HIV Drug Resistance Assessment in the Western Pacific Region. A Systematic Review

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Abstract

Antiretroviral therapy is being rapidly scaled-up in Western Pacific region countries. Prevention and assessment of HIV drug resistance is an essential component of successful global antiretroviral therapy scale-up. We performed a systematic review of public health surveys and HIV drug resistance studies conducted in the low- and middle-income countries in the Western Pacific region. A total of 38 publications assessing HIV drug resistance were reviewed. Studies assessing transmitted drug resistance in recently infected individuals or drug resistance among individuals starting antiretroviral therapy found low rates of HIV drug resistance. Assessments of HIV drug resistance emerging in populations receiving antiretroviral therapy demonstrated variable rates of drug resistance, but suggest an urgent need to support antiretroviral therapy adherence and retention in care, ensure the use of quality assured drugs, and guarantee continuous drug supplies. Additionally, programmatic assessment informed by routine standardized surveillance of transmitted and acquired HIV drug resistance is essential to optimize antiretroviral therapy delivery in the Western Pacific region. **(AIDS Rev. 2011;13:214-26)**

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Key words

HIV. Drug resistance. Western Pacific.

Introduction

Since 2003, the international momentum generated by the World Health Organization's (WHO) "3 by 5" initiative has led to greatly expanded efforts to scale-up access to antiretroviral therapy (ART) for people living with HIV in low- and middle-income countries. At the end of 2009, 5.25 million people were receiving ART globally, with an estimated 160,000 receiving ART in the Western Pacific region, representing an almost ten-fold increase over a period of five years[1]. The number of patients on ART in Cambodia, China, Malaysia, Papua New Guinea, and Vietnam accounts for more than 90% of the total number receiving ART in the region. The rapid scale-up of ART in the Western Pacific has been possible because of the use of a public health approach following standardized treatment protocols with decentralized service delivery, enabling treatment to be delivered to large numbers of infected individuals. Treatment outcomes in the Western Pacific are similar to those reported in other regions. In Vietnam, for example, follow-up of 5,184 patients initiating ART at 31 facilities between January and December 2008 showed that 84% remained alive and on treatment after 12 months of therapy[2]. Similar data are available for other countries in the region[3]. In a 2009 WHO survey, 97% of patients who had ever started on ART were still receiving first-line regimens in Cambodia, China, Papua New Guinea, and Vietnam[4], suggesting either long-term efficacy and durability of these regimens, or limited capacity to identify failures and switch patients to second-line regimens.

As ART is scaled-up, some degree of HIV drug resistance (HIVDR) is inevitable[5]. Therefore, global ART

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scale-up should be accompanied by robust, programmatic assessment informed by routine surveillance of acquired and transmitted HIVDR[6]. A major international concern has been that rapid scale-up of ART would lead to the emergence and transmission of HIVDR in resource-limited settings due to poor adherence, treatment interruptions caused by drug stock-outs, and inadequate HIVDR surveillance, thus undermining the success of population-based ART programs[7,8]. In response to these concerns, the WHO developed a global strategy for the prevention and assessment of HIVDR, including methods to estimate transmitted and acquired HIVDR for public health planning purposes[8,9].

Broadly, there are three categories of HIVDR. Acquired HIVDR occurs when resistance mutations are selected for by drug-selective pressure in individuals receiving antiretroviral drugs (ARV). In individuals taking ART, acquired HIVDR may emerge due to suboptimal adherence, treatment interruptions, inadequate plasma drug concentrations, or the use of suboptimal drugs or drug combinations. Transmitted HIVDR occurs when previously uninfected individuals are infected with drug-resistant virus. The term "transmitted HIVDR" is appropriately applied only to HIVDR detected in recently infected individuals. The third category is HIVDR detected in individuals with chronic infection where drug resistance can be either transmitted or acquired.

Transmitted HIVDR may persist for many months or years in the absence of drug selective pressure (i.e. in individuals naive to ARV), although duration varies by mutation. For example, the reverse transcriptase (RT) mutation M184V, which confers resistance to the nucleoside reverse transcriptase inhibitors (NRTI) lamivudine and emtricitabine, reduces viral fitness; while, the K103N and Y181C mutations that cause resistance to the non-nucleoside reverse transcriptase inhibitors (NNRTI) nevirapine and efavirenz have little impact on viral fitness[10]. In an individual infected by a virus with drug resistance mutations that only modestly reduce fitness, most but not all mutant species are likely to persist over long periods of time. Specifically, M41L, T69N, K103N, and some T215 variants show little tendency to revert to wild-type over time. However, it is theoretically possible that some transmitted drug-resistant HIV may have reverted to wild-type prior to genotypic assessment[11-13] or have fallen to levels below the threshold of detection by population-based sequencing, persisting as minority variants or archived resistance in proviral DNA[14]. However, some HIVDR detected in chronically infected patients may in fact be acquired due to previous ARV exposure not elicited at the time of testing due to social desirability bias, desire of individuals to participate in a particular study, or interviewer bias. Nonetheless, there is value in surveying HIVDR in populations starting ART in settings where transmitted drug resistance (TDR) is known to occur at high levels, and results provide data about the likely efficacy of currently available regimens in patients starting ART.

An important consideration in determining the prevalence of HIVDR is the method used to classify mutations. When assessing transmitted HIVDR, the WHO recommends use of the WHO surveillance drug resistance mutations list[15]. Mutations included on this list are recognized as: (i) causing or contributing to HIVDR; (ii) non-polymorphic; (iii) subtype independent; and (iv) emerging under drug selective pressure. Surveys of transmitted resistance using this list may be compared over time and between regions. When assessing acquired HIVDR or HIVDR in chronically infected patients, clinically based algorithms such as the Stanford HIV Drug Resistance database[16,17], which considers the contribution of polymorphisms and mutation combination to overall drug susceptibility, or the IAS-USA HIV mutations list are used[18].

Since the median time from HIV infection eligibility for initiation of ART is estimated to be 7-9 years[19], TDR may have reverted to wild-type prior to assessment[12,13,20] or have fallen to levels below the threshold of detection by population-based sequencing[21,22].

Transmitted drug-resistant HIV has been documented in many countries with established ART programs[23,24]. Surveillance of TDR is important because results support a country's choice of pre- and post-exposure prophylaxis, prevention of mother-to-child transmission of HIV, and future first-line regimens. Additionally, rates of TDR provide direct evidence of the success of a country's prevention for positives programs. Studies have shown that the likelihood of resistance in acute seroconverters was inversely correlated to the presence of undetectable viral loads in chronically infected patients (i.e. the potential sources of infection). Surveillance of TDR should be performed in recently infected populations sampled from a clearly defined geographical region[25]; and ideally, from individuals sharing a common HIV risk exposure (i.e. heterosexual contact, intravenous drug use, men having sex with men)[9,26]. Importantly, accurate estimation of TDR rests upon sampling recently infected populations, which minimizes the likelihood of reversion to wild type or ARV exposure (prevention of mother-to-child transmission, pre- and post-exposure prophylaxis, sharing of ARV, others) prior to drug resistance testing[22].

The WHO has developed a minimum resource method to classify TDR in recently infected populations, which uses epidemiological criteria to define recent

infection in specific populations in defined geographic regions[9]. Additional methods have been described to identify likely incident infection, including incidence rates obtained from observational seroconversion studies and laboratory methods (serologic testing algorithm for recent HIV seroconversion or STARHS), such as BED and antibody affinity assays. Importantly, a proportion of chronically infected individuals will misclassify as "recent" on BED testing and the false recent rate varies substantially between populations; therefore, a local false recent rate is required to properly calibrate the HIV incidence estimate[27].

Just as the assessment of TDR has programmatic implications, population-based surveillance of acquired HIVDR is an important component of ART programmatic assessment. As an important tool for patient monitoring, WHO suggests that ART sites achieve ≥ 70% viral load suppression in patients 12 months after initiation of ART[28]. Importantly, viral load suppression is a direct marker of successful HIVDR prevention as patients with undetectable viral load have no effective HIVDR[28].

The purpose of this review is to evaluate the published literature assessing rates of transmitted, acquired, and HIVDR present in chronically infected patients (naive to ART) in the Western Pacific, identify gaps, and highlight areas for future surveillance activities.

Methods

Sources of information. The primary sources of information used were peer reviewed publications appearing in scientific journals, abstracts, and reports presented at major international conferences.

Search strategy. We performed a systematic literature review to identify all studies, surveys, and reports published in English and Chinese that assessed transmitted and acquired HIVDR in the Western Pacific. We used PubMed, GlobalHealthLibrary (WHO database), NLM Gate Way (National Library of Medicine database for conference abstracts), GoogleScholar, VIP and WanFang (databases for Chinese literature). Abstracts presented at IAS conferences and CROI were identified from the respective conference websites. There was no limitation to years of publication.

The following search terms were used: "HIV" OR "AIDS" OR "human immunodeficiency virus" OR "acquired immunodeficiency syndrome" AND "resistance" OR "drug resistance" OR "genotype*" AND "Asia" OR "China" OR "Vietnam" OR "Papua New Guinea" OR "Malaysia" OR "Cambodia" or "Pacific countries". The search was supplemented by reviewing the bibliographies of key papers.

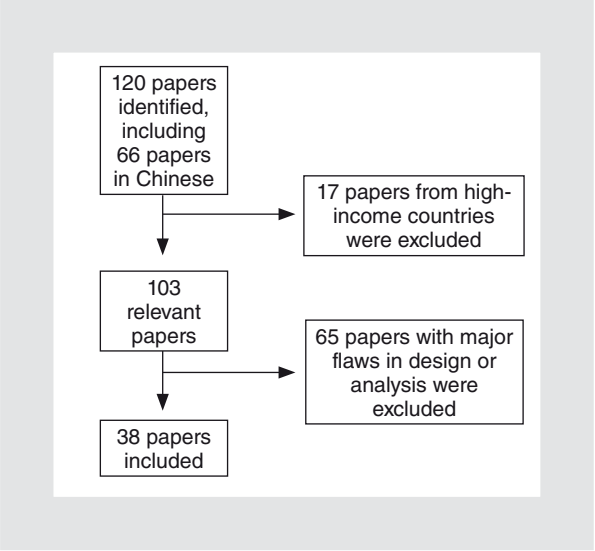

Figure 1. *Flow chart for paper selection.*

Collection of papers and abstraction of data. All English and Chinese language publications assessing transmitted and acquired HIVDR in the Western Pacific were initially included. Dongbao Yu abstracted and translated relevant information from Chinese publications into English. Three reviewers (Yu, Sutherland, and Jordan) independently read and appraised the quality and content of each paper. Publications were included in the review if the surveys/studies clearly identified the population(s) being studied and clearly described the epidemiological and laboratory methods used. Studies/surveys with obvious epidemiological flaws in design or data analysis were excluded from the review. Due to marked heterogeneity in study design, meta-analyses were not appropriate (Fig. 1).

Results

Published studies were divided into three types: (i) assessment of TDR in individuals with recent HIV infection; (ii) assessment of HIVDR among reportedly ARV-naive individuals with chronic infection; and (iii) assessment of acquired HIVDR among individuals receiving ART.

Assessments of transmitted HIV drug resistance

Ten studies from Cambodia, China and Vietnam were included in this review (Table 1).

Table 1. Surveys assessing transmitted drug resistant HIV among individuals with recent infection in Western Pacific region countries

Authors	Country and area	Study design and subjects	Results and drug resistance mutations	Reviewers' comments
Ly, et al. 2005[29]	Cambodia	146 subjects from VCT center (seroconversion < 1 year) and pregnant women (HIV positive for the first time in previous year).	136 isolates successfully genotyped; 5 with DRM to RTI: K70R (1), V75M (3), and K101E (1).	The prevalence of DRM is 3.7%. Noted that all PI mutations are polymorphic and can be ignored.
Yuan, et al. 2009[30]	China: Henan	69 individuals, recruited Nov, 2007 to Aug, 2008, aged 2-25 years; no AIDS; no ART history or ARV exposure.	50 (of the 69) specimens amplified and genotyped. 1 case with K103N, and until 47 specimens, thus classified as < 5% according to WHO method.	Caveats on the mixed population.
Tu, et al. 2009[34]	China: Henan	39 BED-confirmed new infections out of 271 HIV-positive cases from 5,204 specimens, Aug, 2006 to June, 2007 collected from VCT sites.	34 genotyped, 3 cases with DRM to NNRTI.	Caveats on using BED method for confirming individual new infection; sample size not big enough to make conclusions.
Chen, et al. 2008[31]	China: Hunan	86 specimens (68 residue sera after the HIV confirmation test); mean age 21.26 years (range 2-25); mixed routes of transmission.	69 genotyped; DRM in 2 subjects. 5 NRTI DRM: V75M, V118I, T69S; 3 NNRTI DRM: K103N, V181C, K103R; no major PI DRM.	Drug resistance is 2.9% (2/69). Caveats on the mixed routes of transmissions and criteria of new infection.
Zhang, et al. 2010[32]	China: Shandong	53 DBS from VCT sites in 12 cities in Shandong province, subjects with mixed transmission routes.	Consecutively genotyped 47 of the 53 DBS specimens (88.7%); 1 found DRM (K101E).	HIVDR is low at < 5%. Caveats on large geographic catchment area of subjects.
Liao, et al. 2007[33]	China: Xinjiang, Sichuan	25 IDU subjects from Sichuan and Xinjiang provinces identified as recent infections in a cohort study in Mar, 2003 and Nov, 2004. Known cohort of seroconverters.	No major DRM found.	New infections are seroconverters in a cohort study. Caveats on small sample size, subjects from two different provinces.
Wang, et al. 2008[35]	China: Yunnan	64 subjects confirmed as newly infected by BED, out of 1,048 specimens of newly tested HIV infections by the surveillance system in Dehong, Yunnan province during Jul to Dec, 2006.	4 specimens (6.25%) reported DRM to NRTI and NNRTI based on TrueGene OpenGene System; M41L, E44D, T69D/N, V75A/T, K101E/Q, K103N, V108I, V118I, V179D/E, Y181C, M184V, G190A, L210W, T215Y/S.	Generalizable to the area under study. Caveats on BED method for indentifying recent infections.
Feng, et al. 2008[36]	China: Chongqing	33 subjects identified as recent infection by BED assay out of 1,000 specimens from MSM collected by snowballing method.	22 successfully genotyped, 1 subject found major PR DRM (M46L).	Caveats on the small sample size, low genotyping success, and BED method for identifying recent infections.
Nguyen, et al. 2007[37]	Vietnam: Hanoi	VCT sites clients, 18-24 years old; from 2 VCT sites in Hanoi.	49 of the first 52 specimens amplified for genotyping. Both L74V and Y181C found in 1 specimen.	The prevalence of TDR was low in Hanoi according to WHO methodology.
Ayouba, et al. 2009[39]	Vietnam: Ho Chi Minh City	Review limited to subjects of Ho Chi Minh City: 63 cases attending VCT centers with CD4 counts > 500 cells/mm^3, ARV naive.	2 subjects with DRM (out of 63): 1 with G190A, 1 with M46I.	The HIVDR is < 5%. Caveats on sampling and generalizability of the study.

VCT: voluntary counseling and testing; DBS: dried blood spots; IDU: injecting drug user; MSM: men who have sex with men; DRM: drug resistance mutation; RTI: reverse transcriptase inhibitor; NRTI: nucleoside reverse transcriptase inhibitor; NNRTI: nonnucleoside reverse transcriptase inhibitor; PI: protease inhibitor: HIVDR: HIV drug resistance; TDR: transmitted drug resistance.

Cambodia. Ly, et al.[29] assessed TDR in 146 individuals with self-reported "recent infections". Specimens from 22 individuals from voluntary counseling and testing centers with suspected seroconversion within the previous year and 124 pregnant women with their first positive HIV test attending antenatal care were tested. Five specimens (4.9%) were found to have HIVDR: three with V75M, one with K70R and one with K101E.

China. Seven studies were identified assessing TDR among individuals with "recent infection". Yuan, et al.[30] assessed TDR in 69 individuals (age 2-25 years) from Henan province. Overall, 72% of specimens were successfully sequenced and TDR was reported as < 5%. Using specimens obtained from routine HIV surveillance, Chen, et al.[31] assessed TDR in Hunan province, genotyping 86 specimens from individuals aged 2-25 years (mean 21 years) having mixed risk factors for HIV acquisition (injecting drug use [IDU], heterosexual, men who have sex with men [MSM], and mother-to-child transmission). Two of 69 (2.9%) specimens successfully genotyped had NNRTI drug resistance mutations (K103N, V181C)[13]. Zhang, et al.[32] reported a survey in Shandong province using dried blood spots obtained from patients with mixed modes of HIV acquisition from 12 different voluntary counseling and testing sites within the province. One of 47 specimens was found to have K101E; the authors concluded that TDR was low for NRTI, NNRTI, and protease inhibitors (PI). Using a prospective cohort, Liao, et al.[33] found no TDR in a survey of 25 IDU identified as having seroconverted between March, 2003 and November, 2004 in Sichuan and Xinjiang provinces.

Using laboratory methods to identify likely recent infection, Tu, et al.[34] identified 39 HIV-1 BED assay defined "recent infections" from a total of 271 HIV-positive cases out of 5,204 specimens tested from voluntary counseling and testing sites in Henan province. Three of the 34 successfully genotyped specimens (8.8%) had NNRTI drug resistance. Similarly, Wang, et al.[35] assessed TDR in 64 patients reported as "recently infected" by BED assay, out of 1,048 specimens tested for HIV infection collected from routine HIV surveillance in Dehong Prefecture, Yunnan Province between July and December, 2006. Four specimens (6.25%) had NRTI and/or NNRTI drug resistance. No false recent rate calibration was reported. Feng, et al.[36] assessed HIVDR among 33 MSM judged as recent infection by BED; one specimen (4.55%) had the PI mutation M46L. False recent rate calibration was not reported.

Vietnam. In 2007, Nguyen, et al.[37] assessed TDR in Hanoi, Vietnam using the WHO suggested method[38]. Subjects aged < 24 years from two voluntary counseling and testing sites in Hanoi were assessed. One specimen had one NRTI mutation (L74V) and one NNRTI mutation (Y181C); TDR was classified as < 5% for both NRTI and NNRTI in Hanoi. In a multicenter study, Ayouba, et al.[39] documented 2/63 patients attending voluntary counseling and testing centers in Ho Chi Minh City with the following presumed NNRTI and NRTI TDR mutations: G190A and M46I, respectively.

Assessing HIV drug resistance in chronically infected populations starting antiretroviral therapy

Seventeen studies assessing HIVDR in chronically infected patients initiating ART are summarized in table 2.

Cambodia. Nouhin, et al.[40] reported a prevalence of "baseline HIVDR" of 1.49% among 67 patients (18-30 years with CD4 > 350/mm^3) starting ART from Phnom Penh and other provinces.

China. In 2010, Liao et al.[41] reported results of a large-scale survey of HIVDR among 1,194 chronically infected ARV-naive individuals from 28 provinces. Out of 676 isolates, 26 (3.8%) were found to have one or more drug resistance mutation (DRM). Si, et al.[42] reported aggregate results of "baseline HIVDR" among patients with different risk factors from 21 different provinces; 1/164 specimens had the PI mutation M64I; 10/138 specimens were observed to have one or more NRTI or NNRTI mutation: eight and two specimens with NRTI and NNRTI mutations, respectively. Zhou, et al.[43] collected 84 specimens from Sichuan, Yunnan, Xinjiang, and Hunan between 2005 and 2006 and reported two cases with NRTI (A62V; V179D) and one case with NNRTI (G190A) mutations.

Additional surveys assessing HIVDR in chronically infected individuals have been performed in different Chinese provinces. Liu, et al.[44] surveyed 66 subjects, including 52 reportedly ARV-naive patients in Fujian province, with mixed modes of transmission. Two specimens (4.0%) of ARV-naive subjects were found to have NRTI and NNRTI mutations. Tang, et al.[45] genotyped 135 specimens collected from Hubei during 2004/2005; 97% of the cohort had been infected via selling blood/plasma. Of the specimens successfully sequenced, 13/115 (11.3%) had evidence of HIVDR. He, et al.[46] reported a survey of 51 HIV-1-infected individuals in Hunan. Of the 47 specimens genotyped, one specimen

Table 2. Surveys of HIV drug resistance in reportedly antiretroviral therapy-naive patients starting antiretroviral therapy in Western Pacific region countries

Authors	Country and area	Study design and subjects	Results and drug resistance mutations	Reviewers' comments
Nouhin, et al. 2009[40]	Cambodia	Multicenter ANRS study (ANRS 12134): 67 Cambodian patients: aged 18-30 years, no ARV exposure, CD4 > 350/mm^3; from Phnom Penh and provinces; 65 cases infected by heterosexual and 2 unknown.	Only 59 (56 CRF01_AE and 3 B) of 67 could be amplified. No DRM to PI. One subject with DRM to RTI.	Prevalence of HIVDR was 1.49%. High amplification failure; results not generalizable to the country.
Liao, et al. 2010[41]	China	1,194 ARV-naive HIV-infected individuals from 28 provinces, with mixed routes of transmission: plasma/blood donation, sexual contact, injecting drug use.	Of the 1,194 subjects, 227 with VL < 1,000 copies/mm^3 excluded; of the remaining 967 samples, 676 (69.9%) were successfully sequenced. 26 of 676 isolates found DRM. 3 samples with DRM to PI: M46I, N88D, V82A; 23 with DRM to RTI: M184V/I the most frequent to NRTI; K103N the most frequent to NNRTI.	Large-scale survey on baseline HIVDR in China found HIVDR prevalence at 3.8%.
Liu, et al. 2007[44]	China: Fujian	66 subjects, including 52 ARV-naive patients, with mixed routes of transmission.	2/52 specimens (4.0%) of the ARV-naive subjects found DRM to NRTI or NNRTI (A62V and T69N).	Prevalence of HIVDR was 4% among ARV-naive individuals.
Yu, et al. 2009[49]	China: Guangdong	99 IDU, all males; mean age 31.5 years (22-57 years).	1 DRM (Q151LQ) found out of 97 cases genotyped.	Low baseline HIVDR among IDU in Guangdong. Caveats on generalizability of the study.
Yu, et al. 2009[50]	China: Guangdong	63 subjects from the Detoxification Center of Guangzhou City.	49 successfully genotyped. No DRM to PI; 3 cases with DRM to RTI: L33I, A71V/T; V179D; Q151LQ.	Baseline HIVDR < 5% among IDU in Guangzhou City Detoxification Center. Note that only Q151LQ is DRM, and others considered polymorphisms.
Si, et al. 2004[42]	China: 23 provinces	20% of specimens collected in 2002 national survey were selected for genotyping.	164 PR and 138 RT gene sequences obtained. 1/164 specimen with PI DRM (M64I); 10/138 specimens with one or more RTI-related mutations, of which 8 cases DR to NRTI and 2 cases DR to NNRTI.	Large-scale survey confirmed the low level of baseline HIVDR in China. Caveats about the random selection of specimens thus may limit the generalizability of the findings in this large study.
Tang, et al. 2007[45]	China: Hubei	135 specimens collected in 2004-2005, mean age 44.5 years (26-65); 97.04% infected by blood transmission.	115 genotyped. 1 specimen with NRTI-related DRM: E44K ; 8 with NNRTI-related DRM: K103I, K103N, K103R, V106I, G190A, G190R.	Caveats about the mixed routes of infections, older ages, and possible exposure of ARV.
He, et al. 2007[46]	China: Hunan	51 HIV-1-infected individuals; 42 male and 9 female.	47 genotyped. 1 specimen with major mutations to PI and NRTI.	Caveats about sampling and generalizability of the survey.
Zhou, et al. 2008[43]	China: Hunan, Yunnan, Sichuan and Xinjiang	84 specimens from Sichuan, Yunnan, Xinjiang and Hunan in Jan, 2005 to Dec, 2006.	3 cases with DRM to RTI: A62V; V179D; G190A.	Caveats about sampling and generalizability of the study.
Han, et al. 2007[70]	China: Liaoning	46 specimens from MSM about to start ART, during period 1999-2007; mean age 36 years (18-70 years).	2 PI-related DRM. No definite RTI-related DRM found.	Caveats on the undisclosed history of PI exposure, sampling, and generalizability of the results to the population.

(continue)

Table 2. Surveys of HIV drug resistance in reportedly antiretroviral therapy-naive patients starting antiretroviral therapy in Western Pacific region countries *(continued)*

Authors	Country and area	Study design and subjects	Results and drug resistance mutations	Reviewers' comments
Han, et al. 2006[51]	China: Liaoning	90 patients during 1999-2004: median age 35; mean infection years 7; mixed routes of transmission.	Major DRM to PI: 2 cases with M64I; 3 cases with DRM to RTI: 1 with M184I, 1 A62V+T69N, 1 A62V.	Caveats about the possible ARV exposure, sampling, and generalizability of the results.
Tu, et al. 2009[52]	China: Yunnan	52 HIV-1-seropositive blood units from Yunnan Kunming Blood Center during Feb, 2005 to Aug, 2006.	49 samples genotyped. 1/49 (2.0%) with DRM of clinical significance.	Prevalence of HIVDR mutations among blood donors in Yunnan was low.
Yao, et al. 2008[48]	China: Zhejiang	104 ARV-naive subjects in 2004-2006, mixed routes of transmission.	83 specimens genotyped. 1/74 with major PI-related DRM (M64I): 14/83 had 17 DRM to RTI: 7 to NRTI; 9 to NNRTI.	Overall prevalence of baseline HIVDR mutations was 6%. Caveats about previous exposure to ART, sampling and generalizability of the findings.
Tee, et al. 2006[53]	Malaysia	100 ARV-naive HIV-1-positive individuals.	Only 1 (1%) found 1 major mutation to NNRTI (Y181C).	Baseline HIVDR < 5% in Malaysia among ARV-naive patients before initiating ART. Caveats about sampling and generalizability of the study findings.
Ishizaki, et al. 2009[54]	Vietnam: Hai Phong	301 HIV-positives of 1,355 tested in Apr to Oct, 2007; mixed routes of transmission.	275 specimens genotyped, 3 (1.1%) cases showed major NRTI DRM: 5 (1.8%) showed major NNRTI DRM: 1 (0.3%) harbored both.	Overall DRM prevalence 2.6%. Baseline HIVDR was low. Caveats about previous exposure to ART and generalizability of the findings.
Lan, et al. 2003[56]	Vietnam: Ho Chi Minh City	Part of ANRS 1257 project. 200 subjects, mean age 23 years, mixed routes of transmission.	9 subjects (4.5%) with major DRM to NRTI (1 M41L, 4 K219Q, and 4 M184I). 4 subjects with PI major DRM: 1 D30N and 3 L90M (2%).	Prevalence of resistant strains to ARV is 6.5%. Limitation in interpretation of such a study regarding prevalence of transmission of HIVDR.
Phan, et al. 2010[55]	Vietnam: Ha Noi, Ninh Binh, Dam Dinh	206 HIV infections (161 men and 45 women) in 2008, mean age 32 years (17-54 years). No ARV exposure.	3 subjects (1.7%) with PI DRM; 7 (4.5%) with RTI DRM.	Usual concerns exist re interpretation, HIVDR in an older age group, unknown infection duration and mixed routes of transmission.

IDU: injecting drug user; MSM: men who have sex with men; DRM: drug resistance mutation; ARV: antiretroviral; ART: antiretroviral therapy; RTI: reverse transcriptase inhibitor; NRTI: nucleoside reverse transcriptase inhibitor; NNRTI: nonnucleoside reverse transcriptase inhibitor; PI: protease inhibitor: HIVDR: HIV drug resistance; TDR: transmitted drug resistance.

was found to harbor PI and NRTI mutations. Han, et al.[47] surveyed 90 patients in Liaoning province between 1999 and 2004. Two patients were observed to have major PI mutations (M64I), and three patients were observed to have NRTI mutations: one with M184V, one with A62V+T69N, and one with A62V. Yao, et al.[48] reported on HIVDR among 99 ARV-naive subjects in Zhejiang: 1/74 patients was found to have the major PI mutation M64I, and 14/83 patients had seven and nine NRTI and NNRTI mutations, respectively.

Additionally, HIVDR has been studied in several most at risk populations and special populations such as pregnant women. Yu, et al.[49] assessed HIVDR in 99 male IDU subjects in Guangdong; one isolate demonstrated resistance (Q151L/Q) among 97 specimens genotyped. In a different study by Yu, et al.[50], among 63 HIV-positive IDU recruited from a detoxification center in Guangdong, three were found to have HIVDR: one case with L33I, A71V/T, one with V179D, and one with Q151LQ. In 2006, Han, et al.[51] reported HIVDR from 46 chronically infected MSM in Liaoning province initiating ART between 1999 and 2007. Two patients had PI mutations (L90M, L10I, A71T; M46I). Finally, one of 49 HIV-positive discarded blood units collected from Yunnan Kunming Blood Center between 2005 and 2006 had HIVDR[52].

Malaysia. In 2006, Tee, et al.[53] published results from a survey of 100 ARV-naive HIV-1-positive individuals

initiating ART. One (1%) was found to have resistance to NNRTI (Y181C); no PI mutations were observed.

Vietnam. Ishizaki, et al.[54] surveyed HIVDR among different chronically infected subpopulations including IDU, sex workers, seafarers, pregnant women, and blood donors in Hai Phong, Northern Vietnam, who had tested HIV positive but claimed no prior ARV exposure. Of 273 cases with genotyping data available, three (1.1%) had NRTI mutations (L74I, M184I, K219E), and five (1.8%) had NNRTI mutations (K103N, G190E). One (0.3%) patient had both NRTI and NNRTI mutations (M184I, K103N); overall HIVDR was estimated as 2.6%. In a separate survey of HIVDR in 206 patients in Hai Phong published in 2010[55], the following PI mutations were observed in three (1.7%) patients: L33F, M46I, and M46L; the following NRTI or NNRTI mutations were observed in seven (4.5%) patients: A62V, K103N, and Y181C. Finally, in 2003, the overall prevalence of HIVDR among 200 ARV-naive patients in a cohort from Ho Chi Minh City was 6.5%; NRTI mutations were noted in nine (4.5%): one M41L, four K219Q, and four M184I, and PI mutations were noted in four (one D30N, three L90M)[56].

Assessment of acquired HIV drug resistance

Assessment of acquired HIVDR at the population level is a useful tool for ART programs to assess success in optimizing patient care and in maintaining the long-term efficacy and durability of available first- and second-line regimens. Despite these undisputed benefits, methodological challenges exist. Two methods are generally used to assess the emergence of HIVDR in populations selected for during a course of ART: (i) prospective surveillance performed on a cohort of patients starting ART derived from a single site (WHO recommended methodology[28]) or multiple sites with assessment of HIVDR at predetermined time points (i.e. 12 and 24 months after ART initiation), or (ii) cross-sectional surveys of patients receiving ART at one or more sites for the same or variable durations of time. Eleven publications assessing the emergence of HIVDR in patients receiving ART were included in this review (Table 3).

Cambodia. Nerrienet, et al.[57] prospectively followed 257 adults initiating ART at Calmette Hospital in Phnom Penh. After 18 months, 198 patients (77%) achieved viral suppression (HIV RNA < 400 copies/ml). A total of 41 patients had HIVDR at the time of initiation of ART, with 26 of them developing additional DRM at 18 months. Of the 18 patients without HIVDR prior to start of ART, six developed DRM at 18 months after ART. Janssens, et al.[58] studied the emergence of HIVDR in a cohort of 212 pediatric patients (median age 6 years) from 2003 to 2005. Of 212 children initiating ART, 92 and 91% were alive and in care after 12 and 24 months, respectively; 156/193 (81%) achieved viral suppression (viral load < 400 copies/ml at 12 months), suggesting overall success in the prevention of HIVDR in this cohort. Of the 36 patients with viral load > 400 copies/ml, 32 (89%) had either NNRTI or NRTI resistance mutations as defined by the ANRS mutations list[58].

China. The majority of studies assessing acquired HIVDR have been conducted in Henan province, where China's HIV public heath response was first established and where the largest number of patients receives ART. In 2007, Li, et al.[59] performed a cross-sectional analysis of 431 patients in Henan, of which 104 were ARV-naive, 97 were on ART for < 6 months, 140 were on ART for 6-12 months, and 90 were on ART for > 12 months. The prevalence of HIVDR was 7.0, 48.6, 70.8, and 72.3% for ARV-naive, < 6 months on ART, 6-12 months on ART, and > 12 months on ART groups, respectively. The results support an earlier cohort study[60] in Henan, which demonstrated high prevalence of HIVDR: 6.76, 13.51, 14.86, and 9.46% for zidovudine, and 9.46, 18.92, 22.97, and 32.43% for nevirapine, respectively, in patients receiving ART for 3, 6, 12, and 18 months. Similarly in Henan, Wang, et al.[61] observed a cohort of patients at six month intervals between 2004 and 2006 and reported rates of HIVDR of 19.0, 35.8, 41.7, and 48.2% at 6, 12, 18, and 24 months, respectively. Also in Henan, in 2005[62], cross-sectional surveys were performed on 138 and 112 patients receiving ART for three and six months, respectively; 45.4 and 62.7% of the patients had developed HIVDR by 3 and 6 months, respectively, with the majority of HIVDR reported for codons 103, 106, and 215.

In contrast to the poor treatment outcomes and high HIVDR in Henan, better outcomes have been reported in other provinces. In Hubei, Tang, et al.[63] surveyed 239 subjects receiving first-line ART for a median 24 months; 67.36% of subjects had viral loads < 1,000 copies/mm^3. Of 78 patients with viral load > 1,000 copies/mm^3, 51 had HIVDR testing: 19, 28, and 1% had resistance to NRTI, NNRTI, and PI, respectively. Also in Hubei, Luo, et al.[64] reported a cross-sectional survey carried out between 2003 and 2005. The overall prevalence of HIVDR among patients with

Table 3. Surveys on acquired HIV drug resistance in Western Pacific region countries

Authors	Country and area	Study design and subjects	Results and drug resistance mutation	Reviewers' comments
Janssens, et al. 2007[58]	Cambodia	Design: prospective; 212 pediatric patients, mean age 6 years, recruited during June, 2003 to March, 2005. In January, 2006, 193 of patients were alive and included in the cross-sectional virologic evaluation.	81% (156/193) with undetectable virus. Of 36 with VL > 400 copies/ml, 31 and 32 (of 34 patients) had NRTI and NNRTI mutations, respectively.	ART program outcome is good but of those who were not suppressed a high percentage had HIVDR.
Nerrienet, et al. 2006[57]	Cambodia	Design: prospective; 257 adults, plasma collected before ARV initiation (M0) and at 18 months (M18) in Calmette Hospital.	At 18 months, 198 patients (77%) achieved viral suppression (HIV < 400 copies/ml). 41 were found DRM at baseline, of which 26 showed additional DRM. For the other 18 patients, 6 developed DRM at 18 months after ART.	The study done in a hospital setting. Treatment outcome is good.
Li, et al. 2007[65]	China: Guangxi	Design: cross-sectional; 133 patients on ART, mean duration 9.7 months on ART.	Of the 133 patients, 113 with good adherence, 7 interrupted and 13 stopped. Of the patients, 90.3% had VL < 1,000 copies/mm^3. Genotyping successfully done on 42 cases, with overall HIVDR prevalence of 11.9% (4 to NRTI, 4 to NNRTI, and 1 to PI).	Cross-sectional study, but showed a satisfactory ART treatment outcome in Guangxi province. Caveats about sampling and generalizability of the finding.
Li, et al. 2007[59]	China: Henan	Design: cross-sectional; 431 patients: 104 ARV-naive; 97 on ART < 6 months, 140 on ART 6-12 months, 90 on ART > 12 months.	DRM were detected in 7.0, 48.6, 70.8, and 72.3% of patients who were ARV-naive, < 6 months, 6-12 months, and > 12 months on ART, respectively. A longer duration on ART was correlated with the emergence of HIVDR ($p < 0.005$).	High occurrence of DRM by 12 months of ART and onwards. A warning sign to ART program in Henan.
Li, et al. 2006[60]	China: Henan	Design: prospective cohort; 74 patients infected by blood/plasma donation. No ART history before entering cohort. Specimens collected 3, 6, 12, and 18 months on ART.	HIVDR were 6.76, 13.51, 14.86, and 9.46% to AZT; 9.46, 18.92, 22.97, and 32.43% for NVP, in patients receiving ART at 3, 6, 12, and 18 months after initiation of ART, respectively.	High occurrence of DRM among patients during ART in Henan province. Caveats about small sample size, risk factors collection and analysis, and generalizability of the findings to the whole Henan Province.
Wang, et al. 2007[61]	China: Henan	Design: prospective cohort; 107 subjects on AZT+ddI+NVP. Follow-up every 6 months from Feb, 2004 until 2006, until 2 years.	37 subjects with DR: shortest time 45 days, longest time 558.5 days, with an average of 409.5 days. The accumulated DR were: 19.0, 35.8, 41.7, and 48.2% for 0.5, 1.0, 1.5, and 2.0 years, respectively.	The 2-year accumulated DR is at a high level in Henan Province.
Li, et al. 2005[62]	China: Henan	Design: cross-sectional survey; 138 (3 months on ART) and 112 (6 months on ART) patients on AZT+ddI+NVP since Nov, 2003.	DRM was 45.4% in 3 months in ART group; and 62.7% in 6-month ART group. Mutation sites primarily at the 103, 106, and 215 codons in the 3-month ART group; increased to 15 codon mutations in 6-month ART group.	The occurrence of HIVDR was high in Henan. Concerns with previous exposure to ART and poor adherence for the high rate of HIVDR among patients in the provinces.

(continue)

Table 3. Surveys on acquired HIV drug resistance in Western Pacific region countries *(continued)*

Authors	Country and area	Study design and subjects	Results and drug resistance mutation	Reviewers' comments
Zhang, et al. 2009[66]	China: Henan	Design: prospective; 83 pediatric patients: 32 with ART and 51 ART-naive; median age 11 yrs.	34 of the 41 patients (25 ART-experienced and 9 previously ART-naive) genotyping success. TAM: M41L, D67 N/G, K70R, L210W, T215F/Y, K219E more found in the ART-experienced patients (46 TAM/25 patients vs. 9 TAM/9 patients; $p < 0.05$). NNRTI mutations Y181C more common in previously ART-naive patients compared with ART-experienced patients (77.8 vs. 36.0%; $p < 0.05$).	Drug resistance is high in this cohort. Program and patient factors needed for analyzing the treatment failure and occurrence of HIVDR.
Tang, et al. 2007[63]	China: Hubei	Design: cross-sectional survey; 239 on ART (first-line ARV regimens) for an average of 24 months; survey done in Nov to Dec, 2006.	161 patients (67.36%) had VL < 1,000 copies/mm^3. Of the 78 subjects with VL > 1,000 copies/mm^3, 51 had HIVDR testing: 19, 28, and 1% had resistance to NRTI, NNRTI, and PI, respectively.	Viral suppression similar to another study in Hubei, which was better than reported in Henan.
Luo, et al. 2009[64]	China: Hubei	Design: two repeated cross-sectional survey; 150 ART-naive (99 received subsequent therapy) between 2003 and 2005, and 288 ART-experienced patients mainly between 2005 and 2006. About 83.5% from rural villages, most infected by plasma/blood donations.	186 patients (64.6%) had undetectable viral load over the course of ART, whereas 102 had detectable viremia. DRM increased among those with detected viremia after ART for 3-6 months (24.3%), 9-12 months (57.1%), and 20-24 months (63.3%).	Viral suppression is sub-optimal but better than reported in Henan, China.
Ruan, et al. 2010[67]	China: Hubei, Anhui, Henan	Design: prospective; 341 subjects from 3 provinces followed up for an average of 12.1 months.	265 patients followed up at 12 months. 227 had VL < 1,000 copies/mm^3 (85.7%). 38 patients HIV-1 > 1,000 copies/ml at 12 months. 13 had DRM to NNRTI (34.2%) and NRTI (23.7%); most frequent NNRTI resistance: K101E, K103N/R/S and G190A/S; major NRTI resistance mutations, K70R and M184V.	Caveats about combination of unknown number of ART sites in 3 provinces, which limits the analysis of site and patient factors for HIVDR.

DRM: drug resistance mutation; NVP: nevirapine; ARV: antiretroviral; ART: antiretroviral therapy; AZT: zidovudine; ddI: didanosine; NVP: nevirapine; TAM: thymidine analogue mutation; VL: viral load; RTI: reverse transcriptase inhibitor; NRTI: nucleoside reverse transcriptase inhibitor; NNRTI: nonnucleoside reverse transcriptase inhibitor; PI: protease inhibitor: HIVDR: HIV drug resistance; TDR: transmitted drug resistance.

viremia was 24.3, 57.1, and 63.3%, at 3-6, 9-12, and 20-24 months on ART, respectively. Similar findings were reported in a cross-sectional survey performed in 2007 in Guangxi[65]. Of 133 patient on ART for a mean of 9.7 months, 90.3% had viral loads < 1,000 copies/mm^3. Genotyping was successfully performed in 42 cases, with an overall prevalence of acquired HIVDR of 11.9% (four to NRTI, four to NNRTI, and one to PI).

In 2009, Zhang, et al.[66] reported a study of acquired HIVDR in a cohort of 83 pediatric patients in Henan, 32 with previous ARV experience and 51 ARV naive). A total of 34 of the 41 patients (25 ART experienced and nine previously ART naive) had successful genotyping. Thymidine analogue mutations (TAM) are more commonly found in the ART-experienced patients (46 TAM/25 patients vs. 9 TAM/9 patients; $p < 0.05$). The NNRTI mutations Y181C were more common in previously ART-naive patients compared with ART-experienced patients (77.8 vs. 36.0%; $p < 0.05$). More recently in 2010, Ruan, et al.[67] assessed acquired HIVDR in a cohort of

341 patients from Hubei, Anhui, and Henan provinces. Of 265 patients, 227 had viral loads < 1,000 copies/mm^3 (85.7%) after a mean of 12.1 months on ART. Among patients with viral load > 1,000 copies/mm^3 at 12 months, 34.2% had NNRTI resistance and 23.7% had NRTI resistance. The most frequently observed mutations were K101E, K103N/R/S, G190A/S, K70R, and M184V.

Discussion

Assessment of HIVDR in the Western Pacific region is an active area of research, which highlights the importance attached to it by ART programs, researchers, and funders in the region. As more patients are maintained on ART for longer periods of time, the routine assessment of transmitted and acquired HIVDR will become even more important in measuring the success of ART delivery in minimizing the emergence and transmission of HIVDR and in guiding selection of current pre- and post-exposure prophylaxis, prevention of mother-to-child transmission regimens, and the composition of future first- and second-line ART.

Globally, a significant challenge in assessing TDR remains the identification of recently infected populations. At present, there are three methods commonly used to identify incident cases: identification of seroconverters in a prospective cohort of HIV-negative individuals, application of globally developed and locally appropriate epidemiological surrogate definitions which account for major determinants of spread, age, and risk factor(s)[8] alone or in combination with an appropriately calibrated BED assay, with or without antibody avidity testing or CD4 cell counts. Given the limitations of BED performance and the difficulty in correctly estimating the false recent rate, the WHO recommends the use of surrogate epidemiological definitions applied to specific populations in a specific geographical region at a specified time[9].

Although current data are limited and studies included in this review may not have adequately captured recent infections or have been limited to a single risk group, rates of transmitted HIVDR appear to remain low in the region (< 5%). However, interpretation and generalizability are limited because published reports lack methodological standardization, including standardized inclusion/exclusion criteria, and often include patients of different ages and HIV risk factors from different geographical regions. The lack of standardization and inclusion of different risk groups limits the utility of the data and makes development of targeted public health action challenging.

A large number of studies have been performed assessing HIVDR among chronically infected populations starting ART. These studies generally used convenience samples from large geographical areas and included reportedly ARV-naive patients infected by mixed modes of transmission. Because patients are chronically infected, observed HIVDR may have been transmitted or acquired and the observed prevalence may over- or underestimate the true TDR. Nonetheless, there is value in surveying HIVDR in populations starting ART especially in settings where TDR is known to occur at high levels, and results provide data about the likely efficacy of currently available regimens in patients starting ART.

Importantly, most studies show low rates of HIVDR, suggesting that available first- and second-line regimens are likely to be effective.

Just as assessment of TDR is important and has programmatic implications, so too does assessment of HIVDR emerging in populations receiving ART. In this review, both cross-sectional and prospective cohort methods have been used to assess the emergence of HIVDR in patients taking ART. Notably, rates of acquired HIVDR varied substantially between countries and regions within countries, which may reflect study methodology or the quality of ARV delivery. Outcome assessments in Cambodia[57,58] and some provinces of China[63-65] are positive in terms of rates of viral suppression at 12 months or longer and the low emergence of HIVDR mutations among those with detectible viral load. However, in China's Henan province, reports have repeatedly demonstrated higher rates of virologic failure and higher levels of HIVDR[60,61,68,69]. Given that TDR is likely to be low, high rates of virologic failure and HIVDR suggest suboptimal population adherence to ART and/or the need to strengthen ART retention and adherence support.

An additional consideration when assessing the emergence of acquired HIVDR is appropriate classification of patients who stop therapy, are lost to follow-up, or who fail to suppress but have no HIVDR detected on genotyping. When considering the emergence of acquired HIVDR, it is vital to also consider HIVDR not measured by standard population-based sequencing, either in the cases where patients fail to suppress, or patients with failure to suppress and no HIVDR observed, or in the case where genotyping is not feasible (patients who are lost from care or who have stopped therapy) as they may serve as the reservoir to transmit HIVDR to others.

Given that over 160,000 people are receiving ART in the Western Pacific region[4], the paucity of data regarding the emergence of HIVDR and more specifically standardized evaluation of programmatic success in

preventing HIVDR in populations taking ART is striking. Although viral load and HIVDR testing are not a routine part of individual patient care in the Western Pacific region, this need never limit the optimization of patient care and the minimization of HIVDR through the use of standardized assessment of patient adherence and assessment programmatic factors associated with the emergence of HIVDR such as: physician prescribing practices, drug supply continuity, and use of quality assured drugs. Failure to proactively identify and address programmatic challenges associated with the emergence of HIVDR are likely to lead to inadequate response at the population level of available first- and second-line regimens.

The WHO has recommended surveys to assess the emergence of HIVDR in populations taking ART for the purposes of identifying program and site factors associated with the emergence of HIVDR. However, few have been implemented in this region. One potential barrier may be the inherent sample size requirements of prospective cohort methods, and the need to abstract large amounts of data from available medical and pharmacy records. As ART expands in the Western Pacific, it is imperative that new, simple, and inexpensive public health methodologies be developed to identify ART sites failing to achieve optimal rates of virologic suppression and thus creating an environment for the selection of HIVDR. One such approach may be the development of an alert method using binomial sequential sampling techniques, which uses small sample sizes and which can be applied to a large number of representative sites in order to assess population-based virologic suppression rates above or below defined thresholds. Additionally, estimation of TDR in most at risk populations and in settings with low-prevalence, generalized epidemics remains a challenge due to sample size and generalizability constraints. New epidemiological and/or laboratory methods to reliably identify incident infection and estimate rates of TDR in these settings are urgently needed.

Conclusions

Current evidence suggests that rates of TDR in the Western Pacific region remain low, attesting to the success of ART and HIV prevention programs. However, ongoing vigilance is required. New methods should be developed to assess transmitted HIVDR using behavioral surveys and/or respondent-driven sampling techniques. Moreover, additional research is required to optimize the use of epidemiological surrogates and laboratory methods to identify recently HIV-infected patients to more accurately assess TDR. The lack of data derived using clearly defined methods makes assessing of acquired HIVDR in the region challenging. It is imperative that ART programs in countries of the Western Pacific implement simple, routine and sustainable, representative standardized surveillance of programmatic success in achieving viral load suppression in order to identify programmatic factors needing adjustment to maximize population rates of viral load suppression, thus minimizing the emergence of HIVDR.

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