Transmission of Drug-Resistant HIV-1 Is Stabilizing in Europe

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The SPREAD Programme investigated prospectively the time trend from September 2002 through December 2005 of transmitted drug resistance (TDR) among 2793 patients in 20 European countries and in Israel with newly diagnosed human immunodeficiency virus type 1 (HIV-1) infection. The overall prevalence of TDR was 8.4% (225 of 2687 patients; 95% confidence interval [CI], 7.4%–9.5%), the prevalence of nucleoside reverse-transcriptase inhibitor (NRTI) resistance was 4.7% (125 of 2687 patients; 95% CI, 3.9%–5.5%), the prevalence of nonucleoside reverse-transcriptase inhibitor (NNRTI) resistance was 2.3% (62 of 2687 patients; 95% CI, 1.8%–2.9%), and the prevalence of protease inhibitor (PI) resistance was 2.9% (79 of 2687 patients; 95% CI, 2.4%–3.6%). There was no time trend in the overall TDR or in NRTI resistance, but there was a statistically significant decrease in PI resistance ($P = .04$) and in NNRTI resistance after an initial increase ($P = .02$). We found that TDR appears to be stabilizing in Europe, consistent with recent reports of decreasing drug resistance and improved viral suppression in patients treated for HIV-1 infection.

Despite successful treatment of human immunodeficiency virus type 1 (HIV-1) infection in Europe [1], there is still a limited group of individuals with treatment failure who are potentially at risk of spreading drug-resistant HIV-1. The objectives of the SPREAD Programme are to determine the prevalence of transmitted drug resistance (TDR) within the participating European countries, to study temporal trends in resistance, and to investigate possible predictors for TDR [2].

Patients and methods. The SPREAD Programme recruited patients in whom HIV-1 infection was newly diagnosed from September 2002 through December 2005 in 20 European countries (Austria, Belgium, Cyprus, Czech Republic, Germany, Denmark, Spain, Finland, Greece, Ireland, Italy, Luxembourg, the Netherlands, Norway, Poland, Portugal, Sweden, Slovenia, Slovakia, and Serbia) and Israel. To obtain representative samples from every country, the investigators selected individuals according to the national distribution of transmission risk groups and the geographical distribution of patients with new diagnoses of HIV-1 infection. For more details on the sampling strategy, see the previous report of the SPREAD Programme [2]. Epidemiological, clinical, and behavioral data were collected using a standardized questionnaire. A thorough data verification process preceded the analysis of the data.

The first available measurements of viral load and CD4 cell counts were collected. A blood sample was taken for genotypic resistance testing within 6 months after diagnosis. Population-

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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. The GenBank accession numbers for the sequences used in this analysis are listed after the text. All countries took part in a blinded quality control program to verify the quality of the genotypic data generated. TDR was defined according to the recently published list of mutations for surveillance of TDR as recommended by the World Health Organization [3].

Either the duration of infection in patients with a new diagnosis was unknown ($n = 1878$ patients with only first confirmed positive antibody test result date) or seroconversion was documented ($n = 809$). For patients with laboratory evidence for acute seroconversion, the date of the first positive reactive laboratory test result before complete seroconversion ($n = 382$) was used as the estimated date of infection. For other documented seroconverters ($n = 427$), the date of infection was estimated as the midpoint between the date of the last documented negative HIV-1 antibody test result and the date of first documented confirmed positive antibody test result, provided that the time interval between the 2 tests was <3 years. For the purpose of analysis, Western Europe was defined to include those countries with a long history of good access to antiretroviral drugs, as follows: 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.

Univariate and multivariate analyses were used to determine predictors of TDR. All statistically significant ($P < .05$) univariate predictors of TDR that were not equally distributed over time were considered as possible confounding factors in the multivariate time trend analysis. 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/) [4]. The data were analyzed using the statistical software R (version 2.3.1). Prevalence values were calculated with a 95% Wilson score confidence interval (CI) on the basis of a binomial distribution. Categorical data were compared by use of the χ^2 test, Fisher's exact test, or logistic regression techniques. Continuous data were investigated by means of Student's *t* test, the Mann-Whitney *U* test, linear regression, or Poisson regression. Phylogenetic analysis was performed to investigate whether some samples belonged to tight clusters (potentially naive transmission clusters).

Results. In total, 2793 patients with newly diagnosed HIV-1 infection met the predefined inclusion criteria: they were HIV-1 drug naive, were ⩾18 years old, provided a sample for genotypic analysis within 6 months after diagnosis, and had viral loads >1000 copies/mL. Sequence analysis was successful for >96% (2687) of 2793 patient samples. The baseline characteristics of the patients are summarized in Table 1. The majority of the Western European patients (964 [60%] of 1607) were men who have sex with men (MSM), whereas almost all (316 [98%] of 323) of the patients originating from sub-Saharan Africa were infected through heterosexual contact. Most of the patients (1878 [70%] of 2687) had an unknown duration of infection. A relatively high proportion of the study patients (1175 [69%] of 1697) reported that the source of infection was unknown. This was significantly more often the case among MSM than among heterosexual patients (541 [44%] of 1230 vs 181 [18%] of 1004, respectively). Among all patients who were infected through sexual contact, 4% (89 of 2234) knew at the time of infection that the source was HIV positive. Most of these patients (66 [73%] of 89) were MSM.

The prevalence of TDR was 8.4% from 2002 through 2006 (225 of 2687 patients; 95% CI, 7.4%–9.5%). The prevalence of mutations associated with transmitted nucleoside reverse-transcriptase inhibitor (NRTI) resistance was 4.7% (125 of 2687 patients; 95% CI, 3.9%–5.5%), that with transmitted nonucleoside reverse-transcriptase inhibitor (NNRTI) resistance was 2.3% (62 of 2687 patients; 95% CI, 1.8%–2.9%), and that with transmitted protease inhibitor (PI) resistance was 2.9% (79 of 2687 patients; 95% CI, 2.4%–3.6%). Dual class resistance occurred in 17 cases (0.63%), and triple class resistance occurred in 12 cases (0.45%). The most prevalent amino acid change was RT215Y/F or RT215 revertant (67 [2.51%] of 225 patients). RT41L was detected in 41 cases (1.51%). Other frequent (found in >0.5% of cases) mutations were RT103N/S (1.42%), PR46I/L (1.17%), RT219Q/E/R/N (0.84%), PR90M (0.67%), PR82A/F/T/S (0.59%), RT210W (0.50%), and RT181C/I/V (0.50%).

Logistic regression showed no time trend (2002–2006) in the overall TDR (odds ratio [OR], 0.89 [95% CI, 0.75–1.05]; $P = .17$) or in transmitted NRTI resistance (OR, 0.84 [95% CI, 0.67–1.04]; $P = .11$) (Figure 1). Transmitted NNRTI resistance showed a statistically significant parabolic time trend with a peak at the end of 2004 ($P = .02$). Finally, there was a statistically significant decrease over time in transmitted PI resistance (OR, 0.75 [95% CI, 0.57–0.99]; $P = .04$) that coincided with a statistically significant decrease in PR90M (OR, 0.39 [95% CI, 0.20–0.76]; $P = .006$).

All potential predictors of TDR were first tested univariately (Table 1). Infection with subtype B virus and MSM transmission route were statistically significantly ($P < .05$) associated with TDR. Being infected with a subtype B virus was the only independent predictor in the multivariate approach. RT215Y/F revertant and RT41L were significantly more prevalent in subtype B compared to non-B (OR, 4.82 [95% CI, 2.31–11.64]; $P < .001$; and OR, 3.10 [95% CI, 1.19–10.28]; $P = .01$; respectively).

Approximately two-thirds of the patients harbored a subtype B virus (1795 [66.8%] of 2687). Subtype A was identified in

Table 1. Comparison of Characteristics between Patients Infected with Human Immunodeficiency Virus Type 1 (HIV-1) Harboring Transmitted Drug Resistance and Patients Infected with Wild-Type Virus

				Univariate		Multivariate	
Characteristic	Total	TDR	Wild type	OR (95% CI)	*P*	OR (95% CI)	*P*
Patients	2687	225 (8.4)	2462 (91.6)				
Age, mean years ± SD	36.7 ± 10.8	36 ± 10.7	37 ± 10.8	0.93 (0.82–1.06)	.3		
Sex							
Male	2080 (78)	183 (81)	1897 (77)	1.28 (0.90–1.86)	.18		
Female	598 (22)	42 (19)	556 (23)				
Area of origin							
Western Europe	1607 (60)	143 (64)	1464 (59)	1.19 (0.89–1.60)	.26		
Sub-Saharan Africa	323 (12)	23 (10)	300 (12)				
Eastern Europe and Central Asia	424 (16)	37 (17)	387 (16)				
Other	333 (12)	22 (9)	311 (13)				
Route of transmission							
MSM	1230 (46)	124 (55)	1106 (45)	1.50 (1.13–2.00)	.004[a]	1.30 (0.94–1.80)	.12
Heterosexual contact	1004 (37)	70 (31)	934 (38)				
Injection drug use	208 (8)	8 (4)	200 (8)				
Other	245 (9)	23 (10)	222 (9)				
Area of infection							
Western Europe and United States	1508 (56)	136 (60)	1372 (56)	1.21 (0.91–1.62)	.18		
Other	1179 (44)	89 (40)	1090 (44)				
Duration of infection							
<1 year	733 (27)	74 (33)	659 (27)	1.59 (0.62–5.22)	.42		
1–2 years	76 (3)	5 (2)	71 (3)				
Unknown duration	1878 (70)	146 (65)	1732 (70)				
CDC stage							
A and B	2180 (87)	186 (87)	1995 (87)	0.93 (0.59–1.43)	.83		
C	338 (13)	27 (13)	311 (13)				
Coinfection with hepatitis B virus	109 (4)	13 (6)	96 (4)	1.65 (0.83–3.03)	.10		
Coinfection with hepatitis C virus	242 (9)	14 (6)	228 (9)	0.66 (0.35–1.17)	.17		
STD within previous 2 years	432 (16)	48 (21)	384 (16)	1.40 (0.97–2.00)	.07		
Awareness of source of infection							
Aware	522 (31)	37 (25)	485 (31)	1.38 (0.93–2.09)	.11		
Unaware	1175 (69)	112 (75)	1063 (69)				
CD4 cell count, median cells/mm^3 (IQR)	343 (370)	390 (380)	340 (368)	1.03 (0.98–1.09)	.20		
HIV-1 RNA, mean log copies/mL ± SD	4.82 ± 0.8	4.79 ± 0.8	4.82 ± 0.8	0.93 (0.78–1.12)	.46		
Subtype B	1795 (67)	178 (77)	1599 (65)	1.64 (1.19–2.29)	.002[a]	1.48 (1.22–2.15)	.04[a]
Subtype non-B	892 (33)	53 (23)	856 (35)				

NOTE. 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; CI, confidence interval; IQR, interquartile range; MSM, men who have sex with men; OR, odds ratio; SD, standard deviation; STD, sexually transmitted disease; TDR, transmitted drug resistance.

[a] Statistically significant results ($P < .05$).

265 cases (9.9%). Other non-B subtypes or circulating recombinant forms (CRFs) were C (6.7%), CRF02_AG (5.1%), G (4.2%), F (1.3%), and less frequently (<1%) CRF06_cpx, D, H, and J. There were 110 sequences (4%) that could not be classified because they did not cluster unambiguously with reference sequences of the subtyping tool. Being infected with a subtype B strain was more common among MSM than it was among patients in other transmission groups (OR, 9.2 [95% CI, 7.4–11.4]; $P < .001$). Furthermore, subtype B was statistically significantly more prevalent in people infected in Western Europe or the United States than it was among those infected elsewhere (75% vs 54%; OR, 2.6 [95% CI, 2.2–3.0]; $P < .001$).

Discussion. To our knowledge, the SPREAD Programme is the first prospective, representative surveillance program of TDR, its predictors, and its trends. The representative data provide evidence of a stabilizing trend over time (2002–2006) in the overall transmitted resistance in Europe. This study confirms that over the entire surveillance period, ~1 in 10 patients with newly diagnosed HIV-1 infection in Europe was infected with a drug-resistant strain, as previous reports have already indicated (SPREAD [2], EuroSIDA [5], and Combined Analysis of Resistance Transmission over Time of Chronically and Acutely Infected HIV Patients [CATCH] [6]).

Furthermore, we noticed a significant decrease over time in

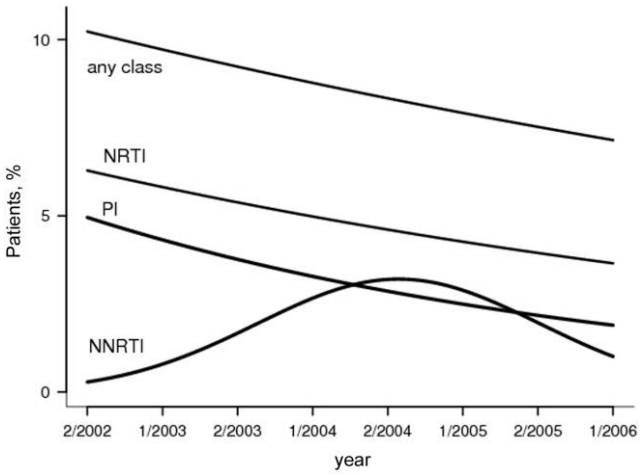

Figure 1. Time trends of transmitted drug resistance among patients with newly diagnosed human immunodeficiency virus type 1 (HIV-1) infection in Europe. NNRTI, nonucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; PI, protease inhibitor. 2/2002, July–December 2002; 1/2003, January–June 2003; 2/2003, July–December 2003; etc.

the prevalence of transmitted PI resistance and a significant up-and-down trend in NNRTI resistance, the latter with a maximum prevalence at the end of 2004. This peak could not be explained by the presence of transmission clusters of NNRTI resistance in the middle of the sampling period (phylogenetic analysis results available upon request). In addition, transmitted NRTI resistance showed a decreasing trend; however, this was not statistically significant. It should be stressed that this study population consisted of patients with new diagnoses, the majority of whom (1878 [70%] of 2687) had an unknown duration of infection. When the analysis was repeated on a subset (733 [27%] of 2687) of recently infected patients (duration of infection, <1 year), statistically significant time trends were obtained (eg, for PI resistance, $P = .01$).

Of all the statistically significant univariate predictors of TDR, only the proportion of patients infected in Western Europe or the United States statistically significantly decreased over time ($P < .001$), which suggested that the increasing number of immigrants in Europe with new diagnoses may be a contributing factor to the decreasing trends of TDR. However, when we corrected for this possible confounding factor, the significance of the trends decreased only marginally, which suggested that the increase in immigrants only slightly affected these trends. We propose a more likely explanation for these trends: on the one hand, resistance is decreasing in the treated population, and therefore the pool from which resistance is transmitted is decreasing, whereas on the other hand, there are fewer patients in whom treatment fails, so the sources of potential transmission are shifting toward untreated patients. Indeed, several published studies have reported a decreasing trend of resistance among patients in whom treatment fails, and more strikingly, one study also reported a parabolic trend in NNRTI resistance (first increasing and subsequently decreasing) [7–12]. Thus, even though caution is needed when comparing prevalence rates between different studies, the trends in TDR (especially for NNRTI and PI) in this study seem to reflect the decreasing trends in prevalence of resistance in the population of patients who experienced treatment failure [7–12]. This concordance is reassuring and indicates that our observed time trends in TDR are a genuine consequence of improved clinical care of treated patients rather than a methodological artifact. We have to note here that some studies have reported increasing prevalence of resistance over time, but we also note that these studies were performed before or soon after highly active antiretroviral therapy was introduced, during which time resistance could accumulate in treatment-experienced patients and subsequently be transmitted to newly infected patients.

In addition, we were able to investigate in a prospective manner the factors associated with the risk for transmission of drug-resistant HIV-1. Our results, consistent with previous reports [5, 13], show that MSM infected with a subtype B virus were more likely than other patients ($P < .01$) to be infected with drug-resistant HIV-1. The most likely explanation for this trend is the long history of antiretroviral drug use in Western Europe and the United States, especially among MSM and patients infected with subtype B virus. In the multivariate analysis, only subtype B proved to be independently associated with TDR ($P = .04$). This could mean that we are seeing, not a major import of resistant non-B strains into Europe, but rather a local spread of resistant B strains within Europe. Future phylogenetic studies might give support to this hypothesis. However, it is also possible that this trend is (partially) attributable to the fact that only for subtype B was there sufficient genuine data to achieve statistical significance. The data on route of transmission, country of infection, and awareness of the source were available only if they were reported in the questionnaire; this may introduce bias. For example, there may be a bias in the patients' declaration regarding sexual behavior (heterosexual contact vs MSM), which could partially contribute to the statistically significant association of subtype B with TDR irrespective of the transmission group.

In the absence of drug selective pressure, viruses with resistance mutations have a tendency to revert to wild type because of lower fitness of the virus carrying resistance mutations [14]. However, in chronically infected, drug-naive patients, these originally transmitted resistance mutations can persist as minor variants below the sensitivity of the genotypic assays [15]. The fact that the mutations observed are not representative of the mutations generally seen in the population with treatment failure might be the result of the reversion phenomenon (see also the previous report from the SPREAD Programme [2]). Thus,

we likely underestimate the calculated prevalence of transmitted resistance. In addition, for patients who show any sign of transmitted resistance, it may be advisable to use antiretroviral drug combinations with a "high genetic barrier," because additional mutations might have been transmitted that subsequently reverted.

In conclusion, the prevalence of overall transmitted resistance among patients in Europe in whom HIV-1 infection was newly diagnosed was found to be stabilizing under 10%. Concomitantly, transmitted NRTI resistance also seems to be stabilizing, whereas transmitted NNRTI and PI resistance decreased over time. Subtype B infection was the strongest predictor of transmission of resistance.

GenBank accession numbers. The following are the GenBank accession numbers for all of the sequences used in this analysis: AJ971091, AJ971093, AJ971096, AJ971102, AJ971103, AJ971106, AJ971107, AJ971109, AJ971110, AJ971113, AJ971114, AJ971117, AJ971122, AJ971128, AJ971133, AJ971140, AJ971143, AJ971144, AJ971268, AJ971271, AJ971274, AJ971276–AJ971281, AJ971283, AJ971285–AJ971287, AJ971289, AM113750, AY694290, AY694313, AY694317, AY694318, AY694321, AY694322, AY694324, AY694328–AY694330, AY694338, AY694339, AY694343–AY694345, AY694350, AY694353, AY694361, AY694362, AY694377, AY694382, AY938435/AY940218, AY938437/AY940236, AY938439/AY940229, AY938440/AY940230, AY938441/AY940315, AY938442/AY940257, AY938443/AY940259, AY938444/AY940243, AY938445/AY940238, AY938446/AY940238, AY938447/AY940237, AY938448/AY940239, AY938449/AY940240, AY938450/AY940245, AY938451/AY940246, AY938452/AY940224, AY938453/AY940264, AY938454/AY940247, AY938455/AY940248, AY938456/AY940222, AY938458/AY940262, AY938459/AY940253, AY938460/AY940219, AY938461/AY940220, AY938463/AY940244, AY938464/AY940216, AY938465/AY940215, AY938470/AY940252, AY938471/AY940254, AY938472/AY940232, AY938473/AY940235, AY938474/AY940225, AY938475/AY940277, AY938476/AY940296, AY938477/AY940282, AY938482/AY940273, AY938484/AY940276, AY938487/AY940293, AY938488/AY940283, AY938489/AY940280, AY938490/AY940275, AY938492/AY940272, AY938498/AY940297, AY938499/AY940249, AY938500/AY940270, AY938501/AY940298, AY938502/AY940251, AY938503/AY940302, AY938504/AY940217, AY938507/AY940279, AY938508/AY940291, AY938509/AY940274, AY938510/AY940271, AY938511/AY940301, AY938512/AY940234, AY938513/AY940263, AY938514/AY940265, AY938515/AY940267, AY938516/AY940268, AY938517/AY940260, AY938519/AY940266, AY938520/AY940250, AY938521/AY940304, AY938522/AY940307, AY938523/AY940305, AY938524/AY940306, AY938525/AY940226, AY938526/AY940311, AY938527/AY940312, AY938528/AY940313, AY938530/AY940294, AY938531/AY940255, AY938532/AY940233, AY938533/AY940269, DQ974841, DQ974844, DQ974845, DQ974847, DQ974848, DQ974850, DQ974853, DQ974854, DQ974857, DQ974858, DQ974863–DQ974865, DQ974867–DQ974873, DQ974875–DQ974877, DQ974880–DQ974882, DQ974887, DQ974890, DQ974892, DQ974893, DQ974895–DQ974897, DQ974899, DQ974902–DQ974906, DQ974908, DQ974910–DQ974912, DQ974922–DQ974924, DQ974927–DQ974929, DQ974931, DQ974932, DQ974941, DQ974944–DQ974947, DQ974951–DQ974953, DQ974955−DQ974963, DQ974965, DQ974966, DQ974968, DQ974982–DQ974991, DQ974996–DQ974998, DQ975003, DQ975011–DQ975015, DQ975018–DQ975021, DQ975024, DQ975032, DQ975034–DQ975036, DQ975044, DQ975136, DQ975139, DQ975140–DQ975147, DQ975156–DQ975159, DQ975161–DQ975163, DQ975165, DQ975169, DQ975172, DQ975173, DQ975187, EU248291–EU248297, EU248299, EU248300, EU248302, EU248303, EU248305–EU248307, EU248309, EU248310, EU248312, EU248314, EU248315, EU248317, EU248320–EU248323, EU248325, EU248327, EU248329, EU248331–EU248337, EU248340, EU248341, EU248343–EU248345, EU248347–EU248360, EU248363–EU248365, EU248368, EU248371–EU248373, EU248376–EU248378, EU248382, EU248383, EU248385–EU248387, EU248389, EU248392, EU248393, EU248396, EU248399, EU248400, EU248401, EU248403, EU248404, EU248406–EU248408, EU248410–EU248412, EU248415–EU248419, EU248421–EU248426, EU248428, EU248431, EU248432, EU248435, EU248439, EU248440–EU248444, EU248446, EU248448, EU248449, EU248451, EU248453, EU248455–EU248457, EU248459–EU248461, EU248463–EU248466, EU248468–EU248474, EU248476, EU248477, EU248479, EU248480, EU248483, EU248485, EU248487–EU248490, EU248492, EU248494–EU248498, EU248500–EU248505, EU248507, EU248509, EU248512, EU248515, EU248517–EU248521, EU248523, EU248526–EU248569, EU248571–EU248582, EU248584–EU248588, EU673374–EU673397, FJ030767, FJ030769, FJ030771, FJ030772, FJ185113–FJ185120, FJ185122, FJ185124, FJ185125, FJ185127, and GQ398826–GQ401023.

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