

## Low circulation of multidrug-resistant *Mycobacterium tuberculosis* strains in Cuba

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### ABSTRACT

The surveillance of drug resistance, using mycobacterial cultures and *in vitro* susceptibility tests, allows the estimation of the regional and worldwide magnitude of this phenomenon in tuberculosis. This study, which is part of 3 international projects organized by WHO/UICTER, determined the prevalence of the resistance to antituberculosis drugs in Cuba, during the 1995-2000 period, in both naïve and previously treated patients. Resistance to first line drugs (isoniazide rifampicin, streptomycin and ethambutol) was evaluated with the method of the proportions for 2140 *Mycobacterium tuberculosis* strains. The levels of resistance or multidrug resistance (MDR) in naïve patients were of 7.1% and 0.4%, respectively, which proved the low levels of circulation of MDR strains in Cuba and led to a worldwide acknowledgement of the efficacy of the National Control Program and the strategy of strictly supervised treatment which has been used in our country since 1971.

### Introduction

Worldwide, the number of patients afflicted from tuberculosis (TB) increases 3% every year, mainly due to the appearance and circulation of multidrug-resistant strains (MDR). According to the data from WHO, around 50 million persons are currently infected with MDR strains, with 300 000 new cases of MDR-TB being reported every year. This situation is considered to be just the beginning of a problem of unpredictable consequences, since the carrier population for this infection may be the source of an intractable epidemic on a global scale. Therefore, an emergency health situation has been declared that takes into account the emergency measures that have to be implemented by each country in the battle against tuberculosis [1].

This work presents the results of the tests for resistance to first line antituberculosis drugs in *Mycobacterium tuberculosis* performed at the National Tuberculosis and Mycobacteria Reference and Research Laboratory (LNR-TB) and the PAHO-WHO Collaborating Center of the "Pedro Kourí" Tropical Medicine Institute. These results belong to 3 worldwide projects (1997, 2000 and 2004) organized by WHO/UICTER, which include the LNR-TB as a working group [2-4].

The results of this research were awarded the National Prize from the National Academy of Sciences of Cuba, in 2004, under the title "Low circulation of multidrug-resistant *Mycobacterium tuberculosis* strains in Cuba".

### Materials and methods

During the period from year 1995 to 2000 the LNR-TB, at IPK, in Cuba, studied 2140 *M. tuberculosis* strains from all over the country. The samples were selected by conglomeration, in proportion to the population of symptomatic respiratory patients with positive bacilloscopy reported for each year of the study. Resistance to isoniazide (INH), rifampicin (RMP),

ethambutol (EMB) and streptomycin (SM) was determined with the proportional method of Canetti in its economical variation type b, using the indirect technique. The critical concentrations recommended for each drug were used: 0.2 µg/mL, 4 µg/mL, 2 µg/mL and 40 µg/mL for INH, SM, EMB and RMP, respectively [5].

The results for each drug were expressed as a percentage and divided on three groups according to the most current classification criteria: resistance in naïve cases (NC), in previously treated cases (PTC) and combined resistance (the sum of resistance in NC and PTC), calculating the 95% confidence intervals.

### Results

Out of the 2 140 strains from the period 1995-2000, 1977 belonged to patients classified as NC. From them, 1 835 were susceptible to all four drugs (92.9%) and 141 (7.1%) were resistant to at least one of the drugs under study. A more detailed analysis reveals that 116 strains (5.8%) were resistant to just 1 drug; most of them to SM (4.9%), followed by INH (0.8%) and only 1 strain (0.05%) was resistant to RMP alone. Nine strains (0.4%) were MDR. In this regard, the most frequent association was INH + RMP + SM in 6 strains (0.3%) (Table 1).

For PTC patients 163 strains were studied, of which 105 were sensitive to all the drugs (64.4%) and 58 were resistant to at least 1 drug (35.6%). Out of the latter, 35 were resistant to only one drug (21.5%); resistance to SM was the most frequently detected with 27 strains (16.6%), followed by INH with 8 strains (4.9%). No strains resistant to RMP alone were detected. MDR was observed in 14 strains (8.6%) (Table 1).

Sensitive strains with combined resistance totaled a number of 1940 (90.7%), and 199 turned out to be resistant strains (9.3%). MDR was detected in 23 strains (1.1%), (Table 1).

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## Discussion

The magnitude of the problem of antituberculosis drug resistance has not been grasped until recently. Although a review of the literature and the reports published before the last decades suggest the existence of high levels of resistance in some areas, many of these studies involve non-representative samples or patients with an unknown treatment record, without a methodological consensus in the assays used for the detection of resistance. These limitations have prompted the organization of studies on a worldwide scale to determine with certainty the dimensions of this phenomenon, using previously agreed methods in order to be able to compare the results of the different participating countries [6].

One antecedent for the studies of resistance to antituberculosis drugs in Cuba is the report by Valdivia and Suárez Méndez in 1968, in which 1022 strains from patients of the (at the time) Antituberculosis Hospital "Julio Trigo", from Havana, were studied. The reported level of resistance in NC against INH, SM and para-aminosalicylic acid (PAS) was 8.6%, with INH as the drug against which most resistant strains were found (3.8%). The incidence of resistance in PTC was 90.8%; in 7.8% of the strains the resistance was against INH. There was resistance to the three drugs under study (INH, SM and PAS) in/48.2% of the analyzed strains [7].

The LNR-TB, from IPK, started in 1982 a longitudinal study on resistance to antituberculosis drugs in parallel with the National Control Program (NCP), with the aim of estimating the magnitude and variations of this phenomenon, which would be related to mass treatment, its efficacy and control. A 6.0% incidence of resistance against four drugs (INH, SM, PAS and thiocetazone [Tb1]) was registered among 430 studied strains from NC (1978-80). Of them, 3.5% were resistant to INH and 5.3% to SM. No strains resistant to PAS or Tb1 were found. The incidence of resistance among strains from PTC was 5.3% [8].

After the implementation of the 3 worldwide projects mentioned above, it was shown that there is detectable circulation of MDR strains in every participating zone and country, although some of the regions where this phenomenon may have the highest incidence have not yet been studied. Another finding of these projects is that the level of resistance of MDR strains is increasing, and therefore most of them are not responsive to current therapies. The rate of appearance of drug-resistant TB is 10 times higher in Eastern Europe and Central Asia than in the rest of the world, with a high concentration of MDR TB

Table 1. Prevalence of antituberculosis drug resistance in Cuba from 1995 to 2000.

| 1995-2000               | Naive cases |      | Previously treated cases |      | Combined resistance |      |
|-------------------------|-------------|------|--------------------------|------|---------------------|------|
|                         | n           | %    | n                        | %    | n                   | %    |
| Total number of strains | 1 977       | 100  | 163                      | 100  | 2 140               | 100  |
| Sensitive               | 1 835       | 92.9 | 105                      | 64.4 | 1 940               | 90.7 |
| Any resistance          | 141         | 7.1  | 58                       | 35.6 | 199                 | 9.3  |
| Resistance to 1 drug    | 116         | 5.8  | 35                       | 21.5 | 151                 | 7.0  |
| INH                     | 17          | 0.8  | 8                        | 4.9  | 25                  | 1.2  |
| RMP                     | 1           | 0.05 | 0                        | 0    | 1                   | 0.05 |
| EMB                     | 0           | 0    | 0                        | 0    | 0                   | 0    |
| SM                      | 98          | 4.9  | 27                       | 16.6 | 125                 | 5.8  |
| MDR                     | 9           | 0.4  | 14                       | 8.6  | 23                  | 1.1  |
| INH+RMP                 | 3           | 0.1  | 3                        | 1.8  | 6                   | 0.3  |
| INH+RMP+SM              | 6           | 0.3  | 6                        | 3.7  | 12                  | 0.6  |
| INH+RMP+EMB             | 0           | 0    | 4                        | 2.4  | 4                   | 0.2  |
| INH+RMP+SM+EMB          | 0           | 0    | 1                        | 0.6  | 1                   | 0.05 |

located at the Community of Independent States. The presence of an association between low levels of circulation of MDR strains and the presence of effective control programs using the DOTS/TAES strategy (strictly supervised shortened treatment) has been shown, with Cuba standing as an example at an international level [2-4, 9, 10].

In conclusion, our findings show that the levels of circulation of MDR *M. tuberculosis* strains in Cuba are low. When compared with the incidence of MDR strains in the American region, our country ranks last, which is far from typical in the current worldwide situation. Cuba is the only Iberoamerican country which has been involved with the three international projects for the surveillance of drug resistance in TB. These studies have allowed the detection of a downward trend in the incidence of drug resistance among Cuban strains, which fits well with the high therapeutic success rates and the low rates of therapeutic failure and treatment dropouts [9-11].

The results and the experience gained on the topic of resistance to antituberculosis drugs place the TB Collaborating Center from IPK at a prominent spot on an international level, and support the proposal of several international organizations for the immediate implementation of a program for the eradication of TB in Cuba. It is widely acknowledged that ours is the only country with a realistic chance for the elimination of this disease in the current situation, thanks to the long and successful trajectory of the NCP, who pioneered the application of the DOTS/TAES strategy.

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