Nosocomial Spread of Human Immunodeficiency Virus–Related Multidrug-Resistant Tuberculosis in Buenos Aires

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A steep upsurge of human immunodeficiency virus (HIV)–associated multidrug-resistant tuberculosis (MDR-TB) was recently observed at a referral treatment center in Buenos Aires City. Between January 1994 and June 1995, TB isolates resistant to at least five drugs were recovered from 101 of 272 HIV-infected inpatients. Highly resistant isolates from 77 patients underwent restriction fragment length polymorphism study with IS6110. After cross-contamination was eliminated, a single TB strain was found to have caused disease in 68 patients with a history of on-site exposure. The frequency of smear-positive pulmonary disease was higher among these patients than among non–MDR-TB HIV-infected patients (50/68 vs. 60/148, P < .001), and the 1-year survival was dramatically reduced (5/68 vs. 92/148). The strain involved in the outbreak was traced back to patients hospitalized in 1992. Institutional infection control policies were and may still be inadequate to contain the spread of TB among immunodepressed subjects, as is the case in other large urban hospitals in Argentina.

Following trends previously observed in other urban areas worldwide, tuberculosis (TB) has reemerged in the city and province of Buenos Aires at a pace 5-fold faster than the average annual increase noticed in Argentina since the beginning of this decade [1]. Buenos Aires produces nearly half of the 14,000 new TB cases in Argentina each year [2]. AIDS plays a major role in this resurgence, as shown by rates of human immunodeficiency virus (HIV) infection and AIDS-related TB in Buenos Aires, which are the highest in the country [3, 4]. Deteriorated TB control policies and poor socioeconomic conditions contribute as well, favoring transmission in underprivileged sectors of the community, particularly among HIV-infected individuals [5].

Lately, a dramatic increase in the number of AIDS patients initially infected with multidrug resistant (MDR) tubercle bacilli has been detected in a nationwide survey endorsed by the World Health Organization, to which Buenos Aires and its suburban area contributed with the heaviest load of HIV-related MDR cultures [6]. In addition, one of the two MDR-TB outbreaks reported so far in Argentina occurred at a large suburban hospital, near Buenos Aires [7, 8].

Muñiz Hospital, in Buenos Aires City, is a referral treatment center for infectious diseases as well as the seat of the TB Department of the University of Buenos Aires. Wards are distributed in several buildings within the hospital grounds. Since the onset of the AIDS epidemic, an increasing number of HIV-infected patients have shared wards and other facilities with nonimmunocompromised patients. In 1993, a special building was devoted to them, but no particular policy was implemented to guard against hospital infection among immunosuppressed patients, who were free to circulate in common areas within the hospital grounds. Whereas HIV-associated TB was already fairly prevalent in the hospital between 1985 and 1990 (~20% of all TB cases), no MDR Mycobacterium tuberculosis culture was obtained from HIV-infected patients during that period [9]. Since then, the incidence of MDR-TB has increased from a single case in 1991 to 5 and 10 cases in 1992 and 1993, respectively.

In 1994, an alarmingly high proportion of isolates found to be similarly resistant to several drugs suggested hospital infection. To investigate this possibility, bacteriologic and clinical records from HIV-TB patients admitted throughout 1994 and the first semester of 1995 were reviewed, and restriction fragment length polymorphism (RFLP) typing with IS6110 was performed on MDR M. tuberculosis isolates from patients with a history of hospital exposure. Available MDR cultures obtained before that period from HIV-infected and non–HIV-infected patients were also subjected to RFLP typing. Clinical and epidemiologic data of patients were analyzed in the light of DNA hybridization patterns of isolates.

Materials and Methods

Mycobacterial isolates and subjects. Bacteriologic and clinical records of HIV-infected patients with culture-proven TB admitted
to Muñiz Hospital between 1 January 1994 and 30 June 1995 were reviewed. *M. tuberculosis* cultures were considered as MDR when showing resistance to at least isoniazid and rifampin, and date of diagnosis was considered the date on which the first MDR *M. tuberculosis* culture was obtained from the patient. MDR cultures displaying additional resistance to at least three drugs were submitted to RFLP typing. Age, sex, place of residence, risk group for HIV, extrahospital exposure to MDR-TB patients, and 1-year survival were determined.

Laboratory cross-contamination was explored for clinical specimens processed on the same day that resulted in cultures with an identical RFLP pattern. Same-day isolates with identical drug susceptibility profiles were also considered, when applicable, if RFLP was not available. The order number of the specimen, laboratory operator, result of acid-fast bacilli (AFB) smear examination, existence of isolates with an identical resistance profile obtained from the patient at another date, and clinical evolution compatible with TB were assessed.

A different group of MDR *M. tuberculosis* isolates from patients hospitalized in 1992 and 1993 at Muñiz Hospital that were still viable was submitted to RFLP typing, irrespective of the resistance profile. The isolates originated from 3 HIV-infected patients with a diagnosis of MDR-TB and 35 non–HIV-infected patients with records of chronic pulmonary disease and treatment failure or non-compliance.

Conventional culture and typing of isolates were carried out as described elsewhere [10]. Drug susceptibility tests were performed on Lowenstein-Jensen slants using the proportion method [11].

**DNA techniques.** The standard protocol for determining *M. tuberculosis* DNA patterns with IS6110 was applied [12, 13]. Briefly, chromosomal DNA was extracted from bacilli suspensions and digested with the restriction enzyme *Pvu*II. DNA fragments were electrophoresed in 0.8% agarose and vacuum-blotted onto a positively charged nylon membrane. The IS6110 probe was a 245-bp DNA fragment amplified by polymerase chain reaction and labeled by the enhanced chemiluminescence gene detection system (Amersham International, Amersham, UK). *M. tuberculosis* Mtb identified in isolates of 66 patients. The Mn variant was recovered from 12 patients and 64 isolates of 74. Patterns of the remaining 8 isolates available for RFLP were not related to the M genotype. Two M variants were named Mn (8 fragments) and Mm (9 fragments). Compared with Mm, variant Mn had an additional single fragment of 1.0 kbp, suggesting a direct lineage between them (figure 2). The Mm variant was identified in isolates of 66 patients. The Mn variant was recovered between March and June 1995 from only 3 patients who had previously shared ward facilities.

**RFLP analysis.** Cultures from 77 of 101 patients with highly resistant TB were available for RFLP analysis. Of the 77, 74 had a history of hospitalization(s) in the previous 12 months. A common RFLP pattern, hereafter identified as genotype M, clustered isolates from 69 of these 74. Patterns of the remaining 8 isolates available for RFLP were not related to the M genotype. Two M variants were named Mm (8 fragments) and Mn (9 fragments). Compared with Mm, variant Mn had an additional single fragment of 1.0 kbp, suggesting a direct lineage between them (figure 2). The Mm variant was identified in isolates of 66 patients. The Mn variant was recovered between March and June 1995 from only 3 patients who had previously shared ward facilities.

**Statistical analysis.** Categorical data from groups of patients were compared by the Mantel-Haenszel χ² test. For the comparison of age means, the Barlett test for homogeneity of variance was applied. Epi Info version 6.02 was used for calculations.

**Results**

During the period 1 January 1994 through 30 June 1995, 1253 patients with culture-proven TB were hospitalized. Of them, 272 were HIV-infected and 981 were HIV-negative or their HIV status was unknown. Isolates from 148 HIV-infected patients were either fully susceptible or resistant to one or two drugs other than the combination of isoniazid plus rifampin. The remaining 124 HIV-infected patients had MDR isolates, of which 101 were resistant to five or more drugs, usually ethambutol, kanamycin, and pyrazinamide in addition to isoniazid and rifampin. In figure 1, frequencies of TB with different levels of drug susceptibility are depicted by semester, showing a steep increase in highly resistant disease throughout this period.

![Figure 1. Frequencies of different levels of drug susceptibility among HIV-infected patients with tuberculosis hospitalized at Muñiz Hospital throughout 1994 and the first semester of 1995: multiresistance (MDR) involving resistance to ≥5 drugs, MDR to <5 drugs (other MDR), or drug susceptibility plus resistance other than MDR (non MDR).](https://academic.oup.com/jid/article-abstract/176/3/637/871997/763/65276079987/473317341703)

**Investigation of laboratory cross-contamination.** Ten pairs of clinical specimens, each pair processed at the laboratory the same day, resulted in *M. tuberculosis* cultures with an identical M RFLP pattern. On the basis of bacteriologic and clinical records, laboratory error was virtually discarded for all but 1 specimen. The suspect sample was a blood culture processed at the laboratory immediately after an AFB smear–positive specimen from another patient with the M strain. Although the blood culture was from a symptomatic TB patient with a previous culture–positive sputum sample (not available for RFLP study), contamination could not be completely ruled out, and the patient was excluded from further analysis.

**Characteristics of patients in the M cluster.** In table 1, characteristics of 68 HIV-infected patients confirmed to be ill with the M strain are compared with those of 148 with non-MDR-TB (126 fully susceptible plus 22 drug-resistant TB other than MDR) who were hospitalized during the same period. Significant differences were observed only in relation to site
of disease, smear positivity, and 1-year survival. Median survival of patients with the M strain was 33 days. No significant difference was found in any of these variables when patients with the M strain were compared with 32 patients whose similarly multiply resistant isolates either had an RFLP pattern other than M or were not studied by RFLP.

At the time of diagnosis of MDR-TB, patients with the M strain were hospitalized in 10 different wards. Table 2 shows the number of patients per ward by semester at diagnosis. Nineteen patients had records of a single previous hospitalization, which occurred 2–7 months prior to the onset of disease with the M strain, in wards 10, 11, 17, 18, and 20 (decreasing order of frequency). The remaining 49 patients had a history of two to five hospitalizations throughout the year before diagnosis. Only 3 patients, who shared a house, had extrahospital exposure to each other in addition to hospital exposure. They contracted MDR-TB within a period of 4 months, as did 2 children in the household (isolates not available for this study).

Twelve patients had been treated at the hospital for fully susceptible TB sometime before the isolation of M bacilli. Two others had records of previous TB with cultures resistant to a single drug. All 14 completed treatment or had negative cultures before the onset of the disease with the M strain. Only 1 of them suffered both episodes of TB within the period of study; a drug-susceptible M. tuberculosis specimen, obtained while he was still under standard antituberculous therapy, showed a completely unrelated genotype (figure 3).

**Table 1.** Characteristics of HIV patients with tuberculosis due to the M strain of *Mycobacterium tuberculosis* and to non–multidrug-resistant tubercle bacilli (non–MDR-TB), hospitalized in Muñiz Hospital, 1 January 1994 to 30 June 1995.

<table>
<thead>
<tr>
<th>Patients with</th>
<th>M strain (n = 68)</th>
<th>Non–MDR-TB (n = 148)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median, years</td>
<td>29</td>
<td>28</td>
<td>NS</td>
</tr>
<tr>
<td>Male, no. (%)</td>
<td>59 (75)</td>
<td>118 (80)</td>
<td>NS</td>
</tr>
<tr>
<td>Risk factor for HIV, no. (%)</td>
<td>Injection drug</td>
<td>51 (75)</td>
<td>104 (70)</td>
</tr>
<tr>
<td>Homosexuality</td>
<td>7 (10)</td>
<td>9 (6)</td>
<td>NS</td>
</tr>
<tr>
<td>Other</td>
<td>8 (12)</td>
<td>19 (13)</td>
<td>NS</td>
</tr>
<tr>
<td>Not determined</td>
<td>2 (3)</td>
<td>16 (11)</td>
<td>NS</td>
</tr>
<tr>
<td>Place of residence, no. (%)</td>
<td>Buenos Aires City</td>
<td>26 (38)</td>
<td>52 (35)</td>
</tr>
<tr>
<td>City suburbs</td>
<td>41 (60)</td>
<td>84 (57)</td>
<td>NS</td>
</tr>
<tr>
<td>Not determined</td>
<td>1 (1)</td>
<td>12 (8)</td>
<td>NS</td>
</tr>
<tr>
<td>Site of disease at diagnosis, no. (%)</td>
<td>Pulmonary</td>
<td>55 (81)</td>
<td>84 (57)</td>
</tr>
<tr>
<td>Extrapulmonary</td>
<td>12 (18)</td>
<td>61 (41)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Both</td>
<td>1 (1)</td>
<td>3 (2)</td>
<td>NS</td>
</tr>
<tr>
<td>AFB positive smear from respiratory secretions, no. (%)</td>
<td>50 (74)</td>
<td>60 (41)</td>
<td>&lt;.001*</td>
</tr>
<tr>
<td>One-year survival, no. (%)</td>
<td>5 (7)</td>
<td>92 (62)</td>
<td>&lt;.001*</td>
</tr>
</tbody>
</table>

**Figure 2.** IS6110 DNA patterns of 3 variants of multidrug-resistant *Mycobacterium tuberculosis* strain isolated from 70 HIV-infected patients (Mm and Mn) and 1 non-HIV patient (Mo) hospitalized at Muñiz Hospital, Buenos Aires, 1992–1995. R: reference strain 14323. Numbers at right indicate sizes (kbp) of standard DNA fragments.

NOTE. %s may not total 100 because of rounding. NS, not significant; AFB, acid-fast bacilli.

**Table 2.** Number of inpatients with disease due to the M strain of *Mycobacterium tuberculosis* in 10 different wards, by semester (Muñiz Hospital, January 1994 to June 1995).

<table>
<thead>
<tr>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>11*</td>
<td>1 (1)</td>
<td>10 (9)</td>
<td>11 (6)</td>
<td>22 (16)</td>
</tr>
<tr>
<td>10*</td>
<td>1 (1)</td>
<td>9 (8)</td>
<td>8 (8)</td>
<td>18 (17)</td>
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<tr>
<td>17*</td>
<td>0</td>
<td>2 (2)</td>
<td>6 (1)</td>
<td>8 (3)</td>
</tr>
<tr>
<td>16*</td>
<td>1 (1)</td>
<td>2 (1)</td>
<td>3 (2)</td>
<td>6 (4)</td>
</tr>
<tr>
<td>18</td>
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<td>0</td>
<td>3 (1)</td>
<td>4 (1)</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
<td>1 (1)</td>
<td>2 (1)</td>
<td>3 (2)</td>
</tr>
<tr>
<td>19</td>
<td>0</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>38</td>
<td>0</td>
<td>1 (1)</td>
<td>0</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Tuberculosis unit</td>
<td>1 (1)</td>
<td>0</td>
<td>1 (1)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Intensive care unit</td>
<td>0</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Total</td>
<td>5 (4)</td>
<td>27 (24)</td>
<td>36 (22)</td>
<td>68 (50)</td>
</tr>
</tbody>
</table>

* No. of patients with acid-fast bacilli–positive smears for pulmonary disease.
* P = .005 (50/56 vs. 60/87) for patients with pulmonary disease with/without extrapulmonary involvement.

* Wards in building for HIV patients.
that of the M strain. Both patients with the M strain had initially resistant TB at admission (ward 18 in September, ward 10 in December). During the months previous to diagnosis, they had been subjected to prolonged hospitalizations in wards 18 and 20 due to other opportunistic infections associated with AIDS.

Of the 35 non-HIV-infected MDR-TB cases included in the study (1992–1993), only 1 presented a culture with a pattern closely related to M. It was recovered in January 1993 from a patient with pulmonary TB who had been unsuccessfully treated at the Tuberculosis Unit throughout 1992 (figure 2, variant Mo). After this MDR isolate was obtained, a new therapeutic scheme was applied and she was discharged, apparently cured. RFLP patterns of MDR M. tuberculosis isolates obtained from the remaining non-HIV-infected patients hospitalized during 1992 and 1993 were polymorphic in comparison with each other and unrelated to the M genotype.

Discussion

Recently, an increased incidence of TB and of HIV infection coincided in producing a strong impact on current hospital practices in Buenos Aires and its suburban area [1, 6]. Inadequate isolation facilities, scarcity of resources, and unawareness at the management level aggravated the situation. Muñiz Hospital, being the referral treatment center for communicable diseases in Argentina, had the worst share of this burden. Severe cases of both TB and AIDS converged there independently, often after having become unmanageable at peripheral health care centers. Such a setting was prone to foster the dissemination of MDR-TB among immunocompromised hosts. Not surprisingly, the frequency of HIV-associated MDR-TB started to increase in this institution at the beginning of this decade and reached outstanding figures in 1995, when it largely outnumbered cases due to non-MDR tubercle bacilli. Data presented herein show that, between January 1994 and June 1995, this steep upsurge was mainly associated with a common resistance profile, which included at least five drugs (figure 1).

When studied by RFLP, a single M. tuberculosis strain, named the M strain, was found to affect 92% (68/74) of the patients with this highly resistant TB and a history of on-site (hospital) exposure. In several cases, including 1 with proven exogenous TB reinfection with the M strain (figure 3), only 2–7 months elapsed between the hospital exposure and isolation of the M strain, suggesting an accelerated progression from infection to disease. As half the patients survived <5 weeks from the onset of disease, and lengthy conventional methods were applied for cultures and susceptibility tests, the diagnosis of MDR-TB was often confirmed postmortem. Besides, 74% of the patients with the M strain had AFB smear-positive pulmonary disease, and the hospital had a limited provision of alternative drugs. Thus, many of them remained infectious until death, and HIV-infected patients suffering from other opportunistic infections were exposed for long periods to the M strain. Nosocomial transmission of TB is documented for the first time in a developing country. The association with AIDS, the accelerated spread, and the high rate of fatal outcome were three features shared with similar outbreaks reported earlier [14–17].

The M strain could be traced retrospectively to 2 of the 5 HIV-infected inpatients with MDR-TB diagnosed at Muñiz Hospital in 1992. As clinical records of both patients strongly suggested nosocomial infection, it is conceivable that this strain, or an ancestor, had been brought into the hospital earlier. Although the available information was inadequate for identification of an index case, a variant of this strain (Mo) was found to coexist about that time at the hospital, and it was presumably a representative of a progenitor strain.

Although at the time of diagnosis >80% of the patients with the M strain were hospitalized in the building set apart for HIV-infected patients, virtually every ward and service at the hospital was involved in the outbreak throughout the period of study (table 2). Several of these patients had received care at outpatient clinics, emergency rooms, or intensive care units shortly before or after diagnosis.

Certainly, personnel at these facilities must have been extensively exposed to infection. To our knowledge, no case of active MDR-TB has been documented among health care workers to date. However, no risk assessment has been conducted in this occupational group, neither have skin-test screenings been routinely performed. Thus, there is no basis for estimating the frequency of recent TB infection among health care workers during the period of study. Educational and skin-testing programs for health staff should be given high priority in this institution.
In the second half of 1995, certain measures were undertaken in an attempt to bring this outbreak to a halt. Bacteriologic diagnosis was expedited, alternative antituberculous drugs became available for all MDR-TB patients, and a new ward separated from the common circulation area has been assigned exclusively to them. For infectious TB patients, isolation rooms with air extractors and high-efficiency particulate air (HEPA) filters became available in HIV buildings, and the use of face masks is prescribed for patients. However, free circulation of patients after hours could not be prevented completely.

With regard to personal respiratory protection, only ordinary surgical masks are available at the institution. Although the effectiveness of different respiratory devices in protection from M. tuberculosis transmission has not been fully assessed, surgical masks do not meet the prevailing criteria for face-seal and filter efficacy. Since they do not prevent inhalation of particles the size of infectious airborne droplet nuclei (1–5 µm), they are not indicated for staff and visitors. On the other hand, by reducing the expulsion of respiratory secretions into the air, surgical masks worn by infectious patients may help to prevent transmission. Therefore, their use by patients with known or suspected infectious TB has been recommended, especially when they are out of TB isolation rooms [18, 19].

According to the statistics at the Mycobacteriology Department, 82 and 76 new cases of MDR-TB occurred among HIV-infected inpatients at Muñiz Hospital in the second semester of 1995 and the first semester of 1996, respectively. The vast majority of these cases had resistance to five to seven drugs. The M strain was isolated from 11 of 14 HIV-infected patients with highly resistant TB chosen randomly among those hospitalized between May and July 1996 (data not shown). These preliminary data show no encouraging indication that the outbreak is being controlled. In addition, evidence of spread of the M strain into several suburban hospitals is currently being gathered. Patients seeking care at different hospitals may carry the disease and should be a matter of concern for the public health system in this metropolis. Even though the study is not comprehensive, results presented here reveal a critical epidemiologic situation. Buenos Aires, after New York City, could indeed be fostering the second largest spread of MDR-TB so far reported [20, 21].

The risk of cross-contamination with the M strain in the daily processing of a large number of M. tuberculosis specimens at the hospital laboratory was evaluated. Laboratory error was convincingly eliminated in all but 1 case, which was itself inconclusive. Even though current facilities at the Mycobacteriology Department are very limited, as is the situation in most hospital laboratories in developing countries, the virtual absence of cross-contamination in this study could be attributed to staff adherence to traditional bacteriologic procedures and strict safety precautions. In fact, the radiometric method for cultures, which has been most frequently blamed for specimen cross-contamination, was only introduced into that laboratory after this study was finished, among other strategies for the containment of MDR-TB [22–24].

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References

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