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HIV-1 drug resistance surveillance among parturient women on anti-retroviral therapy in the Eastern Cape, South Africa: Implications for elimination of mother-to-child transmission

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ABSTRACT

Background: The emergence of HIV drug resistance poses a significant threat to achieving the goal of elimination of mother-to-child transmission.
Objectives: We assessed the extent and patterns of HIV-1 drug resistance mutations (DRMs) within the context of the public sector prevention of mother-to-child transmission (PMTCT) programme in the Eastern Cape, South Africa.
Study design: We conducted analysis of the Pol sub-genomic sequence of RNA extracted from plasma samples of women with probable virological failure at delivery between January and May 2018 from two large maternity centres in the Eastern Cape using standard protocols. Partial pol gene covering 1030bp were amplified and sequenced according to previously reported protocol. DRMs were determined by submitting the generated partial pol sequences to the Stanford drug resistance database for query on mutations associated with drug resistance in HIV viruses. We examined the correlates of DRMs using bivariate analysis.
Results: The age of parturient women ranged from 16 to 43 years. The majority of the parturient women were currently on Efavirenz-based regimen (first line ART) (82.5%) and had been on ART for more than 12 months (65.0%). The prevalence of DRMs was 72.5% (n = 58). The CD4 count demonstrated a negative linear association with the DRMs (p = 0.002). The predominant DRMs were K103 N (n = 43; 74.1%), M184 V (n = 28; 48.3%) and K65R (n = 11; 19%). Among the parturient women on EFV-based regimen treatment; 79.1% already had K103 N while nine patients on protease inhibitor-based regimen still harboured K103 N. The majority of the M184 V mutations were observed in parturient women on first line regimen (n = 23; 82.1%).
Conclusions: We found a high prevalence of DRMs in women delivering their index babies at high viral loads in the study settings. Drug resistance surveillance using point-of-care reverse transcriptase-PCR strategies for the screening of pregnant women on ART could be a game-changer in the resource-constrained settings.

1. Background

With the accelerated roll-out of anti-retroviral therapy (ART) at the population level, especially in pregnant and breastfeeding women, the evolution of HIV drug resistance becomes inevitable [1–4]. It is crucial to ascertain the contribution of drug resistance mutations (DRMs) to ongoing mother-to-child transmission (MTCT) of HIV in sub-Saharan Africa (SSA), home to the largest proportion of pregnant and breast-feeding women living with HIV. Robust evidence from randomised controlled trials proved the efficacy of combination ART in the prevention of mother-to-child transmission (PMTCT) conclusively [4–6]. Findings from these studies informed the World Health Organization

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(WHO) recommendations [7,8] and many other PMTCT guidelines across the world [9–12].

In South Africa, all pregnant and breastfeeding women arriving at health facilities are tested for HIV and offered ART on the same day for those who tested positive [13]. The preferred first-line ART in South Africa is a fixed-dose combination of two nucleoside or nucleotide reverse transcriptase inhibitors (NRTIs) plus non-nucleoside reverse transcriptase inhibitors (NNRTIs) [7,13]. In the event of virological failure, a switch to second-line ART consisting of ritonavir-boosted protease inhibitors (PIs) plus two nucleoside reverse transcriptase inhibitors is recommended [13–15]. This strategy aims to optimise the outcomes of second line ART before accumulation of drug resistance. Many studies support this algorithm-based approach for managing first line virological failure in the resource-constrained settings [16,17].

Available data on HIV drug resistance mutations were obtained from the general population [18–20], but such data are rarely transferable to the pregnant and breastfeeding population. Data-driven evidence on drug resistance mutations is needed for this unique population because of the significant implications for MTCT of HIV. NNRTIs mutations tend to appear first by a change of a single amino acid; for example K103 N, P225H and Y188 L. In this way, they confer resistance to Efavirenz and Nevirapine, and to a lesser extent, Rilpivirine and Etravirine [21]. These NNRTIs mutations are clinically relevant in the context of PMTCT as the majority of pregnant and breastfeeding women on ART in the SSA will commence Efavirenz-based regimen as the first line regimen [13,22,23]. Also, Nevirapine is the cornerstone of infant prophylaxis in many parts of the world [7,9,13]. Therefore, women on a failing Efavirenz-based ART, acquiring these NNRTIs mutations, are at greater risks of transmitting the mutant viral strains to their exposed-infants, irrespective of Nevirapine neonatal prophylaxis [4,24,25].

Within the context of a failing first line-regimen in pregnancy and breastfeeding, parturient women could potentially develop major nucleoside reverse transcriptase inhibitors (NRTIs) mutations which are selected by Tenofovir, Abacavir, Zidovudine, Emtricitabine and Lamivudine. Though, the genetic barriers of some of these drugs differ, however, failure to identify and switch failing regimen would invariably lead to the accumulation of resistance mutations and the emergence of cross-resistance [3,17]. This could then compromise future therapy options for pregnant women and prophylaxis for the exposed-infants.

1.1. Objectives

This study examined the extent and patterns of HIV-1 DRMs from parturient women delivering their index babies at high viral load while on ART in the resource constrained settings of Eastern Cape, South Africa. Findings on the frequency and patterns of resistance mutations in this cohort might give insights into new strategies and innovations in the efforts towards eliminating MTCT.

1.2. Study design

This DRMs Surveillance sub-study was nested within the larger East London Prospective Cohort Study [26], a longitudinal cohort of HIV-infected pregnant women delivering at three of the largest maternity centres in the Amathole/Buffalo City districts of the Eastern Cape, South Africa. All pregnant women on ART attending the maternity centres for delivery of index babies at Frere and Cecilia Makiwane hospitals were included in this sub-study. Frere hospital is located in East London and provides tertiary care services while Cecilia Makiwane hospital is a regional academic centre which provides level one and two services for the entire Buffalo City Metropolitan and Amathole districts, boasting a combined population of about 1.7 million people [27].

The antenatal prevalence of HIV in the central region is 30.2% [13]. All pregnant women receive HIV counselling and testing during their antenatal, delivery and post-natal period. Initiation of ART is fast-tracked in this population with ongoing adherence counselling and support. Viral load monitoring is performed at three monthly intervals during the pregnancy and breastfeeding period to detect virological failure for immediate interventions. The clinicians (mostly nurses and the attending doctors) interpret the results of viral load based on the recommendations by the South African National Department of Health PMTCT Guideline [13]. Patients are offered interventions following the standard of care; intensified counselling and adherence support, and/or switch of treatment to second-line regimen. Viral load assays for the central region of the Eastern Cape are performed by the National Health Laboratory Services (NHLS), East London in accordance with the standard protocols. Turn-around time for viral loads ranges from two – seven days depending on the distance of the facilities to the NHLS, East London.

Using the threshold for HIV drug resistance surveys sampling recommendation [28], a minimum of 52 samples is required. However, the sample number was increased to 102 in anticipation of possible insufficient samples and non-amplification, Participants were included in this sub-study if they were older than 16 years, had been on ART for at least four months and the most recent viral load within two weeks of delivery (peripartum viral load) was greater than 1000 copies/mL, considered as virological failure. Relevant data on demographics (age, marital status, parity and employment status) were obtained through interviews.

We obtained information on the most recent CD4 count, current ART, prior switch of ART, total duration on ART, including duration after the switch of ART regimen. Self-report of adherence was categorized as good (if no missed pills/doses in the previous seven days), sub-optimal (if only one or two pills/doses were missed) and poor (if more than two pills/doses were missed). Each participant underwent a clinical examination, and the WHO Clinical Staging was documented. In total, 102 parturient women with virological failure were recruited sequentially in both study sites to avoid bias selection. Venous blood samples (5 ml) were collected from each participant (N = 102; Frere hospital cohort = 52 and Cecilia Makiwane hospital cohort = 50) into ethylenediaminetetraacetic acid (EDTA) vacutainer tubes. Blood samples were transported on dry ice to the laboratory where the plasma was extracted and stored in RNase- and DNase-free tubes at -80^{0}C on each day between January to May 2018 until RNA isolation.

1.3. Viral RNA purification and reverse transcriptase (RT)-PCR

The viral RNA was extracted and purified from the plasma samples using the QIAGEN Viral RNA Mini Kit (QIAGEN GmbH, Germany) per the manufacturer's instructions. Amplification of the polymerase gene was performed by using a one-tube RT-PCR followed by nested PCR in accordance with validated protocols [29]. In summary, a partial polymerase fragment of 1400 bp was generated using the following primer pairs: RT-RV, 5′-TAT TTC AGC TAT CAA GTC TTT GAT GGG TCA-3′ and Pol1C 5′-GAA GGA CAC CAA TTG AAA GAC TGC AC-3′. For the RT-PCR step, 5′-CAA GGG GAG GCC AGG GAA TTT-3′, and Pol2R, 5′-TGA TGG GTC ATA ATA TAC TCC ATG-3′ for the nested PCR. A 50 µl of PCR mixture containing 5 µl of RNA, 5 µl of 10X buffer, 1 µl each of the RT-RV and Pol 1C primers (10 pmol/µl), 0.5 µl of 10 mM dNTP mix, 0.25 µl each of Taq polymerase enzyme (5U/µl), AMV RT (22U/µl), RNase inhibitor (40U/µl), 3 µl of $MgCl_2$ (25 mM), and PCR-grade water was added to make up the final volume to generate the RT-PCR reaction. The thermal cycling conditions for the RT-PCR are as follows: 42^{0}C for 60 min, then an initial step of 95^{0}C for 3 min, followed by 30 cycles of 94^{0}C for 1 min, 58^{0}C for 1 min and 68^{0}C for 2 min, and a final extension time of 10 min at 68^{0}C.

We performed the nested reactions in 100 µl reaction mixture with 5 µl of the first amplification product, 10 µl of 10X buffer, 2 µl each of the second-round primers, 1 µl of 10 mM dNTP mix, 0.5 µl of Taq polymerase enzyme (5 U/µl), 6 µl of $MgCl_2$ and water to make the final volume. The thermal cycling conditions follow the same steps above,

except for the RT step. In order to detect contamination, negative control was included in all PCR reactions. We confirmed PCR products for the expected band size (1084 base pairs) using 1% agarose gel electrophoresis followed by visualisation under ultraviolet transillumination.

1.4. Sequencing analyses

The automated population-based sequencing was performed on both strands of viral DNA with the PCR nested primers using the dideoxynucleotide chain termination approach on an ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Forward and reverse nucleotide sequences were assembled, edited and translated into predicted amino acid with the SeqMan Pro and Seqbuilder programmes included in the DNAStar software (DNASTART, INC, Madison, Wisconsin, USA).

1.5. Phylogenetic analysis

HIV-1 subtyping for the obtained sequences was performed by using the REGA 4 HIV-1 Genotyping tool [30]. Phylogenetic analyses were conducted for 80 test isolates; protease (PR) and RT genes using neighbour-joining method included in the REGA 4 for sequences with unclassifiable subtypes. Reference sequences were obtained from the Los Alamos Sequence Database (https://www.hiv.lanl.gov/content/index) representing all HIV-1 subtypes: A–D, F–H, J and K.

1.6. Genotypic drug resistance profiling

We performed an analysis of the HIV drug resistance-associated mutations in 80 PR and RT genes by using the Stanford Genotypic Resistance interpretation algorithm (https://hivdb.stanford.edu/hivdb/) [21]. This programme, which is based on subtype B consensus sequences, compares codons of the query sequence with resistance-encoding nucleotides contained in the database. Using the Stanford HIV dbv7.0, HIV drug-associated resistance was categorised into five drug response levels based on net drug score: susceptible (≤9), potential low-level resistance [10–14], low-level resistance [15–30], intermediate resistance (31–59) and high-level resistance (≥60) [31].

1.7. Ethical considerations

The DRMs Surveillance sub-study received ethical approval from the University of Fort Hare (Reference number: OBI021SADE01/2016) and Walter Sisulu University Ethics Committees (Reference number: 085/2017). The Eastern Cape Department of Health and the clinical governance of both hospitals granted permission for the implementation of this study. Participants received information on the purpose and process of the study in IsiXhosa or English, depending on their preferences. Each participant provided written informed consent of her voluntary participation in the study. Participants younger than 18 years gave informed assent in the presence of a parent/guardian, who also provided written informed consent. Participants' rights to privacy and confidentiality were protected during and after the study.

1.8. Statistical analyses

Analyses were limited to samples which were successfully amplified (n = 80/102; 79.4%); 22 viral samples failed to amplify. We used descriptive statistics (mean, median and percentages) to describe the characteristics of the participants, frequency and patterns of resistance mutations. Using the bivariate analysis, we examined the associations between the resistance mutations and the clinical profile of participants (HIV sub-type, duration on ART and type of ART, CD4 count and viral load as well as demographic characteristics such as age, parity, marital status and employment status). A p-value of < 0.05 was considered statistically significant.

Table 1
Clinical and Demographic Characteristics of the Participants.

Variables	Frequency	Percentages
Age*		
16–24	17	21.8
25–34	37	47.4
≥35	24	30.8
Duration on ART (months)		
≤6	16	20.0
7–12	12	15.0
13–24	12	15.0
≥25	40	50.0
Recent Viral Load		
1001–10000	18	22.5
10001–100000	43	53.8
> 100000	19	23.8
Parity*		
1	30	39.0
2	18	23.4
3	23	29.9
4	6	7.8
Marital status		
Single	69	86.3
Married	11	13.8
Employment status		
Unemployed	62	78.5
Employed	15	19.0
Scholar	2	2.5
WHO Clinical Stage**		
I	44	62.0
II	11	15.5
III	14	19.7
IV	2	2.8
ART Regimen		
First Line	66	82.5
Second Line	14	17.5
Adherence***		
Complete	5	7.2
Sub-optimal	15	21.7
Poor	49	71.0
Recent CD4 Count****		
< 200	23	34.3
200–349	24	35.8
350–499	14	20.9
≥500	6	9.0

*(Missing data were not documented); *Age = 2, *Parity = 3; **WHO Clinical Stage = 9; ***Adherence = 11; ****CD4 Count = 13.

2. Results

2.1. Demographic and clinical characteristics of participants

The age of the parturient women ranged from 16 to 43 years, with a mean (± standard deviation) of 30.2 (± 6.2) years. The majority of the participants were single (86.3%), unemployed (78.5%), had a maximum of two children (62.4%), assessed as WHO Clinical Stage 1 (62.0%), currently on Efavirenz-based regimen (first line ART) (82.5%) and had been on ART for more than 12 months (65%) (Table 1). The median duration of treatment in those on first-line and second-line regimens were 21 months and 42 months, respectively. However, the median duration on second-line regimen after switching therapy was 11 months. The mean CD4 count was 273 (interquartile range of 3–759) while viral load was 5.1 logs (interquartile range of 3.0–6.5 logs).

Table 2
Drug Resistance Mutations in Viral Sequences.

Drug Resistance Mutations	Frequency	Percentages (%)
Major NNRTI Mutations		
K103N	43	74.1
V106M	9	15.5
V108I	5	8.6
P225H	10	17.2
K101E	2	3.4
Y188L	4	6.9
Major NRTI Mutations		
M184V	28	48.3
K65R	11	19.0
K70R/E	4	6.9
K219Q	4	6.9
Major PI Mutations		
V82L	1	1.7
L90M	1	1.7

NNRTI = non-nucleoside reverse transcriptase inhibitor;NRTI = nucleoside reverse transcriptase inhibitor; PI = protease inhibitor.

2.2. Drug resistance mutations in the pol gene sequence

Nucleotide sequence of the Pol gene region was analysed using the Stanford HIV genotypic resistance interpretation algorithm to detect the presence of mutations conferring resistance to protease and reverse transcriptase inhibitors. Overall, HIV-1 drug resistance occurred in 58 (72.5%) viral sequences. The prevalence of DRMs in parturient women on Efavirenz-based cART was 69.7%, and protease inhibitors-based cART was 87.7%. In the RT gene, the predominant mutation was K103 N (n = 43/58; 74.1%), which confers resistance to commonly used NNRTIs: Efavirenz and Nevirapine. The prevalence of K103 N in participants who were still on first line regimen (TDF/FTC(3TC)/EFV) was 79.1% (n = 34), while the prevalence in individuals already switched to second line regimen was 20.9% (n = 9). The distribution of K103 N in participants who were on second line regimen are: AZT/3TC/Lop/r = 6; ABC/3TC/Lop/r = 2 and ABC/3TC/ATZ/r = 1. Five of these participants on second line regimen had protease inhibitor-based regimen for about four months while the remaining four were exposed to PI-based regimen for 15, 17, 40 and 52 months, respectively. Other mutations conferring resistance to NNRTIs include: V106 M (15.5%) and P225H (17.2%) (Table 2). A combination of multiple NNRTI-associated resistance mutations conferring cross-resistance to Etravirine and Rilpivirine were also observed.

Major resistance mutations to NRTIs were observed in some of the RNA sequences. M184 V mutation conferring resistance to Lamivudine and Emtricitabine occurred in 28 (48.3%) of the participants. The majority of the M184 V mutations were observed in parturient women on first line regimen (n = 23; 82.1%). The remaining five participants had been switched to second-line regimen, which still includes Lamivudine in the current regimen.

Also, K65R mutation conferring resistance to Tenofovir and Abacavir occurred in 11 participants (19%). The K65R occurred in 10 participants who were still on first line regimen (TDF/FTC/EFV) (90.9%) while the other participant had been switched to PI-based regimen (AZT/3TC/Lop/r) for 15 months at the time of the study. In the PR gene, there were two major protease inhibitors-associated mutations; V82 L and L90 M found in the study. However, the mutations did not accumulate in any individual patient.

2.3. HIV-1 sub-type and drug resistance mutations

Of all the viral isolates sequenced successfully in this study (N = 80), sub-type C accounted for 97.5% (n = 78), and the remaining two sub-types were: each of unique recombinant form (URF) C/D and circulating recombinant form (CRF02_AG). Sub-type C accounted for nearly all the DRMs (n = 57; 98.3%). URF C/D harbours drug-resistant mutations while CRF02_AG did not show any resistant mutation in its Pol gene.

2.4. Dual and triple-class failures

Dual class failures involving nucleoside and non-nucleoside reverse transcriptase inhibitors were mostly observed in some of the patients in the study.

2.4.1. AZT resistance

All the patients with intermediate (n = 5) to high-level resistance (n = 1) also harboured major NNRTI mutations; K103 N and Y188 L.

2.4.2. TDF resistance

High-level resistance occurred in 4 samples, while 8 had intermediate-level resistance and 6 had low-level resistance. All the patients had major NNRTI mutations; K103 N/R, Y188 L, V108I and K103E.

2.4.3. Lopinavir (Atazanavir) resistance

Potentially low-level resistance occurred in 2 samples. The patient with V82I mutation also had major NRTIs mutations (M184I and D67DN) and NNRTIs mutations (K103 N and L100I). There was co-occurrence of L90 M mutation with NRTIs mutations (K65R, M184 V and K219KE) and NNRTIs (K103 N, L100I and P225 H).

2.5. Associations between HIV drug resistance and baseline characteristics

In the bivariate analysis, the resistance mutations did not demonstrate any significant associations with demographic parameters (age, parity, marital status and employment status) ($p > 0.05$). However, the CD4 count demonstrated an inverse relationship with the DRMs ($p = 0.002$). This association was not replicated by the WHO Clinical Stage, viral load and duration on cART (Table 3).

2.6. Drug resistance mutations and individual ART

Though there was a potentially low or low-level resistance to ATZ/r, and LPV/r in two samples, these level of resistance are not clinically relevant. High-level resistance was found against Zidovudine in only one sample. Mutations conferring high level resistance to both

Table 3
Bivariate Analysis of Clinical Correlates of HIV Drug Resistance.

Variables	All	Resistance	No resistance	p-value
CD4 Count (cells/μl)				
< 200	23 (34.3)	23 (95.7)	1 (4.3)	0.002
200–349	24 (35.8)	13 (54.2)	11 (45.8)	
350–499	14 (20.9)	11 (78.6)	3 (21.4)	
≥500	6 (9.0)	2 (33.3)	4 (66.7)	
WHO Stage				
I	44 (62.0)	28 (63.6)	16 (36.4)	0.304
II	11 (15.5)	9 (81.8)	2 (18.2)	
III	14 (19.7)	12 (85.7)	2 (14.3)	
IV	2 (2.8)	1 (50.0)	1 (50.0)	
Duration on ART (months)				
≤6	16 (20.0)	13 (81.3)	3 (18.8)	0.224
7–12	12 (15.0)	6 (50.0)	6 (50.0)	
13–24	12 (15.0)	10 (83.3)	2 (16.7)	
≥25	40 (50.0)	29 (72.5)	11 (27.5)	
Recent Viral Load (copies/mL)				
1001–10000	18 (22.5)	14 (77.8)	4 (22.2)	0.097
10001–100000	43 (53.8)	27 (62.8)	16 (37.2)	
> 100000	19 (23.8)	17 (89.5)	2 (10.5)	

ART = anti-retroviral therapy; WHO = World Health Organization; EFV = Efavirenz; PI = Protease inhibitors.

Table 4
Drug resistance categories and ART.

	High-level resistance	Intermediate resistance	Low level resistance	Potential low level resistance	Susceptible
*ATV/R Resistance (n = 79)			1 (1.1)	1 (1.1)	77 (83.7)
DRV/R Resistance					80 (100.0)
LPV/R Resistance (n = 80)			1 (1.3)	1 (1.3)	78 (97.5)
AZT Resistance (n = 80)	1 (1.3)	5 (6.3)			74 (92.5)
D4T Resistance (79)	6 (7.6)	10 (12.7)	5 (6.3)		58 (73.4)
3 TC Resistance (n = 80)	36 (45.0)				44 (55.0)
FTC Resistance (n = 80)	36 (45.0)				44 (55.0)
ABC Resistance (n = 80)	17 (21.3)	3 (3.8)	16 (20.0)	1 (1.3)	43 (53.8)
TDF Resistance (n = 80)	4 (5.0)	8 (10.0)	6 (7.5)	1 (1.3)	61 (76.3)
EFV Resistance (n = 80)	56 (70.0)	1 (1.3)		1 (1.3)	22 (27.5)
NVP Resistance (n = 79)	57 (72.2)			1 (1.3)	21 (26.6)
RPV Resistance (n = 80)	19 (23.8)	3 (3.8)	7 (8.8)	1 (1.3)	50 (62.5)
ETR Resistance (n = 77)	5 (6.5)	10 (13.0)	2 (2.6)	12 (15.6)	48 (62.3)

ART = anti-retroviral therapy; ATV/R = Atazanavir/ritonavir; LOP/R = lopinavir/ritonavir; AZT = Zidovudine; D4T = Stavudine; 3TC = Lamivudine; FTC = Emtricitabine; ABC = Abacavir; TDF = Tenofovir disoproxil fumarate; EFV = Efavirenz; NVP = Nevirapine; RPV = Rilpivirine; ETR = Etravirine.

Emtricitabine and Lamivudine were found in 36 samples. High-level resistance against Efavirenz and Nevirapine occurred in 56 and 57 samples, respectively. Cross-resistance occurred within the NNRTI class; high-level resistance against Rilpivirine (n = 19) and Etravirine (n = 5) were found in the study (Table 4).

3. Discussions

The emergence of DRMs in the context of South African PMTCT programme could potentially threaten the goal of elimination of MTCT of HIV in view of the high ante-natal seroprevalence of 30.8% and over 95% ART coverage in the country [13,32]. This study examined the extent of and patterns of DRMs, and their potential implications for elimination of MTCT in the resource-constrained Eastern Cape, South Africa. The present study is the first from the region to assess the prevalence and patterns of HIV DRMs from parturient women with peripartum virological failure while on ART. This multi-centre HIV DRMs surveillance study utilised sequential selection of eligible parturient women in order to ensure unbiased sampling. This methodological approach followed the recommendations of the WHO [28] guideline for surveillance of HIV drug resistance and thus, validates the findings of the study. Available data on DRMs from other countries are rarely generalisable because of the variations in the sensitivity assays of the sequencing analysis performed and methodological approaches adopted in those studies [25,33].

We found a high prevalence of DRMs of 72.5% in women who delivered their index pregnancy at high viral load in the study settings. The emergence of acquired resistance is a direct consequence of suboptimal adherence reported by over 90% of the cohort. However, the contribution of primary resistance cannot be ascertained in this study. Future studies should examine the prevalence of primary resistance associated with ART in the Eastern Cape. Of significant interest is the detection of NNRTI- resistance mutations in parturient women who had been switched to second-line ART for at least 11 months. The possibility of archived resistance is a plausible explanation due to a lack of selective pressure of these drugs at the time of this study. These unique findings on DRMs have enormous implications for the elimination of MTCT of HIV in this region and South Africa.

We found a significant association between DRMs and CD4 count of the patients. This is expected, given the inherent relationship between viral replication and depletion of the CD4 cells in HIV-infected individuals. CD4 cells serve as the central mediator of the immune system, which becomes the target of HIV, leading to its destruction [34]. However, the lack of association between the WHO clinical stage and the prevalence of DRMs is not surprising, given that clinical failure often lags behind both virological and immunological failures [35].

We found a predominant NNRTI-associated mutations: K103 N, V106 M, V108I, P225H, K101E, and Y188 L in this study. These mutations confer varying degrees of resistance to Efavirenz and Nevirapine predominantly, and to a lesser extent, cross-resistance to Rilpivirine and Etravirine. Several non-polymorphic mutations (K103 N, V106 M and Y188 L) tend to confer high-level resistance (30 – 50-fold) to Nevirapine and Efavirenz [21]. However, P225H, an accessory mutation, confers high level resistance (> 50-fold) to Nevirapine and Efavirenz only when it co-exists with a K103 N mutation. Irrespective of the reverse transcriptase DRMs, evidence supports the practice of switching Efavirenz-based ART to a protease inhibitors-based regimen [7,13,17]. The presence of NNRTI-associated mutations would render Nevirapine prophylaxis ineffective and increase the risk of vertical transmission of resistant viral strains as reported by previous studies [3,20,21,35]. In the light of our results, supported by previous reports [25,36,37], Zidovudine alone or in combination with Nevirapine will offer better protection for HIV-exposed infants than Nevirapine alone as a prevention strategy.

We found M184 V mutation as the predominant NRTI-associated DRM followed by K65R mutation, classical thymidine analogue mutations (TAMs) (K70R and K219Q) and lastly, K70E. Zidovudine (AZT) is the least likely to develop clinically relevant resistance mutations. This corroborates earlier reports by the EARNEST Study, which demonstrated that AZT is more durable than most of the NRTIs [20]. K65R mutation reduces susceptibility to TDF, ABC, Stavudine (D4T) and rarely, 3TC. However, evidence suggests that K65R increases susceptibility to AZT, except in the presence of Q151 M, which rarely occurs together [21]. As such, the recommendations of switching first line TDF-containing regimen to second-line AZT-containing regimen [7,8,13] is supported by our results. Also, we found dual class failures involving mostly TDF and NNRTI-resistance, which further questions the rationale for Nevirapine prophylaxis in HIV-exposed neonates. As such, our findings highlight the need for revision of South African PMTCT guideline.

M184 V mutation confers high-level resistance to 3TC and FTC (100-fold), and low-level resistance to ABC but increases susceptibility to AZT, D4T and TDF. It also impairs the replicative capacity of the virus. The majority of patients with M184 V mutation would have acquired major NNRTI-associated mutations due to the low genetic barriers of this class of drugs [38]. Of interest is the presence of K70R in the cohort, which could potentially reduce the susceptibility of AZT and to a lesser extent, TDF and D4T. Also, K219Q could potentially reduce the susceptibility of AZT and D4T when present with other TAMs [25]. The two protease inhibitors-associated mutations (V82 L and L90 M) found in this study were not clinically relevant. Protease inhibitors require accumulation of mutations, leading to a progressive increase in the level of resistance [39]. This confirms that the main reason for high viral loads in the patients on protease inhibitors-based regimen is sub-

optimal adherence. It is therefore crucial for clinicians to screen for and address adherence challenges in patients with failing first-line regimen before switching to second-line regimen. This is particularly true for patients on second-line regimen who may have to contend with the gastrointestinal adverse effects of Lopinavir in addition to nausea and vomiting associated with pregnancy.

4. Strength and limitations of the study

Though, this study provides a snapshot of the frequency and patterns of DRMs from women delivering babies at high viral loads in the Eastern Cape, a previously unexplored region of South Africa, the findings may not be representative of the entire Eastern Cape Province. We sequentially select women with virological failure in order to prevent bias sampling. Notwithstanding that we selected the two largest maternity centres, serving the most populous, central region of the province for this study, a larger, multi-centre (across the entire province) HIV resistance surveillance study should follow to further elucidate our findings. In order to properly contextualize the findings of our study, perhaps, a baseline genotyping result would have shed light on the contribution of primary (transmitted) resistance to virological failure in the cohort. As such, we were unable to quantify the risks of baseline DRMs to the PMTCT outcomes. Failure to use point-of-care non-B-sub-type resistance assays did not allow for immediate interventions based on DRMs results.

5. Conclusions

We confirmed a high prevalence of drug resistance mutations in women delivering their index babies at high viral loads in a resource-constrained settings of the Eastern Cape, South Africa. Though, HIV-1 sub-type C is the predominant circulating virus in the region; thus, accounting for nearly all the drug resistance mutations. An effective surveillance system for tracking all pregnant women on cART will assist in identifying those with virological failure and drug resistance during antenatal, labour and delivery for prompt interventions. A nationally representative drug resistance surveillance in pregnant women should be undertaken to guide future policies and management guidelines in the country. Also, a point-of-care reverse transcriptase-PCR strategies for screening for common resistance mutations (K65R, K103 N and Y188 L) should be made available in order to select appropriate neonatal prophylaxis and maternal therapy in resource-constrained settings.

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Authors' credit

OVA, CLO, BI, AO, DTG, JL and AIA conceptualised and designed the study. All authors were involved in the implementation of the project. OVA, AIA, BI and DTG conducted the data analysis. OVA, AIA, DTG, BI, CC and CLO drafted the manuscript. All authors gave intellectual input and approved the final version of the manuscript for submission.

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