

HIV Drug Resistance Patterns at the Epicentre of the HIV-1 Epidemic in Kwazulu-Natal, South Africa 2003-2013

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Abstract

South Africa has the highest number of individuals with HIV-1 infection worldwide with the epicentre of the epidemic in the province of KwaZulu-Natal (KZN). Currently South Africa has the largest antiretroviral treatment (ART) programme with KZN and Gauteng provinces accounting for 56% of all patients on ART. In order to understand transmission dynamics and temporal trends in drug resistance, we performed a comprehensive literature review of primary and acquired drug resistance in adults and children in KZN over a ten year period, 2003-2013.

Transmitted drug resistance (TDR) to non-nucleoside reverse transcriptase inhibitors (NNRTIs) in adults is increasing to moderate levels (5-15%). Acquired drug resistance to first line drug regimens in adults was associated with at least one mutation to nucleoside reverse transcriptase inhibitors (NRTI) or NNRTIs or both, with M184V and K103N the most common mutations. Prevalence of thymidine analogues (TAMs) was low to moderate. There was no published data on acquired drug resistance to second line agents. Children failing first line drug regimens harboured drug resistance mutations to NRTI (M184V and TAMs), NNRTI (V106M and K103N) and protease inhibitors (PIs) (V82A).

Concerns have been raised about the high levels of the K65R mutation associated with tenofovir regimens in adults and children. Continued vigilance is needed for ART failure in both adults and children, so as not to compromise second line treatment options. Drug resistance genotyping is now cost effective and should be considered for inclusion in regional surveillance studies and during routine care.

Keywords: KwaZulu-Natal; HIV; Primary; Acquired; Drug Resistance.

Introduction

According to the UNAIDS statistics (2012) sub-Saharan Africa has the highest numbers of adults and children living with HIV (22.5 million) and those newly infected with HIV (1.8 million) worldwide [1]. South Africa's epidemic remains the largest in the world with an estimated 6 million people living with HIV in 2011 [2]. KwaZulu-Natal (KZN) is the province with the highest HIV burden, with an HIV prevalence of 38% among women participating in ante-natal surveys [3]. However, since 2004 the rapid expansion of the availability of anti-retroviral therapy (ART) in South Africa, has led to the decline in the annual HIV incidence among women 15-24 years old from 5.5 per 100 individuals in 2003-2005 to 2.2 in 2005-2008 [4]. The wide use of ART has been reported to be one of the main interventions responsible for decreasing the incidence of HIV-1 infection in rural KZN [5].

Antiretroviral therapy was implemented after the announcement of the Operational Plan for Comprehensive HIV and AIDS Care and Treatment for South Africa through the public health system with treatment regimens specified by the South African National adult and paediatric guidelines [6]. Currently, South Africa has the largest ART programme [7] with an estimated 1.79 million people on ART [8], the majority (85%) through the public health sector, 4% through non-governmental community treatment programmes and 11% through the private health sector. The ART roll-out has profoundly reduced AIDS-related morbidity and mortality in resource poor settings [9,10]. There

is evidence of an increase in adult life expectancy in South Africans since the initiation of ART [11,12].

KwaZulu-Natal and Gauteng provinces account for 56% of all patients receiving or on ART in South Africa. An inevitable consequence of ART is drug resistance. Virological failure of ART regimens and accumulation of drug resistance mutations in adults and children in South Africa have been reported from several treatment programmes. We performed a comprehensive literature review of primary and acquired resistance in adults and children to both first- and second-line regimens in KZN. This was done to better understand temporal trends and possible transmission dynamics of drug resistance over a ten year period (2003-2013).

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Articles were retrieved from PubMed using the following key search terms: "HIV drug resistance and KwaZulu-Natal" (Figure 1). Permission was requested from the authors to include their published data in this report.

Transmitted Drug Resistance (TDR)

The surveillance of TDR is essential in a public health approach to ART. The levels and patterns of TDR can guide the selection of the first-line regimens and impact on post exposure prophylaxis policies. In resource constrained settings the World Health Organization (WHO) recommends focused surveillance for TDR in individuals likely to be recently infected [13]. A convenient population to sample for TDR is young (<25 years) primigravida attending antenatal clinics (ANC) in areas where ART has been available for at least 3 years. Alternative estimates may be obtained from longitudinal studies of HIV seroconversion with estimated dates. In addition, WHO suggest using HIV threshold survey methods that allows the categorization of TDR as <5%, 5-15% and <15% [13]. A number of these surveys have been

performed in KZN [14,15].

Furthermore, regular cross sectional studies in recent seroconverters and latently infected individuals has provided information on TDR [16-18].

Table 1 summarizes the studies on TDR undertaken in KZN. The first study on drug resistance in treatment naïve patients from KZN was reported by Gordon et al. in 2003 prior to the "roll out" of the public antiretroviral programme [16]. They showed no evidence of TDR in 56 treatment naïve chronically infected patients. A report by Hunt et al. using WHO threshold surveys in KZN between 2005 and 2009 showed TDR levels increasing from < 5% to 5-15% [15]. One of 40 (2.5%) samples genotyped had evidence of surveillance drug resistance mutations (SDRMs) [19] in 2005, whereas none of 34 samples genotyped in 2007, had evidence of TDR. However, 5/37 (13.5%) and 3/47 (6.4%) had SDRMs in 2008 and 2009 respectively [15]. The number of samples successfully genotyped in 2005 and 2008 were less than the recommended 47 when there is at least one SDRM. Thus the estimates of TDR for these two years could have been underestimated. Drug resistance mutations associated with NNRTI resistance were primarily responsible for the increase in TDR for all the years that had TDR levels greater than 5%.

Another WHO threshold survey performed in 2009 detected SDRMs in only one sample out of 47 (2.1%) genotyped [14]. This latter finding is supported by Manasa et al. who did not find any SDRMs in 72 samples from recently infected patients identified in 2010 through a longitudinal population based HIV surveillance programme from rural KZN [17].

The most recent report from a cross sectional study undertaken in semi-rural and urban sites in the greater Durban area of KZN, on women being screened for an HIV prevention trial between 2010 and 2011 found that 26/352 (7.4%) women had at least one SDRM. Twenty of the 26 had resistance to a single class, NNRTI, NRTI or PI. The remaining six had dual class resistance, five (NNRTI + NRTI) and one (NNRTI + PI) resistance. Twenty three of the 26 patients had NNRTI resistance, 19 had the K103N mutation [18].

Most of the studies above show that TDR is still low (<5%) in KZN, similar to other provinces in South Africa [15,20-24]. However, the 2008 and 2009 data by Hunt et al. suggest that TDR might be increasing to intermediate levels predominantly due to NNRTI resistance. This trend is similar to observations from west and central Africa as well as Zambia, who have had more experience with ART [24-27]. NNRTI resistance mutations are stable and have little impact on viral fitness, thus they tend to persist for a longer time than mutations to other drug classes [28]. This and the ease with which they are selected under

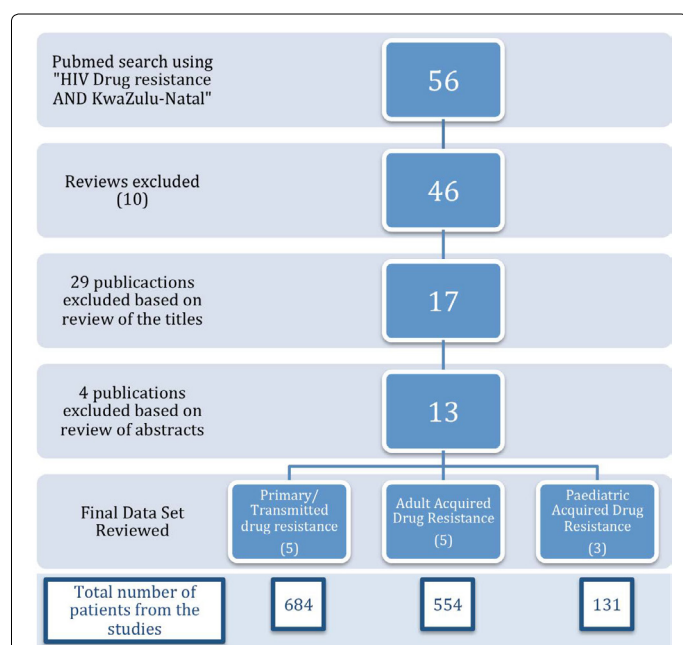


Figure 1: Pubmed search using the key words, "HIV drug resistance AND KwaZulu-Natal". The final analysis was based on 13 publications only. These studies reported drug resistance data from treatment naïve patients as well as adult and pediatric patients failing first line therapy. The treatment naïve patients studies reported data from a total of 684 patients while the adult and pediatric studies reported data from 554 and 131 patients respectively.

Study Period	Patient Population	N	Any mutation (%)	NNRTI (%)	NRTI (%)	PI (%)	Reference
2011	Treatment naïve women	351	7.4	5.7	2.0	0.6	Parikh et al. [13]
2010	HIV surveillance	72	0.0	0.0	0.0	0.0	Manasa et al. [17]
2009	ANC Survey	47	<5.0	<5.0 (2.1)	<5.0	<5.0	Parboosing et al. [14]
2009	ANC Survey	47	5-15	5-15	<5.0	<5.0	Hunt et al. [15]
2008*	ANC Survey	37	13.5	8.1	5.4	2.7	Hunt et al. [15]
2007	ANC Survey	34	<5.0	<5.0	<5.0	<5.0	Hunt et al. [15]
2005*	ANC Survey	40	2.5	2.5	ND	ND	Hunt et al. [15]
2003	Treatment naïve adults	56	<5.0	<5.0 (2.1)	<5.0	<5.0	Gordon et al. [16]

* The threshold level could not be determined because of insufficient numbers of samples genotyped.

N - number of patients genotyped; NNRTI - Non-nucleoside reverse transcriptase inhibitor; ND - not determined; NRTI - nucleoside reverse transcriptase inhibitor; PI - protease inhibitor;

Table 1: Surveillance of transmitted drug resistance (TDR) studies from KwaZulu-Natal (KZN), South Africa.

suboptimal therapy as well as the wide use of first-line agents might be the contributory factors towards the disproportionately higher levels of NNRTI TDR relative to NRTI TDR.

There are several limitations to the TDR data reviewed. Most studies had a small sample size; surveyed limited regions; had short duration of follow up; were retrospective and lacked verification of prior ART exposure which may have inflated the TDR levels by including patients with previous ART exposure.

Acquired Resistance Mutations After Virologic Failure to First-Line Regimens in Adults in KZN

From inception in 2004, the South African national treatment programme has used standardized first-line and second-line regimens. The South African national adult treatment guidelines recommended a first-line regimen consisting of two NRTIs (stavudine (d4T) + lamivudine (3TC)) combined with one NNRTI (efavirenz (EFV) or nevirapine (NVP)) [6]. Stavudine was replaced by tenofovir (TDF) in 2010 due to toxicity [29]. Unlike most resource-constrained settings, South Africa recommended 6 monthly viral load monitoring in its guidelines, however drug resistance testing for patients failing therapy was not recommended. Patients with confirmed virological failure were switched to a standardized second-line regimen consisting of two NRTIs and a boosted PI. Studies in patients failing first-line therapy are not only informative on the performance of ART programmes but also on the appropriateness of the standardized second-line treatment regimens.

Table 2 summarizes the five published studies reporting drug resistance mutations in adult patients failing first-line regimens in KZN [30-34]. All but one recruited participants from urban hospital-

based clinics. The two urban hospital sites used were: the Sinikithemba Outpatient HIV/AIDS clinic, McCord Hospital and iThemba clinic, St Mary's Hospital in Durban [30-33]. All studies showed similar results, with drug resistance in > 80% of patients failing first-line ART. Only two studies reported on their virologic failure rates, which ranged from 6 to 10% [33,34].

All studies reported at least one drug resistance mutation in over 80 percent of patients failing first-line treatment. Murphy et al. reported on patients failing therapy from both urban sites between 2004 and 2006. They showed that 86.5% of the patients failing therapy had one or more drug resistance mutations. Five percent of the patients had the K65R mutation [31]. In 2005 and 2006 Marconi et al. also recruited patients from the same two clinics and found similar levels of drug resistance. One or more drug resistance mutations were detected in 83.5%. More than half of these harboured resistance to two drug classes. Prevalence of thymidine analogue mutations (TAMs) was low. The most frequent mutations detected were M184V/I and K103N [30].

Singh et al. in 2011 detected at least one resistance mutation in 95% of patients failing ART: 91% had evidence of resistance to both NRTI and NNRTIs. The only reported PI mutation; T74S is a subtype C polymorphism [32].

Another study also done at the Sinikithemba clinic between 2010 and 2011 genotyped patients failing a new first-line regimen of TDF + 3TC + EFV/ NVP that had been introduced in 2010 [33]. The study followed up 585 patients over a period of approximately six months. The reported virological failure rate was 6% at median follow up of 5.7 months. Approximately 70% of the virologic failures had the K65R mutation with few additional NRTI mutations. There were fewer M184V mutations and TAMs compared to other reports from KZN or

Study Period	Site	Criteria for failure	N	Median Duration of ART (months)	ART Regimen (%)	≥1 DRM (%)	NNRTI (%)	M184V (%)	Any TAM (%)	TAM ≥3 (%)	K65R (%)	Ref
Dec 2010 – Mar 2012	Hlabisa (17 rural clinics)	1 × VL >1000	222	42	d4T/3TC/EFV (51) d4T/3TC/NVP (24) AZT/3TC/EFV (8) TDF/3TC/EFV (11) Other (6)	86.0	83.0	78.0	40.0	18.0	6.0	Manasa et al. [34]
Sep 2010 to Mar 2011	Urban clinic	1 × VL >1000	33	5.7	TDF/3TC/EFV (89)	>97.0	97.0	27.3	15.2	NR	70.0	Sunpath et al. [33]
NR	Durban (urban hospital)	1 × VL >5000	43	29	d4T/3TC/EFV (51) AZT/3TC/EFV (29) AZT/3TC/NVP (9) Other (10)	95.0	95.0	87.0	55.0	NR	NR	Singh et al. [32]
Aug 2004 to Aug 2006	Urban Clinics	1 × VL >1000	141	NR	D4T/3TC/EFV (43) D4T/3TC/NVP (6) AZT/3TC/EFV (27) AZT/3TC/NVP (12) OTHER (11)	86.5	NR	NR	NR	NR	5.0	Murphy et al. [31]
Jan 2005 – Aug 2006	Durban (two urban hospitals)	1 × VL >1000	115	10.8	d4T/3TC/EFV (49) d4T/3TC/NVP (5) AZT/3TC/EFV (26) AZT/3TC/NVP (11) Other (8)	83.5	78.3	64.3	32.2	13.0	2.6	Marconi et al. [30]

ART, antiretroviral therapy; N, number of patients genotyped; DRM, drug resistance mutation; TAM, thymidine analogue mutation; VL, viral load; NR, Not reported; NA, Not applicable; Ref- Reference.

Table 2: Summary of acquired drug resistance studies in adults treated with first-line antiretroviral therapy in KwaZulu-Natal (KZN), South Africa.

the rest of South Africa [30-32,34]. The early development of K65R in the patients included in this study might have been responsible for the low levels of TAMs due to the reported antagonism between TAMs and the K65R mutation [35,36]. Only 33 patients were genotyped in this first study of resistance after failure of a TDF based first-line regimen. Larger studies with more power are needed to have a clearer understanding of the rate of emergence and patterns of drug resistance in patients failing the new standard first-line regimen [33]. Another report [37], showed that there were no significant differences in virological control between Zidovudine (AZT), d4T or TDF first-line regimens. The preferential selection of K65R in patients on TDF based first-line regimen has been attributed to a more rapid in vivo selection of the K65R mutation in clade C virus [38].

However, TDF based regimens were associated with less single drug substitution (mostly associated with toxicity) as well as mortality when compared to the regimens based on either AZT or d4T [37].

The most recent report on acquired drug resistance on first-line therapy failure was from Manasa et al. Unlike previous studies this report was from a rural decentralized treatment programme in northern KZN [34]. Here patients were recruited from 17 primary health care clinics in the Hlabisa sub-district between 2010 and 2012. They reported a virologic failure rate of 10% over the two year study period. This report only included patients receiving d4T/ AZT based first-line regimen. One or more drug resistance mutations were found in 86% of the patients. One or more TAMs were detected in 40% of the patients while 18% had three or more TAMs. The K65R mutation was detected in 6% of the patients. In addition to the mutations, the authors reported on the total genotypic susceptibility scores (GSS) of the viral isolates to the recommended second-line regimen. Based on the GSS approximately 15% of the patients harboured viruses with resistance levels that could potentially compromise the effectiveness of the standard second-line regimen [34].

The average time on failing regimens among the patients from rural KZN was 26 months. There is limited data from other studies on the time patients spent on failing regimens, however it is anticipated that it will be significantly lower for patients being managed through hospital based programmes in urban centres. Prolonged periods on failing regimens are associated with accumulation of high-level resistance, which may compromise second-line treatment options in both children and adults. This study highlighted that: as the treatment programme expands, there is a need to adhere to treatment and monitoring guidelines more closely in order to identify patients failing therapy early and act quickly to provide the appropriate clinical management. The study also seems to highlight the importance of introducing drug resistance testing for those participants failing first-line therapy, so that they are put on the

most effective second-line therapy available, especially if remaining on a failing first-line regimen for prolonged periods of time compromises second-line therapy.

Most of the above reports are similar to reports from other parts of South Africa as well as the rest of the continent [39-44]. Failure of first-line drug regimens in adults was associated with at least one mutation from either the NRTI or NNRTI, with significant proportions harbouring drug resistance mutations to both classes. The most common drug resistance mutations were M184V and K103N while 15 to 55% of the patients had evidence of one or more TAMs.

There are no published reports on drug resistance mutations on second-line ART failure in KZN.

Acquired Resistance Mutations After Virologic Failure to First-Line Regimens in Children in KZN

There are only three reports of paediatric HIV-1 drug resistance to first-line regimens in KZN as summarized in Table 3. One from the Sinikithemba paediatric HIV-1 cohort at McCord Hospital, Durban, South Africa [45]; the second from the King Edward VIII hospital, Durban [46] and the most recent study from rural clinics in the Hlabisa sub-district of rural KZN [47]. The study from the Sinikithemba Clinic was a case study describing resistance in a child less than 3 years co-infected with *M tuberculosis*. The child had the NRTI mutations K65R, and D67N as well as the PI mutations, M46I, I54V and V82A [45].

Green et al. in 2012 found that in children failing HAART, 85.4% had >1 drug resistance mutation to one drug class and 80.5% to 2 drug classes. The most common NRTI mutations were M184V (70.7%), K219E (43.9%), K70R (36.6%) and D67N (34.1%). The K65R resistance mutation was found in 7.3% of children failing HAART. The most common NNRTI mutations were V106M (41.5%) and K103N (39%), 58.5% had >1 TAM and 39.0% had ≥3 TAMs. Although 14.6% of 41 children had mutations from the TAM1 (M41L, L210W, T215Y) and TAM2 (D67N, K70R, T215F, K219Q/E) pathways, the latter predominated 16 (39.0%) over the former 2 (4.9%) [46]. Fortunately TAM2 pathway mutations are associated with lesser virologic fitness compared to TAM1 mutations. In addition, they are associated with lesser cross resistance such that TDF remains active in the presence of TAM2 mutation [48].

The study from the Hlabisa sub district also showed similar levels of resistance. One or more drug resistance mutations were detected in 91% of the patients failing therapy. The most common mutation was the M184V mutation. Resistance to the NNRTIs was detected in 71.9% of the patients. Any TAM and/or more than three TAMs were detected in 23.4% and 5.5% of the patients respectively [47]. The accumulation of

Study Period	Site	Criteria for failure	N	Median Duration of ART (months)	ART Regimen (%)	≥1 DRM (%)	NNRTI (%)	M184V (%)	TAM (%)	TAM ≥3 (%)	K65R (%)	Ref
Aug 2011- Dec 2012	Rural clinics	1 × VL >1000	89	39.6	d4T/3TC/EFV (72) d4T/3TC/LPVr (14) ABC/3TC/EFV (9) Other (6)	91.0	71.9	78.7	23.4	5.5	5.6	Pillay et al. [47]
Aug 2008 to Jan 2010	Urban Hospital	1 × VL >1000	41	28.6	d4T/3TC/EFV (61) d4T/3TC/LPVr (15) AZT/3TC/NVP (7) Other (10)	85.4	78.0	70.7	58.5	39.0	7.3	Green et al. [46]
2009	Urban Clinic	1 × VL >1000	1	~22	D4T/3TC/RTV AZT/3TC/LPVr	NA	NA	NA	NA	NA	NA	Murphy et al. [45]

ART, antiretroviral therapy; N, number of patients genotyped; DRM, drug resistance mutation; TAM, thymidine analogue mutation; VL, viral load; NR, Not reported; NA, Not applicable; Ref, Reference

Table 3: Summary of acquired drug resistance studies in children treated with first-line antiretroviral therapy in KwaZulu-Natal, South Africa.

TAMs is likely due to the duration on a failing regimen, underscoring the importance of early switching using viral load estimation to monitor the response to ART. Interestingly, only 1/17 patients on PI-based regimen had PI drug resistance mutation. This likely reflects poor adherence to the paediatric lopinavir/ritonavir liquid formulation.

Conclusion

The TDR level in KZN, the province considered to be at the epicentre of the HIV-1 epidemic, is low to medium with a dominance of NNRTI resistance mutations. Further studies to evaluate the impact of the increasing treatment coverage on the levels of TDR are needed.

The patterns and levels of acquired resistance were very similar among patients failing comparable regimens in KZN and did not differ from other cohorts in South Africa and the rest of the continent. However, there remains a need to assess the failure rates and resistance patterns of patients failing the new TDF based first-line regimen.

There is a need to seriously consider integrating drug resistance testing in the continuum of care, particularly in light of a study that has shown that the cost of genotypic drug resistance testing is cost saving for patients failing second-line therapy and cost neutral for patients failing first-line therapy [49].

Genotyping patients failing second-line therapy is recommended by the South African National Treatment Guideline. The PASER study showed that about one third of adults failing first-line regimens have wild type genotypes [50]. In the absence of genotypic resistance testing these patients have poorer outcomes when switched to second-line regimens without accurately assessing antiretroviral adherence.

The appropriate use of HIV drug-resistance testing together with improved quality of care in HIV programmes will ultimately result in the long term reduction of the spread and emergence of drug resistance in low income settings [51].

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