Transmission of a Multidrug-Resistant *Mycobacterium tuberculosis* Strain Resembling “Strain W” among Noninstitutionalized, Human Immunodeficiency Virus–Seronegative Patients

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Since 1990, several outbreaks of multidrug-resistant tuberculosis (MDR-TB) have been described among institutionalized patients infected with human immunodeficiency virus (HIV). We describe a community MDR-TB outbreak among HIV-seronegative patients in Cape Town, South Africa. Isolates were characterized by restriction fragment length polymorphism (RFLP) analysis and dot-blot hybridization analysis of mutations conferring resistance for isoniazid, rifampin, streptomycin, and ethambutol. All isolates were identical on RFLP analysis. In 2 patients, RFLP analysis showed exogenous reinfection during or after treatment for drug-susceptible TB. Mutation analysis confirmed the genotypic identity of the isolates. The infecting strain was genotypically related to strain W, which is responsible for the majority of MDR-TB outbreaks in New York City. Transmission of MDR-TB is thus not limited to HIV-seropositive patients in an institutional setting but occurs within a community.

Clinical drug resistance in tuberculosis can be classified as acquired resistance, when drug-resistant mutants are selected as a result of ineffective treatment, or as primary resistance, when a patient is infected with a resistant strain. Mutations in the genome of *Mycobacterium tuberculosis* that can confer resistance to antituberculous drugs occur spontaneously, with an estimated frequency of $3.5 \times 10^{-6}$ for isoniazid and $3.1 \times 10^{-8}$ for rifampin. Because the chromosomal loci responsible for resistance to various drugs are not linked, the risk of a double spontaneous mutation is extremely small: $9 \times 10^{-34}$ for both isoniazid and rifampin [1]. Multidrug-resistant tuberculosis (MDR-TB), defined by the World Health Organization (WHO) as resistance to, at least, isoniazid and rifampin, will thus occur mainly in circumstances where sequential drug resistance follows sustained treatment failure.

Reports of nosocomial outbreaks of MDR-TB in institutions such as hospitals [2–5] and prisons [6] in the United States, Europe [7], and developing countries [8] have focused attention on MDR-TB as a major health issue. More than 80% of these patients were seropositive for the human immunodeficiency virus (HIV). Infection and a few cases of active MDR-TB in health care workers after exposure to patients with MDR-TB [9–11] and limited spread of a nosocomial outbreak into a community [12, 13] have also been reported. But even in these settings, most patients with active MDR-TB were immunocompromised.

Restriction fragment length polymorphism (RFLP) analysis of *M. tuberculosis* strains was used to study the disease dynamics of most MDR-TB outbreaks [3, 4, 6–8, 10, 12, 13]. This technique not only confirmed transmission between patients with suspected epidemiologic connection but also suggested transmission between patients not suspected to have epidemiologic links.

The most extensive MDR-TB outbreak reported to date consists of 267 patients infected with an isolate of *M. tuberculosis* with an RFLP pattern identical or closely related to strain W. Eighty-six percent of these patients were HIV positive. Seventy percent of these patients could be epidemiologically linked, and the locations of transmission were a hospital (96%), correctional system (2%), and community (3%) [14].

An opportunity for these outbreaks seemed to be created by the cohabitation of infectious MDR-TB patients with highly susceptible immunocompromised patients in settings with inadequate infection control procedures. HIV is postulated as the major factor that amplified, accelerated, and characterized MDR-TB outbreaks [1].

This report documents the genotypic characterization and
spread of a “strain W-like” multidrug-resistant \textit{M. tuberculosis} strain in an urban South African community among noninstitutionalized, HIV-seronegative individuals and challenges the view that MDR-TB outbreaks only occur in very specific population groups.

\section*{Materials and Methods}

The patients described in this paper were given their diagnoses between 1993 and 1997 and resided in 2 neighboring communities of metropolitan Cape Town, South Africa. This 2.5-km² area has a population of \approx 34,000 people, a reported TB notification rate \gt 1000 per 100,000/year [15], and \approx 150 \textit{M. tuberculosis} culture-positive patients/year. Although the socioeconomic living conditions are poor, there is no homelessness and no evidence of intravenous drug use. During the study period, there was a low prevalence of HIV infection in the Western Cape Province (ranging from 0.25\% in 1992 [16] to 3\% in 1996 [17]).

Sputum samples were collected at the primary health care clinics of the 2 communities studied and were sent to the routine laboratory for staining and culture. Drug susceptibility testing was done by the indirect proportion method on Löwenstein-Jensen medium containing critical concentrations of 0.2 \mu g/mL isoniazid, 30 \mu g/mL rifampin, 2 \mu g/mL ethambutol, 5 \mu g/mL streptomycin, and 20 \mu g/mL ethionamide. Kanamycin and thiacetazone were tested on Middlebrook 7H10 agar containing critical concentrations of, respectively, 5 and 2 \mu g/mL. Resistance was defined as \geq 1\% bacterial growth in comparison with a control by use of international criteria. Cultures resistant to isoniazid or rifampin were subsequently tested for resistance to other antituberculous drugs. Resistance to pyrazinamide was not tested.

Since late 1992, as part of a prospective study, positive cultures for \textit{M. tuberculosis} from patients residing in these communities were genotyped by RFLP analysis (IS6110) according to the standardized method [18] and analyzed by use of Gel Compar 4.0 software (Applied Maths BVBA, Kortrijk, Belgium). This study has had a recovery rate of \approx 70\% of positive cultures and resulted in an RFLP database consisting of 650 patients. Cluster analysis (by use of the Dice coefficient and an unweighted pair group method using arithmetical averages [UPGMA] clustering formula) [19] was applied to identify possible clusters of transmission. Since late 1992, 107 clusters of drug-sensitive isolates (average cluster size, 4.7; range, 2–47) and 6 clusters of drug-resistant isolates (cluster size ranging from 2 to 16) have been identified. These data were then combined with a standardized set of clinical data (date of birth, sex, history of previous TB treatment, and drug sensitivity).

A cluster of identical isolates (19 IS6110 insertion elements) was subjected to further genotyping as follows: genomic DNA was restricted with PvuII and HinfI, electrophoretically fractionated, and transferred to Hybond-N+ (Amersham, Amersham, UK). The PvuII digests were sequentially hybridized with enhanced chemiluminescence–labeled IS6110-3 probe (complementary to the 3' IS6110 domain between nucleotides 631 and 875), IS6110-5' probe (complementary to the 5' IS6110 domain between nucleotides 77 and 462), \textsuperscript{32}P-labeled DRr probe, and ISL540 (GenBank accession number U60566). The HinfI digests were hybridized with \textsuperscript{32}P-la-

beled MTB484(1) probe [20]. The respective RFLP patterns were visualized by autoradiography.

This cluster of isolates was analyzed for the following mutations: \textit{katG}315 conferring isoniazid resistance and the silent mutation \textit{katG}463; \textit{rpoB}526; and \textit{rpoB}531 conferring rifampin resistance; \textit{rpsL}43, \textit{rpsL}88, \textit{rrs}513, and \textit{rrs}491 conferring streptomycin resistance; and \textit{embB}306 conferring ethambutol resistance. Mutational analysis was done by polymerase chain reaction amplification of extracted DNA with specific primers and dot-blot hybridization for the different loci with a radiolabeled probe specific for each of the mutations.

Extensive clinical histories of patients infected with a strain with 19 IS6110 copies were reviewed and detailed data concerning medical history, HIV status, previous TB treatment, type of TB, chest radiography, sputum staining and cultures, sensitivity, and treatment were collected. Patients were interviewed in detail by a medical anthropologist for completion of data regarding place of work, school, recreation, focusing on drinking places, church attendance, and treatment supervisor. To maintain patients' confidentiality, other patients' names were never mentioned. To further enhance the elicitation of epidemiologic links, patients were asked for the names of their social contacts in certain streets.

\section*{Results}

From the strains collected between January 1993 and March 1997, 21 patients were identified with isolates resistant to at least 4 front-line drugs (isoniazid, rifampin, streptomycin, ethambutol). In 16 of these patients, genotype identity of the infecting \textit{M. tuberculosis} strain was shown by standardized IS6110 typing (figure 1). These isolates had 19 copies of IS6110 and were arbitrarily called strain U. For 12 patients, >1 isolate with a U-strain pattern on RFLP analysis was available. All 4 remaining patients had clinical evidence of active TB and multiple positive cultures with similar resistance patterns. There was thus no evidence of laboratory cross-contamination [21]. One additional patient was infected with an \textit{M. tuberculosis} strain closely related to strain U (similarity index of 79\% [IS6110-3] by use of the Dice coefficient and UPGMA clustering formula; figure 1). The other 4 patients, with isolates resistant to the 4 front-line drugs, were infected with an isolate characterized by 5, 7, 10, and 12 copies, respectively, of IS6110. During the entire prospective study period (late 1992–mid 1998), no samples with strain U were recovered from 585 patients with drug-sensitive TB.

All 16 patients infected with strain U had pulmonary MDR-TB, 94\% had smear-positive TB, and 80\% had cavities on chest radiograph at the time of diagnosis. Nine patients were female, 7 were male. The average age at diagnosis of MDR-TB was 28 years (range, 11–50; figure 2). All 16 patients were seronegative for HIV, and none had a malignancy or received steroid therapy, which could lead to immunosuppression. None of the patients had been hospitalized for TB treatment or other respiratory diseases prior to the diagnosis of MDR-TB. All patients were treated at the primary health care clinics of the 2
communities; 13 patients also received treatment as inpatients for a median duration of 107 days at a hospital that specialized in TB treatment. All but 1 patient were treated with 4 drugs to which the organism was susceptible (i.e., appropriate treatment). On average, the median time between diagnosis and initiation of appropriate treatment was 94 days (range, 0–555 days). Ten of the 16 patients were compliant (>80% of prescribed dosages). Only 13 patients achieved bacterial conversion. The median time patients were sputum positive was 238 days (range, 35–1102 days). Patients who achieved bacterial conversion were treated for a median duration of 380 days (range, 121–637 days) after conversion.

RFLP analysis by use of the IS6110-3' probe (figure 1) and IS6110-5' probe (data not shown) showed that all 16 samples representing the infecting strain of each patient had an identical pattern of 19 IS elements. In addition to IS6110, 2 additional
Figure 2. Patient characteristics and resistance pattern of strain U *Mycobacterium tuberculosis* isolates. DR-TB, drug-resistant tuberculosis; m, male; f, female; INH, isoniazid; RIF, rifampin; SM, streptomycin; EMB, ethambutol; THIA, thiacetazone; ETH, ethionamide.

*M. tuberculosis* subtyping probes, DRr and MTB484(1), confirmed that the genotypes were identical for all 16 isolates (data not shown). Comparison of the IS6110-3 and IS6110-5 RFLP analyses of strain W [22] and strain U suggested that strain U may be genetically related to strain W (figure 3). The similarity index calculated by use of the Dice coefficient and UPGMA clustering formula was 81.1% (IS6110-3) and 78.9% (IS6110-5). To test further for similarity, a genomic sequence (ISL540.3) present in H37Rv and most clinical isolates but absent in all strain U isolates was hybridized against DNA of strain W (961-0874). No hybridization was detected, suggesting a common ancestry (data not shown).

DNA from the initial isolate of the MDR-TB episode was available for mutational analysis in 9 cases. In all of these isolates, the following mutations could be demonstrated: katG315, katG463, rpoB531, rrs513, and embB306, conferring resistance to, respectively, isoniazid, rifampin, streptomycin, and ethambutol. No mutations were identified in the rpoB526, rpsL43, or rpsL88 loci or in the rrs491 position. In 6 of the 7 patients from whom no initial isolate was available, analysis of a follow-up isolate revealed identical mutations (figure 2). This result confirms the identical genotype of the isolates as suggested by RFLP analysis. Mutation analysis of the strain closely related to strain U revealed identical mutations responsible for resistance to isoniazid and ethambutol but different mutations for rifampin (rpoB526) and streptomycin (rpsL43).

Initially, the outbreak propagated rapidly, with 8 cases in 1 year. Thereafter, the spread continued at a slower rate, with 8 new cases over the following 3 years (figure 4). Figure 4 might suggest a single chain of transmission; however, conventional contact tracing refutes this. Of the 14 patients who could be interviewed, there were only 2 patients who had no apparent epidemiologic link with any of the other patients. Among the 12 patients with social links, there was 1 group consisting of 8 people who were either relatives or friends of each other and another group of 4 people who were close or distant family. No relationship between these 2 groups could be found (figure 2). Social anthropology data suggest that patient 1 was most likely infected by a close friend, an HIV-seronegative MDR-TB patient resistant to isoniazid, rifampin, and ethambutol who was culture positive >1 year prior to his death in May 1993. As this person lived outside the communities studied, his *M. tuberculosis* isolates were not available for RFLP or mutational analysis. Because this person was infectious for such a prolonged period, it is possible that he was the source case for at least 1 of the 8 related patients. As this study was performed in 2 communities with a high population density (14,500 people/km²), casual or unrecognized contact is a likely explanation for the patients with no apparent epidemiologic link. Moreover, all residents attend the primary health care clinic as a first line for all health problems, which makes the clinic itself another possible contact place.

The majority of patients (11/16) received antituberculous treatment prior to the diagnosis of MDR-TB. Four patients...
Figure 3. Southern blot comparison of *Mycobacterium tuberculosis* isolates hybridized with *A*, IS6110-3 (IS6110 nucleotides 631-875) and *B*, IS6110-5 (IS6110 nucleotides 77-462). Lane R, MTB 14323; lane 1, strain W (961-0874); lane 2, strain U.

received an average of 5 months of treatment before drug-sensitivity tests were requested. Seven patients received a prior course of treatment for an episode of TB, predating the MDR-TB episode by an average of 3 years. In 2 cases, DNA was available from a specimen obtained prior to the diagnosis of MDR-TB. Patient 3 received treatment for culture-proven TB 3 months before diagnosis of MDR-TB. These bacilli were sensitive to all drugs and had an RFLP pattern of 15 IS6110 elements not related to the strain responsible for the MDR-TB episode (similarity index of 33% as calculated by use of the Dice coefficient and UPGMA clustering formula). Patient 16 received a full treatment course for drug-sensitive TB 3 years before diagnosis of MDR-TB; the isolate was then characterized by 9 IS6110 elements on RFLP analysis, again not related to the strain responsible for the MDR-TB episode (similarity index of 27% [data not shown]). Both patients were compliant during the treatment for drug-sensitive TB as judged on the basis of attendance of directly observed treatment (taking >80% of prescribed dosages).

**Discussion**

As far as could be ascertained, this is the first report of an MDR-TB outbreak occurring in a community without the involvement of a single HIV-seropositive or institutionalized patient and where transmission was proved by modern molecular biology techniques such as RFLP and mutation analysis.

In the pre-AIDS era, outbreaks of MDR-TB were occasionally reported [23, 24]. Such outbreaks were characterized by a slow spread over a number of years and a limited number of patients. Only 1 of these outbreaks reported before 1990 involved cases resistant to both isoniazid and rifampin, the current WHO definition of MDR-TB [25]. Suspected epidemiologic links could be confirmed only by phage typing [26, 27], a far less precise technique than RFLP analysis. The rare occurrence of outbreaks of drug-resistant TB was ascribed to the conviction that drug-resistant *M. tuberculosis* strains are less virulent [28]. However, recent studies showed that drug-resistant strains do not differ from drug-sensitive strains in their ability to create infection or disease [29, 30]. This report of a cluster of 16 MDR-TB patients confirms that drug-resistant strains of *M. tuberculosis* can be transmitted, and not only to the immunocompromised host.

In 1990, an outbreak of MDR-TB involving 10 epidemiologically related, noninstitutionalized, HIV-seronegative patients was reported by the Centers for Disease Control and Prevention of the United States [31]. However, drug resistance was proved in only 8 of the 10 cases, the initial resistance pattern was the same in only 2 of the cases, and no further analysis (phage typing or genotyping) was performed to establish whether all cases were part of a chain of transmission caused by 1 specific strain.

The results of our study indicate that a difference in resistance
pattern on conventional susceptibility testing of the initial isolate does not exclude recent transmission. The resistance pattern of initial isolates showed resistance to isoniazid, rifampin, streptomycin, and ethambutol in only 6 of the 16 patients. In 3 of these and 6 other patients, the initial isolate was available, and mutational analysis showed the presence of the same 5 mutations conferring resistance to all 4 drugs, even if conventional methods reported the isolates as susceptible to $\geqslant 1$ of those 4 drugs. These false-negative results on conventional susceptibility testing are most likely a consequence of laboratory error. This problem, and the fact that susceptibility testing is not routinely performed, are factors that lead to suboptimal treatment of patients with primary MDR-TB. This can in turn cause prolonged infectivity and further transmission within the community. Efficient molecular procedures such as those described in this study could therefore be valuable in the quick diagnosis and prevention of spread of MDR *M. tuberculosis* strains.

Our study also indicates that history of previous treatment is another clinical parameter that by itself cannot be the sole factor to reject the possibility of recent transmission of MDR-TB. It was believed that exogenous reinfection was rare because of the possibility of protective immunity acquired after a first infection. A person with drug-resistant TB and a history of a previous TB episode was therefore classified as having acquired drug-resistant TB. There have been a few reports of exogenous reinfection in immunocompromised patients [13, 32, 33] and 1 report of exogenous reinfection in an immunocompetent patient [34]. Eleven of the 16 immunocompetent patients reported in our study had a history of previous treatment. In 2 patients, RFLP analysis of both the drug-sensitive and drug-resistant isolates could be performed. This showed that in both these patients the 2 TB episodes were caused by 2 different, unrelated strains, thereby proving exogenous reinfection during treatment (patient 3) or after completion of a previous treatment episode (patient 16). The presence of the 5 identical mutations in genes conferring resistance to antituberculous drugs (*katG*315, *katG*463, *rpoB*531, *rrs*513, and *embB*306) in all 16 patients also supports transmission rather than acquisition as the mechanism responsible for drug-resistant TB in these patients.

MDR-TB outbreaks became an established phenomenon, as hundreds of cases were reported worldwide and transmission between cases was proven by molecular epidemiologic techniques [2–7]. These outbreaks were characterized by rapid spread, accelerated progression from infection to disease, and high mortality rates. The overwhelming majority of the patients were HIV seropositive and/or institutionalized (hospitals, prisons). The characteristics of the outbreak described in this report are very different from the above. This outbreak propagated over a number of years, the mortality rate was low (12%), patients were HIV seronegative, and transmission occurred in the community. However, strain W (New York) and the “strain W-like” strain U (Cape Town) were responsible for $\geqslant 80\%$ of cases resistant to at least 4 drugs, respectively, in New York City and the 2 Cape Town communities. Because these 2 strains...
are not identical, as shown by a related but nonidentical RFLP pattern and a difference in the mutation conferring resistance to streptomycin \( (rpsL43 \text{ in strain W and } rrs513 \text{ in strain U}) \), strain U cannot be the result of a recent transcontinental spread of strain W. These 2 genomically-related strains thus independently arose in 2 different geographic locations and in 2 very different patient groups, suggesting that this \( M. \) \( \text{tuberculosis} \) strain family is prone to the development of mutations conferring resistance.

On the basis of the finding that \( \geq95\% \) of 267 cases of MDR-TB caused by outbreaks of strain W in New York City over a 3-year period were likely to have been nosocomial infections, it was suggested that transmission of MDR-TB was almost exclusively confined to institutional settings, with no significant spread beyond the walls of institutions [14]. This would imply that attention can be focused on hospitals serving at-risk populations as a unique locus of transmission of MDR-TB [35].

The outbreak described in this report challenges that idea. A community can be the location of rapid spread of MDR-TB even if there is a low prevalence of HIV and no homelessness or intravenous drug use. A factor that could have contributed to the extent of this outbreak is the prolonged period of infectiosity in these patients. This study describes an outbreak in an area with a high incidence of TB, where the priority is the application of direct observed therapy short course (DOTS) to treat all smear-positive (drug-sensitive) cases effectively to reduce the infectious pool and prevent the acquisition of drug resistance. The DOTS program for low-income countries does not require sensitivity testing on a routine basis and gives no priority to effective personalized treatment for patients with MDR-TB. This results in delayed diagnosis and a delay in the initiation of effective MDR-TB treatment, leaving patients infectious for a prolonged period. Transmission of MDR-TB to nonimmunocompromised individuals creates a reservoir of persons infected with an MDR-TB strain, an infection for which there is currently no efficient disease-prevention strategy. To prevent an outbreak of MDR-TB, as well as the creation of a pool of persons infected with MDR-TB strains within a community, prevention of transmission of multidrug-resistant bacilli should be targeted by adequate case investigation (including contact investigation), rapid and correct diagnosis of drug resistance, and adequate treatment of patients with MDR-TB.

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