J Antimicrob Chemother 2015; **70**: 1146–1149
doi:10.1093/jac/dku499 Advance Access publication 18 December 2014

Journal of Antimicrobial Chemotherapy

Transmitted HIV drug resistance at the Thai Red Cross Anonymous Clinic in Bangkok: results from three consecutive years of annual surveillance

Sunee Sirivichayakul[1], Rami Kantor[2], Allison K. DeLong[2], Rapeeporn Wongkunya[3], Suwanna Mekprasan[1], Kiat Ruxrungtham[1,3], Annette H. Sohn[4] and Praphan Phanuphak[1,3]*

[1]*Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand;* [2]*Alpert Medical School, Brown University, Providence, RI, USA;* [3]*The Thai Red Cross AIDS Research Centre, Bangkok, Thailand;* [4]*TREAT Asia, amfAR, The Foundation for AIDS Research, Bangkok, Thailand*

*Corresponding author. The Thai Red Cross AIDS Research Centre, Pathumwan, Bangkok 10330, Thailand. Tel: +66-22541511; Fax: +66-22547577; E-mail: praphan.p@chula.ac.th

Received 12 July 2014; returned 2 September 2014; revised 4 November 2014; accepted 15 November 2014

Objectives: The aim of this study was to prospectively survey transmitted drug resistance (TDR) among recently infected individuals (mostly MSM).

Methods: TDR was determined in prospective annual cohorts of recently HIV-1-infected individuals consecutively recruited from 2008 to 2010. Resistance interpretation was carried out using Stanford Database tools and the WHO surveillance drug resistance mutation list. Kruskal–Wallis and Fisher's exact tests were used to compare demographic and laboratory outcomes.

Results: A total of 299 subjects were enrolled, with 89% MSM. Median viral load was significantly higher in 2010 than in 2008 ($P=0.004$). Of the 284 analysable reverse transcriptase/protease sequences, TDR to any drug was found in 14/284 (4.9%); 4.0% in 2008, 5.9% in 2009 and 5.3% in 2010, with an increasing trend of TDR to NRTIs and NNRTIs from 2008 to 2010 ($P=0.07$). Good correlation was found between our data and the WHO threshold surveillance method. Only rilpivirine had significantly higher ($P<0.05$) predicted resistance in 2010 than in 2008 and 2009.

Conclusions: A trend towards an increase in TDR in Thailand where the major epidemic is among MSM was observed, but did not reach the WHO-defined high-level threshold (>15%). Attention to prevent the development and spread of drug resistance is needed.

Keywords: Thailand, recent infection, transmitted drug resistance, surveillance drug resistance mutation

Introduction

The HIV epidemic in Thailand started in 1985 among MSM,[1] then expanded to include intravenous drug users and sequentially spread to female sex workers, their male clients and then heterosexual women and newborns.[2] Though overall HIV incidence in Thailand is decreasing, prevalence and incidence among MSM are increasing.[3]

In developing countries where HIV drug resistance (HIVDR) testing prior to ART is not generally available due to cost and infrastructure constraints, the prospective surveillance of transmitted drug resistance (TDR) among recently infected individuals can inform policies regarding first-line ART regimens and the cost-effectiveness of HIVDR testing. A meta-regression analysis by the WHO of TDR trends in developing countries showed that TDR, especially to NNRTIs, increased significantly after ART rollout (2001–11) in sub-Saharan Africa to as high as 7.4% (4.3%–12.7%). Less evidence to support determination of trends exists in Asia.[4]

Low prevalence (<5%) of TDR was reported among individuals recently infected with HIV in Bangkok in 2005–06[5] using WHO threshold surveillance methods. However, increasing HIV prevalence among MSM suggests this as an appropriate target group for continued TDR surveillance. Here, we report findings from a prospective, annual TDR survey in recently infected, treatment-naive individuals at the Anonymous Clinic of the Thai Red Cross AIDS Research Centre (TRC-ARC) in Bangkok, Thailand during 2008–10.

Methods

Study population

As part of a regional multicentre study on HIVDR in Asia (TREAT Asia Studies to Evaluate Resistance-Surveillance), ~80 recently HIV-1-infected, antiretroviral-naive individuals were consecutively recruited each year from 2008 to 2010 at the TRC-ARC. Only newly infected MSM were consecutively

recruited in 2010, as MSM represented the majority of newly infected cases in that year. Recent HIV infection was defined as a new HIV diagnosis in subjects between 15 and 25 years old. For those who were >25 years old, previous HIV-negative documentation within the past 12 months was required for study inclusion.

Determination of TDR and drug susceptibility

HIVDR testing was done with an in-house assay covering amino acids 1–99 of the protease (Pr) gene and amino acids 20–260 of the reverse transcriptase (RT) gene. TDR was defined by the presence of at least one amino acid mutation as listed in the 2009 WHO surveillance drug resistance mutation (SDRM) list.[6] Predicted susceptibility scores to specific antiretroviral drugs were estimated using the Stanford HIV Sequence Database drug susceptibility tool (version 7.0, sierra2.stanford.edu/sierra/servlet/JSierra, calculated on 6 September 2014). These scores were used to classify participants into three resistance levels per drug: low- (<30), intermediate- (30–59) and high-level (≥60) resistance, respectively. The WHO's cost-saving method of determining TDR prevalence in small sample sizes[7] was tested in our larger sample set in order to evaluate its compatibility. The WHO method as well as the methods of HIV testing, HIV subtyping, Stanford drug susceptibility scoring, data collection and statistical methods used are described in the Supplementary data (available at *JAC* Online).

Results

Demographic and laboratory characteristics

A total of 299 recently infected subjects with a mean age of 23 years were consecutively enrolled from 2008 ($n=130$), 2009 ($n=89$) and 2010 ($n=80$). Among these, 264 (88%) were MSM and 34 (11%) were heterosexuals (Table 1). Median viral load (VL) in 2008 was significantly lower than in 2010 among MSM ($P=0.006$) as well as among all subjects ($P=0.004$). There were no significant differences in age, CD4 and VL between the 29 non-MSM and 101 MSM enrolled in 2008.

Genotypic drug resistance

Of the 299 enrolled subjects, 284 RT/Pr sequences were analysable (124 from 2008, 84 from 2009 and 76 from 2010): 5 were only amplifiable in either RT or Pr and 10 were not amplifiable, all with VLs between 39 and 1200 copies/mL.

Fourteen of 284 sequences (4.9%, 95% CI 2.8%–8.3%) had at least one HIV SDRM; 7 (2.5%, 95% CI 1.0%–5.0%) had NRTI SDRMs; 8 (2.8%, 95% CI 1.2%–5.5%) had NNRTI SDRMs; and 6 (2.1%, 95% CI 0.8%–4.5%) had PI SDRMs (Table S1). In 2008, 2009 and 2010, a total of 5/124 (4.0%), 5/84 (6.0%) and 4/76

Table 1. Demographic and laboratory characteristics of all participants

	Participants			
	all participants	2008	2009	2010
Number	299	130	89	80
Male, *n* (%)	280 (94)	115 (88)	85 (96)	80 (100)
Female, *n* (%)	19 (6)	15 (12)	4 (4)	0
Age at enrolment (years), median (range)	23 (17–47)	23 (17–46)	22 (18–47)	23 (17–45)
Thai ethnicity, *n* (%)	297 (99)	130 (100)	87 (98)	80 (100)
Exposure, *n* (%)				
MSM	264 (88)	100 (77)	84 (94)	80 (100)
MSM and intravenous drug users	1 (0)	1 (1)	0 (0)	0 (0)
heterosexual	34 (11)	29 (22)	5 (6)	0 (0)
HIV RNA (copies/mL), median (range)[a]	37020 (39–10000000)	28340 (376–948200)	40660 (375–10000000)	64040 (39–2459000)
CD4 count (cells/mm^3), median (range)[b]	348 (9–1007)	357 (9–1007)	350 (31–666)	345 (89–842)
CD4%, median (range)[b]	18 (1–42)	18 (1–42)	18 (3–34)	17 (7–35)
Subtype (with sequences), *n* (%)				
B	25 (8)	12 (9)	7 (8)	6 (8)
CRF01_AE	232 (78)	102 (78)	68 (76)	62 (78)
A	10 (3)	2 (2)	5 (6)	3 (4)
AB	9 (3)	4 (3)	2 (2)	3 (4)
BA	3 (1)	2 (2)	0 (0)	1 (1)
BD	2 (1)	0 (0)	2 (2)	0 (0)
recombinant[c]	3 (1)	2 (2)	0 (0)	1 (1)
unsequenced	15 (5)	6 (5)	5 (6)	4 (5)

[a]*P* value comparing 2008 and 2010: <0.01.
[b]CD4 count data were missing in 7, 43 and 14 patients in 2008, 2009 and 2010, respectively, and 1 additional patient in 2009 was missing the CD4%.
[c]Not defined further by the Rega subtyping tool. Manual examination of all three sequences suggested they are BA recombinants.

Table 2. Prevalence of TDR classified by drug classes and years and comparison of the odds of having at least one resistance mutation between years

	NRTI	NNRTI	PI	Any
Prevalence, % (95% CI)				
2008	0.8 (0–4.4)	0.8 (0–4.4)	3.2 (0.9–8.1)	4.0 (1.5–9.6)
2009	2.4 (0.3–8.3)	3.6 (0.7–10.1)	1.2 (0–6.5)	5.9 (2.2–14.0)
2010	5.3 (1.5–12.9)	5.3 (1.4–12.9)	1.3 (0.3–7.1)	5.3 (1.7–13.6)
Comparison, OR (95% CI)				
2009 versus 2008	3.0 (0.2–178.2) $P=0.57$	4.5 (0.4–240.7) $P=0.31$	0.4 (0–3.8) $P=0.65$	1.5 (0.33–6.8) $P=0.53$
2010 versus 2008	6.8 (0.7–338.6) $P=0.07$	6.8 (0.7–338.6) $P=0.07$	0.4 (0.1–4.2) $P=0.65$	1.3 (0.25–1.3) $P=0.73$
2010 versus 2009	2.3 (0.3–25.8) $P=0.42$	1.5 (0.2–10.6) $P=0.71$	1.1 (0.01–87.8) $P=1.0$	0.88 (0.17–4.3) $P=1.0$

(5.3%) subjects had any SDRM, respectively (Table 2). The most prevalent mutations were NRTI-associated T215D/F/I/Y (1.8%) and M184V/I (1.4%), NNRTI-associated K103N (1.1%) and Y181C (1.8%) and PI-associated V82A (1.1%) (Table S1).

The frequency of TDR to NRTIs and NNRTIs, but not to PIs, showed an increasing trend from 2008 to 2010 ($P=0.07$; Table 2). NRTI mutations increased from 0.8% in 2008 to 2.4% in 2009 and 5.3% in 2010. Similarly, NNRTI mutations increased from 0.8% in 2008 to 3.6% in 2009 and 5.3% in 2010. The odds of having an NRTI or NNRTI mutation were 6.8 times higher from 2008 to 2010 (95% CI 0.7–338.6, $P=0.07$). The number of PI mutations did not increase significantly over the study period.

Mutation scoring per year by drug class

Only efavirenz showed significant differential predicted resistance over the years ($P<0.01$; Figure S1), with more high-level resistance in 2008 and 2009 compared with 2010 and more intermediate-level resistance in 2010. The newer generation NNRTIs, etravirine and rilpivirine, had somewhat higher prevalence of intermediate versus low resistance in 2010 compared with the earlier years ($P=0.08$ and 0.03, respectively; Figure S1). No significant changes in predicted PI resistance (lopinavir/ritonavir and atazanavir/ritonavir) by year were seen.

Comparison of TDR prevalence with WHO-recommended methods

The WHO surveillance threshold method to estimate TDR prevalence by drug class (NRTI, NNRTI, PI and any) and year performed well using our data (Table S2) and corresponded to 10 of our 12 estimates (Table S2). The two discordances were: (i) any TDR in 2008, 4% (low) by our data and intermediate (5%–15%) by WHO algorithm; and (ii) NRTI TDR in 2009, 2.4% (low) by our data and intermediate by WHO algorithm. In each case, our calculated 95% confidence intervals overlapped with the WHO algorithm.

Discussion

Our study highlights the changes in TDR in an MSM-predominant population over three consecutive years in a resource-limited setting. Of the 284 analysable RT/Pr sequences from the 299 subjects enrolled from 2008 to 2010, 14 (4.9%) had at least one SDRM, distributed almost equally among the three drug classes (NRTI, NNRTI and PI). TDR to NRTIs and NNRTIs, but not to PIs, showed an increasing trend from low prevalence in 2008 to intermediate prevalence in 2010 ($P=0.07$). Only rilpivirine had greater predicted resistance with time. Good, though not complete, correlation was found between our results and the WHO methods.

Temporal changes in TDR have been inconsistent in different parts of the world. Initially, high TDR prevalence (6.2%–21%) was reported in Western countries,[8–10] which then stabilized or declined. A review of time trends of TDR prevalence by Frentz *et al*.[11] across three time periods (<2001, 2001–03 and 2003–09) showed that NRTI TDR declined over time in North America ($P=0.03$), Europe ($P<0.001$) and Latin America ($P<0.001$), but increased in Asia ($P=0.047$) and Africa ($P=0.001$). However, NNRTI TDR increased in all parts of the world. Gupta *et al*.[4] also reported significant global increases of NNRTI TDR over time since ART rollout (2001–11).

TDR in this study was associated mainly with zidovudine (T215D/F/I/Y), lamivudine (M184V/I) and NNRTIs (K103N and Y181C). These are the drugs and drug classes that have been used in first-line regimens since the start of universal access to ART in Thailand in 2004.[12] These findings and the observed trends in the study years warrant evaluation of resistance to the new NNRTIs before their use in subsequent regimens. HIVDR testing prior to ART initiation as used in the developed world[13] is difficult to implement in resource-limited settings due to cost and infrastructure constraints unless cost-effectiveness can be proved.[14] In this study, comparability of the WHO threshold surveillance method of using small sample sizes was compared with results from our larger sample set. Although actual testing from a larger sample size should theoretically be more accurate than estimation from a more limited number, we found that the WHO's cost-saving method compared well with the actual testing in most circumstances.

The main limitation of our study is the inclusion of patients from only one HIV testing site in Bangkok, comprised mainly of patients from urban areas and who were self-reported MSM. Additional surveys of different target populations within a range of geographic and demographic settings would be needed to confirm national trends in increasing TDR. However, the data complement other epidemiologic studies of the prevalence and incidence of HIV in Bangkok.

In summary, our results show that the prevalence of TDR may be increasing in Bangkok, Thailand, where the scaling up of ART has been ongoing since 2004. Strategies to prevent treatment failure and secondary transmission of HIV should target those at highest risk of infection and drug resistance. Greater efforts to support the

development and implementation of accessible and affordable genotypic drug resistance testing are needed in this setting.

Acknowledgements

We would like to thank all the study volunteers for their kind contribution. The TREAT Asia Studies to Evaluate Resistance (TASER) is an initiative of TREAT Asia, a programme of amfAR, The Foundation for AIDS Research.

Funding

The major support of this project was provided by the Dutch Ministry of Foreign Affairs through a partnership with Stichting Aids Fonds, and with additional support from amfAR. S. S. and K. R. have received partial support from the National Research University (NRU) Project of the Commission of Higher Education (CHE) and the Ratchadaphiseksomphot Endowment Fund (HR1161A), Thailand. K. R. has also received partial support from the Senior Researcher Scholar, the Thailand Research Fund (TRF). A. K. D. and R. K. were supported by the US NIAID-funded Lifespan/Tufts/Brown Center for AIDS Research (P30AI042853) and R01AI066922.

Transparency declarations

None to declare.

Disclaimer

The content of this publication is solely the responsibility of the authors and does not necessarily represent the official views of any of the governments or institutions mentioned above.

Supplementary data

Supplementary Materials and methods, Table S1, Table S2 and Figure S1 are available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

References

1 Phanuphak P, Locharernkul C, Panmuong W *et al*. A report of three cases of AIDS in Thailand. *Asian Pac J Allergy Immunol* 1985; **3**: 195–9.

2 Weniger BG, Limpakarnjanarat K, Ungchusak K *et al*. The epidemiology of HIV infection and AIDS in Thailand. *AIDS* 1991; **5** Suppl 2: S71–85.

3 van Griensven F, Varangrat A, Wimonsate W *et al*. Trends in HIV prevalence, estimated HIV incidence, and risk behavior among men who have sex with men in Bangkok, Thailand, 2003–2007. *J Acquir Immune Defic Syndr* 2010; **53**: 234–9.

4 Gupta RK, Jordan MR, Sultan BJ *et al*. Global trends in antiretroviral resistance in treatment-naïve individuals with HIV after rollout of antiretroviral treatment in resource-limited settings: a global collaborative study and meta-regression analysis. *Lancet* 2012; **380**: 1250–8.

5 Sirivichayakul S, Phanuphak P, Pankam T *et al*. HIV drug resistance transmission threshold survey in Bangkok, Thailand. *Antivir Ther* 2008; **13** Suppl 2: 109–13.

6 Bennett DE, Camacho RJ, Otelea D *et al*. Drug resistance mutations for surveillance of transmitted HIV-1 drug-resistance: 2009 update. *PLoS One* 2009; **4**: e4724.

7 Bennett DE, Myatt M, Bertagnolio S *et al*. Recommendations for surveillance of transmitted HIV drug resistance in countries scaling up antiretroviral treatment. *Antivir Ther* 2008; **13** Suppl 2: 25–36.

8 Ross L, Lim ML, Liao Q *et al*. Prevalence of antiretroviral drug resistance and resistance-associated mutations in antiretroviral therapy-naïve HIV-infected individuals from 40 United States cities. *HIV Clin Trials* 2007; **8**: 1–8.

9 Descamps D, Chaix ML, Montes B *et al*. Increasing prevalence of transmitted drug resistance mutations and non-B subtype circulation in antiretroviral-naïve chronically HIV-infected patients from 2001 to 2006/2007 in France. *J Antimicrob Chemother* 2010; **65**: 2620–7.

10 Cane P, Chrystie I, Dunn D *et al*. Time trends in primary resistance to HIV drugs in the United Kingdom: multicentre observational study. *BMJ* 2005; **331**: 1368.

11 Frentz D, Boucher CA, van de Vijver DA. Temporal changes in the epidemiology of transmission of drug-resistant HIV-1 across the world. *AIDS Rev* 2012; **14**: 17–27.

12 Sungkanuparph S, Techasathit W, Utaipiboon C *et al*. Thai national guidelines for antiretroviral therapy in HIV-1 infected adults and adolescents 2010. *Asian Biomed* 2010; **4**: 515–28.

13 Hirsch MS, Gunthard HF, Schapiro JM *et al*. Antiretroviral drug resistance testing in adult HIV-1 infection: 2008 recommendations of an International AIDS Society-USA panel. *Clin Infect Dis* 2008; **47**: 266–85.

14 Sax PE, Islam R, Walensky RP *et al*. Should resistance testing be performed for treatment-naïve HIV-infected patients? A cost-effectiveness analysis. *Clin Infect Dis* 2005; **41**: 1316–23.