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Short communication

First Susceptibility Testing of *Mycobacterium tuberculosis* for Second-line Anti-tuberculosis Drugs in Ghana

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Abstract: We performed drug susceptibility testing on first- and second-line drugs in *Mycobacterium tuberculosis* (*M. tuberculosis*) for the first time in Ghana to obtain preliminary data on drug-resistant tuberculosis. Of 21 isolates (4 new cases and 17 treated cases), 5 (23.8%) were multi-drug resistant tuberculosis (MDR-TB) and 19 (90.5%) were resistant to at least one drug, but no extensively drug-resistant TB (XDR-TB) was identified. Since the target patients were Category II, IV or smear positive at follow-up microscopy, it is understandable that there were many drug-resistant TB cases. Six isolates were resistant to one or two second-line drugs, but the second-line drugs were not approved in Ghana. It is considered that the bacilli were imported from abroad. Preventing the import of drug-resistant TB bacilli is probably one of best ways to control TB in Ghana.
Key words: Ghana, Drug susceptibility testing, MDR/XDR-TB, second-line drugs

INTRODUCTION

The Republic of Ghana is a west African country with a population of 23 million [1]. The incidence of tuberculosis (TB) declined from 231 in 2000 to 106 per 100,000 in 2010 [2]. Since human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) prevalence is not very high (89/100,000, 2007) [1], TB incidence has also remained low compared to other sub-Saharan African countries such as Nigeria and Kenya [3, 4].

Drug resistant TB is difficult to treat. That is especially true for MDR-TB, which is resistant to isoniazid (INH) and rifampicin (RIF), the two major bactericidal anti-TB drugs. Any drug(s) to which the bacilli show resistance must be replaced to avoid creating further drug resistance and to ensure successful treatment results. Therefore, it is important to determine the susceptibility of *M. tuberculosis* to various anti-TB drugs.

Approval for the purchase and use of second-line drugs by the Green Light Committee was in the pipeline as of 2011, meaning that MDR-TB patients cannot readily receive treatment. The main concern is to implement proper measures to avoid the emergence of XDR-TB, which is defined as resistance of *M. tuberculosis* to INH and RIF as well as any fluoroquinolone and at least one of the injectable anti-TB drugs (such as amikacin (AMK), kanamycin (KM) and capreomycin (CPM)). One such measure to ensure is that the central TB laboratory has the capacity to perform second-line drug susceptibility testing.

In this study, therefore, we performed first- and second-line drug susceptibility testing on the isolates from previously treated TB patients to obtain preliminary data on MDR/XDR-TB.

MATERIALS AND METHODS

Specimen collection

The targeted patients were under WHO category II regimen or smear positive in follow-up microscopy at 2, 4, 5, 6, 8 and 9 months, including some new TB patients with positive smear as a reference. The category classification was at the time of study. Sputum specimens were collected

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from Kaneshie Polyclinic (33 specimens), Korle-bu teaching hospital (24 specimens), La General Hospital (19 specimens), Nsawam Government Hospital (6 specimens), and one patient who came directly to the Noguchi Memorial Institute for Medical Research. Specimens were collected between 2008 and 2009. Collected specimens were stored in a refrigerator from 1–7 days and transported to the laboratory in a cooler box.

Isolation of *M. tuberculosis* and drug susceptibility testing

Sputum was collected in a 50-mL centrifuge tube and kept in a refrigerator until inoculation. It was decontaminated with 4% NaOH and inoculated on 2% Kudo medium. The recovered *M. tuberculosis* colonies were then subcultured in Middlebrook 7H9 (Becton Dickinson) broth to obtain the bacterial suspension at OD 0.2 (530 nm). Recovered bacilli were tested with Capilia TB (TOUNS, Numazu, Japan) to identify *Mycobacterium tuberculosis* complex (MTC). The drug susceptibility testing (DST) was performed by the standard proportion method on Lowenstein-Jensen (L-J) medium. In short, each bacterial suspension was diluted by 1/100 and inoculated on L-J medium containing each anti-tuberculosis drug. For control, bacilli diluted by 1/100 and 1/10,000 were inoculated on drug-free L-J medium. The drugs and concentrations used for DST were INH (0.2 μg/mL), RIF (40 μg/mL), streptomycin (SM) (4 μg/mL), ethambutol (EB) (2 μg/mL), KM (30 μg/mL), AMK (40 μg/mL), CPM (40 μg/mL), ethionamide (ETH) (20 μg/mL), para-aminosalicylic acid (PAS) (1 μg/mL), cycloserine (CS) (40 μg/mL) and levofloxacin (LVFX) (1 μg/mL). The initial reading was done after 4 weeks and the final at 6 weeks. The colonies were counted and determined as resistant when more colonies were observed on the drug-containing medium than on the 1/10,000 control.

Ethical consideration

The research protocol was approved by the Ethics Committee of the Noguchi Memorial Institute for Medical Research (NMIMR – IRB CPN 003/04-05). Each sputum specimen was collected after obtaining written informed consent from the patients.

RESULTS

A total of 83 sputum specimens were collected from 62 males, 18 females and 3 people of unknown gender. The mean age was 42.0 ± 12.0 for males and 33.6 ± 16.6 for females. The number of specimens from WHO category I, II and IV were 60, 15 and 3, respectively.

Of the 83 specimens, 39 were culture positive (35 MTC and 4 non-tuberculous mycobacteria), 40 were culture negative and 4 were contaminated. Thirty-five culture positive MTC samples were then sub-cultured in Middlebrook 7H9 broth for DST. However, 14 samples did not grow sufficiently. Finally, 21 isolates were subjected to DST.

The DST results are shown in Table 1. Of 21 *M. tuberculosis* complex isolates, 5 (23.8%) were MDR-TB, 19 (90.5%) were resistant to at least one drug, and 17 (81.0%) were resistant to SM. As for the second-line drug 6, one strain each was resistant to ETH CPM and LVFX. However, no isolate was resistant to KM, AMK, PAS or CS and no isolate belonged to the category of XDR-TB.

DISCUSSION

Of the 83 specimens, 40 (48.2%) were culture negative. Although all specimen used in this study were smear positive, some patients may have been carrying dead bacilli because most of the patients were under treatment. The duration of sample collection and inoculation did not affect the recovery. But it is possible that a power disruption occurred during storage at the clinic. It cannot be denied that the condition of sample storage may affect the recovery ratio. It is understandable that there are many drug-resistant TB cases because the target patients were Category II, IV or smear positive at follow-up microscopy. We found many isolates resistant to SM as compared to other drugs. Although we suspected the inactivation of the drug, a similar result was reported by Forson et al. [5]. Since second-line drugs were not approved in Ghana at the time of the study, second-line drug-resistant TB bacilli may have been imported from abroad because as the second-line anti-TB drugs, in general, cannot be found in private pharmacies or hospitals in Ghana. Fortunately, no XDR-TB was detected in this study. However, the total number of collected specimens was low because most of the patients who came to the hospitals were new cases and we could not obtain a large number of retreatment cases. If more samples are collected in future studies, it may be possible to detect XDR-TB cases. In this study, the specimens with numbers 056, 066 and 074 were identified as MDR-TB by MGIT 960 at a different laboratory, but one was not MDR-TB. This may be due to a difference in methods used by the two laboratories.

In conclusion, it was indicated that some second-line drug-resistant TB bacilli may be imported from abroad. Since XDR-TB is prevalent in neighboring countries [6, 7], preventing the import of drug-resistant TB is probably one of best ways to control TB in Ghana.

Table 1. Result of DST of *M. tuberculosis* isolated in Ghana.

	Drug	INH	RFP	SM	EB	KM	AMK	CPM	TH	PAS	CS	LVFX	Status
	(μg/mL)	0.2	40	4	2	30	40	40	20	1	40	1	
1	NSW2	S	S	R	S	S	S	S	S	S	S	S	Cat I
2	KB3	S	S	R	S	S	S	S	S	S	S	S	Cat II
3	KB12	S	S	R	S	S	S	S	S	S	S	S	Cat II
4	KB16	S	R	R	S	S	S	S	S	S	S	S	Cat II
5	KB17	R	R	R	R	S	S	S	R	S	S	S	Identified as MDR-TB at PHL*
6	KB18	S	S	S	S	S	S	S	S	S	S	S	Identified as MDR-TB at PHL
7	KB20	S	S	R	S	S	S	S	S	S	S	S	Identified as MDR-TB at PHL
8	056	S	R	R	R	S	S	S	R	S	S	S	Identified as MDR-TB at PHL
9	066	R	R	R	R	S	S	S	R	S	S	S	Identified as MDR-TB at PHL
10	074	R	R	R	R	S	S	S	R	S	S	R	Identified as MDR-TB at PHL
11	KN14	R	R	R	R	S	S	R	S	S	S	S	Cat I
12	KN17	S	S	S	S	S	S	S	S	S	S	S	Cat I
13	KN26	R	R	S	R	S	S	S	R	S	S	S	Cat I
14	KN30	S	S	R	S	S	S	S	S	S	S	S	New
15	KN31	S	S	R	S	S	S	S	S	S	S	S	New
16	LG1	R	S	R	S	S	S	S	R	S	S	S	Cat I
17	LG7	S	S	S	S	S	S	S	S	S	S	S	Cat I
18	LG13	S	S	R	S	S	S	S	S	S	S	S	Cat I
19	LG14	S	S	R	S	S	S	S	S	S	S	S	Cat I
20	LG17	S	S	R	S	S	S	S	S	S	S	S	New
21	LG18	S	S	R	S	S	S	S	S	S	S	S	New
Susceptible		15	14	4	15	21	21	20	15	21	21	20	
Resistant		6	7	17	6	0	0	1	6	0	0	1	
Resistant (%)		28.6	33.3	81.0	28.6	0	0	4.8	28.6	0	0	4.8	

S: Susceptible, R: Resistant
*PHL: Public Health Reference Laboratory

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