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Recent HIV-1 Outbreak Among Intravenous Drug Users in Romania: Evidence for Cocirculation of CRF14_BG and Subtype F1 Strains

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Abstract

Since 2011, Romania has faced an HIV outbreak among injecting drug users (IDUs). Our aim was to identify and describe clinical and epidemiological patterns of this outbreak. A cross-sectional study enrolled 138 IDUs diagnosed with HIV infection between 2011 and 2013 with 58 sexually infected individuals included as the control group. The IDUs had a long history of heroin abuse (10 years) and a recent history of new psychostimulant injection (3–4 years). Classical epidemiological data and molecular techniques were used to describe the transmission dynamics. A high prevalence of hepatitis C virus (HCV) coinfection was noted (98.6%) compared to the control group (10.3%) ($p < 0.001$). IDUs had initially been infected with HCV. HIV infection was more recent, linked to starting injecting stimulants. HIV subtype analysis showed a predominance of the local F1 strain in both IDUs and sexually infected patients; in IDUs it also identified 28 CRF14_BG recombinants and six unique recombinant forms (URFs) between F1 and CRF14_BG. A few patients from both risk groups were infected with subtype B. Among IDUs, CRF14_BG was associated with a lower CD4 cell count and more advanced stages of disease, which correlated with CXCR4 tropism. Phylogenetic analysis revealed the spread of HIV through three major IDU clusters of recent date. Among IDUs with CRF14_BG, some reported travel abroad (Spain, Greece). By identifying clusters of IDUs with related viruses, molecular epidemiologic methods provide valuable information on patterns of HIV transmission that can be useful in planning appropriate harm reduction interventions.

Introduction

Despite significant progress in therapy and prevention HIV-1 infection remains one of the most important public health challenges: in 2012, more than 29,000 new HIV cases were diagnosed in the European Union and European Economic Area Member States.[1] Injecting drug use represents another major international public health issue and transmission of bloodborne viruses is very common within this population. The magnitude of the HIV epidemic among injecting drug users (IDUs) varies worldwide, reaching a high prevalence (more than 40%) in countries from Southeast Asia, Eastern Europe, and Latin America.[2] Furthermore, AIDS-related diseases represent a major cause for mortality among IDUs.[3]

The HIV epidemic in Romania started in the late 1980s in adults and spread extensively soon after in newborns and children. The overuse of parenteral treatments with improper sterilized needles or syringes in children from orphanages is thought to have contributed to this situation.[4] The pediatric population represents half of the accumulated HIV cases reported in Romania (9,931 of 18,797).[5] The young adults infected in the late 1980s as infants and young children still account for half of the people currently living with HIV.[5] The other distinctive feature of this epidemic is the high prevalence of subtype F1. Previous phylogenetic studies consistently described a well-supported monophyletic clade including all the analyzed strains from Romania, closely related to sequences from Angola.[6,7] In recent years, other subtypes have been increasingly described in newly diagnosed patients.[8]

Until early 2011, the major risk behavior for the spread of HIV-1 in the Romanian adult population was unprotected heterosexual contact. Annually, only a few new cases of

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infection were reported among men who have sex with men (MSM) and sporadic cases in IDUs.[9] The incidence of HIV infections among IDUs increased in recent years, accounting for one-third of new HIV cases. This is correlated with the rising number of young IDUs as a result of the introduction of ''legal highs'' to the drug market.[10] These new psychoactive substances (NPS) were very accessible, cheaper, and caused more serious psychological and behavioral alterations, leading to greater risk taking (sharing needles and syringes, multiple sexual partners).[11] The most affected region is the Bucharest metropolitan area, which in 2013 accounted for more than 80% of the new HIV cases among IDUs.[12]

To better understand the recent HIV outbreak among IDUs in Bucharest, we have analyzed epidemiological factors and the patterns of HIV-1 spread in this particular population by means of molecular epidemiology.

Materials and Methods

Study population

We have enrolled 138 IDUs who were diagnosed with HIV infection from 2011 to 2013 in the National Institute for Infectious Diseases ''Matei Bals,'' Bucharest. During this time span 616 new cases of HIV infection were diagnosed among IDUs in Romania, out of which 494 were located in the Bucharest area. For comparison, 58 patients infected by sexual contact, diagnosed during the same time interval and in the same location as the IDUs, were also included in this study. Epidemiological, demographic, and clinical data were collected through a questionnaire survey (age, gender, residence, socioeconomic status, imprisonment, risk factors—sexual and injecting drug use, travel abroad, clinical stage of HIV) and laboratory tests [CD4 count at baseline, viral load at diagnosis, coinfections—hepatitis C virus, hepatitis B virus (HCV, HBV), syphilis] were performed.

All the patients were naive to antiretroviral drugs at the moment of blood sampling. The plasma samples were obtained from blood drawn on EDTA by centrifugation at 3000 rpm for 15 min and stored at $-80°C$ prior to genotypic testing. The study has been conducted according to the current local ethical regulations. Informed consent was signed by all participants.

HIV sequencing

HIV-1 RNA was extracted from plasma using the automated NucliSens easyMAG nucleic acid extraction system (BioMerieux) and specific magnetic extraction reagents, according to the manufacturer's instructions. The first part of the *pol* gene (coding for protease and two-thirds of reverse transcriptase) was amplified using the reagents included in the Viroseq HIV-1 Genotyping System (Celera Diagnostics, Alameda, CA) and sequenced on the ABI 3500 Instrument (Applied Biosystems). The sequences were analyzed primarily using Sequencing Analysis Software Version 3.7 (Life Technologies) and assembled with ViroSeq 2.8 HIV-1 Genotyping System Software (Celera Diagnostics, Alameda, CA) to generate a consensus sequence of about 1,300 bp long. HIV-1 subtype assessment was done with the REGA HIV-1&2 automated subtyping tool version 2.0.[13] SimPlot v3.5.1 software[14] was used to identify the recombination breakpoints (sliding window, 400 nt; T:t ratio = 2.0; model of evolution, Kimura two-parameter; bootstrap, 1,000 replicates). All sequences were screened for hypermutation using the Hypermut 2.0 algorithm.[15]

Tropism testing

Forty-five HIV-1 samples isolated from IDUs (9 CRF14_BG and 36 F1 subtype) were further analyzed for viral tropism using a genotypic test. The V2-V3 region of the *env* gene was amplified as described previously.[16] Cycle sequencing was performed with the BigDye Terminator system v 1.1 (Life Technologies). The sequencing products were analyzed on the ABI 3500 instrument (Applied Biosystems) and the resulting sequences were assembled with Seqscape version 2.7 (Applied Biosystems). The tropism prediction was performed using the geno2pheno$_{(coreceptor)}$ 2.5 algorithm.[17] The significance level was defined by a false-positive rate threshold of 10% as recommended by the European guidelines for tropism testing.[18]

Phylogenetic analysis

Phylogenetic analysis was performed using the maximum likelihood method as implemented in PAUP*,[19] using the GTR (general time reversible) as the model of evolution and gamma (Γ) distribution of variability of rates between sites, calculated empirically from the data with four categories of rates. Bootstrapping was performed on the neighbor-joining trees (1,000 replicates) to assess the robustness of the obtained topologies.

The reference sequences used for subtype F1 subtype analysis were sequences from Romanian patients infected heterosexually and diagnosed in recent years (JQ083067, JQ083056, JQ083041, JQ083077, JQ083074, JQ083062, JQ083051, JQ083048, JQ083044, JQ083026, JQ083025, JQ083018, JQ083014), several sequences corresponding to the children infected in the late 1980s in Romania (EU553014, EU552988, EU552998, EU553003, EU553097, EU553080, EU553082, EU553175, EU552942, EU553143, EU553113, EU553196, EU553251, EU553210, EU553235), as well as F1 sequences from Angola (FJ688211, FJ688212, JN937026, JN937080, JN937089, JN937113, JN937051, JN937039, JN937068, JN937092). HIV-1 subtype F2 sequences from Cameroon used as the outgroup have the following accession numbers: AY444279, AY444280, FJ688211, and FJ688212.

Transmission networks were assigned as those clades consisting of at least four sequences of Romanian origin receiving $>75\%$ bootstrap support. Phylogenetic trees were visualized using FigTree software version 1.2.3.[20]

Phylodynamic analysis

Molecular clock analysis for the three IDU clusters (two of subtype F1 and one of CRF14_BG) was performed using the Bayesian method as implemented in BEAST (version 1.7.4).[21] The datasets for subtype F1 consisted of IDU and reference sequences from Romania sampled between 2011–2013 and 1993–2003, respectively. For CRF14_BG we assembled a data set with IDU sequences from Romania and reference CRF14_BG sequences available in the HIV sequence database, isolated in Spain and Portugal (AF423756, AF423757, AF423758, AF423759, AF450096, AF450097, FJ670518, FJ670522, FJ670528, GU230137, JX140649). The sampling window for CRF14_BG was 14 years (1999–2013). Molecular clock analysis was performed using the GTR + G nucleotide substitution model and uncorrelated lognormal

relaxed clock model with TipDates. For coalescent tree priors we used Bayesian skyline plots. Markov chain Monte Carlo (MCMC) was run for 30×10^6 generations sampled every 1,000 generations with a burnin of 30×10^5. We assessed convergence and sufficient mixes of the Markov chains [effective sample size (ESS) >100] using the program Tracer v1.4.[22] The consensus for each run was inferred by the TreeAnnotator program.

Statistical analysis

Statistical analysis was performed using SPSS Statistics v.17.0 (IBM, New York, NY). All variables were tested for normality using the Shapiro–Wilk test; parametric variables were expressed as mean ± SD and nonparametric variables as median (IQR). To analyze differences between different subgroups we used the Mann–Whitney *U* test for continuous nonparametric variables and the independent sample *t*-test for continuous parametric ones. For categorical variables the Chi-square or Fischer test was used. The significance level was set at 0.05.

Results

The present study investigates the clinical and epidemiological features of HIV-1-infected IDUs as well as the transmission chains among these patients. Our results showed that the majority of the IDUs were young men (median age of 29 years) living in the Bucharest metropolitan area. Coinfection with HCV was present in 98.6% of them with a median duration of diagnosis of 6 years, as documented in the hospital database. Hepatitis B coinfection (HBsAg positive) was detected in 19 (13.8%) IDUs. Most of the IDUs (126 of 138) had been heroin users (with a median abuse duration of 9 years) and they also started injecting NPS within the past 2–3 years. Ninety heroin users (71.4%) were on substitution therapy at the moment of evaluation, but 72 (80%) of them had had relapses under the opioid substitution treatment, showing its poor efficiency. Most of these patients had no HIV/AIDS-related symptoms (clinical stage A 73.9%) and had good immunological status, both consistent with relatively recent HIV acquisition: about 20% of the IDUs had an HIV-negative test in the past 2 years. One-third of the IDUs (47 of 138) were diagnosed with HIV while in detention. By comparison, the group infected by heterosexual contact consisted of patients with more advanced disease and lower CD4 counts. Several other statistically significant differences that were identified between IDUs and heterosexual patients are presented in Table 1. More IDUs reported travel to Spain as compared to the heterosexuals (26.1% vs. 5.2%, $p<0.001$).

Table 1. Comparison Between Intraverous Drug Users and Patients Infected by the Sexual Route

Characteristic	*IDUs* N = *138*	*Sexual contact* N = *58*	p-*value*	*OR (95% CI)*
Age (years)				
Median [range]	29 [16–69]	34 [20–64]	0.014 (Mann–Whitney)	
IQR	7	14		
Gender—Male	113 (81.9%)	37 (63.8%)	0.009	2.5 (1.2–5.1)
Clinical stage				
A	102 (73.9%)	17 (29.3%)	<0.001	6.8 (3.4–13)
B	26 (18.8%)	28 (48.3%)	<0.001	0.2 (0.1–0.4)
C	10 (7.2%)	13 (22.4%)	0.006	0.2 (0.1–0.6)
CD4 count (cells/mm^3)				
<200	22 (15.9%)	18 (31%)	0.002	0.4 (0.2–0.8)
200–500	62 (44.9%)	29 (50%)	0.5	
>500	54 (39.1%)	11 (19%)	0.008	2.7 (1.3–5.7)
In prison				
Yes	47 (34.1%)	1 (1.7%)	<0.001	29 (3.9–219)
No	91 (65.9%)	57 (98.3%)		
HCV coinfection	136 (98.6%)	6 (10.3%)	<0.001	589 (115–3013)
HBV coinfection	19 (13.8%)	11 (19%)	0.3	
VDRL	7 (5.1%)	3 (5.2%)	1	
TPHA	12 (8.7%)	14 (24.1%)	0.001	0.3 (0.1–0.7)
Opportunistic infections	10 (7.2%)	13 (22.4%)	0.006	0.2 (0.1–0.6)
Travels abroad	56 (40.6%)	24 (41.4%)		1
Greece	15 (10.9%)	3 (5.2%)	0.1	
Spain	36 (26.1%)	3 (5.2%)	<0.001	7.7 (2.2–26.3)
HIV subtype				
F1	94 (68.1%)	51 (87.9%)	0.004	0.2 (0.1–0.6)
CRF14_BG	28 (20.3%)	1 (1.7%)	<0.001	14 (2–109)
B	8 (5.8%)	6 (10.3%)	0.3	
CRF02_AG	1 (0.7%)	0 (0.0%)		
CRF14BG_F1 recombinant	6 (4.3%)	0 (0.0%)		
B_F1 recombinant	1 (0.7%)	0 (0.0%)		
Negative HIV test in the past 2 years	26 (18.8%)	2 (3.4%)	0.003	6.5 (1.4–28)

IDUs, injecting drug users; OR, odds ratio; CI, confidence interval; IQR, interquartile range; HCV, HBV, hepatits C and B virus.

Table 2. Characteristics of Intravenous Drug Users with CRF14_BG

Patients N *(%)*	*IDUs with CRF14_BG* N = *28*	*IDUs with other subtypes* N = *110*	p-*value*	*OR (95% CI)*
CD4 count (cells/mm^3)				
<200	11 (39.3%)	11 (10%)	0.001	5.8 (2.1–15)
200–500	10 (35.7%)	52 (47.3%)	0.2	
>500	7 (25%)	47 (42.7%)	0.1	
Travels abroad	16 (57.1%)	40 (36.4%)	0.05	2.3 (1–5.4)
Spain	7 (25%)	29 (26.4%)	0.8	
Greece	9 (32.1%)	6 (5.5%)	0.001	7.5 (2.3–23)
Clinical stage				
A	14 (50%)	88 (80%)	0.003	0.2 (0.1–0.6)
B	10 (35.7%)	16 (14.5%)	0.016	3.2 (1.2–8.3)
C	4 (14.3%)	6 (5.5%)	0.1	
CXCR4 tropism	7 (77.8%)	8 (22.2%)	0.003	12.2 (2–70)
CCR5 tropism	2 (22.2%)	28 (77.8%)		

IDUs, injecting drug users; OR, odds ratio; CI, confidence interval.

HIV-1 subtyping analysis indicated that subtype F1 was present in 68.1% of the IDUs and in a greater proportion (87.9%) in the heterosexual group. Rather unexpectedly, as many as 28 IDUs (20.3%) and one heterosexual (sexual partner of an IDU) were found to be infected with the CRF14_BG recombinant. Statistical data indicated that the drug-injecting patients infected with this recombinant form have lower CD4 counts and clinically more advanced disease at the moment of diagnosis (Table 2). The geno2pheno$_{(coreceptor)}$ algorithm predicted that seven of nine CRF14_BG samples tested had CXCR4 tropism whereas the majority of the F1 subtype strains tested (28 of 36) were CCR5 tropic; the difference is statistically significant ($p = 0.003$). Travel outside Romania, especially to Greece, was reported more frequently for IDUs infected with CRF14_BG strains than for those infected with other subtypes (Table 2).

We have identified six potentially new intersubtype recombinant forms between subtype F1 and CRF14_BG (recombination analysis of full-genome sequences is pending). Distinct phylogenetic analyses were performed for each subtype observed in the IDUs: subtype F1, CRF14_BG, and subtype B. The phylogenetic tree of Romanian subtype F1 sequences is presented in Fig. 1. The sequences analyzed are distinctly marked in the figure: IDUs in red and sexually infected patients in blue. We have also included in the analysis previously studied sequences from heterosexual adults diagnosed in 2007–2009 (black) and sequences from children infected in the late 1980s and diagnosed in early childhood (green). This analysis pointed to the existence of two major transmission groups among IDUs infected with subtype F1. The first cluster consists of 23 sequences isolated exclusively from IDUs and highlighted in blue in Fig. 1;

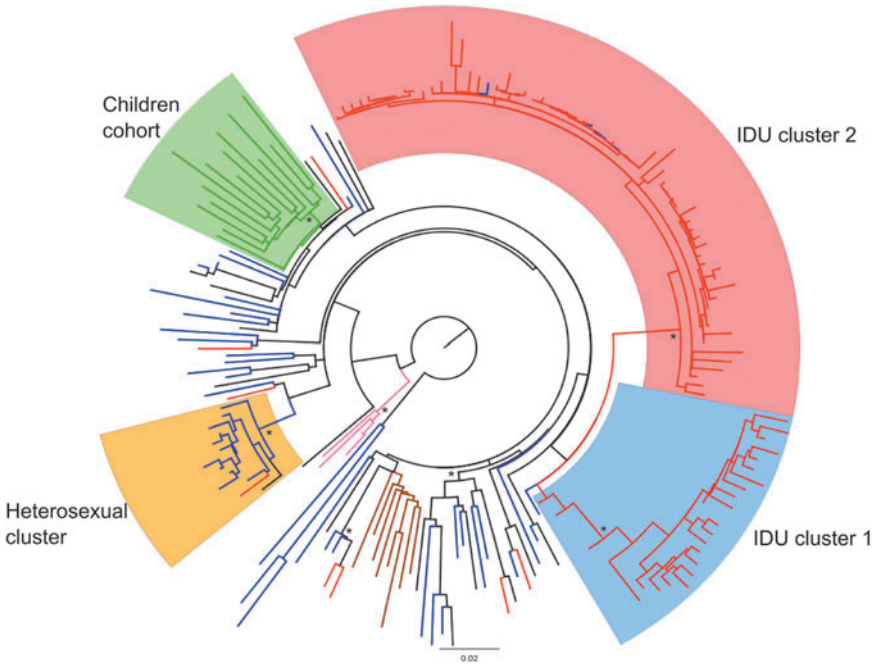

FIG. 1. Maximum likelihood phylogenetic analysis of Romanian F1 subtype sequences. The sequences corresponding to intravenous drug users (IDUs) are in *red* and those from sexually infected patients are in *blue*. The F1 reference sequences were marked as follows: Angola (*brown*), Romanian nosocomial infected children (*green*), and Romanian heterosexuals diagnosed in 2007–2009 (*black*). The tree was rooted using subtype F2 sequences (*pink*) as the outgroup. The bootstrap support values greater than 0.75 are indicated by an *asterisk* at the nodes. Different clusters were specifically marked on the tree.

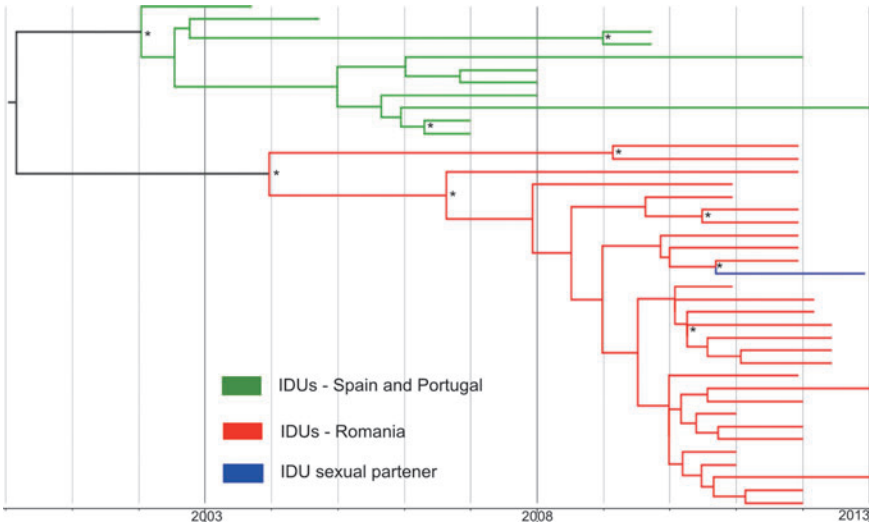

FIG. 2. Molecular clock analysis of CRF14_BG sequences isolated from IDUs. The tree was generated as described under Materials and Methods. The internal node labels represent the posterior probability support (only values higher than 0.9 are indicated by an *asterisk*). The scale is in years.

almost 50% of them (11/23) originate from men diagnosed in the same detention center. One-third of the sequences ($n=8$) in this cluster correspond to persons infected recently (HIV-negative test within 1 year) and almost all the IDUs from this group (22 of 23) are HCV coinfected.

The second F1 cluster identified is larger and includes 62 sequences from IDUs and six sequences from sexually infected patients. This cluster is highlighted in red in Fig. 1. A total of 25 persons in this group were diagnosed during detention and five of them were seroconverters as documented by a negative HIV test within the previous 6 months. Sequences from persons infected by heterosexual contact were more scattered in the tree; only one group of 11 sequences clustered together with one sequence from IDUs and one reference sequence (from a Romanian heterosexual adult diagnosed in 2008). This group is distinctly marked in Fig. 1 (orange).

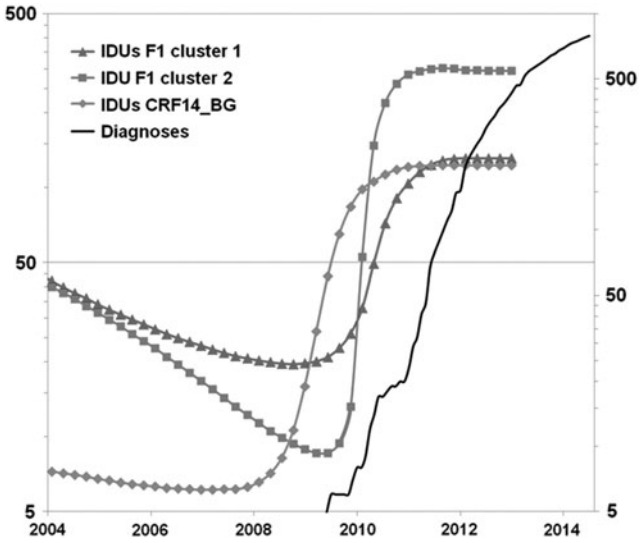

FIG. 3. Population dynamics for the three IDU specific transmission clusters in Romania as estimated by phylodynamic analyses. On the *y*-axis estimated value is the effective number of infections (Ne) × generation time (T). The numbers of reported cases among IDUs (Diagnoses) over the past years are plotted in the figure.

Molecular clock analysis of CRF14_BG sequences from Romanian IDUs was performed to estimate the time to the most recent common ancestor (tMRCA) of this group. The result is presented in Fig. 2. The tMRCA inferred for the CRF14_BG transmission network among Romanian IDUs was 2004 [95% higher posterior density (HPD): 1999–2008], whereas the time of the split of lineages for most infections within this cluster was more recent, estimated approximately after 2010. The dynamics of CRF14_BG and the two subtype F1 subepidemics among IDUs in Romania was also estimated by coalescent analysis using the Bayesian sky plot model. All the three IDU clades coalesced at a very recent time interval of 1–3 years. These findings are also supported by epidemiological data. When compared to sexually infected patients, larger numbers of IDUs were seroconverters (negative HIV test less than a year before diagnosis) ($p=0.003$). The results are presented in Fig. 3.

The three IDU transmission networks identified by phylogenetic analysis included 114 persons in total, representing 82.6% of the IDU patients analyzed. By comparison, only 11 of the 58 heterosexuals analyzed (18.9%) were grouped in a transmission cluster. A total of 32% of IDUs from F1 transmission groups and 57% from the CRF14_BG cluster had a history of recent foreign travel during which they engaged in risk-taking activities (injecting drugs, unprotected sex).

Discussion

Romania and Greece reported an increase in the number of HIV-1 infections among IDUs since 2011, as opposed to the overall declining trend in newly diagnosed cases among IDUs in other European countries.[23,24] In 2011, Romania reported more than a 6-fold increase in IDU as a risk factor in newly reported HIV-1 infections (18.1%, 131 of 720) as compared to each of the previous two years (2.6%, 14 of 531 newly diagnosed HIV-1 persons in 2010; 1.4%, 7 of 497 new cases in 2009). The increasing trend continued throughout 2012 and 2013, reaching 30.6% (252 patients) and 29.23% (233 patients), respectively.[9,25]

Most of the IDUs recently diagnosed with HIV-1 were young men living in Bucharest and the suburbs, with low socioeconomic status and present or past detentions; in 2013, 83% of HIV-1 cases among IDUs were Bucharest residents.[12]

HCV coinfection was present in more than 90% of the HIV-1-positive patients. The majority had been diagnosed with HCV infection several years before (shortly after starting injecting heroin). The time of HIV-1 infection was more recent for many of them, closely related to the initiation of NPS use. Hence, the HIV-1 epidemic among IDUs in Romania spread in a population with a high prevalence of HCV infection. It has been shown that among IDUs the potential for an HIV-1 epidemic is greater in settings with a high prevalence of HCV.[26] Moreover, mathematical models of HIV and HCV transmission among IDUs who share needles showed that the minimum duration of injecting required to induce an epidemic is 11.6 years for HIV and only 2.3 years for HCV.[27] Travel to European countries, mainly to Spain and Greece, was reported by 40% of the IDUs studied and the proportion was higher in CRF14_BG-infected IDUs than in those infected with other subtypes. The majority admitted injecting drugs and/or engaging in unprotected sex while traveling. Hence, some of the new HIV cases among IDUs with CRF14_BG were likely imported from Spain and Portugal and spread to Romania and then probably to Greece[28] as suggested by phylogenetic analysis as well.

Differences among IDUs' clinical pictures and laboratory findings were observed to correlate with the subtype of the infective strain: those infected with the F1 subtype were frequently asymptomatic, with higher CD4 counts, and the tropism of the virus was CCR5, while CRF14_BG-infected IDUs were diagnosed in more advanced stages of disease, with lower CD4 counts and with CXCR4 tropic viruses. This observation is consistent with previous studies that showed, using both genotypic and phenotypic testing, that the CXCR4 phenotype was very common at baseline among CRF14_BG-infected patients.[29] This leads to more rapid disease progression in patients infected with CRF14_BG.[30]

Among newly diagnosed patients infected heterosexually, the late presenters (CD4 counts <250 and/or CDC stage C) carrying F1 subtype strains were frequent. These observations correlate with the data reported for the last 2 years by the Romanian HIV/AIDS National Committee.[31] Bucharest and its suburbs are facing a recent and dramatic increase in the number of IDUs who are using both NPS and heroin: more than 90% of the IDUs are concentrated in this region.[32]

This particular situation emerged due to changes in the drug market: so called legal highs (NPS) were introduced in Romania in 2009. Due to their broad accessibility, the estimated number of IDUs rose from 17,000 in 2008 to about 20,000 in 2011.[33] In addition, IDUs switched from heroin use to injecting legal highs; these changes in drug use patterns constitute the major cause for the dramatic increase in the number of new HIV cases, resulting from the fact that injection of amphetamine-type stimulants (NPS) is associated with higher risk behavior than heroine use. Stimulants are injected more frequently than heroine (6–10 times a day as compared to 3–5 times a day for heroine) and there are reports of high levels of risk behavior related to NPS use, mainly due to mental disturbances: increased syringe sharing and unprotected sex.[25] Unfortunately, no substitution therapy is currently available for NPS users. In addition, opioid substitution therapy for heroin users and needle-exchange programs are inefficient. The last reports from 2012 showed that the number of IDUs started to decrease, although the number of new HIV cases among them continuously increased. [9,33]

This HIV outbreak among IDUs coincided with the cessation of activity of several international programs and funding available from the Global Fund to Fight HIV/AIDS, Tuberculosis and Malaria and United Nations Office on Drugs and Crime (UNODC) that had offered harm reduction services until 2010.

The HIV epidemic in Romania has distinctive traits: the F1 subtype is responsible for nearly all of the HIV-1 infections in the country.[34] This subtype was most likely introduced into Romania from Angola in the early 1980s and spread in adults and children within the borders due to the almost closed status of the country. With only a few exceptions (some cases of infections with subtype C), all the children infected in the late 1980s were carrying F1 subtype viruses. This subtype is also frequent among sexually infected adults. Subtypes other than F1 reported in adults are subtype B, more frequent among MSMs, and subtype C, detected in heterosexuals.[35]

Our study showed that in IDUs, CRF14_BG was the second most encountered HIV-1 type after the F1 subtype. Before 2011, only a single patient infected with this recombinant was reported in Romania.[36] Although it had been initially isolated in the early 2000s in Spanish IDUs from Galicia, near the border with Portugal, recent phylodynamic analysis of CRF14_BG indicated a Portuguese origin; in Portugal, subtypes B and G are cocirculating.[29] In Romania, it was identified almost exclusively in drug-injecting patients, the same risk category as in the origin countries, Spain and Portugal. Estonia, Italy, and Germany also reported HIV cases with CRF14 strains, but fewer accounts of this particular CRF were recorded in recent years in Europe.[37] Recombinant forms between subtype F1 and CRF14_BG were found in IDUs. Due to their risk-taking behavior superinfections and recombination events are to be expected, potentially leading to the selection of more virulent strains.

This study has a number of limitations: the studied sample represents 22.4% of all new reported cases among IDUs during the study period. We did not have access to all the patients diagnosed in Bucharest. Furthermore, complete data sets including HIV sequences could be generated for only 138 persons. Tropism testing was performed only for one-third of IDUs and the full-genome sequencing data to confirm the new recombinant forms are not available at this point.

We have identified by phylogenetic approaches three major transmission networks among IDUs: two infected with F1 subtype strains and one with the recombinant form CRF14_BG. Some of the patients in the two F1 transmission clusters identified were diagnosed in the same detention center.

Epidemiological data indicated that Romania was the probable origin of the strains in these patients. More than half of CRF14_BG-infected IDUs indicated travel to Greece and Spain. Recent data show that CRF14_BG is also involved in the HIV-1 outbreak among IDUs in Greece.[28]

Using molecular epidemiology tools to characterize the HIV subepidemics in IDUs proves to be of significant value for public health: molecular typing of circulating strains and predicting some biological characteristics (e.g., virulence) may influence disease control strategies accordingly.

Sequence Data

Nucleotide sequences reported in this article are available in the GenBank and have the following accession numbers: KJ194636–KJ194831

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Contributions of the authors: I.N., study design, patients selection, clinical data collection, and statistical analysis; S.P., study design, molecular testing, phylogenetic analysis, and drafting of the manuscript; D.P., phylodynamics analysis and drafting of the manuscript; A.A., patient selection and clinical data collection; I.B., molecular testing and clinical data collection; L.B., clinical data collection and drafting of the manuscript; D.O., study design and drafting of the manuscript.

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Author Disclosure Statement

No competing financial interests exist.

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