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Research Paper

Trends in Pretreatment HIV-1 Drug Resistance in Antiretroviral Therapy-naive Adults in South Africa, 2000–2016: A Pooled Sequence Analysis

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ABSTRACT

Background: South Africa has the largest public antiretroviral therapy (ART) programme in the world. We assessed temporal trends in pretreatment HIV-1 drug resistance (PDR) in ART-naïve adults from South Africa.
Methods: We included datasets from studies conducted between 2000 and 2016, with HIV-1 *pol* sequences from more than ten ART-naïve adults. We analysed sequences for the presence of 101 drug resistance mutations. We pooled sequences by sampling year and performed a sequence-level analysis using a generalized linear mixed model, including the dataset as a random effect.
Findings: We identified 38 datasets, and retrieved 6880 HIV-1 *pol* sequences for analysis. The pooled annual prevalence of PDR remained below 5% until 2009, then increased to a peak of 11·9% (95% confidence interval (CI) 9·2-15·0) in 2015. The pooled annual prevalence of non-nucleoside reverse-transcriptase inhibitor (NNRTI) PDR remained below 5% until 2011, then increased to 10.0% (95% CI 8.4–11.8) by 2014. Between 2000 and 2016, there was a 1.18-fold (95% CI 1.13–1.23) annual increase in NNRTI PDR ($p < 0.001$), and a 1.10-fold (95% CI 1.05–1.16) annual increase in nucleoside reverse-transcriptase inhibitor PDR ($p = 0.001$).
Interpretation: Increasing PDR in South Africa presents a threat to the efforts to end the HIV/AIDS epidemic. These findings support the recent decision to modify the standard first-line ART regimen, but also highlights the need for broader public health action to prevent the further emergence and transmission of drug-resistant HIV.
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Research in context

Evidence before this study

We searched PubMed for systematic reviews and meta-analyses of pretreatment or transmitted HIV drug resistance in South Africa. We used the search terms "HIV" AND "South Africa" AND "drug resistance" AND "(systematic review OR meta-analysis)". We found two meta-analyses exploring regional prevalence of pretreatment or transmitted HIV drug resistance, where data from South Africa were combined with data from other countries in a regional analysis (southern Africa or sub-Saharan Africa). We found a meta-analysis of pretreatment HIV drug resistance in children younger than 12 years, which included data from South Africa. We also had a systematic review from our own group which analysed transmitted drug resistance in South Africa up to 2010. We did not identify any studies that focused on South Africa and incorporated sequences collected since 2010, when scale-up of antiretroviral therapy accelerated.

Added value of this study

In this pooled analysis of 6880 HIV-1 sequences from 38 datasets, we provide up-to-date estimates of the prevalence of pretreatment HIV drug resistance (PDR) in South Africa. We present evidence of increasing PDR, particularly since the acceleration of ART scale-up in 2010. We demonstrate that the increase is largely driven by non-nucleoside reverse-transcriptase inhibitor (NNRTI) PDR, but that levels of nucleoside reverse-transcriptase inhibitor (NRTI) PDR are also rising. In particular, we note a concerning increase in the prevalence of tenofovir resistance-associated mutations (TRAMs), which could have important implications for current treatment and prevention strategies.

Implications of all the available evidence

Our findings provide clear evidence that PDR in South Africa has reached the threshold at which the World Health Organization recommends urgent public health action (NNRTI PDR > 10%). Whilst our data provide support for the decision to move to a new dolutegravir-based first-line regimen, they also highlight the broader need to improve quality of HIV treatment and prevention if South Africa is to achieve the Joint United Nations Programme on HIV/AIDS goal of ending AIDS by 2030.

1. Introduction

The roll-out of antiretroviral therapy (ART) has been a major breakthrough in the global response to HIV, helping to reduce HIV-related deaths by 48% between 2005 and 2016, and new HIV infections by 11% between 2010 and 2016 [1]. Despite these impressive public health gains, substantial expansion of access to ART will be required to achieve the target of ending the HIV epidemic by 2030 [1]. The emergence and transmission of HIV drug resistance (HIVDR) pose a threat to the successful treatment and prevention of HIV, and there is now strong evidence that levels of HIVDR are increasing substantially in southern Africa [2], the region that faces the greatest challenges to ending the HIV epidemic.

Pretreatment HIV drug resistance (PDR) is drug resistance in a person initiating or re-initiating ART (i.e. with or without prior ART exposure) [3,4]. PDR can arise in one of three ways: transmission of drug-resistant HIV from a person with acquired drug resistance (ADR); transmission of primary drug-resistant HIV from another ART-naïve person; or ADR resulting from prior exposure to antiretroviral drugs for treatment or prevention. The presence of PDR is associated with poorer virological outcomes on first-line ART [5,6].

South Africa, with over seven million people living with HIV (PLHIV) in 2016, accounts for almost one in five PLHIV globally [1]. The country has the largest public ART programme in the world, with more than four million people on ART by early 2018 [7]. In the first few years of ART rollout, the levels of PDR were low (<5%) [8]. More recent studies, conducted since the accelerated expansion of ART coverage in 2010, have suggested higher levels of PDR [9,10].

Given this evidence of rising levels of PDR in the country and the wider region, and the continued expansion of ART for treatment and prevention, we performed a pooled analysis of HIV sequence data from South Africa, firstly to determine the annual trends in PDR and secondly to explore in detail the patterns of observed drug resistance mutations (DRMs).

2. Methods

2.1. Search Strategy and Selection Criteria

This study was a systematic review and pooled analysis aimed at determining trends in PDR amongst ART-naïve adults in South Africa. We conducted and reported this in line with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (checklist included in Appendix, p 1) [11]. To identify relevant studies we first searched for published articles in MEDLINE using the OvidSP interface on 12 September 2017 (Appendix, p 3). We then scanned the reference lists of all articles selected for inclusion and conducted forward citation searches using Google Scholar. Finally, we searched South African HIV-1 sequence datasets not linked to a published article, using the PopSet database on the National Center for Biotechnology Information website [12].

We included studies involving adults (defined for the purpose of this analysis as 15 years or older) in South Africa with recent or chronic HIV infection and no documented prior ART exposure. We obtained information about prior ART exposure from either the article or the sequence annotation in GenBank. We excluded studies that enrolled women with documented exposure to antiretrovirals for prevention of mother-to-child transmission (pMTCT). We excluded studies with fewer than ten HIV-1 *pol* sequences; and studies where the sequences were generated from samples collected prior to 2000. Where articles reported on multiple separate cross-sectional studies (for example a series of annual antenatal surveys), we separated the sequences into individual datasets according to the sampling year. If results from the same study were presented in more than one publication, we pooled the sequences into a single dataset. We included sequences from one multi-national study [13], as South African sequences could be identified through the sequence annotation in GenBank.

From the articles, we retrieved a core set of information, including the year(s) of sample collection, province, study type, study population, proportion of participants that were female, and method for determining prior ART exposure.

2.2. Sequence Analysis

We downloaded publicly available sequences for the included studies from GenBank [12]. Where sequences were not publicly accessible, we contacted the study authors to request the sequences. We aligned and visually inspected the sequences in AliView v1.18 (http://ormbunkar.se/aliview/) [14]. We manually edited the sequences until perfect codon-based alignments were produced. We assessed sequences for their completeness and quality using the Calibrated Population Resistance (CPR) tool (http://cpr.stanford.edu/cpr.cgi) [15]. Stop codons, frameshift mutations, APOBEC3G/F hyper-mutations, highly unusual mutations and highly ambiguous nucleotides (B, D, H, V and N), were all used as indicators of poor sequence quality. We excluded from the analysis any

sequence that did not meet the sequence inclusion criteria of the CPR tool [14]. We included all sequences that had complete reverse transcriptase gene (*RT*) sequences (codons 40 to 240), with or without complete protease (*PR*) sequences. Where multiple sequences were identified from the same study participant (for example in cohort studies), we only included the sequence from the earliest time point. Most sequences were not annotated with information about participant sex or age, so we did not include this information in the datasets.

We defined PDR as the presence of any of 101 DRMs. The mutation list included the 93 mutations from the World Health Organization (WHO) 2009 list of surveillance drug-resistance mutations (SDRMs) [16]; and eight additional tenofovir (TDF) resistance-associated mutations (TRAMs) characterised in a recent international collaborative analysis (A62V, K65N, S68GDN, K70QT, and V75L) [17], (Appendix, p 4). Overall, the mutation list encompassed 42 nucleoside reverse-transcriptase inhibitor (NRTI)-resistance mutations at 17 *RT* positions, 19 non-nucleoside reverse-transcriptase inhibitor (NNRTI)-resistance mutations at ten *RT* positions, and 40 protease inhibitor (PI)-resistance mutations at 18 *PR* positions. We used the CPR tool to calculate the proportion of sequences with overall and drug class-specific PDR [15].

2.3. Trends in Pretreatment Drug Resistance

To assess the annual increase in overall and drug class-specific PDR, we pooled sequences from different studies by year of sample collection and performed a generalized linear mixed regression model using the R package (v3.3.1) lme4. We used the presence or absence of PDR (or drug class-specific PDR) as the binary outcome variable and the sampling year as the explanatory variable. Where samples from the same study had been collected over more than one year and where the sequence annotation did not include year of sample collection, we allocated the sequences to the median sampling year. To account for heterogeneity between studies, we included the dataset as a random effect in the model. Given the relatively small number of sequences with specific mutations, we also pooled the sequences into three periods (2000–2008, 2009–2012, and 2013–2016) and checked for any trend in prevalence of specific NRTI- and NNRTI-resistance mutations using the chi-squared test for trend.

3. Results

We initially identified 856 articles through our database search and nine articles through other sources. After removing duplicate publications, we screened 790 abstracts and assessed 46 full-text articles for eligibility. We excluded 14 articles on the basis of our eligibility criteria: eight contained fewer than 10 HIV-1 *pol* sequences; two had only PR sequences with no RT sequences; one reported on a duplicate sequence dataset; one contained only sequences generated from samples collected prior to 2000; one was based on targeted sequencing for a single mutation (K65R); and sequences were unavailable for one study (Appendix, p 5). From the 32 articles, we identified 38 datasets with at least ten HIV-1 *pol* sequences from ART-naïve adults (Fig. 1, Table 1, Appendix,

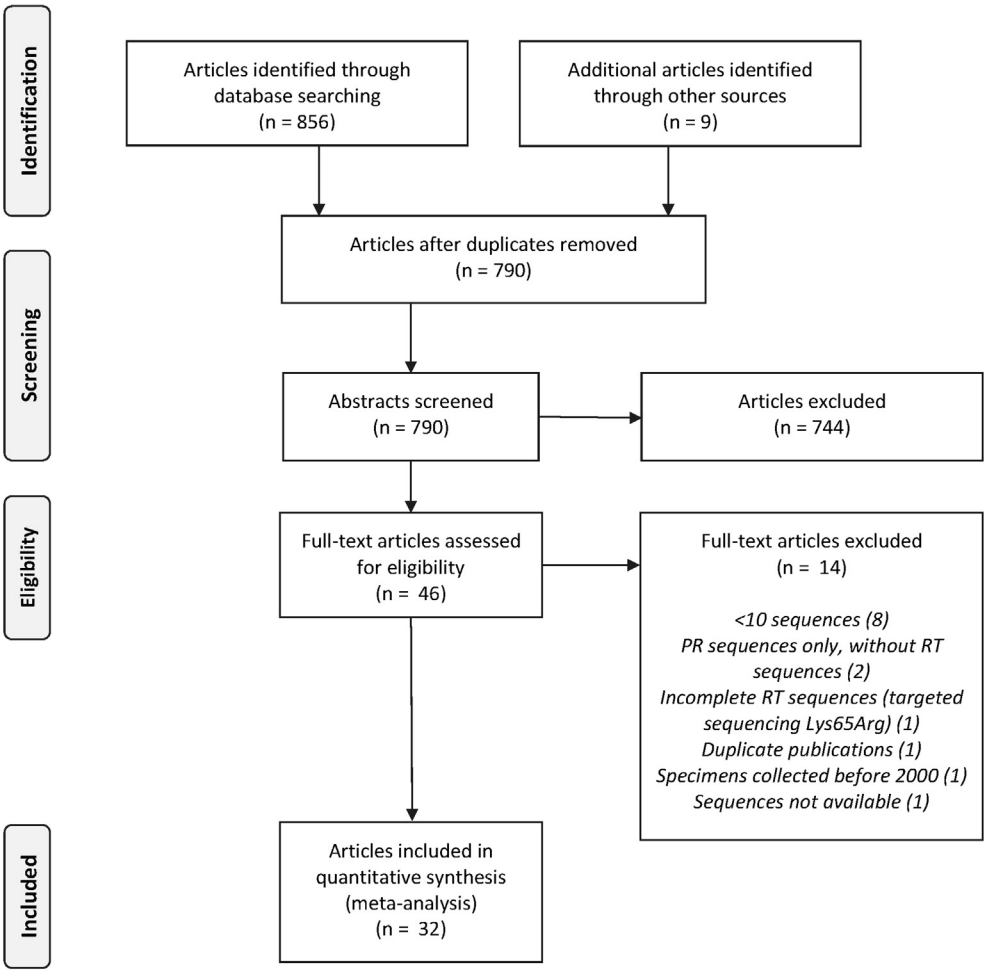

Fig. 1. Flow diagram of articles and datasets identified and selected for the pooled sequence analysis of PDR in antiretroviral therapy-naive adults in South Africa.

Table 1
Characteristics of included datasets with ten or more RT sequences from ART-naïve adults.

Dataset ID	Source	Sampling years	Province(s)	Study type	Study population	Proportion females	Method for determining prior ART use	Met criteria for WHO TDR/PDR survey
1	Bessong	2001	LP	Genetic diversity	ART-naïve adults	79%	NS	No
2	Bessong	2001–2004	GT, LP	TDR	ART-naïve adults	68%	NS	Yes
3	Chimukangara	2013	KZN	Population HIV surveillance	HIV-positive adults >15 years	73%	Linkage to public sector records	No
4	Chimukangara	2014	KZN	Population HIV surveillance	HIV-positive adults >15 years	75%	Linkage to public sector records	No
5	Chimukangara	2014–2015	KZN	Population HIV surveillance	HIV-positive adults 15–49 years	66%	Self-report	No
6	Gordon	2001–2002	KZN	Genetic diversity	ART-naïve adults	66%	NS	No
7	Hamers	2007–2008	GT, MP	PDR	Adults eligible for ART	62%	Self-report	Yes
8	Huang	2006	FS	TDR	ART-naïve adults	NS	Self-report	Yes
9	Hunt	2005	GT, KZN	ANC survey	Primigravid female <25 years	100%	NS	Yes
10	Hunt	2006	GT, KZN	ANC survey	Primigravid female <25 years	100%	NS	Yes
11	Hunt	2007	GT, KZN	ANC survey	Primigravid female <25 years	100%	NS	Yes
12	Hunt	2008	GT, KZN	ANC survey	Primigravid female <25 years	100%	NS	Yes
13	Hunt	2009	GT, KZN	ANC survey	Primigravid female <25 years	100%	NS	Yes
14	Hunt	2010	GT, KZN	ANC survey	Primigravid females ≤21 years	100%	NS	Yes
15	Hunt	2011	EC, FS, GT, KZN, WC	ANC survey	Primigravid females ≤25 years	100%	NS	Yes
16	Hunt	2012	EC, FS, GT, KZN, LP, MP, NC, NW, WC	ANC survey	Primigravid females ≤21 years	100%	NS	Yes
17	Iweriebor	2007–2008	LP	Genetic diversity	ART-naïve adults	90%	Self-report	No
18	Jacobs	2002–2004	WC	Genetic diversity	ART-naïve adults	66%	Self-report	No
19	Jacobs	2008–2010	WC	Neurocognitive study	ART-naïve females	100%	NS	No
20	Manasa	2010	KZN	Population HIV surveillance	HIV-positive adults >15 years	85%	NS	No
21	Manasa	2011	KZN	Population HIV surveillance	HIV-positive adults >15 years	76%	Linkage to public sector records	No
22	Manasa	2012	KZN	Population HIV surveillance	HIV-positive adults >15 years	71%	Linkage to public sector records	No
23	Matthews	2000–2004	KZN	Chronic infection cohort	ART-naïve adults	92%	Self-report	No
24	Msimanga	2009	MP	Genetic diversity	ART-naïve adults	95%	Self-report	No
25	Musyoki	2007	GT	Genetic diversity	Adults initiating ART	NS	Self-report	No
26	Nwobegahay	2008	LP	TDR	ART-naïve adults	70–73%	Self-report	Yes
27	Papathanasopoulos	2006–2007	GT	Genetic diversity	ART-naïve adults	74%	Self-report	No
28	Parboosing	2009	KZN	TDR	Primigravid female <22 years	100%	NS	Yes
29	Parikh	2010–2011	KZN	Trial screening (HIV prevention)	Females 18–40 years first positive test	100%	NS	No
30	Pillay	2000	GT	Trial screening (pMTCT)	ART-naïve pregnant females	100%	NS	No
31	Pillay	2002	GT	ANC survey	Primigravid females <22 years	100%	NS	Yes
32	Pillay	2004	GT	ANC survey	Primigravid females <22 years	100%	NS	Yes
33	Seoighe	2003–2005	GT, KZN	Trial baseline (pMTCT)	Pregnant females	100%	NS	No
34	Steegen	2013–2014	EC, FS, GT, KZN, LP, MP, NC, NW, WC	PDR	Adults initiating ART or in pre-ART care	59%	Self-report	Yes
35	Treurnicht	2004–2005	KZN	Acute infection study	Females with documented acute infection	100%	NS	No
36	van Zyl	2016–2017	WC	PDR	ART-naïve adults initiating ART	52%	Self-report	Yes
37	Wilkinson	2000	WC	Phylogenetic study	ART-naïve patients	NS	NS	No
38	Wilkinson	2004	WC	Phylogenetic study	ART-naïve patients	NS	NS	No

ANC, antenatal care; ART, antiretroviral therapy; EC, Eastern Cape; FS, Free State; GT, Gauteng; KZN, KwaZulu-Natal; LP, Limpopo; MP, Mpumalanga; NC, Northern Cape; NS, not stated; NW, North West; PDR, pretreatment drug resistance; pMTCT, prevention of mother-to-child transmission; TDR, transmitted drug resistance; WC, Western Cape.

pp. 6–9) [8–10,13,18–44]. Seventeen datasets were from formal surveys of PDR or transmitted drug resistance.

We retrieved 7025 *RT* sequences and 6501 *PR* sequences. We excluded 145 RT sequences and 207 PR sequences that did not meet sequence quality criteria. Therefore, we included 6880 *RT* sequences and 6294 *PR* sequences in the analysis (i.e. 6294 sequences with combined *PR* and *RT* and 586 with *RT* only) (Appendix, pp. 10, 11). The majority of sequences were subtype C (99.2%). Overall, 478 of 6880 sequences (6.9%) had at least one DRM. The majority of these sequences had only NNRTI-resistance mutations (289/478, 60.5%); dual class NRTI and NNRTI PDR were present in 79/478 (16.5%) (Appendix, p 12). The prevalence of overall and drug class-specific PDR in each dataset is displayed

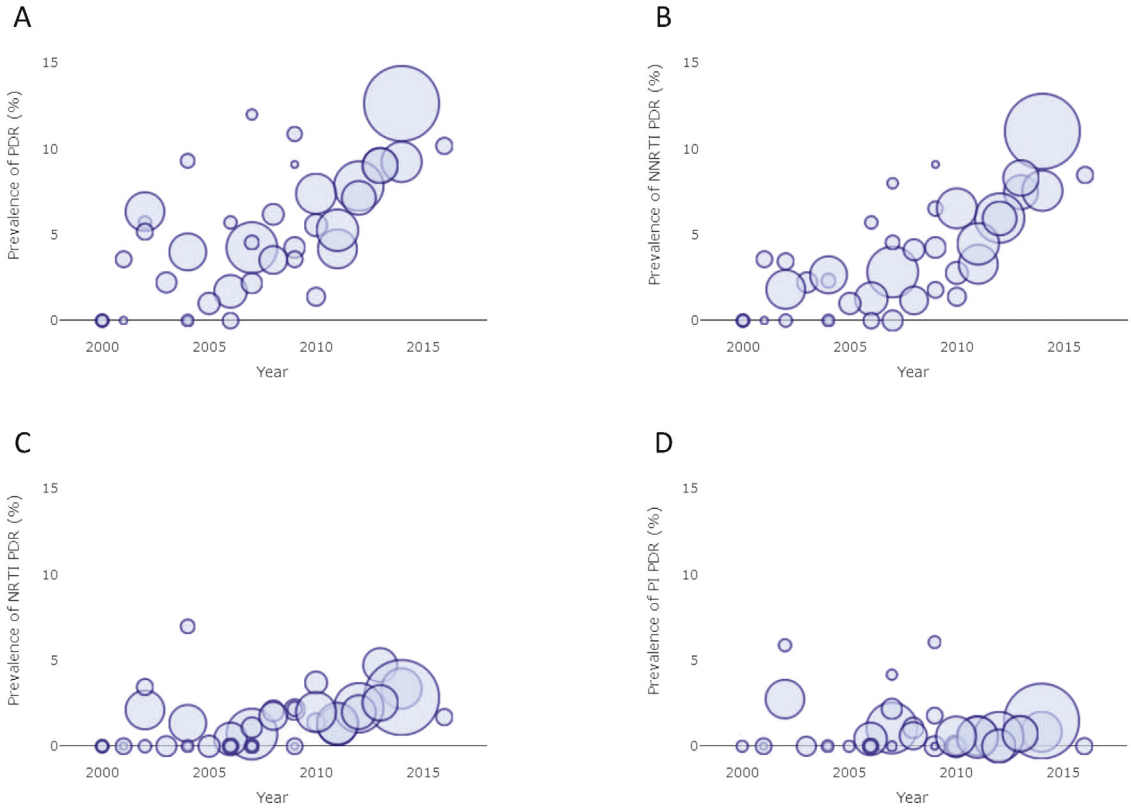

Fig. 2. Prevalence of pretreatment HIV drug resistance by year of sampling. A) Overall, B) non-nucleoside reverse-transcriptase inhibitor, C) nucleoside reverse-transcriptase inhibitor, D) protease inhibitor. Each bubble represents a dataset and the size of the bubble is proportional to the number of sequences in the dataset. The sampling year is shown on the horizontal axis and the percentage PDR on the vertical axis. PDR, pretreatment HIV drug resistance.

in Fig. 2, and the crude pooled prevalence of overall and drug class-specific PDR by year is shown in Table 2. The prevalence of NNRTI PDR remained below 5% until 2011 and then increased rapidly to above 10% by 2014. The pooled prevalence of NRTI PDR and PI PDR remained below 5% across all years. Over the entire study period (2000–2016), there was a 1.10-fold yearly increase in the odds of PDR (95% confidence interval (CI) 1.06–1.15), which was driven by increasing NNRTI PDR (odds ratio (OR) 1.18, 95% CI 1.13–1.23) and NRTI PDR (OR 1.10, 95% CI 1.05–1.16) (Table 3).

Overall, 374 sequences (5.4%) had at least one NNRTI DRM (Appendix, p 13). The most prevalent mutation was K103NS, occurring in 278 sequences (58.2% of sequences with any DRM; 4.0% of all sequences) (Fig. 3). In the majority of these sequences (218/278), K103NS was the only DRM. Other common NNRTI-resistance mutations included V106AM (n = 47), Y181C (n = 34), K101EP (n = 29) and G190ASE (n = 27). Overall, 77/374 (20.6%) had more than one NNRTI DRM, most commonly K103N + P225H (n = 16) and K103N + V106M (n = 12). The prevalence of some specific NNRTI-resistance mutations increased over time. This trend was most marked for the K103NS and V106AM mutations, and less so for the K101EP mutations. There was no evidence of changing prevalence of Y181C or G190ASE (Appendix, p 14).

Table 2
Pooled prevalence of pretreatment HIV drug resistance (PDR), NNRTI PDR, and NRTI PDR, by year.

Year	Number of RT sequences	Any DRM	Any PDR (95% CI)	NNRTI DRM	NNRTI PDR (95% CI)	NRTI DRM	NRTI PDR (95% CI)
2000	66	0	–	0	–	0	–
2001	69	2	2.9 (0.4–10.1)	2	2.9 (0.4–10.1)	0	–
2002	424	26	6.1 (4.0–8.9)	8	1.9 (0.8–3.7)	9	2.1 (1.0–4.0)
2003	90	2	2.2 (0.3–7.8)	2	2.2 (0.3–7.8)	0	–
2004	377	16	4.2 (2.4–6.8)	9	2.4 (1.1–4.5)	7	1.9 (0.7–3.8)
2005	113	1	0.9 (0–4.8)	1	0.9 (0–4.8)	0	–
2006	303	5	1.7 (0.5–3.8)	4	1.3 (0.4–3.3)	1	0.3 (0–1.8)
2007	748	32	4.3 (2.9–6.0)	21	2.8 (1.7–4.3)	5	0.7 (0.2–1.6)
2008	290	13	4.5 (2.4–7.5)	7	2.4 (1.0–4.9)	5	1.7 (0.6–4.0)
2009	172	7	4.1 (1.7–8.2)	6	3.5 (1.3–7.4)	2	1.2 (0.1–4.1)
2010	306	17	5.6 (3.3–8.7)	12	3.9 (2.0–6.7)	6	2.0 (0.7–4.2)
2011	953	54	5.7 (4.3–7.3)	45	4.7 (3.5–6.3)	16	1.7 (1.0–2.7)
2012	788	60	7.6 (5.9–9.7)	47	6.0 (4.4–7.9)	17	2.2 (1.3–3.4)
2013	370	36	9.7 (6.9–13.2)	31	8.4 (5.8–11.7)	16	4.3 (2.5–6.9)
2014	1255	142	11.3 (9.6–13.2)	126	10.0 (8.4–11.8)	38	3.0 (2.2–4.1)
2015	497	59	11.9 (9.2–15.0)	48	9.7 (7.2–12.6)	12	2.4 (1.3–4.2)
2016	59	6	10.2 (3.8–20.8)	5	8.5 (2.8–18.7)	1	1.7 (0–9.1)

CI, confidence interval; DRM, drug resistance mutation; NRTI, nucleoside reverse-transcriptase inhibitor; NNRTI, non-nucleoside reverse-transcriptase inhibitor; PDR, pretreatment drug resistance; RT, reverse transcriptase.

Table 3
Annual change in odds of pretreatment HIV drug resistance, 2000–2016.

Drug class	Odds ratio (95% CI)	p value
NRTI	1.10 (1.05–1.16)	0.0001
NNRTI	1.18 (1.13–1.23)	<0.0001
PI	0.96 (0.89–1.04)	0.3650
Overall	1.10 (1.06–1.15)	<0.0001

CI, confidence interval; NRTI, nucleoside reverse-transcriptase inhibitor; NNRTI, non-nucleoside reverse-transcriptase inhibitor; PI, protease inhibitor.

M184VI was the most common NRTI-resistance mutation, present in 71 sequences (14.9% of sequences with any DRM; 1.0% of all sequences) (Appendix, p 15). Most of the sequences with M184VI had at least one NNRTI DRM (66/71) and just under half had additional NRTI DRMs (31/71). The other NRTI DRMs accompanying M184VI included thymidine analogue mutations (TAMs, n = 11), TRAMs (n = 11), L74VI and/or Y115F (n = 7), and other multi-NRTI mutations (n = 2). Classical TAMs (M41L, D67N, K70R, L210W, T215FY, and K219EQ) were detected in 36 sequences (7.5% of sequences with any DRM; 0.5% of all sequences). The majority of these (30/36) had a single TAM; and eleven sequences had the M41L mutation alone without other DRMs. Overall, TRAMs were detected in 37 sequences (7.7% of sequences with any DRM; 0.5% of all sequences). The TRAM most frequently detected was K65R (n = 21). Twelve sequences had a TRAM not on the WHO SDRM list (A62V, n = 10; K70T, n = 2), although in four of these sequences the mutation was present with the K65R mutation. The prevalence of TRAMs increased in later time periods: 0.1% (3/2480) in 2000–2008, 0.5% (11/2219) in 2009–2012, and 1.1% (23/2181) in 2013–2016, and for the M184VI mutation: 0.2% (4/2480) in 2000–2008, 0.9% (20/2219) in 2009–2012, and 2.2% (47/2181) in 2013–2016 ($p < 0.001$, χ^2 test for trend) (Appendix, p 14).

Fifty-six sequences (0.9%) had at least one PI DRM. The most frequently observed mutation was the relatively non-polymorphic M46IL mutation, which was detected in 35 sequences (0.6%) (Appendix, p 16).

4. Discussion

In this pooled analysis with more than 6000 HIV-1 sequences from ART-naïve adults in South Africa, we observed a sustained increase in pretreatment HIV drug resistance between 2000 and 2016, driven primarily by NNRTI-resistance. The increase in PDR seems to have accelerated since 2010, which coincides with the rapid expansion of ART coverage in the country from just 20% in 2010 to 56% in 2016 [45]. By 2014, the pooled prevalence of NNRTI PDR had reached 10%, the threshold at which the WHO now recommends urgent public health action [46]. There was also some evidence of increasing NRTI PDR, particularly TDF-associated resistance and the M184VI mutation associated with lamivudine (3TC) and emtricitabine (FTC) resistance. However, the pooled prevalence of NRTI-resistance remained low (<5%) in each sampling year.

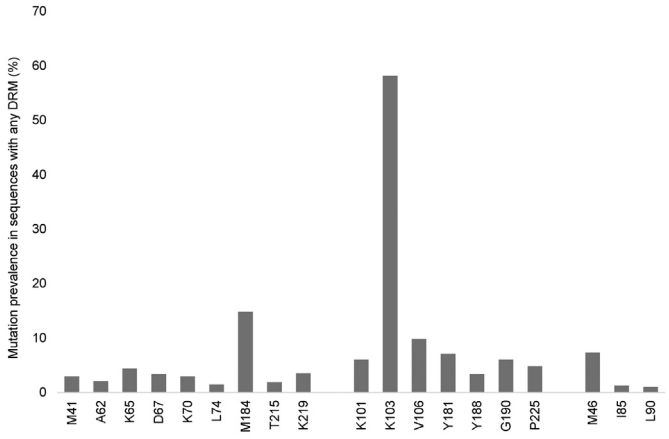

Fig. 3. Prevalence of specific mutations in HIV-1 sequences with any drug resistance mutation. Mutations shown on the horizontal axis include all mutations observed in >1% of the sequences with any drug resistance mutation. DRM, drug resistance mutation.

These findings are consistent with those from recent meta-analyses exploring drug resistance across Africa, which showed levels of resistance rising to moderate levels about ten years into the scale-up of ART in the region [2,47]. The overall 11% annual increase in odds of PDR between 2000 and 2016 in South Africa is comparable to the 12% increase in odds of transmitted drug resistance across sub-Saharan Africa between 2000 and 2013 [47]. The 18% annual increase in odds of NNRTI PDR is somewhat lower than the 24% reported for the southern Africa region in a more recent meta-analysis [2]. That could be explained by the fact that we only included ART-naïve adults, whereas the regional meta-analysis included a small number of sequences from people with prior ART exposure. Alternatively, it could be that the higher rate of increase in PDR in the regional meta-analysis was reflective of higher levels of PDR in other southern African countries.

Our analysis was restricted to ART-naïve individuals and our assumption is therefore that transmitted drug resistance is the primary driver of the increasing PDR prevalence. There are limitations to this assumption, best illustrated by the most prevalent DRM, the K103NS mutation. This mutation, selected by efavirenz (EFV) and nevirapine (NVP), is the most common acquired NNRTI DRM in people with virological failure on standard first-line ART regimens in South Africa [48]. Viruses with the K103NS mutation have transmission fitness similar to wild-type virus [49,50], and can persist for years in the infected host [51]. It's therefore entirely plausible that the high prevalence of this mutation is a consequence of frequent transmission. However, K103NS is also the most common mutation to emerge in women who receive single-dose NVP for the prevention of mother-to-child transmission and, in this context too, the mutation can persist for years in the absence of antiretroviral therapy [52,53]. Although we restricted the analysis to ART-naïve individuals, we could not be certain that participants in the individual studies were truly ART naïve. Most studies relied on self-report of antiretroviral use, which can be unreliable [54–59]. Given that the majority of sequences were from women, it is possible that some of the NNRTI-resistance arose from prior exposure to NVP for pMTCT rather than from transmitted drug resistance.

We also revealed evidence of increasing NRTI-resistance, at a rate similar to that observed in the larger regional meta-analyses [2,47]. We specifically demonstrated increasing prevalence of TRAMs and the M184VI mutation, which is of some concern as TDF and FTC/3TC remain the NRTI backbone of choice for first-line ART regimens. In the latter years (2013–2016), the pooled prevalence of the M184VI mutation was approximately 2% and the prevalence of TRAMs was 1%. TDF and FTC/3TC have been part of the standard first-line ART regimen in South Africa since 2010. The national drug resistance survey in 2013–14 showed that most people with virological failure on first-line NNRTI-based ART harboured the M184VI mutation and about half had TRAMs [48]. Whilst our findings could be a signal of increasing transmission of NRTI-resistant virus, we urge some caution in interpretation. Viruses with the M184VI and K65R mutations are thought to be infrequently transmitted due to low transmission fitness [49,50]. If they are transmitted, the mutations revert rapidly in the absence of drug pressure [51,60]. It is possible that some of the sequences with NRTI resistance were obtained from people who reported themselves to be ART naïve but who had previously been exposed to NRTIs. This is certainly plausible as there is an increasing frequency of cyclical engagement in care as ART programmes have matured [61]. Somewhat against that was the observation that the prevalence of TAMs did not change and remained very low (<1%) throughout the study period, although this may be a reflection of the diminished use of stavudine and zidovudine in first-line regimens.

We included a number of TRAMs that are not currently in the WHO SDRM list, but that are associated with TDF selection pressure [17]. We did identify sequences with these TRAMs, in particular the A62V mutation, which was present both with and without the signature K65R mutation. Further work is required to understand the significance of these mutations and their effect on response to TDF-based regimens.

Without appropriate action, PDR at the levels we have documented would be likely to have a significant impact on the HIV epidemic in South Africa. One mathematical model suggested that with PDR prevalence ≥10% and no change in the rates of resistance acquisition and transmission, 16% more AIDS deaths each year, 9% higher HIV incidence, and 8% higher ART costs would be attributable to drug resistance in Africa between 2016 and 2030 [62]. Once prevalence of NNRTI PDR exceeds 10%, the WHO recommends that national programmes consider switching to an alternative non-NNRTI first-line ART regimen [46]. Many countries, including South Africa, have taken the decision to transition to a new first-line regimen of co-formulated generic TDF, 3TC and dolutegravir (DTG) [63]. This is the option that mathematical models have predicted will mitigate the effects of HIVDR, will produce the greatest health benefits and reduce overall programme costs [64,65]. However, there remain unanswered questions around DTG in the South African context, and strengthening of HIVDR surveillance and response systems will still be important to maximise the impact of the new regimen [66,67].

An alternative approach to the modified first-line ART regimen would be to introduce pretreatment HIVDR testing and shift towards individualised drug regimens [46]. Whilst there is some evidence that HIVDR testing can be implemented in a research setting in South Africa [68], there is no evidence that it can be delivered cost-effectively through the public health system. The shift towards more rapid initiation of ART (including same-day initiation) would make it particularly challenging to deliver pretreatment HIVDR testing. We still lack simple, rapid, and inexpensive HIVDR assays, although there are promising technologies in development [69]. Given the increasing complexity of HIV care and the uncertainty about the long-term effectiveness of DTG-based regimens, there is still a need to develop and evaluate HIVDR assays and pretreatment HIVDR testing strategies.

We believe it would be a mistake to think that modifying the first-line ART regimen is an adequate response on its own to the rising levels of PDR. Whilst there will clearly be a reduced risk of drug resistance emergence with DTG-based regimens, the public health approach to ART creates scenarios where the risk may be higher, particularly where DTG is the only fully active agent in the regimen [66,67]. The increasing prevalence of PDR reflects weaknesses in prevention, treatment, and care. Although South Africa implements routine viral load monitoring for people on ART, there are critical gaps in the viral load testing cascade and long delays in switching people with virological failure to second-line regimens [70]. This means there is probably an expanding pool of people with acquired HIVDR who can then transmit drug-resistant virus to susceptible individuals. Our findings therefore support calls to focus on improving the quality of HIV services [71]. This needs to be rooted within a broader multisectoral response, informed by high quality transdisciplinary research, that addresses the social and structural drivers of the epidemic [72].

Interpretation of our findings should be subject to some limitations beyond those already discussed. Firstly, certain provinces were over-represented in our analysis, particularly KwaZulu-Natal and Gauteng, and estimates from the latter years were dominated by two large population-based surveillance studies from KwaZulu-Natal. Findings from the national PDR survey in 2013–14 suggested substantial heterogeneity between the provinces in levels of PDR, and therefore our estimates may not reflect the situation throughout the country [9]. Secondly, we pooled results from a number of individual studies, not all of which were designed to evaluate PDR. We did not account for individual study design in our analysis and derived only pooled crude estimates of prevalence. Our estimates should therefore not be taken to represent population prevalence. Lastly, we analysed only sequence data and were unable to explore differences by sex, age, CD4+ cell count, and duration of infection, as this information was not available for the majority of sequences.

In conclusion, we present evidence that the prevalence of PDR has risen substantially in South Africa in the past few years. Whilst this is predominantly NNRTI-resistance, there is also evidence of rising levels of resistance to TDF and FTC/3TC, although the absolute prevalence of PDR to these drugs remains low. Our findings support the decision to transition to a new, DTG-based first-line ART regimen. If the association between neural tube defects and DTG is confirmed, and NNRTIs continue to be recommended for women of childbearing age [73], this evidence would suggest the need for additional interventions, such as pre-treatment genotypic resistance testing or early VL testing. These findings also highlight the need for broader strengthening of HIV services within the public health system if we are to eliminate HIV/AIDS as a public health threat by 2030.

Contributors

BC, RJL, S-YR and TDO were responsible for the study conception and design; BC, RJL, S-YR, AK, GH, PK, JM, and TDO were responsible for acquisition of data; BC, RJL, S-YR, JG, KN, LL, RS, AV, and TDO were responsible for data analysis; BC, RJL, S-YR, JG, AK, KN, LL, CC, DK, KAA, KD, RS, GH, AV, BS-P, MG, TM, PK, GR, JL, MK, LM, UMP, JWM, RWS, DK, PM, RKG, DP, SSAK and TDO were responsible for data interpretation, and critically revising the manuscript for important intellectual content. All authors approved the final version of the manuscript.

Declaration of Interests

We declare no competing interests.

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Appendix A. Supplementary data

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