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Moderate to high levels of pre-treatment HIV drug resistance in KwaZulu-Natal Province, South Africa

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Abstract

Introduction: There is evidence of increasing levels of pre-treatment HIV drug resistance (PDR) in Southern Africa. We used data from two large population-based HIV surveillance studies to estimate prevalence of PDR in KwaZulu-Natal, the province with the highest HIV prevalence in South Africa.

Methods: Sanger sequencing was performed on samples obtained from a longitudinal HIV surveillance programme (study A, 2013-2014) and the HIV Incidence Provincial Surveillance System (study B, 2014-2015). Sequences were included for adult HIV positive participants (age ≥15 years for study A, age 15-49 years for study B) with no documented prior exposure to ART. Overall and drug class-specific PDR was estimated using the World Health Organization 2009 surveillance drug resistance mutation (SDRM) list and phylogenetic analysis was performed to establish evidence of drug resistance transmission linkage.

Results: One thousand eight hundred and forty-five (1845) sequences were analysed (611 study A; 1234 study B). An overall PDR prevalence of 9.2% (95% confidence interval (CI): 7.0-11.7) was observed for study A and 11.0% (95% CI 8.9-13.2) for study B. In study B, the prevalence of non-nucleoside reverse-transcriptase inhibitor (NNRTI) PDR exceeded 10% for sequences collected in 2014 (10.2%, 95% CI 7.5-12.9). The most prevalent SDRMs were K103NS (7.5%), M184VI (2.4%) and V106AM (1.4%). There was no evidence of large transmission chains of drug-resistant virus.

Conclusion: High level NNRTI-PDR (>10%) suggests a need to modify the standard first-line ART regimen and to focus attention on improving the quality of HIV prevention, treatment and care.

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Introduction

After approximately two decades of combination antiretroviral therapy (ART), the global response to the human immunodeficiency virus (HIV) is threatened by the development of HIV drug resistance (HIVDR).[1] Pre-treatment drug resistance (PDR) refers to the presence of drug resistance in a person initiating or re-initiating ART, and can therefore be a combination of transmitted and acquired drug resistance (ADR). Such resistance is considered the best indicator to guide the selection of effective first-line ART regimens.[2–4] While the levels of PDR in low- and middle-income countries have been low to moderate historically, there are concerns over increasing levels, given the rapid expansion in ART access and the persistent high incidence of new HIV infections.[5] Once PDR exceeds 10%, modelling suggests that in Africa, HIVDR could account for almost half a million new infections, and $6.5 billion in additional ART costs between 2016 and 2030.[6]

As part of its coordinated approach to prevent, monitor and respond to the emergence of HIVDR, the World Health Organization (WHO) recommends surveys of PDR.[4] As more people receive ART and develop ADR, the risk of transmission of drug-resistant HIV increases.[7] The presence of PDR can lead to inadequate virologic suppression on ART and further accumulation of drug resistance mutations,[8,9] and as a result, the levels of PDR have to be continually monitored to ensure the effective use of ART. At this critical juncture in the global response to HIV, with scale-up of universal test-and-treat and pre-exposure prophylaxis (PrEP) for HIV prevention,[10] it is important to understand the current epidemiology of PDR in high-prevalence settings.

South Africa has the largest ART programme in the world, with approximately 3.9 million people on treatment as of August 2017.[11] Generally, low levels of PDR have been documented in the country,[12,13] but there is recent evidence of higher levels, which raises concern over the continued effectiveness of first-line non-nucleoside reverse-transcriptase inhibitor (NNRTI)-based ART regimens.[14–16] The evidence of increasing PDR is in the context of a growing number of people with virological failure on ART and delayed switching to second-line ART, creating an expanding pool of those with ADR.[17] In this paper, we present

estimates of PDR from two population-based studies in KwaZulu-Natal Province (KZN), South Africa.

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Materials and Methods

Setting

The Africa Health Research Institute (AHRI) has conducted longitudinal population-based HIV surveillance in the uMkhanyakude District Municipality, northern KZN since 2003 (Study A, Figure 1).[18] All individuals 15 years and older in a population of approximately 65,000 resident members are invited to provide dried blood spot (DBS) specimens on an annual basis. For this study, viral reverse transcription PCR was performed on the DBS specimens of the participants with a positive HIV enzyme-linked immunosorbent assay (ELISA) in 2013 or 2014, who had a DBS HIV ribonucleic acid (RNA) ≥10,000 copies/mL. PCR and sequencing were also attempted on some DBS specimens with HIV RNA <10,000 copies/mL, but this was not pursued as the rate of successful amplification was low. We excluded sequences obtained from participants with documented ART initiation prior to the date of specimen collection. Information about ART use was obtained through linkage of the population surveillance data with routine HIV programme data.[19] This did not include information about prior use of antiretrovirals for preventing mother-to-child transmission (pMTCT). We estimated the date of HIV infection using the midpoint between the last negative test date and the first positive test date, and estimated the duration of infection in months, by calculating the time between the estimated date of infection and the sample collection date, as described previously.[14]

Epicentre AIDS Risk Management, The Centre for the AIDS Programme of Research in South Africa (CAPRISA), and the United States Centers for Disease Control and Prevention (CDC) coordinate the HIV Incidence Provincial Surveillance System (HIPSS) in two sub-districts of uMgungundlovu District Municipality in central KZN (Study B, Figure 1).[20–22] In 2014-2015, a representative cross-sectional household survey enrolled 9812 individuals aged 15-49 years. A multistage cluster sampling technique was used to randomly select the households and individuals included in the study, as described previously.[23] One individual was selected per household. From the 14618 households found and occupied,

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9812 individuals were eligible and consented to participate. HIV ELISA was performed on the peripheral blood specimens of the participants,[22] and sequencing was performed on the plasma specimens from participants with positive HIV serology and plasma HIV RNA ≥1000 copies/mL.[21] For the analysis, we excluded sequences from those who self-reported any prior ART use (for treatment or pMTCT). Details on the timing of recruitment and ascertainment of ART status for the two population-based studies, are provided in Supplementary data.

Laboratory methods and data analysis

Genotypic drug resistance testing of the HIV-1 *reverse transcriptase (RT)* and *protease (PR)* genes was done on stored specimens by Sanger sequencing on an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA) using previously described methods.[24] Sequence quality and coverage were assessed using the Calibrated Population Resistance (CPR) tool (http://cpr.stanford.edu/cpr.cgi). Sequences that had quality concerns, such as stop codons, or that did not cover all possible surveillance drug resistance mutation (SDRM) positions, were excluded. We included participants with complete *RT* sequences, with or without the *PR* sequence.

PDR was determined by detecting SDRMs with the CPR tool using the WHO 2009 SDRM list.[25,26] The results were used to estimate the levels of overall and drug class-specific resistance for each study, with the data being analyzed using STATA version 15.1 (StataCorp, College Station, TX) and SAS version 9.4 (SAS Institute, Cary, NC). The chi-square test was used to establish any difference in PDR prevalence across the years, within each study, with a Rao-Scott chi-square test being used for study B to adjust for the survey design. Logistic regression analysis was performed to explore associations between PDR and individual participant characteristics for each study (i.e. sex, age, HIV RNA, and for study A; the estimated duration of infection), and accounted for the survey sample design in study B. Where appropriate, analyses for study B were conducted by applying sampling weights and using survey procedures. The sampling weights adjusted for non-equal probabilities of selection associated with the complex survey design, and for non-response across age and gender categories.[20] The confidence intervals were calculated using Wald

confidence limits, and the Taylor series linearization method was used to estimate standard errors of proportions.

To establish evidence of SDRM transmission, we performed phylogenetic analysis to identify HIV transmission clusters. Sequences with drug resistance mutations identified in this study were aligned with a background dataset of 15,313 HIV-1 subtype C *pol* sequences. This consisted of publicly available sequences from the Los Alamos HIV Database (http://www.hiv.lanl.gov), isolated from Southern African countries, and sequences generated previously from the AHRI surveillance population.[14,17,27] To avoid cluster formation due to convergent evolution under ART pressure, codon positions associated with drug resistance mutations were removed from the alignment.[28]

A maximum likelihood (ML) phylogenetic tree was constructed using FastTree2,[29] and cluster support was assessed with Shimodaira-Hasegawa approximate likelihood ratio test (SH-aLRT) with 1000 pseudo-replicates. HIV-1 transmission clusters were identified from the ML tree using the ClusterPicker software version 1.2.3,[30] where the definitions of a transmission cluster were set to a minimum clade support of 90 SH-aLRT and a maximum within-cluster genetic distance of 4.5%. To identify if sequences clustering together had similar SDRMs, we further submitted the full-length sequences (with all codon positions) to the CPR tool.

Ethics Statement

Approval for the two studies was obtained from the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (UKZN) (reference numbers BF233/09 and BF269/13) the KZN Provincial Department of Health (HRKM 08/14) and the Centre for Global Health, CDC. Written informed consent for use of stored specimens was obtained from all study participants.

Results

A total of 1845 HIV-1 sequences were included in the analysis, and consisted of 611 sequences for study A, with 254 from 2013 and 357 from 2014. From study B, 1234 sequences were included, with 737 from 2014 and 497 from 2015 (Figure 2).

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Overall, 1841 had complete *RT* and *PR* sequences, and four had only the complete *RT* sequence. The characteristics of the participants included in the analysis are summarised in Table 1.

The estimated prevalence of PDR was 9.2% (95% confidence interval (CI) 7.0-11.7) for study A and 11.0% (95% CI 8.9-13.2) for study B. The estimated prevalence of NNRTI PDR was 7.5% (95% CI 5.6-9.9) for study A and 9.2% (95% CI 7.2-11.3) for study B. There was no evidence of an increase in overall PDR or NNRTI PDR across the two years in either study (Figure 3). The estimated prevalence of PDR was higher for women than men in both studies: 9.9% vs. 7.1% for study A (odds ratio (OR) 1.45, 95% CI 0.73-2.87); and 13.6% vs. 8.3% for study B (OR 1.73, 95% CI 1.06-2.81) (Table 3 and Supplementary Figure S1).

The prevalence of PDR peaked at 17.0% (95% CI 11.9-22.1) in women aged 25-34 years (Supplementary Table S1). There was no strong evidence of an association between PDR and age or HIV RNA in either study (see Table 3). In study A, the prevalence of PDR was lower in those with an estimated duration of infection ≤24 months than in those with estimated duration >24 months (3.0% vs. 8.6%), but the analysis was limited by small numbers with recent infection (n=66) (see Table 3).

Of all 1845 sequences across the two studies, 212 (11.5%) had at least one SDRM. The frequency of individual SDRMs by study year is displayed in Table 2. Overall, 182/1845 (10.0%) had NNRTI mutations, 59/1845 (3.2%) had nucleoside reverse-transcriptase inhibitor (NRTI) mutations, and 23/1841 (1.2%) had protease inhibitor (PI) mutations (Table 2). Of those with SDRMs, 162 (76.4%) had single class resistance, 48 (22.6%) dual class resistance, and two (1.0%) triple class resistance. The most frequently observed SDRM was the NNRTI mutation K103NS, occurring in 139 participants (7.5% of all participants, or 65.6% of those with SDRMs). In 100 participants (47.2% of those with drug resistance mutations), the K103NS mutation was the only SDRM detected (Supplementary Table S2), which lists the most frequently observed patterns of mutations).

The most common NRTI mutation was M184VI (2.4%), and in almost all cases (43 of 44) it was detected in combination with at least one NNRTI mutation, while in half the cases (22 of 44) with other NRTI mutations. The K65R mutation associated with tenofovir (TDF)

resistance was detected in 11 participants overall (0.6%), with no evidence of an increase across the two years in either study. Of the 23 with PI mutations, 20 had a single PI mutation. The most common PI mutation was the M46IL mutation, occurring in 18 participants. Two participants with four or more PI mutations had similar patterns of PI resistance (M46I, I54V, L76V, V82A), with one participant having in addition the L90M mutation. In both cases, there was triple class resistance with NRTI mutations (M184V, L74V) and an NNRTI mutation (K103S).

From the phylogenetic analysis, we identified 25 transmission clusters with individuals harbouring at least one PDR mutation in common (Supplementary Figure S2). In total, 57 individuals were grouped in these transmission clusters, 56% (32/57) were from studies A and B, and 44% (25/57) were South African individuals whose sequences were present in the background dataset. Individuals from studies A and B (in the transmission linkages) comprised 15% (32/212) of the sequences with any PDR mutations identified here. From the background dataset, we had information about ART exposure for 18 of the 25 individuals. Sixteen of the 18 (89%) were ART experienced, and were linked to 14 ART-naïve individuals with PDR (Supplementary Figure S2). K103NS was the most common PDR mutation, observed in 72% (41/57) of individuals involved in linked transmissions.

Discussion

Surveillance of HIVDR is a key component of the public health approach to sustainable use of ART. In this analysis, we found moderate to high levels of PDR in two KZN districts between 2013 and 2015, at approximately the turn of the second decade of ART rollout. The results from the AHRI longitudinal population surveillance suggest a continued trend of steadily increasing PDR since 2010.[14] In the HIPSS cross-sectional survey, the level of NNRTI PDR was close to 10%, the current threshold at which the WHO recommends urgent public health action.[2,3] The attempt to exclude people with prior ART exposure means that our findings are likely to reflect predominantly transmitted resistance. The levels of resistance documented and the phylogenetic analysis therefore suggest increasing transmission of HIVDR from people treated with ART. This raises concerns about the

quality of HIV prevention, treatment and care, and should prompt consideration of appropriate public health measures to ensure the long-term sustainability of ART.

The timing of this increase in levels of PDR is consistent with other findings from sub-Saharan Africa, where PDR rose to moderate levels about ten years into ART scale-up.[31,32] To some extent, our findings are consistent with a nationally-representative survey conducted in South Africa in 2013-2014, which estimated the prevalence of PDR at 9.0% and NNRTI PDR at 8.3% nationally.[15] The two sites are similar in terms of demographics, HIV epidemiology and HIV care cascades.[22,33] However, there were some differences in the study populations, particularly the higher HIV RNA levels in study A due to the use of DBS samples for sequencing, as the amplification success rate reduces at lower HIV RNA levels (<10,000 copies/mL) in DBS samples.[34] Using DBS samples which have a higher HIV RNA requirement for genotyping, could have resulted in an underestimation of the levels of resistance for study A, as drug-resistant viruses have a lower replicative capacity than the wild-type virus, which could result in lower HIV RNA levels, although this may depend on the specific profile of mutations.[35,36]

Another difference between the two studies was the method used to determine prior ART use. It is possible that the self-report of ART use in the HIPSS (study B) was less reliable than linkage to health service records as a method for uncovering current or prior use of ART. Significant undisclosed ART use has been documented in other population-based surveys,[37,38] and if people on ART were inadvertently included in the sample for this analysis, then the levels of PDR from the HIPSS (study B) may be overestimated. Given these difficulties, it is possible that future studies should include testing for antiretroviral drug levels to determine the true ART status. Although the PDR prevalence was somewhat higher in women in both studies, the difference was not as marked as that reported for a number of recent national PDR surveys.[5] In addition, we did not observe a clear gradient in PDR across age groups, unlike a recent study in Kenya,[39] although it is notable that PDR levels were particularly high in young women, the group with the highest HIV incidence in these populations.[21]

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The rapid expansion of ART coverage has been a considerable achievement in South Africa, having resulted in substantial gains in life expectancy.[40,41] Routine viral load (VL) monitoring to guide ART switches has been incorporated into the treatment programme since the start of ART roll-out. Despite this, implementation remains inconsistent, and the results are often not appropriately acted upon.[42–45] With the resulting delays in switching to second-line ART regimens, people spend more time viraemic and at risk of accumulating drug resistance.[17] With the growing caseloads of people on first-line ART, this suggests that there will be an expanding pool of people with ADR, creating conditions for an increase in the transmission of HIVDR.[46] Our findings lend support to calls for increased focus on quality improvement within the HIV treatment programme, particularly with respect to adherence support, routine virologic monitoring and timely ART switching.[44,45]

Overall, around one in ten participants had at least one NNRTI SDRM, the most frequent mutation being K103NS, which is consistent with the national PDR survey,[15] and is the most common NNRTI mutation documented in the context of ADR on first-line ART.[47] The persistence of this mutation in the absence of drug pressure [48] may increase the chance of onward transmission, and the levels documented here raise concern about the continued effectiveness of efavirenz (EFV) and nevirapine in first-line regimens. The levels of NRTI resistance were relatively low, and the estimated prevalence of key SDRMs associated with TDF resistance (K65R and K70E) was below 1% in both studies. This was also consistent with the national survey that estimated the prevalence of K65R at 1.4%.[15] At the time of these studies, TDF had still only been in widespread use in first-line ART regimens for under five years, so continued vigilance is required, especially as high levels of K65R have been documented in those with ADR on first-line ART, and with evidence supporting the transmissibility of K65R variants.[47,49] However, these findings provide reassurance for now about the place of TDF in first-line ART and PrEP regimens,[50] and although the levels of PI resistance were low, the two instances of multiple major PI mutations and triple class resistance raise some concern. The information available from the public sector health records suggested that neither had been exposed to ART. However, multiple major PI mutations and triple class resistance suggest the two individuals had prior use of ART. Therefore, these findings should be interpreted with caution, given the potential for data

linkage problems, access to ART in a public sector programme outside the study area, or access to ART in the private sector.

From the phylogenetic analysis, approximately 15% of the individuals with SDRM from study A and B were linked in a transmission cluster with at least one other person with an identical SDRM. Fourteen of 32 ART-naïve individuals were linked with ART-experienced individuals whose sequences were in the background dataset. In these linkages, we can infer that drug-resistance mutations were most likely transmitted from ART-experienced individuals to ART-naïve individuals in the same cluster. For the other 18 ART-naïve individuals with PDR, the source of drug resistance is most likely to be from ART-experienced individuals that were not part of our study sample, although we cannot exclude onward transmission from ART-naïve individuals, and undisclosed ART exposure. K103N was the most common mutation observed in the linked cases, consistent with a study from Aruba, a highly HIV endemic area in the Caribbean.[51] However, unlike the Aruba cluster, where onward transmission of K103N was observed among ART-naive individuals in a large transmission chain, the pattern of small and independent transmission clusters observed here is more suggestive of multiple transmission events from people with ADR on ART.

Our findings suggest that additional public health interventions may now be required in South Africa, as recommended by the WHO.[2,3] One option would be to introduce genotypic resistance testing prior to ART initiation, but this would have substantial cost implications and present considerable operational challenges. The more likely option would be to change the standard first-line ART regimen, probably to an integrase inhibitor-based regimen. Dolutegravir (DTG) has already replaced EFV in first-line ART in neighbouring Botswana and there are plans for it to be introduced for first-line ART in South Africa in 2018.[11,52] While there are some concerns about introducing DTG in the South African context (e.g. with paucity of studies on the use of DTG in pregnancy and dosing in people with TB on rifampicin), cost savings are an additional driver to adopt this for first-line therapy.[52]

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Our findings should be interpreted with certain limitations, as the two studies were not conducted as formal drug resistance surveys for pre-treatment or transmitted drug resistance. Although population-based studies present challenges in accurately determining current and prior ART use, they might have some advantages over facility-based HIVDR surveys in that they include a more broadly representative sample of HIV-positive people in the population, including people not accessing health care. However, this representativeness is diminished if there are low levels of consent in population-based surveys, as seen in the AHRI surveillance. There was substantial attrition in the laboratory processes, particularly with the DBS specimens, and we cannot be certain that the participants whose virus was successfully sequenced were representative of all eligible ART-naïve people in the study populations. Our capacity to uncover linked drug resistant transmissions was limited by the relatively low coverage of people living with HIV in the study areas, particularly for study B. Finally, as these studies were in geographically-restricted populations, these results should not be taken to be representative of the entire province of KZN, or of South Africa more generally.

In conclusion, the high levels of PDR documented here highlight the need for renewed focus on improving the quality in HIV prevention, treatment and care. In particular, the systems for routine VL monitoring for people on ART and switching to second-line ART should be strengthened. These findings should be interpreted together with results of the national drug resistance survey to inform the need for modification of the standard first-line ART regimen or the introduction of other public health measures to prevent the spread of drug resistance.

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Author Disclosure Statement

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Table 1. Study participants' demographic and clinical characteristics

	Total	AHRI surveillance (Study A)		HIPSS (Study B)	
		2013	2014	2014	2015
Participants	1845	254	357	737	497
Sex, female, n (%)	1269 (69)	186 (73)	269 (75)	507 (69)	307 (62)
Age, years, median (IQR)	30 (25-39)	30 (24-40)	33 (26-42)	30 (24-38)	30 (24-36)
HIV RNA, log_{10} copies/mL, median (IQR)[a]	4.50 (4.02-4.96)	4.77 (4.35-5.18)	4.70 (4.36-5.10)	4.39 (3.80-4.87)	4.41 (3.91-4.83)

AHRI, Africa Health Research Institute; HIPSS, HIV Incidence Provincial Surveillance System; IQR, interquartile range; RNA, ribonucleic acid

[a] HIV RNA missing for 84 participants (77 study A, 7 study B)

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Table 2. Surveillance drug resistance mutations detected in two population-based studies in KZN, South Africa

	AHRI surveillance (Study A)		HIPSS (Study B)		Overall
	2013	**2014**	**2014**	**2015**	
NNRTI Mutations	n=254	n=357	n=737	n=497	n=1845
L100I	0	0	0.4	0.2	0.2
K101EP	0.8	0.3	1.1	0.6	0.8
K103NS	**6.3**	**6.2**	**9.2**	**6.6**	**7.5**
V106AM	0.8	0.8	2.0	1.0	1.4
Y181C	0.4	0	0.1	0.6	0.3
Y188LC	0.4	0	0.3	0.6	0.3
G190AS	0	0.8	0.7	0.2	0.5
P225H	1.2	0.8	1.1	0.8	1.0
M230L	0	0.3	0.4	0.2	0.3
Overall NNRTI resistance	**7.5**	**7.6**	**11.9**	**9.7**	**9.9**
NRTI Mutations	n=254	n=357	n=737	n=497	n=1845
M41L	0	0.3	0.3	0.2	0.2
K65R	1.2	0.3	0.7	0.4	0.6
D67N	0.8	0	0.1	0.4	0.3

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T69D	0	0.3	0	0	0.1
K70R	0.8	0	0	0.4	0.2
K70E	0.0	0.3	0.0	0.6	0.2
L74VI	0.4	0.3	0.1	0.2	0.2
Y115F	0	0	0.3	0.2	0.2
M184VI	3.1	2.0	2.6	2.0	2.4
L210W	0	0	0.1	0	0.1
T215DEV	0.4	0	0.1	0	0.1
T215Y	0.4	0	0	0	0.1
K219ENR	0.8	0.6	0.3	0.2	0.4
Overall NRTI resistance	4.7	3.4	3.1	2.4	3.2
PI Mutations[a]	n=254	n=356	n=736	n=495	n=1841
L24I	0	0	0	0.2	0.1
M46IL	0.4	0.6	0.7	2.0	1.0
F53Y	0	0	0	0.2	0.1
I54V	0.4	0.3	0	0	0.1
L76V	0.4	0.3	0	0	0.1
V82A	0.4	0.3	0	0	0.1
I85V	0	0.3	0	0.4	0.2
L90M	0.8	0	0	0	0.1

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Overall PI resistance	0.8	0.8	0.7	2.6	1.2

AHRI, Africa Health Research Institute; HIPSS, HIV Incidence Provincial Surveillance System; NNRTI, non-nucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; PI, protease inhibitor

All figures are percentages; figures in bold and underlined are levels of resistance ≥5%

The following surveillance drug resistance mutations were not detected in either study and are therefore not listed: L23I, D30N, V32I, I47VA, G48VM, I50LV, G73STCA, N83D, I84VAC, N88DS, T69ins, V75MTAS, F77L, F116Y, Q151M, V179F

[a] Denominator for PI mutations based on number of complete *PR* sequences

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Table 3. Association between pre-treatment drug resistance and participant characteristics

		Study A			Study B		
	Category	N	n (%)	OR (95% CI)	N	n (%)	OR (95% CI)
Sex	Male	156	11 (7.1)	1	420	39 (9.3)	1
	Female	455	45 (9.9)	1.45 (0.73-2.87)	814	117 (14.4)	1.64 (1.12-2.41)
Age, yrs	15-24	145	15 (10.3)	1.28 (0.62-2.62)	314	40 (12.7)	0.88 (0.58-1.34)
	25-34	217	18 (8.3)	1	507	72 (14.2)	1
	35-44	125	10 (8.0)	0.96 (0.43-2.15)	319	30 (9.4)	0.63 (0.40-0.98)
	45+	124	13 (10.5)	1.29 (0.61-2.74)	94	14 (14.9)	1.06 (0.57-1.97)
HIV RNA, log_{10} copies/mL	<4	51	4 (7.8)	1	372	61 (16.4)	1
	4-5	309	33 (10.7)	1.40 (0.48-4.15)	619	61 (9.9)	0.56 (0.38-0.82)
	>5	174	14 (8.0)	1.03 (0.32-3.27)	236	33 (14.0)	0.83 (0.52-1.31)
	Missing	77	5 (6.5)	0.82 (0.21-	7	1	0.85 (0.10-

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				3.20)		(14.3)	27 7.18)
Duration of infection[a]	≤24 months	66	2 (3.0)	0.33 (0.08-1.42)	-	-	-
	>24 months	385	33 (8.6)	1	-	-	-
	Unknown	160	21 (13.1)	1.61 (0.90-2.88)	-	-	-

CI, confidence interval; OR, odds ratio; RNA, ribonucleic acid

[a] For those with a prior negative HIV ELISA in the population surveillance, estimated date of infection was calculated as mid-point between last negative HIV ELISA and first positive HIV ELISA. Estimated duration of infection was then calculated from that date of infection to the date the sample processed for sequencing was collected. For those with only prior positive HIV ELISA tests, duration of infection was taken to be >24 months if there was a positive HIV ELISA more than 24 months prior to the sample date. Unknown duration of infection implies no prior negative HIV ELISA and no prior positive HIV ELISA beyond 24 months

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Figure Legends

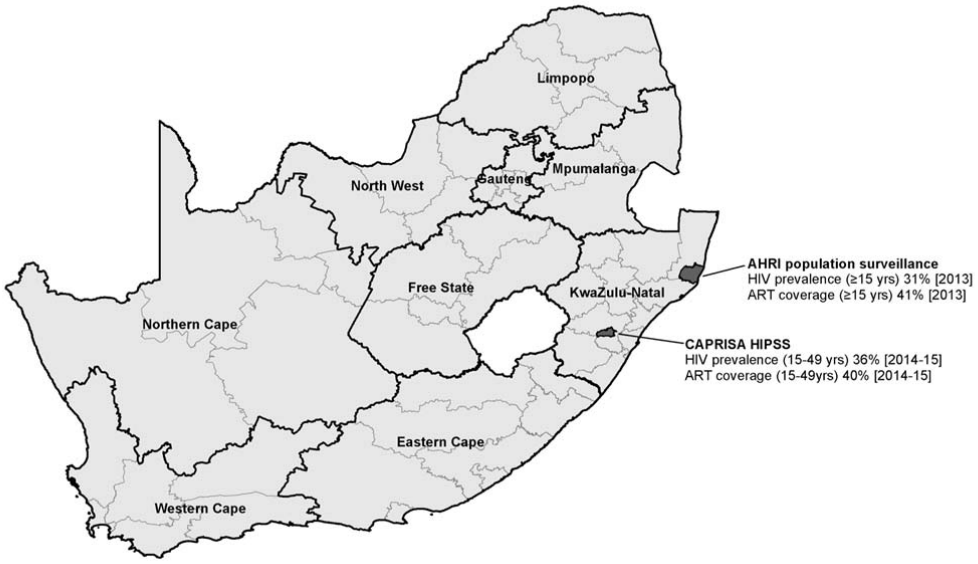

Figure 1. Location of the two population-based studies

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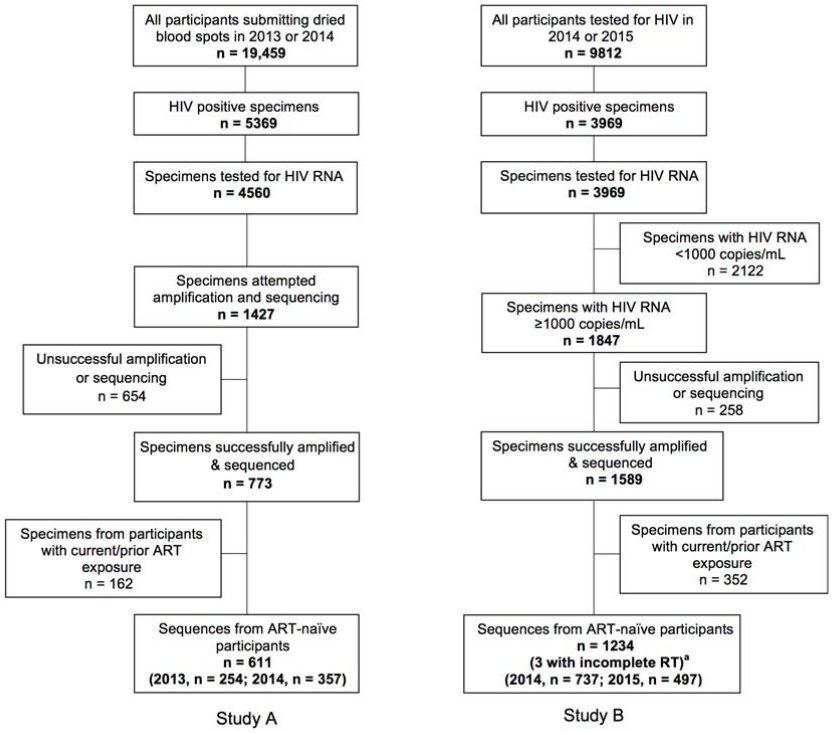

Figure 2. Summary of the specimens and HIV-1 sequences from the two studies in KZN, South Africa

[a] Excluded due to incomplete reverse transcriptase (*RT*) sequences

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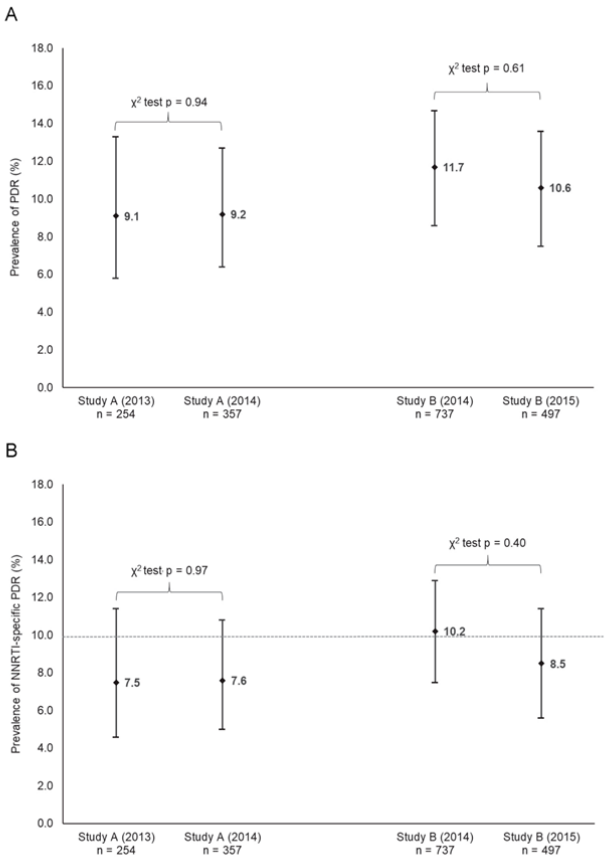

Figure 3. Levels of pre-treatment drug resistance (A) and NNRTI-specific pre-treatment drug resistance (B)

Marker line in Figure 3B corresponds to 10% threshold for NNRTI PDR

Prevalence estimates and confidence intervals for study B were weighted to adjust for the survey design, and for non-response across age and gender categories

Description on timing of recruitment and ascertainment of antiretroviral therapy status for the two population-based studies

Study A

In 2013, samples were collected between 22 January and 27 November, and the median date of sample collection was 5 June 2013, whilst in 2014, samples were collected between 21 January and 30 November, and the median date of sample collection was 18 July 2014.

To complement the population-based surveillance research, the Africa Health Research Institute (formerly Africa Centre for Population Health) has maintained a clinical database for all people treated with antiretroviral therapy (ART) at 17 primary health care clinics and one district hospital in the Hlabisa sub-district. This database holds records for people who have received ART since 2004, the start of the public sector ART roll-out. Data from the clinical database are linked with the population-based surveillance data by deterministic record linkage (using the unique South African ID number, if recorded) or probabilistic record linkage (using first name, surname, date of birth, and sex). The database has a variable for date of ART initiation, so for the purposes of this analysis, we could determine whether there had been any use of ART (for treatment) prior to the date of surveillance sample collection used for genotypic resistance testing. The clinical database does not hold information on antiretroviral regimens for prevention of mother-to-child transmission (pMTCT) prior to 2013, i.e. single-dose nevirapine regimens with or without zidovudine and/or single-dose tenofovir/emtricitabine. It also does not hold information on use of antiretrovirals for pre-exposure or post-exposure prophylaxis, but use in these circumstances was very low over the study period. The database does not hold information about people who accessed ART in the public sector outside Hlabisa sub-district. The database also does not hold information about people who accessed ART in the private sector. Private sector ART use is low in the study area, due to the low levels of private health insurance and good access to ART in the public sector.

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Study B

In 2014, samples were collected between 7 January and 12 December, and the median date of sample collection was 26 August 2014, whilst in 2015, samples were collected between between 4 January and 6 December, and the median date of sample collection was 28 April 2015.

In the HIV Incidence Provincial Surveillance System (HIPSS), the survey included questions about antiretroviral use, which were asked to any participant who reported being HIV positive. The first question asked was 'Has a doctor or nurse told you that you need to take ARVs?' If the answer to this question was yes, then the participant was asked 'Are you still on ARVs?' In addition, female participants were asked the question, 'Have you ever been pregnant while you were HIV positive?' If the answer to this was yes, they were asked 'Which of the following services did you access while HIV positive and pregnant?' One option for this question was 'Medication to prevent mother-to-child transmission'. The survey did not ask questions about use of antiretrovirals for pre-exposure or post-exposure prophylaxis.

From these questions, we determined whether there had been any use of antiretrovirals for treatment or pMTCT prior to the date of sample collection.

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Supplemental Data

Table S1. Estimated prevalence of pre-treatment HIV drug resistance by sex and age

	Pre-treatment HIVDR Prevalence					
	Female		Male		Total	
	n/N	% (95% CI)	n/N	% (95% CI)	n/N	% (95% CI)
Study A						
15-24 years	13/121	10.7 (5.8-17.7)	2/24	8.3 (1.0-27.0)	15/145	10.3 (5.9-16.5)
25-34 years	17/162	10.5 (6.2-16.3)	1/55	1.8 (0.0-9.7)	18/217	8.3 (5.0-12.8)
35-44 years	7/84	8.3 (3.4-16.4)	3/41	7.3 (1.5-19.9)	10/125	8.0 (3.9-14.2)
45+ years	8/88	9.1 (4.0-17.1)	5/36	13.9 (4.7-29.5)	13/124	10.5 (5.7-17.3)
Study B[a]						
15-24 years	34/248	14.1 (8.6-19.5)	6/66	7.9 (0.9-14.9)	40/314	12.2 (7.9-16.5)
25-34 years	52/299	17.0 (11.9-22.1)	20/208	8.1 (4.2-12.0)	72/507	11.9 (8.6-15.1)
35-44 years	22/203	8.5 (4.4-12.6)	8/116	7.5 (1.8-13.2)	30/319	8.0 (4.5-11.5)
45+ years	9/64	11.2 (1.1-21.3)	5/30	15.0 (1.2-28.7)	14/94	13.0 (4.9-21.1)

[a] Data for study B have been weighted to adjust for the survey design and for non-response across age and gender categories

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Table S2. Most frequently observed patterns of surveillance drug resistance mutations

Mutations	Study A	Study B	Overall
K103NS	24 (42.9)	76 (48.7)	100 (47.2)
M46IL	1 (1.8)	11 (7.1)	12 (5.7)
V106AM	1 (1.8)	8 (5.1)	9 (4.2)
M184V, K103NS, P225H	4 (7.1)	3 (1.9)	7 (3.3)
M184V, K103NS	3 (5.4)	2 (1.3)	5 (2.4)
G190AS	1 (1.8)	3 (1.9)	4 (1.9)
K101EP	0	3 (1.9)	3 (1.4)
Y181C	0	3 (1.9)	3 (1.4)
M230L	0	3 (1.9)	3 (1.4)
K103NS, P225H	1 (1.8)	2 (1.3)	3 (1.4)
M41L	1 (1.8)	2 (1.3)	3 (1.4)
Other	20 (35.7)	40 (25.6)	60 (28.3)
Total	56	156	212

Specific SDRM patterns are listed if observed in three or more participants overall

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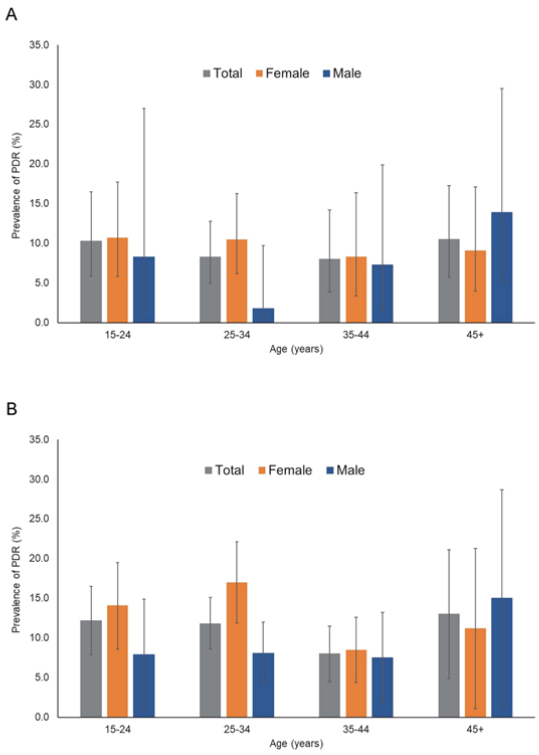

Figure S1. Estimated prevalence (with 95% confidence intervals) of pre-treatment HIV drug resistance by sex and age in the AHRI population-based surveillance study (A) and the CAPRISA HIPSS (B)

Prevalence estimates and confidence intervals for study B have been weighted to adjust for the survey design and for non-response across age and gender categories

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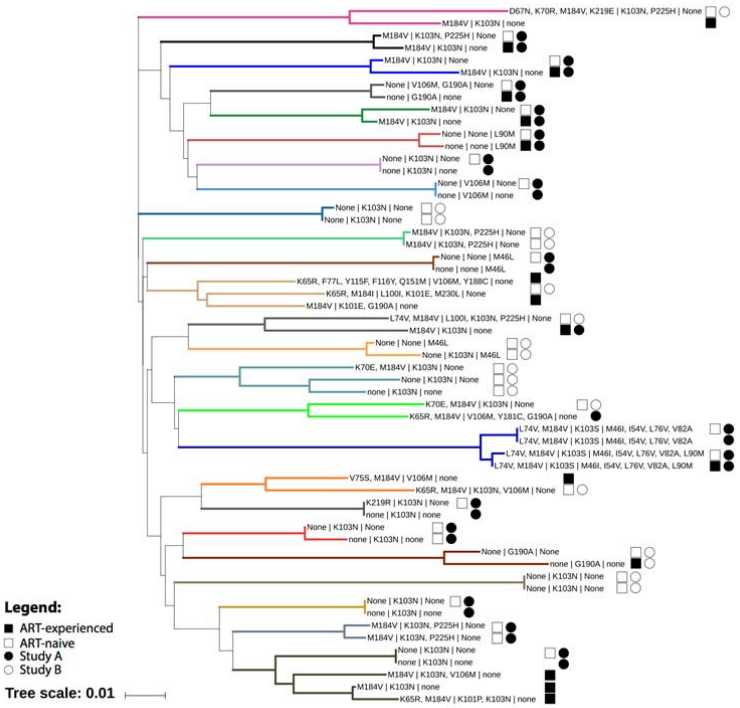

Figure S2. Maximum likelihood phylogenetic tree of clusters involving drug-resistance