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Determinants of HIV-1 transmission clusters and transmitted drug resistance in men who have sex with men: A multicenter study in Portugal (2014-2019)

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ABSTRACT

Introduction: In the EU/EEA, men who have sex with men (MSM) is a priority group for the prevention and control of HIV-1 infection. In Portugal, the 2023 HIV incidence rate was 8.2 per 100,000 inhabitants, with 876 new infections, 41.7% in MSM. We aim to characterize HIV-1 transmission clusters (TC) and transmitted drug resistance (TDR) and its sociodemographic, behavioral, clinical, and viral genomic determinants in MSM newly diagnosed in Portugal between 2014 and 2019.
Methods: A total of 340 MSM newly diagnosed with HIV-1 infection at 17 hospitals in Portugal were included. TC was identified with branch support ≥90% and 1.5% genetic distance. Logistic regression models were used to examine factors associated with TC and TDR.
Results: We identified 38 TC with 104 MSM, which includes 81 (26.6%) of the 305 MSM from our sample included in cluster analysis. The overall prevalence of TDR was 8.2%. Only HIV-1 subtype C was significantly associated with TDR. Overall, 10.5% of the clusters had at least 1 surveillance drug resistance mutation. There was no significant difference in the prevalence of TDR or the proportion of Portuguese and migrant MSM inside and outside clusters. Age at diagnosis, district of residence, unprotected sex with a woman, HIV testing, presenter status, and HIV-1 subtype were significantly associated with TC.
Conclusion: Specific subgroups of MSM are contributing to HIV-1 clustered transmission in Portugal. However, no association was found between TDR and sociodemographic or behavioral factors. Directed prevention measures should focus on those subgroups.

1. Introduction

HIV adversely affects public health in the WHO European Region, reaching an incidence rate of 12.7 per 100,000 inhabitants in 2023. Specifically, men who have sex with men (MSM) remain the main mode of transmission in the EU/EEA. Overall, 46.7% of diagnoses with known route of transmission were in MSM. Thus, this group continues to be a priority for HIV prevention and control [1]. In Portugal, the 2023 HIV incidence rate was 8.2 per 100,000 inhabitants, with 876 new infections, 41.7% in MSM [2].

In recent years, a new approach to direct precise HIV-1 prevention and treatment services based on molecular epidemiology allows the identification HIV-1 transmission clusters (TC) and associated factors [3,4]. TC extracted from phylogenetic trees constructed with HIV genomic sequences from newly diagnosed people living with HIV (PLWH) signals transmission linkage within people in each TC [5]. These analyses allow for direct and substantially improved HIV public health measures identifying HIV transmission within or into vulnerable populations or specific communities at risk which may not be apparent from other surveillance sources, like classical epidemiology, and may allow for fine-grain determinants of transmission. Ultimately, this new approach can guide timely interventions to specific groups and to study HIV-1 transmitted drug resistance (TDR) patterns [6].

Recently, highly effective measures were recommended to decrease new HIV infections, namely treatment for all, pre-exposure prophylaxis (PrEP) use, and mass screening for high-risk groups [7]. With broader access to antiretroviral therapy (ART) and PrEP, more people are taking antiretroviral (ARV) drugs [8]. In 2023, 30.7 million people were on ARV treatment globally [9]. Therefore, an increase in resistance is likely, which may decrease the effectiveness of these drugs [10]. ART reduces HIV-related morbidity, mortality, and transmission [11,12], and an increase in TDR may also contribute to an increase in the incidence of HIV-1 infections [10]. Specifically in Portugal, there was an overall proportion of ARV resistance mutations of 9.4% between 2001 and 2017, with an increasing trend from 7.9% in 2003 to 13.1% in 2017 [13].

In our analysis, we aim to characterize HIV-1 TC and TDR, and its sociodemographic, behavioral, clinical, and viral genomic determinants in MSM newly diagnosed in Portugal between 2014 and 2019.

2. Materials and methods

2.1. Ethics

The Ethics Committees of all hospitals approved this research (Supplementary Material—Ethical committee approvals). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

2.2. Research sample

Data on sociodemographic, behavioral, clinical, and viral genomic factors was collected from newly diagnosed MSM with HIV-1 infection at 17 hospitals in Portugal between September 2014 and December 2019 as part of the BEST HOPE Project, upon HIV-1 diagnosis. This database included comprehensive information on the demographic, behavioral, clinical, and genotypic resistance characteristics. A total of 340 MSM above the age of 18 were recruited, were HIV drug naïve, and tested for resistance to ARV drugs before starting treatment, with the viral genomic sequence available (2.4. Data collection). This sample represents 17% of the total number of MSM diagnosed with HIV infection in Portugal in this period.

2.3. BEST HOPE project

To better understand the dynamics and behavioral determinants of HIV-1 transmission, the BEST HOPE project was an observational cross-sectional study using socio-behavioral and clinical questionnaires, and genomic sequences of newly diagnosed PLWH in Portuguese hospitals across the country.

2.4. Data collection

Sociodemographic and behavioral data were collected using a questionnaire and clinicians provided clinical information (Supplementary Material—Variables description). Partial HIV-1 pol sequences were obtained in the hospital setting (Sanger populational sequencing with consensus generated with ambiguity codes defined by IUPAC (https://www.dnabaser.com/articles/IUPAC%20ambiguity%20codes.html)) for routine TDR identification before the start of ART and were available for each patient in their clinical records. The partial HIV-1 pol sequences included amino acid positions 1-99 in the protease (PR) (HXB2 position: 2253 → 2549), 1-323 in the reverse transcriptase (RT) (HXB2 2550 → 3516). The consensus FASTA file sequence was sent by clinicians for each patient and was used in the analysis. The database was encoded and anonymized.

2.5. HIV-1 subtyping

HIV-1 subtype analyses used the consensus of three distinct algorithms (REGA V.3.0, Comet, and Scuel. If there was no agreement, the majority assignment was considered (when at least two algorithms reached concordance) (Supplementary Material—List of references: Methods section).

2.6. Analysis of TDR

The partial HIV-1 pol sequences obtained in the hospital setting (Sanger populational sequencing with consensus generated with ambiguity codes defined by IUPAC sequences) were submitted to the Calibrated Population Resistance (CPR) analysis tool at HIV Drug Resistance Database at Stanford University (https://hivdb.stanford.edu/cpr/) to evaluate TDR, defined as the presence of one or more surveillance drug resistance mutations (SDRMs) (WHO 2009 surveillance list).

2.7. Late presenters for care

A CD4 count <350 cells/μL or an AIDS-defining event, regardless of CD4 count at presentation for care, was defined as late presentation (LP) (Supplementary Material—List of references: Methods section).

2.8. Recentness of infection

The rate of ambiguity in viral genomic sequences assessed infection recency. Chronic infection was defined by a rate above 0.45% and recent infection as 0.45% or below (Supplementary Material—List of references: Methods section).

2.9. Cluster analysis

The dataset of *pol* sequences was divided into HIV-1 subtypes A1, B, C, F, and G to identify TC, including 305 sequences from the 340 MSM of the initial sample. Control sequences were extracted from the Los Alamos database (https://www.hiv.lanl.gov/content/sequence/HIV/mainpage.html). Three reference sequences (HIV-1 subtypes B and C) were used as an outgroup. Using VIRULIGN, each dataset was aligned against the global background dataset selected as the control. Sequences with ≤1050 nucleotides and ≤90% nucleotides identified were excluded. Codon positions associated with drug resistance were removed from the alignment. Duplicates were removed with the Seqkit and Los Alamos tools. Maximum likelihood phylogenies were constructed using FastTree with the generalized time-reversible evolutionary model. Statistical support of clades was assessed using the Shimodaira–Hasegawa-like test (SH-test). Putative TC was identified using ClusterPicker v1.2.3 and were defined as clades with branch support ≥90% in the likelihood ratio test and a genetic distance ≤1.5% [14,15]. Sensitivity analyses were performed using different genetic distances (2%, 2.5%, 3.5%, 4.5%) (Supplementary Material—Sensitivity analysis and interpretation). A thorough description of the analyses per HIV-1 subtype is available (Supplementary Material—Cluster analysis per HIV-1 subtype (counts)) (Supplementary Material—List of references: Methods section).

2.10. Statistical analysis

We estimated medians and proportions for continuous and qualitative variables, respectively, with 95% confidence intervals for proportions and interquartile ranges for medians.

To compare sociodemographic and behavioral characteristics of MSM inside and outside TC, we used Student's *t*-test, Mann–Whitney's *U*-test, Chi-square test, and Fisher's exact test. A description of the variables is available in the Supplementary Material—Variables description.

Logistic regression models were computed to examine factors associated with TC and TDR. All variables were included in the univariate logistic regression models. Variables directly associated with each other because they were either computed using values of one another or because they measure the same characteristic were not included in the same multivariate model because of multicollinearity issues. For example, "B vs Non-B" was not included because "HIV subtype" was. "Ambiguity Rate" and "Recentness" were not included because "Presenter status" was.

We considered that sociodemographic, behavioral, testing, and STI factors should be analyzed separately from clinical and viral genomic factors, as the first are strictly linked to the host, while the latter are linked to the host and the virus. Sociodemographic, behavioral, testing, and STI factors are related merely to the host and can help guide future HIV prevention programs. Clinical and genomic factors are related to the interplay between HIV and the host's immune system and can provide insights to guide the preparedness of clinical settings for HIV and therapeutic guidelines. Additionally, they relate to two different time points: before and after presentation to care. Both are crucial for public health but serve different purposes at distinct moments of the HIV cascade.

As such, two separate logistic regression models were calculated: one with sociodemographic, behavioral, testing, and STI factors, and another with clinical and viral genomics factors (Figure 1).

To analyze factors associated with TDR, all 340 MSM were included in the model. To analyze the factors associated with TC, 305 MSM (with the most frequent HIV-1 subtypes: A1, B, C, F1, and G) were included in the model. Factors in the univariate models with a *P*-value <0.2 were included in the multivariate models.

We evaluated multicollinearity by computing modified generalized variance-inflation factors ($GVIF(1/(2 \times Df))$; Fox and Monette) (threshold of 2) and tested the goodness of fit using the Hosmer–Lemeshow test.

The alpha level was 5%. The data analysis was conducted using R (v4.2.2).

3. Results

3.1. Cluster analysis

We identified 38 TC (Figure 2) with 104 MSM, which included 81 (26.6%) of the total 305 MSM from our database used for cluster analysis. The mean branch support was 96.8%, and the mean genetic distance was 0.9%. The mean cluster size was 2.7, ranging

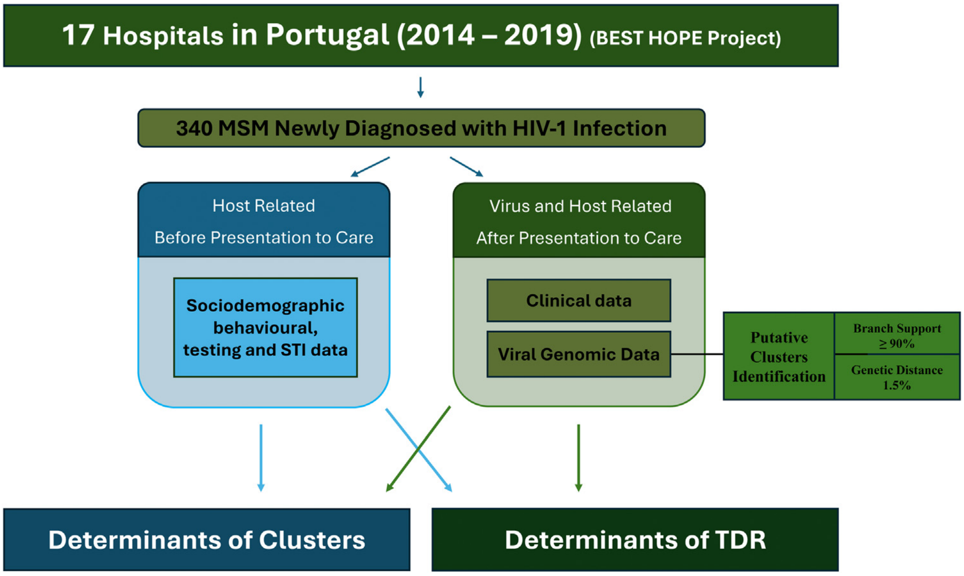

Figure 1. Conceptual model for analyses. STI, sexual transmitted infections; TDR, transmitted drug resistance.

from 2 (23 clusters) to 9 (1 cluster). Overall, 50% of the clusters were composed of Portuguese native MSM only, whereas 16% had exclusively migrants. Regarding geographic region, 66% of the clusters had at least 1 MSM from Lisbon. Concerning drug resistance, 4 (10.5%) clusters had at least 1 member with 1 resistance (K103N, L90M (2x) or N88S) (Supplementary File—Cluster characteristics).

3.2. Sociodemographics

The median age at diagnosis was 32 (IQR: 25-39) years, with MSM in TC significantly younger (30, IQR: 24-36) than outside TC (33, IQR: 26-40). The majority were Portuguese (74%, 95% CI [69%, 79%]), followed by Latin American (19%, 95% CI [15%, 24%]). No significant difference was found between the proportion of Portuguese and migrants inside and outside TC. Globally, most men lived in Lisbon district (51%, 95% CI [46%, 57%]), Porto (16%, 95% CI [12%, 21%), Faro (12%, 95% CI [8.3%, 16%]) and Setúbal (8.6%, 95% CI [5.8%, 12%). Regarding occupation, 76% (95% CI [70%, 80%]) were employed, and 49% (95% CI [43%, 55%]) were on minimum wage (501-1000€) (Table 1). No significant associations with TC were found for these factors.

3.3. Sexual behavioral, testing and STIs

87% (95% CI [82%, 90%]) of men reported having sex only with other men, while 13% (95% CI [9.9%, 18%]) with both men and women. The overall proportion of unprotected sex with other men (last 12 months) was 65% (95% CI [59%, 70%]). Overall, 15% (95% CI [11%, 20%]) reported unprotected sex with a woman in that same period. Significantly more MSM inside TC (23%, 95% CI [15%, 35%]) reported unprotected sex with a woman (last 12 months) than those outside TC (12%, 95% CI [8.3%, 17%]). Within the previous year, 60% (95% CI [52%, 67%]) consumed alcohol during unprotected sexual activity, whereas 47% (95% CI [40%, 55%]) consumed illegal drugs. However, those behavioral factors were not associated with TC.

Although 41% (95% CI [36%, 47%]) tested more than once per year and 36% (95% CI [31%, 42%]) tested once per year, there were a worrying proportion of 23% (95% CI [18%, 28%]) who had never tested for HIV infection before diagnosis. HIV testing frequency significantly differed between those inside and outside TC (Table 1). Overall, 41% (95% CI [36%, 47%]) had at least one STI in the past (Table 1), but this was not associated with TC.

3.4. Clinical information

The median CD4 count was 392 cells/μL (IQR: 239-572) and significantly higher for those inside TC (470 cells/μL, IQR: 342-640) compared to those outside TC (359 cells/μL, IQR: 172-533). Overall, 80% (95% CI [75%, 85%]) were in stage A of HIV infection. Consistently, the proportion of LP was significantly higher outside TC (48%, 95% CI [42%, 55%]) than inside TC (28%, 95% CI [19%, 40%]) (Table 1).

3.5. Viral genomics

3.5.1. HIV-1 subtypes

Among the total 340 MSM included in the sample, HIV-1 subtype B was the most prevalent (58%, 95% CI [52%, 63%]) with 42% [37%, 48%] of non-B strains: HIV-1 subtype A1 (13%, 95% CI [9.7%, 17%]), Recombinant forms (8.2%, 95% CI [5.6%, 12%]), HIV-1 subtype F1 (7.9%, 95% CI [5.4%, 11%]), C (5.3%, 95% CI [3.3%, 8.4%]) and G (5.3%, 95% CI [3.3%, 8.4%]) (Supplementary Material—HIV-1 subtypes).

Among the 305 MSM included in the cluster analysis, most were infected with HIV-1 subtype B (64%, 95 CI [59%,70%]), which had a significantly higher proportion outside TC (69%, 95% CI [62%,75%]) than inside TC (52%, 95% CI [41%, 63%]). Non-B strains were more frequent inside TC (48%, 95% CI [37%, 59%]) than outside TC (31%, 95% CI [25%, 38%]) (Table 1).

3.5.2. TDR analysis

For the TDR analysis, all 340 MSM were included. The prevalence of TDR was 8.2% (95% CI [5.6%, 12%]). The SDRM's more frequent were L90M (2.6%, 95% CI [1.3%, 5.1%]), M41L (1.5%, 95% CI [0.54%, 3.6%]) and K103N (1.5%, 95% CI [0.54%, 3.6%]). Considering the ARV class, 4.4% (95% CI [2.6%, 7.3%]) had resistance to PIs; 2.4% (95% CI [1.1%, 4.8%]) to NRTIs; and 2.1% (95% CI [0.91%, 4.4%]) to NNRTIs. Regarding ARV resistance and its clinical impact level, 4.1% (95% CI [2.4%, 7%]) had resistance to Atazanavir (PI) (SDRM: L90M), mainly low level (86%, 95% CI [56%, 97%]); 3.5% (95% CI [1.9%, 6.2%]) to Lopinavir (PI) (SDRM: L90M), mainly low level (92%, 95% CI [60%, 100%]); 2.1% (95% CI [0.91%, 4.4%]) to Efavirenz (NNRTI)

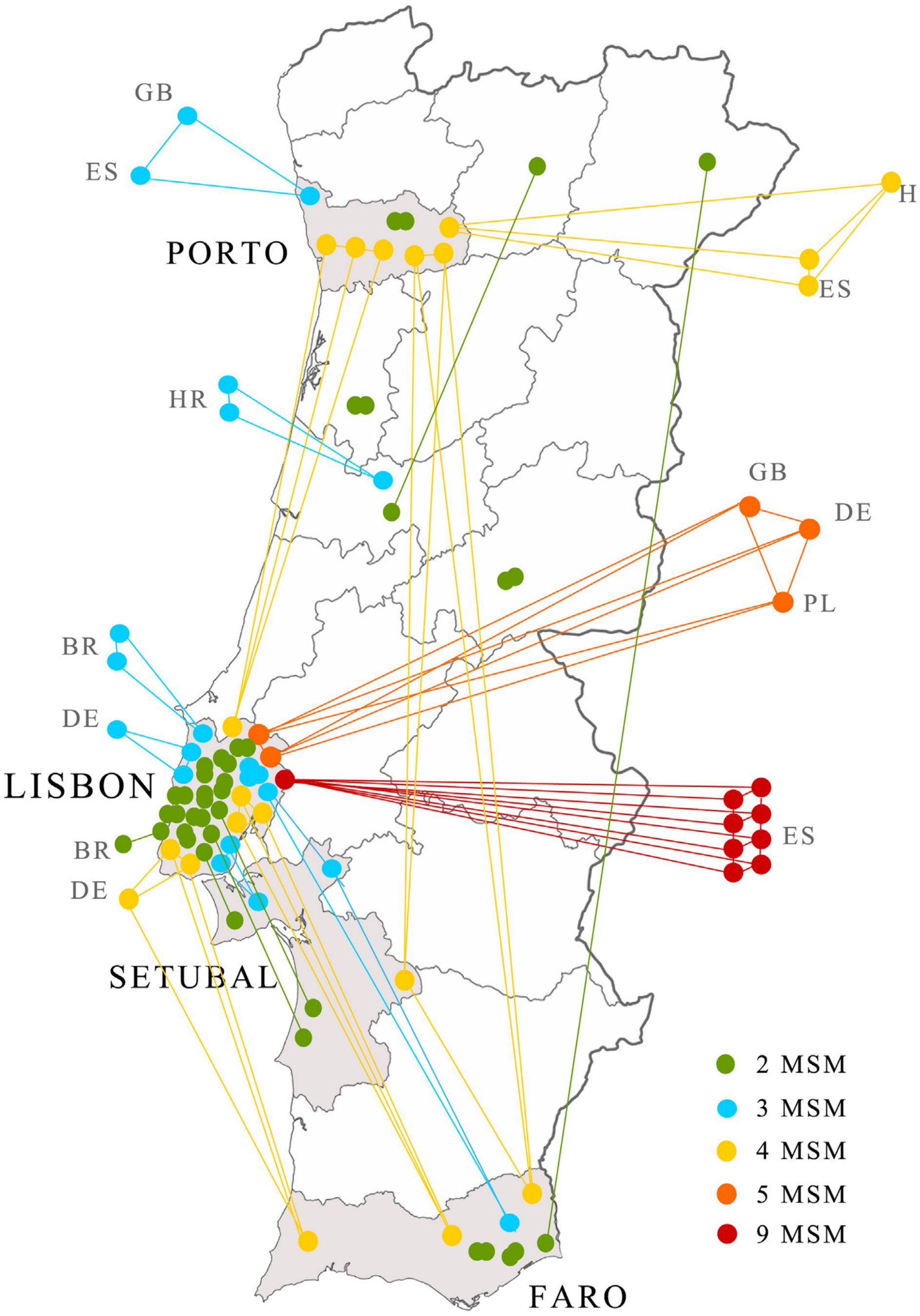

Figure 2. Transmission clusters (TC) identified. Map of Portugal with districts. Clusters are represented by size. MSM, men who have sex with men. Members of the clusters represented outside the country's frontier are migrants (country of birth other than Portugal).

(SDRM: K103N), mainly high level (86%, 95% CI [42%, 99%]); and 2.1% (95% CI [0.91%, 4.4%]) to Nevirapine (NNRTI) (SDRM: K103N), all high level (100%, 95% CI [56%, 100%]) (Supplementary File—TDR analysis).

Among the 305 MSM included in the cluster analysis, there was no significant difference in the prevalence of TDR inside (9.9%, 95% CI [4.7%, 19%]) and outside TC (7.6%, 95% CI [4.6%, 12%]) (Table 1).

3.6. Factors associated to transmitted HIV drug resistance (TDR)

None of the sociodemographic, behavior, testing, and STI factors was significantly associated with TDR (Supplementary File—Factors associated with TDR).

In the analysis with clinical and genomic factors, only HIV-1 subtype was significantly associated with TDR (C compared to B, OR = 13.9, 95% CI [4.15, 49.9]) (Supplementary File—Factors associated with TDR).

3.7. Sociodemographic, behavioral, testing, and STI factors associated with HIV-1 TC

The sociodemographic, behavioral, testing and STIs factors (Table 2) significantly associated with TC were age at diagnosis (25-34 compared to 18-24, OR = 0.42, 95% CI [0.19, 0.89]; 35-44 compared to 18-24, OR = 0.31, 95% CI [0.13, 0.75]; 45-54 compared to 18-24, OR = 0.19, 95% CI [0.04, 0.7]), indicating that younger

Table 1
Sociodemographic, sexual behavior, HIV testing, STIs, clinical and genomic characteristics of the newly diagnosed MSM included in the cluster analysis.

	N	**Overall** *N* (%) [95% CI] Median (IQR)	**Noncluster (*N* = 224)** *N* (%) [95% CI] Median (IQR)	**Cluster (*N* = 81)** *N* (%) [95% CI] Median (IQR)	***P*-value**[a]
Sociodemographics					
Diagnosis year	305				0.47
2014-2016		202 (66%) [61%, 71%]	151 (67%) [61%, 73%]	51 (63%) [51%, 73%]	
2017-2019		103 (34%) [29%, 39%]	73 (33%) [27%, 39%]	30 (37%) [27%, 49%]	
Age at diagnosis	305	32 (25, 39)	33 (26, 40)	30 (24, 36)	**0.02**
Age at diagnosis (groups)	305				0.22
18-24		58 (19%) [15%, 24%]	37 (17%) [12%, 22%]	21 (26%) [17%, 37%]	
25-34		126 (41%) [36%, 47%]	91 (41%) [34%, 47%]	35 (43%) [32%, 55%]	
35-44		78 (26%) [21%, 31%]	60 (27%) [21%, 33%]	18 (22%) [14%, 33%]	
45-54		29 (9.5%) [6.6%, 14%]	25 (11%) [7.5%, 16%]	4 (4.9%) [1.6%, 13%]	
≥55		14 (4.6%) [2.6%, 7.8%]	11 (4.9%) [2.6%, 8.9%]	3 (3.7%) [0.96%, 11%]	
Region of origin[b]	305				0.89
Portugal		227 (74%) [69%, 79%]	165 (74%) [67%, 79%]	62 (77%) [66%, 85%]	
Latin America		57 (19%) [15%, 24%]	44 (20%) [15%, 26%]	13 (16%) [9.2%, 26%]	
Africa		11 (3.6%) [1.9%, 6.5%]	8 (3.6%) [1.7%, 7.2%]	3 (3.7%) [0.96%, 11%]	
Other		10 (3.3%) [1.7%, 6.1%]	7 (3.1%) [1.4%, 6.6%]	3 (3.7%) [0.96%, 11%]	
Migrant status[c]	305				0.61
Native		227 (74%) [69%, 79%]	165 (74%) [67%, 79%]	62 (77%) [66%, 85%]	
Migrant		78 (26%) [21%, 31%]	59 (26%) [21%, 33%]	19 (23%) [15%, 34%]	
District of residence	303				0.24
Lisboa		156 (51%) [46%, 57%]	113 (51%) [44%, 58%]	43 (53%) [42%, 64%]	
Porto		48 (16%) [12%, 21%]	39 (18%) [13%, 23%]	9 (11%) [5.5%, 21%]	
Faro		35 (12%) [8.3%, 16%]	25 (11%) [7.6%, 16%]	10 (12%) [6.4%, 22%]	
Setúbal		26 (8.6%) [5.8%, 12%]	15 (6.8%) [4.0%, 11%]	11 (14%) [7.3%, 23%]	
Other		38 (13%) [9.1%, 17%]	30 (14%) [9.4%, 19%]	8 (9.9%) [4.7%, 19%]	
School level	303				0.60
Third level (up to 9th degree)		50 (17%) [13%, 21%]	39 (17%) [13%, 23%]	11 (14%) [7.4%, 24%]	
Secondary (12th degree)/technical specialization		131 (43%) [38%, 49%]	93 (42%) [35%, 48%]	38 (48%) [36%, 59%]	
Higher education (bachelor, master, PhD)		122 (40%) [35%, 46%]	91 (41%) [34%, 48%]	31 (39%) [28%, 50%]	
Current occupation	295				0.60
Employed		223 (76%) [70%, 80%]	160 (74%) [68%, 80%]	63 (80%) [69%, 88%]	
Unemployed		50 (17%) [13%, 22%]	39 (18%) [13%, 24%]	11 (14%) [7.5%, 24%]	
Other		22 (7.5%) [4.8%, 11%]	17 (7.9%) [4.8%, 13%]	5 (6.3%) [2.4%, 15%]	
Current income	276				0.77
Insufficient (≤500€)		54 (20%) [15%, 25%]	38 (18%) [14%, 25%]	16 (23%) [14%, 35%]	
Minimum wage (501-1000€)		136 (49%) [43%, 55%]	102 (50%) [43%, 57%]	34 (49%) [37%, 61%]	
Average (1001-2000€)		61 (22%) [17%, 28%]	48 (23%) [18%, 30%]	13 (19%) [11%, 30%]	
Above average (>2000€)		25 (9.1%) [6.1%, 13%]	18 (8.7%) [5.4%, 14%]	7 (10%) [4.5%, 20%]	
Sexual behaviors					
Sexual partners[d]	298				0.63
Men		258 (87%) [82%, 90%]	190 (87%) [82%, 91%]	68 (85%) [75%, 92%]	
Men and women		40 (13%) [9.9%, 18%]	28 (13%) [8.8%, 18%]	12 (15%) [8.3%, 25%]	
Meets sexual partners in coffee shops/bars/discos	279	148 (53%) [47%, 59%]	104 (51%) [44%, 59%]	44 (57%) [45%, 68%]	0.40
Meets sexual partners in saunas	268	62 (23%) [18%, 29%]	47 (24%) [19%, 31%]	15 (20%) [12%, 31%]	0.45
Meets sexual partners in cruising circuits	272	72 (26%) [21%, 32%]	51 (26%) [20%, 33%]	21 (28%) [18%, 39%]	0.79
Meets sexual partners online	277	173 (62%) [56%, 68%]	122 (61%) [54%, 68%]	51 (66%) [54%, 76%]	0.42
Meets sexual partners in mobile apps	276	181 (66%) [60%, 71%]	126 (63%) [56%, 70%]	55 (71%) [60%, 81%]	0.20
Unprotected anal sex (last 12 months)[e]	280	181 (65%) [59%, 70%]	135 (66%) [59%, 72%]	46 (62%) [50%, 73%]	0.60
Unprotected insertive anal sex (last 12 months)[e]	174	138 (79%) [72%, 85%]	100 (78%) [69%, 84%]	38 (84%) [70%, 93%]	0.32
Unprotected receptive anal sex (last 12 months)[e]	176	148 (84%) [78%, 89%]	114 (86%) [78%, 91%]	34 (79%) [64%, 89%]	0.30
Unprotected anal or vaginal sex with a woman (last 12 months)	299	45 (15%) [11%, 20%]	27 (12%) [8.3%, 17%]	18 (23%) [15%, 35%]	**0.018**
Condom use in trios/group sex (last 12 months)	298				0.39
Didn't have sex in group		177 (59%) [54%, 65%]	135 (62%) [55%, 68%]	42 (53%) [42%, 64%]	
Yes		72 (24%) [19%, 30%]	49 (22%) [17%, 29%]	23 (29%) [20%, 41%]	
No		49 (16%) [13%, 21%]	35 (16%) [12%, 22%]	14 (18%) [10%, 28%]	
Asked steady partner his serologic status for HIV (last unprotected sex)	204	67 (33%) [27%, 40%]	48 (33%) [25%, 41%]	19 (33%) [22%, 47%]	0.93
Asked occasional partner his serologic status for HIV (last unprotected sex)	146	31 (21%) [15%, 29%]	23 (23%) [16%, 33%]	8 (17%) [8.1%, 31%]	0.39

(*continued on next page*)

Table 1 (*continued*)

Sociodemographics	***N***	**Overall** *N* (%) [95% CI] Median (IQR)	**Noncluster (*N* = 224)** *N* (%) [95% CI] Median (IQR)	**Cluster (*N* = 81)** *N* (%) [95% CI] Median (IQR)	***P*-value**[a]
Consumed alcohol in unprotected sex	178	107 (60%) [52%, 67%]	77 (58%) [49%, 66%]	30 (67%) [51%, 80%]	0.30
Consumed illegal substances in unprotected sex	178	84 (47%) [40%, 55%]	61 (46%) [38%, 55%]	23 (50%) [36%, 64%]	0.66
Testing					
HIV testing frequency	292				**0.020**
More than once per year		121 (41%) [36%, 47%]	84 (39%) [33%, 46%]	37 (47%) [36%, 58%]	
Once per year		105 (36%) [31%, 42%]	72 (34%) [28%, 41%]	33 (42%) [31%, 53%]	
Never got tested		66 (23%) [18%, 28%]	57 (27%) [21%, 33%]	9 (11%) [5.7%, 21%]	
STIs					
STI ever diagnosed[f]	294	122 (41%) [36%, 47%]	88 (41%) [34%, 48%]	34 (44%) [33%, 55%]	0.66
Clinical					
CD4 count (cells/mm^3)	295	392 (239, 572)	359 (172, 533)	470 (342, 640)	**<0.001**
CD8 count (cells/mm^3)	260	924 (638, 1239)	903 (626, 1264)	996 (648, 1203)	0.5
Viral load (copies/mL)	292				0.34
≤10,000		35 (12%) [8.6%, 16%]	24 (11%) [7.5%, 16%]	11 (14%) [7.6%, 24%]	
10,000-100,000		128 (44%) [38%, 50%]	90 (42%) [35%, 49%]	38 (49%) [37%, 60%]	
≥100,000		129 (44%) [38%, 50%]	100 (47%) [40%, 54%]	29 (37%) [27%, 49%]	
Infection stage	289				0.08
A		232 (80%) [75%, 85%]	165 (78%) [72%, 83%]	67 (87%) [77%, 93%]	
B		32 (11%) [7.8%, 15%]	24 (11%) [7.5%, 17%]	8 (10%) [4.9%, 20%]	
C		25 (8.7%) [5.8%, 13%]	23 (11%) [7.1%, 16%]	2 (2.6%) [0.45%, 9.9%]	
Presenter status	295				**0.002**
NonLP		168 (57%) [51%, 63%]	112 (52%) [45%, 58%]	56 (72%) [60%, 81%]	
LP		127 (43%) [37%, 49%]	105 (48%) [42%, 55%]	22 (28%) [19%, 40%]	
HLA-B57	256				>0.99
Negative		248 (97%) [94%, 99%]	182 (97%) [93%, 99%]	66 (97%) [89%, 99%]	
Positive		8 (3.1%) [1.5%, 6.3%]	6 (3.2%) [1.3%, 7.1%]	2 (2.9%) [0.51%, 11%]	
Genomics					
Any SDRM	305	25 (8.2%) [5.5%, 12%]	17 (7.6%) [4.6%, 12%]	8 (9.9%) [4.7%, 19%]	0.52
PIs resistance (yes)	305	14 (4.6%) [2.6%, 7.8%]	8 (3.6%) [1.7%, 7.2%]	6 (7.4%) [3.0%, 16%]	0.21
NRTIs resistance (yes)	305	5 (1.6%) [0.61%, 4.0%]	5 (2.2%) [0.82%, 5.4%]	0 (0%) [0.00%, 5.6%]	0.33
NNRTIs resistance (yes)	305	6 (2.0%) [0.80%, 4.4%]	4 (1.8%) [0.57%, 4.8%]	2 (2.5%) [0.43%, 9.5%]	0.66
HIV-1 Subtype	305				**0.003**
B		196 (64%) [59%, 70%]	154 (69%) [62%, 75%]	42 (52%) [41%, 63%]	
A1		44 (14%) [11%, 19%]	29 (13%) [9.0%, 18%]	15 (19%) [11%, 29%]	
C		20 (6.6%) [4.2%, 10%]	11 (4.9%) [2.6%, 8.9%]	9 (11%) [5.5%, 21%]	
F1		27 (8.9%) [6.0%, 13%]	14 (6.3%) [3.6%, 10%]	13 (16%) [9.2%, 26%]	
G		18 (5.9%) [3.6%, 9.3%]	16 (7.1%) [4.3%, 12%]	2 (2.5%) [0.43%, 9.5%]	
B vs non-B	305				**0.007**
B		196 (64%) [59%, 70%]	154 (69%) [62%, 75%]	42 (52%) [41%, 63%]	
Non-B		109 (36%) [30%, 41%]	70 (31%) [25%, 38%]	39 (48%) [37%, 59%]	
Ambiguity rate	305	0.31 (0.08, 0.71)	0.39 (0.15, 1.00)	0.11 (0.00, 0.31)	**<0.001**
Recentness[g]	305				**<0.001**
Chronic		122 (40%) [35%, 46%]	111 (50%) [43%, 56%]	11 (14%) [7.3%, 23%]	
Recent		183 (60%) [54%, 65%]	113 (50%) [44%, 57%]	70 (86%) [77%, 93%]	

LP, late presenters; NNRTI, non-nucleoside reverse transcriptase inhibitors; NonLP, nonlate presenters; NRTI, nucleoside reverse transcriptase inhibitors; PI, protease inhibitors; STI, sexual transmitted infection. The bold values refer to p value ≤ 0.05.

[a] Student's *t*-test, Mann–Whitney's *U*-test, Chi-square test, and Fisher's exact test.

[b] Place of birth.

[c] Migrant if place of birth other than Portugal.

[d] Usual sexual partners.

[e] With other men.

[f] Lifetime (Yes/No answer).

[g] Recent infection defined as an ambiguity level value ≤0.45.

MSM had a higher chance of being in TC than the older ones. Also, newly diagnosed MSM living with HIV living in the district of residence Setúbal had a higher chance of being in TC (Setúbal compared to Lisbon, OR = 2.63, 95% CI [1.00, 6.89]). MSM reporting to have had unprotected anal or vaginal sex with a woman (last 12 months) had a higher chance of being inside TC (Yes compared to No, OR = 2.22, 95% CI [1.02, 4.81]). Finally, those who reported that they never got tested had a lower chance of being in TC (Never got tested compared to being tested more than once yearly, OR = 0.27, 95% CI [0.1, 0.67]).

3.8. Clinical and genomic factors associated with HIV-1 TC

The clinical and genomic factors (Table 3) significantly associated with TC were presenter status and HIV-1 subtype. LP had a lower chance of being inside TC (LP compared to NonLP, OR = 0.45,

Table 2
Sociodemographics, sexual behaviors, HIV testing, and STI factors associated with HIV-1 transmission clusters (TC).

Univariate and multivariate logistic regression analysis of sociodemographic, behavior, testing, and STI factors associated with HIV-1 transmission clusters (TC)

	Unadjusted		Adjusted ($N = 312$)	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Sociodemographics				
Diagnosis year				
2014-2016	1			
2017-2019	1.22 (0.71-2.06)	0.47		
Age at diagnosis	0.97 (0.95-1)	**0.039**		
Age at diagnosis (groups)				
18-24	1		1	
25-34	0.68 (0.35-1.32)	0.25	0.42 (0.19-0.89)	**0.024**
35-44	0.53 (0.25-1.12)	0.10	0.31 (0.13-0.75)	**0.010**
45-54	0.28 (0.08-0.85)	**0.036**	0.19 (0.04-0.7)	**0.022**
≥55	0.48 (0.1-1.75)	0.30	0.4 (0.07-1.98)	0.28
Region of origin[a]				
Portugal	1			
Latin America	0.79 (0.38-1.52)	0.49		
Africa	1 (0.21-3.57)	>0.99		
Other	1.14 (0.24-4.24)	0.85		
Migrant status[b]				
Native	1			
Migrant	0.86 (0.46-1.53)	0.61		
District of residence				
Lisboa	1		1	
Porto	0.61 (0.26-1.31)	0.22	0.73 (0.29-1.7)	0.47
Faro	1.05 (0.45-2.32)	0.90	1.35 (0.49-3.52)	0.55
Setúbal	1.93 (0.8-4.51)	0.13	2.63 (1.00-6.89)	**0.049**
Other	0.7 (0.28-1.59)	0.42	1.08 (0.37-2.89)	0.88
School level				
Third level (up to 9th degree)	1			
Secondary (12th degree)/technical specialization	1.45 (0.69-3.24)	0.34		
Higher education (bachelor, master, PhD)	1.21 (0.56-2.73)	0.64		
Current occupation				
Employed	1			
Unemployed	0.72 (0.33-1.44)	0.37		
Other	0.75 (0.24-1.98)	0.58		
Current income				
Insufficient (≤500€)	1			
Minimum wage (501-1000€)	0.79 (0.4-1.62)	0.51		
Average (1001-2000€)	0.64 (0.27-1.5)	0.31		
Above average (>2000€)	0.92 (0.31-2.59)	0.88		
Behaviors				
Sexual partners[c]				
Men	1			
Men and women	1.2 (0.56-2.44)	0.63		
Meets sexual partners in coffee shops/bars/discos				
No	1			
Yes	1.26 (0.74-2.14)	0.40		
Meets sexual partners in saunas				
No	1			
Yes	0.78 (0.38-1.47)	0.45		
Meets sexual partners in cruising circuits				
No	1			
Yes	1.09 (0.59-1.95)	0.79		
Meets sexual partners online				
No	1			
Yes	1.25 (0.73-2.2)	0.42		
Meets sexual partners in mobile apps				
No	1		1	
Yes	1.45 (0.83-2.6)	0.20	1.06 (0.55-2.09)	0.86
Unprotected anal sex (last 12 months)[d]				
No	1			
Yes	0.86 (0.5-1.51)	0.60		
Unprotected insertive anal sex (last 12 months)[d]				
No	1			
Yes	1.57 (0.67-4.18)	0.33		
Unprotected receptive anal sex (last 12 months)[d]				
No	1			
Yes	0.63 (0.27-1.58)	0.30		
Unprotected anal or vaginal sex with a woman (last 12 months)				
No	1		1	
Yes	2.2 (1.12-4.26)	**0.020**	2.22 (1.02-4.81)	**0.042**

(*continued on next page*)

Table 2 (*continued*)

Univariate and multivariate logistic regression analysis of sociodemographic, behavior, testing, and STI factors associated with HIV-1 transmission clusters (TC)

	Unadjusted		Adjusted ($N = 312$)	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Condom use in trios/group sex (last 12 months)				
Didn't have sex in group	1		1	
Yes	1.51 (0.82-2.75)	0.18	1.54 (0.75-3.14)	0.24
No	1.29 (0.62-2.58)	0.49	1.63 (0.67-3.85)	0.27
Asked steady partner his serologic status for HIV (last unprotected sex)				
No	1			
Yes	1.03 (0.5 3-1.96)	0.93		
Asked occasional partner his serologic status for HIV (last unprotected sex)				
No	1			
Yes	0.68 (0.26-1.6)	0.39		
Consumed alcohol in unprotected sex				
No	1			
Yes	1.45 (0.72-3.01)	0.30		
Consumed illegal substances in unprotected sex				
No	1			
Yes	1.16 (0.59-2.29)	0.66		
Testing and prophylaxis				
HIV testing frequency				
More than once per year	1		1	
Once per year	1.04 (0.59-1.83)	0.89	1.06 (0.56-2.02)	0.86
Never got tested	0.36 (0.15-0.77)	**0.012**	0.27 (0.1-0.67)	**0.007**
STIs				
STI ever diagnosed[e]				
No	1			
Yes	1.12 (0.66-1.89)	0.66		

STI, sexual transmitted infection. The bold values refer to p value ≤ 0.05.

[a] Place of birth.

[b] Migrant if place of birth other than Portugal.

[c] Usual sexual partners.

[d] With other men.

[e] Lifetime (Yes/No answer).

95% CI [0.24, 0.82] and MSM infected with HIV-1 subtype C or F1 had a higher chance of being inside TC (C compared to B, OR = 6.12, 95% CI [1.69, 23.8]; F1 compared to B, OR = 2.99, 95% CI [1.25, 7.12]). The presence of TDR was not significantly associated with TC.

4. Discussion

The present study analyzed sociodemographic, sexual behavioral, testing frequency, STI history, clinical and viral genomic characteristics of MSM diagnosed with HIV-1 in Portugal from 2014 to 2019 to identify factors associated with HIV-1 TC and TDR.

The analyses indicated that TC were associated with age at diagnosis, district of residence, unprotected sex with women, testing frequency, presenter status, and HIV-1 subtype.

MSM inside TC were significantly younger. Therefore, younger MSM are more associated with TC than older MSM, consistent with studies from other countries [16,17].

We found no association between migration status and TC, reinforcing that migrants are not linked to clustered transmission, which aligns with two European studies showing natives primarily responsible for these networks [16,17].

The district of residence was significantly associated with TC, with MSM from Setúbal having higher odds of clustering than those from Lisbon (OR = 2.63, 95% CI]1.00, 6.89]). This finding was surprising as the incidence of HIV-1 infection in the period of this study in Setúbal was lower compared to Lisbon: 11.9-19.1 per 100,000 inhabitants vs 22.4-30.9 per 100,000 inhabitants, respectively [2]. Contrary to our findings, a study on HIV TC in Los Angeles County found that larger urban centers have more dynamic transmission networks due to geographic mixing patterns in urban areas, which increase the likelihood of cluster formation due to high mobility and dense social networks [18]. This underlines the importance of cluster analysis at a national level, as local outbreaks with fewer cases may be missed in national surveillance data. The social and sexual networks of MSM in Setúbal may be smaller and more tightly interconnected compared to those in Lisbon. In smaller communities, such networks can facilitate clustering, as clustered transmission is more likely to occur within closed or semi-closed groups [19]. Differences in healthcare access and HIV testing in MSM between the two districts may impact this result. In Setúbal, early identification of cases and targeted testing within social or sexual networks may have increased the likelihood of detecting clusters. Conversely, in Lisbon, broader testing coverage may have diagnosed more nonclustered cases, diluting the apparent odds of clustering in relation to those in Setúbal [20]. Another aspect to consider is that the district of residence (Setúbal, with OR = 2.63, $p = 0.049$ at 1.5% threshold) was significant in the primary analysis but did not appear as a significant factor in the sensitivity analyses at higher thresholds. This suggests that the effect of geographical location on cluster membership may be more sensitive to the definition of genetic distance, warranting cautious interpretation.

Strikingly, MSM who engaged in unprotected sex with women (birth sex) had higher odds of clustering (OR = 2.22, 95% CI [1.02, 4.81]). To our knowledge, this is the first paper to analyze the association between a comprehensive set of sexual behaviors and TC, suggesting that a subgroup of MSM with female (birth sex) partners are involved in HIV transmission hotspots and linking transmission networks between different risk groups. Note that the variable "Unprotected anal or vaginal sex with a woman (last 12 months)" is an objective Yes/No variable while "Sexual partners" refers to the birth sex of usual sexual partners, and these variables are not mutually exclusive. Unprotected sexual contact between

Table 3
Clinical and genomic factors associated with HIV-1 transmission clusters (TC).

Univariate and multivariate logistic regression analysis of clinical and genomic factors associated with HIV-1 molecular transmission cluster (TC)				
	Unadjusted		Adjusted ($N = 325$)	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Clinical				
Age at diagnosis (groups)				
18-24	1		1	
25-34	0.68 (0.35-1.32)	0.25	0.77 (0.39-1.57)	0.47
35-44	0.53 (0.25-1.12)	0.10	0.65 (0.29-1.48)	0.31
45-54	0.28 (0.08-0.85)	**0.036**	0.2 (0.04-0.77)	**0.032**
≥55	0.48 (0.1-1.75)	0.30	0.55 (0.08-2.46)	0.47
CD4 count (cells/mm^3)	1.00 (1.00-1.00)	**<0.001**		
CD8 count (cells/mm^3)	1.00 (1.00-1.00)	0.18		
Viral load (copies/mL)				
≤10,000	1			
10,000-100,000	0.92 (0.42-2.13)	0.84		
≥100,000	0.63 (0.28-1.48)	0.28		
Infection stage				
A	1			
B	0.82 (0.33-1.85)	0.65		
C	0.21 (0.03-0.75)	**0.040**		
Presenter status				
NonLP	1		1	
LP	0.42 (0.24-0.73)	**0.002**	0.45 (0.24-0.82)	**0.010**
HLA-B57+				
Negative	1			
Positive	0.92 (0.13-4.1)	0.92		
Genomic				
Any SDRM				
No	1			
Yes	1.33 (0.53-3.14)	0.52		
PIs resistance				
No	1		1	
Yes	2.16 (0.69-6.41)	0.17	1.22 (0.27-4.79)	0.78
NRTIs_resistance				
No				
Yes				
NNRTIs_resistance				
No	1			
Yes	1.39 (0.19-7.28)	0.71		
HIV-1 subtype				
B	1		1	
A1	1.9 (0.92-3.82)	0.08	1.94 (0.89-4.11)	0.09
C	3.00 (1.14-7.73)	**0.023**	6.12 (1.69-23.8)	**0.006**
F1	3.4 (1.47-7.84)	**0.004**	2.99 (1.25-7.12)	**0.013**
G	0.46 (0.07-1.70)	0.31	0.6 (0.09-2.38)	0.52
B	1			
Non-B	2.04 (1.21-3.44)	**0.007**		
Ambiguity rate	0.15 (0.06-0.31)	**<0.001**		
Recentness[a]				
Chronic	1			
Recent	6.25 (3.26-13)	**<0.001**		

LP, late presenters; NNRTI, non-nucleoside reverse transcriptase inhibitors; NonLP, nonlate presenters; NRTI, nucleoside reverse transcriptase inhibitors; PI, protease inhibitors. The bold values refer to p value ≤ 0.05.

[a] Recent infection defined as an ambiguity level value ≤0.45.

MSM and women (birth sex) can function as an important channel, connecting two epidemiologically different transmission networks: the MSM transmission network and the heterosexual transmission network [21]. This connectivity facilitates the spread of HIV across otherwise segregated transmission networks and interventions to minimize transmissions involving MSM are likely to reduce HIV acquisition among other risk populations [22]. Future research should focus on understanding HIV transmission in diverse gender partnership networks. Education should focus on reducing stigma around diverse gender orientation to increase dialogue, improve health-seeking behavior, and partner notification among persons in this demographic. This was an unexpected finding, and we will explore it in future studies.

Regarding HIV testing frequency and presenter status, we showed in a previous study that MSM who never tested before diagnosis had higher odds of being LP [23]. In the present study, consistent with the latter study, we have concluded that those who never tested before diagnosis (OR = 0.27, 95% CI [0.1, 0.67]) and LP (OR = 0.45, 95% CI [0.24, 0.82]) are both at lower odds of clustering. These two variables were included in separate regression models, and multicollinearity was not a concern. It is known that HIV testing frequency is associated with the perception of risk and the ones who test less are expected to be less involved in high-risk networks of HIV transmission [24]. Our results reinforce Huang's findings, showing MSM who do not test with lower odds of clustering, as do MSM classified as LP. Besides the risk factors inherent to LP, a potential alternative explanation for this result is the time between infection and diagnosis. Because the virus in LP had more time to develop genetic mutations due to the high mutation rate of HIV-1 [25], when it was sequenced at diagnosis, a higher genetic distance with members of a potential TC can exist at the time of infection. Thus, these MSM could be artificially excluded from original clusters later at the time of diagnosis. As expected, in our sensitivity analysis, for genetic distances higher than 2.5% late presenters were not significantly associated (lower odds compared to nonlate presenters) with clustered transmission. As such, we must acknowledge that, in this present study, the lower odds of LP clustering may be simply an effect of the longer time between infection and diagnosis.

Historically, HIV infection in MSM was linked to HIV-1 subtype B. However, there has been a shift in Europe toward greater diversity with an increased prevalence of non-B strains [26]. Our findings, with 42% [37%, 48%] of non-B strains in MSM in Portugal, are consistent with earlier research. The most prevalent non-B strain was A1 (13% [9.7%, 17%]), and according to a previous study, A1 clusters in Portugal are connected to other European countries and share a recent common ancestor with the Greek A1 outbreak [27]. Regarding association between subtype and TC, we found that HIV-1 subtypes C and F1 were at higher odds of clustering (OR = 6.12, 95% CI [1.69, 23.8] and OR = 2.99, 95% CI [1.25, 7.12], respectively), indicating potential hotspots for transmission. But due to the low number of C and F1 sequences (Table 1), further studies are needed to interpret these results. In this analysis between HIV-1 subtypes and the definition of a TC it is important to mention that inherent differential tempo evolution of different HIV-1 subtypes could result in different levels of genetic diversity in transmission networks per HIV-1 subtype [28]. This can have implications in the definition of genetic diversity used to define thresholds to detect TC. A study showed that subtypes C and A1 presented the fastest substitution rates, and subtype D was the slowest evolving group [29]. We addressed that issue by calculating the pairwise genetic distances for each HIV-1 subtype, and we obtained very comparable values (Supplementary Material—Pairwise genetic distances). Consequently, we used the same genetic distances as thresholds for all HIV-1 subtypes.

According to our study, the estimated prevalence of TDR was 8.2% [5.6%, 12%]. Similar prevalence has been observed by other European studies, such as France (10.8%) [30] and the UK (7.5%) [31]. In the TDR analysis of associated factors, no associations were identified between the presence of TDR and factors such as sociodemographic characteristics, sexual behaviors, HIV testing frequency, or STI history. To our knowledge, this study innovated by examining in the same model the relationship between sociodemographic data, sexual behaviors and behaviors toward testing, and the presence of TDR in MSM. Some might assume that engaging in high-risk sexual activities increases the chance of contracting drug-resistant HIV [32]. Our study refutes this hypothesis, showing no association between TDR and socio-behavioral factors. The diversity of risk behaviors present within the MSM population [33] adds a lot of variation and can dilute the association between any particular sexual practices and TDR. So, MSM may have different frequencies of condomless intercourse, number of partners, and substance use, among other factors, creating overlapping risk patterns. Regarding migrant status, a Portuguese study reported higher TDR in newly diagnosed PLWH from Mozambique [34], reinforcing a Swedish study that showed increased TDR in sub-Saharan African migrants [35]. In contrast, we found no association between TDR and migrant status in MSM. Migrant and nonmigrant MSM may have overlapping sexual networks, resulting in comparable amounts of exposure to drug-resistant HIV strains. Social and sexual integration among MSM communities may reduce the impact of migratory status as a predictor of TDR. In fact, the majority of migrants in this sample were from Brazil, a Portuguese-speaking country with no language barriers, and there is evidence that among Brazilian migrants, the majority are infected by Portuguese natives [36]. Additionally, there are community-based HIV and other STI screening programs are available to migrant MSM. These services provide free, anonymous testing and counseling for MSM improving timely access to sexual health care for key populations, including migrants [37].

Regarding clinical and viral genomic factors, we found an association between TDR and HIV-1 subtype C. No other clinical or genomic factors were associated with TDR (Supplementary Material—Factors associated with TDR). In a clinical trial [38], pretreatment resistance and HIV-1 subtype C were independently associated. with virologic failure, which was significantly associated with TDR. Due to the low number of HIV subtype C in our sample, we will address this association in future studies.

Also, we found no association between TDR and TC, which is in line with other European studies [39]. This means that TDR are likely to arise independently in various individuals rather than being amplified or sustained within particular TC.

There are some limitations to be acknowledged. Sensitive self-reported data, such as sexual behavior, can be subject to reporting bias. Thus, the results might be influenced by how participants reported their behavior. Also, the threshold selection in cluster analysis depends on parameters given by the researcher, which significantly influence the number and composition of the inferred clusters. Therefore, the analysis of clusters should be interpreted with prudence and consider the sensitivity analysis interpretation (Supplementary Material—Sensitivity analysis and interpretation). Moreover, 67% of the MSM included in the cluster analysis lived in the two major cities in Portugal, Lisbon and Porto. Additionally, the low number of HIV-1 subtype C, F1, and G sequences may impact the logistic regression results and interpretation.

5. Conclusion

Specific subgroups of MSM are contributing to HIV clustered transmission in Portugal, particularly nonlate presenters, younger MSM, those who test for HIV-1 more frequently, MSM from Setúbal (district of residence), MSM who had unprotected anal/vaginal sex with a woman (last 12 months), and those infected with HIV-1 subtype C and F1. However, no association was found between sociodemographic/behavioral traits and TDR. The results of our study, combining socio-behavioral questionnaires linked with clinical and genomic data to detect HIV-1 transmission networks, support an integrated molecular epidemiology approach as crucial to developing direct measures to prevent and control HIV infection.

Ethical approval and informed consent

The study was approved by the Ethics Committees of all participating hospitals. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

RA, VP, MP, and AA: conceptualization and writing—original draft and writing—reviewing and editing. RA, VP, CS, MNM, and MP: data curation and validation. RA, VP: formal analysis. MP and AA: funding acquisition, project administration, and supervision. MP, VP, PG, and MROM: investigation. SS, MP, VP, MROM, and AA: methodology. PG, ARS, AD, BA, CP, CK, CR, CC, CM, DF, EGS, ET, FMo, FaR, FMa, FeR, GG, HR, IC, ID, IG, JSi, JO, JF, JP, JSC, JSo, KM, LP, MJA, MJM, MM, MS, MC, NM, OC, PPa, PPr, PR, RP, RT, RCA, RCR, RS, RSC, SN, TF, TB, DS and LM: resources, data collection, and approval of the publication contents. All authors contributed to the article and approved the submitted version.

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In memoriam

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Declarations of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2025.107888.

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