Transcontinental Spread of Multidrug-resistant *Mycobacterium bovis*

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Globally, the proportion of all cases of tuberculosis (TB) caused by drug-resistant strains is increasing. We report the case of a Canadian citizen who acquired a highly drug-resistant strain of *Mycobacterium bovis* while visiting a relative with AIDS-related tuberculosis in Spain. The origin of the strain was traced using spoligotyping, a polymerase chain reaction (PCR)-based fingerprint technology, and the European DNA database. The level of primary drug resistance—all five first-line drugs and 19 of 21 second-line drugs—in this case was unprecedented in Canada. Isolation of this strain from a Canadian citizen represents the first report of its appearance in this hemisphere. The infection was contained and combined medical–surgical treatment delivered. **Long R, Nobert E, Chomyc S, van Embden J, McNamee C, Rey Duran R, Talbot J, Fanning A. Transcontinental spread of multidrug-resistant *Mycobacterium bovis*.** AM J RESPIR CRIT CARE MED 1999;159:2014–2017.

The proportion of all cases of tuberculosis (TB) caused by drug-resistant strains is increasing (1). The problem is not confined to developing countries where there is greater opportunity for misuse of the drugs. Human immunodeficiency virus (HIV) co-infection, immigration from high prevalence to low prevalence regions, and greater world travel in general have resulted in a global increase in the likelihood of encountering drug-resistant strains of TB. In the Province of Alberta, Canada (population 2,696,826 in 1996), drug susceptibility testing is performed on isolates of *Mycobacterium tuberculosis* complex from all new cases. Between January 1989 and June 1998 there were eight initial isolates with multidrug resistance (resistance to at least isoniazid and rifampin), six from foreign-born individuals and two from Canadian-born individuals who had traveled abroad (2). In one of the latter, presented here, we document the transmission of a strain of *Mycobacterium bovis* of unprecedented resistance from an HIV-seropositive patient in Spain to a healthy HIV-seronegative contact. The HIV-seronegative contact then returned to her home in Canada where she developed active disease. Isolation of this strain from a Canadian citizen represents the first report of its appearance in this hemisphere. Containment of the infection and cure of her disease required an extraordinary expenditure of energy and resources.

CASE REPORT

In the spring of 1996 a 49-yr-old white female resident of the Province of Alberta, Canada traveled to Spain to be with a relative who was terminally ill with acquired immunodeficiency syndrome (AIDS)-related pulmonary TB. After visiting for 5 wk she returned to Canada where she felt well until September 1997 when she developed a cough. She saw her physician who described a well-nourished (body mass index 26 kg/m^2) healthy-appearing woman who had no past history of tuberculosis, no travel history other than the previously mentioned trip to Spain, and no history of having been visited by anyone from Spain, nor anyone known to have TB. Her only immunocompromising condition favoring TB was noninsulin-dependent diabetes; HIV serology was negative, and the CD4 lymphocyte count normal (1.0×10^9/L). A plain chest radiograph revealed a few well-defined nodules, mainly in the upper lung zones. A computed tomogram (CT) of the thorax confirmed the presence of at least seven widely distributed nodular lesions, two with small central areas of cavitation (Figures 1A and 1B). Direct microscopy of sputum was negative for acid-fast bacilli (AFB). Tuberculin tests (5 tuberculin units [TU] of purified protein derivative [PPD]) performed before and 3 mo after travel and again in November 1997 were negative (0 mm induration). A thoracoscopic biopsy of the right-sided lesions, performed in November 1997, demonstrated caseating granulomas on histopathology. Tissue and subsequent sputum cultures were positive for *M. tuberculosis* complex on DNA probe (Gen-Probe Accuprobe). Standard antituberculous drug therapy was begun; isoniazid 300 mg, rifampin 600 mg, pyrazinamide 1,500 mg, and ethambutol 1,200 mg together with vitamin B6, 25 mg once daily.

When it became evident that the isolate was extremely slow-growing in both Bactec 12B and conventional culture medium and could possibly be an *M. bovis*, further subcultures from all isolates that grew in Bactec 12B medium were inoculated to Marks pyruvate (modified Lowenstein-Jensen with pyruvic acid but without glycerol), Lowenstein-Jensen, Middlebrook 7H11 plates, and commercial BBL Middlebrook 7H10/7H11 biplates with aspartic acid and pyruvate. Growth was 2–3+, no growth, 1–2+, and 3+ (best on 7H11 with aspartic acid and pyruvate), respectively. Biochemical tests (niacin production, ox-
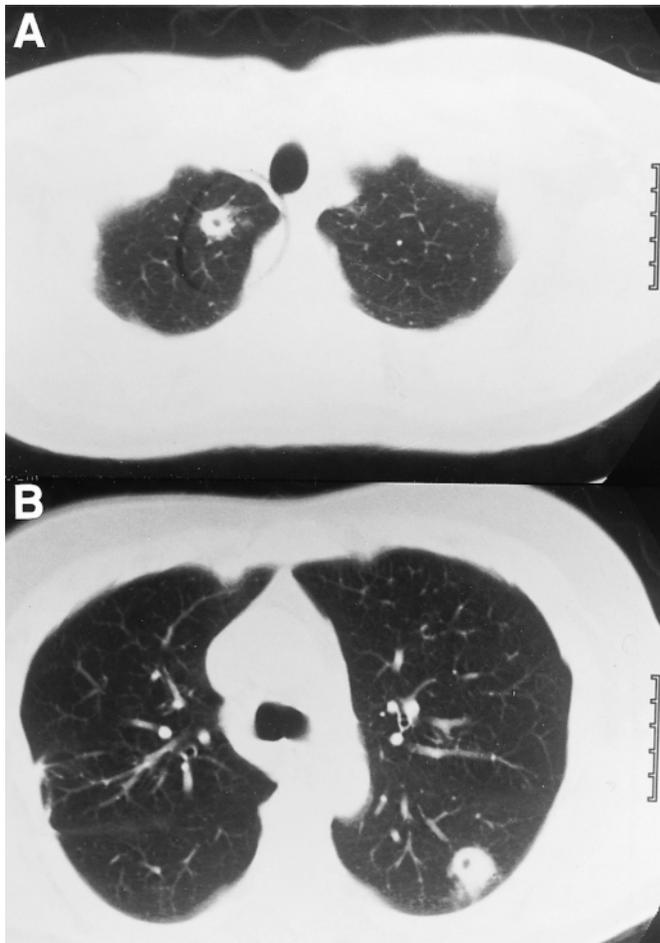
Figure 1. Conventional CT scan demonstrating the two largest nodules, each with small central cavitation; one in the apical segment of the right upper lobe (*A*) and one in the superior segment of the left lower lobe (*B*).

ygen preference, and thiophene-2-carboxylic acid hydrazide susceptibility testing) confirmed the identity of the isolate as *M. bovis*. All microbiological work with the isolate was carried out in a Biosafety Level III facility by experienced technologists wearing high-efficiency particulate air (HEPA) filter masks (3).

Drug susceptibility testing, performed using Bactec 12B medium and the radiometric modified proportion method (Bactec 460; Becton-Dickinson Diagnostic Instruments Systems, MD) indicated resistance to all first-line drugs (4, 5). Extensive further testing, performed in collaboration with the Mycobacteriology Laboratory, National Jewish Center for Immunology and Respiratory Medicine in Denver, Colorado, determined that the isolate was resistant to all available antimycobacterial drugs except cycloserine and clofazimine (Table 1) (6, 7). *M. bovis* is characteristically resistant to pyrazinamide.

To trace the origin of the strain of *M. bovis*, heat-killed cells were sent to a central European DNA database for spoligotyping, a polymerase chain reaction (PCR)-based DNA fingerprint technology that is superior to restriction fragment length polymorphism when the IS6110 copy number is low, as is the case with *M. bovis* (8, 9). The strain matched identically that isolated from her relative in Spain, a strain that was in turn identical to that reported in several large outbreaks of multidrug-resistant (MDR) *M. bovis* in HIV-seropositive patients in Spain (Figure 2) (10–13).

The patient was placed in respiratory isolation. Multiple sputa (n = 12) collected over 5 mo (mid-November 1997 to mid-April 1998) were culture-positive for the same isolate. All were negative for AFB on direct microscopy. A repeat CT scan of the thorax was performed in January 1998. Though disease remained localized, with cavitation that was minimal and limited to only two nodules—perhaps explaining the

sputum smear negativity—there no doubt had been progression when compared with the November 1997 scan. A fourth tuberculin test along with a skin test to mumps antigen, in consideration of possible anergy, were performed in January 1998. Both were positive (tuberculin test = 11×14 mm of induration). Standard drug therapy was discontinued and a regimen of cycloserine 500 mg twice daily, clofazimine 300 mg once daily, and trimethoprim–sulfamethoxazole two single-strength tablets twice daily was commenced in mid-February 1998 when second-line antituberculous drug susceptibility test results became available. The trimethoprim–sulfamethoxazole was included as the estimated achievable serum level approximated the minimal inhibitory concentration. Two-hour postdose serum levels of cycloserine and clofazimine were determined to be therapeutic. The relatively weak regimen, together with the extraordinary degree of primary drug resistance, however, provided little promise that medical therapy alone could achieve a cure. Accordingly, after ensuring the adequacy of her lung function (FEV$_1$ and FVC = 107 and 109% predicted, respectively, and Pa$_{O_2}$ and Pa$_{CO_2}$ while breathing room air of 82 and 37 mm Hg, respectively), plans were made to resect her disease in two, staged procedures while administering those drugs to which her isolate was susceptible.

Through regular meetings which included hospital administration, infection control, occupational health and safety, the Provincial Laboratory of Public Health, the operating room, the critical care unit, the thoracic surgery ward, the TB ward, the physical plant and involved physicians, nurses, respiratory therapists, and laboratory technologists, the collective resources of the hospital were marshalled to the task of performing the surgery while preventing nosocomial spread of the organism. Biosafety level III laboratory conditions were introduced into the operating room (3). This consisted of the primary barrier (the specific personal protective wear, including HEPA filter masks) and the secondary barrier (the directional airflow from the entry/exits to the areas of increased hazard, and the air exchanges per hour). Real or possible high-risk postoperative activities were also identified. In mid-March and mid-April 1998, respectively, left and right thoracotomies were performed and all disease visible on preoperative CT scans was resected. Before each procedure a bronchoscopy was performed and secretions were removed. The airway was then secured with a double-lumen endotracheal tube. Reexploration for bleeding was required after the second thoracotomy.

AFB were present on smear and *M. bovis* was cultured from the right apical and the left superior segmental lesions demonstrated in Figure 1 as well as from a right lower lobe lesion. In May 1998, after a hospitalization that lasted 4 mo, the patient was discharged on directly observed therapy. All sputa collected after her second thoracotomy remain negative for AFB on smear and culture, and no new lesions are demonstrable on CT scans of the thorax. (Between April 14, 1998 and January 15, 1999, 22 specimens of sputum have been collected; 21 are smear- and culture-negative, one—collected at the time of writing—is smear-negative, culture pending.) Among 80 known contacts in Canada (22 friends and coworkers and 58 hospital staff) no tuberculin test converter or secondary case has so far been identified.

DISCUSSION

We have presented a case of pulmonary TB caused by a highly drug-resistant strain of *M. bovis* acquired by a Canadian citizen traveling abroad to Spain. The level of primary drug resistance in this case was unprecedented in Canada (Laboratory Center for Disease Control, Ottawa, Canada), and, to our knowledge, in the United States. Out of a total of 26 antimycobacterials of varying efficacy the isolate was fully susceptible to only one, cycloserine, and intermediately susceptible to only one, clofazimine. Presumably, the multidrug-resistant phenotype in these organisms arose by sequential acquisition of resistance-conferring mutations in several genes. Most likely this occurred as a consequence of antibiotic selection of randomly occurring mutants in concert with inadequately treated infections. Despite assurances that sputum-smear-negative cases of pulmonary TB are not likely to infect others

TABLE 1
DRUG SUSCEPTIBILITY TEST RESULTS: MDR *M. bovis*

	Methodology*			
Drug	Radiometric Modified Proportion Method	Radiometric MIC Method	Proportion Method	E_{TEST}
First-line				
Isoniazid	R		R	
Rifampin	R	R (> 8.0)	R	
Pyrazinamide	R			
Ethambutol	R	R (> 8.0)	R	
Streptomycin	R	R (> 8.0)	R	
Second-line				
Amikacin		R (> 8.0)		
Capreomycin		R (> 8.0)	R	
Kanamycin		R (> 8.0)	R	
Ethionamide			R	
Cycloserine			S	
Para-aminosalicylic acid			R	
Clofazimine		S (0.12)		
Rifabutin		R (> 2.0)		
Ciprofloxacin		R (> 4.0)		
Ofloxacin		R (> 8.0)		
Sparfloxacin		R (> 2.0)		
Clarithromycin		R (32.0)		
Azithromycin		R (> 64.0)		
Amoxicillin-clavulanic acid		R (> 16/8)		
Trimethoprim–sulfamethoxazole		R (76/4)		
Thiacetazone		R (> 1.2)		
Imipenem				R
Synercid				R
Trovalfloxacin				R
Grepafloxacin				R
Meropenum				R

Definition of abbreviations: MIC = minimal inhibitory concentration; R = resistant; S = susceptible.

* The radiometric modified proportion method used Bactec 12B medium except for pyrazinamide where Bactec pyrazinamide medium was used. The radiometric method for determining MICs used Bactec 12B medium. The proportion method used conventional Middlebrook 7H10 medium. The E_{TEST} methodology used a modified protocol suggested in E_{TEST} *News*, No. 13, August 1995; this methodology has not been standardized.

(14), the level of anxiety that followed notification of this case was remarkable; the effort made to contain the infection and cure the disease, prodigious.

Once identified as MDR *M. bovis* the process of tracing the strain to its source was facilitated by the systematic typing of MDR-TB strains in Spain and the existence of a European DNA database. Until as recently as 1987 majority opinion held that human-to-human transmission of *M. bovis* must be a rare event if it ever did take place (15, 16). Fingerprint technology has now established as certain that such transmission does indeed occur, especially among those that are HIV-seropositive. All of the outbreaks of MDR *M. bovis* in HIV-seropositive patients in Spain are believed to have resulted from human-to-human transmission. Our patient along with five other patients in Spain, two of whom are health care workers, are the only HIV-seronegative patients known to have developed disease from this strain of MDR *M. bovis* (17). Carriage of this strain outside of Spain (to Holland) has been reported in only one other case (9). Disease from this strain of *M. bovis* has been fatal in all those co-infected with HIV. Of the five HIV-seronegative patients in Spain with disease caused by this strain, two have died and one has undergone a pneumonectomy (R. Rey Duran, personal communication).

In cases of disease resulting from highly drug-resistant *M. tuberculosis*, where the chances of a medical cure are slim, surgery, either resectional (18) or collapse (19), is a recognized treatment option. In preparation for resectional surgery most authorities recommend that sputum-smear- and culture-nega-

tive status be secured preoperatively with at least 3 mo of anti-tuberculous drugs (18). Clearance of bacilli from the airway is believed to minimize the chances of intraoperative dissemination or postoperative breakdown of the bronchial stump (20). In our patient the weak regimen of drugs available to us and the existence of localized, nodular disease that was both operable and resectable—but not likely to remain so—led us to conclude that the best chance for cure was the early performance of bilateral wedge resections.

Why our patient's tuberculin test was negative 3 mo after her return from Spain, where she was definitely infected, and why her test remained negative in November 1997 when she was diagnosed with active disease, is unknown. All tests were performed with the same antigen (5 TU of purified protein derivative–standard [PPD-S], Connaught Laboratories) and read by qualified staff. Nor were there obvious host factors such as concomitant disease, drug therapy (21), or "serious illness" (22) to explain the falsely negative tests. Delayed-type hypersensitivity (DTH) is known to be associated with but not identical to protective immunity. In experimental systems, separate cell populations transfer DTH and protective immu-

Figure 2. Comparison of the spoligotype of the MDR *M. bovis* isolated in our Canadian patient (1) and the outbreak strain isolated from several patients, including her relative, in Spain (2).

nity (23), an observation that appeared to be supported by the fact that our patient had localized nodular disease together with what appeared to be a normal granulomatous tissue response despite being tuberculin-negative. At the skin test site, T cells are known to proliferate in response to mycobacterial antigens and produce predominantly T helper cell, type 1 (Th1) cytokines (24). Impaired Th1 responses have been reported in HIV-seronegative patients with MDR-TB and low CD4 cell counts but not in those with counts over $0.5 \times 10^9/L$ (25). Perhaps, as has been suggested by Ellner (26), the negative tuberculin tests were the result of circulating suppressor adherent cells.

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