นิพนธ์ต้นฉบับ Original Article

Use of dried blood spot specimens for in-house HIV drug resistance genotyping assay in HIV-exposed infants in Thailand

การใช้กระดาษซับเลือดเพื่อตรวจเชื้อเอชไอวีดื้อยาต้านไวรัสด้วยวิธีที่พัฒนาขึ้นในทารกในประเทศไทย

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Abstract

The prevention of mother-to-child transmission (PMTCT) of HIV through highly active antiretroviral therapy (HAART) administered to infants in Thailand presents challenges for HIV drug resistance (HIVDR) testing due to limited access to infant blood samples and limited assay capacity for detecting HIV treatment failure. This study aims to develop and evaluate the in-house HIVDR genotyping assay using DBS and to determine the prevalence of HIVDR mutations in newly diagnosed HIV-infected infants. DBS specimens were collected from infants following the national guidelines for early infant diagnosis (EID) of HIV from January 2014 to January 2017. A total of 12,385 DBS specimens were collected, and only 111 samples from 69 individuals with HIV-positive results were genotyped for protease (PR) and reverse transcriptase

(RT) genes. The sensitivity of this DBS assay was 2.5 copies per reaction, and the specificity of DBS samples was 100%. The success rate of genotyping was 79.3% (111 out of 140 specimens) for DBS samples, regardless of viral load. Ten (14.4%) of 69 infants exhibited major drug resistance-associated mutations in the PR and RT genes. The T215A, T215S, and T215I substitutions are revertant mutations at codon 215 mutations that confer increased risk of virological failure of zidovudine, and these mutations were frequently observed. Mutations conferring resistance to non-nucleoside reverse transcriptase inhibitors (NNRTIs), including Y181C, K103N, and G190A, were found. The predominant HIV subtype observed was CRF01_AE, accounting for 91.9% of cases. The limitation of this study is the lack of viral load information. Nonetheless, our results provide reassurance regarding the use of the in-house genotyping assay with DBS specimens for monitoring HIVDR among infants in Thailand. The identified major mutations conferring resistance to NRTIs and NNRTIs are relevant to the PMTCT regimen in infants and their mothers. This study highlights the importance of implementing HIVDR testing in newly diagnosed infants to enhance the effectiveness of treatment.

บทคัดย่อ

การป้องกันการแพร่เชื้อเอชไอวีจากแม่สู่ลูก ในเด็กทารกด้วยการใช้ยาต้านไวรัส highly active antiretroviral therapy (HAART) เป็นความท้าทายในการตรวจเชื้อเอชไอวีดื้อยาต้านไวรัส เนื่องจากการได้มาของเลือดเด็กทารกและข้อจำกัดในการตรวจวิเคราะห์เชื้อเอชไอวีที่ดื้อยา วัตถุประสงค์การศึกษานี้เพื่อพัฒนาและประเมินวิธีตรวจเชื้อดื้อยาที่พัฒนาขึ้นบนกระดาษซับเลือด (dried blood spot, DBS) และเพื่อเฝ้าระวังความชุกของเชื้อดื้อยาในทารกที่พึ่งติดเชื้อเอชไอวี วิธีการศึกษา นำ DBS จากทารกที่เก็บนำส่งในปี พ.ศ. 2557-2560 เพื่อส่งตรวจวินิจฉัยเชื้อเอชไอวีในทารกระยะเริ่มแรก ผลการศึกษา มี DBS ทั้งหมด 12,385 ตัวอย่าง และมีเพียง 111 ตัวอย่างจากเด็กทารก 69 คน ที่ให้ผลบวกต่อการตรวจเอชไอวี และต่อมาได้นำไปตรวจหาเชื้อเอชไอวีดื้อยาต้านไวรัสที่จำเพาะต่อยีน protease และยีน reverse transcriptase ความไวสำหรับการตรวจเชื้อเอชไอวีบนกระดาษซับเลือดวิธีนี้ คือ 2.5 copies/ปฏิกิริยา และความจำเพาะคือร้อยละ 100 อัตราความสำเร็จของการตรวจเชื้อดื้อยาบน DBS คือ ร้อยละ 79.3 (111 จาก 140 ตัวอย่าง) โดยไม่คำนึงถึงปริมาณไวรัส ความชุกของเชื้อดื้อยาในเด็กทารกพบร้อยละ 14.4 (10 จาก 69 คน) การกลายพันธุ์ที่ตำแหน่ง T215A, T215S และ T215I เป็นตำแหน่งที่พบได้บ่อยและสัมพันธ์กับการดื้อยา zidovudine นอกจากนั้นพบการกลายพันธ์ที่ตำแหน่ง Y181C, K103N และ G190A ซึ่งสัมพันธ์กับยา non-nucleoside reverse transcriptase inhibitors (NNRTIs) สายพันธุ์ของเชื้อเอชไอวีที่พบมากที่สุด คือ CRF01_AE พบร้อยละ 91.9 ข้อจำกัดของการศึกษานี้คือไม่ทราบค่าปริมาณไวรัส ผลการศึกษานี้ให้ความมั่นใจการตรวจเชื้อดื้อยาที่พัฒนาขึ้นสามารถนำมาใช้กับกระดาษซับเลือดเพื่อติดตามเชื้อเอชไอวีดื้อยาในทารกในประเทศไทย การพบเชื้อกลายพันธ์ที่ดื้อต่อยา NRTI และ NNRTI ซึ่งเป็นยาต้านไวรัสที่ใช้การป้องกันการแพร่เชื้อจากแม่สู่ลูกทั้งในทารกและมารดา การศึกษานี้แสดงให้เห็นถึงความจำเป็นในการตรวจเชื้อดื้อยาในทารกเพื่อให้การรักษาด้วยยาต้านไวรัสมีประสิทธิภาพมาก

คำสำคัญ
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Keywords
HIV drug resistance, dried blood spots, HIV-exposed infants

Introduction

The Thai national policy for the prevention of HIV transmission from mothers to children was established in 2014, aligning with World Health Organization (WHO) recommendations.[1,2] This policy emphasizes the use of lifelong highly active antiretroviral therapy (HAART) regimens. Since the launch of this program, the prevalence of HIV-positive infants has dramatically decreased from 20.0% in 1990 to 1.8% in 2016. Antiretroviral resistance is observed among HIV-1-infected individuals who have received suboptimal treatment regimens and in those initially infected with HIV-1 strains carrying resistance mutations. Genotypic drug resistance testing plays a crucial role in selecting optimal treatment regimens for HIV-infected individuals. In Thailand, such testing is routinely employed to guide second-line antiretroviral therapy (ART) following first-line treatment failure. As the use of drug resistance (DR) testing in clinical practice increases, the reproducibility of drug resistance-associated mutations (DRMs) is vital for accurately assessing clinical resistance to HIV. WHO recommendations and national policies on HIV prevention of mother-to-child transmission (PMTCT) of HIV are continuously updated, with the most recent guideline[2] widely adopted since 2021.

Genotypic assays detect drug-resistance mutations in relevant viral genes; in general, these assays require a plasma viral load of at least 500 to 1,000 copies/mL. Most genotypic assays involve conventional Sanger sequencing of the reverse transcriptase (RT), protease (PR), and integrase (IN) genes of circulating RNA in plasma to detect mutations that are known to confer drug resistance. However, standard genotypic resistance assays have low success rates in specimens with low viral loads.

Dried blood spots (DBS) are widely accepted for the collection, transportation, and storage of blood samples to detect HIV proviral DNA in pediatric patients. HIV-1 proviral DNA is present in 33-80% of DBS samples. The interference of proviral DNA may vary according to treatment status and viral load level. Several publications have showed that genotypic drug resistance testing using DBS are feasible and comparable with those plasma samples.[3,4] Nevertheless, limitations remain with the lower limit of quantification than that of plasma and the interference of intracellular DNA, which depend on the method used for nucleic acid extraction or the assay along with the overall experience of the laboratory using DBS. Amplification efficiency was reduced in DBS specimens with low viral loads.

Our HIVDR genotyping laboratory has been accredited by ISO15189 and WHO since 2009. We developed an in-house HIV drug resistance (HIVDR) genotyping assay to detect various subtypes, including CRF01_AE, CRF02_AG, B, C, D, CRF07_BC, CRF15_01B, CRF33_01B, and CRF34_01B. While plasma with a viral load (VL) >750 copies/mL was used, obtaining plasma from infants is technically challenging. Therefore, we utilized DNA aliquots from DBS samples, which were used for both HIV diagnosis and HIV genotyping testing. This study aimed to investigate the use of DBS as a replacement for plasma to determine the prevalence of HIVDR mutations. Our objective is to monitor the patterns of HIV drug resistance in newly diagnosed infants, a task that has not previously been undertaken in Thailand. Additionally, this study aimed to validate the feasibility of employing DBS for an in-house HIVDR genotyping assay and to assess the prevalence of HIVDR mutations in HIV-infected infants.

Materials and Methods

1. Ethical statement.

Patient information was de-identified and re-coded to prevent any linkage to individual participants. The study protocol received review and approval from the Ethics Committee of the Department of Medical Sciences, Thailand (Study Code No. 2/2560).

2. Patients and samples.

A retrospective cross-sectional study was conducted from June 2014 to February 2017, involving children under 18 months old born to HIV-1-infected mothers. The national PMTCT guidelines[5] for 2014 are summarized in Table 1.

Table 1. Thailand Prevention of mother-to-child transmission (PMTCT) guidelines in 2014

	Risk of mother-to-child transmission	
	Standard risk	**High risk**
PMTCT regimen in pregnant women	First line: TDF+3TC+EFV Second line: AZT+3TC+LPV/r or TDF+3TC+LPV/r	First line: TDF+3TC+EFV Second line: AZT+3TC+LPV/r or TDF+3TC+LPV/r
Mother	VL ≤50 copies/mL before delivery	VL >50 copies/mL before delivery
	If no VL, use history of ANC and regularly takes HAART >12 weeks before delivery	If no VL, use history of not regularly takes HAART ≤12 weeks before delivery If unidentified, but no ART treatment (additional)
Early infant diagnosis (HIV DNA PCR test)	1, 2-4 months (2 times)	At birth, 1, 2, 4 month (4 times)
Infant prophylaxis	AZT for 4 weeks after birth	AZT/3TC/NVP for 6 weeks after birth

Abbreviations: TDF, Tenofovir; 3TC, Lamivudine; EFV, Efavirenz; LPV/r, Ritonavir-Boosted Lopinavir; AZT, Zidovudine; TDF, Tenofovir; NVP, Nevirapine; ANC, Antenatal care; HAART, Highly Active Antiretroviral Therapy.

3. Clinical DBS specimens and DNA extraction from DBS.

DBS samples were collected from HIV-infected mothers and sent from various locations across Thailand to the Medical Life Science Institute, Department of Medical Sciences, within 30 days of blood collection. Blood collection and storage followed precise instructions, including the use of desiccants, opaque plastic zip-lock bags, and drying overnight at room temperature. All infants received an early infant diagnosis using real-time HIV DNA PCR (6) and confirmation through conventional nested PCR (7) at the Department of Medical Sciences. DNA was extracted from two circles of DBS (approximately 100 µl of whole blood) using the QIAsymphony SP and QIAsymphony DSP DNA kit (QIAGEN, Valencia, CA, USA). All DBS samples were extracted within the first month of storage at -20°C and were subsequently transported to the HIVDR genotyping laboratory for analysis.

4. Sensitivity and specificity of DBS standard panel.

The 8E5 cells (ATCC CRL-8993), which contained a single copy of the HIV genome per cell, were used as the DBS standard sample to validate HIVDR testing. These 8E5 standard cells also served as test material for an external quality assessment

(EQA) scheme of HIV DNA PCR testing in Thailand. Briefly, the 8E5 cells were diluted to 0, 100, 500, 1000, and 5000 HIV copies per mL with HIV-1-negative whole blood. A 50 µl aliquot of the 8E5 cells at each dilution was spotted onto a single spot on a DBS card. Two blood spots were then used for HIV DNA extraction.

5. HIVDR genotyping and validation method.

All positive HIV DNA PCR remnant were tested for the in-house HIVDR testing. Briefly, a 5µl volume of DNA was added to SuperScript III one-step RT-PCR cocktail with Platinum™ Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA) with outer primers: PRO5 (5'- AGA CAG GYT AAT TTT TTA GGG A-3') and 3501_R (5'-TCT TTT GAT GGG TCA TAA TA-3'). Cycling conditions for the first RT-PCR were 30 min at 55°C, 2 min at 94°C followed by 40 cycles of 15 s at 94°C, 15 s at 55°C, and 1 min 30 s at 68°C. After a first round of RT-PCR amplification, 4 µl was subjected to a second round of PCR amplification with KOD-Plus-Neo kit (TOYOBO, Japan) using internal primers: PRO1M (5′- AGA GCC AAC AGC CCC ACC AG -3′), and DRRT4L_R (5′- TAC TTC TGT TAG TGC TTT GGT TCC -3′). The second amplifications cycling conditions were 2 min at 92°C followed by 30 cycles of 10 s at 94°C, 4 s at 60°C, and 15 s at 74°C. The nested PCR products of the pol (pol-RT) were 1277 base pairs (bp) in length. The nucleotide sequences of the protease and reverse transcriptase regions were performed as described elsewhere [8] and submitted to the Stanford HIV Drug Resistance Database web service with an algorithm (HIVdb: Genotypic Resistance Interpretation Algorithm version HIVDB_9.4 and https://hivdb.stanford.edu/). Antiretroviral (ARV) susceptibility, and drug resistance mutations (DRMs) were generated.

Phylogenetic trees were constructed using the Neighbor-joining method with 1000 bootstrap replicates. These analyses were conducted using MEGA version 10.0 software. Subtypes were determined using the Stanford drug resistance database and the Rega tool.

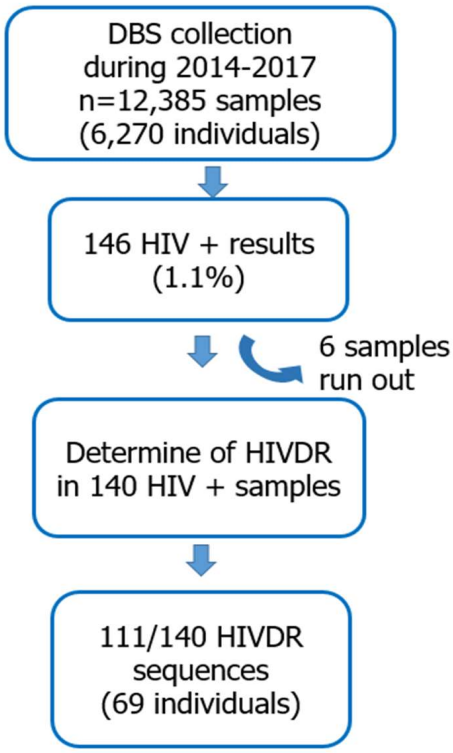
Additionally, 14 DNA samples were included for sequence analysis due to the same patient set, referred to as "the inconclusive HIV DNA PCR group" which was determined to have positive real-time HIV DNA PCR results but negative conventional HIV DNA PCR results. These samples were not included in the sensitivity analysis for DR genotyping because the results of HIV DNA PCR testing were inconclusive.

Results

1) MTCT rate and successful amplification rate among infants during 2014-2017.

Total of 12,385 DBS samples from 6,270 individuals were collected between 2014 and 2017. The rate of mother-to-child-transmission (MTCT) in this study based on HIV DNA PCR results of HIV-infected infants, was 1.14% (72 out of 6,270 individuals). Only 140 samples had left over DNA for HIVDR genotyping. The success rate of genotyping was 79.3% (111 out of 140 samples), representing 69 individuals (see Figure 1).

Figure 1. Recruitment of HIV positive samples for HIVDR assay



2) Demographic and clinical data of 69 HIV-infected infants (140 HIV-positive DBS samples).

All 69 HIV-infected infants were less than 1 year old and had received ART for prevention based on their risk profile. The average age of infants in this study was 63 days. Notably, viral load testing was conducted before ART initiation. A geographical breakdown revealed that 28 infants (40.6%) were from Bangkok and central region, 14 infants (20.3%) were from the southern region, and 11 infants (15.9%) were from the eastern region (see Table 2). Additionally, the majority of infants (92.8%) were of Thai ethnicity (see Table 2).

Table 2. Demographic, clinical and genotypic characteristics of 69 HIV-infected infants.

Details		Number	%
Age (day)	**Median (Range)**	**63 (2-556)**	
Sex	Male	34	49.3
	Female	35	50.7
Region	Bangkok	14	20.3
	Central	14	20.3
	North	3	4.4
	North East	6	8.7
	East	11	15.9
	West	7	10.1
	South	14	20.3
Ethnicity	Thai	64	92.8
	Non-Thai	5	7.2
Infant risk	Standard risk	4	5.8
	High risk	43	62.3
	unidentified	22	31.9
ART for mother	Complete ART	26	37.8
	Incomplete ART	28	40.6
	Unidentified	15	21.7
Breastfeeding	Non-breastfed	44	63.8
	Unidentified	25	36.2
Symptomatic	Symptomatic	9	13.0
HIV infection	Asymptomatic	44	63.8
	Unidentified	16	23.2
Subtype	CRF01_AE	62	90.0
	non-AE	7	10.0
ARV drug resis-	Resistance	10	14.5
tance	Susceptible	59	85.5

Abbreviations and definitions: ART = antiretroviral therapy, ARV = antiretroviral, complete ART for mother = regularly takes HAART >12 weeks before delivery, incomplete ART for mother = irregularly takes HAART ≤12 weeks before delivery.

3) Sensitivity, specificity, and validation of HIVDR genotyping assay in the DBS standard panel.

The sensitivity of PCR amplification of DBS was 100% with a limit of detection of 2.5 copies per reaction. Both precision and reproducibility were 99.8%, and 90% similarity of the nucleotide sequences. These tests were performed in seven replicates, and it is important to note that DBS samples without 8E5 cells could not be amplified.

4) Results of genotyping from phylogenetic tree analysis.

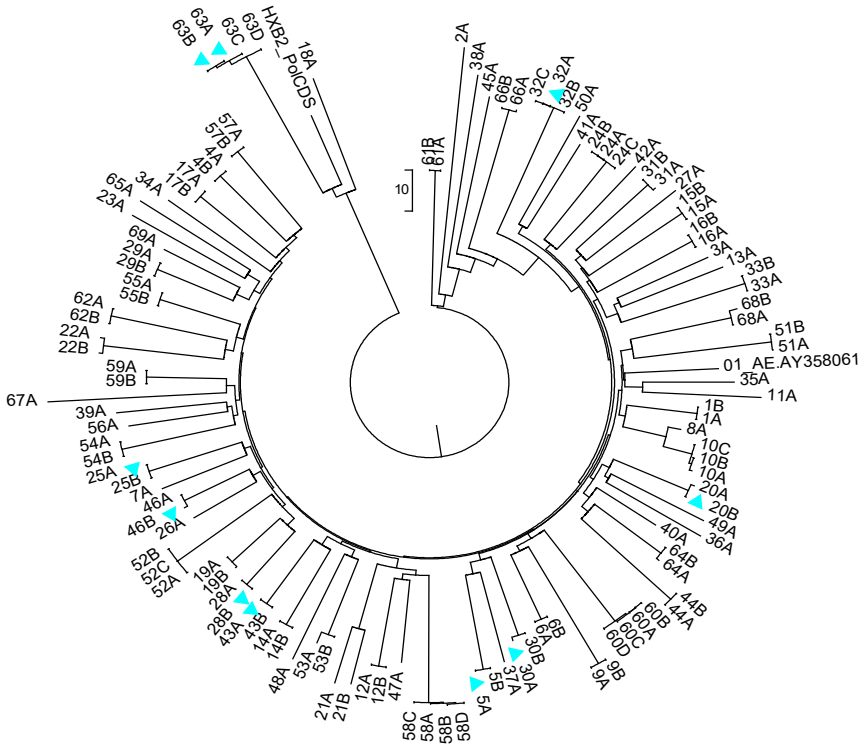
We employed the primer set with satisfactory results on plasma specimens from HIV-1 drug resistance EQA panels from WHO. This panel

consisted of CRF01_AE, CRFI5_01B, CRF33_01B, CRF34_01B, B, C, CRF07_BC, F, and CRF02-AG. Contamination was checked for version 10.0. the phylogenetic tree using MEGA.

There were 111 pol sequences from 69 infants located in distinct clusters on the phylogenetic tree (refer to Figure 2). All intra-patient sequences formed phylogenetic clusters with high bootstrap values, and no hyper-mutations were identified. In the pol trees, most sequences belonged to the monophyletic cluster of HIV-1 CRF01_AE, distinct from other subtypes (see Figure 2). Out of 69 infants, the prevalence of HIV subtypes from the pol gene were as follows: CRF01_AE (90.0%), B+CRF01_AE (4.3%), B (2.9%), A (1.4%), and CRF02_AG (1.4%). 10 of 14 (71.4%) specimens with inconclusive results by HIV DNA PCR were successfully tested for HIVDR assay. Consequently, the nucleotide sequence results were compared with other samples to ensure concordance.

Regarding phylogenetic tree analysis (see Figure 2), ten sequences (marked with blue triangles) were derived from patients with inconclusive HIV DNA PCR results. These sequences were numbered, with No. 61 belonging to subtype A, No. 63 to subtype B, and No. 2 to CRF02_AG. The remaining samples exhibited CRF01_AE as per the phylogenetic tree analysis (see Figure 2).

Figure 2. The result of genotyping from the phylogenetic tree analysis.



(Note: Blue triangles represent ten sequences from patients with inconclusive HIV DNA PCR results. The same numbers indicate identical samples. Patient codes: A, B, C, D show different patient visit. In the context of phylogenetic tree analysis, a neighbor-joining tree was constructed using sequences from our study, with bootstrap values above 95/100 indicated at the nodes of the tree.) HXB2 represents subtype B and 01_AE.AY358061 represents CRF_01AE.

5) History of 10 HIV-infected infants harboring major HIV drug resistance mutations.

According to the International Antiviral (formerly AIDS) Society-USA (IAS-USA, 2022)[9], most sequences showed no mutations. However, among the 69 infants successfully genotyped, 10 (14.4%) exhibited major drug resistance-associated mutations in the protease and reverse transcriptase (RT) genes. Of these, four had resistance to NNRTIs, and five had resistance to nucleoside reverse transcriptase inhibitors (NRTIs). The most frequent RT mutations observed were T215A (n=2) and Y181C (n=2), resulting in NRTI and NNRTI resistance, respectively, as detailed in Table 3. The T215A/I/S substitutions are revertant mutations at codon 215 that confer increased risk of virological failure of zidovudine. We found D30N which conferring to Nelfinavir in the patient code 53A and later this mutation disappeared. Notably, the predominant subtype was CRF01_AE, followed by subtype B.

Remarkably, each patient's dataset or serial samples displayed the same cluster and drug resistance mutations, possibly reflecting continued drug resistance among these patients.

Table 3. History of 10 HIV-infected infants harboring major HIV DR mutations.

No.	Patient code	Age (days)	Treatment history of mother	Infant prophylaxis	Breastfeeding	Symptom	HIV subtype	DR mutation (major only)	Resistance to ARV
1	2A	83	3TC	/	x	-	CRF02_AG	M184V	3TC
2	9A	201	AZT	/	x	x	CRF01_AE	D67G, K70R, T215I, K219E	AZT
	9B	210	AZT	/	x	x	CRF01_AE	D67G, K70R, T215I, K219E	AZT
3	21A	30	AZT	/	x	x	CRF01_AE	T215S	AZT
	21B	54	AZT	/	x	x	CRF01_AE	none	
4	44A	34	AZT	/	x	x	CRF01_AE	T215A	AZT
	44B	68	AZT	/	x	x	CRF01_AE	T215A	AZT
5	46A	2	NVP/EFV	-	-	-	CRF01_AE	Y181C	NVP/EFV
	46B	25	NVP/EFV	-	-	-	CRF01_AE	Y181C	NVP/EFV
6	47A	82	NVP/EFV	x	-	x	CRF01_AE	G190A	NVP/EFV
7	53A	3	-	-	-	-	CRF01_AE	D30N	Nelfinavir
	53B	25	-	/	x	x	CRF01_AE	none	
8	54A	84	NVP/EFV	/	x	x	CRF01_AE	K103N	NVP/EFV
	54B	110	NVP/EFV	/	x	x	CRF01_AE	K103N	NVP/EFV
9	56A	365+	NVP/EFV	/	x	/	CRF01_AE	Y181C	NVP/EFV
10	63B	30	AZT	/	x	x	B	T215TA	AZT
	63C	138	AZT	/	x	x	B	none	
	63D	170	AZT	/	x	x	B	none	

Abbreviations: / = presence,x = not presence, - = Not known, 3TC= Lamivudine, AZT = Zidovudine, NVP = Nevirapine, EFV = Efavirenz, NRTIs = Nucleoside Reverse Transcriptase Inhibitors, Patient codes: A, B, C, D mean different patient visit.

Discussion

Our study revealed the mother-to-child transmission rate of 1.14%, which is consistent with previous data[5], where 1.9% was reported in 2015. A total of 79.3% (111 out of 140) of DBS samples were successfully amplified and sequenced, regardless of viral load. Our results suggest that this in-house genotyping assay on DBS specimens can be effectively used to monitor HIVDR among infants in Thailand. Notably, the average age of infants in this study, 63 days, aligns with previous report[10], indicating that it may take at least two months for HIV to amplify in infants with naive immune systems.

The overall prevalence of major HIVDR mutations in our study was 14.4% (10 out of 69 children), which is lower than the 28.1% reported in a study in Tanzania.[11] However, comparing our results to the Tanzanian study is challenging because they conducted their research under the guideline of single nevirapine in 2011-2012. Conversely, drug resistance mutations were more readily detected in HIV-infected infants, with 21% of Zambian infants infected in 2007, increasing to 40% in 2014.[12] Nine out of the ten cases in our study had the CRF01_AE subtype, suggesting that CRF01_AE DR is prevalent among pregnant women in Thailand. Unfortunately, information about the mothers' profiles, such as drug resistance testing and viral load, is limited, making it unclear whether the DR mutation emerged in the infant after birth or was likely acquire DR mutation. These major mutations confer resistance to NRTIs and NNRTIs, which are relevant to the PMTCT regimen in both infants and their mothers. Regarding recent treatment changes related to dolutegravir (DTG), the Thailand national guidelines on HIV/AIDS treatment and Prevention 2021/2022[13] recommended first-line regimen-TDF (or TAF) +3TC (or FTC) + DLG for HIV-infected pregnant women. In theory, such circumstances may lead to resistance to all drugs in the same class.

This study highlights the urgent need for HIVDR testing in newly diagnosed infants to ensure effective treatment, even in the absence of viral load data due to limitations in blood volume. Our research shows that dried blood spots are highly valuable for laboratory tests, sequencing, subtype confirmation, and drug resistance analysis. We found that the sensitivity of using DBS was 79.1%. Another study reported a sensitivity of 89.5%; however, successful analysis was only achieved in 16.1% of cases with viral loads exceeding 400 copies/mL.[11] Our study has limitations in investigating viral load, making direct comparisons challenging.

Our DBS method has been validated according to WHO recommendations, with slight differences in designed primers. We employed nested amplification to enhance our detection sensitivity. The total cost of reagents and supplies for this assay is approximately 65 US dollars. Our assay, optimized and validated, may prove useful in confirming HIV DNA PCR assay results. It is worth noting that we demonstrated that the sensitivity of this HIVDR genotyping assay is as effective as the real-time PCR DNA assay of early infant HIV diagnosis. Nonetheless, our study underscores the utility of dried blood spots for HIVDR genotyping, particularly its advantages in blood storage and transportation, making it particularly valuable in remote tropical areas. DBS can serve as an alternative sample to plasma for in-house HIVDR genotyping in infants newly diagnosed with HIV.

Conclusions

Our results provide confidence of the in-house genotyping assay using DBS specimens for monitoring the prevention of HIV drug resistance (HIVDR) among infants in Thailand. The major mutations conferring resistance to NRTIs and NNRTIs are particularly relevant to the PMTCT regimen for both infants and their mothers. The Thai government may need to reconsider the balance between ensuring children's longevity with costly treatments and the importance of drug resistance testing. This study underscores the necessity of implementing HIVDR testing in newly diagnosed infants to enhance the effectiveness of their treatment.

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