase.

Nederlandse samenvatting

Naar schatting waren er in 2009 33,3 (31,4-35,3) miljoen mensen wereldwijd geïnfecteerd met HIV. Hoewel het virus zich blijft verspreiden, is het aantal nieuwe infecties gedaald van ongeveer 3,2 (3,0-3,5) miljoen in 1997 naar 2,6 (2,3-2,8) miljoen in 2009. Een mogelijke verklaring van deze daling is het toenemend gebruik van HIV-remmers.

Het HIV virus heeft een hoge genetische verscheidenheid. Hierdoor is een patiënt besmet met een zwerm van genetisch verschillende virus varianten. De virussen die zich het beste repliceren zullen de dominante virus populatie worden. Een HIV-remmer is een geneesmiddel dat de productie van het virus kan afremmen. HIV medicatie wordt altijd in combinatie gebruikt (highly active antriterovral terapy of HAART) om resistentie te voorkomen. De bekende HIV-remmers zijn ingedeeld bij de nucleoside reverse transcriptaseremmers (NRTIs), de non-nucleoside reverse transcriptaseremmers (NNRTIs), en de proteaseremmers (PIs). In patiënten die behandeld worden met HIV-remmers kan in bepaalde omstandigheden nog steeds resistentie ontstaan. Bijvoorbeeld als patiënten niet altijd even trouw hun HIV-remmers slikken. In dat geval kan het virus zich repliceren in aanwezigheid van de geneesmiddelen en daardoor snel varianten selecteren die resistent zijn tegen de cocktail van HIV-remmers die de patiënt gebruikt. Deze resistente virussen kunnen worden overgebracht naar andere (dit noemen virologen transmissie van resistentie). In patiënten die geïnfecteerd zijn geraakt met een resistent virus is er een verhoogd risico dat het virus onvoldoende wordt onderdrukt en virus deeltjes meetbaar blijven (dit heet virologisch falen). Daarom is het belangrijk om in kaart te brengen hoe vaak transmissie van geneesmiddelen resistentie gerelateerde mutaties optreedt.

In het eerste deel van dit proefschrift ligt de focus op het inzicht verkrijgen van de verspreiding van resistente virussen. Hoofdstuk 2 geeft een overzicht van de beschikbare literatuur op het gebied van transmissie van resistent HIV in de verschillende delen van de wereld. Dit overzicht bevat 215 studies en 43.170 onbehandelde HIV-geïnfecteerde patiënten. In Noord-Amerika (12.9%) en Europa (10.9%) werd de hoogste prevalentie van transmissie van resistente HIV-stammen gevonden. Het merendeel van de resistentie kan worden toegeschreven aan resistentie tegen NRTIs (in >55% in alle continenten).

Vervolgens hebben we in de WATCH studie beschikbare data verzameld en geanalyseerd van studies naar transmissie van resistent HIV vanuit de hele wereld. Hierdoor was het mogelijk om de mutatiepatronen tussen verschillende continenten te onderzoeken (hoofdstuk 3). Resistentie tegen de NRTI klasse liet de hoogste prevalentie zien in alle continenten. De meest voorkomende NRTI mutaties waren de thymidine-analoog geassocieerde mutaties (TAMs). Deze TAMs werden dikwijls gevonden in Noord-Amerika (7,2%) en Europa (5,8%) en minder frequent in andere continenten (≤2,4%). Deze mutaties zijn geassocieerd zijn met resistentie tegen zidovudine en stavudine die beide niet meer worden gebruikt in de huidige HIV-therapie.

In hoofdstuk 4, 5, en 6 hebben we onderzoek gedaan naar de prevalentie van resistentie mutaties in Europa door middel van het SPREAD-project. Dit project

includeerde nieuw gediagnosticeerde patiënten die representatief zijn voor de HIV epidemie uit de deelnemende landen in Europa. Het SPREAD-project is opgestart in september 2002 en includeert op dit moment data tot december 2007, waarbij 4.317 patiënten uit 27 landen zijn ingeschreven. Eén op de negen patiënten vertoont tekenen van een resistent virus. De meeste mutaties waren geassocieerd met NRTI resistentie in 5,0%, waarvan 84,4% TAMs waren. De prevalentie van transmissie van resistent HIV liet een stabiele trend over de tijd zien. Echter, veranderingen over tijd waren wel waargenomen in de onderliggende prevalenties van resistentie tegen de individuele klasses van HIV-remmers. De prevalentie van NNRTI resistentie verdubbelde van 2,1% in 2002 naar 4,1% in 2007. Echter, de PI resistentie daalde van 3,9% naar 1,6%. Een statistische analyse toonde aan dat deze toe- en afname niet op toeval berustte. Deze veranderingen kunnen worden verklaard door de verandering van het gebruik van HIV-remmers over de tijd.

Verschillen tussen de prevalentie van transmissie van resistente virussen werden ook aangetoond tussen de HIV transmissie groepen. Het grootste verschil werd gevonden voor NRTI resistentie, waar de prevalentie hoger was in mannen-die-seks-hebben-met-mannen (MSM) (6,6%) in vergelijking tot heteroseksuele patiënten (3,3%) en injecterende drugs gebruikers (2,0%). Omdat heteroseksuele patiënten vaak een Afrikaanse afkomst hebben, volgt de resistentie prevalenties in de verschillende HIV-remmers klasses in deze patiënten dat van studies uitgevoerd in Afrika. In MSM zien we een prevalentie en trends over de tijd die kunnen worden verklaard door de geschiedenis van het medicijn gebruik in de verschillende klasses van HIV-remmers in de westerse landen.

In hoofdstuk 6 behandelen we de impact van reizen op de transmissie van HIV. Met fylogenetische analyses (het uitwerken van het virus in een stamboom) tonen we hier aan dat één derde van de nieuw gediagnosticeerde patiënten deel uitmaakten van een cluster (zeer dichte takken op de stamboom). Deze patiënten waren vaker geïnfecteerd met een resistent virus dan patiënten die niet clusterden (8,3%). Het merendeel van de patiënten maakte deel uit van een cluster die alleen bestond uit patiënten uit hetzelfde land afkomstig. Dit suggereert dat een groot deel van de verspreiding van HIV in Europa kan worden verklaard door de transmissie van infecties die plaats vindt tussen patiënten afkomstig van hetzelfde land. Omdat reizen niet van groot belang blijkt te zijn in de transmissie van HIV, is het niet nodig om veel aandacht te geven aan dit aspect van de HIV transmissie vanuit de volksgezondheid.

In het tweede deel van dit proefschrift bestuderen we de interpretatie van mutaties die verworven zijn door therapiefalen of transmissie. In hoofdstuk 7 vergelijken we de interpretatiesystemen die zijn ontwikkeld om de keuze van nieuwe therapie in patiënten die falen op hun huidige HAART te begeleiden. We hebben kunnen aantonen dat de drie meest gebruikte interpretatiesystemen – ANRS, Stanford HIVdb en Rega – niet verschillen in het voorspellen van de positieve resultaten van therapie op alle tijdspunten (week 12, 24 en 48).

De interpretatie van transmissie van resistent HIV kan gecompliceerd worden door natuurlijk voorkomende mutaties, wat kan leiden tot een overschatting van de resistentie prevalentie (hoofdstuk 8). We vonden een tegenovergestelde relatie tussen de kans op het correct berekenen van de prevalentie van transmissie van resistentie en de aanwezigheid van natuurlijk voorkomende mutaties: hoe

hoger de prevalentie van de natuurlijk voorkomende mutaties, hoe lager de kans op het correct berekenen van de resistentie prevalentie. Bovendien kan een kleine steekproefgrootte de positieve voorspellende waarde (het deel van de patiënten met een positieve testuitslag dat ook daadwerkelijk met een resistent virus is besmet) zelfs meer verlagen. Daarom zal de aanwezigheid van de natuurlijk voorkomende mutaties en het aantal patiënten dat geanalyseerd wordt effect hebben op de nauwkeurigheid van de classificatiemethode aanbevolen door de wereldgezondheid organisatie (WHO- World Health Organisation) wat kan leiden tot een incorrecte categorisatie van de prevalentie van transmissie van resistentie. Dit is in het bijzonder relevant in ontwikkelingslanden, waar de WHO aanbevelingen doet voor prevalenties hoger dan 5% wat dient te leiden tot verschillende acties zoals het uitvoeren van extra onderzoek en meer frequent surveillance studies. Deze bevindingen suggereren om sommige resistentie mutaties van de consensus mutatielijst te verwijderen, om zo een overschatting van de prevalentie van transmissie van resistentie te voorkomen.

Naast een overschatting van de transmissie van resistente virussen, kan de prevalentie ook worden onderschat, namelijk door de aanwezigheid van minderheid varianten. Deze minderheid varianten kunnen niet worden gedetecteerd met populatie resistentiebepaling, een techniek die in alle studies van dit proefschrift is gebruikt. Deze minderheid varianten zijn geassocieerd met het falen van therapie, in het bijzonder bij de NNRTI resistentie. Dit steunt de aanbeveling voor het gebruik van NNRTI minderheid testen als routine test bij resistentiebepalingen.

De hoge prevalentie van TAMs heeft weinig klinische relevantie aangezien deze mutaties over het algemeen geen resistentie veroorzaken tegen NRTIs die op dit moment populair zijn in eerstelijns combinatietherapieën. In het derde deel van dit proefschrift laten we zien dat met de huidige prevalentie van transmissie van resistent HIV in Nederland, een resistentietest van het HIV virus bij nieuw gediagnosticeerde patiënten niet kosteneffectief is (hoofdstuk 9). De kosteneffectiviteit van de resistentietest was berekend als de kosten die nodig zijn als door de test de levensverwachting wordt verlengd met één jaar in goede gezondheid. De grootte van de kosteneffectiviteitratio nam af wanneer een resistentiebepaling meer falen op therapie deed voorkomen (€1,2 miljoen, €220.000, en €94.000 per verkregen levensjaar in goede gezondheid wanneer het falingspercentage was afgenomen met 1, 5, en 10%, respectievelijk). De kosteneffectiviteit nam niet af naar redelijke waardes tenzij het verschil in falingspercentages tussen patiënten met- en zonder baseline resistentiebepaling de 20% overschreed (€30.000 per verkregen levensjaar in goede gezondheid). Desalniettemin, een baseline resistentietest in patiënten met een laag CD4 cel waarde (weinig afweercellen) verlaagde de kosteneffectiviteit naar €65.000 per verkregen levensjaar in goede gezondheid bij een daling van het falingspercentage van 5%. Deze ratio was veel hoger in patiënten met een hoge CD4 cel waarde (€330.000 per verkregen levensjaar in goede gezondheid). Het is daarom een goede mogelijkheid om de kosten van baseline resistentietesten te verlagen door middel van een gerichte strategie waarbij een baseline resistentietest alleen wordt uitgevoerd in patiënten met een lage CD4 cel waarde.

We hebben verder aangetoond dat de prevalentie van de transmissie van PI resistentie laag was in alle continenten. Dit zal daarom weinig impact hebben op de effectiviteit van eerstelijns combinatietherapieën. Deze bevindingen suggereren het beperken van de resistentiebepaling tot alleen het reverse transcriptase gen van het

virus. We hebben laten zien dat de kosteneffectiviteitratio af nam met 50% wanneer dit geïmplementeerd zou worden.

Als conclusie kunnen we stellen dat de prevalentie van resistent HIV relatief laag blijft in alle continenten. De meeste voorkomende mutaties die we vinden zijn de TAMs. Deze TAMs geven geen resistentie tegen de medicijnen die worden voorgeschreven in de huidige eerstelijns combinatietherapieën. Uit onze resultaten blijkt ook dat de prevalentie van resistentie tegen NNRTI over de tijd verdubbeld is in Europa. Ook laten we zien dat baseline genotypering alleen dan kosteneffectief is wanneer het zou leiden tot een afname van het falingspercentage van 20%. Daarom suggereren we tot slot dat de kosten van resistentiebepalingen zouden kunnen worden verlaagd door het alleen uit te voeren in een specifieke groep van patiënten of door het bepalen van de resistentie in alleen het reverse transcriptase gen.

About the author

Curriculum Vitae

Dineke Frentz was born on May 7th 1985 in Apeldoorn, the Netherlands. In 2003 she finished high school at the Jacobus Fruytier School in Apeldoorn and started her study in Biomedical Sciences at the Utrecht University. During her bachelor internship her research focussed on the effect of air pollution on birth weight at the Institute for Risk Assessment Sciences (IRAS) in Utrecht, which is an interdepartmental research institute within the faculties Veterinary Medicine and Medicine Sciences of Utrecht University. During the Master Epidemiology, she specialized further into epidemiology of infectious diseases. She did a research project on transmission of HIV drug resistance at the department of Virology, Medical Microbiology at the University Medical Centre in Utrecht. In 2008, she started as a PhD student at the department of Virology in the Erasmus Medical Centre in Rotterdam under supervision of Professor Charles Boucher and Dr. David van de Vijver. This project, focussing on the epidemiology of HIV-1 transmitted drug resistance, has resulted in the present thesis.

Publications

Frentz D, Boucher CAB, van de Vijver DAMC.
Temporal Changes in the Epidemiology of Transmission of Drug-Resistant HIV-1 across the World.
AIDS Reviews 2012,14:17-27.

Frentz D, van de Vijver DAMC, Boucher CAB, Albert J.
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Journal of Acquired Immune Deficiency Syndromes 2011,58:e135-137.

Frentz D, Boucher CAB, Assel M, De Luca A, Fabbiani M, Incardona F, Libin P, Manca N, Müller V, Ó Nualláin B, Paredes R, Prosperi M, Quiros-Roldan E., Ruiz L, Sloot PMA, Torti C, Vandamme AM, Van Laethem K, Zazzi M, van de Vijver DAMC.
Comparison of HIV-1 genotypic resistance test interpretation systems in predicting virological outcomes over time.
PLoS One 2010,5:e11505.

Assel M, van de Vijver D, Libin P, Theys K, Harezlak D, O Nualláin B, Nowakowski P, Bubak M, Vandamme AM, Imbrechts S, Sangeda R, Jiang T, **Frentz D**, Sloot P.
A collaborative environment allowing clinical investigations on integrated biomedical databases.
Studies in health technology and informatics 2009, 147:51-61.

Phd Portfolio

Name PhD student:	Dineke Frentz
Erasmus MC Department:	Virology
Research School:	Post-graduate Molecular Medicine
PhD period:	September 2008 - September 2012
Promotor(s):	Prof. dr. C.A.B. Boucher
Copromotor:	Dr. D.A.M.C van de Vijver

In-depth courses

2011	Course in Epidemiology. Three-week course in Modern Statistical Methods provided by the Netherlands Institute for Health Sciences, Rotterdam, the Netherlands.
2010	Course in modeling. Two-week course in Epidemiology and Control of Infectious Diseases provided by the Imperial College London, England.
2010	Course in Virology. One-week international training course in General Virology provided by the Post-graduate School Molecular Medicine and the Department of Virology, Erasmus Medical Center, Rotterdam, the Netherlands.
2009	Course in phylogeny. The 15th International BioInformatics Workshop on Virus Evolution and Molecular Epidemiology provided by the Erasmus Post-graduate School Molecular Medicine, Rotterdam, the Netherlands and the Katholieke Universiteit Leuven, Belgium.
2008-present	Internal and external presentations at the department of Virology twice a week.
2008-present	HIV Journal club presentations at the department of virology once every three months.

General courses

2010	Writing Scientific English (short), provided by the Post-graduate School Molecular Medicine, Rotterdam, the Netherlands.
2011	Professional presenting (in Dutch), provided by the Netherlands School of Public & Occupational Health, Amsterdam, the Netherlands.

National and international collaborations

- Prof. Jan Albert, Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, and Department of Clinical Microbiology, Karolinska University Hospital, Stockholm, Sweden. Collaboration on HIV natural sequence polymorphisms.
- SPREAD clinicians, virologists and epidemiologists from 26 European countries. Collaboration of HIV transmitted drug resistance within the European Society for Antiviral Resistance.
- Prof. Peter Sloot, Department of Computational Science, University of Amsterdam, Amsterdam, the Netherlands. Collaboration on the development

of a virtual laboratory for infectious diseases and new paradigm of computing through Dynamically Changing Complex Networks reproducing the way nature processes information within the European projects Virolab and DynaNets.
- Dr. Eric van Gorp, dr. Bart Rijnders and dr. Ineke van der Ende, Department of Internal Medicine, Erasmus Medical Center, Rotterdam, the Netherlands. Collaboration on modelling cost-effectiveness of HIV baseline genotyping.

Miscellaneous
- Member of the European Society Antiretroviral Resistance (ESAR).
- Supervision of MSc student during the course of my PhD project.

Presentations
- 5th Netherlands Conference on HIV Pathogenesis, Prevention and Treatment, Koninklijk Insituut voor de Tropen, Amsterdam, the Netherlands (Oral presentation) (2011).
- 9th European Workshop on HIV & Hepatitis, Coral Beach Hotel, Paphos, Cyprus (Oral presentation) (2011).
- 8th European HIV Drug Resistance Workshop, Hilton Sorrento Palace Hotel, Sorrento, Italy (Oral presentation) (2010).
- 7th European HIV Drug Resistance Workshop, Norra Latin Building, Stockholm, Sweden (Oral presentation) (2009).

Attended
- 19th Conference on Retroviruses and Opportunistic Infections, Washington State Convention Center, Seattle, United States (2012).
- 10th European Workshop on HIV & Hepatitis, Fira Palace Hotel, Barcelona, Spain (2012).
- 1st Infection Dynamics Symposium, Railway Museum, Utrecht, the Netherlands (2011).
- 14th Nationaal Congres SOA HIV Aids, RAI Congress centre, Amsterdam, the Netherlands (2010).
- 13th Nationaal Congres SOA HIV Aids, RAI Congress centre, Amsterdam, the Netherlands (2009).

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