American Journal of
Health, Medicine and Nursing Practice
(AJHMN)

Mother-to-Child Transmission of Drug Resistant HIV-1 in Selected PMTCT Clinics in Rwanda

Gad Rutayisire, Roman Saba Ntale, Fred Kyeyune, Nsereko Vincent, Uwera Marie Grace, Tumusiime Musafiri, Mike Rurangwa, Innocent Ishami, Enock Wekia, Richard Mulondo, Noah Kiwanuka, Bernard Ssentalo Bagaya

AJP

American Journal of Health, Medicine and Nursing Practice
ISSN 2520-4017 (Online)
Vol.11, Issue 3, pp 11- 29, 2025

www.ajpojournals.org

Mother-to-Child Transmission of Drug Resistant HIV-1 in Selected PMTCT Clinics in Rwanda

Short Running Title: Drug Resistance Profiles of Mother-to-Child transmitted HIV

Gad Rutayisire[1], Roman Saba Ntale[2], Semwaga Emmanuel[3], Fred Kyeyune[4], Nsereko Vincent[5], Uwera Marie Grace[6], Tumusiime Musafiri[7], Mike Rurangwa[8], Innocent Ishami[9], Enock Wekia[10], Richard Mulondo[11], Noah Kiwanuka[12], Bernard Ssentalo Bagaya[13]

[1]Department of Biomedical Laboratory Sciences, University of Rwanda, Kigali, Rwanda, [2]Department of Microbiology, Soroti University, Soroti, Uganda, [3]Department of Gynaecology, Mediheal Hospital, Kigali, Rwanda, [4]Molecular Virologist and Senior Research Scientist at CFAR Laboratory, JCRC, Lubowa Hill, Entebbe Road, Kampala, Uganda, [5]Department of Epidemiology and Biostatistics, School of Public Health, Makerere University College of Health Sciences, Kampala, Uganda, [6]Rwanda Biomedical Center, Kigali, Rwanda, [7]Department of Biomedical Laboratory Sciences, University of Rwanda, Kigali, Rwanda, [8,9]Rwanda Biomedical Center, Kigali, Rwanda, [10]National Tuberculosis Reference Laboratory/Supranational Reference Laboratory, Uganda National Health Laboratory and Diagnostic Services, Kampala, Uganda, [11]Genexpert, Uganda LTD, [12]Department of Epidemiology and Biostatistics, School of Public Health, Makerere University College of Health Sciences, Kampala, Uganda, [13]Department of Immunology and Molecular Biology, School of Biomedical Sciences, Makerere University College of Health Sciences, Kampala, Uganda.

Article History

Submitted 07.10.2025 Revised Version Received 06.11.2025 Accepted 04.12.2025

Abstract

Purpose: This study aims at determining the prevalence of high and low frequency HIV drug resistance mutations and determine the HIV-1 genetic diversity distributions with aim of generating new findings, in the era of option B and B+, towards informed decision-making for more efficient ART strategies and therapeutic monitoring in the PMTCT-Clinics in Rwanda, it is then crucial to set-up bold and innovative approaches in the pediatric AIDS response tailored to the specific needs of the local HIV epidemic.

Materials and Methods: Mother-baby baseline viral load was performed on plasma samples and/or Dried Blood Spots(DBS) using the COBAS® AmpliPrep/ COBAS® TaqMan® HIV-1 Test, v2.0 on a COBAS® AmpliPrep Instrument (Roche Molecular Systems) and Early Infant Diagnosis was by HIV DNA PCR using the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test v2.0. While the HIV drug resistance was analyzed using HIV Genome Pro Software from MGI Tech, which was also used to identify HIV subtypes based on phylogenetic analysis.

Findings: Study revealed that the HIV drug resistance mutations detected or observed constituted 9 out of 32(28%) mother-baby pairs with at least one of HIV drug resistance mutations observed. While 23 out of 32(72%) of mother-baby pairs had no HIVDRMs detected or observed. M184V mutations were observed in mothers by 25% and babies by 30%, with 83% being transmitted to their babies. While K103N, mutations were found in mothers by 22% and 19% among their babies, with 100% being transmitted to their babies. It was also observed that the HIV-1 subtype A/A1 were predominant with 26% being reported among the identified subtypes in mother-baby pairs.

Unique Contribution to Theory, Practice and Policy: The findings emphasize the importance of starting antiretroviral therapy (ART) early ideally before conception and maintaining consistent viral suppression throughout pregnancy and breastfeeding to minimize the risk of mother-to-child HIV transmission. They also highlight the need for regular monitoring of drug resistance and viral subtypes among both mothers and infants to guide effective treatment choices and prevent the spread of resistant strains. Strengthening postpartum follow-up systems is equally critical, including supporting maternal adherence, ensuring timely viral load and infant testing, and providing appropriate prophylaxis. Furthermore, the growing diversity of HIV-1 subtypes and the emergence of recombinant forms should be considered when developing regionally tailored ART regimens and vaccine strategies.

Key words: *PMTCT-clinics, HIV-1, Reverse transcriptase, transmitted and acquired Drug resistance*

INTRODUCTION

The advent of Highly Active Antiretroviral Therapies (HAART), the subsequent improvements in drug regimens and treatment guidelines has not only significantly extended but also improved the quality of life for HIV patients [1-3]. Globally, new HIV infections among children have declined by 62% since 2010, with approximately 120,000 new infections in 2024, down from 310,000 in 2010, about 84% of pregnant women living with HIV had access to antiretroviral medicines to prevent transmission to their children in 2024[4, 5]. Eastern and Southern Africa accounted for 79% of new child HIV infections averted, while Western and Central Africa accounted for 14%. Rwanda has made significant strides in reducing transmission rates, with a notable decrease from 2% in the past to 0.9% in 2024[4-6]. In fact, between July 2022 and June 2023, 389,531 pregnant women were tested for HIV, with 99% of those diagnosed receiving ART to minimize transmission risk. Differences in sampling might explain the varying MTCT rates[5, 7].

Our study was restricted to only 10 PMTCT sites across the five provinces, whereas rates reported by Rwanda Biomedical Center(RBC) include all national data[7]. However, our findings may still uncover site-specific challenges in addressing the HIV drug resistance mutations and HIV-1 subtype distribution [4, 6, 7]. The number of HIV-1 patients on HAART in Rwanda has been rising, currently estimated to be 97.5% of those thought to be infected[8]. As is the case with most HIV-1 epidemics among low and middle income countries, Rwanda's epidemic too disproportionately affects girls and young adult females of child bearing age [5, 9, 10]. Inevitably, given a lease on life by HAART, this affected age group lives a normal life, enjoying their right be sexually active, get married and start families. HAART, combined with innovations in management of opportunistic infections has given HIV patients an opportunity to live a normal life. However, the emergence of drug resistance to HAART has become a major public health concern, threatening to reverse successes so far achieved in the management of HIV patients [11-14].

Majorly, poor adherence to the HAART by those on treatment is the major driver of emergence of drug resistance in the patient. The emerging drug resistant HIV-1 can be transmitted to others as a result of failed viral suppression that increases the infectiousness of patients [12-15]. More so, among expectant mothers with unsuppressed drug resistant HIV, there is an increased risk of not only mother-to-child-transmission (MTCT) but also transmission of drug resistant virus to the unborn child [12-15]. Recently in 2024, we showed a MTCT of HIV of 3.7% among 862 mothers selected from 10 PMTCT clinics in Rwanda[7]. While there are other virological factors to explain such high odds of MTCT in Rwanda, such as a multiclade complexity of the HIV-1 epidemic, underlying drug resistance in the face of PMTCT interventions seemed most plausible. This particular study aimed to determine both the prevalence of high and low frequency HIV drug resistance mutations, while also determining the HIV-1 genetic diversity distributions with aim of generating new findings, in the era of option B and B+, towards informed decision-making for more efficient ART strategies and therapeutic monitoring in the PMTCT clinics in Rwanda, it is then crucial to set-up bold and innovative approaches in the pediatric AIDS response tailored to the specific needs of the local HIV epidemic in mothers and their babies infected through MTCT during the 18 months of follow up.

Problem Statement

Mother-to-child transmission(MTCT) of HIV-1 and associated mortality remain un acceptably high despite all efforts that have been in place for decades such as the implementation of PMTCT-programs in 1999/2000 in Rwanda including the modified versions of option B and B+ in 2010 and 2012, respectively, where option B recommends ART for the pregnant mothers

living with HIV from 14weeks' gestation until birth or the cessation of breastfeeding and the use of NVP for the infant until 4-6weeks of age, while option B+ advocates lifelong ART and NVP for the infant as for option B. More worrying is virological factors sustaining MTCT in Rwanda that remain inconclusively explored such as the emergence of HIV drug resistance and high HIV-1 genetic diversity that continue to pose significant challenges to preventing vertical transmission, particularly in resource-limited settings. The problem is further compounded by the lack of effective monitoring and tracking systems, making it difficult to identify and respond to drug resistance and genetic diversity in a timely manner. This knowledge gaps hinders the development of effective interventions to prevent MTCT of HIV, threatening to undermine progress made in reducing Pediatric HIV-infections. Therefore, this study aims at determining the prevalence of high and low frequency HIV drug resistance mutations and determine the HIV-1 genetic diversity distributions with aim of generating new findings, in the era of option B and B+, towards informed decision-making for more efficient ART strategies and therapeutic monitoring in the PMTCT-clinics in Rwanda, it is then crucial to set-up bold and innovative approaches in the pediatric AIDS response tailored to the specific needs of the local HIV epidemic.

MATERIALS AND METHODS

Study Design and Area

A prospective cohort study[16]was conducted among mother-baby pairs where both PMTCT clinics and participants were selected through a two-stage sampling strategy described under the section of sampling strategy below. The study was conducted in ten PMTCT clinics distributed across the five provinces of Rwanda. These included Northern, Southern, Western and Eastern provinces and in Kigali City. In every study province, one urban and one rural (lower- level)-based health facility were included in the study. All hospitals in the study were referral-level health facilities for a number of other medical services, including prevention of mother-to-child transmission/ voluntary counselling and Testing (PMTCT/VCT) of HIV.

Study Population

Expectant mothers living with HIV-1 and participating in PMTCT program, who were to deliver their child at any of the ten health facilities selected for the study were eligible. To be selected for the study, a mother had to be HIV positive, be enrolled in PMTCT, and be under ART. However, mothers whose child died before being discharged from the labor ward or did not consent to the study were excluded.

Sampling Strategy and Sample Size Determination

All five Rwanda's administrative provinces were included in the sample. We used a two-stage sampling strategy; at the first stage, within each province, clinics were first stratified as urban (within the boundaries of provincial or capital cities) and rural (outside city boundaries) to ensure equitable geographical and contextual representation across both urban and rural health systems. A fixed probability sampling (stratified random sampling) was used to select one urban and one rural facility per province to arrive at a total of 10 facilities. A fixed probability sampling (simple random sampling) was used to achieve a total of 862 pregnant mothers living with HIV from the selected PMTCT clinics. Eligible women were those actively enrolled in the PMTCT program and presenting at the selected facilities for childbirth. At delivery, their babies were also enrolled forming mother-baby pairs that were prospectively followed to assess HIV transmission. From the 862-HIV-1-positive pregnant women, 32 cases of vertical HIV transmission were identified, By DNAPCRtest (babies' Dried Blood Spots/DBS) were done immediately at birth, at 4 weeks,6weeks, then at 6-12 weeks or 6-18 months, together with

HIV-1 antibody testing(babies 'blood) by Laboratory technologists under the supervision of the principal investigator to confirm if they were HIV positive, and the results were published[7], constituting the primary analytical cohort for this prospective cohort study.

Sample/Data Collection Procedures

Placental Cord Blood

With the use of needle and Syringe the placental cord blood was sampled from the umbilical cord and placed into EDTA tubes. Plasma was separated in the Laboratory and frozen until tested.

Dried Blood Spots (DBS)

DBS samples were taken from the babies at the time of delivery and at follow-up visits. A finger prick was performed and 3 blood spots placed on the designated areas on the Whattman filter papers.

Venous Blood

Venous blood was collected from mothers and from babies (during follow-up visits). EDTA blood was used for plasma separation.

HIV-1 RNA Extraction

The HIV-1 viral RNA was extracted following manufacturer's instructions using Zymo Quick II viral RNA kit from Zymo Research which is designed for rapid purification of viral RNA from various samples with viral load of less than 2000 copies per ml. The detailed procedures of how HIV-1 RNA viral extraction was done is under the section of supplementary materials.

HIV-1 Viral Load and HIV DNA PCR Testing

All HIV viral load and DNA PCR testing were centralized and conducted at the Rwanda National Reference Laboratory (RNRL), the designated national hub for HIV diagnostic and monitoring services. The DNAPCR tests (babies' dry blood spots) were conducted immediately at birth and at 18 months, together with HIV-1 antibody testing (babies' blood) by Laboratory technologist under the supervision of Principal investigator to confirm if they were HIV positive and the results were published[7].

HIV-1 Genotyping for Drug Resistance and HIV-1 Subtypes

Testing for HIV drug resistance and HIV-1 Subtypes were done using AToplex HIV-1 amplification kit following manufacturer's instructions where the kit is designed for amplifying the HIV-1 genotypic drug resistance target genes for the MGI high through put sequencing platform series. The kit adopts one-step RT-PCR method to quickly amplify the Protease (PR), Reverse transcriptase (RT), and Integrase (IN) regions within *pol gene* of HIV-1 in one tube. Then the MGIEasy Fast PCR-FREE Enzyme Digestion is used for specific library preparation. The combination of one Step RT-PCR amplification method and Fast PCR-FREE library preparation simplifies the library preparation process and shortens the total operation time. All reagents provided within this kit have passed strict quality control and functional verification procedures, ensuring the stability and reproducibility of the experiment. The detailed procedures of how Testing for HIV-1 drug resistance and HIV-1 Subtypes were done are under the section of supplementary materials.

Data Analysis

Mother-baby baseline viral load was performed on plasma samples and/or Dried Blood Spots(DBS) using the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 on a

COBAS® AmpliPrep Instrument (Roche Molecular Systems) and Early Infant Diagnosis was by HIV DNA PCR using the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test v2.0. While the HIV drug resistance was analyzed using HIV Genome Pro Software from MGI Tech, which was also used to identify HIV subtypes based on phylogenetic analysis. All the quality control of the samples in the library preparation passed and show quality fastq files through the spike quality control score, and gapdh. All the details are the on the section of supplementary materials.

Ethical Considerations

Study Hospitals gave administrative permissions. Ethical approval was obtained from Makerere University School of Biomedical Sciences Higher Degrees Research Ethics Committee **(SBS-HDREC No: 738)** and from University of Rwanda Institutional Review Board **(UR-IRB No: 228)**. All mothers/participants provided written informed consent prior to being enrolled into the study. Mothers had liberty to consult their husbands provided consent on behalf of their babies/infants. All participants' documents were labelled with identification numbers, rather than names. The research team collected the completed consent forms from participants. These forms were reviewed for completeness, accuracy, and legibility, and participants' identities and consent were verified as necessary. The consent forms were then documented in the participants' research records. The completed consent forms were stored in a secure, locked location, with access restricted to authorized research team members. The forms were stored in a manner that maintained participant confidentiality and were retained for a specified period as required by study protocols.

FINDINGS

Table 1: Socio-Demographic Characteristics of Mothers Attending the PMTCT Clinics

Mothers IDs	Age	ART-Regimens	Duration on ART-Regimens in months	Baseline VL(RNA copies/mls	PPVL(RNA copies/mls	Babies IDs	Form of MTCT	Baseline VL(RNA copies/mls
19309	41	TDF+3TC+EFV/ TDF+3TC+ATV/r	36	6,634	4,234	20602381	IU	4,800
19222	32	TDF+3TC+DTG	34	10,000	8,648	2167	IU	8,768
19306	42	TDF+3TC+DTG	48	10,000	7,584	602209	IU	6,780
19227	38	TDF+3TC+EFV/ TDF+3TC+ATV/r	56	9,853	6,784	7171	IU	7,680
19491	38	TDF+3TC+DTG	56	10,000	8,685	602181	IU	6,980
19048	33	TDF+3TC+EFV	56	6,665	4,280	2176	IU	4,878
19049	34	ABC + 3TC + DTG	25	10,000	8,784	1534	IU	6,846
19488	42	ABC + 3TC + EFV	49	9,843	6,790	602122	IU	7,864
19230	28	TDF + 3TC + DTG	48	8,400	5,785	620	IU	6,654
43806	30	ABC + 3TC + FTC	58	1,000	9,795	1970661	IU	1,600
19303	37	TDF + 3TC + EFV/ TDF + 3TC + ATV/r	44	6,504	3,970	601526	IU	5,848

19221	33	ABC + 3TC + DTG	48	1,000	1,500	5914	IU	4,600
19044	33	TDF + 3TC + EFV	48	9,666	8,700	4557	IU	5,895
19047	37	ABC + 3TC + EFV	98	5,000	4,900	1526	IU	4,800
19302	38	TDF + 3TC + FTC	48	1,000	1,200	601688	IU	6,984
19054	30	ABC + 3TC + DTG	55	9,669	7,800	8181	IU	5,874
19218	26	TDF + 3TC + EFV	57	6,487	4,684	1812	IU	7,648
19492	32	ABC + 3TC + FTC	48	6,308	5,280	602187	IU	9,984
19308	32	TDF + 3TC + EFV	98	5,452	UVL	20602356	PP	UVL
19061	26	TDF + 3TC + FTC	55	6,400	4,860	2076	IU	6,846
19305	30	ABC + 3TC + EFV	94	5,848	3,800	600940	IU	5,800
1970670	25	TDF + 3TC + EFV	57	9,500	6,700	1749753	IU	1,400
19304	33	ABC + 3TC + FTC	36	1,546	UVL	25139495	PP	UVL
18122404	23	TDF + 3TC + EFV	48	1,000	1,200	3232183	IU	1,100
602866	38	TDF + 3TC + EFV	27	600	UVL	78975	PP	UVL
602950	40	ABC + 3TC + FTC	38	1,200	UVL	1985	PP	UVL
600404	43	TDF + 3TC + EFV	56	1,400	UVL	601934	PP	UVL
20121763	38	ABC + 3TC + FTC	66	890	UVL	141608205	PP	UVL

601725	25	TDF + 3TC + EFV	48	1,200		251394950	PP	UVL
1989	28	ABC + 3TC + FTC	50	800	UVL	602576	PP	UVL
1739706	25	TDF + 3TC + FTC	98	6,000	UVL	47614	PP	UVL
19120	27	ABC + 3TC + EFV	30	<20	UVL	30884045	PP	UVL

PP=Post-partum, IU=In-Utero, UVL=Undetectable Viral load/No PCR, Nucleoside Reverse Transcriptase Inhibitors (NRTIs): 3TC = Lamivudine, ABC = Abacavir, AZT = Zidovudine, d4T = Stavudine, ddI= Didanosine, FTC = Emtricitabine, TDF= Tenofovir. **Non-Nucleoside Reverse Transcriptase Inhibitors (**NNRTIs**):** EFV= Efavirenz, ETR= Etravirine, NVP= Nevirapine, RPV= Rilpivirine

Protease Inhibitors (PIs): ATV/r = Atazanavir/ritonavir, **Integrase Strand Transfer Inhibitors** (**INSTIs):** DTG = Dolutegravir.

Characteristics of Maternal Study Participants

Most of the pregnant mothers living with HIV-1 attending those PMTCT clinics had average age of 33 years and were all reported to be on the first line ART with only 3/32 (9.38%) of mother-baby pairs who failed the first line ART regimens and were switched to second line ART regimens after 6-12 months with persistent high viral load while adhering to first-line ART regimens, the mean and median duration on ART-Regimens being 54 and 33 months respectively and the mean base line maternal viral load was 5,309RNA copies/mls. Among the 32 mother-baby pairs, 10 out of 862 babies (1.25%) had post- partum infection of HIV, while 22 out of 862 babies (2.55%) were infected in-utero and the mean viral load for the babies were 4,051RNA copies/mls. Despite the majority of pregnant mothers being on antiretroviral therapy (ART) for extended durations ranging between 25 and 98 months, MTCT of HIV still occurred. A significant proportion of these mothers had baseline maternal viral loads suppressed to ≤1,000 RNA copies/ml, which is typically considered optimal for reducing the risk of vertical transmission. This finding implies that while virological suppression is critical, it may not be solely sufficient to eliminate the risk of transmission. Conversely, the in-utero transmissions (2.55%) were observed even among mothers on long-term ART regimens, including both first-line (e.g., TDF + 3TC + EFV) and more potent integrase inhibitor-based regimens (e.g., TDF + 3TC + DTG). This raises concern regarding early maternal viral suppression during pregnancy. Demographic characteristics of maternal participants are summarized in Table 1 above.

Table 2: HIV Drug Resistance Patterns and Subtype among Mother-Baby Pairs

Mother/ Baby pair	IDs	NRTIs	NNRTIs	PIs	INSTIs	Subtype
1.	19309	NMD	K103N (85.29) E138A (99.29)	M46L (93.81)	NMD	A1
	20602381	M41L(91.86), M184V(94.69)	K103N (98.38) V108I (92.07) E138A (4.95)	M46L (93.81)	NMD	A1
2.	19222	NMD	NMD	NMD	NMD	B
	2167	NMD	NMD	NMD	NMD	19_cpx
3.	19306	NMD	NMD	NMD	NMD	A1D
	602209	NMD	NMD	NMD	NMD	URF_CD(C/D)
4.	19227	M41L (5.72), M184V (9.65)	K103N (86.13) V108 I (7.36) E138A (90.62)	M46L (7.79)	NMD	A1
	7171	M184V (9.65)	K103N (86.13) V108I (7.36) E138A (90.62)	M46L (7.79)	NMD	A1
5.	19491	NMD	NMD	NMD	NMD	A1
	602181	NMD	NMD	NMD	NMD	D
6.	19048	M41L (5.72), M184V (9.65)	K103N (86.13) V108I (7.36) E138A (90.62)	NMD	NMD	19_cpx
	2176	M184V (9.65)	K103N (86.13) V108I (7.36), E138A (90.62)	NMD	NMD	19_cpx
7.	19049	NMD	NMD	NMD	NMD	D
	1534	NMD	NMD	NMD	NMD	01_AE
8.	19488	NMD	K103N (97.79)	NMD	NMD	A

	602122	NMD	K103N (99.57)	NMD	NMD	A1
9.	19230	NMD	K103N (99.66)	NMD	NMD	URF_08C(08_BC/C)
	620	NMD	K103N (99.58)	NMD	NMD	A1
10.	43806	NMD	NMD	NMD	NMD	B
	1970661	NMD	NMD	NMD	NMD	B
11.	19303	M41L (99.52), M184V (99.58)	K103N (99.62) V108I (99.86)	M46L (99.61)	NMD	A1
	601526	M41L (92.53), M184V (95.58)	K103N (98.43) V108I (91.53) E138A (4.25)	M46L (93.51)	NMD	A1
12.	19221	NMD	NMD	NMD	NMD	25_cpx
	5914	NMD	NMD	NMD	NMD	25_cpx
13.	19044	M41L (99.52), M184V (99.58)	K103N (99.62) V108I (99.86)	NMD	NMD	50_A1D
	4557	M41L (92.53), M184V (95.58)	K103N (98.43), V108I (91.53) E138A (4.25)	NMD	NMD	50_A1D
14.	19047	M41L (99.52), M184V (99.58)	K103N (98.43) V108I(91.53) E138A(4.25)	NMD	NMD	A1
	1526	M41L (99.52), M184V (99.58)	NMD	NMD	NMD	A1
15.	19302	NMD	NMD	NMD	NMD	A1CD
	601688	NMD	NMD	NMD	NMD	A1CD
16.	19054	NMD	V179E (99.33)	NMD	T97A (92.93)	URF_02G (02_AG/G)
	8181	NMD	V179E (99.33)	NMD	T97A (92.93)	URF_02G (02_AG/G)
17.	19218	NMD	NMD	NMD	NMD	A1D

	1812	NMD	NMD	NMD	NMD	A1D	
18.	19492	NMD	NMD	NMD	NMD		URF_CD(C/D)
	602187	NMD	NMD	NMD	NMD		URF_CD(C/D)
19.	19308	UVL	UVL	UVL	UVL		UVL
	20602356	UVL	UVL	UVL	UVL		UVL
20.	19061	NMD	NMD	NMD	NMD		A
	2076	NMD	NMD	NMD	NMD		A
21.	19305	NMD	NMD	NMD	NMD		URF_08C(08_BC/C)
	600940	NMD	NMD	NMD	NMD		URF_08C(08_BC/C)
22.	1970670	NMD	NMD	NMD	NMD		01_AE
	1749753	NMD	NMD	NMD	NMD		01_AE
23.	19304	UVL	UVL	UVL	UVL		UVL
	25139495	UVL	UVL	UVL	UVL		UVL
24.	18122404	NMD	NMD	NMD	NMD		A1D
	3232183	NMD	NMD	NMD	NMD		A1D
25.	602866	UVL	UVL	UVL	UVL		UVL
	78975	UVL	UVL	UVL	UVL		UVL
26.	602950	UVL	UVL	UVL	UVL		UVL
	1985	UVL	UVL	UVL	UVL		UVL
27.	600404	UVL	UVL	UVL	UVL		UVL
	601934	UVL	UVL	UVL	UVL		UVL
28.	20121763	UVL	UVL	UVL	UVL		UVL
	141608205	UVL	UVL	UVL	UVL		UVL
29.	601725	UVL	UVL	UVL	UVL		UVL
	251394950	UVL	UVL	UVL	UVL		UVL
30	1989	UVL	UVL	UVL	UVL		UVL
	602576	UVL	UVL	UVL	UVL		UVL
31.	1739706	UVL	UVL	UVL	UVL		UVL
	47614	UVL	UVL	UVL	UVL		UVL
32.	19120	UVL	UVL	UVL	UVL		UVL
	30884045	UVL	UVL	UVL	UVL		UVL

NMD= No mutation Detected, **UVL=** Undetectable Viral Load /No PCR, K103N=**K:** Lysine (a.a), **N:** Asparagine (a.a), E138A=**E:** Glutamic acid (a.a), **A:** Alanine (a.a), M41L=**M:** Methionine (a.a), **L:** Leucine (a.a), V108I=**V:** Valine (a.a), **I:** Isoleucine (a.a), M46L= **M:** Methionine (a.a), **L:** Leucine (a.a), M184V= **M:** Methionine (a.a), **V:** Valine (a.a), V179E= **V:** Valine (a.a), **E:** Glutamic acid (a.a), T97A=**T:** Threonine (a.a), **A:** Alanine (a.a).

American Journal of Health, Medicine and Nursing Practice
ISSN 2520-4017 (Online)
Vol.11, Issue 3, pp 11- 29, 2025
www.ajpojournals.org

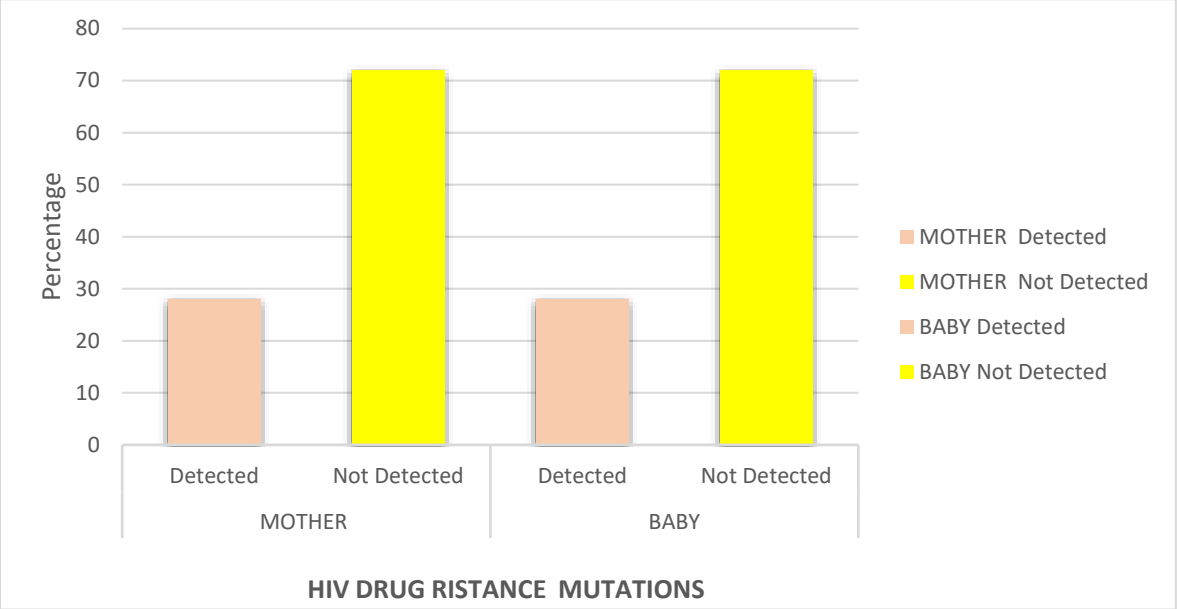

Figure 1: Shows mother-baby pairs with HIV drug resistance mutations detected (observed) constituting 9 out of 32(28%) with at least one of HIV drug resistance mutations observed. While 23 out of 32(72%) of mother-baby pairs had no HIVDRMs detected or observed.

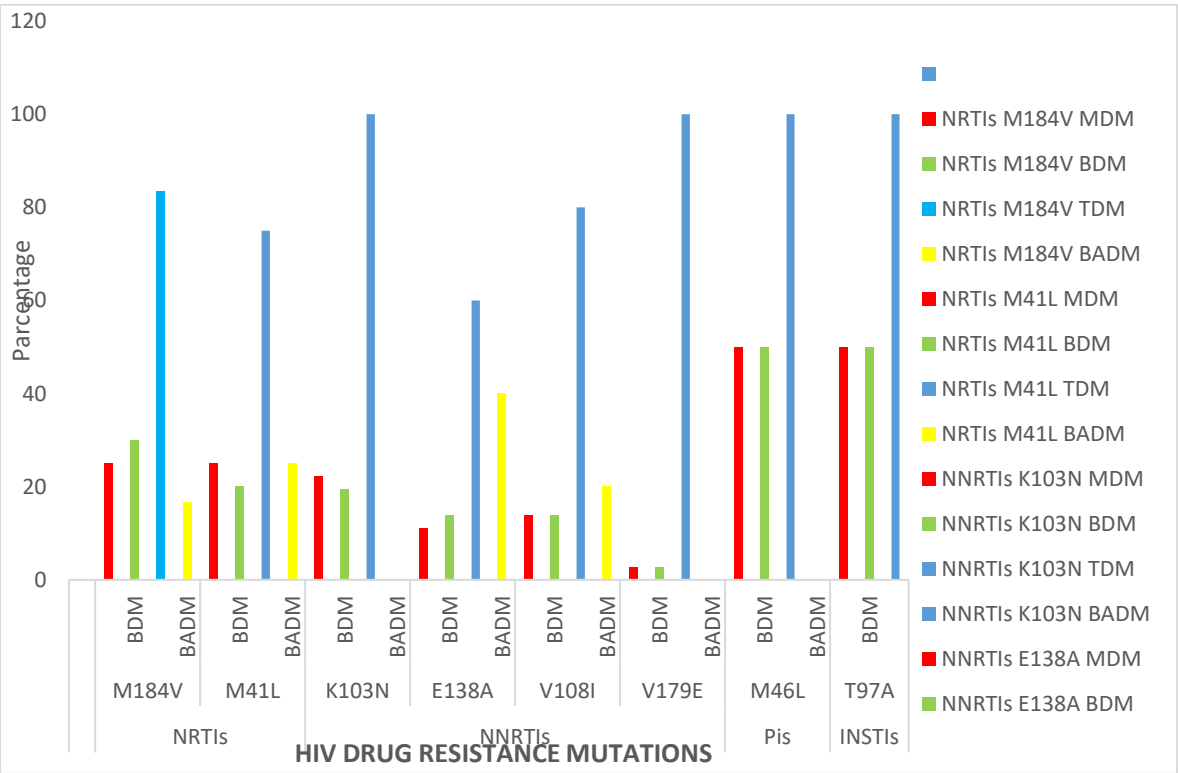

Figure 2: Shows the percentage of each HIVDRMs transmitted or acquired for both mother-baby pairs

***MDM**: Mother drug mutation, **BDM**: baby drug mutation, **TDM:** Transmitted drug mutation, **BADM**: baby acquired drug mutation.*

American Journal of Health, Medicine and Nursing Practice
ISSN 2520-4017 (Online)
Vol.11, Issue 3, pp 11- 29, 2025
www.ajpojournals.org

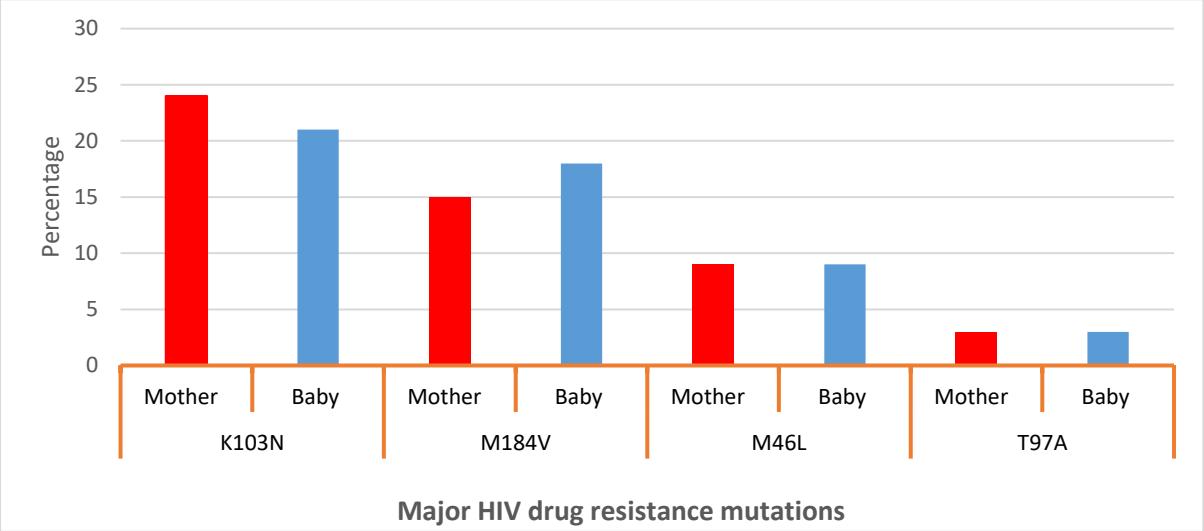

Figure 3: Shows the percentage of major HIVDRMs for the mother-baby pairs in each class of HIV ART.

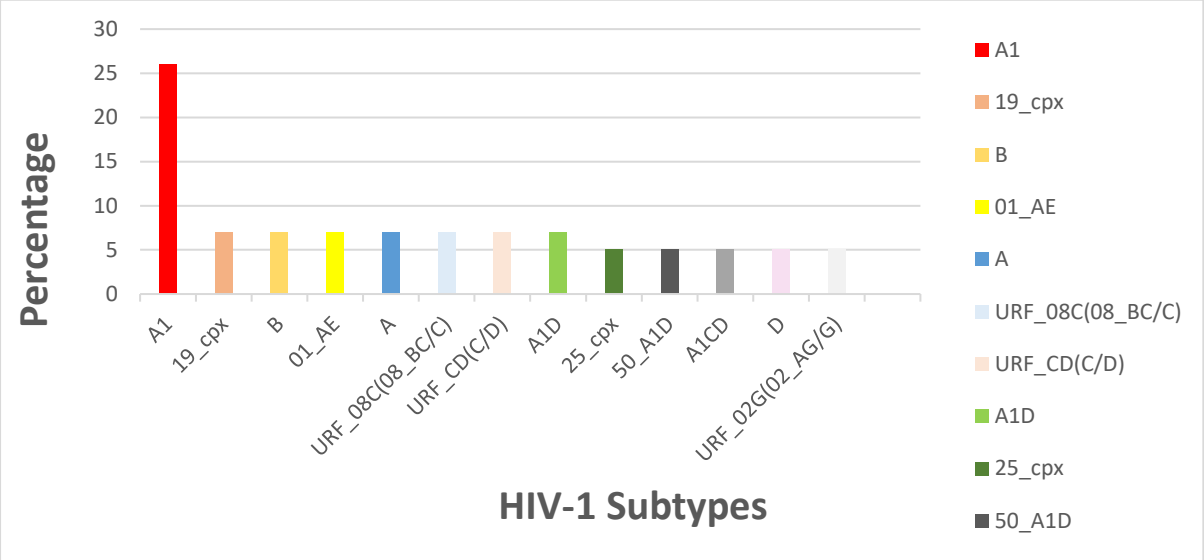

Figure 4: Shows the percentage of HIV-1 Sub types

Table 2 and Figures (A-C) presents the HIV-1 drug resistance mutations (HIVDRMs) profiles and HIV-1 subtypes respectively, among 16 mother-baby pairs. The analysis reveals notable patterns in resistance mutations across Nucleoside reverse transcriptase inhibitors (NRTIs), Non-nucleoside reverse transcriptase inhibitors (NNRTIs), Protease inhibitors (PIs), and Integrase strand transfer inhibitors (INSTIs), alongside the distribution of HIV-1 subtypes. A significant number of mutations were observed among both mothers and infants, particularly in the NNRTI and NRTI classes. The most common NNRTI mutation was K103N, detected in 15 mothers and their corresponding infants, often at high frequencies 15/34 (24%), suggesting sustained virological pressure and potential resistance to first-line NNRTI-based regimens such as Efavirenz and Nevirapine. Other recurrent NNRTI mutations included V108I**,** E138A and V179E observed in several mother-baby pairs, indicating a pattern of vertical transmission of NNRTI resistance representing (28%), (25%) and (5.6%) respectively. For NRTIs, the M184V mutation associated with high-level resistance to lamivudine (3TC) and emtricitabine (FTC) was commonly detected in both mothers and infants across multiple pairs, suggesting resistance

development to core NRTIs used in most ART regimens with (78%). The M41L mutation, linked to thymidine analogue mutations (TAMs) and conferring resistance to AZT and d4T, was also frequently noted with (45%).

Regarding PIs, the M46L with (18%) mutation was detected in a few pairs, suggesting moderate resistance to lopinavir and atazanavir, which could compromise the effectiveness of PI-based regimens. While most participants had no detectable INSTI resistance mutations, T97A (6%) was found in 3 mother-baby pairs, indicating emerging resistance to integrase inhibitors like Dolutegravir.

While Table 2 and Fig D; Shows HIV-1 Subtype analysis with a predominance of subtype A1 and its recombinants, especially among mothers with extensive DRM profiles. Subtype A1 or A1 recombinants (e.g., A1D, A1CD, 50_A1D) appeared in 11 out of 42 mothers –baby pairs, highlighting its circulation within this population. Other subtypes included D, B, 19_cpx, 25_cpx, URF_CD, and URF_08C, reflecting a genetically diverse viral population with multiple recombinant forms. The HIV-1 Subtype distribution revealed that A1 had high frequency 11 out of 42HIV-1 subtypes with 26% followed by subtypes B,01_AE, A, URF_08C(08_BC/C), URF_CD(C/D), A1D and 19_cpx representing 7% respectively, while subtype D, 25_cpx, 50_A1D, A1CD, URF_02G(02_AG/G) had (5%) respectively.

Importantly, in many cases, the infants' resistance patterns mirrored those of their mothers, particularly in the case of high-frequency mutations, indicating perinatal or in-utero transmission of resistant strains rather than de novo resistance development in the infant. This suggests that maternal resistance mutations may significantly influence infant treatment outcomes.

Discussion

Persistent Mother-to-Child Transmission despite ART Coverage

The study highlights a persistent occurrence of mother-to-child transmission (MTCT) of HIV-1, even among mothers on long-term antiretroviral therapy (ART). Despite ART durations ranging from 25 to 98 months and most mothers achieving viral suppression (≤1,000 RNA copies/ml), HIV transmission was observed in all 16 mother-infant pairs. These findings are consistent with previous studies from sub-Saharan Africa, which report residual MTCT among women on ART particularly when viral suppression is not sustained throughout pregnancy and breastfeeding[17, 18]. This persistent transmission underscores significant challenges in current prevention strategies for MTCT. The findings align with those of[19, 20], who similarly reported MTCT among women on ART, especially when viral suppression is intermittently lost. The complexity of vertical transmission is further amplified by contributing factors such as high maternal viral loads, advanced HIV disease, and mixed infant feeding practices, which collectively increase the risk of HIV transmission. These insights reveal critical gaps in prevention of mother-to-child transmission (PMTCT) strategies that must be addressed to improve maternal and child health outcomes.

Timing of Transmission: Antenatal and Postnatal Periods

The distribution of HIV transmission 2.55% in utero and 1.25% postpartum demonstrates that both the antenatal and postnatal periods are critical windows of vulnerability. In utero transmissions occurred despite relatively long durations of ART and were frequently associated with high baseline maternal viral loads ranging from 6,634 to 9,853 RNA copies/ml. These findings are consistent with existing literature showing that maternal viral loads above 1,000 copies/ml are strongly associated with increased MTCT risk [21-23]. Additionally, late ART initiation during pregnancy was observed among several mothers, a factor known to

significantly increase transmission risk. This corroborates findings by[21, 24], who emphasize the importance of initiating ART early ideally before conception. The evidence supports the need for regular viral load monitoring and timely ART initiation to mitigate the risks of virologic failure and delayed suppression. The observed cases suggest virologic failure or delayed suppression may have occurred due to late ART initiation during or shortly before pregnancy, reinforcing the necessity of sustained viral suppression throughout the antenatal period.

Postpartum Transmission and Breastfeeding Risks

Postpartum transmission was slightly more prevalent, echoing earlier studies that identify breastfeeding as a significant route of HIV transmission in resource-limited settings [25-27]. In such contexts, where breastfeeding is essential for babies' survival, transmission risk increases if maternal viral suppression is not consistently maintained. Contributing factors likely include suboptimal ART adherence during breastfeeding, inadequate infant prophylaxis, and potentially unsafe feeding practices.

The findings also raise concerns about the robustness of postpartum PMTCT support systems. Some mothers may have experienced viral rebound due to adherence lapses, while lack of follow-up or poor prophylaxis adherence in infants may have further increased transmission risk. These results underscore the need for strengthened postpartum follow-up systems, comprehensive infant HIV screening, and integrated maternal-child health services to minimize HIV transmission risks while supporting the nutritional needs of babies.

High Prevalence of Drug Resistance Mutations

The study revealed widespread resistance to first-line ART drugs in both mothers and babies. Particularly notable was the NNRTI mutation K103N, observed in 15/34(24%) of mother-baby pairs. This mutation confers resistance to Efavirenz and Nevirapine, drugs commonly used in PMTCT regimens. These findings are consistent with those of[28] and[29] . Other NNRTI mutations identified including V108I, E138A, and V179E further reflect extensive exposure and reduced efficacy of NNRTI-based therapies[29]. Additionally, the M184V mutation, which confers high-level resistance to Lamivudine and Emtricitabine, was detected alongside Thymidine Analogue Mutations (TAMs) such as M41L. These mutations indicate prolonged ART exposure and may be suggestive of prior treatment failure or poor adherence [28, 30]. The mirroring of resistance mutations between mothers and babies suggests the vertical transmission of resistant strains rather than mutation development post-infection an observation also reported in studies by[29, 31] and[14].

Resistance to Protease inhibitors (PIs) and Integrase strand transfer inhibitors (INSTIs) was rare, with only M46L and T97A mutations detected. This scarcity likely reflects limited use of these drug classes in pediatric and antenatal settings in the country. Nevertheless, even low-level emergence of resistance mutations underscores the importance of routine resistance testing where feasible, particularly for infants requiring immediate ART. These findings call for pretreatment drug resistance screening and a shift toward Protease inhibitor- and Integrase inhibitor-based regimens to enhance treatment efficacy for both mothers and babies.

HIV-1 Subtype Diversity and Its Implications

HIV-1 subtype analysis revealed a predominance of subtype A1, consistent with previous reports from East Africa. Additionally, a notable presence of recombinant forms such as A1D, A1CD, CRF19_cpx, and CRF25_cpx was observed, reflecting the region's viral genetic heterogeneity[32, 33]. This diversity results from both the high replication rate and error-prone nature of HIV-1 reverse transcriptase, as well as frequent recombination events noted in

Uganda and Tanzania [33-35].The presence of multiple viral variants and recombinants, such as CRF10_CD, underscores the dynamic nature of HIV-1 evolution. This genetic variability has important implications for ART response and vaccine development [33-36]. The concordance of subtypes within mother-baby pairs suggests direct vertical transmission and raises concerns about the transmissibility and mutability of certain subtypes[32]. These findings highlight the importance of molecular surveillance in understanding transmission dynamics, optimizing ART regimens, and informing regionally tailored vaccine strategies. Additionally, the naturally occurring HIV-1 variants polymorphisms confer resistance to Antiretroviral therapy (ART) leading to multiple drug resistance, overlapping resistance and cross resistance to number of HIV-1 drug classes. Polymorphisms at drug target site may affect drug susceptibility, where there is wide variability in the susceptibility of HIV-1 viral isolates to entry inhibitors; up to a 1000-fold difference in 50% inhibitory concentrations have been demonstrated. Studies of resistance profiles that emerge in non-B subtypes in patients receiving antiretroviral therapy indicates that polymorphisms present in these subtypes before therapy may provide a background for the emergence of subtype-specific pathways to secondary resistance. Continuous monitoring of subtype diversity, recombinant forms and drug resistance profile trends is essential for effective public health responses and intervention planning.

CONCLUSION AND RECOMMENDATIONS

Conclusion

In summary, the findings from this study highlight the complex interplay between ART coverage, drug resistance, and viral genetic diversity in the context of MTCT. While ART has significantly reduced HIV transmission rates, the persistence of both in-utero and post-partum transmissions, especially in the presence of drug-resistant strains, demands a more comprehensive approach, in case of high viral load (VL(s) without resistance, it emphasize the need to address the underlying issues (e.g., improving adherence, adjusting the regimen or managing interactions) can help achieve better Viral Suppression (VS) and improve health outcomes.

Recommendations to Policy Makers

Include early initiation of ART for women of reproductive age, routine HIV-1 drug resistance and subtype testing during pregnancy, enhanced postnatal follow-up, improving adherence, adjusting the regimen, or managing interactions) which can help achieve better VS and improve health outcomes and individualized treatment strategies for HIV-exposed babies.

Implications for Policy and Practice

Taken together, these findings have several important programmatic and policy implications. First, they underscore the critical need for early initiation of ART, ideally prior to conception and the consistent maintenance of viral suppression throughout pregnancy and the breastfeeding period. Ensuring early and sustained viral suppression is essential for reducing the risk of mother-to-child transmission.

Second, the results highlight the necessity of enhanced drug resistance and subtype monitoring for both mothers and babies, especially within ART-experienced populations. Regular drug resistance and subtype testing can inform more effective regimen choices and prevent the transmission of resistant viral strains.

Third, the findings call for the strengthening of postpartum follow-up systems. This includes reinforcing maternal adherence support, ensuring timely viral load testing, and guaranteeing

that babies receive appropriate prophylactic treatment and routine HIV testing during the breastfeeding period.

Finally, the observed HIV-1 subtype diversity including the emergence of complex recombinant forms should be considered in future PMTCT and treatment strategies. This diversity may influence treatment efficacy and underscores the need for regionally adapted approaches to ART regimen selection and vaccine development.

Limitations

Although our samples included clinics from all five provinces, the disproportionate recruitment from more populous provinces like Western and the Eastern may introduce geographic sampling bias. Future studies could employ longitudinal designs with biomarkers confirmation to more accurately capture adherence trajectories, while monitoring and surveillance of HIVDRMs and HIV-1 Subtypes.

Acknowledgements

Thank the research participants for consenting to participate in this research. We also acknowledge the selfless efforts of the staff of the Rwanda Biomedical Center (RBC) for their various contributions to the research in this manuscript.

Authors' Contribution

Bernard S. Bagaya, Gad Rutayisire, Noah Kiwanuka, Roman Saba Ntale, Emmanuel Semwaga, Fred Kyeyune, Enock Wekia, Nsereko Kalisa Vincent: Conceptualization, Methodology, Supervision, Data analysis, Manuscript writing and Review.

Gad Rutayisire, Uwera Marie Grace, Tumusime Musafiri, Innocent Ishami, Richard Mulondo: Data collection, Laboratory Assays, Review of Manuscript.

Funding Statement

We acknowledge the contribution of the Africa Center of Excellence in Materials, Product Development, and Nano Technology, MAPRONANO ACE, College of Engineering, Design, ART, and Technology, Makerere University, Supported by World Bank Project **ID/Number:(P151847 IDA number: 5797-UG)** for the support of this research work.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper

REFERENCES

1. Amone, A., et al., *Enhanced Peer-Group strategies to support prevention of Mother-to-Child HIV transmission leads to increased retention in care in Uganda: A Randomized controlled trial.* medRxiv, 2023: p. 2023.04. 15.23288495.
2. Mukose, A.D., *Implementation of lifelong ART (Option B+) for Prevention of Mother-to-Child Transmission (PMTCT) of HIV in Uganda*. 2024, University of Antwerp.
3. Ruel, T., et al., *Novel Approaches to Postnatal Prophylaxis to Eliminate Vertical Transmission of HIV.* Global Health: Science and Practice, 2023. 11(2).
4. van Schalkwyk, C., et al., *Updated data and methods for the 2023 UNAIDS HIV estimates.* JAIDS Journal of Acquired Immune Deficiency Syndromes, 2024. 95(1S): p. e1-e4.
5. Rwanda Biomedical Center, *Rwanda Population-based HIV Impact Assessment (RPHIA) 2018-2019: Final Report.* Kigali: RBC, 2020.
6. UNAIDS, *AIDSInfo: Global Data on HIV Epidemiology and Response.* 2020.
7. Rutayisire, G., et al., *In Utero Mother-to-Child Transmission of HIV-1 and the Associated Factors in Rwanda, Africa.* AIDS Research and Human Retroviruses, 2024.
8. Rwanda Biomedical Center, *Rwanda Population-based HIV Impact Assessment (RPHIA) 2018-2019*. 2020, RBC Kigali, Rwanda.
9. Deynu, M. and J.J. Nutor, *Determinants of comprehensive knowledge on mother-to-child transmission of HIV and its prevention among childbearing women in Rwanda: insights from the 2020 Rwandan Demographic and Health Survey.* BMC Public Health, 2023. 23(1): p. 5.
10. Nsanzimana, S., et al., *Household survey of HIV incidence in Rwanda: a national observational cohort study.* The Lancet HIV, 2017. 4(10): p. e457-e464.
11. Aliyu, G.G., et al., *Prevalence of HIV drug resistance in Nigeria: results from a cross-sectional, population-based survey of Nigerian adults with unsuppressed viral load.* AIDS, 2023. **37**(2): p. 333-339.
12. Bishop, M.D., et al., *Brief Report: Characterizing HIV Drug Resistance in Cases of Vertical Transmission in the VESTED Randomized Antiretroviral Treatment Trial.* JAIDS Journal of Acquired Immune Deficiency Syndromes, 2024. 96(4): p. 385-392.
13. Dambaya, B., et al., *HIV-1 drug resistance and genetic diversity among vertically infected Cameroonian children and adolescents.* Exploratory Research and Hypothesis in Medicine, 2020. **5**(2): p. 53-61.
14. Yan, L., et al., *Transmitted and acquired HIV-1 drug resistance from a family: a case study.* Infection and drug resistance, 2020: p. 3763-3770.
15. Dambaya, B., J. Fokam, and E. Ngoufack, *HIV-1 Drug Resistance and Genetic Diversity among Vertically Infected Cameroonian Children.* 2019.
16. Mann, C., *Observational research methods. Research design II: cohort, cross sectional, and case-control studies.* Emergency medicine journal, 2003. 20(1): p. 54-60.
17. Abuogi, L., et al., *Development and implementation of an interdisciplinary model for the management of breastfeeding in women with HIV in the United States: experience from the Children's Hospital Colorado Immunodeficiency Program.* Journal of Acquired Immune Deficiency Syndromes (1999), 2023.

18. Behrens, G.M., K. Aebi-Popp, and A. Babiker, *Close to zero, but not zero: What is an acceptable HIV transmission risk through breastfeeding?* JAIDS Journal of Acquired Immune Deficiency Syndromes, 2022. 89(4): p. e42.

19. Namara-Lugolobi, E., et al., *Twenty years of Prevention of Mother to Child HIV Transmission: research to implementation at a national referral hospital in Uganda.* African health sciences, 2022. 22(2): p. 22-33.

20. Teasdale, C.A., B.J. Marais, and E.J. Abrams, *HIV: prevention of mother-to-child transmission.* BMJ clinical evidence, 2011. 2011: p. 0909.

21. Duri, K., et al., *Mother-to-child transmission of HIV within 24 months after delivery in women initiating lifelong antiretroviral therapy Pre/Post-Conception or postnatally; Effects of adolescent girl and Young Woman Status and plasma viremia late in pregnancy.* Frontiers in Virology, 2022. 2: p. 906271.

22. Gill, M.M., et al., *Detectable viral load in late pregnancy among women in the Rwanda option B+ PMTCT program: enrollment results from the Kabeho study.* PloS one, 2016. 11(12): p. e0168671.

23. Mock, P.A., et al., *Maternal viral load and timing of mother-to-child HIV transmission, Bangkok, Thailand.* Aids, 1999. 13(3): p. 407-414.

24. Myer, L., *Initiating antiretroviral therapy in pregnancy: the importance of timing*. 2011, LWW. p. 125-126.

25. Davis, N.L., *Adherence-Related Mechanisms of Breastmilk HIV-1 Transmission.* 2014.

26. Davis, N.L., et al., *Maternal and breastmilk viral load: impacts of adherence on peripartum HIV infections averted—the Breastfeeding, Antiretrovirals, and Nutrition Study.* JAIDS Journal of Acquired Immune Deficiency Syndromes, 2016. **73**(5): p. 572-580.

27. Mofenson, L.M., *Prevention of breast milk transmission of HIV: the time is now*. 2009, LWW. p. 305-308.

28. da Silveira Gouvêa, M.I.F., et al., *Resistance rates among antiretroviral regimens in pregnant people living with HIV.* HIV medicine, 2023. 24(9): p. 1020-1025.

29. Bennett, S.J., et al., *High nonnucleoside reverse transcriptase inhibitor resistance levels in HIV-1-infected Zambian mother–infant pairs.* Aids, 2020. 34(12): p. 1833-1842.

30. Pillay, S., et al., *Drug resistance in children at virological failure in a rural KwaZulu-Natal, South Africa, cohort.* AIDS research and therapy, 2014. 11(1): p. 3.

31. Inzaule, S.C., et al., *High levels of resistance to nucleoside/nucleotide reverse transcriptase inhibitors in newly diagnosed antiretroviral treatment-naive children in sub-Saharan Africa.* AIDS, 2020. 34(10): p. 1567-1570.

32. Kiwelu, I.E., et al., *HIV-1 subtypes and recombinants in Northern Tanzania: distribution of viral quasispecies.* PLoS One, 2012. **7**(10): p. e47605.

33. Paraskevis, D. and A. Hatzakis, *Global molecular epidemiology of HIV-1: The chameleon challenge.* The Lancet Infectious Diseases, 2019. 19(2): p. 114-115.

34. Nyombi, B.M., et al., *Diversity of human immunodeficiency virus type 1 subtypes in Kagera and Kilimanjaro regions, Tanzania.* AIDS research and human retroviruses, 2008. 24(6): p. 761-769.

35. Shao, E., et al., *HIV-1 diversity in tanzania and its implication toward development of effective vaccines: a review article.* 2014.

36. Nsubuga-Nyombi, T., et al., *Increasing HIV-free survival of infants: reorganizing care using quality improvement for the optimal health and nutrition of HIV-positive women and their exposed infants in Uganda.* Journal of the International Association of Providers of AIDS Care (JIAPAC), 2019. 18: p. 2325958219857724.

License