# Characterising HIV-1 transmission in Victoria, Australia: a molecular epidemiological study

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## Summary

**Background** In Australia the incidence of HIV has declined steadily, yet sustained reduction of HIV transmission in this setting requires improved public health responses. As enhanced public health responses and prioritisation of resources may be guided by molecular epidemiological data, here we aimed to assess the applicability of these approaches in Victoria, Australia.

**Methods** A comprehensive collection of HIV-1 *pol* sequences from individuals diagnosed with HIV in Victoria, Australia, between January 1st 2000 and December 31st 2020 were deidentified and used as the basis of our assessment. These sequences were subtyped and surveillance drug resistance mutations (SDRMs) identified, before definition of transmission groups was performed using HIV-TRACE (0.4.4). Phylodynamic methods were applied using BEAST (2.6.6), assessing effective reproductive numbers for large groups, and additional demographic data were integrated to provide a high resolution view of HIV transmission in Victoria on a decadal time scale.

**Findings** Based on standard settings for HIV-TRACE, 70% (2438/3507) of analysed HIV-1 *pol* sequences were readily assigned to a transmission group. Individuals in transmission groups were more commonly males (aOR 1.50), those born in Australia (aOR 2.13), those with probable place of acquisition as Victoria (aOR 6.73), and/or those reporting injectable drug use (aOR 2.13). SDRMs were identified in 375 patients (10.7%), with sustained transmission of these limited to a subset of smaller groups. Informative patterns of epidemic growth, stabilisation, and decline were observed; many transmission groups showed effective reproductive numbers ($R_e$) values reaching greater than 4.0, representing considerable epidemic growth, while others maintained low $R_e$ values.

**Interpretation** This study provides a high resolution view of HIV transmission in Victoria, Australia, and highlights the potential of molecular epidemiology to guide and enhance public health responses in this setting. This informs ongoing discussions with community groups on the acceptability and place of molecular epidemiological approaches in Australia.

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## Research in context

### Evidence before this study

Increasingly sophisticated methods are being used to generate actionable information from virological sequence data. Approaches to characterise HIV transmission using sequence data may be transformative for public health responses. Many of these approaches are yet to be applied in Australia, a country with comparatively low-rates of HIV transmission, and high-rates of treatment coverage and linkage to care. In Australia, HIV sequence data are prepared following an initial diagnosis as part of routine care, for the purposes of identifying anti-retroviral resistance. A search of primary research articles published in PubMed prior to August 22, 2022, using the search terms "HIV" or "human immunodeficiency virus" and "molecular epidemiology" or "phylodynamics" or "phylogenetics" and "Australia" returned 436 results, with 25 relevant studies having Australian cohorts. These studies typically place a focus on a single demographic, transmission group, or HIV subtype, with few modelling transmission for the broad population.

### Added value of this study

In this observational molecular epidemiological study, we apply molecular approaches to characterise HIV transmission in Victoria, Australia. Based on a comprehensive longitudinal collection of available HIV-1 pol gene sequences for the state, we define and describe transmission groups, and highlight those which may warrant prioritisation based on integrated epidemiological data. These findings may guide population level preventative strategies, informing the prioritisation of HIV-related public health responses, as well as individual patient management and focused transmission containment.

### Implications of all the available evidence

Our findings indicate that strategies employing these approaches have the potential to be valuable in guiding public health responses, providing an evidence base and foundation for considering the application of these routinely in Australia and comparable settings. Our observation that molecular transmission groups often have a common associated exposure can support the continued use of tailored prevention strategies. The observation of SDRM transmission in this setting warrants monitoring to ensure the continued efficacy of first-line therapies and antiretroviral preexposure prophylaxis (PrEP). Establishing molecular epidemiological approaches as a routine aspect of the HIV public health response holds promise for preventing onward transmission.

## Introduction

Australia has introduced world-leading models of care for the prevention and management of human immunodeficiency virus (HIV) infection. These include high treatment coverage of people living with HIV (PLWH), access to HIV self-testing, and the widespread uptake of HIV pre-exposure prophylaxis (PrEP) in relevant populations.[1,2] Together, these have contributed to a lower incidence of HIV in Australia than comparable high-income and Asia–Pacific countries.[1,3] There remain challenges to the continued reduction of HIV infections in Australia, however, the notification rate of HIV in Aboriginal and Torres Strait Islander people remains more than double that of the Australian born non-Indigenous population.[4] Further, notification rates have remained high in migrant subpopulations,[5,6] and more recently, interruptions in access to medical advice and testing due to the COVID-19 pandemic have posed further challenges.[7] A concerning 44% of Australian HIV notifications were classified as late diagnoses in 2020, based on a commonly used marker for immune function in HIV infection, CD4+ T-cell count, higher than any year in the preceding decade.[8] A renewed effort to reduce new HIV infections in Australia is required, with the prevention of local transmission highlighted as fundamental to these efforts in Australia's eighth national HIV strategy.[9]

A central strategy to the prevention of local transmission is contact tracing, enabling the identification of individuals who may have been exposed and allowing for appropriate provision of care and public health response. Timely contact tracing represents a key opportunity for prevention through the rapid initiation of antiretroviral therapy (ART), as early or acute HIV infections account for a considerable amount of onward transmission.[10] Contact tracing can be strengthened by molecular epidemiological analyses, as shown for many infectious diseases. For example, molecular epidemiology has successfully been used to investigate HIV outbreaks amongst people who inject drugs (PWID) in Europe and North America,[11–13] confirming suspected outbreaks and supporting secondary case finding and contacting of at-risk individuals. In Australia, molecular epidemiological methods have infrequently been applied to HIV surveillance or control; state-based studies have described changing demographics and patterns of transmission in New South Wales and Victoria,[14–16] with one national study estimating subtype prevalence and transmission between states and territories, as examples.[17] Contemporary methods are able to

provide more granular and actionable information regarding transmission to guide routine public health activities, as seen internationally,[11] with the assessment or routine use of these approaches yet to occur in our setting. The generation, interpretation and handling of data of this nature should only occur within a robust ethical framework, given well described ethical implications and potential harms for individuals and communities.[18,19]

In recent years, mathematical models have been applied to molecular epidemiological data to estimate informative epidemiological parameters, such as the effective reproductive number ($R_e$), indicating the average number of secondary cases per infectious case in a population. Such inferences can inform whether an epidemic is growing, stabilising or decreasing.[20] These models have been used to assess patterns of HIV transmission in both the general population and specific at-risk groups, although are yet to be applied in Australia.[21–23] Accordingly, we applied molecular epidemiology and related models to a comprehensive collection of HIV-1 *pol* sequences to reveal patterns of HIV transmission in Victoria, Australia. If identified in near real-time, we hypothesise that the inferring patterns of HIV transmission could greatly inform public health responses.

## Methods

### Study design and datasets

In Australia, all new HIV cases are notified to public health authorities in each state or territory. As part of routine care, plasma samples from HIV-positive individuals are forwarded to public health laboratories for genotyping and *in silico* inference of antiviral drug resistance. In this study, sequences generated from the earliest samples available for each individual diagnosed in the state of Victoria between January 1st 2000 and December 31st, 2020 were included. Additional data were available from the Victorian Department of Health, including each individual's year of diagnosis, initial CD4+ T-cell count and exposure risk group, alongside state notification data[24] and state population data.[25]

### Sequence generation and analyses

A contiguous HIV *pol* sequence was generated for each sample, spanning the protease (PR, amino acid positions 1–99 in the HXB2 reference genome) and reverse transcriptase region (RT, amino acid positions 1–246) as described previously.[26] Of these sequences, those with ≥5% ambiguous bases were excluded from further analysis. Samples that met our study inclusion criteria were retained, namely those from individuals 18 years of age or older at diagnosis, diagnosed in the state of Victoria, and having complete and available data relating to these criteria. Subtypes were determined using *HIVdb* (9.0)[27] and *REGA* (3.0),[28] requiring concordant results. Drug resistance-associated mutations were determined using the Stanford HIV-1 database and the *HIVdb* (9.0). Surveillance drug resistance mutations (SDRMs) were defined according to the World Health Organisation (WHO) definitions[22] and heteroresistance considered as resistance for the purposes of this study.

### Molecular transmission analysis

HIV-TRACE (0.4.4) was used to construct molecular transmission networks, as previously described.[29] Briefly, sequences were aligned to the HXB2 *pol* reference sequence (positions 2253–3749) and to minimise potential bias due to convergent evolution, codons associated with HIV-1 drug resistance were masked based on a commonly used subtype B-optimised list. Pairwise genetic distances were then calculated and a genetic distance threshold (GDT) of 1.5% was applied to define molecular transmission groups as standard for HIV *pol* gene data. We further constructed molecular transmission groups with more conservative and permissive genetic distance thresholds (0.5–2.0%) using molecular transmission groups suggestive of transmitted drug resistance as an *a posteriori* exploration of the effects of changing genetic distance thresholds on sequences in these priority groups. Transmission groups and associated metadata were visualised using MicrobeTrace (0.8.2) and R (v3.6.1) with the packages ggplot2 (3.3.3) and ggridges (0.5.3). Sequence data are available within GenBank (PP199486–PP202992).

### Modelling effective reproductive number within transmission groups

The effective reproductive number ($R_e$) of transmission groups was estimated as the median of the posterior distribution using a Birth Death Skyline Serial (BDSKY) model within a Bayesian framework, as implemented in BEAST (2.6.6).[30] A group size of 30 was chosen to ensure that at least three samples were available on average per interval of the $R_e$ parameter (10–20 intervals). Alignments of *pol* gene sequences for each transmission group were used, with three partitions to represent each codon position. An HKY substitution model was specified with a strict molecular clock model, calibrated through inclusion of the date of diagnosis for each sequence (Supplementary Methods). Temporal signal was explored and confirmed using TempEst (1.5.3).

### Statistical analyses and outcomes

Statistical analyses were performed in R (3.6.1). Categorical variables were presented as number of relevant cases and percentages and continuous variables were expressed as the mean ± standard deviation. To assess variables associated with (a) inclusion in a molecular transmission group, or (b) carriage of one or more SDRMs, we used two multivariable logistic regression models to calculate adjusted odds ratios using mixed-effects models with *glm* (base R). Placement of an individual within a transmission

group was considered as an outcome for (a), and carriage of one or more SDRMs as defined according to the World Health Organisation (WHO) definitions as an outcome for (b). The following *a priori* specified variables were included in both models as fixed-effects: age group, sex, country of birth, probable place of acquisition and risk group, while for the SDRM-focused model, an additional variable for transmission group status was included. For exposure risk groups, country of birth and probable place of acquisition, an 'Unknown' category was included to accommodate missing data; all other covariates were complete. Confidence intervals (95% CI) for proportions were calculated using prop.test (base R).

### Ethics

Ethical approval for this project was obtained from the University of Melbourne Office of Research Ethics and Integrity (2021-21263–17820-4).

### Role of the funding source

Funding for this project was provided through a Partnership Grant from the National Health and Medical Research Council of Australia (APP1198800), with partnership co-funding from the Victorian Department of Health. The Victorian Department of Health was involved in project conception and data acquisition, and was not involved in the analysis or interpretation of results for this work. The remaining funders had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

## Results

### Study population and sequence diversity

Between January 1st 2000 and December 31st 2020, there were 5303 HIV notifications in Victoria, Australia, of which 90.6% (4807/5303) of notifications were males and 9.4% (496/5303) were females (Supplementary Figure S1). Of these, 66.0% (3507/5303) of individuals had an available HIV-1 *pol* sequence and matched inclusion criteria for this study (Supplementary Figure S1). The majority of included individuals were male (3226/3507; 92.0%), and the exposure risk group was predominantly male-to-male sex (2757/3507; 78.6%) (Table 1). Available HIV-1 *pol* sequences were largely from diagnoses occurring after 2005 (3019/3,507, 86.1%). Patient samples forming the basis of HIV-1 *pol* genotyping were typically collected shortly after diagnoses (mean 38 days following diagnosis, SD = 196 days), representing samples typically from treatment-naïve individuals and those who have recently initiated therapy. Of included individuals with reported country of birth, the majority were born in Australia (2180/3413; 63.9% (95% CI 62.2–65.5%)), in line with broader HIV notification data for Victoria at this time (3358/5303; 63.3% (95% CI 62.0–64.6%)).[31] Individuals commonly had probable place of acquisition listed as Victoria (2364/3032; 78.0% (95% CI 76.5–79.4%)). A subset of individuals reported injectable drug use (221/3507; 6.3% (95% CI 5.5–7.2%)), with this as either a standalone exposure (85/3507; 2.4% (95% CI 2.0–3.0%)), or alongside another exposure (136/3507; 3.9% (95% CI 3.3–4.6%)) (Table 1).

All HIV-1 *pol* sequences matching inclusion criteria were genotyped to identify common subtypes or circulating recombinant forms (CRF), alongside surveillance drug resistance mutations (SDRMs). Of sequences for which a subtype or CRF was identifiable (3259/3507; 92.9% (95% CI 92.0–93.7%)), subtype B sequences were the majority (2327/3259; 71.4% (95% CI 69.8–72.9%)), followed by CRF01_AE (526/3259; 16.1% (95% CI 14.9–17.4%)) and subtype C (277/3259; 8.5% (95% CI 7.6–9.5%)). The frequency of major subtypes and CRFs fluctuated throughout the study period (Supplementary Figure S2), with CRF01_AE HIV-1 increasing from

| Characteristic | Study population (n = 3507) | | Individuals in molecular transmission groups (n = 2438) | |
|---|---|---|---|---|
| | n | % | n | % |
| **Sex** | | | | |
| Male | 3226 | 92.0 | 2305 | 94.5 |
| Female | 281 | 8.0 | 133 | 5.5 |
| **Age** | | | | |
| <20 years | 64 | 1.8 | 43 | 1.8 |
| 20–29 years | 1062 | 30.3 | 745 | 30.6 |
| 30–39 years | 1150 | 32.8 | 815 | 33.4 |
| 40–49 years | 727 | 20.7 | 509 | 20.9 |
| 50–59 years | 356 | 10.2 | 238 | 9.8 |
| 60–69 years | 119 | 3.4 | 76 | 3.1 |
| ≥70 years | 29 | 0.8 | 12 | 0.5 |
| **Risk factor** | | | | |
| Male–female sex | 602 | 17.2 | 289 | 11.9 |
| Male-to-male sex[a] | 2757 | 78.6 | 2046 | 83.9 |
| Injecting drug use[a] | 221 | 6.3 | 180 | 7.4 |
| Other | 16 | 0.5 | 9 | 0.4 |
| Unknown | 47 | 1.3 | 26 | 1.1 |
| **Country of birth** | | | | |
| Australia | 2180 | 62.2 | 1706 | 70.0 |
| Other | 1228 | 35.2 | 653 | 26.8 |
| Unknown | 99 | 2.8 | 79 | 3.2 |
| **Probable place of acquisition** | | | | |
| Victoria | 2364 | 67.4 | 1908 | 78.3 |
| Interstate | 80 | 2.3 | 49 | 2.0 |
| International | 588 | 16.8 | 181 | 7.4 |
| Unknown | 475 | 13.5 | 300 | 12.3 |

[a]A subset of individuals reported both male-to-male sex and injectable drug use as risk factors and have been included in both categories–136 individuals for the broader study population and 112 individuals for those in molecular transmission groups. Race and/or ethnicity data were not collected.

***Table 1:*** **Characteristics of individuals included in this study with HIV from Victoria, Australia (2000–2020), and those grouped in molecular transmission groups.**

7.5% (83/1101) of typable sequences between 2000 and 2009 to 20.5% (443/2158) of typable sequences between 2010 and 2020.

SDRMs were identified in 10.7% (375/3507; 95% CI 9.7–11.4%)) of sequences; specifically, 57 (1.6%; 95% CI 1.3–2.1%) protease inhibitor-associated SDRMs (PI-SDRMs), 183 (5.2%; 95% CI 4.5–6.0%) nucleoside reverse transcriptase inhibitor-associated SDRMs (NRTI-SDRMs) and 176 (5.0%; 95% CI 4.3–5.8%) non-nucleoside reverse transcriptase inhibitor-associated SDRMs (NNRTI-SDRMs) were observed. Combinations of two or more classes of SDRMs were observed in 1.0% (36/3507; 95% CI 0.7–1.4%) of sequences.

## Local transmission groups

Through HIV-TRACE, a total of 359 molecular transmission groups were identified, varying in size from 2 to 301 individuals (median 3; IQR 2–5) and with 69.5% (2438/3507; 95% CI 68.0–71.0%) of notifications included, collectively (Supplementary Data A). Associations between demographic and epidemiological data were investigated for large groups (≥20 individuals, 18 groups) (Fig. 1). The exposure risk group for individuals in these groups was typically male to male sex (923/1089; 84.8%), although for one group, a majority of individuals had injecting drug use as an exposure risk (26/49; 53.1%); this group (Group 7) also had male-female sex as a common risk factor (19/49; 38.8%), particularly for individuals with diagnoses before 2014 (15/24; 62.5%) (Supplementary Figure S3). Male–female sex was a commonly listed risk factor for one other large group (Group 18) (9/21; 42.9%). Notably, these two groups also had place of acquisition more commonly listed as overseas; place of acquisition was listed as overseas for 31.7% (13/41; Group 7) and 41.2% (7/17; Group 18) of individuals (Fig. 1), respectively. A minority (47/953; 4.9%) of individuals across large transmission groups had overseas as probable place of acquisition.

A predominant HIV-1 subtype or CRF was observed for each large transmission group, with subtype B being the most common (14/18 groups; 77.8%) (Fig. 1), broadly in line with the rates that subtypes were observed in the broader population (Supplementary Figure S2). One transmission group was comprised primarily of sequences for which a subtype or CRF was not assigned (Group 5; 71/75; 94.7%), these being classed as novel recombinants of subtypes B and D by the REGA HIV subtyping tool (71/75; 94.7%) and as subtype B by the HIVdb subtyping tool (75/75; 100%). The number of individual-to-individual links within groups, termed the connectivity,[22] was high for large groups (median of 20 links per individual for groups ≥20 individuals; IQR 8–36), although individual groups ranged from comparatively sparse (median 5 links per

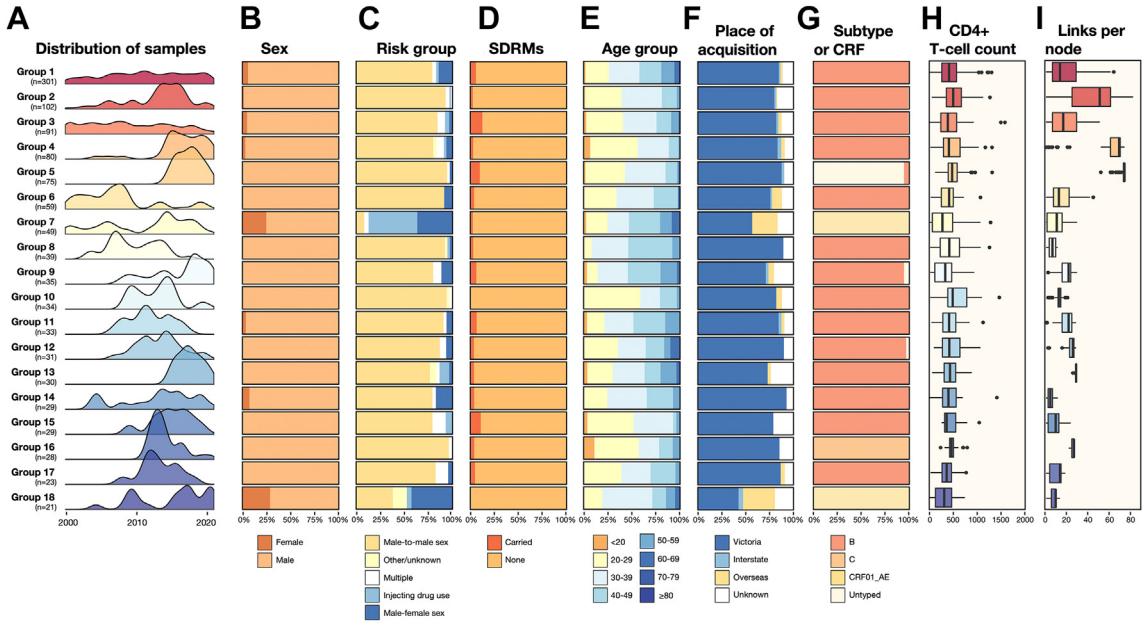


***Fig. 1:* Characteristics of large HIV-1 transmission groups within Victoria, Australia.** (A) Distribution of samples for molecular transmission groups. B–E) Summary of the proportion of different sexes (B), risk groups (C), SDRM (D), age groups (E), probable place of acquisition (F) and subtype or CRF (G) within major transmission groups. (H) Distribution of CD4+ T-cell counts within major transmission groups. Box plots indicate median and IQR, with whiskers representing highest and lowest values within 1.5 × IQR of the upper and lower quartiles, and dots representing outliers. (I) Distribution of per node links within major transmission groups. Box plots indicate median and interquartile range (IQR), formatted as above.

individual; IQR 3–7) to dense (median 74 links per individual; IQR 73–74) (Fig. 1, Supplementary Data B).

Through multivariable logistic regression, we show that individuals were more likely to be a part of a transmission group if they (a) were male (aOR 1.50; 95% CI 1.05–2.15), (b) were born in Australia (aOR 2.13; 95% CI 1.80–2.53), (c) had place of acquisition listed as Victoria rather than overseas (aOR 6.73; 95% CI 5.40–8.41), and/or (d) had injectable drug use as a listed exposure (aOR 2.13; 95% CI 1.18–4.02) (Supplementary Table S1). To focus on contemporary associations, we then restricted the model to only include diagnoses since 2018, representing when PrEP became available through the Australian Pharmaceutical Benefits Scheme (PBS). Notably, this showed an increasing association between individuals being a part of a molecular transmission group if born in Australia (aOR 3.09; 95% CI 2.03–4.73, increasing from aOR 2.13), if place of HIV acquisition was Victoria rather than overseas (aOR 11.93; 95% CI 6.75–21.97, from aOR 6.73), and if the listed exposure was injectable drug use (aOR 3.87; 95% CI 1.06–19.30, from aOR 2.13) (Supplementary Table S3). Additionally, SDRM carriage was more likely for individuals not associated with a transmission group (aOR 1.39; 95% CI 1.07–1.78), as well as older individuals (aOR 1.86; 95% CI 1.04–3.18 for individuals aged 60–69) (Supplementary Table S2).

### Transmission of surveillance drug resistance mutations

To assess transmitted drug resistance (TDR), we identified transmission groups carrying a shared SDRM or set of SDRMs at ≥80% frequency. Fifteen transmission groups putatively represented TDR with these criteria, being a small proportion of the data set (143/3507; 4.1% (95% CI 3.5–4.8%)), although including more than one third of SDRM-carrying sequences (143/375; 38.1% (95% CI 33.4–43.1%)). Four of the transmission groups representing TDR had ten or more individuals, between them carrying SDRMs for NNRTIs (K101E, K103N,

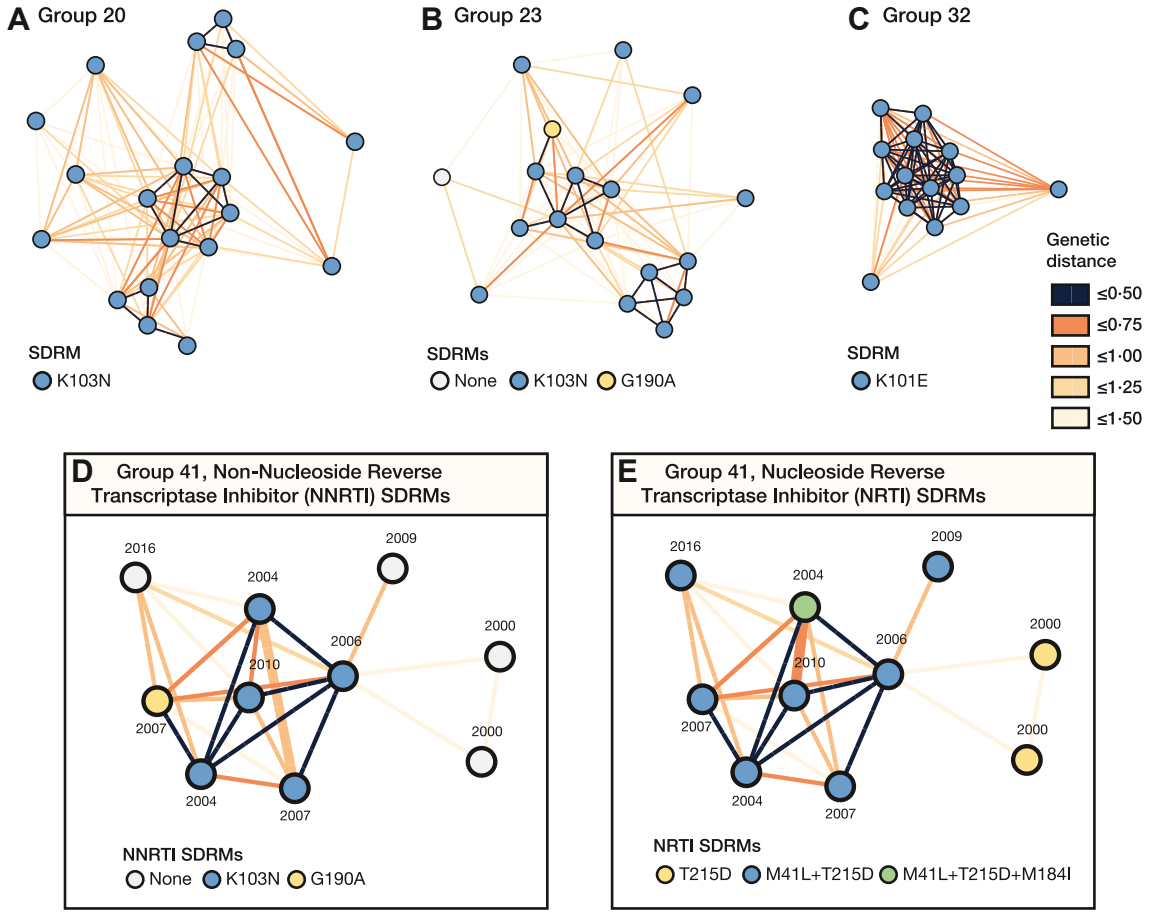


*Fig. 2:* **Transmission of surveillance drug resistance mutations (SDRMs) in Victoria, Australia**. Examples of Victorian transmission groups matching criteria for transmitted drug resistance (TDR) and including ten or more individuals are shown (A–C, with D–E reflecting one group), carrying SDRMs associated with resistance to non-nucleoside reverse transcriptase (NNRTI) and nucleoside reverse transcriptase inhibitors (NRTIs). Links are colored according to the genetic distance threshold required to link nodes, following the HIV-TRACE approach described.

Y181C or G190A) and NRTIs (M41L, M184I, T215A and/or T215D) (Fig. 2). The suitability of the applied GDT was further assessed using TDR groups; the above four groups remained intact at conservative GDTs (1.0% and 1.25%), and increasing GDTs (>1.5%) led to a decrease in the proportion of individuals sharing the relevant SDRM or set of SDRMs, suggesting the applied threshold was suitable (Supplementary Figure S4). A subset of individuals carried multiple SDRMs simultaneously for NNRTIs and NRTIs (30/3507; 0.9% (95% CI 0.6–1.2%)).

### Estimating effective reproductive number of transmission groups

Modelling epidemic growth and decline can be useful in guiding public health responses. In this study, a Bayesian phylodynamic approach was used to infer the effective reproductive number ($R_e$) for groups with 30 or more individuals through the study period (2000–2020) (Fig. 3, Supplementary Data B). Although many groups maintained $R_e$ values less than 2.0 (5/13; 38.5%), the majority showed periods of epidemic expansion, with $R_e$ values reaching >2.0 for eight groups (8/13; 61.5%). Analysed groups appear to have emerged in recent years, with seven groups (7/13; 53.8%) having an estimated most-recent-common-ancestor (MRCA) between 2000 and 2020 (Supplementary Data B), and two (2/13; 15.4%) emerging between 2010 and 2020 (Supplementary Figure S5). As an example, transmission group 5 showed considerable epidemic expansion reaching an $R_e$ peak of 5.6 (median) in early 2014, although with noted uncertainty around these estimates, and had an MRCA estimated at July 2013 (95% highest posterior density (HPD) August 2012–March 2014). To provide added confidence in the model used, a sensitivity analysis specifying reasonable and fixed become uninfectious rates (0.25–2.0) corresponding to infectivity periods of 6 months to 4 years was performed, revealing that this parameter impacted the magnitude but not the timing of $R_e$ change (Supplementary Figure S6).

## Discussion

In this study, a comprehensive collection of HIV-1 *pol* sequences are analysed to provides a high resolution view of HIV transmission in Victoria, Australia. The majority of HIV *pol* sequences (70%; 2438/3507) were

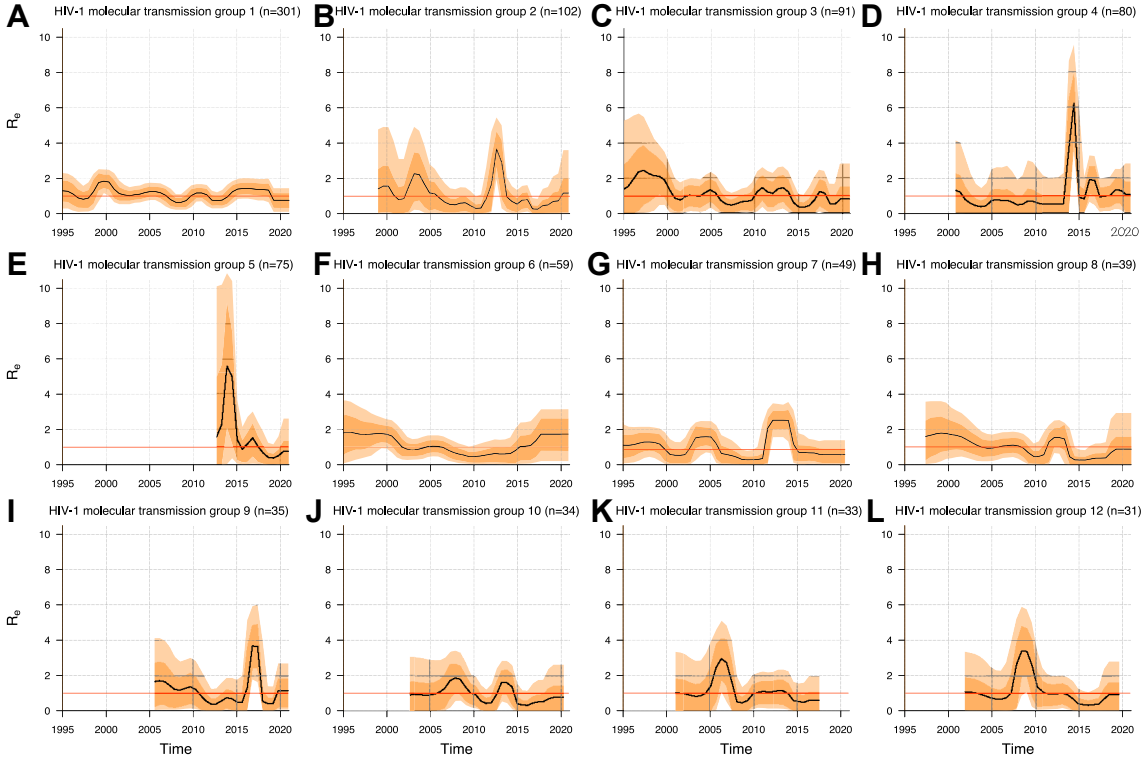


***Fig. 3:* Effective reproductive number ($R_e$) of major HIV molecular transmission groups in Victoria, Australia.** (A–L) The numbers inferred from Birth Death Skyline Serial (BDSKY) models reveals changing patterns of epidemic growth, stabilisation and contraction for the twelve largest HIV transmission groups identified in this study. Median estimate of $R_e$ over time is shown as a black line, the darker orange shading corresponds to the 75% and lighter orange shading the 95% credible intervals. Shading begins at the first credible interval for MRCA (95% HPD) for groups where this is later than 1995, representing the earliest credible point where these transmission group began.

assigned to a transmission group, with some of these groups persisting across the duration of the study. The characteristics of these are detailed by integrating extensive molecular and epidemiological data. Importantly, informative patterns of epidemic growth, stabilisation, and decline were observed for transmission groups. As highlighted by the Australian National HIV Strategy recommended use of high-quality timely data and surveillance systems,[9] the effective introduction of molecular approaches may enable public health services to identify and respond to local transmission, addressing transmission groups displaying resistance to treatment[32] increased virulence,[33] or those linked by shared epidemiological characteristics as shown here.

The introduction of these approaches in routine practice will likely come with further challenges; for HIV, population-level molecular epidemiology likely cannot be real-time given inherent delays between infection, symptom onset, diagnosis and genotyping ahead of analysis. Routine use would require close working between public health laboratories and the relevant response arm. That said, the main public health utility of these approaches may be achieved in countries such as Australia, compared to highly endemic countries, as we approach elimination and are able to focus public health resources toward enhanced contact tracing and relevant support services.[1] Added utility may also come in identifying rapidly growing transmission groups, especially in relatively closed networks; this was seen in Indiana, where molecular analyses were able to reconstruct an outbreak of HIV in PWID and identify individuals at greatest risk for onward transmission.[34]

In line with previous Australian studies, subtype B was commonly observed in this study, comprising approximately 70% of HIV-1 sequences here compared to 74.5% (3631/4873) of sequences in a previous national study.[17] The increasing contribution of global travel and immigration to HIV-1 diversity within Australia may in part explain the observed transition from an early epidemic predominantly characterised by subtype B to a more varied group of subtypes later in the study period.[35] As an example, CRF01_AE increased from 7.5% to 20.5% of typable sequences through the study, with the global dispersal of this subtype centralised to South East Asia.[36] This could further be explored with the inclusion of international HIV-1 *pol* sequences, although the inclusion of such sequences may impact inferences from local data such as collapsing identified transmission groups, say if a close common ancestor was included. Further, transmission within Victoria remains significant, with reported place of acquisition commonly Victoria (67.4%) compared to interstate (2.3%) or international (16.8%) and the remainder unknown (13.5%). Individuals were more likely to be part of a transmission group when born in Australia (aOR 2.13) and/or having a probable place of acquisition as Victoria (aOR 2.83), with this association growing in recent years. Local prevention strategies, therefore, may continue to make significant contributions to reduce transmission.

Transmitted drug resistance was observed, including to more than one therapeutic class. For example, for a subtype B transmission group of ten individuals, the thymidine analogue mutation (TAM) M41L was observed in eight sequences, as was the revertant T215D mutation (10/10 sequences) suggesting possible selective drug pressure from ART. In one sequence from this group, M184I was also observed, being an NRTI-resistant mutation that reduces the susceptibility of L-nucleotide analogs lamivudine (3 TC) and emtricitabine (FTC). Rather than being restricted to the earlier years of the HIV epidemic, this TDR group was observed as recently as 2016. As combination tenofovir/emtricitabine is used as PrEP in this setting, each an NRTI class therapeutic, timely identification of transmitted resistance to these therapies may justify additional resources for secondary case finding and follow up to maintain population level preventative strategies. The prevalence of TDR in Victoria (4.1%) was shown to be similar to comparable nations,[37] with TDR remaining a global challenge.

Estimating temporal changes in HIV transmission patterns may help understand the impacts of preventive strategies and public health policies. Through Bayesian modelling, considerable epidemic expansions and collapses were observed for HIV transmission groups in this study. This did not appear to correlate with major public health initiatives, such as the introduction and increased availability of PrEP for HIV infection prevention, although this has been seen in other settings; in Portugal, estimated declines in $R_e$ values coincided with the introduction of ART and the scale-up of harm reduction for PWID.[21]

Although the public health benefits of genomic epidemiological approaches are increasingly recognised, there remain many ethical considerations around the use of sequence data to characterise disease transmission.[18,19] Individuals and communities may have valid concerns around the lack of informed consent for their data to be used in this way, and, for many countries including Australia, there remains the potential for these data to be used outside of public health settings where criminalisation of HIV transmission remains in place. Further, although state and national integration of sequence data has been achieved for other infectious diseases,[38,39] this may be hampered by the varied legislation in Australian states and territories.

This study has a number of limitations. Inclusion of sequences was dependent on linkage to care, which may underrepresent some groups, although linkage is relatively high throughout Australia. Secondly, sequences are assumed to be from treatment naïve individuals, or those recently initiating therapy. A subset of individuals may, however, have had a previous positive diagnosis,

especially for those emigrating to Australia. In addition, we had limited ability to observe phylodynamic trends in small transmission groups, as these inferences are dependent on adequate sample sizes and sampling frames. This study was also based on partial *pol* sequences; further work could explore the application of whole-genome sequencing. This may provide additional data to define molecular transmission groups and identify HIV anti-retroviral resistances encoded outside of the partial *pol* sequenced routinely at present, such as those involved in integrase-inhibitor resistance.

## Conclusion

The introduction of molecular epidemiological approaches as part of routine HIV surveillance, with community support, has the potential to greatly support HIV prevention in Victoria and Australia more broadly, as one component of a concerted national HIV strategy.

### Contributors

GT, DC, JH, SRL and DAW contributed to study conceptualisation. GT, DC, SH, MG, JD, RS, NR, EL, LM and NH contributed to data generation and curation. GT, SH, MT, DJP, SJL, ES and SD contributed to formal analyses. GEM, MAM, CKF, EPFC and MYC contributed clinical expertise. JP, SRL and DAW contributed to project administration. GT and MT contributed to visualisation. SRL and DAW contributed to project supervision. GT and DAW drafted the initial manuscript and all authors contributed to the final manuscript.

### Data sharing statement

Sequence data arising from this project are made available at GenBank (PP199486–PP202992).

### Declaration of interests

Professor Sharon Lewin has received consulting fees from Abivax, Geovax, ViiV, Tetralogic, Vaxxinity, and Esfam, as well as honoraria from Gilead and Merck Sharpe & Dohme (Merck). The Victorian Department of Health co-funded this research and were involved project conception and data acquisition, although was not involved in the analysis or interpretation of results for this work. The authors declare no other conflicts.

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### Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.lanwpc.2024.101103.

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