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Short Communication

The Pan Degenerate Amplification and Adaptation (PANDAA) assay: a solution for HIV-1 drug resistance testing in a resource limited setting?

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Introduction

In resource limited settings (RLS), the resources and capacity to perform standard genotype resistance testing for the management of antiretroviral therapy (ART) drug resistance in People Living With HIV/AIDS (PLHIV) are limited [1,2]. Therefore, in most instances, ART drug switches are based on unreliable clinical and laboratory data [3-5]. Thus, such switching may occur unnecessarily or individuals may be switched to sub-optimal treatment, leading to the accumulation of drug resistance mutations (DRMs) [6,7]. To address the issues (limited laboratory capacity, high costs of genotyping and shipping amplicons to commercial laboratories for sequencing) and the growing problem of HIV DRMs; several point mutation assays (PMAs) have been developed and implemented for the detection of HIV DRMs against non-nucleotide reverse transcriptase inhibitor (NNRTI)-based first-line ART regimens, following the 2017 WHO HIV resistance network recommendations [8]. The implementation of PMAs, with reduction in cost, complexity and time may help to expand access to HIV resistance testing for the clinical care of PLHIV in many RLS, including Zimbabwe.

The oligonucleotide ligation-based assay (OLA), developed to target specific NNRTI DRMs of high clinical relevance (K103N, Y181C,V106M and G190A) [9] was previously implemented in Kenya and Zimbabwe [10,11]. In Zimbabwe, OLA was successfully used for detecting nevirapine (used as prophylaxis for prevention of mother-to-child-transmission of HIV) associated HIV DRMs from infected infants participating in the Zimbabwean Early Infant Diagnosis Programme [11]. Mutsvangwa et al. concluded that the use of OLA for the detection of HIV DRMs from dried blood spots (DBS) could improve HIV care among HIV-1 infected infants in RLS. Since then, the OLA has been effectively used in the country to address the extent of HIV DRMs in children with the intention of improving the prevention of mother-to-child-transmission and reducing the HIV burden.

Another innovative and focused HIV genotype resistance testing technique, developed to identify specific mutations affecting susceptibility to the HIV reverse transcriptase (K65R, M184VI, K103NS, Y181C, V106M and G190AS) in RLS is the Pan Degenerate Amplification and Adaptation (PANDAA) assay [12]. The PANDAA assay, using quantitative polymerase chain reaction (qPCR) technology, was first used at the Botswana Harvard AIDS Institute, Gaborone, Botswana [12]. Similarly, we have recently demonstrated that PANDAA was successful in detecting these acquired DRMs (K65R, M184VI, K103NS, Y181C, V106M and G190AS) among plasma samples from adolescents and young adults (aged 10 to 24 years) failing ART in Harare, Zimbabwe [13]. In our study, PANDAA showed high sensitivity (98%) and specificity (94%) and a strong agreement with the gold standard Sanger sequencing.

The potential advantages of the PANDAA assay, compared to standard Sanger sequencing for RLS, include assay simplicity, faster turnaround time, lower cost, and more manageable equipment requirements. The PANDAA assay and analysis software are user-friendly. Hence, we concluded, in our study that PANDAA could represent a simple and rapid alternative HIV resistance assay in RLS.

Additionally, in our lab, PANDAA also showed a good agreement (kappa(95%CI) = 0.93 (0.78-0.98)) compared to Sanger sequencing, when screening for pre-treatment drug resistance (K65R, M184VI, K103NS, Y181C, V106M and G190AS) among HIV-1 infected ART-naïve individuals or in people reinitiating first-line ART (18 years or older) in Harare, Zimbabwe (Kouamou V et al., manuscript in preparation).

We recognize however that in our study [13], PANDAA was successfully carried out on plasma specimens in a well-resourced, urban Zimbabwe laboratory. The need for a qPCR instrument and a stable power supply remains a challenge for rural areas in Zimbabwe. These regions rely on centralized, urban laboratories to perform assays such as viral load (VL) and HIV genotype resistance testing [5]. In these rural areas, the challenges of electricity availability for plasma preparation and storage, and the status of the roads for satisfactory transportation make plasma an unreliable specimen of choice for these assays [5]. To mitigate the problem of plasma samples in these regions, DBS offer an alternative specimen type for HIV VL measurement and genotype resistance testing.

Dried blood spots have previously been used as an alternative sample type to plasma for HIV-1 ribonucleic acid quantification in Zimbabwe [14-16]. However, DBS specimens have not yet been assessed for genotype resistance testing using the PANDAA assay. Therefore, optimizing the PANDAA assay for DBS samples would address one of the real problems that remote areas in RLS face day-to-day.

To fully address these real-world setting challenges, an ideal HIV genotype resistance assay for use in RLS should be designed to integrate VL and genotype resistance testing into point-of-care (POC) PMAs, in which the VL is measured first and drug resistance testing is triggered automatically in individuals with viral failure, as per the 2017 WHO HIV resistance network report [8]. The assay should be rapid, simple, affordable, inexpensive and with easy-to-interpret results. In line with this, a promising and commendable POC HIV genotype resistance assay has recently been developed and validated [17]. The OLA-Simple method is a lateral flow detection system, designed to be read manually, with in-house software which provides guidance for inexperienced users [17]. Such novel technologies could reduce costs, encourage decentralization and represent a potentially important step forward for HIV genotyping in remote areas.

However, there are concerns which arise with the implementation of these PMA or POC HIV genotyping assays, which target NNRTI DRMs. These concerns include the minimal role that they may play in the context of the HIV integrase inhibitor dolutegravir (DTG) as many RLS, including Zimbabwe, are transitioning to a dolutegravir-based treatment regimen, as per the recent WHO guidelines [18]. Consequently, these assays could have become less relevant in the context of DTG, which has a high genetic barrier to resistance [19]. However, it is crucial in the future for these PMAs to include primers targeting the integrase region so as to assess virologic failure on a DTG-based regimen, especially in RLS where VL monitoring is limited.

Additionally, based on the recent findings from the ADVANCE study, these PMAs may still remain crucial as the authors raised a concern about the implications of these DRMs for DTG efficacy [20]; they noted the significant contribution pre-treatment NNRTI resistance may have for the efficacy of DTG. These findings call for urgent consideration in the many RLS, with high levels of pre-treatment NNRTI resistance, who are transitioning to DTG-based regimens. Such is the case for Zimbabwe where we have recently demonstrated high levels (29%) of pre-treatment NNRTI resistance among ART-naive adults initiating or re-initiating first-line ART containing DTG [21]. Therefore, more studies are needed to understand the impact of circulating pre-treatment NNRTI resistance on virologic outcomes among people on DTG-based regimens. If the findings from the ADVANCE study are confirmed there will be an urgent need for pre-treatment NNRTI resistance testing, using PMAs, to optimize first-line regimens containing DTG in many RLS.

Conclusion

Point mutation assays, including PANDAA, present opportunities for rapid and simplified HIV genotyping. However, an ideal HIV genotype resistance testing system for use in RLS would be a low-cost POC assay, which uses DBS specimens, so allowing de-centralization of DRM analysis.

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