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PRE TREATMENT HIV-1 DRUG RESISTANCE IN TRANSMISSION CLUSTERS OF THE COLOGNE-BONN REGION, GERMANY

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Abstract

Objectives: In Germany, previous reports have demonstrated transmitted HIV-1 drug resistance mutations (DRM) in 11% of newly diagnosed individuals, highlighting the importance of drug-resistance screening prior to initiation of antiretroviral therapy (ART). Here, we sought to understand the molecular epidemiology of HIV DRM transmission in the Cologne-Bonn region of

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Contribution: AC, MS, MH, SM and JV designed the study, AC, MS, MH and SM analysed and interpreted the data. JV, MS, AE, CL, GF, JW, and EK provided the data, and contributed critically important ideas on how to interpret the data. MS, AC and MH drafted the primary draft of the manuscript. SM, JV, AE, CL, GF, JW, and EK revised the manuscript critically for important intellectual content. All authors revised and approved the final version of the manuscript.

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Germany, given one of the highest rates of new HIV diagnoses in Western Europe (13.7 per 100,000 habitants).

Methods: We analysed 714 HIV-1 ART naïve infected individuals diagnosed at the University Hospitals Cologne and Bonn between 2001 and 2016. Screening for DRM was performed according to the Stanford University Genotypic Resistance Interpretation. Shared DRM were defined as any DRM present in genetically linked individuals (<1.5% genetic distance). Phylogenetic and network analyses were performed to infer putative relationships and shared DRMs.

Results: The prevalence of any DRM at time of diagnosis was 17.2% (123/714 participants). Genetic transmission network analyses showed comparable frequencies of DRM in clustering vs. not clustering individuals [17.1% (85/497) versus 17.5 % (38/217)]. The observed rate of DRMs in the region was higher than previous reports 10.8% (87/809) (<0.001), revealing the need to reduce onward transmission in this area. Genetically linked individuals harbouring shared DRMs were more likely to live in suburban areas (24/38) than in central Cologne (1/38) (p<0.001).

Conclusion: The rate of DRMs was exceptionally high. Network analysis elucidated frequent cases of shared DRMs among genetically linked individuals, revealing the potential spread of DRMs and the need to prevent onward transmission of DRM in the Cologne-Bonn area.

Keywords

HIV; Drug Resistant Mutations; Cluster; Phylogenetic Analysis; Network Analysis

INTRODUCTION

HIV treatment options have improved considerably in recent years, but transmitted HIV-1 drug resistance mutations (DRM) remain a matter of concern. Hofstra and colleagues (1) have studied transmitted HIV-1 drug resistance mutations in 26 European countries and detected an overall prevalence of 8.3%. Transmitted DRM not only affect response to antiretroviral therapy (ART), but also viral escape from pre- and post-exposure prophylaxis strategies. In Germany, nation-wide estimates of DRM are not available, and reported DRM prevalence for specific regions ranges between 10.4% and 12.5% (2, 3). Importantly, the occurrence of transmitted DRM varies between geographic regions and risk groups, with the highest prevalence among men having sex with men (MSM) (12.5%), heterosexuals (HTS) (10.3%) and people with injection drug use (PWID) (4.8%) (4). Geospatial analyses may help to explain the differing DRM rates between risk groups and regions.

Here, we sought to determine the dynamics and spread (i.e. geospatial diffusion) of HIV DRM in the Cologne-Bonn region, an area with one of the highest rates of new HIV infections in Europe (13.7 per 100,000 habitants) (5). Furthermore, we sought to determine risk groups and population correlates of DRM transmission to guide future public health responses.

METHODS

Phylogenetic and molecular network analysis were performed on 714 HIV-1 infected and ART naïve individuals, receiving care at the University Hospitals of Cologne (n= 558) and Bonn (n= 156) between 2001 and 2016. HIV-1 partial *pol* sequences (HXB2 position 2550 → 3356) were obtained from blood plasma at the time of diagnosis prior to ART initiation. Sequencing technology evolved from Sanger sequencing on an ABI 3130×l Genetic Analyser (Applied Biosystems, CA, USA) between 2001–2014 to Next-Gen Sequencing (NGS) (Illumina MiSeq, CA, USA) in 2015–2016 (HIV *pol* consensus sequences representative of at least 15% of each participant's HIV NGS data were considered for the latter, consistent with Sanger sequencing sensitivity (6)). Partial *pol* sequences were subtyped, and the genetic transmission network inferred as described before (7–10). DRM screening was performed according to the Stanford University Genotypic Resistance Interpretation (https://hivdb.stanford.edu/). Shared DRM were defined as any DRM present in genetically linked individuals.

Geospatial analysis was performed using the 3-digit zip code of the residential address from each participant (11). In these analyses, we compared individuals who were living in the city centre of Cologne, to those living in the surrounding/suburban areas according to the zip codes, as described previously (9). To identify possible correlates of DRM, the sociodemographic characteristics of individuals who harboured any DRM and shared DRM were assessed. This project was approved by the ethics committee of each partner sites.

RESULTS

Of the 714 participants the majority were male (582/714; 81.5%), and reported MSM as main risk factor (408/714; 57.1%) (Table 1).

The prevalence of any DRM at time of diagnosis was 17.2% (123/714 participants) and remained stable over time [2010–2016: 17.4% (44/253) vs. 2001–2009: 17.1% (79/461); p=0.931; OR per year 0.96, 95% CI 0.26–3.46]. Nucleoside-and non-nucleoside reverse transcriptase inhibitor (NRTI/NNRTI) resistance mutations were detected in 97/714 (13.6%) and 49/714 (6.9%) individuals, respectively. Of the DRMs, E138A (36/714; 5.0%) and K103N (23/714; 3.2%) were the most frequently observed. The likelihood that individuals harboured any DRM did not differ between groups (age, gender, HIV-1 subtype, risk group, residential area, and country of origin).

Transmission network analysis found 217/714 (30.4%) genetically linked individuals forming 77 clusters and ranging in size from 2 to 8 (Figure 1A). Individuals living in the suburban areas of Cologne or Bonn were more likely to cluster compared to people living in the city centre of Cologne (25/95, 26.3%) (OR 1.70; 95% CI 1.01–2.86; p=0.04). The frequency of DRM did not differ between clustering and non- clustering individuals [17.5% (38/217) versus 17.1% (85/497); p=0.89]. Further details of the transmission network analysis are shown in Supplementary Table 2.

Of the 123 sequences harbouring DRM, we identified 38 (30.9%) that were members of 19 different clusters. Of those, 25/38 (65.8%) were shared by HIV genetically linked partners

living in suburban areas of Cologne (24/38; 65.8%) and (1/38; 2.6%) living in the city centre of Cologne ($p< 0.001$), suggesting DRM transmission among ART naïve individuals (Figure 1A&B). HIV-1 subtype B was associated with a higher proportion of shared DRM (24/539; 4.5%) compared to non-B subtypes (1/175; 0.6%; $p=0.02$). HTS (5/184; 2.7%) were less likely to harbour shared DRM compared to MSM (15/408; 3.7%) and PWID (3/19; 15.8%; $p=0.03$). All observed DRM in PWID were identified in clustering individuals (3/3; 100%) (Table 1). The largest cluster including individuals with shared DRM (seven individuals) was comprised predominantly of MSM and/or suburban residents (Figure 1B).

DISCUSSION

Here we explored the dynamics of DRM transmission among HIV-1 infected ART naïve individuals in the metropolitan area Cologne-Bonn in Germany, a high-incidence region in Western Europe (5). We observed a higher prevalence (17.2%) of transmitted DRM compared to previous reports from Germany (2, 4) and other European countries (1). This may be due to local onward transmission from individuals failing ART or among drug-naïve individuals with transmitted DRM. Also, a long history of ART availability for early HIV stages may have contributed to the high prevalence of DRM in the Cologne-Bonn region. The relatively high proportion of DRM that were shared (30.9%) in Cologne-Bonn might be the result of several factors. In clustering individuals, shared DRMs were most prevalent among PWID who were living in suburban areas and were part of transmission clusters with HTS (12). The two clusters including PWID were diagnosed in 2015 and 2016 and might represent sources of local onward transmission among PWID and transmission to other risk groups. The largest cluster of shared DRM was identified among MSM living in suburban areas, suggesting that these individuals contribute significantly to the local epidemic.

This finding may inform future allocation of HIV testing and prevention services, which are currently mainly concentrated in the city centre of Cologne (6 out of 7), and may therefore insufficiently reach suburban populations at risk for transmitted DRM.

Our findings have several limitations, including a limited sample population and a convenience sampling approach, both of which could lead to bias. Based on the national surveillance report, an estimated number of 2,401 persons were newly diagnosed with HIV in the Cologne-Bonn region between 2001 and 2016 (13). We used a conservative approach, which made the dataset smaller but cleaner to preclude the possibility that transmitted DRM was in fact an acquired DRM. As a result we did not reach the suggested 50% sampling for phylogenetic studies (14), but our study population still represented approximately 30% and is therefore comparable to previous studies (15). Also our risk factors assessments over time focused on the primary risk factor for HIV. The influence of substance use in MSM and chemsex on likelihood of DRM transmission could therefore not be assessed.

In conclusion, we were able to show that the rate of transmitted DRMs was high in the Cologne/Bonn area, with the highest prevalence among MSM and HTS. Network analysis elucidated numerous cases of shared DRMs among genetically linked individuals most frequent in PWID and MSM living in suburban areas. Our findings highlight the necessity to

implement effective prevention interventions targeting suburban populations and groups at risk in the Cologne-Bonn area.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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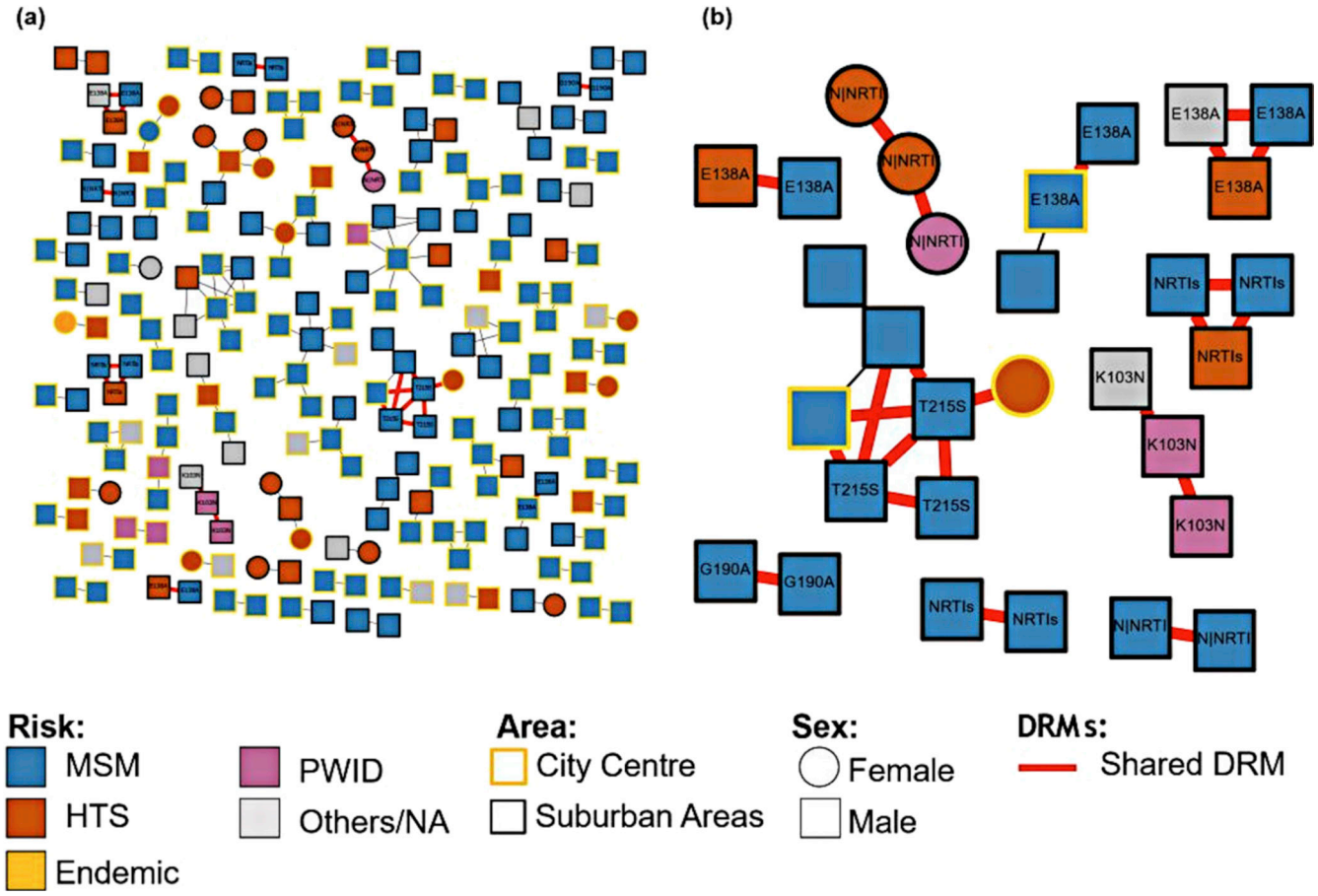

Fig. 1.

(a) Human immunodeficiency virus type 1 (HIV-1) transmission cluster of pretreatment drug resistance in the Cologne-Bonn region. The colour indicates the reported risk group and the frames indicate individuals living in the city centre of Cologne (in yellow) or suburban areas (in black) of Cologne-Bonn. Squares and circles indicate male and female. All edges represent a genetic distance of ≤1.5%. Lines in bold red indicate individuals who shared drug-resistant mutations (DRM). (b) Enlargement of clustering individuals harbouring shared DRM labelled with each node. N/NRTIs indicate the presence of one or more nucleoside or non-nucleoside reverse transcriptase inhibitor resistance(s).

Table 1.

Population Characteristics. Baseline demographic, risk- and viral characteristics in individuals harboring DRM and shared DRM.

	Study population N (%)	DRM, N (%)	Shared DRM, N (%)	*p-value***
Total	714 (100)	123 (17.2)	25 (3.5)	
Age				NS
<30	137 (19.2)	30 (24.4)	9 (36.0)	
31–40	240 (33.6)	39 (31.7)	8 (32.0)	
41–50	211 (29.6)	35 (28.5)	6 (24.0)	
>50	126 (17.6)	19 (15.4)	2 (8.0)	
Gender				NS
Male	582 (81.5)	101 (82.1)	22 (88.0)	
Female	129 (18.1)	22 (17.9)	3 (12.0)	
HIV subtype				**0.02**
B	539 (75.5)	98 (79.7)	24 (96.0)	
non-B	175 (24.5)	25 (20.3)	1 (4.0)	
Risk*				**0.03**
MSM	408 (57.1)	74 (60.2)	15 (60.0)	
HTS	184 (25.8)	29 (23.6)	5 (20.0)	
PWID	19 (2.7)	3 (2.4)	3 (12.0)	
ENDEMIC	51 (7.1)	11 (8.9)	-	
Others/Unknown	52 (7.3)	6 (4.9)	2 (8.0)	
Residential area				**<0.001**
Suburban areas[a]	619 (86.7)	106 (86.2)	24 (96.0)	
City center Cologne[b]	95 (13.3)	17 (13.8)	1 (4.0)	
Region of origin				**0.02**
Germany	517 (72.4)	89 (74.2)	23 (80.0)	
Foreign	187 (26.2)	31 (25.8)	2 (13.2)	
Unknown	10 (1.4)			

*MSM, men who have sex with men; HTS, heterosexuals; PWID, persons who inject drugs; ENDEMIC, recent immigration from a country with a HIV prevalence >1%.

**Chi-square and Fisher's exact test. P-values given in bold depict significant results.

[a]Zip codes (including the suburban areas of Cologne or Bonn): 501, 502, 503, 507, 508, 509, 510, 511, 513, 514, 515, 520, 521, 531, 532, 533, 534, 535, 536, 537, 538

[b]Zip code: 506