



THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

National HIV Drug Resistance Prevention, Monitoring and Surveillance Activities, National Status Report

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REPUBLIC OF UGANDA

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Acronyms and Abbreviations

AAVP	African AIDS Vaccine Programme
ACP	AIDS Control Programme
AFENET	African Field Epidemiology Network
AIDS	Acquired Immune Deficiency Virus
ART	Antiretroviral Therapy
ARV	Antiretroviral
CRS	Catholic Relief Services
DART	Development of Antiretroviral Therapy
DBS	Dried Blood Spots
DR	Drug Resistance
DRM	Drug Resistance Mutation
EDCTP	European Developing Countries Clinical Trials Partnerships
EID	Early Infant Diagnosis
EVF	Early Virologic Failure
EWI	Early Warning Indicators
HIV	Human Immune Deficiency Virus
IAEA	International Atomic Energy Agency
IAVI	International AIDS Vaccine Initiative
IDI	Infectious Diseases Institute
JCRC	Joint Clinical Research Centre
MARPs	Most at Risk Populations
MJAP	Makerere Mbarara Joint AIDS Program
MoH	Ministry of Health
MRC	Medical Research Council
MSF	Medicines Sans Frontières
MSH	Management Sciences for Health
NDA	National Drugs Authority
NGOs	Non-Governmental Organizations
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitors
NRTI	Nucleoside Reverse Transcriptase Inhibitors
NUMAT	Northern Uganda Malaria, AIDS and Tuberculosis Program
PASER	PharmaAccess Studies to Evaluate Resistance
PEPFAR	Presidential Emergency Fund for AIDS Relief
PHAs	People Living with HIV/AIDS
PIDC	Pediatric Infectious Diseases Clinic
PMTCT	Prevention of Mother to Child Transmission
QA	Quality Assurance
QC	Quality Control
RNA	Ribonucleic Acid
RRH	Regional Referral Hospital
SDRM	Surveillance Drug Resistance Mutations
SURE	Securing Uganda's Rights to Essential Medicines
TASO	The AIDS Support Organization
TB	Tuberculosis
TDR	Transmitted Drug Resistance
TRM	Transmitted Resistance Mutations
TS	Threshold Survey
TWG	Technical Working Group
UGATM	Uganda Global Fund for AIDS, Tuberculosis and Malaria
UNAIDS	United Nations AIDS Programme
UVRI	Uganda Virus Research Institute
VCT	Voluntary Counselling and Testing
VS	Viral suppression
VLS	Viral load suppression
WHO	World Health Organization

Foreword by the Minister of Health

Since the early 2000's, the Ministry of Health (MOH) has progressively rolled out the public sector anti-retroviral treatment (ART) programme that provides free ART through accredited health facilities. The number of health facilities has progressively risen from 26 in 2003 to 1,803 by end of 2017 while the number of people on ART has increased from 20,000 in 2003 to over 1,000,000 by the end of 2017. In 2014, the MOH adopted the WHO/UNAIDS 2020 targets that aim at 90% of people with HIV knowing their status; 90% of people with HIV infection receiving antiretroviral therapy (ART) and 90% of people with HIV on ART achieving sustained viral suppression (VS). By the end of 2017, it was estimated that there were 1,324,685 people living with HIV in Uganda, of these 1,189,811 (90%) knew their HIV status, of those who knew their HIV status, 1,140,420 (96%) were on treatment and of those on treatment, 992,165 (87%) had viral suppression (VS). The figure for those suppressing however is lower than the WHO target. From these, it indicates that the country is moving towards meeting all these targets.

The MOH is well aware that a multi-pronged approach to prevention and access to care and treatment including meeting the above targets and sustaining them is not going to be easy. There will be challenges and sacrifices that we need to accept but we have no choice if we are to end the epidemic.

One of the serious threats to the effectiveness of the current ART programme that could compromise our efforts to meeting the targets especially the last 90 is HIV drug resistance. In 2007, we adopted the WHO strategy for the prevention, monitoring and surveillance of HIVDR, and came up with a five-year plan. With the support from different partners, I am pleased to see that this plan, which was extended for an additional 5 years has generated data that has contributed to improving of our ART programmes. For example, the early warning indicator (EWI) surveys have continued to monitor the programmatic factors required for prevention of HIVDR. Surveys of transmitted, pre-treatment and acquired drug resistance have given us an idea of the extent of the problem in different populations. Through this work, Uganda has been shown to be one of the countries where there is a serious pre-treatment HIVDR prevalence that necessitated changes to our first line regimens.

I am pleased to note that Uganda is one of the few countries that has been able to deliver on all the different aspects of the WHO HIVDR strategic plan as shown in the different WHO/UNAIDS reports.

I would like to thank all the institutions and individuals that have participated in these activities and have provided the necessary data in this report and other published work. I thank the different members of the Technical Working Group (TWG) who have coordinated these efforts. I thank the Uganda Virus Research Institute (UVRI), which has hosted the secretariat for these activities. We thank the national HIVDR reference laboratories at UVRI and Joint Clinical Research Centre (JCRC) that have provided the resistance data. As we look forward and start to revise the strategic plan for HIVDR prevention, monitoring and surveillance, the MOH pledges its continued support and will give priority to these activities. The activities will continue to be

part of, and integrated into our ART programme activities. We also endorse the WHO Global Action Plan on HIV DR, which describes activities that will be required to prevent HIVDR from undermining efforts to achieve the global targets on health and HIV.

Finally, I wish to thank all our development and collaborating partners for the support provided in the implementation of this Strategic Plan.

Dr Jane Ruth Aceng
MINISTER OF HEALTH

Executive summary

In 2007, Uganda adopted the WHO strategy for HIV DR prevention, monitoring and surveillance with the development of a country plan. Different stakeholders came together to harmonize priorities for gathering the necessary crucial information.

The main elements of our plan were aimed at promoting the use of standard ART regimens; supporting use of standardized individual treatment records; support for and active monitoring of adherence; quality assurance/control for drugs, including adequate and continuous drug supply; prevention programs to reduce HIV transmission for persons receiving ART; use of program monitoring for EWIs for HIVDR; tracking success by drug resistance lab-based surveillance for HIVDR transmission, pre-treatment DR and by monitoring HIVDR emergence in treated populations. A number of specific objectives were set in our plan in order to deliver the above aims.

In this report, we provide some of the results from these activities and recommendations made over the years at the various stakeholder meetings held. I wish to summarize some of the achievements.

A national secretariat for HIVDR was set up at the Uganda Virus Research Institute, with some designated personnel to coordinate some of these activities. A national HIVDR Technical Working Group (TWG) was constituted with individuals from different institutions and disciplines to guide, coordinate and share information. Some members of this TWG participate in various ART subcommittees and the National ART committee. UVRI and other institutions have participated in the raising of funds from different donors and agencies to implement these activities.

We have conducted five EWI surveys, whose results have greatly contributed towards the strengthening of ART programmes and ensuring that some of these EWI become part of the routine monitoring of programmes.

Nine different transmitted drug resistance (TDR) surveys have been conducted by different partners to provide information on the extent of transmitted resistance in the various populations and guiding on the appropriate response required.

Surveys for pre-treatment and acquired HIVDR have been conducted in adults and children, including one that has used nationally representative sampling to estimate pre-treatment and acquired DR. A key finding has been the very high prevalence of pre-treatment DR to NNRTIs in adults and both NNRTIs and NRTIs in children especially those exposed to PMTCT.

A national HIVDR reference laboratory was designated at UVRI, later obtaining WHO certification and the laboratory at JCRC was also certified. The two laboratories have recently

been assigned to perform genotyping for all second-line ART treatment failures in order to guide on third-line ART treatment. The laboratory teams have been very actively involved the WHO/HIV ResNet HIV laboratory activities.

The WHO HIV DR database was used in the different surveys; and this is being expanded to create a national data base for HIV genotypes beyond resistance genotypes.

This report provides some of the recommendations made at the different stakeholder meetings, some key ones include, inclusion of EWI in routine monitoring, introduction of viral loads in treatment monitoring, use of protease inhibitors in children as first-line and the introduction of dolutegravir as part of adult first-line regimen. We are happy to note that most recommendations made have been implemented.

Although not implemented through this plan, the report also provides some of the HIVDR prevention activities that have been conducted by the different stakeholders.

Uganda has been very active in providing relevant information to WHO/UNAIDS contributing to the Global HIVDR reports.

Other participation has been at International HIVDR resistance meetings where key results have been shared and discussed. Some of our results have contributed to global multi-country meta-analysis, and some of these analyses are included in this report.

I wish to thank all implementing partners, funders, the TWG, the secretariat at UVRI and the laboratories at UVRI, JCRC and CPHL for their contributions.

We are now well positioned to embark on the next five-year strategic plan, knowing that prevention of HIVDR will be crucial if we are to meet the 2020 WHO/UNAIDS 90-90-90 targets and ending of the epidemic by 2030.

THANK YOU

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Director, Uganda Virus Research Institute

Director, MRC/UVRI & LSHTM Uganda Research Unit

CHAIR, HIV DR TECHNICAL WORKING GROUP

Chapter One: Introduction

1.1 Overview

The purpose of this report is to summarize key HIV drug resistance prevention, monitoring and surveillance activities, which have been undertaken in Uganda under the auspices of the HIV Drug Resistance (HIVDR) Secretariat at the Uganda Virus Research Institute (UVRI) and associated partners or stakeholder institutions, for the period 2008 – 2018. The report also summarizes some of the published work from other related studies. This report is not comprehensive enough to provide all HIVDR activities by all other stakeholders, except for work that feeds into this national plan. The report also provides recommendations including those with policy implications. The report is structured in eleven chapters as shown below:

- Chapter one: Introduction
- Chapter two: Management structures for HIVDR
- Chapter three: Monitoring HIV drug resistance Early Warning Indicators
- Chapter four: Threshold surveys of transmitted HIV drug resistance
- Chapter five: Pre-treatment and Acquired drug resistance
- Chapter six: HIV drug resistance Genotyping Laboratories
- Chapter seven: The HIV drug resistance database
- Chapter eight: HIV drug resistance prevention activities
- Chapter nine: Experience with second line resistance
- Chapter ten: Discussions
- Chapter eleven: Recommendations
- Chapter twelve: Conclusion and way forward

1.2 Background

Combination antiretroviral therapy (ART) for HIV infection has saved millions of lives since its introduction and scale up. As coverage of ART continues to expand, some degree of emergence and transmission of HIVDR is inevitable. Significant population level HIVDR could potentially restrict future therapeutic options and increase treatment costs by requiring new and more expensive antiretroviral (ARV) regimens. HIVDR could also hinder progress towards the global goal of ending the epidemic by 2030 and the goal of meeting the 90-90-90 targets by 2020 especially the last 90. These targets envisage 90% of people with HIV, knowing their status, 90% of people diagnosed with HIV infection receiving ART, and 90% of people with HIV on ART achieving sustained viral load suppression (VLS).

However, as the experience of many countries demonstrates, HIVDR can be monitored and steps can be taken to minimize its emergence. In simple terms, HIVDR refers to the ability of HIV to replicate in the presence of drugs that usually suppress its replication. HIVDR is caused

by changes (mutations) in the virus's genetic structure. Mutations are very common in HIV because the virus replicates very rapidly and does not contain the proteins needed to correct the mistakes it makes during this process. Even before treatment is initiated, the high rate of HIV replication, combined with the high mutation rate that occurs during each cycle of replication, ensures that patients have a complex and diverse mixture of viral quasiespecies, each differing by one or more mutations. The emergence of these resistant viruses can occur in a matter of weeks in case of inadequate suppression. In addition, high levels of resistance can also occur with gradual accumulation of additional mutations [1]. Some degree of HIVDR is anticipated to occur among people receiving treatment even when appropriate regimes are provided and optimal adherence is achieved [2].

The WHO definitions of HIV drug resistance are [3]:

1. Acquired HIVDR (ADR) develops when HIV mutations emerge due to viral replication in individuals receiving ARV drugs.
2. Transmitted HIVDR (TDR) is detected in ARV drug naïve people with no history of ARV drug exposure. TDR occurs when previously uninfected individuals are infected with virus that has drug resistance mutations.
3. Pretreatment HIVDR (PDR) is detected in ARV drug naïve people initiating ART or people with prior ARV drug exposure(s) initiating or reinitiating first-line ART. PDR is either transmitted or acquired drug resistance, or both. PDR may have been transmitted at the time of infection (i.e. TDR), or it may be acquired by virtue of prior ARV drug exposure(s), such as in women exposed to ARV drugs for the prevention of mother-to-child transmission (PMTCT) of HIV, or in people who have received pre-exposure prophylaxis (PrEP), or in individuals re-initiating first-line ART after a period of treatment interruption without documented virological failure.

ARV drug naïve: This term is applied to people with no history of ARV drug exposure(s).

Reducing the high mortality rate associated with HIV/AIDS requires provision of quality HIV early diagnosis, care and treatment, and follow-up coupled with appropriate measures for preventing the unnecessary emergence of drug resistant HIV in individuals receiving therapy and strategies to minimize transmission of HIVDR in communities. Measures to minimize the emergence of HIVDR require high quality care which includes: greater access to diagnosis, care and treatment, support for adherence to ART, strengthening of ARV procurement and supply chain management and establishment of national and regional HIVDR strategies. National strategies for monitoring and prevention of HIVDR include the collection of strategic information using standardized methodologies to provide data to national ART programmes for use in evidence based decision making to minimize the emergence of HIVDR. These measures are necessary to maximize the long-term efficacy and durability of available antiretroviral regimens [4].

Although ART scale-up measures designed to achieve universal access include many aspects which will minimize HIVDR, the World Health Organization (WHO) recommends that a conscious HIVDR prevention strategy be fully integrated into all national and regional ART scale-up initiatives [5]. About ten years ago, the Ministry of Health (MoH) with support from the WHO developed a national plan for HIVDR prevention, monitoring and surveillance through an extensive consultative process that involved the several stakeholders in the country. The plan provided a common reference point of priorities for the different stakeholders and partners in Uganda, to implement the HIVDR activities as ARVs are rolled out countrywide. The overarching goal of the national HIVDR prevention, monitoring and surveillance plan is to minimize preventable emergence of HIVDR, and to restrict the extent to which ARV resistance jeopardizes the effectiveness of the limited ART regimens available, within the context of the national HIV prevention and treatment plan. The specific objectives of the national plan as agreed upon by the different stakeholders in 2007 are shown below:

- a) To develop and support capacity for HIVDR prevention, monitoring and surveillance activities at both the national and institutional level, particularly for programmes providing access to ARVs.
- b) To develop a list of early warning indicators (EWIs) that will be regularly evaluated from all potential sites; to monitor whether ART programs are functioning to optimize prevention of HIVDR.
- c) To support and coordinate surveillance of HIVDR transmission in different geographical settings using Threshold Surveys (TS)
- d) To support and coordinate the monitoring of HIVDR arising in adult and pediatric populations starting and continuing ART.
- e) Accredited and support in country genotyping laboratories with adequate capacity to support HIVDR surveillance and monitoring activities in the country.
- f) Develop and maintain an HIVDR database.
- g) Disseminate key program findings and results for evidence based HIVDR containment strategies.



Figure 1 National HIV Drug Resistance Prevention Monitoring and Surveillance Workshop, 23rd -24th January 2007, Kampala, Uganda

1.3 Current Status of HIV in Uganda

Uganda is among the countries in the African region that have been severely affected by the HIV epidemic for over three decades. According to the recent Uganda Population-Based HIV Impact Assessment (UPHIA), the HIV prevalence among adults aged 15-49 years was 6.0%; among females 15-64 years it was 7.6% while among males it was 4.7% [6].

Since 2014, Uganda has implemented the Test and Treat policy for all HIV-infected children, pregnant and breastfeeding women, HIV and TB, Hepatitis B co-infected people, the HIV-infected partner in a sero-discordant relationship and HIV-infected individuals among key populations. In 2016, the Test and Treat policy was expanded to include all people living with HIV irrespective of CD4 count or clinical stage. The 2016 guidelines further recommended that pre-exposure prophylaxis be given to HIV-negative individuals at high risk of acquiring HIV [7].

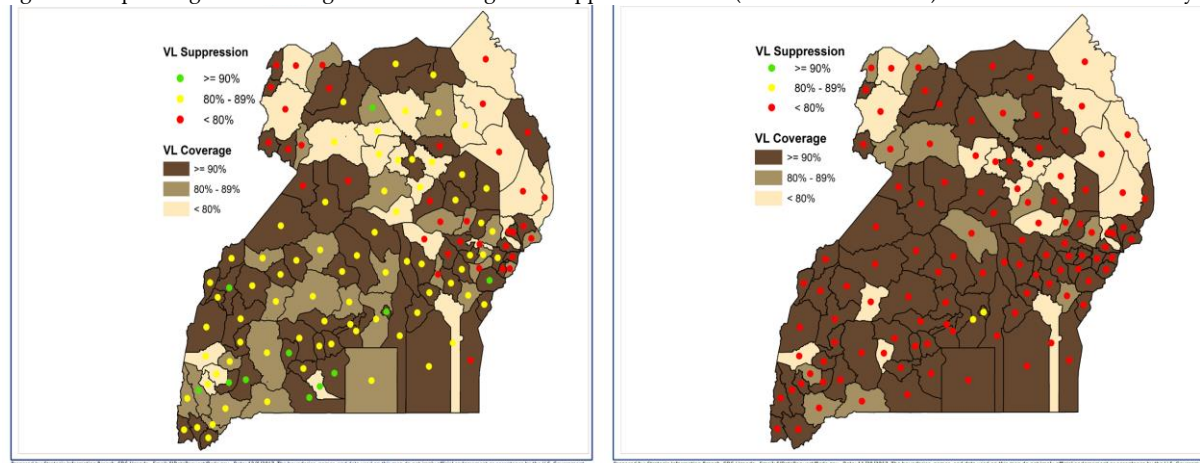
By June 2018, it was estimated that there were 1,324,685 people living with HIV, 1,189,811 (90%) knew their HIV status, of those who knew their HIV status, 1,140,420 (96%) were on treatment and of those on treatment, 992,165 (87%) had viral suppression (VS) [8]. Since adherence is key for a successful ART program, the national treatment guidelines do provide guidance on how to measure and improve on adherence. They provide for how to prepare patients for ART, monitor and support them to adhere to ART.

Viral load (VL) is one of the measures for adherence and confirming treatment response. All HIV-infected patients are currently required to receive a viral load test 6 months after initiating treatment. For adults, another VL is performed at 12 months and thereafter, annually. For

children and adolescents under 19 years of age, VL is performed every six months. There are also guidelines for pregnant women. Following an initial high VL (>1000 copies/mL), enhanced/intensive adherence counseling should be carried out before conducting a second VL test.

The VL coverage of all PLHIV increased from 47% in September 2016 to 75% by end of September 2017. However, while coverage had improved and overall viral suppression (VS) was 87%, it was 69% for children and adolescents.

Figure 2: Maps of Uganda showing 2017 VL coverage and suppression for all (adults and Children) on ART and children <15 years



From Fig 2 (Program data from CPHL2017) it is noted that the West Nile, North Eastern and Eastern regions of the country have suppression rates less than 80%. In addition, children <15 years generally have low suppression rates (Fig 2). The low suppression rates among children could be due to infant HIVDR coupled with suboptimal regimens, adherence challenges and inadequate psychosocial support. It is also anticipated that there are cross border mobile populations who may be having ART adherence challenges. More work needs to be done in these regions to ascertain the actual reason for the noted observations.

The recent UPHIA survey [6] looking at viral suppression among HIV-positive people by age and sex indicated that the prevalence of VLS among HIV-positive people in Uganda was highest among older adults: 80.3% among HIV-positive females aged 55 to 64 and 70.2% among HIV positive males aged 45-54. In contrast, the prevalence of VLS was lower among younger adults: 44.9% among HIV-positive females and 32.5% among HIV positive males aged 15-24 years.

Resistance testing is not recommended for initiation of treatment and for switching to second line. The 2016 and 2018 Uganda HIV consolidated guidelines provide for HIV resistance testing for clients failing on second line who have two consecutive viral loads >1000 copies/ml after three sessions of intensive adherence counselling each one month apart.

The following are some of the challenges to the sustained roll out of ART services in the country:

- a) Limited health infrastructure with inadequate human resources including numbers and skills for ART service delivery especially for children
- b) Ensuring constant supply of commodities especially ARVs, HIV test kits, cotrimoxazole and other drugs for treating opportunistic infections
- c) Sustainability of programs that are currently heavily supported by donors and other global health initiatives. The numbers of individuals that are eligible for treatment as well as the attendant costs are high, yet we are operating in a resource constrained environment.
- d) Poor coordination of the multiple programmes implemented by various implementing partners with different modes of service delivery
- e) Uptake and coverage for early Infant HIV diagnosis is still low, but this is improving
- f) Inadequate district funding to support training in HIV care and ART to address the high levels of staff attrition
- g) Supporting and monitoring adherence to treatment is highly subjective to attending clinician as self-report is widely used in Uganda. Limited community ART literacy, education and mobilization
- h) Limited child- and adolescent-friendly services especially reproductive health issues for adolescents and family centered care

Some of the important lessons learned from the ART programme implementation in Uganda include the following:

- Informal task-shifting is being practiced in a number of facilities and this has freed up the professional health workers to attend to roles that are more critical. People living with HIV/AIDS (PHAs) (expert clients) are involved in sorting out of files and pre-packaging cotrimoxazole allowing the clinical staff to concentrate on technical activities.
- Redistribution of resources from facilities and other implementing partners that have large stocks to those that have stocked out.
- Triage systems have been introduced in many sites thereby improving the efficiency of clinics. Nurse and drug pick up visits have been introduced allowing clinical staff to attend to patients with medical problems only.
- Integration of Family planning, PMTCT, TB and ART services has been implemented by many sites allowing patients to be served better.
- Involvement of District Health Teams in ART services has improved coordination and reporting.
- Differentiated services delivery model implementation improves adherence, retention and viral suppression.

1.4 Summary of national guidelines for standard first-line and second-line ART

The public health approach to ART in Uganda recommends standard first-line and second-line ART regimens comprising of triple ARV therapy. The table below extracted from the treatment guidelines shows the current regimens including those recommended to switch to. Recently, we reported high levels of pre-treatment resistance to NNRTI-containing combinations in Uganda [8] estimated at 15.9%, and exceeding the threshold of 10.0% set by WHO for first-line ARVs. This has led to the introduction of dolutegravir as part of first line regimen.

Table 1: Recommended ART regimens

FIRST-LINE ARV REGIMENS			
PATIENT CATEGORY		PREFERRED REGIMEN	ALTERNATIVE REGIMEN
1. Adults and adolescents aged ≥10 years and ≥35 kg			
1.1 Adult men and adolescent boys	TDF+3TC+DTG	If DTG is contraindicated: TDF+3TC+EFV	
1.2 Adult women and adolescent girls on effective contraception		If TDF is contraindicated: ABC+3TC+DTG	
1.3 Adult women and adolescent girls not of childbearing potential			
1.4 Adult women and adolescent girls of child bearing potential who are pregnant, intend to get pregnant or not on effective contraception	TDF+3TC+EFV	If EFV is contraindicated: TDF+3TC+ATVr If TDF is contraindicated: ABC+3TC+EFV	
2. Children aged 0 to <10 years and <35 kg			
2.1 Children <3 months	ABC+3TC+LPV/r(Syrup)	ABC+3TC+RAL	
2.2 Children ≥ 3 months to < 3 years	ABC+3TC+LPV/r(pellets)	ABC+3TC+RAL	
2.3 Children ≥ 3 years to < 10 years	ABC+3TC+LPV/r(tablets)	ABC+3TC+DTG or ABC+3TC+RAL	
2 ND AND 3 RD LINE ART REGIMENS			
POPULATION	FAILING 1 ST LINE REGIMEN	2 ND LINE REGIMEN	3 RD LINE REGIMEN
Adults, pregnant/breastfeeding women and adolescents	TDF+3TC+EFV	AZT+3TC+ATV/r (recommended) AZT+3TC+LPV/r (alternative) AZT+3TC+DTG (alternative)	All 3 rd line regimen to be guided by resistance testing.
	TDF+3TC+DTG		
	ABC+3TC+DTG		
	ABC+3TC+EFV		
	ABC/3TC/NPV		
	TDF/3TC/NPV		

	AZT/3TC/NPV AZT/3TC/EFV	TDF+3TC+ATV/r (recommended) TDF+3TC+LPV/r (alternative) TDF+3TC+DTG (alternative)	
	TDF/3TC/ATVr	AZT/3TC/DTG	
Children 3 to <10 years	ABC/3TC/LPV/r	AZT+3TC+DTG AZT+3TC+RAL	or
	ABC+3TC+EFV	AZT+3TC+DTG AZT+3TC+LPV/r	
	ABC+3TC+NVP		
	AZT+3TC+NVP	ABC+3TC+LPV/r+ RAL	
	AZT/3TC/EFV		
	AZT+3TC+LPV/r	ABC+3TC+DRV/r+ RAL	
Children under 3 years	ABC+3TC+LPV/r(Pellets)	AZT+3TC+RAL	
	AZT+3TC+LPV/r(Pellets)	ABC+3TC+DRV/r+ RAL	
	AZT+3TC+NVP	ABC+3TC+LPV/r+ RAL	

Chapter Two: Management Structures and funding for HIVDR activities in Uganda

2.1 National Secretariat for HIVDR activities

This was established at the Uganda Virus Research Institute with staff who have supported the coordination of the HIVDR prevention, monitoring and surveillance plan at national and institutional level. The secretariat works closely with the AIDS Control Programme (ACP) of the Ministry of Health and key HIV partners, treatment centers, other research institutions in the country and with the Technical Working Group (TWG). The key staff at the secretariat are comprised of the Project Coordinator, Laboratory Director, Scientists, Technicians and a Data Manager/Analyst. Part of the staffing reflects the other roles at UVRI in hosting related laboratory activities. Staffing levels are also dictated by the resource envelope since more personnel are required for efficient and timely implementation of activities.

2.2 National HIVDR Technical Working Group

This is a multi-disciplinary and multi stakeholder national TWG on HIVDR. It is one of the sub-committees of the National ART committee with the overall mandate of guiding implementation of the HIVDR strategy. The membership is from different institutions such as the MOH e.g ACP, CPHL and UVRI, Development partners/ International Organizations such as WHO and CDC Treatment Programmes/ Non-governmental organizations such as, Mildmay, TASO, MJAP and MSF. Research Institutions such as UVRI, MRC/UVRI, JCRC, Rakai Health Sciences and Regulatory authorities such as the National Drug Authority (NDA). It is also multidisciplinary to include epidemiologists, clinicians, ART monitoring officers, virologists, laboratory specialists, data management specialists, researchers and regulatory officers among others. Over this period membership has changed with new partners joining and others leaving (see list page 63-64).

The terms of reference of the National HIVDR TWG, as prescribed by the Director General of Health Services, are as follows:

- a) To coordinate implementation of the national HIVDR prevention, surveillance and monitoring plan.
- b) To collect and analyze HIVDR Early Warning Indicators.
- c) To develop and coordinate the implementation of the country protocol for monitoring HIVDR in representative sentinel ART sites.
- d) To regularly perform HIVDR threshold surveys to evaluate transmitted resistance in specific geographic areas.
- e) To continue capacity building for genotyping and other activities to support HIV drug resistance surveillance and monitoring with in the country.

- f) To provide to other countries an example of implementation of the national HIVDR strategy, including elements recommended by WHO.
- g) To develop and collect information on activities and programs which will contribute public health and research activities in the country.
- h) To ensure all activities follow country and international ethical standards designed to ensure the well-being and health of individuals and communities
- i) To prepare and disseminate annual HIVDR reports and recommendations.

The HIVDR TWG conducted several meetings that guided implementation of the activities of the national plan including organizing national workshops and drafting policy documents.

2.3 Funding for HIVDR activities

The largest funder of the national plan activities has been through the CDC-UVRI cooperative agreement (Co Ag). Since October 2009, about 2 million USD have been awarded for these activities through this agreement. The other major funders have been MRC-UVRI supported by MRC-UK, which has supported the genotyping laboratory through staffing and facilities. The Pharma Access (PASER) funded related activities, especially studies in children, TDR, pre-treatment and laboratory support to JCRC. In addition, the Case Western Reserve University Centers for AIDS Research (CFAR) has provided support staff and purchased equipment for the HIVDR laboratory at JCRC. PEPFAR through USAID and now CDC has also supported the resistance work at JCRC from 2015. The other sources of funding for HIVDR activities in Uganda included the following:

- In 2006 and 2008 Gates Foundation through WHO provided 67,000 and 60,000 USD respectively for HIVDR activities (national plan development, sensitization workshops, early warning indicator surveys and laboratory supplies).
- In 2006, WHO/AAVP/IAEA provided funds for TDR survey among ANC attendees in Entebbe).
- MRC-UK contributed to support the genotyping laboratory including purchase of one Beckman Coulter and two ABI sequencers 3030 and 3031).
- ABI sequencer at JCRC by CFAR
- Global Fund provided funds in 2008 to purchase a Beckman sequencer, DNA extractor and some small equipments. An additional 446,500 USD have been approved for the years 2018 and 2019.
- In 2009, The European Drug Clinical Trials Partnership (EDCTP) provided a sequencer (ABI sequencer 3030).
- In 2010, The PharmaAccess African Studies to Evaluate Resistance (PASER) program contributed 62,080 Euros for TDR surveillance in Kampala.
- MRC-UK has provided funds for laboratory certification, staff, dried blood spots and monitoring studies.

- WHO and CDC also supported a study to determine the best way to store and use DBS for HIVDR.
- Since 2009, most of the activities as mentioned above have been supported through the CDC/PEPFAR.
- Other partners such as PASER, MRC, EDCTP, IAVI, IDI, NIH and Rakai Health Sciences supported relevant studies.

Chapter Three: Monitoring HIV drug resistance Early Warning Indicators

The Early Warning Indicators (EWIs) for HIVDR are factors (facility practices and client behaviours) that are associated with a high likelihood of emergence and transmission of ARV resistance. The monitoring process involved tracking of EWIs during five survey rounds at a sample of ART sites and information was obtained which was used to take action for optimizing ART programme performance for HIVDR prevention.

3.1 List of EWIs assessed

The Uganda MoH ACP and the HIVDR TWG conducted an assessment of the following WHO recommended set of EWIs in 2007, 2008/2009, 2012, 2014 and 2017:

- a) *ARV drug prescribing practices*: The proportion of patients who are prescribed a standard regimen at the start of ART (suggested target: 100%). It has been established that incorrect ARV prescription can lead to the emergence of drug-resistant HIV strains.
- b) *Percentage of patients lost to follow-up*: The proportion of patients lost to follow up during the first 12 months after ART initiation (suggested target: <20%). Patients are termed 'lost to follow up' based on persistent failure to attend clinic appointments for at least 90 days since the last missed clinic appointment. The causes of patients lost to follow up include unreported death, unreported transfer to another centre and discontinuation of ART.
- c) *Patient retention on first-line ART*: The proportion of patients starting an appropriate first-line ARV regimen who are still on first-line treatment 12 months later (suggested target: >70%). Due to the high cost and limited availability of second-line drugs, prolonging the clinical efficacy of first-line ARV regimens in resource-limited settings is critical.
- d) *ARV drug pickup*: The proportion of patients picking up all prescribed drugs on time i.e. before the previously prescribed and dispensed drugs have run out (suggested target: 90%). On-time ARV drug pickup is one of the measures of ART adherence. Appointment keeping and drug pick up has been shown to be associated with medication adherence(22). Also lack of on-time ARV drug pick-up has been shown to be linked to viral failure and HIV drug resistance (23-27).
- e) *ART Clinical Review Appointment Keeping*: The proportion of patients starting ART in a defined period, who attended all appointments (including drug pick up and clinical review) during the year (suggested target: 80%). Appointment keeping is another measure of adherence and is considered within 7 days of a scheduled appointment.

- f) *Drug supply continuity*: The proportion of months in a year in which there were no drug stock outs (suggested target: 100%). Continuity of ARV supplies is vital to ensure uninterrupted adherence. Data are abstracted for each ARV drug in regular use at the site (not ARV drugs being used for clinical trials or for very few patients). Irregular drug supplies often result in sub-therapeutic drug levels in patients due to treatment interruptions, or switching of medication, both of which can lead to the emergence of HIVDR. Many countries in sub-Saharan Africa have experienced significant bottlenecks with the supply of drugs.
- g) *Viral Load Suppression*: In 2010 WHO revised the guidelines for EWI by identifying 5 key EWI which were used in the 2017 survey discussed later (*Page 23*) including viral load suppression.

3.2 Study designs for the 2007, 2008/9, 2012 and 2014 surveys.

Cross-sectional surveys were done which involved abstraction of retrospective client data from clinical records to ascertain the HIVDR-EWIs. Both paper-based and electronic clinical records of all or a sample of clients that initiated treatment during a defined twelve months' period were eligible. For indicators of loss to follow-up, drug pick up, clinical appointment keeping and drug continuity supply retrospective longitudinal data was collected from facility held clinical and pharmacy records of this treatment group (cohort) of patients that had been on treatment for at least one year. For prescribing practices at ART start and retention on first-line regimen cross-sectional data was collected for the same treatment group (cohort) of ART patients.

Study Design for the 2017 survey

The 2017 EWI survey was implemented following the revised WHO guidance. The following indicators were collected:

- a) On-time ARV Drug Pick-up: percentage of patients (adults) that pick-up ART no more than two days late at the first pick-up after the baseline pick-up.
- b) Retention in Care: Percentage of adults and children known to be alive and on treatment 12 months after initiation of ART.
- c) Pharmacy Stock-Outs: Percentage of months in a designated year in which there were no ARV drug stock-outs.
- d) Dispensing Practices: Percentage of adults and children prescribed or picking up mono or dual ARV therapy.
- e) Virological suppression: Viral load suppression; Percentage of patients with viral load <1000 copies/mL 12 months after ART initiation

- f) Viral load coverage: Percentage of patients with a 12-month viral load test result available

The EWIs are divided into cross sectional indicators, (On-time pill pick up and dispensing practices) and indicators based on a 12-month reporting period (Retention, Stock-outs and Virological suppression).

The timeline for sampling of patients was based on a 12 months reporting period required for retention, viral load suppression and drug stock-out indicator. Individuals initiating ART in the period 1 July 2015 – 30 June 2016, constituted outcomes for retention and virological suppression 12 months after ART initiation. Data for pharmacy stock-outs was obtained for the period 1 July 2016 – 30 June 2017.

Assessment of on time pill pick-out and prescribing practices occurred over a period of time that allowed a site to cross-sectionally abstract data on a baseline ART pick-up and 1 subsequent pick-up. This constituted the period 1 July 2016 – 30 June 2017. The EWI sampling started with the first patient picking up ART at the pharmacy. Data was subsequently abstracted on consecutive patients until the required sample size is reached.

Data was abstracted from a random sample of 305 ART service outlets that are nationally and geographically representative of the country to obtain data on these ART programmatic factors and client behavior.

3.3 Sampling of health facilities and client records

Different survey samples were assessed during the 5four rounds of data collections (41 health facilities in 2007, 75 health facilities in 2008/2009, 95 health facilities in 2012,96 health facilities in 2014 and 305 in 2017. The ART sites were chosen randomly from all regions of the country and type of facilities.

The sampling frames comprised of health facilities which had been providing ART for at least one year.

Field teams obtained lists of clients at each facility, and consecutively selected records of clients until the required sample was attained. The following categories of patients were excluded from the assessment: i) patients with prior ART, ii) patients transferring in from other ART facilities and iii) Patients with crucial variables missing e.g. patient ART number and ART start date; however, the number and therefore the proportion of such client's records was recorded to facilitate assessment of quality record keeping in the analysis.

3.4 Data sources

During the survey rounds, the variables that were used to obtain the numerators and denominators for the various EWIs at site level are summarized in table 2 below:

Table 2. Sources of data for the various variables that were used to calculate the EWIs			
EWI	Numerator/Denominator	Sources of data	Variables/ fields extracted
Prescribing Practices	<u>Numerator:</u> Number of individuals initiating first-line who are prescribed the correct first line regimen during the study period <u>Denominator:</u> Number of individuals starting ART during the same period	ART register or database, HIV care/ ART card	ART start date, date of first ARV drug pick up, first line ARV combination <u>prescribed/picked</u> ART start date
Patient loss to follow up	<u>Numerator:</u> Number of individuals starting ART during the study period who are subsequently classified as lost to follow up during the first 12 months of ART <u>Denominator:</u> Individuals starting ART during the same period	ART register or database, HIV care/ ART card	Follow up status at 12 months
Patient retention on first-line ART	<u>Numerator:</u> Number of individuals starting ART during the study period who are still on first line ART 12 months later <u>Denominator:</u> Individuals starting ART during the same period	ART register or database, HIV care/ ART card	ARV combination prescribed on or just before completion of 12 months of ART start, duration of ARVs
On-time ARV pick-up	<u>Numerator:</u> Number of patients picking up all prescribed drugs on time i.e. before the previously prescribed and dispensed drugs have run out <u>Denominator:</u> Individuals starting ART during the same period	ART register or database, Pharmacy stock cards, dispensing logs	Dates recorded of patients ARV drug pick up, the number of days of ARVs drugs picked up, No. of pills picked
ART appointment keeping	<u>Numerator:</u> Number of individuals who kept all appointments in the first year of ART until the time they were classified as lost to follow up, dead, transferred out or stopped ART <u>Denominator:</u> Individuals starting ART during the same period	HIV care/ ART card or database	Dates recorded of patients appointments and actual visits over the 12 months period or till censured in the event of death, transfer out, loss to follow up
Adherence	<u>Numerator:</u> Number of individuals demonstrating 95% ARVs have been taken during the first 12 months of ART <u>Denominator:</u> Individuals starting ART during the same period	HIV care/ ART card or database	% adherence to treatment (and where available method of assessment)
Drug supply continuity	<u>Numerator:</u> Number of months in the year in which there were no ARV stock outs for any ARV in any of the standard ART regimens supplied by the site <u>Denominator:</u> 12	Stock cards	ARV drugs, number of days with drug stock outs per month
Viral load suppression	<u>Numerator:</u> Number of patients with viral load <1000 copies/mL 12 months after ART initiation <u>Denominator:</u> Number of patients alive and on ART 12 months after treatment initiation who have a viral load test result available.	Patient files, ART cards, result slips and viral load registers or database	ART start date, date of viral load, viral load result
Viral load coverage	<u>Numerator:</u> Number of patients with a 12-month viral load test result available <u>Denominator:</u> Number of patients alive and on ART 12 months after treatment initiation, who are therefore, consistent with the policy, expected to have a viral load test result available in the primary medical record.	Patient files, ART cards, result slips and viral load registers or database	ART start date, date of viral load, viral load result

3.5 Data abstraction process

To assess prescribing practices, data for clients recruited during a specified period was abstracted. For indicators requiring retrospective longitudinal data, 12 months follow-up data for all clients initiating treatment during the specified reference period was abstracted. Data was censored at the end of 12 months, or at the time of occurrence of events such as transfer out, death or loss to follow-up during the 12 months' period.

At the facility, the team together with the facility staff appointed to assist them, identified the sampling frame and produced a list of patients that started ART during a specified period. The number of clients who transferred into the facility during the selected period were also tallied and recorded on the data collection forms. Records for eligible clients at start of ART and follow up and other forms and electronic registries were subsequently used to abstract data. Patient data was entered into the standard WHO HIVResNet excel-based tool.

Facility characteristics were abstracted using the WHO site profile data collection tool. Variables that were collected included, facility level, geographical location, number of patients on ART, number and cadre of ART care givers, ARVs dispensed and dispensing locations, clinic waiting time, days clinic is open and number of hours the clinic is open, average distance travelled to the clinic by the patients.

3.6 Data validation process

Following the data abstraction by field teams, a separate centrally constituted team conducted field visits to validate the data in a subset of facilities. Facilities with incoherent data were selected for validation after descriptive summaries had been produced. During the validation, facility records for specific patients were reviewed. If there were more than 30 records, the validation team randomly selected 30 records. If the health facility had less than 30 records, all the records were reviewed again. The results obtained by the validation team were compared with those obtained during actual field data collection and it was indeed found that the data collected were valid in most cases. The discrepancies found were arising from poor quality data at these facilities. Missing data was usually due to misinterpretation of what was required, the health workers had reported that the data was not available. In such cases, data was re-abstracted by either the validation team or the team that had originally collected the data.

3.7 Data management and analysis

Patient-specific data from the WHO HIVResNet excel-based files was exported to Stata and merged into a single dataset containing patient-level data. Facility level data was entered into Ms Access databases and the excel files were exported into Stata files and merged with patient-level data before analysis. Patient level performance for each EWI was assessed against pre-determined targets. For facility level analysis, that is, the percentage of patients who met a particular indicator was determined and national level performance for each EWI was

determined by the proportion of clinics that met the WHO criteria for each indicator. ARV supply continuity was analysed separately to generate the percentage of months in a year in which there were no ARV drug stock-outs at each facility. All statistical analyses were performed using STATA version 12 (StataCorp LP, Texas, USA). Chi-square tests were used to determine associations between facility characteristics and EWIs.

3.8 Ethical Consideration

For all survey rounds, protocols were developed, discussed with stakeholders and received ethical approval/non-research determination from the UVRI Research and Ethics Committee, CDC Associate Director for Science and Technology and were registered with the Uganda National Council of Science and Technology (UNCST).

For all the survey rounds, there was no primary data collection from patients and there was no direct contact between data collection teams and ART patients. General consent was requested from the head of each facility before data collection. Confidentiality and anonymity of all data was observed during the assessments. All field workers received training in data confidentiality and signed a data confidentiality agreement. Only ART clinic data routinely recorded was used for the assessments. Patient names were not recorded on the data collection forms. The only personal identifier collected were the patients' ART number, which is necessary to link patient data across different forms and files within a facility. These ID numbers were removed after information from different data sets, registries, or files were entered. The HIVDR-EWI databases were located at UVRI, protected by a password and only study investigators had access to the data for the different survey rounds. All data extraction forms used during the assessments were locked in a file cabinet and subsequently stored in secure cabinets after data entry.

3.9 Summary results and conclusions from the 2006, 2008, 2012 and 2014 survey rounds

Figure 3 shows the percentage of ART sites which met the targets for the EWIs during the four survey rounds.

- a) There was an improvement in the percentage of ART sites that met the target for appropriate prescribing practices in the first three survey rounds, with a reduction of about 5 percentage points in the survey round done in 2014.
- b) There was a reduction in the percentage of ART sites which met the target for patient loss to follow up (loss of less than 20%) during the first 12 months, for the first three survey rounds. No data was collected on loss to follow up during 2014.
- c) There was steady increase in the percentage of ART sites which the target for retention on first-line ART in the first 12 months, from 71% in 2007 to 100% in 2014.
- d) Less than 10% of the sampled ART sites met the target for on time ARV drug pick up during the survey rounds done in 2012 and 2014. No data was collected for this indicator in 2007.

- e) Less than 15% of the sites met the target for ART clinical appointment keeping in the first three survey rounds. No data was collected in 2014.
- f) Less than 50% of the sites met the target for ARV drug supply continuity during all the four survey rounds.

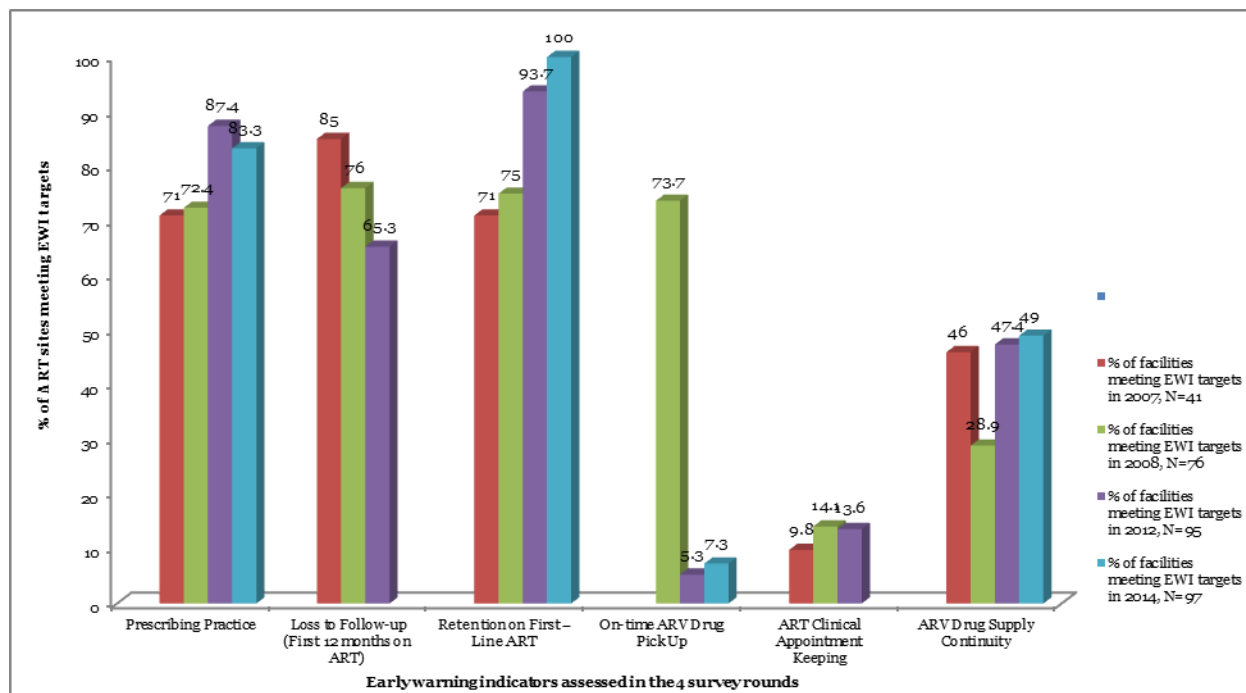


Figure 3: Percentage of ART sites which met the targets for the EWIs during the four survey rounds.

- g) There was heterogeneity in facility and client practices regarding the risk of HIVDR in Uganda for all the four survey rounds. For instance, during 2008/2009, nearly one quarter of facilities had not prescribed standard first-line regimen to all their new clients and; nearly one quarter of facilities had lost more than 20% of their clients during the first year on treatment. In addition, nearly one quarter of facilities had not retained at least 70% of their new clients on first-line ART during the first 12 months, and approximately three quarters of facilities had experienced ARV stock outs of varying duration during a period of 12 months. Furthermore, among approximately one-eighth of facilities, at least 80% of clients kept all their clinical review appointments during the first 12 months of treatment.
- h) The aspects of ART that had the most weaknesses were ARV drug supply continuity, on-time ARV drug pick up and clinical review appointment keeping even among new clients
- i) Additionally, private clinics had high rates of loss-to-follow up, poor retention on first line and clients least likely to keep all appointments. Facilities in the northern region had high loss-to-follow-up poor retention on first line and interruption of ARV supplies. Well-resourced facilities especially those with significant donor support performed better on all indicators.

Our surveys of 2006, 2007 and 2012 contributed to the Global report [10]. In this Global report, overall, no regions globally reached the target. The worst performance was on time pill pick up,

retention, drug stock outs and LTFU, with the lowest overall performance for all indicators in Western Africa.

3.10 Results for the assessment of EWIs for HIVDR, 2017

In 2017, we assessed EWI among 305 facilities in the country following revised WHO guidance for EWI surveys where the set of indicators reviewed were defined differently from the earlier surveys (see table 3). The EWIs assessed included: On-time ARV drug pickup (EWI 1), Retention in care (EWI 2), pharmacy stock outs (EWI 3), dispensing practices (EWI 4) and viral load suppression (EWI 5).

During this assessment, only 35 (11.5%) of the facilities met the indicator of on-time pill pick up. Out of the 305 facilities, 280 had complete data on this indicator. (Table 3). Seventy-nine, (25.9%) of the facilities had at least 80% of their patients known to be alive and on treatment 12 months after initiation of ART (EWI 2). The total number of facilities with 100% of months in the year in which there were no ARV drug stock-outs (EWI 3), was 101 (33.1%). Most of the facilities recorded a stock out of any of the ARVs prescribed during the year of assessment.

The indicator on dispensing practices, that is, the percentage of adults and children prescribed or picking up mono or dual ARV therapy; was met by all (100.0%) of the facilities. This was the indicator at which the facilities performed best.

Table 3: Uganda 2017 HIVDR EWI Target Summary.

WHO-recommended EWIs of HIVDR	Target (green: good; amber: fair; red: poor)	Facility Performance n(%)
On time ARV-drug pick up; % of patients that pick-up ART no more than two days late at the first drug pickup after a defined baseline pick-up	Green:>90	35(11.5)
	Amber:80-90	46(15.1)
	Red:<80	199(65.2)
	No results	25(8.2)
Retention in care; % of patients retained on ART 12 months after ART initiation	Green:>80	79(25.9)
	Amber:75-85	73(23.9)
	Red:<75	153(50.2)
Pharmacy stock outs; % of months with any day(s) of stock-out of any routinely dispensed ARV drug	Green:100	101(33.1)
	Red:<100	204(66.9)
Dispensing Practices; % of patients prescribed or picking up mono or dual ARV therapy	Green:0	305(100.0)
	Red:>0	0(0.0)
Viral load suppression; % of patients with viral load <1000 copies/mL 12 months after ART initiation	Green: ≥90	153(51.3) ⁱ
	Amber:80-<90	105(35.2) ⁱⁱ
	Red:<80	40(13.5)
	No results	7(2.3)
Viral load coverage; % of patients with a 12-month viral load test result available	Green: ≥70	156(51.1)
	Red:<70	149(48.9)

ⁱThe denominator for the viral load suppression indicator is the number of patients alive and on ART 12 months after treatment initiation who have a viral load test result available.

ⁱⁱThe denominator for the viral load completion indicator is the number of patients alive and on ART 12 months after treatment initiation, who are therefore, consistent with the policy, expected to have a viral load test result available in the primary medical record.

All the facilities dispensed the recommended triple therapy to all their patients, which was commendable. Retention in care was sub-optimal in all the surveys conducted. More effort is needed to track all patients to ensure that they are maintained on ART even if they change facility where they receive their drugs.

During the 2017 survey, data was collected on viral load (VL). Despite most patients having VS, there remains a challenge in ensuring that all patients who are eligible for VL testing have the test performed.

It is therefore of high importance that the national ART program ensures intensified follow-up for patients, ensuring continuous uninterrupted supply of ARV drugs at all facilities, and scaling up of VL testing, in order to improve ART adherence and minimize emergence of HIVDR.

3.11 Challenges and lessons learned during EWI monitoring

The following challenges were encountered during the assessment of the EWIs:

- a) Poor record keeping in some facilities that resulted in difficulties in obtaining follow up data such as loss to follow up. This resulted in poor scores as lack of data was considered as poor performance on some indicators. In instances where the facility had completely no data, the facility was excluded from the final analysis.
- b) Multiple data collection systems at facility level, especially for electronic patient registries made it difficult to standardize queries required to yield the required data. In some instances, it was not even possible to obtain data on some indicators.
- c) The indicator related to adherence could not be determined in line with international standards that require it to be based on adherence assessed only through objective methods such as pill counts. In Uganda, multiple methods of adherence assessment are used across facilities and the methods used are often not recorded. In addition, adherence is categorized >95%, 85 – 95% and less < 95%. In view of this, we adjusted the threshold to >95%, and used any adherence data irrespective of how it was determined. These results therefore may not readily be comparable to international standards for this indicator.

Chapter Four: Threshold Surveys for Transmitted HIV Drug Resistance

4.1 Overview of the threshold surveys

Threshold Survey (TS) for transmitted HIVDR uses the minimum-resource method recommended by WHO, to assess whether TDR is sufficiently prevalent in specific geographic areas of the country where ART is already in use or being rapidly scaled up. Threshold surveys do not give a precise estimate of prevalence of drug resistance, but rather a classification (for each drug or drug class) of the prevalence of TDR into one of three categories; low prevalence=5%, high prevalence=15%, moderate prevalence 5-15%. If the prevalence of HIVDR is classified as < 5% to all relevant drugs, the HIVDR TS should be repeated two years later. If the prevalence is classified in the higher categories, additional surveys or more resource-intensive surveillance may be required, as well as additional public health actions.

Nine different HIV drug resistance threshold surveys have been conducted to date under this plan by different partners, targeting groups of individuals recently infected with HIV or confirmed ART naïve patients. This included specimens from VCT sites or from recent sero-converters in longitudinal cohorts. The targeted sites had sufficient numbers of HIV positive persons with a high proportion of persons recently infected and unlikely to be ART experienced. In some of the studies, remnants of eligible HIV positive specimens in antenatal clinics and in target groups of interest were used for the threshold surveys.

HIVDR genotyping was performed at the national certified laboratories at UVRI and in South Africa. The survey population and patient selection criteria were designed to predict the likelihood of recent HIV infection.

4.2 Accomplished threshold surveys

The abstracts of published threshold surveys are described below:

a) Transmitted antiretroviral drug resistance surveillance among newly HIV type 1-diagnosed women attending an antenatal clinic in Entebbe, Uganda [11].

To evaluate transmitted HIV-1 drug resistance and study the natural polymorphism in *pol* of HIV-1 strains of newly diagnosed women attending an antenatal clinic in Uganda we sequenced the *protease* and *reverse transcriptase* genes for 46 HIV-1 strains from the threshold surveillance. Of the 46 sequences analyzed, 48.0% were subtype A1 (n 22), 39.0% subtype D (n 18), 2.0% subtype A2 (n 1), 2.0% subtype C (n 1), and 9.0% inter-subtype recombinant A1/D (n 4). Overall, many minor mutations were identified in the *protease* sequences. None of the strains had major associated mutations to any RTI drug or drug class of interest after genotyping 37 samples of our cohort. The HIV drug resistance prevalence estimate in Entebbe following the HIVDR-TS methodology was less than 5% as set out by WHO guidelines.

b) Transmitted antiretroviral drug resistance among newly HIV-1 diagnosed young individuals in Kampala [12].

This survey was conducted to assess the emergence of transmitted HIV-1 drug resistance (TDR) in Kampala after 10 years of rolling-out antiretroviral treatment (ART) and to compare with a previous survey among antenatal clinic attendees in 2007 (reporting 0% TDR). A cross-sectional survey was conducted among newly diagnosed HIV-1, antiretroviral-naïve young adults attending two large voluntary counselling and testing centres with the geographic area of Kampala. Proxy criteria of recent HIV-I infection were used as defined by WHO. Population sequencing of the *pol* gene was performed on plasma samples with HIV-1 RNA of at least 1000 copies/ml. Surveillance drug resistance mutations (SDRMs) were identified according to the 2009 WHO list for surveillance of TDR. HIV-1 sub types were designated using maximum likelihood phylogenetic reconstruction.

Genotypic test results were obtained for 70 out of 77 (90.9%) participants. SDRMs were identified in six samples yielding a prevalence of TDR of 8.6% (95% confidence interval 3.2-17.7%) Two had SDRMs to nucleoside reverse transcriptase inhibitors (D67G and L210W), three had SDRMs to non-nucleoside reverse transcriptase inhibitors (G190A, G190S and K101E), and one had SDRMs to protease inhibitors (N88D). Frequencies of HIV-1 sub types were A (36/70, 51.4%) C (2/70, 2.9%), D (23/70, 32.9%) and unique recombinant forms (9/70, 12.9%).

The study concluded that there was an increase in TDR in Kampala, compared with the previous survey. The study findings justified increased vigilance with respect to surveillance of TDR in areas in Africa where ART programs are rolled out.

c) Transmitted HIV type 1 drug resistance among individuals with recent HIV infection in east and southern Africa [13].

This study aimed to characterize the WHO-defined transmitted HIV drug resistance mutation (TDRM) data from recently HIV-infected African volunteers. Specimens from ARV naïve volunteers were evaluated for TDRM within 1 year of their estimated date of infection at eight research centers in sub-Saharan Africa including Uganda. Specimens were obtained from seroconverting partners in discordant relationships.

TDRMs were detected in 19/408 (5%) volunteers. The prevalence of TDRMs varied by research center, from 5/26 (19%) in Entebbe, 6/78 (8%) in Kigali, 2/49 (4%) in Kilifi, to 3/106 (3%) in Lusaka. One of five volunteers from Cape Town (20%) had TDRMs. Despite small numbers, the data suggested an increase in DRMs by year of infection in Zambia ($p=0.004$). The prevalence observed in Entebbe was high across the entire study. ARV history data from 12 (63%) HIV-infected sexual partners were available; 3 reported ARV use prior to transmission. Among four partners with sequence data available, transmission linkage was confirmed and two had the same TDRMs as the newly infected volunteer (both K103N). This study concluded that there is need to prioritize the monitoring of incident virus strains for the presence of TDRMs as ART continues to be rolled out in Africa. This study concluded that early HIV infection cohorts provide an excellent

and important platform to monitor the development of TDRMs to inform treatment guidelines, drug choices, and strategies for secondary prevention of TDRM transmission.

d) Transmitted antiretroviral drug resistance among drug-naïve female sex workers with recent infection in Kampala, Uganda [14].

During 2006-2007, transmitted human immunodeficiency virus (HIV) drug resistance (TDR) among drug-naïve women with newly diagnosed HIV infection and likely to be recently infected when attending antenatal clinics in Entebbe was found to be <5% with use of the World Health Organization (WHO) survey method. Using the same method, we attempted to classify TDR among women who seroconverted during 2008-2010 and who were identified from a cohort of recently infected sex workers in Kampala, Uganda. TDR mutations were identified using the 2009 WHO TDR mutations list. The WHO survey method could not be used to classify TDR because the necessary sample size was not reached during the survey period. However, a point prevalence estimate of 2.6% (95% confidence interval, 0.07%-13.8%) non-nucleoside reverse-transcriptase inhibitor TDR was determined.

e) HIV type 1 transmitted drug resistance and evidence of transmission clusters among recently infected antiretroviral-naïve individuals from Ugandan fishing communities of Lake Victoria [15].

This study was conducted among the fishing communities on Lake Victoria with a high incidence and prevalence of Human immunodeficiency virus type 1 (HIV-1). This population may play a role in driving the HIV epidemic in Uganda including the spread of transmitted drug resistance (TDR). Data was reported on TDR in this population among antiretroviral (ARV)-naïve, recently infected individuals about 5 years after ARV scaling-up in Uganda. Phylogenetic transmission clusters were identified and combined with volunteer life histories in order to understand the sexual networks within this population.

From a prospective cohort of 1,000 HIV-negative individuals recruited from five communities, 51 sero-converters were identified over a period of 2 years. From these, whole blood was collected and population sequencing of the HIV-1 *pol* gene (*protease/reverse transcriptase*) was performed from plasma. Drug resistance mutations (DRMs) were scored using the 2009 WHO list for surveillance of TDR. TDR prevalence categories were estimated using the WHO recommended truncated sampling technique for the surveillance of TDR for use in resource-limited settings (RLS). Of the samples, 92% (47/51) were successfully genotyped. HIV-1 subtype frequencies were 15/47 (32%) A1, 20/47 (43%) D, 1/47 (2%) C, 1/47 (2%) G and 10/47 (21%) unique recombinant forms. Non-nucleoside reverse transcriptase inhibitor (NNRTI) drug resistance mutation K103N was identified in two individuals and V106A in one (6%) suggesting that the level of TDR was moderate in this population. No nucleoside/tide reverse transcriptase inhibitor (NRTI) or protease inhibitor (PI) DRMs were detected. Five transmission clusters supported by high bootstrap values and low genetic distances were identified. Of these, one pair included the two individuals with K103N. Two of the genotypic clusters corresponded with reported sexual partnerships as detected through prior in-depth interviews. The level of TDR to NNRTIs in these ARV-naïve

individuals was moderate by WHO threshold survey categorization. The transmission clusters suggested a high degree of sexual partner mixing between members of these communities.

f) Low drug resistance levels among drug-naïve individuals with recent HIV type 1 infection in a rural clinical cohort in southwestern Uganda [16].

This study aimed to investigate the prevalence of transmitted drug resistance (TDR) among individuals with recent HIV-1 infection between February 2004 and January 2010 in a rural clinical cohort, samples from 72 participants were analyzed. Results from the 72 participants showed no protease inhibitor and nucleoside reverse transcriptase inhibitor-associated mutations. One participant (1.4%, 95% CI: 0.04-7.5%) had two non-nucleoside reverse transcriptase inhibitor mutations (G190E and P225H). HIV-1 subtype frequencies were A 22 (30.6%), D 38 (52.8%), and C 1 (1.4%); 11 (15.3%) were A/D unique recombinant forms. Seven years after the scale up of antiretroviral therapy (ART) in a rural clinical cohort in Uganda, the prevalence of TDR among recently HIV-1-infected individuals was low at 1.4%.

Since our findings from an HIV study cohort may not be generalizable to the general population, routine TDR surveys in specific populations may be necessary to inform policy on the magnitude and prevention strategies of TDR.

g) Prevalence and Virologic Consequences of Transmitted HIV-1 Drug Resistance in Uganda. AIDS Res Hum Retroviruses. 2014 Sep 1; 30(9): 896–906 [17].

In this study, they examined TDR prevalence in Kampala and Mbarara, Uganda and assessed its virologic consequences after antiretroviral therapy initiation. We sequenced the HIV-1 protease/reverse transcriptase from n=81 and n=491 treatment-naïve participants of the Uganda AIDS Rural Treatment Outcomes (UARTO) pilot study in Kampala (AMU 2002–2004) and main cohort in Mbarara (MBA 2005–2010). TDR-associated mutations were defined by the WHO 2009 surveillance mutation list. Post-treatment viral load data were available for both populations. Overall TDR prevalence was 7% (Kampala) and 3% (Mbarara) with no significant time trend. There was a slight but statistically non-significant trend indicating that the presence of TDR was associated with a worse treatment outcome. Virologic suppression (≤ 400 copies/ml within 6 months' post-therapy initiation) was achieved in 87% and 96% of participants with wild type viruses versus 67% and 83% of participants with TDR (AMU, MBA $p=0.2$ and 0.1); time to suppression (log-rank $p=0.3$ and $p=0.05$). Overall, 85% and 96% of study participants achieved suppression regardless of TDR status. Surprisingly, among the TDR cases, approximately half still achieved suppression; the presence of pre-therapy K103N while on nevirapine and fewer active drugs in the first regimen were most often observed with failures. The majority of patients benefited from the local HIV care system even without resistance monitoring. Overall, TDR prevalence was relatively low and its presence did not always imply treatment failure.

**** However, in the above study, 4 individuals had detectable drug concentration measured by HPLC mass spectrometer.**

h) Low Rates of Transmitted Drug Resistance Among Newly Identified HIV-1 Seroconverters in Rural Rakai, Uganda [18].

The study team investigated the rate of transmitted drug resistance (TDR) among HIV-1 seroconverters identified from the Rakai Community Cohort Study (RCCS) survey, a population-based cohort in Rakai District, Uganda. Participants aged 15-49 are interviewed at study visits approximately every 12-18 months and provided a serological sample. Antiretroviral therapy (ART) had been provided free of charge since 2004. RCCS participants with documented negative HIV-1 serology between January 2011 and August 2012 and confirmed seroconversion between November 2012 and October 2013 were included in this analysis. Serum was genotyped for HIV drug resistance mutations in *reverse transcriptase* and *protease* genes. Mutations were classified according to the 2009 World Health Organization surveillance of transmitted HIV-1 drug resistance update. Seventy-five (75) seroconverters were identified and genotyped. The mean age was 28 years (range 18-49) and the majority were male, n=44 (58%). The HIV-1 subtype frequencies were A=19 (25%), D=44 (59%), C=4 (5%), A/D recombinant=5 (7%), and C/D recombinant=3 (4%). The majority (72/75, 96%) of individuals were infected with wild-type virus with no evidence of TDR. Two individuals had a single non-nucleoside reverse transcriptase inhibitor mutation each, K101E and K103N, and one had a single protease inhibitor mutation, M46I. No mutations were identified involving nucleoside reverse transcriptase inhibitors. In conclusion, almost 10 years after the introduction of ART in rural Uganda, rates of TDR remain low. Ongoing surveillance for TDR remains an important public health priority and should be conducted among known seroconverters to estimate TDR.

i) HIV-1 Drug Resistance Among Ugandan Adults Attending an Urban Out-Patient Clinic [19].

This study was conducted in the adults' out-patient clinic of the Infectious Diseases Institute, Kampala, Uganda. This was a cross-sectional, observational study between June and September, 2015.

HIV genotyping was performed in ART-naïve patients and in treatment-experienced patients on ART for ≥6 months with virological failure (≥1000 copies/mL).

A total of 152 ART-naïve and 2430 ART-experienced patients were included. Transmitted drug resistance was detected in 9 (5.9%) patients. After a median time on ART of 4.7 years [interquartile range: 2.5-8.7], 190 patients (7.8%) had virological failure with a median viral load of 4.4 log₁₀ copies per milliliter (interquartile range: 3.9-4.9). In addition, 146 patients had a viral load between 51 and 999 copies per milliliter. Most patients with virological failure (142, 74.7%) were on first-line ART. For 163 (85.8%) ART-experienced patients, genotype results were available. Relevant drug-resistance mutations were observed in 135 (82.8%), of which 103 (63.2%) had resistance to 2 drug classes, and 11 (6.7%) had resistance to all drug classes available in Uganda.

The prevalence of transmitted drug resistance was lower than recently reported by the WHO. With 92% of all patients virologically suppressed on ART, the prevalence of virological failure was low when a cutoff of 1000 copies per milliliter is applied, and is in line with the third of the 90-90-90 UNAIDS targets. However, most failing patients had developed multiclass drug resistance.

Table 4: Published Transmitted DR

Author	Year of sample collection	Location	Total samples tested	% with TDR	Resistance drug class
Ndembi et al. 2008 [11]	2006-2007	ANC- Entebbe	46	0%	
Price et al. 2011 [13]	2005-2009	Recently infected in discordant couples, Masaka and Entebbe	26 Entebbe 65 Masaka	Entebbe 19% Masaka 1.5%	<u>Entebbe:</u> 2 NRTI, 2 NNRTI, 2 PIs, 1 both PI and NRTI <u>Masaka:</u> 1 PI
Ndembi et al. 2011 [12]	2010	Newly HIV-1 diagnosed young individuals in Kampala.	70	8.6%	2 NRTI; 3 NNRTI; 1 PI
Ssemwanga et al. 2012 [14]	2008-2010	Recently infected commercial sex workers in Kampala	38	2.6%	1 NNRTI
Nazziwa et al. 2012 [15]	2009	Recently infected in fishing community in Wakiso and Masaka	47	6%	3 NNRTI
Ssemwanga et al. 2012 [16]	2004-2010	Recent seroconverters in an MRC cohort in SW Uganda	72	1.4%	NNRTI
Lee GQ et al. 2014* [17]	Kampala 2002-2004 Mbarara 2005-2010	Treatment naïve participants in Kampala and Mbarara	81 Kampala 491 Mbarara	7% Kampala 3% Mbarara	<u>Kampala:</u> 3 NRTI, 1 NNRTI, 1 PI, 1 both NRTI and NNRTI <u>Mbarara:</u> 4 NNRTI, 9 NNRTI, 2 both
Reynolds SJ et al. 2014 [18]	2012-2013	Recently infected individuals in Rakai	75	4%	2 NNRTI-1PI
van Braun et al. 2018*[19]	2015	ART clinic at IDI in Kampala	152	5.9%	5 NRTI; 8 NNRTI 4 both

* Treatment naïve should be confirmed not to have been exposed to any treatment. However, in the Lee et

al study, 4 individuals had detectable drug concentration measured by HPLC mass spectrometer.

4.3 Conclusions on TDR

Apart from the IAVI funded study above where the TDR prevalence was 19% in Entebbe, which included seroconverting partners in discordant relationships and where some of the positive partners were known to be on ART, the rest of the studies show low to moderate TDR (0/46 (0%), 1/65 (1.5%) at the Masaka site in the IAVI study, 6/70 (8.6%), 1/38 (2.6%), 3/47 (6%), 1/72 (1.4%); 6/81 (7%) and 15/491 (3%) in Mbarara and Kampala respectively; 3/75 (4%) and 9/152 (5.9%). Following WHO recommendations this means where moderate rates have been identified there should be repeat surveys within the subsequent five years (low prevalence $\leq 5\%$; moderate 5-15%, high prevalence $\geq 15\%$). Like in other countries the most frequently observed mutations are NNRTI. Transmitted PI mutations were very low.

Some of the challenges faced in survey implementation include the following: (i) Minimum resource strategy e.g use of remnant specimens from sero-surveys was not possible in most studies; (ii) Achieving the required sample size within the planned recruitment period was challenging; (iii) Laboratory evidence of recent infections was difficult and (iv) Finally these studies are expensive to conduct.

Within the UPHIA cross-sectional household based survey, samples from recent seroconverters (based on the LAg assay) have been genotyped at UVRI and the results are being analysed. This will provide more information on countrywide TDR.

Chapter Five: Surveys for Pre-Treatment and Acquired HIV Drug Resistance

5.1 Overview

The 2004 WHO generic protocol for monitoring HIVDR prevention during treatment in sentinel sites utilizes a standardized, minimum-resource prospective survey methodology to assess the success of ART programmes in preventing HIVDR during the first year of treatment and identifies factors associated with the emergence of HIVDR which can be successfully addressed at the level of the ART site and programme.

Like EWIs, WHO HIVDR prevention surveys were designed to be integrated easily into ongoing, routine HIV-related evaluation activities. Performed regularly at representative sites, the data generated informs the evidence-base for national and global ART regimen selection and promotes efforts to minimize the emergence of HIVDR at a population level by effecting positive programmatic change if necessary.

This initial WHO protocol of baseline and acquired HIVDR were not nationally representative but more site specific. In order to obtain nationally representative surveillance of HIVDR in populations initiating a standard triple-drug ART, WHO in 2014 came up with new protocols for pre-treatment DR (PDR) and for acquired DR (ADR) [20], which we have also implemented. PDR are cross-sectional surveys that employ a two-stage cluster sampling design. The first stage involves the selection of ART clinics using probability proportional to size (PPS) sampling, a method by which the probability of selecting an ART clinic is proportional to the size of the population initiating ART at a given clinic. The second stage involves consecutive enrolment of eligible individuals initiating ART at the sampled clinics until the pre-determined sample size for each is achieved. This approach estimates nationally representative prevalence of HIVDR among all ART initiators, regardless of their prior exposure to ARV drugs; and nationally representative prevalence of HIVDR among ARV drug-naïve initiators.

On the other hand, nationally representative surveys of ADR are designed to yield nationally representative prevalence estimates of HIVDR in populations receiving ART for 12 (± 3) months (referred to as early time point surveys) and in populations receiving ART for 48+ months (referred to as late time point surveys), in addition to estimates of VS in these respective populations. ADR surveys provide an indication of the proportion of individuals on ART at 12 months and 48+ months who are failing treatment and should be switched to second-line ART. ADR survey results provide critical information to assess programme performance in achieving VS, and to inform the optimal selection of second- and potentially third line regimens, based on prevalence of resistance in individuals failing treatment.

There were also surveys among children, one conducted by the TWG looking at the initial HIV drug resistance among young children under 2 years and recently diagnosed with HIV and

another by PASER to determine the prevalence of PDR and ADR. However, the latter study didn't provide nationally representative resistance in children and adolescents. Plans are underway to conduct nationally representative surveys among children and adolescents to close this gap.

5.2 Some of the completed studies in both adults and children

a) Virologic response and antiretroviral drug resistance emerging during antiretroviral therapy at three treatment centers in Uganda [21]

The objective of this study was to monitor antiretroviral therapy (ART) scale up programme performance in order to maximize ART efficacy and limit HIV drug resistance (HIVDR). WHO HIVDR prospective survey protocol was implemented at three treatment centers between 2012 and 2013. Data were abstracted from patient records at ART start (T1) and after 12 months (T2). Genotyping was performed in the HIV *pol* region at the two time points.

Of the 425 patients enrolled, at T2, 20(4.7%) had died, 66(15.5%) were lost to follow-up, 313 (73.6%) were still on first-line, 8 (1.9%) had switched to second-line, 17 (4.0%) had transferred out and 1(0.2%) had stopped treatment. At T2, 272 out of 321 on first and second line (84.7%) suppressed below 1000 copies/ml and the HIV DR prevention rate was 70.1%, just within the WHO threshold of $\geq 70\%$. The proportion of participants with potential HIVDR was 20.9%, which is higher than the 18.8% based on pooled analyses from African studies. Of the 35 patients with mutations at T2, 80% had M184V/I, 65.7% Y181C, and 48.6% (54.8% excluding those not on Tenofovir) had K65R mutations. 22.9% had Thymidine Analogue Mutations (TAMs). Factors significantly associated with HIVDR prevention at T2 were: baseline viral load (VL) <100,000 copies/ml [Adjusted odds ratio (AOR) 3.13, 95% confidence interval (CI): 1.36-7.19] and facility. Independent baseline predictors for HIVDR mutations at T2 were: CD4 count <250 cells/ μ l (AOR 2.80, 95% CI: 1.08 -7.29) and viral load $\geq 100,000$ copies/ml (AOR 2.48, 95% CI: 1.00-6.14).

Strengthening defaulter tracing, intensified follow-up for patients with low CD4 counts and/or high VL at ART initiation together with early treatment initiation above 250 CD4 cells/ μ l and adequate patient counselling would improve ART efficacy and HIVDR prevention. The high rate of K65R and TAMs could compromise second line regimens including NRTIs

The above study contributed to the Global analysis of DR after first- line regimen failure
Global epidemiology of drug resistance after failure of WHO recommended first-line regimens for adult HIV-1 infection: a multicentre retrospective cohort study [22] below.

This analysis included 1,926 patients from 36 countries with treatment failure between 1998 and 2015. Prevalence of tenofovir resistance was highest in sub-Saharan Africa (370/654 [57%]). Pre-ART CD4 cell count was the covariate most strongly associated with the development of tenofovir resistance (odds ratio [OR] 1.50, 95% CI 1.27-1.77 for CD4 cell count <100 cells per μ L). Use of lamivudine versus emtricitabine increased the risk of tenofovir resistance across regions

(OR 1.48, 95% CI 1.20-1.82). Of 700 individuals with tenofovir resistance, 578 (83%) had cytosine analogue resistance (M184V/I mutation), 543 (78%) had major NNRTI resistance, and 457 (65%) had both. The mean plasma viral load at virological failure was similar in individuals with and without tenofovir resistance (145,700 copies per mL [SE 12 480] versus 133,900 copies per mL [SE 16 650; $p=0.626$]).

We recorded drug resistance in a high proportion of patients after virological failure on a tenofovir-containing first-line regimen across low-income and middle-income regions. Effective surveillance for transmission of drug resistance is therefore crucial

b) Nationally representative survey of HIV drug resistance in adults initiating antiretroviral therapy (Pre-treatment) HIV Drug Resistance

We conducted a cross sectional survey of HIVDR among adults initiating or re-initiating antiretroviral therapy. This enabled us assess the prevalence of pretreatment HIVDR among adults. Briefly, we conducted a cross-sectional survey of adults 18 years or older initiating or re-initiating ART at 23 randomly selected sites using the probability proportional to size (PPS) sampling method between August 2016 and March 2017.

DBS or plasma samples prepared from whole blood were shipped to Entebbe for sequencing and identification of HIVDR mutations (DRMs) in the MRC/UVRI genotyping laboratory. Four hundred ninety-one participants were enrolled into the survey. Three hundred and fifty-eight (73%) were successfully genotyped. Participants' median age was 32 years (Interquartile range: 25 – 41) and 61.3% were female. Forty-eight or 17.4% (95% confidence interval: 12.1-24.3) had any DRMs: 43 (or 15.4%) were non-nucleoside reverse transcriptase inhibitor (NNRTI), 11 (5.1%) nucleoside reverse transcriptase inhibitor (NRTI), 2 (1%) protease inhibitor (PI) DRMs and 8 (4.1%) had NNRTI+NRTI mutations. Nine patients reported prior exposure to ART (6 full ART, 1 prevention of mother-to-child transmission, 2 unspecified) and among these, 2 had NNRTI mutations. Common NNRTI mutations were K103N ($n=32$), Y181C (7) and G190A (6); NRTI mutations were M41L (5), M184V (4) and K70R (3). PI mutations were I85V and M46L; there were five thymidine analogue mutations, all at position T215.

The level of pretreatment HIVDR that we observed in this survey was high. Based on the updated WHO recommendations in settings of high PDR levels above 10%, the Ministry guidelines have been revised accordingly from Tenofovir+Lamivudine+Efavirenz (TLE) to Tenofovir +Lamivudine+ Dolutegravir (TLD) as first line regimen for adults. Results from this survey contributed to the HIV Drug Resistance Report 2017. The study above also contributed to the Global systematic review and meta-regression analysis [23] below.

HIV-1 drug resistance before initiation or re-initiation of first-line antiretroviral therapy in low-income and middle-income countries: a systematic review and meta-regression analysis

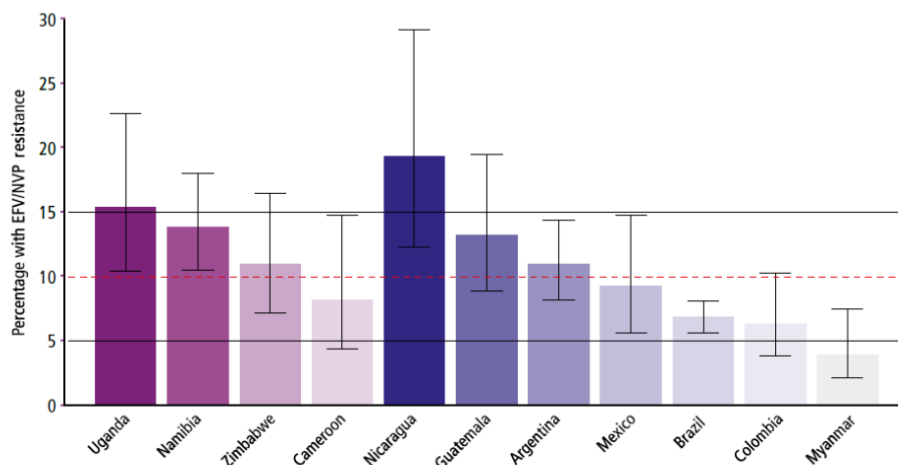
In this analysis, 358 datasets were identified that contributed data to the analyses, representing 56 044 adults in 63 countries. Prevalence estimates of pretreatment NNRTI resistance in 2016

were 11.0% (7.5-15.9) in southern Africa, 10.1% (5.1-19.4) in eastern Africa, 7.2% (2.9-16.5) in western and central Africa, and 9.4% (6.6-13.2) in Latin America and the Caribbean. There were substantial increases in pretreatment NNRTI resistance per year in all regions. The yearly increases in the odds of pretreatment drug resistance were 23% (95% CI 16-29) in southern Africa, 17% (5-30) in eastern Africa, 17% (6-29) in western and central Africa, 11% (5-18) in Latin America and the Caribbean, and 11% (2-20) in Asia. Estimated increases in the absolute prevalence of pretreatment drug resistance between 2015 and 2016 ranged from 0.3% in Asia to 1.8% in Southern Africa.

This review showed that Pretreatment drug resistance is increasing at substantial rate in LMICs, especially in sub-Saharan Africa. In 2016, the prevalence of pretreatment NNRTI resistance was near WHO's 10% threshold for changing first-line ART in Southern and Eastern Africa and Latin America, underscoring the need for routine national HIV drug-resistance surveillance and review of national policies for first-line ART regimen composition

In the above analysis, levels of PDR were driven by NNRTI resistance, which exceeded 10% in six out of the 11 countries (Fig 4)

Figure 4: Pretreatment HIV drug resistance to EFV or NVP in first-line ART initiators (pre-treatment HIV-drug resistance national surveys, 2014-2016) [24]



Resistance to EFV/NVP is defined by the Stanford HIVdb algorithm (version 8.3); sequences with predicted low-, intermediate- or high-level resistance are considered as resistant.

c) Increasing Prevalence of HIV Pretreatment Drug Resistance in Women But Not Men in Rural Uganda During 2005-2013 [25].

In this recently published study, the team sought to describe correlates of PDR and evaluate effects of PDR on clinical outcomes in rural Uganda. They analyzed data from the Uganda AIDS Rural Treatment Outcomes study, a cohort of ART-naïve adults with HIV (2005-2015). They performed resistance testing on pre-ART specimens. They defined PDR as any WHO 2009 SDRM and classified PDR level using the Stanford algorithm. They fitted unadjusted and sex-

stratified log binomial regression and Cox proportional hazard models to identify correlates of PDR and the impact of PDR on viral suppression, loss to follow-up (LTFU), and death. They analyzed data from 738 participants (median age 33 years, 69% female). Overall, prevalence of PDR was 3.5% (n=26), owing mostly to resistance to NNRTIs. PDR increased over time in women (1.8% in those enrolling in clinic in 2001-2006, vs. 7.0% in 2007-2013; $p=0.006$), but not in men (1.15% vs. 0.72%, $p=0.737$). Lower pre-ART log₁₀ HIV RNA was also associated with higher prevalence of PDR. They identified longer time to viral suppression among those with PDR compared with without PDR (0.5 and 0.3 years, respectively, $p=0.023$), but there was no significant relationship with mortality or LTFU ($p=0.139$). They observed increasing rates of PDR in women in southwestern Uganda. Implications of this trend, particularly to prevention of mother-to-child transmission programs in the region, require attention due to delayed viral suppression among those with PDR

d) Nationally representative Acquired HIV drug resistance (ADR) prevalence in adults receiving antiretroviral therapy after 12 months (manuscript in preparation)

We conducted a cross-sectional survey of acquired HIV drug resistance among adult patients on first-line ART for 12 months between August 2016 and March 2017 at 23 sites randomly selected using probability proportional to size sampling. This approach enabled us obtain a nationally representative prevalence estimate. At the sites, eligible and consenting patients who had been on ART for 12 months were enrolled into this survey.

Blood samples (both plasma and DBS) were obtained for viral load (VL) testing and genotyping for drug resistance. A total of 547 patients were enrolled; VL results were available for 97.4% (533/547) patients of which only 7.5% (40/533) had virological failure (VF); Genotypic ADR testing was available for 30 (75%) of the 40 VFs (28 patients were genotyped in reverse transcriptase alone). Patients with any SDRM were 28/30 (93.3%) of which one patient (1/30, 3.3%) had the (I85V) PI mutation, 23/28 (82.1%) had NRTI mutations, 26/28 (92.9%) had NNRTI mutations and 22/28 (78.6%) had both NRTI and NNRTI mutations. No patient was found with all three-drug class mutations.

In this cross-sectional study, we found low rates of VF on first-line ART, however, the prevalence of ADR mutations is high among the VFs putting into question the benefit of delayed switching after adherence counselling and repeat viral load. The mutations identified could compromise second line regimens especially NRTIs.

e) Nationally representative Acquired HIV drug resistance (ADR) prevalence in adults receiving antiretroviral therapy after 48 months (manuscript in preparation).

We enrolled 1,064 persons from September to November 2017 at 23 sites across the country using a two-stage cluster design. Viral load testing and HIVDR genotyping for those with VF were performed on either DBS or plasma samples from each participant. DRMs were analysed

using the Stanford HIVdb Program using the 2009 WHO mutation list. Pearson's chi-square tests, Kruskal Wallis tests and multivariate logistic regression were used to determine independent predictors of VF and HIVDR with level of significance at $p < 0.05$.

The median age was 44 years, IQR 43-45, and 34.7% of the participants were males. By survey enrollment, 853 (80.2%) of the patients were still maintained on their initial first-line regimen and 211 (19.8%) had their regimen substituted including all the 101 who had been initiated on d4T. The median time on ART was 82 months (IQR: 79-85). All patients had VL results and the prevalence of VL suppression ($n=926$) was 87.0%, 95% (CI 84.1-90.0). Of the 138 patients with VF, 95 (68.8%) were successfully genotyped and 88 (63.8%) had DRMs as follows; 85(96.5%) had NRTI mutations, 86(97.7%) had NNRTI mutations, 83(94.3%) had NRTI and NNRTI mutations, none had PI mutations. Of the 88 patients with mutations, 83(94.3%) had M184V/I, 43(48.9%) had K103N/S, 24(27.3%) had Y181C and 41(46.6%) had TAMs. Factors significantly associated with VF were initiation on AZT-based regimen adjusted odds ratio 1.79, CI: 1.14-2.80 and ART duration of less than 82 months AOR 1.53 CI: 1.02-2.29. Independent predictors for HIVDR were initiation on AZT-based regimen AOR 2.63, CI: 1.47-4.78 and age less than 43 years AOR 1.79, CI: 1.12-2.86.

These findings suggest successful treatment outcomes for about 87% of the patients after 48 months on ART. However, patients with VF have high prevalence of HIVDR, with patients initiating AZT-based regimens being more susceptible to both VF and HIVDR. There is therefore need for close monitoring of patients with VF and access to affordable genotypic monitoring to facilitate early switching to second line regimen.

f) HIV-1 drug resistance in antiretroviral-naïve individuals in sub-Saharan Africa after rollout of antiretroviral therapy: a multicenter observational study [26].

The objective of this study was to assess the prevalence of primary resistance in six African countries after ART roll-out and if wider use of ART in sub-Saharan Africa was associated with rising prevalence of drug resistance. This study did not follow the exact WHO protocol but provided important information earlier on, on the extent of PDR.

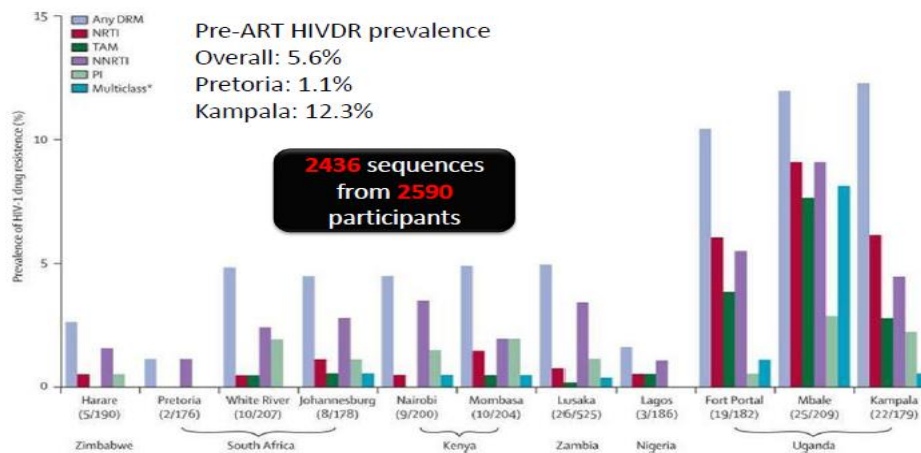
Cross-sectional study was done in ARV-naïve adults infected with HIV-1 who had not started first-line ART and were recruited between 2007 and 2009. Population-based sequencing of the *pol* gene was done on plasma specimens with greater than 1000 copies per mL of HIV RNA. Drug-resistance mutations were identified with the WHO list for transmitted resistance. The prevalence of sequences containing at least one drug-resistance mutation was calculated accounting for the sampling weights of the sites. Risk factors of resistance were assessed with multilevel logistic regression with random coefficients.

436 (94.1%) of 2590 participants had a pretreatment genotypic resistance result. 1486 participants (57.4%) were women, 1575 (60.8%) had WHO clinical stage 3 or 4 disease, and the median CD4 count was 133 cells per μL (IQR 62-204). Overall, sample-weighted drug-resistance prevalence

was 5.6% (139 of 2436; 95% CI 4.6-6.7). The pooled prevalence for all three Ugandan sites was 11.6% (66 of 570; 8.9-14.2). Drug class-specific resistance prevalence was 2.5% (54 of 2436; 1.8-3.2) for nucleoside reverse-transcriptase inhibitors (NRTIs), 3.3% (83 of 2436; 2.5-4.2) for non-NRTIs (NNRTIs), 1.3% (31 of 2436; 0.8-1.8) for protease inhibitors, and 1.2% (25 of 2436; 0.7-1.7) for dual-class resistance to NRTIs and NNRTIs. The most common drug-resistance mutations were K103N (43 [1.8%] of 2436), thymidine analogue mutations (33 [1.6%] of 2436), M184V (25 [1.2%] of 2436), and Y181C/I (19 [0.7%] of 2436). The odds ratio for drug resistance associated with each additional year since the start of the ART roll-out in a region was 1.38 (95% CI 1.13-1.68; $p=0.001$).

The higher prevalence of primary drug resistance in Uganda than in other African countries is probably related to the earlier start of ART rollout in Uganda. Resistance surveillance and prevention should be prioritized in settings where ART programmes are scaled up.

Figure 5: Pre-therapy HIVDR by region and drug class [26]



Hamers et al., *LID* 11, 750-9, 2011

g) Monitoring Antiretroviral Resistance in Children (MARCH) in Uganda [27].

The objectives of this study were to: Measure baseline HIVDR prevalence in children initiating first- or second-line ART; monitor virological response to treatment, determine prevalence and patterns of HIVDR in children with detectable VL and identify risk factors for virologic failure and HIVDR. It was prospective, observational cohort study of HIV- positive children ≤ 12 years and eligibility was when initiating first-line ART or switching to second-line ART due to treatment failure.

At three Ugandan clinics, children (age <12 years) requiring ART were recruited between January 2010 and August 2011. Before starting ART, blood was collected for VL and *pol* gene

sequencing. Drug resistance mutations were determined using the 2010 International AIDS Society-USA mutation list. Risk factors for HIVDR were assessed with multivariate regression analysis.

Three hundred nineteen HIV-infected children with a median age of 4.9 years were enrolled. Sequencing was successful in 279 children (87.5%). HIVDR was present in 10% of all children and 15.2% of children <3 years. Nucleoside reverse transcriptase inhibitors (NRTIs), non-NRTI (NNRTI), and dual-class resistance was present in 5.7%, 7.5%, and 3.2%, respectively. HIVDR occurred in 35.7% of prevention of mother-to-child transmission (PMTCT)-exposed children, 15.6% in children with unknown PMTCT history, and 7.7% among antiretroviral-naïve children. History of PMTCT exposure [adjusted odds ratio (AOR): 2.6, 95% CI: 1.3-5.1] or unknown PMTCT status (AOR: 3.8, 95% CI: 1.1-13.5), low CD4 (AOR: 2.2, 95% CI: 1.3-3.6), current breastfeeding (AOR: 7.4, 95% CI: 2.6-21), and current maternal ART use (AOR: 6.4, 95% CI: 3.4-11.9) emerged as risk factors for primary HIVDR in multivariate analysis.

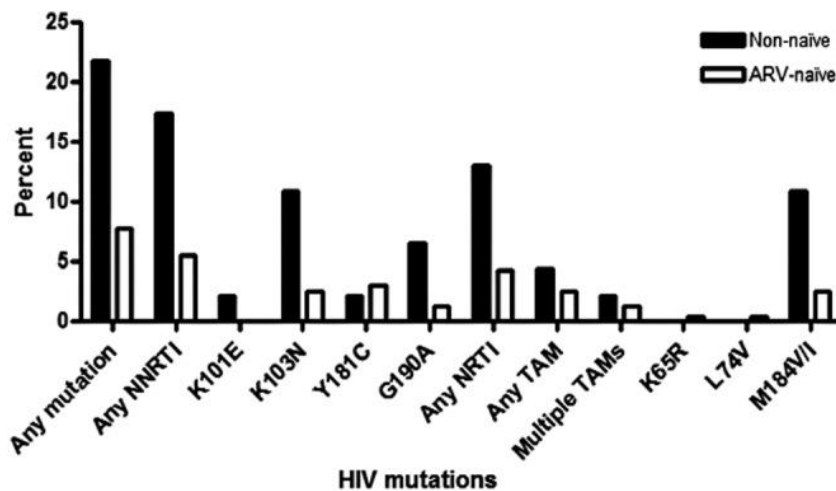


Figure 6 presents the mutational patterns among the enrolled patients initiating first-line ART stratified by previous ARV exposure. [26]

Pretreatment HIVDR was high, especially in children with PMTCT exposure (Fig 7). Protease inhibitor (PI)-based regimens are advocated by the World Health Organization, but availability in children is limited. Children with (unknown) PMTCT exposure, low CD4 count, current breastfeeding, or maternal ART need to be prioritized to receive PI-based regimens.

h) Multicounty analysis study reported Human Immunodeficiency Virus (HIV) Drug Resistance in African Infants and Young Children Newly Diagnosed with HIV: A Multi-country Analysis [28].

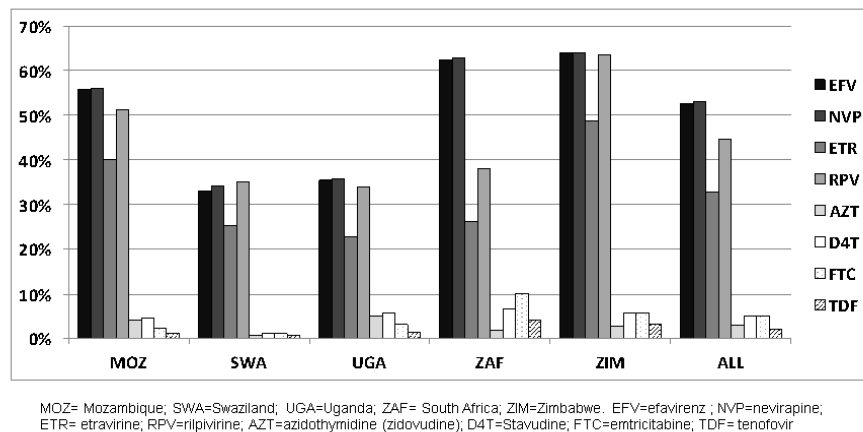
Programs for the prevention of mother-to-child transmission (PMTCT) of human immunodeficiency virus (HIV) have been scaled up in many low- and middle-income countries. However, HIV drug resistance (HIVDR) data among HIV-1-infected young children remain limited.

Surveys of pretreatment HIVDR among children aged <18 months who were diagnosed with HIV through early infant diagnosis were conducted in 5 sub-Saharan African countries (Mozambique, Swaziland, South Africa, Uganda, and Zimbabwe) between 2011 and 2014 following World Health Organization (WHO) guidance. De-identified demographic and clinical data were used to explore risk factors associated with nonnucleoside reverse transcriptase inhibitor (NNRTI) resistance.

Among the 1450 genotypes analyzed, 1048 had accompanying demographic and clinical data. The median age of children was 4 months; 50.4% were female. HIV from 54.1% showed resistance to 1 or more antiretroviral (ARV) drugs, with 53.0% and 8.8% having resistance to 1 or more NNRTI or nucleoside reverse transcriptase inhibitors, respectively. NNRTI resistance was particularly high in children exposed to ARV drugs through PMTCT; adjusted odds ratios were 1.8 (95% confidence interval [CI], 1.3-2.6) for maternal exposure only and 2.4 (CI, 1.6-3.6) for neonatal exposure only.

In the subset of Uganda children, the overall prevalence rate was 38.4%, (95%CI:32.0-44.8); prevalence to NRTI's was 8.5% (95% CI 4.8-12.1) and to NNRTIs at 35.7% (95% CI 29.4-42.0).

Figure 7: Predicted levels of NRTI and NNRTI resistance by drug [27].



In conclusion, protease inhibitor-based regimens in children aged <3 years are currently recommended by WHO, but the implementation of this recommendation is suboptimal. These results reinforce the urgent need to overcome barriers to scaling up pediatric protease inhibitor-based regimens in sub-Saharan Africa and underscore the need to accelerate the study and approval of integrase inhibitors for use in young children.

5.3 Conclusions on PDR and ADR

Adults

We have observed that strengthening defaulter tracing, intensified follow-up for patients with low CD4 counts at ART initiation together with early treatment initiation above 250 CD4 cells/ul and adequate patient counselling would improve ART efficacy and HIVDR prevention. In one survey, we have also observed high rates of K65R and TAMs which could compromise second line regimens including NRTIs. The global analysis of DR after first line failure showed that prevalence of tenofovir resistance was highest in sub-Saharan Africa, and this is of concern.

In the nationally representative study, the level of PDR that we observed in Uganda was high, prompting the recommendation to introduce dolutegravir in the first line regimen for adults. The systematic review and meta-analysis indicates that PDR is increasing at a substantial rate in LMICs, especially in sub-Saharan Africa.

A recent study has also shown increasing rates of PDR in women in southwestern Uganda between 2005-2013 though lower than the above study.

Nationally representative surveys have shown that:

- i) While there is good VL suppression at 12 months, the prevalence of ADR mutations at 12 months is high among the VF putting into question the benefit of delayed switching after adherence counselling and repeat VL. The mutations identified could compromise second line regimens especially NRTIs.
- ii) At 48 months, there is good VS (87%) and the prevalence of HIVDR was about 10.1% in the studied population. Of the patients with VF and genotype data, 63.8% had DRMs as follows; 96.5% had NRTI mutations, 97.7% had NNRTI mutations, 94.3% had NRTI and NNRTI mutations and none had PI mutations.

Pediatric surveys

Pretreatment HIVDR was high, in children with PMTCT exposure and the use of Protease inhibitor (PI)-based regimens as advocated by the WHO should be supported, unfortunately availability in children is limited.

Chapter Six: HIV Drug Resistance Genotyping Laboratories

For the implementation of surveys to monitor HIVDR prevention and threshold surveys for surveillance of TDR, countries should select one or more reference genotyping laboratories from the list of the WHO Global HIVDR Laboratory Network [29]. The WHO HIV DR laboratory strategy involves supporting HIVDR surveillance efforts by providing accurate and timely genotyping results that meet WHO specifications. The strategy promotes the proper collection, handling, shipment and storage of specimens and the availability of quality assured HIV genotyping laboratory services at the national, regional and global level. Additionally, the national HIV DR working groups coordinating WHO recommended surveys must use a WHO designated genotyping laboratory to provide quality assured testing services for the surveys.

To be designated as a laboratory for WHO surveys, a detailed assessment is performed, which includes a site inspection and annual external proficiency testing. The UVRI laboratory at Entebbe was officially designated as a national reference laboratory by the MOH in 2007 and was certified by WHO as a national reference laboratory for genotyping using plasma specimens in 2008 and using dry blood spots (DBS) in 2014. Furthermore, the laboratory was also certified by WHO as a regional reference laboratory for genotyping in 2011 in order to serve other countries in the region. This being one of the three regional reference laboratories in Africa.

The UVRI genotyping laboratory has successfully undertaken the following functions:

- Represented Uganda in the WHO HIVResLab Network
- Conducted genotyping for WHO HIVDR surveillance and monitoring
- Served as a key point of contact between WHO and the country on all questions relating to HIV genotyping sequencing, virological and epidemiological surveillance.
- Participated in the WHO recognized quality assurance programs for genotyping
- Tested samples from other countries such as Zimbabwe and Tanzania
- The laboratory has continued to train both local and international technicians.
- The laboratory also participated in studies to come up with DBS protocols for storage and transportation [30].

JCRC was also evaluated by WHO HIVResNet and certified as national reference laboratory for genotyping using plasma in 2014. This laboratory has undertaken the following activities:

- Offers HIV drug resistance services to Case Western Reserve University studies in Uganda, PharmAccess African studies and Earnest studies.
- Provides HIV-1 drug resistance services to HIV-1 patients seeking care through the different clinics and hospitals in Uganda. This has been done since the early 2000's up to today (July 2018).
- Participates in various quality assurance programs including those of WHO and College of American Pathologist (CAP) and prior the TREAT Asia quality assurance program.

Chapter Seven: HIV Drug Resistance Database

WHO and CDC developed a HIVDR database for use in management of data in the context of surveys to monitor HIVDR prevention and threshold surveys for surveillance of transmitted HIVDR. Two officials from MOH ACP and one WHO official attended a training workshop on the new version of the HIVDR resistance database supported by WHO in Harare, Zimbabwe in August 2010. The overall aim of the workshop was to train key staff from the region in all aspects of the use of the updated version of the antiretroviral drug resistance database, (version 2.0), and to acquaint them with the knowledge and skills to set up a national electronic database.

The HIVDR database was developed by WHO and CDC for countries to use as a data repository during the implementation and reporting of information obtained during surveys on transmitted HIVDR as well as surveys for tracking HIVDR emerging during ARV treatment.

Electronic tools for facility level data collection of EWIs have now replaced the paper based data tools and all the data is uploaded onto templates provided by WHO. Similarly, both clinic and laboratory data including sequences for the acquired and pre-treatment drug resistance surveys are uploaded to the WHO database.

WHO runs quality checks on the data uploaded into the database and works with the secretariat to address any quality issues.

Data analyses were performed at central level in order to generate individual site reports, or cumulative regional or national reports, according to the specific design of the survey.

Plans are under way to create a national database for HIV genotypes beyond resistance genotypes.

Chapter Eight: HIV Drug Resistance Prevention Activities

There are other activities focused on HIV drug resistance assessment and prevention relevant to this plan which were undertaken by other key players but not supervised by the TWG. Some of these activities include the following:

8.1 Promoting standard prescribing practices for ART regimens

ACP and the ART subcommittee have spearheaded provision of treatment guidelines. Different partners have contributed to capacity building and ensuring these guidelines are followed.

8.3 Adequate and continuous ARV drug supply and monitoring of supply shortages at site and regional levels

The pharmacy division conducts annual forecasting and quantification for all ARVs needed to meet the needs of patients estimated/projected to be enrolled on treatment. The national stock status of ARVs is reviewed regularly and where necessary, emergency procurements are planned.

8.4 Standard ART patient records or minimum standard of recorded data:

A system based on the WHO generic recommendations was developed in the country. According to the national system, once a patient is started on ART or chronic AIDS care, a chronic HIV care /ART card is opened at the facility with clients identified using a unique facility-level patient number. On this card, basic demographic information, data on point of entry into care, HIV test results, biological assessment results (CD-4 cell counts, hemoglobin, etc.), ART information (e.g. eligibility, ARV regimen, etc.) and clinical, laboratory, therapeutic, and psychosocial information is recorded. The follow up section of the card records information on ART duration, ARV prescription (status, regimen, substitutions and reasons for it, switch or stop), clinical stage, and functional state, clinical appointments and adherence.

On a monthly or quarterly basis, some clinical variables are abstracted from the HIV care/ART cards and recorded on patient ART and pre-ART registers. Efforts have been made to ensure treatment centres transition to electronic data capture. The standard ART data management and reporting forms have been updated recently to provide for better inter-linkage of chronic HIV care / ART information systems with that of tuberculosis, PMTCT and maternal and child health programme. There is a concerted effort by all stakeholders to harmonize the various HIV care/ ART patient monitoring systems with the Ministry of Health recommendations.

8.5 ART patient cohort monitoring

Uganda has adapted the WHO cohort monitoring system. At the end of each month, longitudinal follow up information of treatment groups (cohorts) of patients on ART is

summarized in the ART register. The monthly follow up variables recorded in the registers include ARVs dispensed and if the client does not pick ARVs, then one of the follow up status including lost, transferred out, died, stopped treatment, lost to follow up is recorded. Every 6 months, data pertaining to WHO clinical stage, body weight and CD-4 T-cell counts where available are also updated on the register. At the end of each quarter, selected variables from the ART register are summarised on the cohort analysis form for those cohorts completing, 6, 12, 24, 36 etc months during that quarter. Both baseline and outcome information is summarised for specific indicators.

8.6 Promotion and on-going support of patient adherence

At each follow up visit, patients are expected to be assessed about adherence to treatment. In most programs, patients pick up medication on a monthly basis, regardless of whether clinical follow up is conducted every 2-3 months. There is a range of methods being used to assess adherence, but most programs use a combination of self-report of pills missed during the last 3 days, and pill counts. Pill counting is most often performed by the pharmacist dispensing the medication, or by outreach/community health workers for programs in which some follow up and adherence support are done in the community. Adherence may also be assessed by clinicians (nurse or physician). Pharmacists are required to maintain records on drugs dispensed and clients' adherence to treatment. The standardization of adherence measurement has been problematic. There are currently no standardized guidelines, and therefore programs and facilities use a combination of methods, and/or apply them somewhat differently.

8.7 Removal of barriers to continuous access to care

The government of Uganda is committed to increase access to ARV drugs, including mobilization of national and international resources for the provision of ART services. The government through the Uganda AIDS commission, developed a National Strategic Plan (NSP) with clear objectives for care and treatment; these include increasing access to ART and non-ART care; scaling up HIV counseling and testing to facilitate universal access to treatment and also integrating HIV prevention into all care and treatment services.

The ART policy provides a strategy for initiating and expanding public sector provision ART services with increased equity. Strategies of reduction of barriers to continuous access to care have included moving services closer to the people through a phased scale-up of services to lower level health facilities and provision of free ARVs among others. Currently over 80% of all HC IV are providing ART. Provision of free ARVs has been achieved through support from both international and local development partners. This has been enhanced by the steady reduction in the costs of ARVs resulting from negotiations with pharmaceutical companies and competition from generic drug manufacturers. The government also allocates funds for procurement of ARVs.

Chapter Nine: Experience with Resistance to Second-line ART

From other studies elsewhere, approximately 22% of patients receiving second-line therapy do not achieve HIV RNA suppression by 6 months, 23.1% at 12 months, 26.7% at 24 months and 38% at 36 months. Poor adherence, rather than HIV DR is driving most of the VF to second line. It is estimated that major protease inhibitor resistance at the time of second line VF ranges between 0 -50%, but studies are limited in Africa.

We conducted a cross sectional study in an MRC longitudinal cohort (COLTART) where we had 956 patients on ART for > 6 months from 2 MRC cohorts. 81% were on ART for more ≥ 9 years. We found that 119 had VL ≥ 1000 copies/ml. We successfully genotyped 110 (74 on 1st line, 36 on 2nd line) [31]. For the 36 who were on second line, only 7 had PI resistance. In another study, in collaboration with colleagues at MSF-Arua (Epicentre-MSF) we studied HIVDR associated with 2nd line failure. There were 78 pts failing 2nd line ART (2 consecutive VL ≥ 1000 copies/ml), we genotyped 70 and found, 18.5% had ≥ 1 major PI mutation, 82.8% ≥ 1 NRTI mutations. 30 (42.8%) were switched to 3rd line composed of integrase inhibitor and PI (60% darunavir) +/- NRTI [32].

Another PASER study looked at HIV-1-infected adults on PI-based second-line ART for >180 days [33]. They assessed risk factors for having a detectable viral load (VL, ≥ 400 cps/mL) using Cox models. If VL was ≥ 1000 cps/mL, genotyping was performed. Of 227 included participants, 14.6%, 15.2% and 11.1% had VLs ≥ 400 cps/mL at 12, 24, and 36 months, respectively. Risk factors for a detectable VL were as follows: exposure to nonstandard nonnucleoside reverse-transcriptase inhibitor (NNRTI)-based (hazard ratio, 7.10; 95% confidence interval, 3.40-14.83; $P < .001$) or PI-based (7.59; 3.02-19.07; $P = .001$) first-line regimen compared with zidovudine/lamivudine/NNRTI, PI resistance at switch (6.69; 2.49-17.98; $P < .001$), and suboptimal adherence (3.05; 1.71-5.42; $P = .025$). Among participants with VLs ≥ 1000 cps/mL, 22 of 32 (69%) harbored drug resistance mutation(s), and 7 of 32 (22%) harbored PI resistance. They conclude that although VL suppression rates were high, PI resistance was detected in 22% of participants with VLs ≥ 1000 cps/mL. To ensure long-term ART success, intensified support for adherence, VL and drug resistance testing, and third-line drugs will be necessary.

The above studies therefore confirm previous reports from elsewhere that many patients failing 2nd line do not have resistance to PIs and it may be an issue of adherence or resistance to NRTIs. These studies also highlight the need for resistance testing to guide treatment and unnecessary switch to 3rd line. The WHO guidelines are silent on resistance testing after 2nd line failure in LMIC. "In resource-constrained countries, where drug resistance tests are commonly not available, clinicians are supposed to follow the WHO recommendation which proposes the combination of darunavir/ritonavir and one HIV integrase inhibitor as "empirical" third-line ART". However, in Uganda based on our findings and from elsewhere, new guidelines indicate that before anyone is switched to 3rd line ART, they should have resistance profiling test done to confirm PI resistance and to determine the most optimal regimen.

For the above reason the UVRI and JCRC laboratories have been requested to genotype all patients in the country before switch to 3rd line. Viral load is centrally performed at Central Public Health Laboratories using DBS.

In the UVRI laboratories, DBS samples from 277 participants were received between April 2017 and March 2018. One hundred and ninety-four (194, 70.0%) were successfully genotyped and data from all of them were included in the analysis. About fifty-one percent (51.1 %) were female and median age was 24 years (IQR=12 – 38). 167 (86.1%) had any DRMs, 161 (83.0%) had NNRTI, 148 (76.3%) NRTI and 51 (26.3%) PI DRMs. About 70% had both NRTI and NNRTI mutations and 22% had multi-class DRMs. For those with PI mutations, darunavir was the only PI with the highest remaining susceptibility; majority had high-level resistance to atazanavir and lopinavir, the PIs currently used in second-line regimens.

These patients are only tested for VL after intensive adherence counselling, 3 sessions at least one month apart and the patients should have more than 95% (good) adherence.

Individualized genotype resistance testing is therefore necessary for optimization of treatment regimens. Sub-optimal adherence in more than 10% of patients with virological non-suppression without DRMs could be contributing to treatment failure. One other challenge is the use of DBS which has less genotype success than plasma especially with low VL.

At JCRC 1471 patient plasma samples were received of which 1159 were successfully tested. Of these, 400 had resistance to protease inhibitors. Those which failed to amplify were 312 (around 50% of these had a viral load of less than 1000 cp/ml, the cut off point for HIV-1 drug resistance testing). About 922 (79.6%) samples had resistance to at least one antiretroviral drug.

The results accumulated will also be used to estimate the national need for third - line ARVs in Uganda and to determine the prevalence of HIVDR among patients failing second-line ART in Uganda.

There may also be a need to learn from other countries as we implement the third-line committees that advice on when to switch and to which regimens, including Stanford scores as used in S. Africa for efficient patient management.

Chapter Ten: Discussions

In this chapter, we discuss the implications of the findings from the past 10 years of the implementation of the HIVDR prevention, monitoring, surveillance activities.

As described in this report, we have succeeded in implementing the major components of the National plan with coordination at the UVRI and guided by the TWG, MOH, WHO, CDC and other partners. We have conducted five EWI surveys and eight TDR surveys, while one other TDR survey has been conducted by other partners. Surveys for pre-treatment and acquired HIVDR have been conducted in adults and children, including one that has used a nationally representative sampling to estimate pre-treatment and acquired DR. A national HIVDR reference laboratory was designated at UVRI, later obtaining WHO certification and the laboratory at JCRC was also certified. We have participated in different global activities including contributing to the WHO Global reports.

We have regularly held stakeholder meetings where results have been presented, recommendations made some resulting into new policy formulations and influencing ART programme practice. Below are some of the implications of our work.

Monitoring Early Warning Indicators

Though ARV resistance is inevitable, there are specific drivers of accelerated resistance some also termed as EWI. The most important driver of resistance selection and spread in Uganda is attributable to intermittent drug supply that leads to stock outs; use of sub-optimal regimens; ARV associated toxicity, weak health systems (human resources, quality systems) and inadequate virological monitoring. Prominent drivers at the individual level include; poor adherence to treatment, poor retention and previous use or exposure to ARVs. Among pediatric cases, HIVDR before first line ART initiation is associated with history of PMTCT exposure through preventive therapy and maternal ART during breast-feeding as well as sub-optimal regimens in children.

Data from the five EWI surveys shows that clinic review appointment keeping, on-time drug pick up and continuity of drug supply manifest the most profound weaknesses at the programme level. Furthermore, the 2017 survey indicated that though most patients were suppressing, there remains a challenge in ensuring that all patients who are eligible for VL testing have the test performed. These challenges arise from constraints affecting the entire health system e.g. inadequate human resources, information systems, supply chain management systems and partnerships. These will require increased attention.

Pre-treatment and Acquired HIVDR

In drug naïve individuals, with no history of ARV drug exposure, TDR is the main reason for DR. At population level, Uganda is experiencing moderate levels of transmitted DR (i.e. 5%-<15% to NNRTI and NRTI, <5% to PIs) especially around Kampala. There was however one

study that showed a high NNRTI prevalence in Entebbe. Well-functioning ART programmes should result in TDR remaining in the category of <5 to each drug in the first line therapy [34]. The way to address this is first preventing new infections in both adults and children, but also ensuring DR is prevented. It is however also important to note that in some of these studies, drug exposure could not be ruled out. Furthermore, some of the WHO protocols, used proxy criteria for recent infection e.g young prime gravidae in ANC and young adolescents in VCT clinics; some may not be recently infected.

The survey method using truncated sequential sampling which provided a prevalence classification of TDR in a specific geographic area is no longer recommended by WHO. WHO and partners are working to develop a new concept note which will provide a national statistic of TDR in recently infected populations.

On the other hand, the prevalence of pre-treatment HIVDR (PDR) is substantially higher in Uganda, where ARVs were first available, compared with other African countries. Overall, PDR appears to be increasing in LMIC. PDR is detected in ARV drug naive people initiating ART or people with prior ARV drug exposure(s) initiating or reinitiating first-line ART. PDR is either transmitted or acquired drug resistance, or both.

Our early experience on second line failures indicates that in addition to intensified adherence support and VL, genotyping is useful. Most of those failing do not have resistance to the PIs which could have unnecessarily been substituted. However, this program is challenged by DBS sample handling and the higher VL required for successful DBS genotyping.

The results presented in this report indicate that the development of ARV resistance in Uganda has led to a reduction in drug options for individual patients. Further still, the transmission of drug resistant strains is of growing concern for it represents a threat to effective use of low cost ARVs for treatment, PMTCT regimes for both mothers and babies and post exposure prophylaxis. The rapid emergence of resistance always complicates further efforts to control viral replication especially when therapeutic options are limited. When resistance occurs, it takes longer to reach VS.

Whereas clinical and immunologic monitoring using CD4 counts was used in the identification of failure to first line ART during scale up years, it was not a reliable approach. This is because it is associated with prolonged duration of virological failure, which in turn, is associated with higher frequencies and complexity of drug resistance mutation (DRM) patterns. The country is currently making progress in using the preferred method of VL monitoring.

HIV DR testing in HIV care

Currently in Uganda, DR testing is not routinely done for people initiating ART. PDR surveys will continue to provide information on the best regimens to use as first line. For patients failing first line, a public health approach is used to switch them to second line. However, for patients failing second line drugs, HIVDR testing is done prior to switching to third line. The current

consolidated guidelines for prevention and treatment of HIV recommend genotyping for patients failing on second –line antiretroviral therapy. Samples from patients with VF confirmed from the national viral load program laboratory at CPHL are shipped to UVRI and JCRC for testing on a monthly basis and results are submitted to the national committee responsible for switching patients to third-line regimens. The national viral load scale-up program started in August 2014 while the second line genotyping program started in September 2017.

Since the HIVDR plan was initiated, there have been a number of steps and policies that have come on board relevant for prevention of HIVDR with the most recent being the use of viral load monitoring. With the roll out of viral load monitoring in the country, clinicians are encouraged to make timely switches in order to avoid accumulation of drug resistance mutations.

Research on HIVDR

There has been a rise in HIVDR research carried out by the different HIV partners in Uganda. The HIVDR TWG provides a platform for reviewing data from completed research projects and developing policy recommendations. However, some research findings have not been shared on this platform there is therefore, a need for developing an inventory of all on going HIVDR monitoring and prevention activities in the country and coordinate dissemination of findings to stakeholders.

Finally, as Uganda implements the Test and Treat together with the roll out of PrEP, the number of people on treatment is on the increase. The national HIVDR activities will therefore require more attention and support but also to ensure that data acquired from these activities is shared and where appropriate translated into better service delivery.

Chapter Eleven: Recommendations

In this chapter, some of these recommendations have already been implemented and a few others are less relevant due to the changes to the treatment guidelines.

Table 5: Recommendations and status of implementation

Recommendation	What has been implemented	Comments
Medical records to facilitate routine HIVDR Early Warning Indicator abstraction		
Recommendation: Harmonize routine medical records to facilitate routine HIVDR Early Warning Indicator abstraction at ART facilities	EWIs Indicators: <ul style="list-style-type: none"> Retention in care; % of patients retained on ART 12 months after ART initiation <ul style="list-style-type: none"> 12-month Retention data is now routinely collected in DHIS-2 and HMIS 106a Viral load suppression; % of patients with viral load <1000 copies/mL 12 months after ART initiation <ul style="list-style-type: none"> Incorporated into the HMIS 106a for national reporting A non-suppressed viral load register is used to track patients with non-suppressed viral loads for follow-up Viral load coverage; % of patients with a 12-month viral load test result available 	<ul style="list-style-type: none"> In addition, CPHL has a VL dashboard that captures the number of samples received, tested, suppression and rejection rate This is also captured in HMIS form 113 at facility level and uploaded into DHIS-2

Recommendation	What has been implemented	Comments
	<ul style="list-style-type: none"> ○ Incorporated into the HMIS 106a for national reporting. In addition, CPHL has a dashboard accessible to all • Pharmacy stock outs; % of months with any day(s) of stock-out of any routinely dispensed ARV drug <ul style="list-style-type: none"> ○ Stock outs are captured through the Real time ARV stock-status (RASS) which has been rolled out in selected districts but will be rolled out nationally 	<ul style="list-style-type: none"> • A web-based ARV ordering system (WAOS) has been implemented at district level to enable timely ordering of drugs
Routine VL monitoring for all individuals on ART		
<ul style="list-style-type: none"> • Routine viral load monitoring for all on ART. • VL monitoring at 6 and 12 months after ART initiation instead 	<ul style="list-style-type: none"> • Routine VL monitoring started in Aug 2014 • The MOH revised the VL algorithm to provide for 12 monthly VL monitoring for newly 	<ul style="list-style-type: none"> • Data are now captured on the HMIS 106a and the CPHL dashboard • VL campaigns spearheaded by MOH with support from PEPFAR improved coverage from 47% in

Recommendation	What has been implemented	Comments
of 18 months	initiating individuals in the revised 2018 guidelines	<p>2016 to 75% in 2017</p> <ul style="list-style-type: none"> This recommendation follows WHO guidance on global reporting of EWIs
Provision of Standard Medical records for capturing data to enhance ART monitoring		
<ul style="list-style-type: none"> Provision of adequate clinic records and tools for ART monitoring for private for profit and public health facilities 	<ul style="list-style-type: none"> MOH has printed data monitoring and capturing tools for all facilities (private and public) providing HIV services 	<ul style="list-style-type: none"> These include:- the blue ART card, ART registers, VL/HIVDR requisition form etc.
<ul style="list-style-type: none"> Roll out of standardized and harmonized electronic medical records system 	<ul style="list-style-type: none"> The MOH with support from Development partners rolled out the use of electronic medical records (OpenMRS) in high volume sites to capture client information 	<ul style="list-style-type: none"> Lower level health facilities (low volume sites) are to be supported by Implementing partners
Engage the private and public sectors to align with national guidelines		
<ul style="list-style-type: none"> Strengthen private and public sector training on regulation, use of optimal regimens and sensitization of new drugs 	<ul style="list-style-type: none"> MOH /ACP is in charge of writing and revising HIV treatment guidelines as well as sensitize the public on the use optimal regimens and new drugs MOH has National ART committee that advises the MOH on the benefits of new drugs 	<ul style="list-style-type: none"> MOH with support from Implementing partners roll out revised guidelines and train both private and public sectors
<ul style="list-style-type: none"> The MOH should intensify efforts to harmonize the practices of all 	<ul style="list-style-type: none"> The National ART committee meets regularly to discuss upcoming issues, 	<ul style="list-style-type: none"> Committee has academia, MOH, researchers, Implementing partners,

Recommendation	What has been implemented	Comments
programmes to align with national recommendations	provides recommendation and updates the guidelines regularly	and Development partners
<ul style="list-style-type: none"> Standardized guidelines for assessment of adherence for tracking HIVDR and treatment outcomes 	<ul style="list-style-type: none"> The MOH developed an adherence strategy that clearly outlines how ART adherence should be assessed and documented on the ART card 	<ul style="list-style-type: none"> Unfortunately, much as this indicator is collected it is not captured on the HMIS 106a for national reporting
Provision of human resource to support the HIV program		
<ul style="list-style-type: none"> Strengthen human resources through recruitment; training, mentoring and task shifting 	<ul style="list-style-type: none"> The national and district health service commissions have hired health care workers in an effort to fill vacant positions to attain the recommended staffing norms. PEPFAR implementing partners have also beefed up these efforts Task shifting has taken root and nurses have been trained to prescribe ARVs and treat opportunistic infections. In addition, partners have built capacity of expert clients to be lay counselors Village health teams are facilitated by implementing partners track defaulters, DSDM have been 	<ul style="list-style-type: none"> The MOH ACP team as well district health teams provide supportive supervision to ensure the program runs smoothly

Recommendation	What has been implemented	Comments
	adopted, guidelines developed and is being rolled out	
Timely provision of HIV commodities to facilities		
<ul style="list-style-type: none"> The National Medical Stores should intensify efforts to ensure timely delivery of commodities to facilities 	<ul style="list-style-type: none"> PSM has been rationalized to provide for three channels of distribution i.e. NMS for the public sector, MAUL and JMS for the private sector The three warehouses conform to the supply chain rationalization ensuring continuity of supplies at the facilities under their jurisdiction 	
Funding for ARV drugs		
<ul style="list-style-type: none"> Focused advocacy efforts to raise funding for ARV drugs used in the country. 	<ul style="list-style-type: none"> There has been an increment in funding for HIV drugs in Uganda from several donors e.g. GF, PEPFAR, CHAI, and GOU. However, there is still a gap in the public sector 	<ul style="list-style-type: none"> More advocacy is still required to increase funding for ARVs
Appropriate ARV regimens for different subpopulations		
<ul style="list-style-type: none"> Use of PI based regimens as first line for HIV infected infants especially with prior PMTCT exposure 	<ul style="list-style-type: none"> MOH rolled out use of Lopinavir pellets as first line for HIV positive infants <3 years country - wide in 2016 	<ul style="list-style-type: none"> This recommendation follows high prevalence of HIVDR especially in infants with prior exposure to PMTCT
<ul style="list-style-type: none"> Use of Tenofovir in standard first 	<ul style="list-style-type: none"> The MOH revised the national treatment 	<ul style="list-style-type: none"> Due to its tolerability and cost-effectiveness,

Recommendation	What has been implemented	Comments
regimens	guidelines and adopted use of Tenofovir as first line in 2013	TDF has been found to be safe in 2-12 years old and has good safety profiles in pregnancy. It has a high genetic barrier to resistance and is recommended in non-sub type C dominated countries like Uganda
<ul style="list-style-type: none"> Use of Integrase strand inhibitors (Dolutegravir) as first line due to high Pre-Treatment HIVDR to NNRTIs in the country 	<ul style="list-style-type: none"> MOH has adopted DTG-based regimens as the preferred first line ART regimen in women >50 years, adolescent and adult men; and as an alternative second line in the above eligible groups 	<ul style="list-style-type: none"> Women of reproductive age are currently not eligible for DTG regimens until further guidance is obtained
Routine HIVDR testing for clients failing second line		
<ul style="list-style-type: none"> Routine HIVDR testing for individuals failing 2nd line ART regimens 	<ul style="list-style-type: none"> The MOH included routine HIVDR testing in the revised 2016 national guidelines at UVRI and JCRC In addition, MOH has constituted a 3rd line committee that reviews genotype results and patient history notes to determine appropriate 3rd line drugs 	<ul style="list-style-type: none"> Capacity building for clinicians at Regional referral hospitals is ongoing to empower them switch clients to 3rdline ART
Pharmacovigilance and registration of ARV drugs		
<ul style="list-style-type: none"> Integrate Pharmacovigilance, and drug quality assurance 	<ul style="list-style-type: none"> NDA conducts Inspection, Verification, Mandatory testing and Registration of ARVS. In addition, the NDA 	

Recommendation	What has been implemented	Comments
into the ART program	<p>has a post market surveillance unit that collects drugs on the market to test for quality</p> <ul style="list-style-type: none"> The National Drug Authority has a pharmacovigilance unit that is responsible for monitors and tracks adverse events to ARVs 	
Validating Point of Care VL assays		
<ul style="list-style-type: none"> Validate simple and affordable POC VL assays, including (semi) quantitative test that can identify virologic failure 	<ul style="list-style-type: none"> There are on-going validation studies for POC VL platforms in country 	

Table 6: HIVDR TWG Recommendations yet to be implemented.

Recommendation		Comment
Early Warning Indicator abstraction at ART facilities		
<ul style="list-style-type: none"> Appointment keeping and On time ARV-drug pick up; % of patients that pick-up ART no more than two days late at the first drug pickup after a defined baseline pick-up 	<ul style="list-style-type: none"> The MoH blue ART card, the appointment book and ART register have a provision for client appointments. The client blue ART card has a provision for number of pills and days of ARVs dispensed 	Despite the efforts stated there is no national record to capture appointments and on time ARV pick up

Chapter Twelve: Conclusion and Way Forward

In conclusion, with the widespread use of ART, there is a need to routinely monitor and prevent HIVDR. HIVDR prevention requires strong programmatic implementation to ensure commodity security, drug adherence and patient retention. The country now has better capacity for DR prevention including routine laboratory VL monitoring.

The emergency of first line DR among ART pre-treatment patients has transformed the landscape of HIV treatment that has required us to change ART guidelines. Without strengthening systems, we risk losing effectiveness of our new drug regimens.

As a way forward, we need to endorse as a country the WHO Global Action Plan on HIV DR which describes activities that will be required to prevent HIVDR from undermining efforts to achieve the global targets on health and HIV. To achieve this, HIVDR prevention, monitoring and surveillance activities will have to be part of the ART scale up country plan and this will require financial commitment from government and development partners.

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