

# HIV drug resistance transmission threshold survey in Bangkok, Thailand

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**Background:** Antiretroviral therapy (ART) began in Thailand in the Bangkok Metropolitan Area (BMA) in 1988 and scale-up began in 2001. The national first-line regimen is stavudine, lamivudine and nevirapine in fixed-dose combination, which is a regimen with a low genetic barrier for resistance. Because viral load and resistance testing are not widely available, unidentified HIV drug resistance (HIVDR) may occur during treatment and could be transmitted.

**Methods:** We undertook a threshold survey to assess HIVDR transmission in two subsets of recently infected individuals in the BMA. The first group consisted of returning blood donors tested at the Thai Red Cross National Blood Centre who seroconverted within the past 12 months. The second group comprised recently infected

(as defined by BED assay) clients of the Thai Red Cross voluntary counselling and testing centre (VCT).

**Results:** Genotyping of 50 consecutive specimens each from blood donors and VCT clients during 2005–2006 showed no mutations associated with HIVDR in the reverse transcriptase or protease regions of the HIV *pol* gene. These results are categorized by the WHO HIV drug resistance threshold survey method as representing a low prevalence (<5%) of transmitted HIV drug resistance.

**Conclusions:** Every effort should be made to minimize the emergence of resistance in treated individuals and to prevent primary and secondary HIV transmission. To continue to monitor HIVDR transmission, Thailand has planned additional surveys – including longitudinal surveys – in these and additional groups of individuals.

## Introduction

In 2004, Thailand had a population of 64 million. Of this number, 580,000 were estimated to be living with HIV/AIDS by 2006 [1] and an equal number of Thais have already died since the HIV epidemic began in 1984/5 [2]. HIV in Thailand was initially identified in men having sex with men (MSM), then in intravenous drug users (IDU), female sex workers, their clients and, finally, in housewives and newborns [3]. This represents a well-known epidemic pattern reported in many countries.

Antiretroviral therapy (ART) started in Thailand around 1988 when the first antiretroviral (ARV), zidovudine (ZDV), was registered in Thailand. From 1988 to 1994 less than 2,000 patients in the large cities, including Bangkok, were treated out of their own financial resources. From 1994 onwards, the Ministry of Public Health (MOPH) of Thailand began to provide free ART and had treated 10,000 patients by 2003, this number increased to 50,000 by 2004. Of the 580,000 people living with HIV/AIDS in Thailand

by 2006, it is estimated that a total of 100,000 patients are being treated with ART. Of these, 80% receive ART from the MOPH universal coverage programme; the remaining 20% buy their own ARV treatment. An estimated 40% of those who are in need of ART are currently treated [4].

Since 2003, Thai ART guidelines have followed international guidelines, in particular the 2003 World Health Organization (WHO) Guidelines for Resource-limited Settings [5]. In early years, patients received single or double nucleosides from teaching hospitals and large private hospitals, as well as from a few clinical trial settings [6–8]. Highly active antiretroviral therapy (HAART) started in Thailand around 1998 when protease inhibitors (PIs) became available; initially, it was used as ‘salvage therapy’ for those who had failed on double nucleoside treatment. However, large-scale use of HAART as first-line therapy did not begin until 2001, when the Thai Government Pharmaceutical Organization (GPO) manufactured

fixed-dose combinations of stavudine (d4T), lamivudine (3TC) and nevirapine (NVP) in a single tablet called 'GPO-Vir S' at a cost of US\$1 per day. GPO also produces cheap generic ZDV, 3TC, ZDV/3TC, d4T, didanosine (ddI), NVP and nelfinavir (NFV). With the exception of NFV, all these ARVs are commonly used in Thailand and resistance patterns commonly seen in treatment reflect mutations conferring resistance to one or more of these drugs (Sirivichayakul S, *et al.* unpublished data).

GPO-Vir S is the most commonly prescribed first-line HAART regimen in Thailand due to its low cost. It is expected that the newly available fixed-dose combination of ZDV/3TC/NVP (GPO-Vir Z250) will soon replace GPO-Vir S as first-line regimen in concordance with the new WHO guidelines [9]. Efavirenz (EFV) is available in the national ARV programme for those who cannot tolerate NVP or those with tuberculosis (TB) co-infection. EFV is also the non-nucleoside reverse transcriptase inhibitor (NNRTI) of choice in private practice. PIs are seldom used in first-line regimens, except in private hospital patients, owing to their extremely high prices; however, they are required in second-line therapy. Boosted lopinavir is currently the most commonly used PI, whereas indinavir (IDV) or boosted IDV was the preferred PI in the early years. Supply has been uninterrupted for all of the ARVs in standard regimens in Thailand, except for EFV which was in short supply for a few months in 2005. During this time, NVP in combination with rifampicin was prescribed for TB coinfection.

Initially, ART was prescribed by experts in teaching hospitals as well as in large government and private hospitals. In response to the national policy of ART scale-up, an infrastructure was set up and large-scale training of healthcare workers was carried out by MOPH. As of 2006, ART can be prescribed at district hospitals. Measurements of CD4<sup>+</sup> T-cell count by flow cytometry can be made in nearly all of the 75 provincial hospitals. ART has been included in the universal coverage healthcare package since 2006, which theoretically means that every Thai citizen can have access to ART if needed. The government also plans to include free viral load and genotypic resistance testing in the 2007 national ART package. This is intended to detect early treatment failure so that emergence of HIV drug resistance (HIVDR) associated with undetected virological failure can be minimized. Drug resistance to nucleoside reverse transcriptase inhibitors (NRTIs) and NNRTIs is increasingly seen after the wider introduction of GPO-Vir S, particularly in those with prior double nucleoside failure [10] (Ruxruntham K, unpublished data). Additional concerns include patients who might have suboptimal adherence to care or who might have interrupted access to ART, such as drug users,

inmates, migrant workers and illegal immigrants. Women who have received single-dose NVP in conjunction with ZDV as prevention of mother-to-child transmission (PMTCT) also have a higher risk of HIVDR in Thailand [11]. The recent increase in the incidence rate of sexually transmitted diseases in the general population, and the increased incidence of pregnancy in treated HIV-infected individuals, heightens the concern of transmitted HIVDR in Thailand.

To address concerns about the emergence and transmission of HIVDR, and following the recommendation from the WHO Resistance Network (ResNet), a Thai national working group on HIVDR has been established and Thailand's first threshold survey of HIVDR transmission was planned and completed in 2006 in the Bangkok Metropolitan Area (BMA) – an area where ART was begun early and is most extensive [6]. This report will describe the results of threshold surveys for transmitted HIVDR among recently seroconverting regular blood donors and in recently infected clients of a large voluntary counselling and testing centre (VCT), all in the BMA.

## Methods

### Recently seroconverting returned blood donors from the Bangkok Metropolitan area

Consecutive returning blood donors of the Thai Red Cross National Blood Centre (TRC-NBC) were prospectively identified for the survey between July 2005 and April 2006 and were included if there was evidence that they had seroconverted. Seroconversion was defined by a negative anti-HIV test at the preceding donation no longer than 12 months previously. All blood donations in Thailand are non-paid donations with consents. Individuals with HIV risk behaviours are screened out during pre-donation counselling. TRC-NBC is Thailand's largest blood bank serving the BMA. The Centre receives 440,000 units of blood a year, which is one-third of the national supply. Of these blood donors, 30% are regular donors (that is, more than one donation per year). HIV infection is screened by anti-HIV ELISA and p24 antigen testing. If the sample turned out to be HIV-positive, plasma was separated within 24 h of blood donation, aliquoted and frozen at -70°C for genotypic drug resistance assay if records showed that the donor was a recent seroconverter.

### Thai Red Cross Anonymous Clinic clients with evidence of recent HIV infection

Thai Red Cross Anonymous Clinic (TRC-AC) is Thailand's largest stand-alone HIV VCT serving the population of BMA with an average of 9,000 clients a year. The overall prevalence of HIV infection in 2005 was 14.6%. The clients represent the average population

of Bangkok who perceive a higher risk of HIV infection. No HIV risk group predominates among those who present for HIV testing [12]. Residual plasma specimens from confirmed HIV-positive samples obtained during July to September 2005 were prospectively collected, aliquoted and frozen at  $-70^{\circ}\text{C}$  within 24 h after blood draw. One aliquot was tested for recent HIV infection using Calypte<sup>®</sup> HIV-1 BED capture enzyme immunoassay (Calypte Biomedical Corporation, Maryland, USA). In samples testing positive, the other aliquot was then analysed using a genotypic drug resistance assay. Data were correlated with the biographic data obtained during pre-test counselling.

Both HIVDR surveys were carried out in an unlinked anonymous manner, for this reason no informed consent was obtained.

### Genotyping methods

Eligible residual specimens identified by the processes described above were used for genotyping in the Vaccine and Cellular Immunology (VCI) Laboratory, Faculty of Medicine, Chulalongkorn University, using an in-house genotypic drug resistance assay. Acid citrate dextrose (ACD) plasma aliquoted from the ACD blood bag and EDTA plasma from Anonymous Clinic clients were frozen within 24–48 h after blood drawing and stored as aliquots at  $-70^{\circ}\text{C}$  until use. HIV RNA was extracted from 500  $\mu\text{l}$  of selected plasma. cDNA was generated by using primer RT-2955 (5'-GCTTTACCT-TAATCCCTGCATAAAA-3'). Reverse transcriptase (RT) sequences were generated with primers B887-2 (5'-CTGTACCAGTAACATTAAAGCCAGG-3') and RT-2923 (5'-GCCCAATTTAGTTTTCCCACTAAT-3'); protease (Pr) sequences were generated with primers Pr-1780 (5'-CGAAGCAGGAGCAGAAAGACAAGG-3') and Pr-2172 (5'-CCATTCCTGGCTTTAATGT-TACTGGTAC-3'). The generated products were sequenced using BigDye dideoxy dye terminator (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol.

### Analysis

Sequences were evaluated for resistance mutations using the WHO mutation list [13]. The Stanford University algorithm (<http://hivdb.stanford.edu>) was used to evaluate the presence of any additional polymorphic mutations potentially associated with resistance, though not with transmitted resistance. This algorithm was used to identify the HIV-1 subtype, based on the RT and PR sequences. The WHO HIV drug resistance threshold survey analysis method was used to categorize prevalence to each relevant drug and drug class [14]. In brief, this is a public health approach used in resource-limited settings to categorize (not to estimate) the prevalence as above or below a threshold

level based on a set of stringent criteria such as specific location, specific timing and consecutively collected samples from a specific population. The in-house genotypic drug resistance assay has been used in our service laboratory for over 6 years, as it is considerably cheaper than assays using commercial kits. The laboratory has participated in the quality assurance programme offered by TREAT Asia through the National Serology Reference Laboratory (NRL), Australia (TREAT Asia External Quality Assurance Scheme or TAQAS; <http://www.nrl.gov.au>).

## Results

### Recently seroconverted returned blood donors

From July 2005 to April 2006, 503 of 286,821 blood donors were found to be HIV-positive, a calculated prevalence of 0.18%. Among these 503 HIV-infected donors, 50 were returned blood donors whose HIV test results from donations during the previous 12 months were HIV-negative. Thirty-eight were males and 12 were females with a mean age of  $31.3 \pm 8.1$  (range 20–54). These 50 individuals had donated between 1 and 77 times before the final anti-HIV-positive donation, mean  $12.8 \pm 14.5$  previous donations. The time intervals between the last HIV-negative donation and the HIV-positive donation range from 3 to 12 months, mean  $6.4 \pm 3.6$  months. HIV infection in all of these 50 returned donors was diagnosed by confirmed anti-HIV testing. Among the 50 seroconverters, 46 (92%) specimens were amplifiable for genotyping.

### Thai Red Cross Anonymous Clinic Clients with evidence of recent HIV infection

From a total of 3,338 clients of the Thai Red Cross Anonymous Clinic during July to December 2005, 552 were confirmed anti-HIV-positive, a prevalence of 16.5%. Fifty BED-positive samples were detected from the screening of the first consecutive 342 anti-HIV-positive specimens during this period. Of these 342 HIV-infected individuals, 222 were males and 120 females. Among the 50 individuals with positive BED assay, 36 were males and 14 females. Among these 36 BED-positive males, the proportion of homosexuals/bisexuals was significantly higher than that in BED-negative males, that is, 23/36 (63.9%) in BED-positive males versus 60/186 (32.3%) in BED-negative males,  $P < 0.05$ . This confirms the previous findings in Thailand that HIV transmission among MSM is on the rise [15].

Of the 50 BED-positive specimens, 46 (92%) were amplifiable for genotyping, 50% each from the homosexual/bisexual and the heterosexual groups. Of the 46 amplifiable sequences, 38 could be genotyped for both RT and PI, seven could be genotyped for only PI

and another one for only RT. The reason why it was not possible to completely genotype the samples is uncertain. It could be due to technical error, inappropriate specimen handling and storage or low viral load. It is interesting to note that all five randomly selected samples from the seven PI-only samples had relatively low viral load (63, 217, 1,203, 1,207 and 1,505 copies/ml).

### Genotypic resistance testing results

Among the seroconverting blood donor specimens, 37 (80%) were subtype CRF01\_AE, 8 (17%) were subtype B, and 1 (2%) was subtype CRF01\_AG. No major resistance-associated mutations were found in any of the specimens. No mutations associated with transmitted resistance were seen among the first 34 consecutively drawn specimens from recently seroconverting blood donors, so the analysis ended for classification purposes after the 34th specimen was examined. The prevalence of overall HIV drug resistance, and drug resistance to each anti-HIV drug class, was classified as <5% among seroconverting blood donors at this site in Bangkok.

Among the Anonymous Clinic specimens from recently infected individuals, 40 (87%) were subtype CRF01\_AE and 6 (13%) were subtype B. Again, no major resistance-associated mutations were found in any of the specimens including the seven PI-only and one RT-only samples as mentioned above. Using the same categorization as in seroconverting blood donors, the prevalence of overall HIV drug resistance, and drug resistance to each anti-HIV drug class, was classified as <5% among these recently infected Anonymous Clinic clients at this site in Bangkok.

## Discussion

This work represents the first study of the prevalence of transmitted HIV drug resistance in Thailand, a country where ART has been available for 18 years with scaling up during the past 6 years. Although resistance to NRTI and NNRTI drug classes has been commonly documented in patients on ART, the prevalence of transmitted drug resistance, as evaluated through these surveys, appears to be low (that is, <5%) in both seroconverting blood donors and recently infected Anonymous Clinic clients in the BMA. These findings imply that the fixed-dose combination of ZDV(d4T)/3TC/NVP remains appropriate as first-line therapy in Thailand without the need for resistance testing prior to initiation of ART. The population selected from the National Blood Centre in this study represents a low-risk general population in BMA, whereas the population selected from the Thai Red Cross Anonymous Clinic (TRC-AC) represents those

who perceive themselves at a higher risk. Such a study from the TRC-AC allows us to correlate recent infection with a particular risk group, that is, MSM in this case. Our findings support the results of recent surveys among MSM who were not commercial sex workers in several large cities in Thailand that prevalence of HIV was increasing in this group [15].

It will be of interest to perform HIVDR threshold surveys in other risk groups with recent infection, such as female sex workers or intravenous drug users. Although BMA is the best location to perform a threshold survey, because ART has been available here for a longer time period than elsewhere in Thailand, it will also be important to assess recently infected individuals from the general population and from specific risk groups in other large cities in Thailand, as risk behaviours and levels of drug adherence may vary in different regions and different cultures. In addition, longitudinal study is needed in order to monitor the trend in BMA. MOPH of Thailand has plans to do follow-up studies.

Transmission of HIVDR can be minimized if emergence of HIVDR can be prevented in ARV-treated individuals and if prevention of primary and secondary HIV transmission can be strengthened. Drug adherence counselling is important and should be performed by all professional healthcare workers. A good example of enhancing adherence is provided by several HIV clinics in Thailand, where people living with HIV/AIDS have been encouraged to work as volunteers. These volunteers are properly trained and patients are able to contact them by mobile phone for consultations 24 h a day. From the start of 2007, the Thai MOPH has made available viral load and resistance testing to monitor ART. This represents additional measures to minimize the emergence of HIVDR. For primary HIV prevention, education, condom campaigns, VCT and good services for the treatment of sexually transmitted diseases (STD) are among the effective measures, although some still need strengthening in Thailand. Disclosure, consistent condom use, appropriate family planning counselling and effective STD services are among the measures to prevent transmission of HIVDR from infected individuals to those who are infected or uninfected. Experiences such as those learned from the MTCT-Plus program of the Thai Red Cross AIDS Research Centre, which showed a high disclosure rate and high HIV testing in male partners, should be shared (Phanuphak N, *et al.* personal communication).

## Conclusions

Although ART has been established in Thailand for almost two decades, a HIVDR threshold survey in the setting of a blood bank and VCT centre in the capital

of Thailand still shows a low prevalence of transmitted HIVDR. A longitudinal survey in a larger setting has been planned by the MOPH of Thailand. Measures to prevent drug resistance in ARV-treated individuals and measures to prevent primary and secondary HIV transmission are of vital importance to prevent or delay the emergence of high prevalence of transmitted HIVDR. Initiatives to minimize the emergence and transmission of HIV drug resistance are ongoing in Thailand.

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## Disclosure statement

The authors declare no conflict of interest.

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