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PREVALENCE OF HIV-1 DRUG RESISTANCE AMONG EARLY DIAGNOSED HIV-INFECTED CHILDREN ENROLLED THROUGH THE NATIONAL PROGRAM FOR PREVENTION OF MOTHER-TO-CHILD TRANSMISSION IN SOUTHERN AND CENTRAL HIGHLAND PROVINCES — VIETNAM WITHIN 2017–2021

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Background. In Vietnam, there has been an increase in efforts to monitor and prevent mother-to-child transmission of HIV since 2013. However, data on HIV drug resistance among HIV-1 infected children younger than 18 months of age remain limited. The study fills a critical knowledge gap by providing important insights into the prevalence of resistance among this population in the Southern and Central Highland provinces of Vietnam.
Objective. This study aimed to determine the prevalence of HIV drug resistance and patterns of mutation among treatment-naïve children under the age of 18 months who had been recently diagnosed with HIV.
Material and methods. Between May 2017 and May 2021, stored remnant DBS samples were collected from children under the age of 18 months who had been diagnosed with HIV through routine Early Infant Diagnosis testing in Central Highland and Southern Vietnam. HIV drug resistance tests were performed, and interpretation was done using the Stanford algorithm.
Results and discussion. Overall, 111 samples with eligible viremia for sequencing (ct value <31) were collected for genotyping, in which 110 protease sequences and 106 complete reverse transcriptase regions were generated. Males were 61.3%, 52.3% were aged from 6 weeks to <9 months and 37.0% were breastfed. Access to mother-to-child intervention was reported in 60.3%. The accumulation of major drug resistance mutations was found in 43.8% of infants and most of them were resistant to Non-nucleoside Reverse Transcriptase Inhibitors (NNRTI) (37.7%). High-level resistance to Nevirapine was present in 40% of cases. The risk factors associated with NNRTI resistance were antiretroviral prophylaxis (aOR: 3.68, 95% CI: 1.83–7.45) and breast-feeding history (aOR: 2.16, 95% CI: 1.03–4.53). CRF01-AE was the predominant subtype.
Conclusion. The study identified a high prevalence of resistance to NNRTIs among HIV-1 infected infants less than 18 months of age in the Southern and Central Highland provinces of Vietnam. This highlights the need for further investigation and a potentially larger national survey to confirm the extent of this issue in Vietnam. Our findings support the current actions of the Vietnam Ministry of Health, which prioritize integrase inhibitor-based regimens as the preferred first line of ART for children to achieve durable viral suppression and minimize treatment failure. This approach aligns with the recommendations of the World Health Organization.

Keywords: HIV-1, children, prevention of mother-to-child transmission, early infant diagnosis, drug resistance

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РАСПРОСТРАНЕННОСТЬ ЛЕКАРСТВЕННОЙ УСТОЙЧИВОСТИ ВИЧ-1 СРЕДИ ДЕТЕЙ, ВКЛЮЧЕННЫХ В НАЦИОНАЛЬНУЮ ПРОГРАММУ ПРОФИЛАКТИКИ ПЕРЕДАЧИ ИНФЕКЦИИ ОТ МАТЕРИ РЕБЕНКУ, В ПРОВИНЦИЯХ ЦЕНТРАЛЬНОГО НАГОРЬЯ И ЮЖНОГО ВЬЕТНАМА В 2017–2021 гг.

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Введение. Во Вьетнаме с 2013 г. возрос мониторинг профилактики передачи ВИЧ от матери к ребенку. Однако данные об устойчивости к антиретровирусным препаратам среди ВИЧ-инфицированных детей в возрасте до 18 месяцев по-прежнему отсутствуют.

Цель. Исследование было направлено на определение распространенности резистентности ВИЧ к лекарственным препаратам и видов мутаций среди детей в возрасте до 18 месяцев, ранее не получавших антиретровирусные препараты, у которых недавно был диагностирован ВИЧ.

Материалы и методы. В период с мая 2017 по май 2021 г. в Центральном нагорье и Южном Вьетнаме были собраны остаточные образцы сухих пятен крови у детей в возрасте до 18 месяцев, у которых был диагностирован ВИЧ в результате планового тестирования в ходе ранней диагностики у младенцев. Проведены тесты на устойчивость ВИЧ к лекарственным препаратам с помощью секвенирования с интерпретацией полученных результатов в соответствии с базой данных Стэнфордского университета.

Результаты и их обсуждение. Всего для генотипирования было собрано 111 образцов с подходящей вирусной нагрузкой для секвенирования (значение ct<31), из которых было получено 110 последовательностей протеазы и 106 фрагментов обратной транскриптазы. Пациенты мужского пола составляли 61,3%, 52,3% были в возрасте от 6 недель до <9 месяцев и 37,0% находились на грудном вскармливании. О доступе к профилактике передачи ВИЧ от матери к ребенку сообщили 60,3%. Основные мутации лекарственной устойчивости были обнаружены у 43,8% детей раннего возраста, и большинство из них были устойчивы к ненуклеозидным ингибиторам обратной транскриптазы (ННИОТ) (37,7%). Резистентность высокого уровня к невирапину присутствовала в 40% случаев. Факторами риска, связанными с устойчивостью к ННИОТ, были антиретровирусная профилактика (OR: 3,68, 95% ДИ: 1,83–7,45) и грудное вскармливание в анамнезе (OR: 2,16, 95% ДИ: 1,03–4,53). CRF01-AE был преобладающим генетическим вариантом ВИЧ.

Заключение. Исследование выявило высокую распространенность резистентности к ННИОТ среди ВИЧ-инфицированных младенцев в возрасте до 18 месяцев в провинциях Центрального нагорья и Южного Вьетнама. Это подчеркивает необходимость дальнейшего исследования и потенциально более масштабного национального исследования для подтверждения масштабов этой проблемы во Вьетнаме. Наши результаты подтверждают текущие действия Министерства здравоохранения Вьетнама, которое отдает приоритет схемам на основе ингибиторов интегразы в качестве предпочтительной первой линии АРТ у детей для достижения стойкого подавления вируса и минимизации неэффективности лечения. Такой подход соответствует рекомендациям Всемирной организации здравоохранения.

Ключевые слова: ВИЧ-1, дети, профилактика передачи ВИЧ от матери ребенку, ранняя диагностика у новорожденных, лекарственная устойчивость

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Introduction. Prevention of Mother-to-child transmission (PMTCT) of HIV has achieved significant success since the large roll-out of the «Global Plan towards the Elimination of New HIV Infections among Children and Keeping their Mothers Alive» in 2011, in which the provision of antiretroviral treatment (ART) to both HIV-infected pregnant/postpartum women and prophylaxis treatment to exposed babies has been implemented. According to UNAIDS, the number of new HIV infections among children aged under five strongly declined from 320,000 [220,000–480,000] in 2010 to 160,000 [110,000–230,000] in 2021, representing a 50% decline all over the world [1]. However, high ART coverage in mothers together with prophylaxis in newborns for PMTCT, particularly for regimens containing nonnucleoside reverse transcriptase inhibitor (NNRTI) can lead to the selection of drug-resistant mutations. These mutations can be transmitted from the mother or can arise de novo after transmission among children, and can potentially affect both PMTCT prophylaxis and future treatment [2–4].

In Vietnam, the PMTCT of HIV program has been introduced since 2005. To meet the target of reducing the proportion of MTCT, the Vietnam Ministry of Health has taken significant steps to expand and improve services related to PMTCT. This has been done through both maternal and neonatal health programs, including strengthening PMTCT infrastructure, improving PMTCT guidelines which are now based on the latest World Health Organization (WHO) recommendations, increasing access to PMTCT services, and strengthening PMTCT monitoring and evaluation. The strategies have led to an increase in PMTCT coverage from 44% in 2010 to 73% in 2017, and up to 90% in 2020. However, the COVID-19 pandemic has had a significant impact on access to PMTCT services, resulting in a decline in coverage to 75% in 2021. The access to early infant diagnosis (EID) tests, which is an important component of PMTCT, has also shown a similar trend. The percentage of access to EID tests increased from 31% in 2017 to 54.6% in 2020, but dropped to 27.9% in 2021, again due to the impact of the COVID-19 pandemic. As a result of this intervention, the final vertical HIV transmission rate also declined from 18.6% in 2010 to 9.7% in 2020 but was still relatively high at 18.5% in 2021 [5–7].

During the study period of 2017–2021, Vietnam's national guidelines for PMTCT intervention adopted Option B+ per WHO recommendations, which provides lifelong ART to all HIV-infected pregnant and breastfeeding women, irrespective of CD4 cell count or clinical WHO stages. All guidelines recommended a triple regimen of tenofovir disoproxil fumarate (TDF), lamivudine (3TC), and efavirenz (EFV) for HIV-infected pregnant women to prevent transmission of the virus to their neonates. This regimen is consistent with the WHO's recommendation of a simplified and standardized ART regimen for pregnant and breastfeeding women living with HIV [8, 9]. By the end of 2017, women detected HIV positive later than the 24th week of pregnancy were advised to take a regimen of TDF+3TC+RAL (raltegravir). Since 2020, a fixed-dose combination of TDF+3TC+DTG (Dolutegravir) has been recommended as the preferred regimen for all pregnant women living with HIV. In practice, however, EFV was continually used due to the shortage of regimens containing DTG or RAL in Vietnam. For infant prophylaxis, a single dose of nevirapine (sdNVP) within 6 to 12 weeks of birth was recommended for HIV-exposed infants. Since 2018, sdNVP for 6 weeks postpartum has been applied for cases with a low risk of vertical transmission, and a combination of AZT (Zidovudine) plus NVP has been used for 6 weeks among infants with a high-risk transmission or 12 weeks if high-risk transmitted and being breastfed [10–12].

The impact of HIV drug resistance (HIVDR) on treatment failure in adults has been extensively studied worldwide. However, limited information is available about HIVDR among children and adolescents, especially in resource-limited settings. Furthermore, data on the role of PMTCT drug exposure in the occurrence of resistance are primarily restricted to HIV-infected newborns and infants. To address this gap, the WHO published a generic concept note outlining methods for assessing the prevalence of HIVDR in children under 18 months of age who are newly diagnosed with HIV and have been exposed to

PMTCT. The note recommends the use of remnant dried blood spot (DBS) specimens from EID for this purpose. Analysis of such data could provide essential information to support the choice of appropriate ART for children [13]. Despite these guidelines, data on pre-treatment drug resistance (PDR) among young children in resource-limited countries, particularly in Asia, remain scarce.

Vietnam boasts one of the lowest rates of HIV drug resistance before treatment, acquired drug resistance, and the highest rate of viral suppression in people on ART in the world. As of 2022, the country has conducted five national surveillances on HIV drug resistance among adults followed the WHO technical guidance [14]. These surveys found that the prevalence of PDR of ART was 2.7% in 2009–10 and 5.8% in 2017 [15, 17]. Regarding acquired drug resistance (ADR), 4.6% of people on ART for at least 36 months in 2013, 2.5% and 3% of people already on ART for 12 months, and 2.7% and 3.4% of people who had been on ART for at least 48 months in 2017 and 2019, respectively, showed any drug resistance [16–18]. However, contrary to studies in adults, there is limited information available on HIV drug resistance among children in Vietnam, particularly as it relates to PMTCT interventions. A prospective cohort of children younger than 15 years old initiating ART in Ho Chi Minh City, Vietnam (2011–2014) showed the prevalence of PDR was 5.15%, and ADR at 12 months of ART was 7.0% [19]. Unfortunately, there is no published literature about HIV drug resistance on infants younger than 18 months of age.

The purpose of our study was to determine the prevalence of PDR and patterns of mutation among treatment-naïve children under the age of 18 months who had been recently diagnosed with HIV. This study, conducted in Central Highland and South Vietnam, is the first of its kind. By analyzing stored remnant DBS samples from routine EID activities, we sought to understand the current status of drug resistance due to PMTCT intervention among young children in Vietnam. Our findings will be vital in helping the Ministry of Health (MOH) to consider planning and implementing representative national surveillance in the context of large-scale PMTCT programs in Vietnam.

Material and methods

Ethical consideration. The study protocol received approval from two ethics committees: The Independent Ethics Committee of Saint Petersburg Pasteur Institute and The Institutional Ethics Committee of Pasteur Institute in Ho Chi Minh City. The authorization numbers for the approval were 54/29.10.2019 and 19/CN_HĐĐĐ, dated October 10, 2019, and December 12, 2019, respectively.

Study design and sampling. The methodology is the retrospective study leveraging the stored remnant DBS samples enrolled from Central Highland and Southern Vietnam which were positive by routine EID test of HIV among children less than 18 months of age between May 2017 and May 2021. Demographic data were abstracted from Appendix 1C about the laboratory requisition form for EID of the national guideline for HIV testing [20].

Specimen handling and genotyping. DBS samples were collected, handled, and transported per the Vietnam national guideline for EID testing [20]. Specimens were sent to the national reference laboratory of HIV at Pasteur Institute in Ho Chi Minh City. All DBS with positive HIV confirmation (by COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test v2.0) were stored at −20° C until used for this study. All tests were performed at the HIV laboratory — Pasteur Institute in Saint Petersburg — Russia. The total nucleic acid samples were extracted and purified using the AmpliSens® RIBO-prep (Moscow, Russia) and performed real-time PCR by AmpliSens® HCV/HBV/HIV-FL (For HIV: Sensitivity =200 cps/mL, Specificity =100%). Samples with Real-time PCR ct value less than 31 were genotyped on *pol* gene for determining HIVDR using AmpliSens® HIV-Resist-Seq. Analyzed sequences were interpreted for drug-resistant mutations (DRMs) using the Stanford HIVdb version 9.1 updated on June 2, 2022 (https://hivdb.stanford.edu/hivdb/).

Data analyses. Data were entered using EpiData V3.1 and analyzed using Stata software V16.0 (StataCorp LP, College Station, Texas). HIVDR prevalence was defined as low-level, intermediate, and high-level resistance according to the Stanford HIVdb. Sequences classified as susceptible and potential low-level resistance were considered to have no HIVDR. A logistic regression model was used for both univariate and multivariate analysis. Factors associated with the HIVDR situation were statistically significant if the p-values were less than 0.05. The phylogenetic tree was created by Geneious Prime software (V2022.2.2) to determine the distribution of subtypes among the study population.

Results

Study population and antiretroviral exposure. Out of 111 stored remnant DBS samples confirmed

HIV positive from EID testing with eligible viremia for sequencing (ct value <31), 106 complete reverse transcriptase sequences and 110 protease sequences were generated and included as study participants in the final dataset for analysis. Sixty-eight (61.3%) were female, and fifty-eight (52.3%) were 6 weeks to less than 9 months of age at the time of specimen collection. The mean age of the study population was 13.2 weeks. A large number of children were late detected HIV, including 37.0% of infants whose mothers knew HIV positive situations in labor and 36.9% of children found their HIV infection until being hospitalized with HIV symptoms or being adopted in orphanages. Thirty- seven (37.0%) of the cases reported having been raised by breastfeeding, among whom 20.4% were still breastfed at the time of sample collection (Table 1). A total of 67 (60.3%) had reported access to PMTCT, among whom 46 cases received both maternal and neonatal antiretroviral therapy, 14 cases only had neonatal prophylaxis only, and 7 were only given maternal therapy (Table 2). There were 75.9% of infants treated with a single dose of NVP syrup and 15.5% given with daily received daily NVP+AZT for 6 weeks in the postpartum period (Table 1).

HIV Drug Resistance. The development of any major drug-resistance mutations was found in 43.8% (95% CI: 34.1–53.8%) of infants and the overall prevalence of HIVDR was largely driven by NNRTI resistance, which was presented in 37.7% (95% CI: 28.5–47.7%). In contrast, the percentage of NRTI resistance was substantially lower (3.8%; 95% CI: 1.0–9.4%). High-level resistance to NVP was found in 40% of cases. Most of the study participants harbored the virus with a single mutation, in which the frequency of mutation at position 181 on the reverse transcriptase region was the highest (26.4%). Other major mutations were K103N, G190A, V106I resistant to NNRTI, and M184I/V resistant to NRTI. Resistant mutations harboring among recently infected infants are shown in Figure 1.

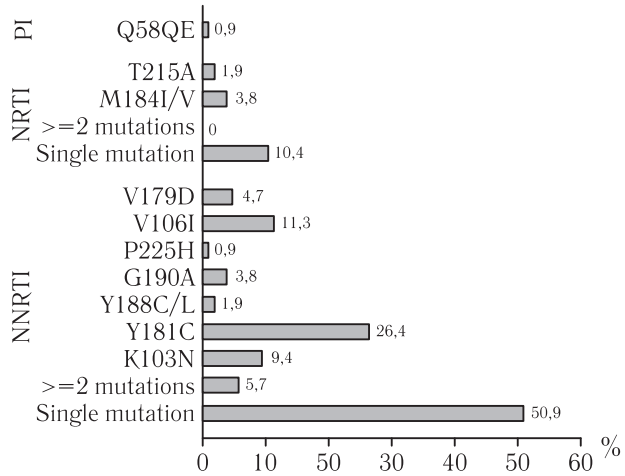

Fig. 1. Frequency of resistant mutations observed in children aged <18 months and diagnosed with HIV through EID testing (n=106 for NNRTI and NRTI, n=110 for PI). Mutations are defined per Stanford HIVdb algorithm version 9.1

Further analysis of the HIVDR situation among infants with and without antiretroviral drug (ARV) exposure is presented in Table 2. Among 46 cases who reported receiving both maternal and neonatal ART, 58.7% developed mutations related to resist-

Table 1

History of breastfeeding and antiretroviral prophylaxis among recently HIV-infected infants younger than 18 months of age

Characteristics	Total (N)	n	%
Babies were raised by	**111**		
Breastfeeding only		24	21.6
Formula milk only		81	73.0
Both breastfeeding & Formular milk		3	2.7
Unknown		3	2.7
Current mother's ART regimen	**53**		
TDF/3TC/EFV		40	75.4
AZT/3TC/EFV		2	3.8
TDF/3TC/LPVr		11	20.8
Children's prophylaxis regimens	**58**		
sdNVP		44	75.9
NVP/AZT		9	15.5
AZT/3TC/NVP		5	8.6
Duration of antiretroviral prophylaxis for children	**58**		
4 weeks		3	5.2
6 weeks		52	89.6
12 weeks		3	5.2

ance to any drug, and most of them were resistant to NNRTI, while all children with only neonatal prophylaxis were resistant to just NNRTI.

there is limited data on children, especially infants below 18 months old. This is the first study that has investigated the drug-resistant situation among early

Table 2

History of PMTCT exposure among recently HIV-infected infants aged <18 months and the proportion of HIVDR situation

History of antiretroviral exposure	Drug resistance	Prevalence	95% CI
Without PMTCT exposure or unknown (n=44)	Any drug	22.7	11.5–37.8
	NNRTI only	20.5	9.8–35.3
With PMTCT exposure (n=67)	Any drug	55.2	42.6–67.4
	NNRTI only	50.7	38.2–63.2
Maternal + neonatal PMTCT (n=46)	Any drug	58.7	43.2–73.0
	NNRTI only	52.2	36.9–67.1
	NRTI only	2.2	0.1–11.5
	NRTI+NNRTI	2.2	0.1–11.5
	NRTI+NNRTI+PI	2.2	0.1–11.5
Neonatal PMTCT only (n=14)	Any drug	50.0	23.0–77.0
	NNRTI	50.0	23.0–77.0
Maternal PMTCT only (n=7)	Any drug	42.9	9.9–81.6
	NNRTI only	14.3	0.4–57.9
	NRTI only	28.6	3.7–71.0
	NRTI+NNRTI	0.0	

Data from the multivariate analysis showed the two factors associated with NNRTI resistance among HIV-infected infants aged <18 months. Among the children who had received antiretroviral prophylaxis, the risk of NNRTI resistance increased with any exposure (adjusted odds ratio [aOR] 3.68, 95% CI 1.83–7.45, p-value <0.001). Babies that were historically raised by breastfeeding were also associated with a higher risk of resistance (aOR 2.16, 95% CI 1.03–4.53, p-value =0.041).

The phylogenetic tree was created by Geneious Prime software (Version V2022.2.2) using Tamura-Nei genetic distance model, Neighbor-Joining method and bootstrap value =1000 replicates. Nucleotide sequences of HIV-1 consensus subtypes A1, A2, B, C, D, F, G, H, BC, and some recombinant subtypes downloaded from the international GenBank database [21] were used as the references to generate the phylogenetic tree and determine the distribution of subtypes among our study population. HIV-1 CRF01_AE was the predominant subtype and was identified in 99.1% of samples, subtype C presented in 0.9% (Fig. 2).

Discussion. The HIVDR Report 2021 by WHO reveals that only 10 countries reported data from their surveys of pretreatment HIVDR among ART-naïve infants younger than 18 months between 2012 and 2020, all from Africa and South Africa [18]. In Vietnam, although national surveillance and research about HIVDR are well documented among adults, HIV-diagnosed infants. The HIV laboratory at the Pasteur Institute in Ho Chi Minh City is the primary entity responsible for conducting all EID tests from Central Highland to Southern Vietnam. Thus, despite it did not involve national surveillance efforts in Vietnam, the DBS samples collected in the study represent two out of three regions of the country.

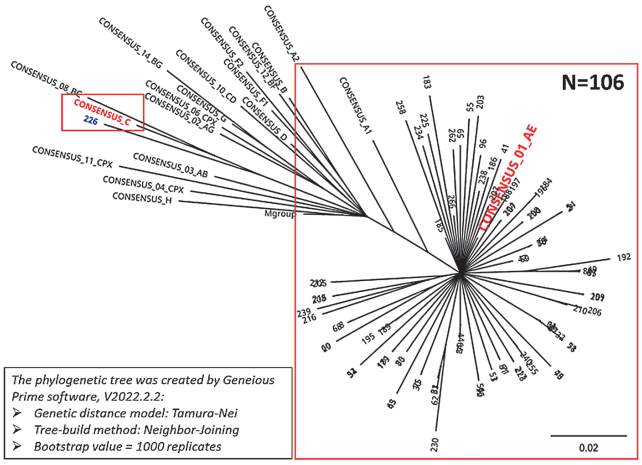

Fig. 2. The circulation of HIV-1 subtypes among HIV-infected children younger than 18 months of age (Tamura-Nei genetic distance model, Neighbor-Joining tree-build method, Bootstrap value =1000 replicates)

The results of this study indicated a high prevalence of HIVDR among HIV-infected children aged less than 18 months, of whom 43.5% presented any-drug resistance. This resistance was mainly caused by mutations associated with NVP contained in ARV

prophylaxis in all cases. Common major mutations positions 103, 181, and 190 on the reverse transcriptase region could be attributed to the wide use of option B+ PMTCT intervention in Vietnam. These mutation positions were also reported in other studies featuring NNRTI-based regimens in the PMTCT program [2, 22, 23]. The most prevalent mutation, Y181C, detected in this study, confers high-level resistance to NVP, again consistent with findings from previous surveillances in Zambia (2014) [23], South Africa (2010, 2011–12, 2012–13) [22], Cameroon (2014), Mozambique (2012), Togo, and Zimbabwe (2012) [18]. The results emphasize the rationality of choosing the preferred integrase inhibitor-based regimens that avoid regimen components with NNRTI for the first line of ART for children in Vietnam's current national guideline. However, DRMs may threaten the effectiveness of HIV treatment for children who are coinfected with tuberculosis and HIV receiving NNRTI-based regimens. The high level of resistance to NVP or EFV among infants living with HIV was unexpected and our study's results further emphasize the need for a pretreatment HIVDR national survey among newly diagnosed infants in Vietnam. This would help to generate a nationally representative estimation of drug resistance prevalence and provide influential data to support the country's recommendation on optimal evidence-based guidelines for HIV prevention and treatment.

Mutations associated with drug resistance were more commonly detected among those with exposure to maternal ARV treatment and/or neonatal PMTCT prophylaxis. In these cases, 55.2% of infants presented resistance to any antiretroviral drug, whereas the percentage of resistant mutations in infants with unknown or no documented ARV exposure was 22.7%. These findings suggest that previous PMTCT interventions may be underreported. The results were consistent with a multi-country analysis conducted in five sub-Saharan African countries (Mozambique, Swaziland, South Africa, Uganda, and Zimbabwe) between 2011 and 2014, which also showed that exposure to ARV led to a higher prevalence of HIVDR resistance (56.2%) compared to non-exposure (23.5%) [2]. The prevalence of HIVDR found in our study was lower than those from pooled analyzed data of the three nationally representative surveys conducted in 2010, 2011–12, and 2012–13 among South African infants aged 4–8 weeks. NNRTI resistance was detected in 68 out of 97 (70%) cases with maternal plus neonatal PMTCT and in 17 out of 26 (65%) cases with neonatal ARV intervention only [22], compared to the findings of our own study, which revealed NNRTI resistance in 52.2% and 50% of the same groups respectively. The difference in NNRTI resistance among these research may be attributed to non-similar ages, as the proportion of NNRTI resistance might be underestimated as children became older due to possible reversion to wild type from NNRTI mutation or discontinuation of postnatal prophylaxis or breastfeeding [2]. In general, after stopping antiretroviral therapy, mutations are likely to be archived in latently infected cells, making them seem to disappear in a matter of weeks and then proliferate again under NNRTI selective pressure, thus impacting long-term treatment [24–26]. In the Africa surveys, DBS samples were collected early after PMTCT exposure, within 4–8 weeks, while the mean age of specimens in our study was 13.2 weeks, with 60.4% of those aged 6 weeks to 18 months. As up to 89.6% of infants received ARV prophylaxis for 6 weeks in our study, the absence of selective pressure from ARV at the time of DBS collection, a few weeks after prophylaxis cessation, may have influenced the disappearance of mutations from the dominant detectable quasi-species. The data from HIV-infected children included in this study also revealed a high proportion who accessed EID tests late and did not receive ARV prophylaxis, which could negatively impact mortality rates and disease progression among infants. Previous studies have shown that initiating treatment in infants immediately after diagnosis, particularly during the first few weeks of life, can help restrict the formation of viral reservoirs [27–29]. However, in our study, a significant proportion of individuals were diagnosed with HIV at a later stage, which could potentially increase the size of viral reservoirs, and may also include quasi-species that could have drug-resistant mutations. The Ministry of Health in Vietnam, therefore, should undertake a critical review of the early diagnosis program's current performance and explore opportunities for strengthening it to maximize the potential benefits of birth testing.

We did not find any correlation between PMTCT access and any-drug resistance; however, as expected, infants with maternal ARV exposure and/or neonatal prophylaxis had a significantly higher prevalence of NNRTI resistance than those without, matching the data reported in the 5 sub-Saharan African countries' surveillances mentioned earlier [2]. Other studies found strongly high resistance to any drugs in infants with documented PMTCT exposure [22][23]. Our study also found a correlation between NNRTI resistance and a history of breastfeeding

among infants, which was previously reported in some studies [30–32].

The results from our study may be interpreted in light of some limitations. Our report only reflects the remnant DBS samples collected from Central Highland and Southern Viet Nam, therefore, it may not be fully representative of the entire population of children under 18 months infected with HIV in the country. Additionally, at the time of the study, early infant diagnosis (EID) coverage was intermediate (around 60% according to UNAIDS) [5], resulting in the potential underdiagnosis of HIV in many children. Although previous studies have shown a strong correlation between mutation detection using Sanger sequencing and Next Generation Sequencing (NGS), it should be noted that low-abundance mutations may only be detectable through NGS [33]. We assumed that the Sanger sequencing technique applied in our study was unable to detect some major and minor drug-resistant mutations, thus the prevalence reported in this study may be underestimated. An additional limitation of our study was unable to determine the situations of HIV drug resistance among the mothers because its methodology relying on the stored remnant DBS samples and their data used in the EID routine national activity for children less than 18 months of age. The drug-resistant mutation may occur either through the development of de novo resistance during the prophylaxis period or via transmission virus harbor drug-resistant mutations from the mothers. Given these limitations, we propose conducting further studies in Vietnam to determine whether HIV mutations develop in children or are transmitted from mothers. Additionally, it would be valuable to investigate the persistence of these mutations using Sanger and/or NGS techniques. Greater understanding from such future studies would improve treatment and prevention strategies, which could in turn minimize the occurrences of mother-to-child HIV transmission and the accumulation of drug resistance.

Despite these limitations, the findings of the study provide valuable insights into the prevalence of drug resistance in HIV-exposed infants and highlight the potential role of maternal ARV exposure, neonatal prophylaxis, and breastfeeding in the development of drug resistance. By identifying these factors, the study contributes to our understanding of mother-to-child HIV transmission and the accumulation of drug resistance and provides a foundation for future research aimed at improving treatment and prevention strategies. Overall, while the limitations of the study should be taken into consideration when interpreting the results, the study's findings remain important and informative for advancing our understanding of HIV transmission and drug resistance.

Conclusion. In conclusion, our study demonstrates a high prevalence of HIV drug resistance to NNRTIs among HIV-positive infants less than 18 months of age in the Southern and Central Highland provinces of Vietnam as a result of the PMTCT program. It highlights the necessary to conduct nationally representative surveys to confirm this phenomenon. Future investigations should explore the patterns of drug resistance among not only children but also their mothers to better understand the impact of de novo or transmitted resistant mutations. The correlation of HIVDR with a number of factors such as maternal ART history, feeding options, and duration of breastfeeding should also be investigated to provide support for the current national guidelines. Since integrase inhibitor-based regimens are widely used for both PMTCT among mothers and ART treatment for children, resistance to integrase inhibitors should also be investigated. Next-generation sequencing may be useful for evaluating the presence of mutations with a low threshold. Our study's results strongly confirm the rationality of the Vietnam Ministry of Health's current guideline on switching to integrase inhibitor-based regimens as the preferred optimal first-line ART for children to prevent drug resistance to NNRTIs.

Author contributions:

H. K. T. Huynh — conducted laboratory experiments, analyzed and interpret HIVDR data and statistical data, prepared the manuscript; *D. E. Valutite* — conducted laboratory experiments, analyzed sequence data; *A. N. Schemelev* — conducted laboratory experiments, analyzed sequence data; *V. S. Davydenko* — conducted laboratory experiments; *Yu. V. Ostankova* — provided guidance and technical support; *T. X. L. Truong* — provided recommendation for the study; *T. Tran* — provided guidance and technical support; *V. T. Nguyen* — provided guidance and technical support, and *A. V. Semenov* — involved in the conception and overall development of the study.

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