ANTI-TUBERCULOSIS DRUG RESISTANCE AND HIV CO-INFECTION IN PHNOM PENH, CAMBODIA

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Abstract. The objective of this study was to observe the prevalence of drug resistance in *Mycobacterium tuberculosis* isolates in HIV associated tuberculosis co-infected patients in Phnom Penh City. The isolates of *M. tuberculosis* were collected during active laboratory-based surveillance. Of the 98 isolates studied, *M. tuberculosis* resistance to isoniazid was seen in 23.5%, resistance to rifampicin was seen in 16.3% and multidrug-resistance (MDR-TB) was seen in 5.1%. Our findings reveal an alarmingly high level of resistance to isoniazid and rifampicin, and confirms the need for drug susceptibility testing to guide treatment in patients with culture positive tuberculosis.

INTRODUCTION

Tuberculosis is one of the largest public health problems in Cambodia with immense consequences. The estimated new sputum smear positivity rate for Cambodia is 220/100,000 population, and the mortality rate is 92/100,000 population (WHO, 2008). Cambodia achieved nationwide DOTS coverage at the district level in 1998. By the year 2003 more than 145 tuberculosis units and 700 health centers were implementing the DOTS strategy. For control measures to succeed, early detection and treatment of patients are essential (Saly *et al*, 2006). Sometimes patients fail to respond to treatment with anti-tuberculosis drugs (Yoshiyama *et al*, 2004).

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The HIV epidemic has caused explosive increases in TB incidence and may contribute to increases in MDR-TB prevalence (Wells *et al*, 2007). The reasons for this could be many, one of them being drug resistance. Two recent developments have worsened the tuberculosis pandemic: an increase in multi-drug resistant bacilli resulting from inadequate treatment, the indiscriminate use of antibiotics, and the presence of AIDS which renders individuals more susceptible to the development of tuberculosis. The prevalence of HIV in tuberculosis patients in Cambodia is 10% based on a WHO report (2008).

The emergence of MDR-TB has further exacerbated the tuberculosis problem. Mycobacterial drug susceptibility profiles are performed to give adequate information regarding the treatment of individual patients and to improve strategies applicable to large populations. In this era of emerging drug resistance, there is a lack of information regarding drug susceptibilities in Cambodia (Docze *et al*, 2004). The gold standard for determining drug susceptibilities is the proportion

method using LJ medium, which is easy to perform and does not require expensive equipment, making it an attractive option for use in our mycobacterial laboratories (Espinal *et al*, 2001) when testing for *M. tuberculosis* in HIV infected patients in Phnom Penh, Cambodia.

MATERIALS AND METHODS

Origin of isolates

During active laboratory surveillance over a period of 24 months, from March 2003 to February 2005, 529 respiratory samples were received from 295 HIV patients with pulmonary infections and cultured for mycobacteria. One hundred sixty-eight specimens were culture positive for *M. tuberculosis* in 98 patients, and *Mycobacterium* other than *tuberculosis* was found in isolates from 12 specimens from 9 patients. Two isolates were isolated from two patients with a previous history of treatment for tuberculosis. All the *M. tuberculosis* clinical isolates were stored and frozen at -70ºC in Middlebrook 7H9 medium with ADC enrichment medium (Becton Dickinson) until drug susceptibity testing (DST) was performed.

Drugs susceptibility test (DST)

DST was performed against isoniazid (0.2 mg/l), rifampicin (40 mg/l), streptomycin (4 mg/l), and ethambutol (2 mg/l) (Bio-Rad) for all 98 *M. tuberculosis* clinical primary isolates using the proportion method on LJ slants as described elsewhere (Canetti *et al*, 1969). An initial reading of the tubes was performed on Day 28 of incubation, while the final reading was done after 40 days of incubation. Resistance was defined as growth in the drug containing tubes greater than 1% of the growth in the drug free control medium.

Quality control

The *M. tuberculosis* isolates from the Research Institute of Tuberculosis in Tokyo, V-38, V-58, V-74, V-88, and V-100, were used as the control strains to check for reproducibility of the results.

RESULTS

Drug susceptibility

Forty-senven (48%) of the 98 *M. tuberculosis* clinical primary isolates tested were susceptible to all the drugs tested (Table 1). The remaining 51 (52%) isolates were resistant to at least one drug. The proportion of the patients with resistance to isoniazid was 23 (23.5%), 16 (16.3%) to rifampicin, 10 (10.2%) to streptomycin, and 2 (2%) to

Table 1
The results of susceptibility testing using the proportion method to evaluate *M. tuberculosis* strains isolated from 98 HIV seropositive patients.

Drugs	*M. tuberculosis* (*n*=98)	%
Any resistance		
INH	23	23.5
STR	10	10.2
RIF	16	16.3
EMB	2	2.0
Resistance to one drug		
INH	12	12.3
STR	5	5.1
RIF	11	11.2
EMB	0	0
Resistance to two drugs		
INH, STR	4	4.1
INH, EMB	1	1
Resistance to three drugs		
INH, STR, EMB	1	1
Multi drug resistance (MDR)		
INH, RIF	5	5.1

INH, isoniazid; STR, streptomycin; RIF, rifampicin; EMB, ethambutol.

ethambutol. Mono-resistance to isoniazid was found in 12 (12.3%), streptomycin in 5 (5.1%) and rifampicin in 11 (11.2%). MDR-TB (*ie,* resistance to at least isoniazid and rifampicin) was observed in 5 (5.1%).

DISCUSSION

The emergence and spread of drug resistant *M. tuberculosis* is a serious threat to the tuberculosis control program. A previous study of drug resistance nationally carried out at the National Tuberculosis Control Program of Cambodia in 2001, showed primary mono-resistance of *M. tuberculosis* to isoniazid, streptomycin, and rifampicin in 4.6, 3.5, and 0.50%, respectively, and MDR-TB was observed in 0.3% (Yamada *et al*, 2007). National surveillance in Thailand 1997 showed primary mono-resistance of *M. tuberculosis* to isoniazid, streptomycin, rifampicin, and ethembutol, in 6.2, 5.6, 2.0 and 3.0%, respectively (Espinal *et al*, 2001). Surveillance in Ho Chi Minh City, Vietnam from 1998 to 2000, demonstrated primary mono-resistance to isoniazid, streptomycin, rifampicin, and ethambutol in 6.5, 11.2, 0.1, and 0%, respectively (Quy *et al*, 2006).

Of the 98 isolates of tuberculosis in HIV infected patients, 2 gave a history of previous treatment of pulmonary tuberculosis. The isolates of *M. tuberculosis* in our study had mono-resistance to isoniazid, streptomycin, and rifampicin in 12.3, 5.1, and 11.20 respectively. MDR-TB was present in 5.1%. Punnotok *et al* (2000) evaluated the prevalence of drug resistance in *M. tuberculosis* isolates in HIV infected patients in Bangkok, Thailand; their findings were similar to our findings. A recent retrospective cohort study in Thailand, also demonstrated mono-resitance to isoniazid, rifampicin and MDR-TB was found, similar to our findings (Sungkanuparph *et al*, 2007). Their study also found that MDR-TB reduced survival among patients co-infected with HIV and TB. HIV co-infection appears to be a risk factor for isoniazid and rifampicin mono-resistance and MDR TB. A recent study in Vietnam found the prevalence of MDR-TB in HIV patients in Haiphong City (Khê *et al*, 2008) was similar to our findings. These studies support that HIV is a risk factor for drug resistant tuberculosis. Wells *et al* (2007) found the HIV epidemic has caused explosive increases in the incidence of TB and may be contributing to increases in the prevalence of MDR-TB. Moore *et al* (1999) found that drug resistance, in TB patients with HIV co-infection in the United States, particularly multidrug resistance and rifampicin mono-resistance, were more common among TB patients with AIDS than among other TB patients. HIV infection may lead to malabsorption of anti-TB drugs and acquired rifampicin resistance (Wells *et al*, 2007).

Our study found a high incidence of drug monoresistance and 5% had MDR-TB in HIV coinfected tuberculosis patients. This study had a number of limitations, but the sample is probably representative for smear positive tuberculosis patients with HIV coinfection in Phnom Penh City during the 24-month study period. Monitoring of drug resistance with DOTS is needed. Our findings underscore the urgent need to assure adherence to complete, effective tuberculosis treatment regimens for this group of patients.

ACKNOWLEDGEMENTS

We are grateful to Dr William N Grut for his constructive suggestions and helpful comments regarding the manuscript. This work was supported in part by a grant from Agence Nationale de Recherches sur le SIDA (ANRS 1260) and an internal grant from the Institut Pasteur du Cambodge, Phnom Penh, Kingdom of Cambodia.

REFERENCES

Canetti G, Fox W, Khomenko A, *et al.* Advance in techniques of testing mycobacterial drug sensitivity and the use of sensitivity tests in tuberculosis control programs. *Bull WHO* 1969; 41: 21-43.

Docze A, Benca G, Augustin A, *et al.* Is antimicrobial multi-resistance to antibiotic in Cambodia HIV-positive children related to prior antiretroviral or tuberculosis chemotherapy? *Scand J Infect Dis* 2004; 36: 779-80.

Khê PM, Phoc TQ, Hung NV, Jarlier V, Robert J. Drug resistance and HIV co-infection among pulmonary tuberculosis patients in Haiphong City, Vietnam. *Int J Tuberc Lung Dis* 2008; 12: 763-8.

Espinal MA, Laszlo A, Simosen L, *et al.* Global trends in resistance to antituberculosis drugs. *N Engl J Med* 2001; 344: 1294-303.

Moore M, McCray E, Onorato IM. Cross-matching TB and AIDS registries: TB patients with HIV co-infection, United States, 1993-1994. *Public Health Rep* 1999; 114: 269-7.

Punnotok J, Shaffer N, Naiwatanakul T, *et al.* Human immunodeficiency virus-related tuberculosis and primary drug resistance in Bangkok, Thailand. *Int J Tuberc Lung Dis* 2000; 4: 537-43.

Quy HT, Buu TN, Cobelens FGJ, Lan NTN, Lambregts CSB, Borgdorff MW. Drug resistance among smear-positive tuberculosis patients in Ho Chi Minh City, Vietnam. *Int J Tuberc Lung Dis* 2006; 10: 160-6.

Saly S, Onozaki I, Ishikawa N, *et al.* Decentralized DOTS shortens delay to TB treatment significantly in Cambodia. *Kekkaku* 2006; 81: 467-74.

Sungkanuparph S, Eampokalap B, Chottanapund S, Bed SD, Manosuthi W. Impact of drug-resistant tuberculosis of the survival of HIV-infcted patients. *Int J Tuberc Lung Dis* 2007; 11: 325-30.

Wells CD, Cegielski JP, Nelson LJ, *et al.* HIV infection and multi-drug resistant tuberculosis: the perfect storm. *J Infect Dis* 2007; 196 (suppl): S86-107.

World Health Organization Global Tuberculosis Control. Country profile, Cambodia. WHO Report. Geneva: World Health Organization, 2008: 93-6.

Yamada N, Saorith K, Yamkami K, *et al.* The national tuberculosis drug resistance survey in Cambodia, 2000-2001. *Int J Tuberc Lung Dis* 2007; 11: 1321-7.

Yoshiyama T, Yanai H, Rhiengtong D, *et al.* Development of acquired drug resistance in current tuberculosis patients with various treatment outcome. *Int J Tuberc Lung Dis* 2004; 8: 31-8.