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# Multidrug-resistant tuberculosis in Port-au-Prince, Haiti

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## Abstract

**Objectives—**Determine the prevalence of MDR-TB among patients with new smear-positive pulmonary TB in Port au Prince, Haiti

**Methods—**Sputum samples were cultured from 1006 patients diagnosed with new tuberculosis in 2008. The core region of the *rpoB* gene that is associated with resistance to rifampin was sequenced. All isolates with *rpoB* mutations were sent to the NY State reference laboratory for conventional drug susceptibility testing (DST). All isolates were also tested with the GenoType MTBDRplus line probe assay.

**Results—***M.tubercuosis* was isolated from 906 patients. 26 (2.9%) of the isolates had mis-sense mutations or deletions in *rpoB* and were resistant to rifampin by DST. All 26 were also resistant to isoniazid and classified as MDR-TB. 46 control isolates without *rpoB* mutations were all found to be rifampin-sensitive by DST. The GenoType MTBDRplus line probe assay correctly identified 26 MDR-TB strains. It misclassified 1 pansusceptible isolate as RIF-resistant.

**Conclusions—**This study shows an MDR-TB prevalence of 2.9% in newly diagnosed TB patients in Haiti and also suggests that rpoB sequencing or hybridization assays are good screening tools for early detection of MDR-TB.

### Keywords

Tuberculosis; Multidrug-Resistant; Haiti

## INTRODUCTION

Haiti has the highest rate of tuberculosis (TB) in the Western Hemisphere with an estimated prevalence rate of 331 per 100,000 population. (1) Because of longstanding poverty and political instability, a significant number of Haitians emigrate to countries around the world. In 2010, the World Bank reported that approximately 1 million Haitians, or nearly ten

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percent of Haiti's resident population, were living abroad in countries that include the Dominican Republic, the Bahamas, the United States, Canada, France, and other parts of the francophone world. (2) Haiti was the most frequent country of birth among the foreign-born TB patients in Montreal where Haitians account for 18.5% of all TB cases and the fifth most frequent in New York with 5% of all TB cases. (3,4) Therefore, an understanding of *Mycobacterium tuberculosis* strains circulating in Haiti, especially drug-resistant strains, has both country-specific and global health significance. (5,6)

Findings from the three *M. tuberculosis* drug resistance prevalence studies conducted over the past twenty years suggest that the rate of drug resistance may be increasing in Haiti. In a 1990 study, the rate of isoniazid resistance was 19% but multidrug resistant tuberculosis (MDR-TB), defined as resistance to both isoniazid and rifampin, was not found in any patient with new tuberculosis. (7) In a 1991 study of recent migrants from Haiti to Cuba, single drug resistance to isoniazid was found in 22% of TB patients but MDR-TB was not found. (8) However, a study in 2002 of patients presenting to an HIV/AIDS center in Port au Prince with new onset tuberculosis found a rate of primary multidrug-resistant tuberculosis of 6%. (9) This 2002 study was small and limited to a single HIV testing center but suggested an increase in MDR-TB.

In 2010 the World Health Organization estimated that 3.4% of the new TB cases globally and 2.1% of the new cases in the Americas were MDR-TB. (1) In the Caribbean, rates of MDR-TB range from < 1% of new cases in Cuba to 6.6% in the Dominican Republic. (10,11) Due to the limited data on drug resistance, the World Health Organization has not provided a definitive assessment of trends of MDR-TB over time globally or in the Americas. It is hoped that with recent advances in molecular techniques, more drug resistance data will be forthcoming and the WHO will be able to determine if the prevalence of MDR-TB is increasing.

Therefore, a survey of tuberculosis drug resistance at the five largest TB treatment centers in Port-au-Prince Haiti was conducted, to determine the prevalence rates of primary MDR-TB and to determine if there has been an increase since 1990. A secondary objective of this study was to validate the use of molecular tests for the diagnosis of MDR-TB in Haiti, prior to their clinical application.

## METHODS

### Study design

This was a cross-sectional prevalence study of *M. tuberculosis* drug resistance among patients presenting with new AFB smear-positive active pulmonary tuberculosis at the five largest tuberculosis treatment centers in the metropolitan Port-au-Prince area in 2008. Sputum samples were cultured from these 5 centers for *M. tuberculosis*. Isolates were then examined by sequence analysis of the *rpoB* gene, which is known to be associated with > 95% of rifampin (RIF) resistance. (12) *M. tuberculosis* isolates resistant to RIF by *rpoB* DNA sequence analysis were sent to the Mycobacteriology Laboratory of the New York State Department of Health for drug susceptibility testing (DST). A subset of isolates negative for rifampin resistance by *rpoB* gene analysis was also sent to the NY reference laboratory. The prevalence of multidrug-resistant isolates is reported, defined as isolates that were resistant to RIF by *rpoB* gene analysis with confirmation of resistance to RIF and isoniazid by DST at the NY reference laboratory.

### Study setting and population

The five tuberculosis centers in Port-au-Prince and its environs included Grace Children's Hospital, The Haitian State Sanatorium, GHESKIO Centers, Sigueneau Sanatorium, and the

Mennonite Mission of Croix des Bouquets. These centers treat ~75% of all TB cases treated in the Port au Prince area. The patients were consecutively diagnosed with acid-fast bacilli (AFB) positive smear in 2008. They did not report a prior diagnosis of tuberculosis.

A laboratory technician placed a 2–5 cc aliquot of sputum from each patient into a 50 ml plastic tube. The tube was labeled with the patient's age, gender, and HIV status (positive, negative, unknown). The sample was refrigerated at 4°C and transported within 2 days to a central laboratory at the GHESKIO Centers.

## Laboratory studies

Sputum samples were decontaminated with sodium lauryl sulfate and cultured on Lowenstein-Jensen (LJ) slants, (Becton Dickenson, Franklin Lakes, NJ, USA). For positive cultures, a loopful of mycobacteria was resuspended in 300 μl of nuclease-free water, heat-killed by 20 minutes incubation at 95°C and disrupted by 3 cycles of freezing at −70°C and heating at 95°C. DNA was separated from debris using Spin-X centrifuge filter tubes, 0.22 μm pore size (COSTAR, Corning Inc, Lowell, MA, USA). The 329 bp *rpoB* fragment was amplified in 35 cycles of polymerase chain reaction (PCR). PCR parameters were 30 sec denaturation at 95°C, 1 min annealing at 55°C and 1 min extension at 72°C. Each 50 μl reaction consisted of 5 μl of DNA extract, 0.2 μM each of primers rpoB-F (5′-CCA-CCC-AGG-ACG-TGG-AGG-CGA-TCA-CAC-3′) and rpoB-R (5′-CGT-TTC-GAT-GAA-CCC-GAA-CGG-GTT-GAC-3′), 200 μM dNTPs and 1.25 U of HotStart Taq Polymerase (QIAGEN) in nuclease-free water.

PCR fragments were purified with QIAquick PCR purification kit (QIAGEN, Hilden, Germany), pre-mixed with the primers used for PCR amplification and sent for Sanger (3730XL) sequencing at Cornell University Bio Resource Center (Ithaca, NY, USA). The sequences obtained were compared to the wild type rpoB sequence of H37Rv for *Mycobacterium tuberculosis* complex (MTBC) identification and for detection of mutations. Isolates were provisionally classified as rifampin-resistant if they had a polymorphism previously described as conferring resistance. (12)

DNA extracts from all isolates were also tested for mutations associated with resistance to INH and RIF by a commercially available line-probe assay using the manufacturer's instructions (GenoType MTBDRplus, Hain Life Sciences, Nehren, Germany). The operator performing the test was blinded to the results of *rpoB* sequence analysis.

All isolates with *rpoB* mutations, along with a subset of isolates without *rpoB* mutations were sent to the Laboratory of Clinical Mycobacteriology, Wadsworth Center, New York State Department of Health, (Albany, NY, USA). All specimens were confirmed as MTBC by real-time PCR assay. (13) In New York drug sensitivity testing to first line anti-tuberculosis drugs was performed in Mycobacteria Growth Indicator Tubes (MGIT) (BACTEC 960, Becton Dickenson, Franklin Lakes, NJ, USA) in accordance with the manufacturer's instructions. For isolates resistant to at least one first-line drug DST to isoniazid, rifampin, ethambutol, streptomycin, capreomycin, cycloserine, ethionamide, kanamycin, para-aminosalicylc acid, amikacin and ofloxacin was peformed with proportion method on 7H10 agar as recommended by Clinical and Laboratory Standards Institute. (14)

## Analysis

The prevalence, with 95% confidence intervals, of MDR-TB at five major TB treatment centers around Port-au-Prince is reported. Proportions were compared using Fisher's exact test and medians with the Wilcoxon rank sum test. This study was approved by the institutional review board (IRB) at GHESKIO and Weill Cornell Medical College, NY.

# RESULTS

## Study population

The five sites sent sputum samples from 1006 patients to the central laboratory at GHESKIO for analysis. Of these, 909 grew *Mycobacteria* on Lowenstein-Jensen media and yielded DNA extracts that were suitable for PCR-sequencing. Of the 909 isolates, 906 were found to belong to the *M. tuberculosis* complex. The characteristics of the 906 patients from whom *M. tuberculosis* was isolated are presented in Table 1. The median age was 30 years, 49.1% were female and 77.9% of patients were HIV-1 seronegative. Each of the five sites contributed approximately the same number of samples.

## Prevalence of MDR-TB

The 81-bp core region of *rpoB* gene was sequenced for all isolates. Of the 906 *M. tuberculosis* isolates, 27 (3.0%) contained mis-sense mutations or deletions in the *rpoB* gene that are known to be associated with RIF resistance. One additional isolate had a silent mutation or synonymous single nucleotide polymorphism in *rpoB* codon T508 (ACC -> ACT).

The 27 *M. tuberculosis* isolates with mis-sense mutations or deletions in *rpoB* were sent to the New York State TB Laboratory. Twenty-six isolates grew in New York, and one isolate was non-viable upon subculture. All 26 of the isolates that grew in New York were confirmed as *M. tuberculosis*. All 26 isolates tested resistant to RIF by conventional culture DST on solid and liquid media. All 26 were also resistant to INH and hence were classified as MDR-TB. The complete drug sensitivity profile of the 26 multidrug-resistant isolates is shown in Table 2.

In total, 26 of 906 (2.9%) *M. tuberculosis* isolates were confirmed to be multidrug-resistant. Of note, no statistically significant associations were found between patient age, gender, or HIV status and multi-drug resistance.

The one isolate that had a silent mutation or synonymous single nucleotide polymorphism in *rpoB* codon T508 (ACC -> ACT) was also analyzed by DST in the reference laboratory and found to be sensitive to rifampin. An additional 46 isolates, which were negative for any mutations in “core region” of *rpoB*, were also sent to the New York State Laboratory for control DST. All 46 isolates were sensitive to RIF.

## Screening with GenoType MTBDRplus line-probe assay

A line-probe assay was performed on DNA extracts from the 906 *M. tuberculosis* isolates. The MTBDRplus assay identified 28 (3.1%) samples as resistant to rifampin. This included all 27 isolates identified by sequencing as having a mis-sense mutations or deletions in the *rpoB* and the one isolate that had a synonymous single nucleotide polymorphism in *rpoB* codon T508 (ACC -> ACT). The remaining isolates were sensitive to RIF by the MTBDRplus assay.

# DISCUSSION

This study documents that at least 2.9% of patients newly diagnosed with smear positive pulmonary tuberculosis in Port-au-Prince had multidrug-resistant *M. tuberculosis*. This is an increase from two studies from the 1990s, when MDR-TB was not detected in any new TB case in Haiti. This study also suggests that *rpoB* sequencing or hybridization assays are good screening tools in this population for the early detection and appropriate treatment of MDR-TB.

Our data document that the rates of MDR-TB have increased in Haiti since studies in the 1990s showed that less than 1% of patients with newly diagnosed tuberculosis were resistant to isoniazid and rifampin. (7,8) Studies from the neighboring Dominican Republic, which shares the island of Hispanola with Haiti, also have shown high MDR-TB rate of 10.2% in all cases and 6.6% of new TB cases. (11) Given the large amount of immigration between countries throughout the western hemisphere, the results of these studies are of particular concern for the spread of previously localized disease.

The results of this study have become even more relevant after the January 12, 2010 earthquake, when the headquarters of the National TB Program and several large tuberculosis treatment centers were destroyed, including three of the five centers that participated in this study. (15) Thousands of TB patients in Port-au-Prince defaulted on therapy and are now living in crowded tent cities. Rates of MDR-TB could climb even higher under these catastrophic conditions.

This study was limited in that gold-standard culture-based drug sensitivity testing was performed only on the samples that tested positive for *rpoB* mutations and only on a subset of the isolates that were negative for the *rpoB* mutations. Therefore, the prevalence of MDR-TB may be underestimated. However, all 46 isolates without *rpoB* mutations tested negative for rifampin resistance by standard culture method, and other studies suggest a > 95% correlation between *rpoB* sequence analysis and phenotypic drug sensitivity testing. Therefore, it is unlikely that the actual rates of MDR-TB are significantly higher than 3%.

At least 2.9% of patients newly diagnosed with smear positive pulmonary tuberculosis in Port-au-Prince have multidrug-resistant *M. tuberculosis*. Sequencing or hybridization assays of the *rpoB* gene are good screening tools in this population for the early detection and appropriate treatment of MDR-TB.

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**Table 1**

Characteristics of 906 Patients from Port-au-Prince, Haiti with a Diagnosis of New Onset Sputum Smear-Positive Tuberculosis

| Characteristic | Value |
|---|---|
| Age, years | |
| Median | 30 |
| Interquartile range | 20 – 41 |
| Female, number (%) | 445 (49.1) |
| HIV status, number (%) | |
| Positive | 115 (12.7) |
| Negative | 706 (77.9) |
| Unknown | 85 (9.4) |
| Site, number (%) | |
| Grace Children's Hospital | 226 (24.9) |
| State TB Sanatorium | 190 (21.0) |
| GHESKIO Centers | 182 (20.1) |
| Sigueneau Sanatorium | 120 (13.2) |
| Mennonite Mission of Croix des Bouquets | 188 (20.7) |

**Table 2**

Drug Resistance of *M tuberculosis* Isolates with Mutations in *rpoB* Gene from 26 Patients with New Diagnosis of Active Pulmonary Tuberculosis in Haiti

| **Drug Resistance**** | ***M tuberculosis* isolates with rpoB mutations* (*n=26)* *number* (%)** |
|---|---|
| Isoniazid | 26 (100.0%) |
| Rifampin | 26 (100.0%) |
| Isoniazid and rifampin (MDR) | 26 (100.0%) |
| Ethambutol | 18 (69.2%) |
| Pyrazinamide | 14 (53.8%) |
| Streptomycin | 12 (46.1%) |
| Ethionamide | 3 (11.5%) |

* This includes mis-sense mutations or deletions in the 81-bp core region of *rpoB*, but does not include isolates with silent mutations or synonymous single nucleotide polymorphisms.

** There were no strains resistant to Para-aminosalicylic acid, Ofloxacin, Capreomycin, Cycloserine, Kanamycin or Amikacin.