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MAJOR ARTICLE

A Recent Human Immunodeficiency Virus Outbreak Among People Who Inject Drugs in Munich, Germany, Is Associated With Consumption of Synthetic Cathinones

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Background. Needle and syringe sharing among people who inject drugs (PWID) can result in a rapid regional spread of a human immunodeficiency virus (HIV) variant. Such outbreaks have been identified recently in several countries and have raised public health attention because of an association with new psychoactive substances (NPS).

Methods. Dried serum spots from approximately 60% of newly diagnosed HIV cases in Germany in 2013–2018 were received together with statutory notification data. Samples were sequenced in the *pol*-region, genotyped, and viral phylogenies were analyzed. For selected samples, the hepatitis C virus (HCV) status and the presence of NPS were determined.

Results. An outbreak of closely related 27 subtype C infections with a core of 11 cases with almost identical sequences was identified using phylogenetic analyses. The first case of the outbreak was diagnosed in 2015, and the last one was in 2018. With exception of 3 infections, all were reported from Munich, the capital of the federal state of Bavaria. Of 26 analyzed outbreak members, 24 (92.3%) had a resolved or viremic HCV coinfection. In 8 of 18 (44%) cases, α-pyrrolidinopentiothiophenone and/or the related substance α-pyrrolidinoheptiophenone was identified.

Conclusions. Despite harm reduction services in place, HIV outbreaks of considerable size can occur in PWID. The establishment of a real-time molecular surveillance is advised to rapidly identify outbreaks and target prevention measures.

Keywords. dried serum spots; HIV-1 outbreak; new psychoactive substances; people who inject drugs; synthetic cathinones.

One of the most efficient ways to transmit human immunodeficiency virus (HIV) and hepatitis C virus (HCV) is by multiperson use of drug injection equipment. In a recent report, it has been estimated that PWID are up to 28 times more likely to be living with HIV than people in the general population [1].

In Germany as well as in most other European countries, harm reduction interventions have been established including low threshold services providing drug use paraphernalia, counseling services, and drug consumption rooms [2]. Nevertheless, in a survey conducted between 2011 and 2014, between 5% and 22% of more than 2000 PWID from 8 large German cities reported to have shared needles and syringes in the previous 30 days [3], and an estimated 7.8% (250 of 3200) of HIV infections in 2015 in Germany were associated with injection drug use [4].

Consumption of psychoactive substances has been changing over time. By 2015, approximately 2.8% of adults in Germany had used at least 1 substance from the broad range of stimulant drugs containing novel chemical compounds collectively called new psychoactive substances (NPS), which are designed to mimic established and often more costly illicit drugs [2]. A substantial proportion of these synthetic drugs are derivatives of cathinone (the β-keto analog of amphetamine), naturally present in the Khat plant [5]. Most of the more than 80 synthetic cathinones that were detected by the European Union Early Warning System between 2005 and 2014 have, for varying periods of time, been legally available in European countries coining the designation "legal highs." Often sold as "research chemicals," "bath salts", or mixtures labeled with fancy street names, users are seldom aware of exactly which substance or combination they have obtained [6]. Although most cathinone consumption occurs via snorting or oral use, some groups of users administer them by injection [7, 8]. Despite significant health hazards, widespread injection of synthetic cathinones has been reported among substance users from Hungary and

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Romania [7, 9, 10], although it appears to be restricted to particular groups of high-risk users or novice users in other European countries including Germany [2]. In comparison to opioids, the stimulating effects of most designer stimulants are rather short-lived, necessitating a much higher frequency of injections. This increases the need for sterile equipment and may also lead to a higher risk of unsafe sexual behavior and therefore increased likelihood of HIV and HCV acquisition if the supply of sterile equipment and prevention of sexual transmission are insufficient. Recent reports on HIV outbreaks among PWID in large cities in Romania, Israel and Ireland have associated these outbreaks with injection of synthetic cathinones [11, 12].

In this study, we report the identification and phylogenetic analyses of a similar HIV outbreak. It started in 2015 among PWID in Munich, Bavaria and is associated with the consumption of 2 related synthetic cathinones.

METHODS

Sampling Strategy

In 2013, a molecular HIV surveillance program linked to the German statutory reporting of new diagnoses to the Robert Koch Institute (RKI) was established [13, 14]. Its foremost objectives are the monitoring of transmitted drug resistance, the surveillance of circulating HIV-1 subtypes, exploration of transmission networks, and outbreaks. Within the frame of this program dried serum spots (DSS) prepared from residual blood of approximately 60% of newly diagnosed and notified cases are received from routine laboratories together with a notification form that provides sociodemographic data. The DSS are linked to an anonymized subset of the notification data. The molecular HIV surveillance program has been approved by the data protection officer of the RKI and the Federal Commissioner for Data Protection and Freedom of Information (II-401/008#0016). One outbreak case with a diagnosis in 2015 was sampled by the German HIV-1 Seroconverter Cohort Study [15]. Coverage rates to estimate the outbreak size were calculated by geographic region and transmission group (Supplement 1).

Serology

The HCV serostatus was determined by using the Monolisa HCV Ag/Ab ULTRA V2 kit (Bio-Rad) [16, 17]. Recency of HIV infection was analyzed utilizing a BED-CEIA assay (Sedia Biosciences Corporation) [18, 19].

Ribonucleic Acid Extraction, Polymerase Chain Reactions, Sequencing, and Genotyping

Extraction of viral ribonucleic acid (RNA) from the DSS (Whatman 903 filter paper) and complementary deoxyribonucleic acid (cDNA)-synthesis are described elsewhere [14, 20]. The cDNA was used to amplify protease and reverse-transcriptase (RT) regions of the *pol* gene (*HXB2* positions protease [amino acids {aa} 9–99] and RT [aa 1–252]) [20]. Amplicons were sequenced by next-generation sequencing (NGS) using the Nextera XT kit (Illumina) and the Illumina MiSeq platform with 2 × 300-base pair (bp) paired-end sequencing mode [14, 21]. The NGS reads were processed by an in-house python pipeline including the tools Trimmomatic (version 0.36) [22], FLASH (version 1.2.11) [23], and BWA (version 0.7.15) [24]. As validated previously [14], a 20% threshold was applied to consider mixed bases in the generated consensus sequences.

THe HIV subtype was assigned with the REGA HIV Subtyping Tool (3.0) [25] and COMET HIV-1 (1.0) [26]. In cases in which a subtype could not be assigned, a maximum likelihood (ML) tree was calculated using the HIV-1 subtype reference panel from the Los Alamos HIV sequence database. Presence of resistance-associated mutations was analyzed using the World Health Organization (WHO) Surveillance Drug Resistance Mutation (SDRM) list [27] and the Stanford HIV Drug Resistance Database 8.4 algorithm [28].

Hepatitis C virus-RNA was detected by an in-house quantitative RT-polymerase chain reaction (RT-PCR) assay [29]. Hepatitis C virus genotyping was done by a nested RT-PCR targeting a 674-bp fragment in NS5B (nucleotide positions 7962–8636 based on AF 009606).

Phylogenetic Analysis

To detect clusters, ML analyses were conducted using 261 subtype C sequences (GenBank accession numbers MN658938–MN659181, MK064323, MK064367, MK064367, MK064370, MK064428, MK064475, MK064481, MK064495, MK064496, MK250696–MK250700, MK250702–MK250705) sampled all over Germany between January 2012 and December 2018 [13, 14]. Ten published subtype A sequences (MF124702–MF124711 [30]) were used as the outgroup. All positions related to SDRMs according to the updated WHO SDRM list were removed from the sequences before analyses [27, 28]. For ML analysis, the identification of putative transmission clusters was carried out with the software Transmic using a >99% bootstrap value and a median pairwise patristic distance of 4.5% as cutoff values [30, 31].

Phylogeographic and phylodynamic analyses were conducted as described previously [30]. In short, 95 related German subtype C sequences neighboring the cases that constitute the outbreak were selected from the ML trees. The Basic Local Alignment Search Tool (BLAST) was used to find the 5 closest related sequences in the Los Alamos HIV database. The final dataset consisting of 516 unique sequences was computed with Bayesian Evolutionary Analysis Sampling Trees (BEAST) using the BEAGLE library for 1 000 000 000 steps. An empiric tree was generated using the General Time-Reversible (GTR)+I+G nucleotide substitution model, a lognormal relaxed clock model, and a skygrid coalescence model with 50 grids [32]. The

resulting empiric tree consisting of 2500 subtrees was used for phylogeographic trait analysis concerning risk groups and geography using asymmetric diffusion models.

To further assess the direction of transmission, deep sequence phylogenetic analysis was performed on samples from outbreak individuals with phyloscanner [33]. On the sequenced partial *pol* gene, a series of overlapping 250-bp genomic windows were defined; reads of outbreak cases falling into the region that overlapped each window were aligned with Multiple Alignment Using Fast Fourier Transform (MAFFT). Deep sequence phylogenies were reconstructed with Randomized Axelerated Maximum Likelihood (RAxML). In each tree, the patristic distance between all pairs of individuals was recorded along with the topological relationship between the viral subtrees of each individual.

Analysis of Dried Serum Spots for Designer Stimulants and Opioids

The DSS were extracted with 3 mL methanol using ultrasonication for 15 minutes. After evaporation (40°C, N_2, 100 µL propan-2-ol/HCl [v/v; 3/1]), samples were reconstituted in 100 µL mobile phase and analyzed using an liquid chromatography-electrospray ionization-tandem mass spectrometry system consisting of a Shimadzu Nexera LC system and a Sciex QTRAP 5500 mass spectrometer. Analysis was done in positive scheduled multiple reaction monitoring (sMRM) mode using a Phenomenex biphenyl column for chromatographic separation. More details are given in Supplement 2.

RESULTS

Molecular Surveillance Reveals a Subtype C Outbreak Among People Who Inject Drugs in Bavaria, Germany

In June 2017, genotyping and an initial phylogenetic analysis of new HIV cases from 2013 to 2016 revealed that an increase among PWID in Bavaria [34, 35] was driven by subtype C infections. In the 5-year period before 2015, 11 PWID infections were reported from Bavaria on average compared to 21 in 2015 and 46 in 2016. Although no subtype C was among the genotyped infections in 2013 and 2014, 2 were present in 2015 and 16 in 2016 (Figure 1). Subtype C is relatively rare in Germany with 5.2% of all new diagnoses in 2013 to 2016 [14]. Later, among notified HIV diagnoses from Bavaria in 2017 and 2018, 3 additional PWID subtype C cases were present in samples from 2017 (Figure 1).

These findings prompted a targeted countrywide phylogenetic investigation. All available 263 partial HIV *pol* sequences of subtype C infections diagnosed in Germany between 2012 and 2018 were included. As shown in Figure 2A, the already known 21 Bavarian PWID cases formed a cluster together with an additional 6 cases reported between 2015 and 2018. Five of the additional cases were submitted with the following self-reported transmission group: men who have sex with men (MSM) (n = 1), heterosexual (HET) (n = 2), or without

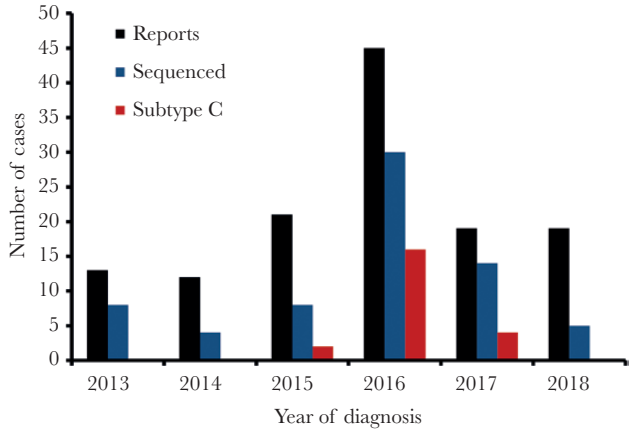

Figure 1. Subtype C in human immunodeficiency virus new diagnoses of people who inject drugs (PWID) in Bavaria. Total numbers of statutory reports from PWID living in Bavaria are shown as black columns (data are obtained from https://survstat.rki.de). Numbers of sequenced cases from PWID in Bavaria are shown as blue columns, and red bars inform about the number of subtype C among sequenced samples.

transmission group data (not reported, n = 2). The sixth additional case belonging to the cluster has been reported from a PWID participating in the German HIV-1 Seroconverter Cohort [15]. The 27 identified cases form an explicit cluster with a very short median pairwise patristic distance of only 0.48%. This indicates a swift transmission. Therefore, we regard this rapidly grown cluster of HIV-1 subtype C infections, defined by the specified ML phylogenetic criteria, as an outbreak.

Month and year of diagnosis of all 27 outbreak cases are shown in Figure 2B. In total, 23 of 27 (85.1%) cases were classified as recent infections (155 days or less between infection and diagnosis), in comparison to 33.1% of non-outbreak cases from Bavaria in the same reporting period (data not shown).

Next, we estimated the potential outbreak size because only a fraction of reported new diagnoses were submitted with a DSS and could be sequenced. Assuming that reported cases were sequenced at random, we estimate that the outbreak may have comprised an additional 21 diagnosed, reported, but not sequenced cases (Supplement 1). This estimate does not account for individuals that were not reported until December 2018, suggesting that the HIV outbreak contained at least 48 individuals (95% confidence interval [CI], 34–60).

Sociodemographic Characteristics, Human Immunodeficiency Virus Drug Resistance, and Hepatitis C Virus Coinfection

Table 1 summarizes demographic and social characteristics of the 27 identified outbreak cases as well as the reported transmission group and the HCV status. In 24 cases, the reported digits of the zip code indicated a residency in Munich. The remaining 3 infections were diagnosed in residents from the nearby Munich metropolitan area. None of the 27 outbreak

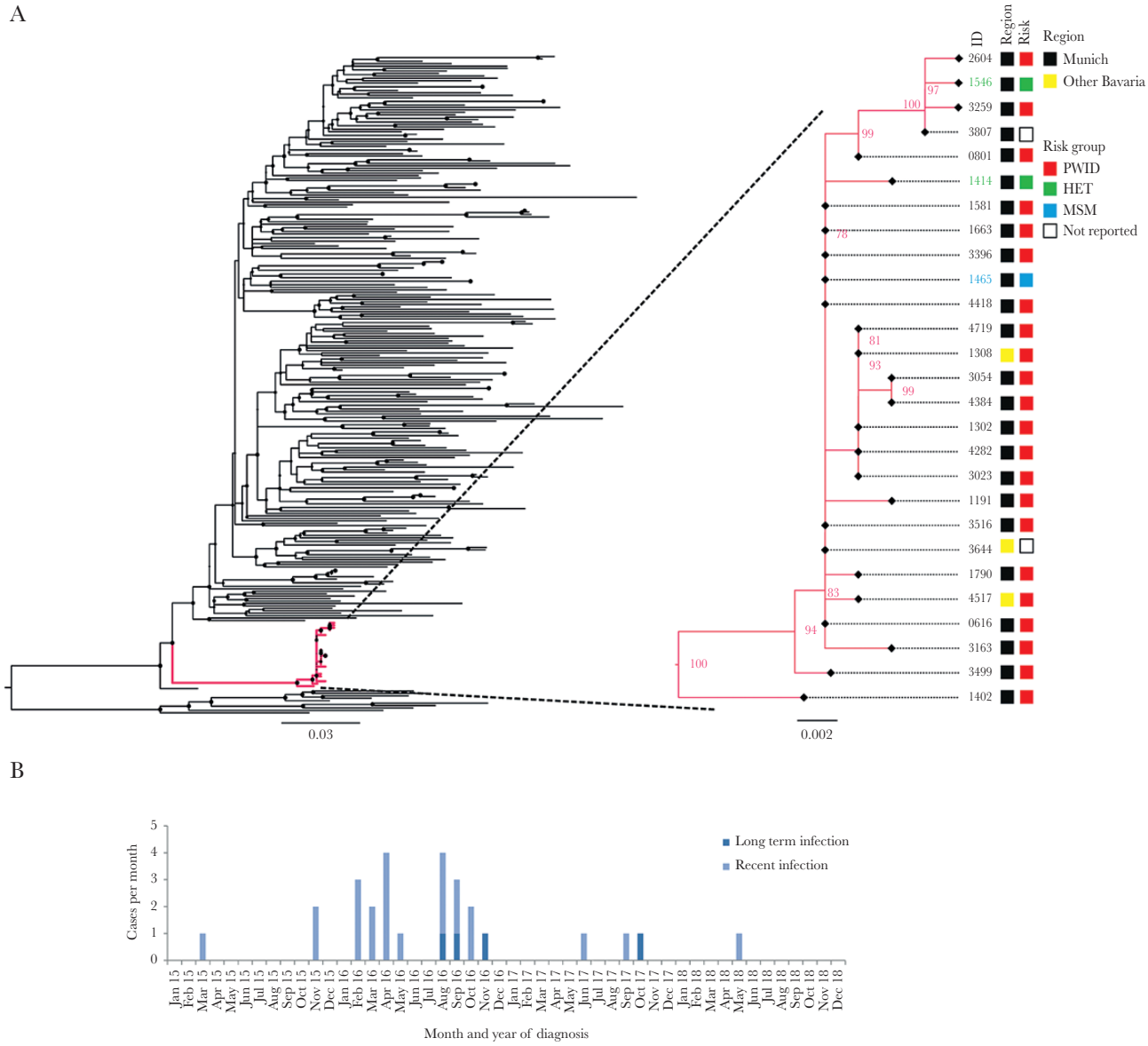

Figure 2. Detection of a subtype C outbreak. (A) Maximum likelihood analyses of 263 German subtype C sequences collected between 2012 and 2019. Ten subtype A sequences were used as outgroup. Node size reflects bootstrap value. The cluster defining the outbreak is highlighted in red and as enlargement. Enlargement: Nodes are depicted as circles according to bootstrap values. Only bootstrap values >80 are depicted by numbers. Tips are highlighted by black diamonds. Transmission groups and living regions are color-coded. (B) Time scale of outbreak cases according to diagnosis dates. Light blue columns represent infections classified as recent by a serological recency test and dark blue columns represent long-term infections.

individuals had a virus with known transmitted drug resistance mutations in the protease and RT (data not shown).

Similar to other countries, HCV infection is significantly more prevalent among PWID in Germany in comparison to the general population. To provide further evidence that this is a PWID outbreak, we determined the HCV serostatus and viremia of the outbreak individuals for which sufficient material was remaining. Anti-HCV antibodies were detected in 24 of 26 (92.3%) cases, and HCV RNA was detected in 17 of 24 (70.8%) cases (Table 1). All 5 outbreak cases for whom injection drug use was not reported on the statutory report form were HCV seropositive. Moreover, HCV genotyping was performed on the 17 RNA-positive samples. Genotype 1a was most common, followed by genotype 3a (Table 1). The HCV sequences of the outbreak cases infected with the same HCV genotype did not cluster in HCV phylogenies (data not shown).

Estimated Origin and Onset of the Outbreak

To assess the geographic and temporal origins of the outbreak, we considered the HIV consensus sequences of the outbreak cases in conjunction with genetically closely related international sequences available from the Los Alamos HIV database,

Table 1. Sociodemographic Characteristics of the Outbreak Cases Newly Diagnosed and Reported in 2015–2018

Demographics and Social Characteristics	Totals (%)
Age (Years)	
<25	1 (3.7)
25–54	8 (29.6)
35–44	15 (55.6)
>45	2 (7.4)
Not reported	
Median age: 35.5	
Sex	
Male	18 (66.7)
Female	8 (29.6)
Not reported	1 (3.7)
Country of origin	
Germany	22 (81.5)
Eastern Europe and Central Asia	2 (7.4)
Western and Central Europe	2 (7.4)
Not reported	1 (3.7)
Country of infection	
Germany	25 (92.6)
Not reported	2 (7.4)
Place of residence	
Munich	24 (88.9)
Outside of Munich	3 (11.1)
Transmission group	
PWID	22 (81.5)
MSM	1 (3.7)
HET	2 (7.4)
Not reported	2 (7.4)
HCV coinfection	
HCV antibodies	24/26 (92.3)
HCV RNA	17/24 (70.8)
Genotype 1a	6/17 (35.3)
Genotype 1b	1/17 (5.9)
Genotype 3a	5/17 29.4)
Genotype 4a	3/17 (16.6)
Genotype 4d	2/17 (11.8)

Abbreviations: HCV, hepatitis C virus; HET, heterosexual; MSM, men who have sex with men; PWID, people who inject drugs; RNA, ribonucleic acid.

and we performed time-resolved Bayesian phylogeographic analyses. The outbreak virus belongs to a Southern African subtype C clade (Figure 3). The most closely related international sequences were all sampled in Austria (Figure 3, red), and the ancestral location of the outbreak virus was attributed to Austria with a geographic posterior probability of 81%. The onset of the outbreak in Germany was estimated to have occurred in 2012/2013 (95% highest posterior density interval, 2005–2013).

Transmission Dynamics Within the Outbreak

To further characterize and resolve the direction of spread within the outbreak, we reconstructed deep sequence viral phylogenies from the primary NGS data of the outbreak cases (Figure 4). One hundred seventy-eight (51%) of the 351 pairs of individuals in the outbreak were classified as phylogenetically linked, suggesting that many transmission histories are compatible with the phylogenetic data. In particular, 11 cases had near identical ancestral virus and formed a core network. Such deep sequence phylogenetic patterns suggest that transmission occurred very rapidly, or at the same exposure event between multiple individuals. The remaining 16 cases were phylogenetically linked to only a limited number of individuals in the core network. Six individuals could be phylogenetically classified as recipients of infection without evidence for onward spread (dead-end recipients), 2 of whom were part of the core network. Three individuals could be phylogenetically classified as sources of infection without evidence for earlier transmission (potential index cases), suggesting that either some individuals remained unsampled or that some of the inferred transmission directions were incorrect.

Study for Designer Stimulants and Opioids

In some of the previously reported HIV outbreaks among PWID, an association with injection of NPS has been identified [36, 37]. Therefore, we examined the anonymous DSS material for the presence of 138 designer stimulants and 73 opioids (Supplementary 2) by tandem-mass spectrometry using 18 DSS samples from the outbreak cases and 11 DSS of non-outbreak cases with reported HIV transmission via injection drug use diagnosed in the same period of time (2015–2016). The 11 DSS controls were from Bavaria (all that were collected), including 5 from Munich. Diverse opioids were detected in 11 of the outbreak cases and in 2 of the control cases (Table 2). Moreover, in 8 of 18 (44.4%) of the DSS from outbreak cases, the alpha-pyrrolidinophenone derivatives alpha-pyrrolidinopentiothiophenone (α-PVT) and/or the closely related drug alpha-pyrrolidinoheptiophenone (PV8) were detected, whereas none of the control samples tested positive for these designer stimulants (Table 2).

DISCUSSION

Molecular examination of new HIV diagnoses combined with routine analysis of linked anonymous statutory reports led to the identification of an outbreak comprising 27 cases diagnosed between March 2015 and March 2018 in Germany. Accounting for incomplete sequence sampling among diagnosed and reported HIV-infections, the HIV outbreak may have comprised up to 48 cases (95% CI, 34–60) by the end of 2018. All observed cases were reported from Munich or nearby Bavarian cities, indicating regional confinement. Injection drug use as the probable mode of HIV transmission in a high proportion of cases (81.5%) together with high HCV seroprevalence (92.3%) among observed cases strongly indicate that this is an outbreak among PWID that shares similarities with a series of recently published occurrences in several European countries, Israel, and the United States [36–40]. A total of 70.8% of the outbreak cases were HCV-RNA positive, imposing a significant risk for future HCV spread

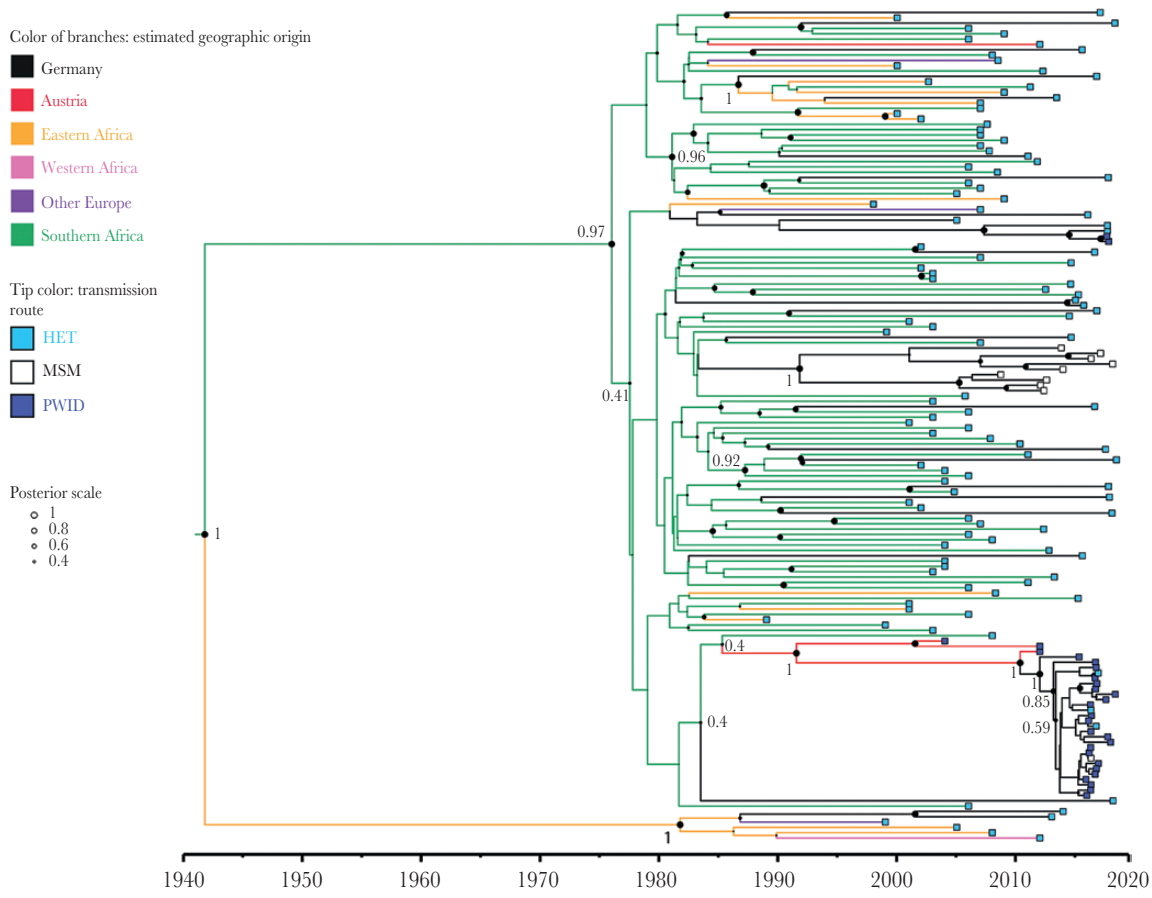

Figure 3. Phylogeographic reconstruction of the subtype C outbreak clade reveals a southern African root with a passage via Austria. Time-scaled Bayesian analyses of 27 outbreak sequences, 67 neighboring German subtype C sequences found by maximum likelihood analyses, and 424 related reference sequences found by BLAST search of the Los Alamos HIV database. Colored branches represent the most likely origin of the common ancestor. Black circles represent posterior values of the nodes according to their size. Numbers represent posterior values of selected nodes. Tip shapes are color-coded according to the risk group. MSM, men who have sex with men; PWID, people who inject drugs.

in this region. Hepatitis C virus infection still causes a very high burden to PWID and PWID living with HIV in Germany, although very effective treatment for this condition is available.

The Munich outbreak is caused by an HIV-1 subtype C virus and is monophyletic. A virus of the same subtype has recently been reported in an outbreak among PWID in Scotland that started in approximately 2003 with 104 assigned cases by mid-2016 [40]. This virus bears 2 resistance mutations, which are not present in the genetically distant Munich strain. The closest identified relatives to our Munich strain are 3 GenBank sequences (GQ400143, KU574426, and KU574391) from Austria (the oldest collected in 2004). A deeper analysis of the NGS data that allows inference of the direction of a transmission and results of the recency testing indicate that the outbreak case with the earliest diagnosis (from March 2015) did not spark the surge with 11 infections that form a core network and presumably occurred at 1 event or in a very short period of time. This finding points to injection in larger group settings, which is 1 of 4 known key factors for rapid transmission [11]. Another finding that fits to the low sequence diversity is the high proportion of recently infected individuals within the outbreak of 85.1% compared to an average rate of 33.1% of non-outbreak cases in this region. The reason for this is not known. However, it is possible that involved PWID received the information about recent HIV diagnoses from members of their injecting group and decided to get tested soon.

Another key factor for rapid transmission among PWID is a limitation to access sterile injection paraphernalia. Outbreaks reaching a substantial size can develop when free access is not or not sufficiently established. This is best exemplified by the HIV epidemic in countries of the former Soviet Union [41, 42]. Without provision of sterile injection equipment, an outbreak can develop a high initial dynamic within networks of connected persons as demonstrated in an outbreak in Scott County, Indiana [38]. Limited prevention programs paired with economic instability were among the factors leading to rapid transmissions of HIV among PWID in Greece and in Romania [43, 44]. In Munich, prevention programs are in

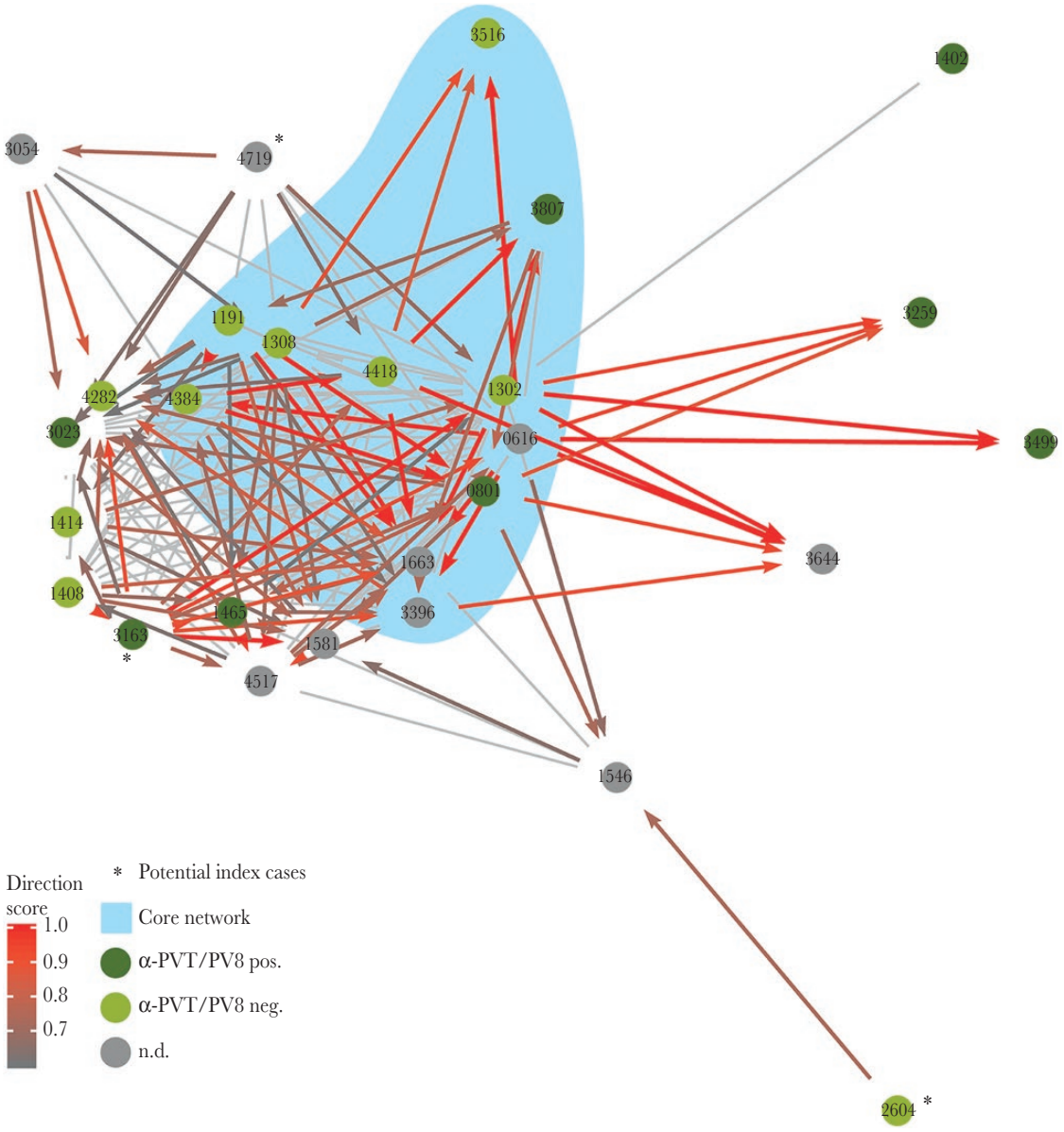

Figure 4. Deep sequence analysis of the outbreak clade using phyloscanner. All individuals of the Munich cluster are depicted as nodes according to their relationship to the other sequences in the outbreak. The epidemiologic relationship that was most frequently inferred in up to 52 overlapping phylogenies is depicted in edges (directed edge: from—to shown in red or orange; undirected edge: direction of transmission not clear show in gray). Individuals with >95% nearly identical ancestral virus are connected by red edges, and individuals with 85%–95% nearly identical ancestral virus are connected by by orange edges. Potential index cases are marked by an asterisk. α-PVT, alpha-pyrrolidinopentiothiophenone; PV8, alpha-pyrrolidinoheptiophenone.

place and access to sterile needles and syringes by vending machines and by other means were provided [29]. However, a change of consumption habits might result in insufficient provision of safe injection material, particularly if drugs are injected in group settings. In addition, new injectors who only recently started injecting drugs may be less attached to services providing them with sterile equipment, and they may not know about the risk of acquiring infection. In addition, almost no German prison runs needle and syringe programs, and, as a consequence, HIV transmission may occur in prison settings [29, 45].

A third key factor is the change in the injection behavior often resulting in an increased frequency of injections. This did play a role in the epidemic in Scott County and most of the other recent HIV outbreaks among PWID [9, 37, 38, 46]. The higher frequency of up to 30 times a day [37] was typically prompted by a shift of the injected drug towards a more affordable one with a shorter stimulating effect and increased severity of mental and physical withdrawal symptoms. In the Munich outbreak, we provide evidence that such a new drug was involved as well and contained α-PVT and/or PV8. In the recent outbreaks in Dublin, Ireland and in

Table 2. Results of Drug Testing From DSS of Outbreak and Non-Outbreak Cases Diagnosed in 2015 and 2016

Drugs	Outbreak (%) (n = 18)	Non-Outbreak (%) (n = 11)	*P*-Value[a]
Opiates[b]			
Negative	7 (38.9)	9 (81.8)	.052
Methadon	4 (22.2)	1 (9.1)	.622
Buprenorphin	4 (22.2)	1 (9.1)	.622
Fentanyl	3 (16.7)	0 (0)	.269
NPS			
Negative	9 (50.0)	11 (100)	.005
α-PVT	7 (38.9)	0 (0)	.026
PV8	4 (22.2)	0 (0)	.268

Abbreviations: α-PVT, alpha-pyrrolidinopentiothiophenone (International Union of Pure and Applied Chemistry (IUPAC) name: 2-(pyrrolidin-1-yl)-1-(thiophen-3-yl)-pentan-1-one); DSS, dried serum spots; NPS, new psychoactive substances; PV8, alpha-pyrrolidinoheptiophenone (IUPAC name: 1-phenyl-2-(pyrrolidin-1-yl)heptan-1-one).

[a]Two-sided Fisher's exact test.

[b]Only those drugs are listed that were detected in at least 3 samples of the outbreak cases or within the control group.

Tel Aviv, Israel, an association with injection of similar substances has been demonstrated [36, 37].

The remaining key factor associated with rapid transmission is the absence of knowledge that HIV is a local threat for PWID [46]. Successful interventions of large HIV outbreaks among PWID in the past targeted the key factors mentioned flanked by additional measures including upscaled HIV testing opportunities occasionally associated with contact tracing [37, 38, 47]. However, under circumstances in which the key factors apply only partially, outbreaks are expected to be smaller and grow slower after an initial burst comprising 1 or a few connected injecting groups. These groups remain local and are often self-limiting. Considering the lower numbers of reported new diagnoses in Bavarian PWID in recent years, the Munich outbreak seems to belong to this category. This does not mean that new infections related to the outbreak did not happen since we diagnosed the last outbreak case reported here. Some might have occurred but remained below the radar of our surveillance. A political measure that may have contributed to limit the size of this outbreak was the implementation of the "Neue-psychoaktive-Stoffe-Gesetz (NpSG)" (law regulating NPS) in Germany in November 2016. This law rendered dealing and possession of α-PVT and other designer stimulants, often called "bath salts," illegal.

CONCLUSIONS

A limitation of our outbreak examination is the incomplete sampling and the unavailability of additional material for full genome NGS to analyze the transmission events in more detail. Moreover, the DSS are linked with an anonymized subset of the notification data. Consequently, outbreak investigation using approaches that require identification and contacting of individuals are precluded. Therefore, specific risks of acquiring infection and contributing factors, for example homelessness, could not be investigated. Nonetheless, the Bavarian authorities, infectiologists, and HIV specialist physicians as well as low-threshold harm reduction services in Munich were informed. Furthermore, we are aware that a more timely identification of the outbreak by an implemented "real time molecular surveillance", as suggested by Poon et al [48], might have provided the opportunity to raise local awareness and increase prevention measures in this region to limit the outbreak at an earlier stage. We are currently working on the implementation of such a system as an additional tool for prevention and reduction of the HIV incidence among vulnerable groups in the population.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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