The Epidemiology of Transmission of Drug Resistant HIV-1

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1. Introduction

The use of highly active antiretroviral therapy has dramatically reduced morbidity and mortality among patients infected with HIV-1 (1,2). But the success of antiretroviral treatment is frequently limited by the emergence of HIV drug resistance (3-5). Importantly, drug resistant viruses can be transmitted to newly infected individuals (6-8). Transmission of drug resistant HIV is a major public health concern, as it could lead to a situation in which no effective drugs are available for the treatment of HIV.

A large number of epidemiological studies have addressed the important issue of transmission of drug resistant HIV. Unfortunately, the results of different studies are difficult to compare because of substantial dissimilarities in assay methodology, definitions used to classify drug resistance, time period in which the data were collected, and the population under study. Despite these differences, some conclusions can be made about the epidemiology and the impact of transmitted drug resistance.

This review summarizes the most important findings of epidemiological studies on transmission of drug resistant HIV. This review was limited to studies published in peer-reviewed journals in the last five years (2002-2006). To allow for comparison, we only considered studies that included the proportion of patients infected with drug resistant HIV and that reported the occurrence of transmitted resistance to a particular class of antiretrovirals. (The fusion-inhibitor enfuvirtide was not considered, as the epidemiological studies did not include the gp41 region where resistance to this drug emerges). Topics that will be discussed include the proportion of transmission of resistant strains in different parts of the world, trends over time, risk factors for acquiring drug resistant HIV, and the impact of transmitted resistance on future therapy. Special emphasis will be given to the methodological dissimilarities between the studies and the potential impact of these factors on the reported proportions of transmission of drug resistant HIV.

2. Methodological differences between epidemiological studies

2.1 Sampling strategy

A significant cause of dissimilarity among studies is whether the individuals sampled had a recent infection, were antiretroviral naïve, or were newly diagnosed patients (Table 1a).

Sampling limited to recently infected patients

Limiting the inclusion to recently infected patients has a substantial epidemiological benefit. Epidemiology defines the occurrence of disease as incident (new cases of disease during a specified period of time) or prevalent (number of diseased individuals at a particular point in time) (9). Incident patients acquired the virus recently, whereas antiretroviral naïve individuals who are chronically infected are defined as prevalent. In recently infected patients, the moment of infection can be estimated. Conversely, prevalent patients are a heterogeneous mixture of individuals who were recently infected and those who acquired the virus many years before but who did not yet receive treatment.

Tables 2a and 2b show an overview of, respectively, studies that limited the sampling to patients who recently acquired HIV (Table 2a) or studies that included antiretroviral naïve patients (Table 2b). The latter group consists of both recently and chronically infected individuals. A first remarkable observation is that the studies among recently infected individuals (Table 2a) used differential inclusion criteria as evidence for seroconversion in the recent past, ranging from 6 to 36 months. But of greater importance is the substantial dissimilarity in risk group distribution between studies including either

Table 1a Methodological differences in sampling strategies used in epidemiological studies

Sampling strategy	Strengths	Weaknesses
Recently infected patients	• Potential reversion is limited. • Duration of infection can be estimated. • Trends over time can be established.	• Recently infected patients are difficult to identify. • Some risk groups could be overrepresented, which limits the generalizibility of the results.
Antiretroviral naïve patients	• The largest possible number of patients can be identified. • Comparison between recently and chronically infected patients can be made. • Patients reflect clinical practice, as they are under consideration for therapy.	• Patients are more heterogeneous, as they are a mixture of individuals recently infected and those who acquired the virus many years before but who did not yet receive treatment.
Newly diagnosed patients	• Large number of patients can be identified. • Frequently the earliest sample that is available is drawn at the time of diagnosis. Compared to antiretroviral naïve patients, reversion is therefore minimized. • Local strategies can be made that allow the identification of a sample representative for the risk group distribution and geographical distribution in a particular country by using surveillance systems already in place in many countries. • Comparison between recently and chronically infected patients can be made.	• Representative sampling could depend on quality of national surveillance system. • Patients may have previously been diagnosed elsewhere.

Table 1b Methodological differences in resistance testing technologies used in epidemiological studies

Technology of testing	Strengths	Weaknesses
Genotypic assays	• Most frequently used method for classifying resistance. • Application of genotypic assays to clinical practice has shown to be beneficial in randomized clinical trials. • Less expensive and quicker. • Gives insight into evolution of resistance by detection of revertants.	• Population sequencing does not detect minor virus populations. • No consensus about amino acid substitutions that should be used to classify genotypic resistance. • Validation for particular subtypes could be limited.
Phenotypic assays	• Results are easier to interpret.	• Minor virus populations are not detected. • No consensus about fold changes in IC_{50} that are relevant for resistance to particular drugs. • More expensive and time consuming.

Table 2a Summary of studies using genotypic assays to define drug resistance among recently infected patients

Ref.	Region	Method[a]	Years of sampling	Duration infection (months)	Nr[b]	HIV risk factor (%)[c]			Incident (%)[d]					Risk factor
						MSM	IDU	HSX	any	NRTI	NNRTI	PI	MDR	
	Europe													
(61)	Europe, Canada	IAS (53)	1987–2003	<36 m	438	75	10	12	10.2	6.4	3.2	2.3	1.1	
(62)	Amsterdam, Netherlands	IAS (48)	1994–2002	<18 m	100	61	27	3	13.0	10.0	2.0	1.0	0	
(63)	France	French guidelines (64)	1996–1999	<6 m	204	60	3	34	8.8	7.4	1.0	3.9	0.5	Originating from Europe
(65)	Spain	IAS (38)	1997–2002	< 12 m	198	70	20	10	12.1	9.6	4.0	2.0	3.5	
(66)	France	IAS (47)	1999–2000	<6 m	249	57	2	32	10.4	7.6	4.0	5.6	4.8	
(67)	London, UK	IAS (38)	2000–2004	<6 m	140	91		9	6.4	2.1	4.3	1.4	1.4	
(68)	France	IAS (51)	2001–2002	<6 m	301	58	0	32	14.0	10.3	3.3	4.3	0.6	
	North America													
(57)	North America	IAS (50)	1995–2000	<12 m	301		23		12.3	10.9	3.5	3.3	5.7	
(69)	New York, USA	IAS (38)	1995–2004	<12 m	361	97[e]			18.8	13.0	8.0	5.0	5.8	
(58)	San Francisco, USA	IAS (46)	1996–2001	<12 m	225	86			23.1	16.0	8.0	5.8	6.2	
	Africa													
(70)	Abidjan, Côte d'Ivoire	Not reported	1997–2000	<36 m	99				0	0	0	0	0	
	South-America													
(71)	Argentina	IAS (38)	2004–2005	<9 m	52	45	2	52	7.7	1.9	5.8	0	0	

[a] Definition used for classifying HIV drug resistance. "IAS" means that the mutation-list defined by the IAS was used; the version of the list is given by the reference.

[b] Nr=number of patients included.

[c] The HIV risk factors were classified as MSM (men-having-sex-with-men), IDU (Intravenous Drug Users) and HSX (Heterosexual).

[d] Resistance was subdivided into particular classes of antiretrovirals. The column "Any" is the proportion of patients infected with a virus that contained at least one resistance-associated mutation. "MDR" is multi-drug-resistance or resistance to at least two classes of antiretrovirals.

[e] data from 2003–4 only.

Table 2b Summary of studies using genotypic assays to define drug resistance among antiretroviral naïve patients

Ref.	Region	Method[a]	Years of sampling	Nr[b]	HIV risk factor (%)[c]			Prevalent (%)[d]					Risk factor
					MSM	IDU	HSX	any	NRTI	NNRTI	PI	MDR	
	Africa												
(59)	Abidjan, Côte d'Ivoire	IAS (49)	unreported	20				0	0	0	0	0	
(60)	Nigeria	Not reported	unreported	18				0	0	0	0	0	
(88)	Yaounde, Cameroon	IAS, version not reported	2001–2002	102				7.8	2.9	2.0	2.9	0	
(89)	Lusaka, Zambia	IAS (49)	2000	28				0	0	0	0	0	
(90)	Abidjan, Côte d'Ivoire	IAS, version not reported	2001–2002	107	0	0	100	5.6	0.9	3.7	0.9	0	
(91)	DR Congo	Stanford HIV database	2002	70				4.3	0	1.4	2.9	0	
(88)	Ouagadougou/Bobo Dioulasso, Burkina Faso	IAS, version not reported	2003	97				8.3	2.1	4.1	2.1	0	
(92)	Nkwen/Kumbo, Cameroon[e]	IAS (38)	2004	54				13.0	3.7	5.6	7.4	3.7	
	Europe - West												
(17)	Europe, Israel	IAS (47)	1996–2002	2208	43	15	41	10.4	7.5	2.9	2.5	2.0	Infection <12 m, subtype B
(77)	United Kingdom	Stanford HIVdb algorithm	1996–2003	2357	63	17	2	14.2	9.9	4.5	4.6	3.3	Recent infection, ethnic group
(78)	Israel	IAS (51)	1999–2003	176	10	23	58	14.8	2.8	8.5	5.1	0.6	Infection in Israel
(79)	Sweden	IAS (50)	1998–2001	100	40	2	41	9.0	5.0	3.0	1.0	0	
(80)	Denmark	Not reported	2000	96				2.1	2.1	0	0	0	
(26)	Nordrhein-Westfalen, Germany	IAS (52)	2001–2003	269	48			11.2	8.6	4.1	1.5	1.5	subtype B virus, white ethnicity, shorter duration of infection
(81)	Galicia, Spain	Stanford HIV database	2000–2002	85	18	32	47	7.1	5.9	0	1.2	0	
(82)	London, UK	IAS (50)	1999–2001	72	0	0	100	6.9	1.4	2.8	4.2	2.8	
(83)	Greece	IAS (53)	2002–2003	101	55	3	23	8.9	5.0	4.0	0	0	
(68)	France	IAS (51)	2001–2002	363	31	8	51	9.1	7.2	1.7	1.9	1.7	
	Europe - East												
(94)	Former Soviet-Union	Stanford HIV database	1997–2004	278	3	84	12	16.6	5.4	7.6	4.0		
(93)	Tbilisi, Georgia	Stanford HIV database	1998/2003	48	2	65	27	4.2	4.2	0	0	0	
	Latin-America												
(95)	Porto Alegre, Brazil	Stanford HIV database	unreported	108				2.8	0.9	1.9	0	0	
(96)	Rio de Janeiro, Brazil	IAS (50)	1998	47				8.5	8.5	0	0	0	

Table 2b cont.

Ref.	Region	Method[a]	Years of sampling	Nr[b]	HIV risk factor (%)[c]			Prevalent (%)[d]					Risk factor
					MSM	IDU	HSX	any	NRTI	NNRTI	PI	MDR	
(97)	Brazil, Sao Paolo[f]	IAS (38)	1998–2002	341				6.2	3.5	0.9	1.2	0.6	Recent infection, non-B subtype
(98)	Brazil	IAS (50)	2001	339	27	5	62	6.5	2.1	2.4	2.4	0.3	
(99)	Peru	IAS (51)	2002–2003	359	100	0	0	3.3	2.2	0.8	2.0	1.7	
	North America												
(72)	USA	IAS (47)	1997–2001	1082	45	10	45	8.3	6.4	1.7	1.9	1.3	Caucasian, partner taking HIV medication, MSM>IDU>HSX
(73)	Boston, USA	IAS (50)	1999	88	20	31	51	18.2	13.6	4.6	3.4	2.3	
(74)	USA	IAS (51)	1999–2001	491	56	15		10.8	7.7	3.1	0.6	0.6	Non-Hispanic white,40% increase in risk for acquiring resistant virus per year
(75)	Canada	IAS (48)	2000–2001	715	26	33	17	8.1	4.1	1.4	1.5	1.0	Infection <6 m, lower among African or Caribbean ethnicity
(76)	San Francisco, USA	IAS (33)	2004	118				14.4	6.8	9.3	2.5	2.5	
	Asia												
(100)	Ho Chi Minh City, Vietnam	IAS, version not reported	2001–2002	200		43		6.5	4.5	0	2.0	0	
(101)	Kuala Lumpur, Malaysia	IAS (38)	2003–2004	100			57	1.0	0	1.0	0	0	

[a] Definition used for classifying HIV drug resistance. "IAS" means that the mutation-list defined by the IAS was used; the version of the list is given by the reference.

[b] Nr=number of patients included.

[c] The HIV risk factors were classified as MSM (men-having-sex-with-men), IDU (Intravenous Drug Users) and HSX (Heterosexual).

[d] Resistance was subdivided into particular classes of antiretrovirals. The column "Any" is the proportion of patients infected with a virus that contained at least one resistance-associated mutation. "MDR" is multi-drug-resistance or resistance to at least two classes of antiretrovirals.

[e] Study used proviral DNA.

[f] Study only included blood-donors.

recently or chronically infected patients. Men-having-sex-with-men (MSM) were the predominant risk group (proportion ranging between 57% and 92%) in studies of recently infected patients in Western Europe or North America (Table 2a). In the same geographical regions, MSM was also a common risk group among prevalent antiretroviral naïve patients (Table 2b). But the proportion of MSM had percentages ranging between 18% and 63%, substantially lower than in most studies of recently infected patients. An important explanation for this dissimilarity in risk group distributions between the studies in Tables 2a and 2b is that, in some European countries, patients who acquired HIV through heterosexual contact are more likely to come from regions with a history of limited access to antiretroviral drugs (10-12); amongst them, transmission of resistance will be rare. In addition, most of these patients are expected to have been infected before arrival in Europe and are therefore expected to be underrepresented among recently infected patients. Finally, it has been reported that MSM more frequently take an HIV test (13). As a consequence, they are likely to be identified earlier during the course of their infection. Therefore, studies limited to recently infected patients may not be representative of all HIV-infected patients and are likely to overestimate the true size of the problem of transmitted drug resistance.

Limiting the inclusion to recently infected individuals also has an advantage from a virological point of view, as reversion of transmitted drug resistance will be minimized. Reversion can occur because mutations conferring resistance to antiretroviral drugs commonly - but not always - result in a virus that replicates less efficiently than wild-type HIV (14,15). Thus, the drug resistant virus could be outgrown by faster-replicating revertant viruses. In addition, reversion could result in a viral sequence intermediate between the wild type and a resistance associated substitution. An important example of this phenomenon occurs at codon 215 of reverse transcriptase. Here, 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 receive antiretroviral treatment for their HIV-infection, unusual codons are frequently found that are intermediates between wild type and T215F/Y (16-19). Interestingly, viruses with a reversion at codon 215 have a decreased genetic barrier for the selection of the resistance-associated amino acid substitution T215Y (19).

Intermediates have not been reported for most other codons where resistance associated substitutions can emerge. As a consequence, reversion is expected to frequently result in a susceptible wild-type virus. In this context, it is important to note that drug resistant HIV can persist for decades by establishing a latent infection in resting memory CD4-positive cells, and perhaps other cells (15). Hence, inclusion of chronically infected antiretroviral naïve patients could underestimate the size of the problem, as resistance - still present as a latent infection - may no longer be detected due to reversion to the wild-type sequence in viral RNA isolated from plasma. Several studies have shown a remarkable persistence of particular patterns of transmitted drug resistance in plasma over time (20-23), indicating that reversion of some mutational patterns only occurs to a limited extent. In addition, a recent study (24) proposed compensatory fixation as a possible explanation for the in vivo persistence of some mutational patterns. 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 mutation therefore causes a further reduction in replication capacity and, as a consequence, the route to wild type is blocked (24). A similar phenomenon was observed in transmitted resistance (25).

Sampling extended to antiretroviral naïve and/or newly diagnosed patients

Patients recently infected with HIV are difficult to identify. But, because of the limited extent to which reversion occurs, epidemiological studies could also include patients living with HIV for a longer period of time who have not received treatment at the time they were sampled; these chronically infected antiretroviral naïve patients are easier to identify. Hence, studies also including antiretroviral naïve patients could identify a larger number of individuals during a shorter period of sampling. This is nicely illustrated by comparing the number of patients included in the studies summarized in Tables 2a and 2b; all studies with more than 500 patients also included antiretroviral naïve individuals.

But antiretroviral naïve patients can be sampled using several strategies. For instance, patients who had not been treated with antiretrovirals at the time of inclusion can be sampled (17). In addition,

a resistance test can be done on a sample collected just before treatment is initiated (26). Sampling is preferably limited to newly diagnosed patients, as this approach allows a resistance test to be performed on the earliest available sample, thus minimizing reversion (27). In addition, a substantial number of countries have HIV surveillance systems that are limited to newly diagnosed patients (10-12,28,29). Using these surveillance systems, local sampling strategies can be made that allow for the identification and inclusion of individuals who are representative of the risk group distribution and geographical distribution of local HIV epidemics. Finally, newly diagnosed patients also include individuals who recently acquired HIV.

2.2 Assay methodology and definitions used to classify resistance

Genotypic assays

Both genotypic and phenotypic assays are used for resistance testing, and the strengths and weaknesses of each methodology are summarized in Table 1b. The majority of epidemiological studies have used genotypic testing (Tables 2a and 2b). Genotypic assays identify the mutations that cause amino acid substitutions associated with drug resistance (30). Importantly, most studies have used population sequencing, which fails to detect and quantify minorities of drug-resistant quasi-species below 25% (31). Using methodology that allows the quantification of minor viral populations has demonstrated that conventional population sequencing considerably underestimates the size of the problem of transmitted resistance (31,32).

Studies using genotypic assays define resistance as the presence of one or more amino acid substitutions included in the resistance guidelines published by the International AIDS Society of the United States (IAS-USA) (Tables 2a and 2b). This is an important advantage, as it facilitates the comparison of rates found between studies. But unfortunately, the IAS-USA resistance guidelines are not designed for this purpose. Indeed, the experts who wrote the guidelines indicate that the list should be used cautiously in studies of the transmission of resistance (33).

Several problems could arise when applying the IAS-USA resistance guidelines in epidemiological studies on transmission of resistance. For instance, the guidelines include polymorphic substitutions that occur naturally in HIV-1 sequences obtained from individuals without any previous drug exposure (34-36). Inclusion of these polymorphic substitutions could overestimate the size of the problem. In this respect, it is also important to discuss the distinction the IAS list makes between major and minor protease substitutions. According to the definition provided in the IAS list, major substitutions by themselves reduce drug susceptibility. Minor substitutions improve, in some cases, the replicative capacity of HIV carrying major substitutions (37), but do not by themselves have a significant effect on drug susceptibility (37,38). Most minor protease substitutions are polymorphic, as they are also common in sequences from patients who have not been exposed to antiretrovirals (36,39). Due to the polymorphic nature of most minor substitutions, studies only consider the major ones as evidence of transmission of drug resistance.

Furthermore, the IAS list may not include all relevant substitutions, as the guidelines are based on published data. Current published data rely mainly on isolates obtained from persons treated for a limited period of time with a single inhibitor. Today, therapeutic regimens are increasingly complex, and a large number of novel resistance-associated substitutions have emerged that are not currently included in the IAS list (35,36,40).

The amino acid substitutions included in the IAS-USA resistance guidelines have mostly been identified in sequences of subtype B. Interestingly, particular subtypes have different mutational patterns (41). But codons at positions with major protease and reverse transcriptase drug resistance-associated substitutions are generally well-conserved across the subtypes (42). Nonetheless, some important differences in resistance pathways are found in subtype B and non-B strains. For instance, in patients failing nelfinavir, subtype B strains most frequently develop the D30N amino acid substitution, whereas other subtypes more commonly develop L90M (43,44). Similarly, upon failure to efavirenz, V106M is more frequently observed in subtype C as compared to B (45).

Finally, the IAS list is regularly updated to include novel substitutions that have been identified as relevant for drug resistance (33,38,46-53). But these updates complicate the comparison between stud-

ies performed at different periods in time. In addition, the IAS list does not consistently list the same mutations. For instance, the RT amino acid substitution V118I was included in previous versions of the IAS list (48,52,53), but was omitted from the most recent update of the list (54). Importantly, the V118I substitution is found in 2-3% of subtype B sequences obtained from patients who did not take antiretroviral drugs (55). Hence, inclusion of V118I overestimates the size of the problem of transmitted resistance. Similarly, the protease V32I substitution was classified in previous versions as a minor mutation (48,52,53), but the most recent update of the list classifies this mutation as major (54).

To facilitate any comparison between studies, the methods section of reports on the epidemiology of transmission of drug resistant HIV should include the mutations considered. In addition, the frequency of the most commonly found amino acid substitutions should be listed in the results section.

Phenotypic assays

A small number of studies classified resistance using phenotypic assays (Table 3). All of these studies also defined drug resistance by means of a genotypic assay (and thus are also included in Tables 2a and 2b). A phenotypic assay measures the in vitro susceptibility of HIV to antiretrovirals. For this purpose, a recombinant virus including the protease and RT gene from the patient's plasma is created. Susceptibility to particular antiretrovirals is then calculated as the fold change in drug concentration at which 50% (IC_{50}) of replication is suppressed, as measured by comparing the IC_{50} values of a reference strain and the recombinant virus (16,56).

Unfortunately, the cut-offs used in phenotypic assays for defining drug resistance are not clearly described. All studies defined phenotypic resistance using cut-offs in IC_{50} at 2.5 and 10 fold (57-60). But the cut-offs above which there is detectable impairment in virological response is known to vary by drug. Surprisingly, only one study used various cut-offs for different antiretrovirals based on assay precision, biological variability, and limited clinical experience (58).

Using different cut-offs had a substantial impact on the proportion of patients in whom transmitted resistance was detected (Table 3). All studies reported a prevalence of at least 10% when using a cut-off of 2.5. Conversely, the size of the problem of resistance was limited if only a fold change in IC_{50} above 10 was considered. Importantly, in all studies, the reported prevalence based on phenotypic assays deviated substantially from the result obtained using genotypic resistance (Figure 1).

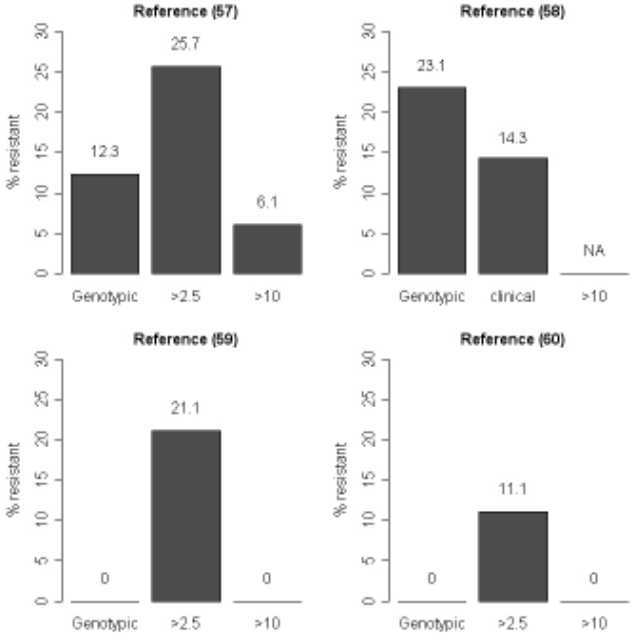

Figure 1. Comparison of genotypic and phenotypic resistance tests. The bars labeled 'genotypic' represent the proportion of patients infected with a drug resistant virus according to the results of genotypic assays. Similarly, the bars >2.5 and >10 represent the proportion of transmitted phenotypic resistance using a fold-change in IC50 of, respectively, at least 2.5 or 10. The study by Grant et al. (reference 58) did not include the fold changes above 2.5, but used clinical cut-offs that varied for each drug.

Table 3 Summary of studies using phenotypic assays to define drug resistance

Ref.	Region	Method	Years of sampling	Nr[a]	IC_{50} fold change Cut-off	Proportion resistant (%)[b] Any	NRTI	NNRTI	PI	MDR
	North America									
(57)	North America	PhenoSense HIV, ViroLogic	1995–2000	377	>2.5	25.7				
					2.5–10	19.6				
					>10	6.1	3.4	3.4	2.7	2.7
(58)	San Francisco, USA	PhenoSense HIV, ViroLogic	1996–2001	210	Clinical cut-offs[c]	14.3	7.6	5.7	3.8	2.4
					>2.5		7.1	24.8	11.9	
					>10		3.3	5.7	2.9	
	Africa									
(59)	Abidjan, Côte d'Ivoire	Antivirogram, Virco	unreported	19	>2.5[d]	21.1	5.3	10.5	15.8	0
					>10	0	0	0	0	0
(60)	Nigeria	PhenoSense HIV, ViroLogic	unreported	18	>2.5	11.1	0	0	11.1	0
					>10	0	0	0	0	0

[a] Nr=number of patients included.

[b] Resistance was subdivided to particular classes of antiretrovirals. The column "Any" is the proportion of patients infected with a virus resistant to at least one antiretroviral drug. "MDR" is multi-drug-resistance or resistance to at least two classes of antiretrovirals.

[c] The clinical cut-offs in IC_{50}-fold change varied per antiretroviral drugs. For the NRTIs these cut-offs ranged between 1.7 (stavudine, didanosine and zalcitabine) and 4.5 (lamivudine, zidovudine and abacavir). The clinical cut-offs for all NNRTIs and PIs were respectively, at least 10 and 4 or greater.

[d] The article mentioned low-level resistance.

3. Summary of the reported results

3.1 Epidemiology of transmitted resistance

Recently infected patients

Studies limited to recently infected patients were predominantly performed in Europe and North America (Table 2a). Interestingly, the incidence figures reported in Europe (ranging between 6.4% and 14.0%) (61-68) were generally lower than those in North America (12.3% to 23.1%) (57,58,69). The magnitude of multi-class resistance, defined as evidence of decreased susceptibility to at least two different classes of antiretrovirals, was also substantially higher in North America (about 6%) as compared to Europe (generally <2%). This indicates that the problem of transmitted resistance is more complex in North America. Furthermore, in both of these parts of the world, resistance was most frequently found for NRTIs (nucleoside reverse transcriptase inhibitors).

The single African study, which only included recently infected individuals, found no evidence of transmitted drug resistance (70). The absence of resistance among these patients most likely reflects the limited availability of antiretrovirals in Africa.

A remarkable study from Argentina reported an incidence of 7.7% (71). The results of this study are interesting because the Argentinean ministry of health has sponsored a policy of universal access to antiretroviral drugs since 1990. It is therefore important to note that the figure reported in this South American country was lower than most estimates from Europe and North America. Genotypic resistance was primarily found for NNRTIs (non-nucleoside reverse transcriptase inhibitors). In addition, transmitted multi-class resistance was not reported.

Antiretroviral naïve and newly diagnosed patients

A considerable number of studies have reported on the epidemiology of transmitted drug resistant HIV among prevalent patients who had not received antiretroviral therapy at the time they were sampled (Table 2b). These studies included antiretroviral naïve patients on almost all continents. Importantly, evidence of transmitted resistance has been observed all over the world. But a consistent finding among antiretroviral naïve patients, irrespective of where they were sampled, is that the prevalence of multi-class resistance is limited.

Studies from North America (72-76) and Western Europe (17,26,68,77-83) generally reported the highest prevalence estimates of transmitted drug resistant HIV (8-18% and 2-14%, respectively). This could be ascribed to the earlier period in time when patients were sampled in these regions. In addition, it could be due to the widespread availability of antiretroviral drugs for a substantial period of time in industrialized countries. A notable exception to the higher prevalence of transmitted resistance in North America and Western Europe is Denmark. A study from this country found evidence of transmitted resistance in only 2.1% of the included individuals (80).

An interesting observation that can be made from the results in Table 2b is that transmitted resistance in North America and Western Europe was most frequently found for NRTIs, irrespective of the years in which patients were sampled. It should be noted that one study from San Francisco (76) was an exception, reporting that transmitted NNRTI resistance was the most common. In other parts of the world, transmitted resistance was generally more homogenously distributed across the various antiretroviral drug classes. A possible explanation for this dissimilarity is that in Western Europe, the USA, and Canada, antiretrovirals were introduced well before HAART (highly active antiretroviral treatment, or the combination of the at least two different classes of anti-HIV drugs) became available in 1996. Before then, treatment of HIV consisted of a single NRTI, usually zidovudine or lamivudine. Resistance to mono-therapy with these drugs emerges rapidly (84-86) and it is thus very likely that a large number of NRTI-resistant viruses circulated at that time. Indeed, the first published case reports in the early 1990s described transmitted zidovudine resistance (6-8). HAART successfully suppresses viral replication making the emergence of resistance less likely. Therefore, in countries where treatment started at the time when HAART had become available, less drug resistant viruses are expected to circulate. This hypothesis is also supported by the observation that rates of transmitted NRTI resistance

were generally highest in Europe and North America (87).

Studies performed in Africa were usually small, with sample sizes ranging between 18 and 107 patients. As could be expected based on the small scale on which antiretrovirals are available in Africa, transmission of drug resistance did not occur frequently, with estimates between 0 and 13.0%. Similarly, the mutational patterns were not complex, as resistance was always observed for only one class of antiretrovirals (59,60,88-92). Nonetheless, one study from Cameroon reported a considerable prevalence of transmitted resistance of 13.0%. But this study used a dissimilar method, as the genotyping was done using proviral DNA. In addition, the researchers analyzed at least four clones per sample. Interestingly, the researchers reported that the drug resistance-associated substitutions were, in all but one case, present as minor populations, as evidence of resistance was only observed in only one of the four clones (92). But these minor viral variants cannot be detected by the population sequencing used in other studies (31). Excluding the drug resistant mutants found as minor populations from the analysis resulted in a decrease of the prevalence of transmitted resistance from 13.0% (7/54) to 1.9% (1/54) (92).

Of particular interest were the two reports from the former Soviet Union (93,94). Since the mid-1990s, this part of the world has experienced a progressively growing epidemic, mostly limited to intravenous drug users. Importantly, Eastern Europe is the region of the world with the fastest growing HIV epidemic (29). One report, from the republic of Georgia, predominantly included intravenous drug users and found a limited prevalence of transmitted resistance of only 4% (93). The other report from Eastern Europe included patients from across the former Soviet Union (94). Surprisingly, the study found a prevalence of 17%, which is among the highest reported anywhere in the world.

In Latin America most reports that limited the inclusion to antiretroviral naïve patients came from Brazil (95-98). This country provides important information, as the Brazilian ministry of health has been sponsoring a policy of universal access to antiretroviral drugs since 1996 (98). The prevalence of transmitted resistance has had values ranging between 2.8 and 8.5% (95-98), generally lower than most reports from Europe and North America. Transmitted multi-class resistance in Brazil was only observed in a very limited fraction (at most 0.6%) of antiretroviral naïve individuals (95-98). Similarly, a study among MSM in Peru found a low prevalence of transmitted resistance of only 3.3% (99).

Two reports from Asia found that the size of the problem of transmitted resistance was limited (100,101). A study from Vietnam found a prevalence of 6.5% among 200 antiretroviral naïve individuals (100). Similarly, a Malaysian study reported evidence of transmitted resistance in only one individual among 100 antiretroviral naïve patients (101).

Comparison of recent vs. chronic infection

A small number of studies that sampled antiretroviral naïve patients also compared the proportion of resistance between recently and chronically infected individuals (Table 4) (17,72,75,83,97,99). All but one of these studies (17,72,75,83,97) found that transmission of drug resistance was most frequent among patients who recently acquired HIV. But two reports found a decreased risk for transmitted resistance among recently infected patients as compared to chronically infected individuals (76,99).

Table 4 Comparison of proportion of transmitted resistance among recently and chronically infected patients

Ref.	Region	Years of sampling	Classification of recent infection	Number of patients		Proportion resistant (%)	
				Recent	Chronic	Recent	Chronic
(17)	Europe, Israel	1996-2002	<12 m	777	607	13.5	8.7
(72)	USA, 10 cities	1997-2001	<6 m	182	767	11.5	7.4
(75)	Canada	2000-2001	<6 m	221	494	12.2	6.3
(76)	San Francisco, USA	2004	Detuned assay (118,119)	42	76	9.5	14.5
(83)	Greece	2002-2003	<12 m	18	79	22.2	6.3
(97)	Sao Paolo, Brazil	1998-2002	Detuned assay (120)	55	280	12.7	5.0
(99)	Peru	2002-2003	Detuned assay (121)	33	326	3.0	3.4

The epidemiological dissimilarity in transmitted resistance between recently and chronically infected patients is due to a complex interplay of various factors. First, the discrepancy is partially explained by the limited degree to which reversion occurs (20,22). In this context, it should be noted that revertants at codon 215 are classified as transmitted resistance. Furthermore, patients who are identified earlier during the course of their HIV-infection generally have a different risk-group distribution. As a consequence, the dissimilarity could be due to the higher prevalence of transmitted resistance in particular risk groups that are more common among patients identified earlier after seroconversion. Finally, both study groups were infected at different moments in time. The lower prevalence among chronically infected patients could therefore reflect a lower prevalence of transmitted resistance in the past.

3.2 Time trends

In this report, the comparison of transmitted resistance over time was limited to patients who were newly infected. Only these patients were considered, as transmission of resistance among chronically infected patients also reflects the risk for acquiring a resistant virus many years ago.

The results from studies that looked at trends are inconsistent, with some studies finding either a substantial increase (57,61,69) or decrease (62,65,67) over time. In addition, other studies reported a slight decrease in transmitted resistance followed by a second peak (17,58,72). The inconsistent findings could be explained by local differences. But the comparison between time periods is complicated, as many studies reported that the population under study also changed over time (61,62). Also, there was a considerable dissimilarity between studies regarding the particular time periods that were compared.

New insights in the treatment of HIV and novel drugs have been developed during the last decade (102-105). As a consequence, it can be expected that treatment has improved over time, and this could have an impact on transmitted resistance. But based on the reports published in recent years, it is not yet possible to conclude whether changes in treatment had a beneficial or detrimental impact on the size of the problem of transmitted resistance over time.

3.3 Risk factors for acquiring drug resistant HIV

Several studies have reported on the risk factors for acquiring a drug resistant virus. Importantly, studies from North America and Europe reporting on risk factors found that transmission of resistance most frequently occurred among Caucasians, as compared to other ethnic groups (26,72,74,75). This dissimilarity is most likely caused by the fact that antiretrovirals have been available for a prolonged period of time in North America and Europe, but have been less accessible in other parts of the world. As non-Caucasians are more likely to carry viruses originating from recent immigrants, they are thus less likely to carry a drug resistant virus.

An interesting observation was that viruses in which drug resistance-associated substitutions were identified were predominantly of subtype B (17,26). HIV-1 subtypes have a distinct geographical distribution, with subtype B predominating the epidemic in North America and Western Europe. Clade B accounts for only a minority of infections in Africa, where subtypes A and C predominate, and a number of other clades are also circulating at a high level (106-108). Subtype B viruses thus predominantly circulate in areas where antiretrovirals are readily available. This explains the higher prevalence of transmitted resistance among subtype B.

In summary, the comparison of reported risk factors suggests that transmission of resistance is most likely in patients originating from areas where antiretrovirals are available on a large scale. As a consequence, immigration from areas with limited access to antiretrovirals could have a profound impact on transmitted resistance.

3.4 Impact of transmitted resistance on treatment efficacy

When treatment of patients who have failed antiretroviral therapy is guided by expert interpretation of genotypic resistance, significantly improved virological outcome is achieved (109-111). These results cannot easily be extrapolated to transmitted resistance, as mutational patterns are more complex among patients failing antiretroviral treatment. For instance, contrary to transmitted resistance, HIV

multi-class resistance is very common in individuals who fail treatment (3-5).

Several of the epidemiological studies discussed in this review analyzed the impact of transmitted resistance on initial antiretroviral treatment. Studies that did not use genotypic information in the initiation of treatment found that antiretroviral naïve patients infected with a drug resistant virus took a longer time to reach viral suppression after starting treatment (57,58,63). In addition, among patients who had a relapse of viremia after viral suppression, the length of time to virologic failure was shorter among individuals with transmitted resistance (57). Complete viral suppression was generally achieved in the vast majority of patients, irrespective of baseline susceptibility patterns (57,58). Nonetheless, the longer time needed to achieve complete viral suppression may permit sufficient further rounds of viral replication to select for additional drug-resistant variants (112), which could be detrimental during a later stage of treatment.

Conversely, studies in which treatment was optimized based on interpretation of resistance found that the time to virological suppression was similar irrespective of baseline susceptibility (26,67,69,109-111). Therefore, guidelines (27,105) recommend resistance testing before initiation of treatment in areas where the prevalence of transmitted resistance is unknown or greater than 5% (105) or 10% (27).

4. Conclusions and summary

The incidence and prevalence of transmitted resistance show substantial variability among studies. The dissimilarities among studies are to some extent ascribed to whether recently or chronically infected patients were sampled. Limiting the inclusion to recently infected patients has some important virological advantages (*i.e.*, potential reversion minimized) and epidemiological advantages (*i.e.*, duration of infection can be estimated), but particular routes of transmission seem to be over-represented. The latter is most likely due to differences in HIV testing behavior between risk groups. As a consequence, studies limited to patients that recently acquired the virus may not be representative of all HIV infections in a particular geographic region. Fortunately, transmitted drug resistant HIV persists for a considerable period. This key observation implies that epidemiological studies on transmitted resistance could also include chronically infected patients who had not received antiretroviral treatment. Notably, most patients are identified when they have entered the asymptomatic phase of AIDS.

As an example of progress in surmounting the problem of differences in study methodologies, we would like to mention the SPREAD program, which is now being implemented by the EuropeHIVResistance Network. This program has investigated transmission of resistance and its determinants across Europe. For practical reasons, the study group consisted of newly diagnosed patients. Sampling newly diagnosed individuals allowed us to obtain the earliest available sample. Importantly, patients were sampled according to a uniform strategy that enabled the identification of those who were representative for the risk group distribution and geographical distribution of the HIV epidemic in each country. Using this strategy, we identified 1083 patients from 17 European countries. A considerable proportion (22%) of the patients had laboratory evidence of recent seroconversion (<1 year) (113). We expect that the results of this large-scale systematic study will shed new light on the transmission of drug resistance in Europe.

The most important risk factor for transmitted resistance seems to be the large-scale availability of antiretrovirals in the area where infection occurred. Immigration from areas with limited or no access to antiretrovirals could therefore have a profound impact on the size of the problem of transmitted resistance (*e.g.*, immigration from Africa to Europe). In theory, the occurrence of transmitted resistance in a risk group sampled in a particular geographical region could increase but, due to immigration, the prevalence could decrease in the population of antiretroviral naïve individuals living in the same area. Therefore, absolute numbers and the occurrence of transmitted resistance should be provided for every risk group.

The vast majority of studies used population sequencing for determining genotypic resistance. This type of genotypic assay sequencing does not allow detection of minor populations present in <25% of the sequences. Virtually all studies therefore underestimate the size of the problem of transmitted resistance. Importantly, there is a surprising lack of consensus with respect to the amino acid substitu-

tions that are of relevance for transmitted drug resistance. Studies using different methods for classifying resistance based on genotypic assays are therefore difficult to compare. Thus, we recommend that the methods section of all epidemiological studies should include the particular amino acid substitutions that were analyzed. In addition, the frequency of the most commonly-found mutations should be listed in the results section.

Transmitted multi-class resistance is rare in all parts of the world. Nonetheless, epidemiological studies that followed patients after the start of antiretroviral treatment showed that therapy guided by resistance testing performed before the commencement of antiretrovirals has a beneficial impact on the length of time to reaching virological suppression. Resistance testing on the earliest available sample is therefore recommended in areas with a prevalence of transmitted resistance that exceeds 5-10%.

Current studies on transmission of drug resistance only consider three classes of antiretroviral drugs (NRTIs, NNRTIs, and protease inhibitors). But since 2003, the fusion inhibitor enfuvirtide is also available in clinical practice (114,115). Transmitted resistance to enfuvirtide does not presently seem to be a problem, as only two cases have been documented (116). A likely explanation for the very limited enfuvirtide resistance is that this drug is used by only a small number of patients. For example, a Dutch online database on drug utilization in the Netherlands reported that in 2005 only 89 patients used enfuvirtide, while almost 9,000 individuals used NRTIs, about 5,000 persons used NNRTIs, and more than 3,000 patients took protease inhibitors (117). Inclusion of enfuvirtide resistance in surveillance programs means that a genetically variable region of the envelope should be genotyped, and this procedure is not currently part of routine clinical practice. Therefore, this test only seems warranted if a substantial number of HIV patients start using this drug. Similarly, transmission of resistance to novel drugs such as CCR5 and integrase inhibitors should only be considered if large numbers of patients start using these drugs. Before large scale epidemiological studies are set up, pilot studies in particular risk groups could be performed to determine if any transmission of resistance to novel classes of drugs is found.

In summary, transmission of resistance has been reported in all parts of the world. The size of the problem varies between 0 and 25% and seems to be the highest in areas where antiretrovirals have been available for a long period of time. Antiretrovirals have shown to dramatically decrease morbidity and mortality among people living with HIV (1,2) and are therefore increasingly provided in many parts of world. Monitoring of transmitted resistance continues to be needed to allow a timely modification of antiretroviral treatment guidelines. Importantly, when comparing results from various studies, the differences in research methodology should be taken into account.

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