Retrospective Detection of Laboratory Cross-Contamination of Mycobacterium tuberculosis Cultures with Use of DNA Fingerprint Analysis

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In 1992–1993, we investigated possible cross-contamination of Mycobacterium tuberculosis cultures as part of a study of tuberculosis in Arkansas by using DNA fingerprint analysis. Of patients whose isolates were matched, those for whom smears were negative and only one culture was positive were identified from laboratory records. Clinical, laboratory, DNA fingerprint, and epidemiological data were reviewed. Of 259 patients, nine (3.5%) were judged to be due to cross-contamination. None of these patients had a clinical course consistent with tuberculosis. All nine specimens were processed with another isolate with a matching DNA fingerprint, and epidemiological connections were not identified among any of the patients. To avoid erroneous diagnoses and unnecessary therapy and public health investigations, specimens from patients with tuberculosis whose smears are negative and only one culture is positive should be investigated for cross-contamination. An inconsistent clinical course and a DNA fingerprint that matches those of other culture-positive specimens processed concurrently, coupled with the lack of an epidemiological connection, suggest cross-contamination.

With the recent resurgence of tuberculosis in the United States, particular attention has been paid to rapid and accurate diagnosis as one of the cornerstones of tuberculosis control [1]. Although tuberculosis is sometimes easy to recognize clinically, it may also present atypically or with nonspecific symptoms, especially in immunocompromised patients [2,3]. In situations in which the diagnosis of tuberculosis is difficult or unexpected, practitioners may rely almost completely on the laboratory for confirmation of the diagnosis. Culture for Mycobacterium tuberculosis is the most specific test used for the diagnosis, but culture reports still require careful interpretation because of false-positive results.

Epidemiological investigations involving nosocomial transmission of tuberculosis have revealed multiple instances where patients who had a single positive culture ultimately were determined not to have tuberculosis [4–7]. The cultures in these instances were positive because the patients’ specimens were contaminated with M. tuberculosis from other patients’ specimens in the clinical mycobacteriology laboratory. Other studies have also described instances of laboratory cross-contamination of specimens [8,9]. In these cases, the cross-contamination was identified because the culture results were not consistent with the clinical courses of the patients or an unusual clustering of positive cultures within the laboratory was noted. Laboratory records in these instances indicated that other specimens containing M. tuberculosis, usually acid-fast bacilli (AFB) smear-positive, were processed in the laboratory concurrently with the suspect specimens.

Various methods have been used to determine that the isolates in the putative contaminating source culture and the suspect culture were related, including biochemical testing [9], phage typing [10], and, most recently, DNA fingerprinting [11]. In the mycobacteriology laboratory, multiple modes of cross-contamination of cultures have been implicated, including the use of pipettes and open containers in dispensing reagents to multiple specimens and serial sampling of multiple culture vials in an automated radiometric culture system such as BACTEC (Becton Dickinson, Sparks, MD) [11,12].

Using clinical, radiographic, laboratory, DNA fingerprint, and epidemiological information, we were able to retrospectively determine the rate of laboratory cross-contamination in M. tuberculosis—positive cultures of specimens from a population of patients for whom tuberculosis was diagnosed in the state of Arkansas over a 2-year period. Because of the retrospective design of this study and the multiple laboratories involved, the specific modes of cross-contamination could not be determined. The methods used and observations made in this study serve as a basis for proposed mechanisms whereby clinicians, tuberculosis control programs, and mycobacteriology laboratories can work in concert to identify cross-contaminated cultures when they occur.
as part of an epidemiological study of tuberculosis in Arkansas, *M. tuberculosis* isolates from patients for whom tuberculosis was diagnosed during the years 1992 and 1993 were analyzed by DNA fingerprinting according to standard methods that use a probe for insertion sequence IS6110 [13, 14]. *M. tuberculosis* isolates that matched one or more other isolates by DNA fingerprint patterns were grouped into clusters. Epidemiological and clinical data for clustered patients were obtained by review of health department records and by individual interviews with patients. The interview consisted of the patient’s history of tuberculosis infection and disease, risk factors for the acquisition of infection and disease, and a detailed lifetime social history. Epidemiological connections among patients in clusters were identified by using this information.

Isolates that had unique fingerprint patterns were assumed not to be due to laboratory cross-contamination. To determine if any isolates from the group of clustered patients were a result of laboratory cross-contamination, we reviewed the laboratory records for each patient. Patients for whom smears of their clinical specimens were all AFB-negative and culture of only one specimen was positive for *M. tuberculosis* were identified for further investigation. In addition, patients from whom two culture-positive specimens were recovered were included in the investigation if the specimens were initially processed on the same day. The positive cultures for these patients were considered suspect for laboratory cross-contamination. For each of these isolates, laboratory records were reviewed to identify other culture-positive specimens processed in the laboratory on the same day. Such specimens were considered potential sources of cross-contamination.

For each patient for whom an isolate was identified as potentially due to cross-contamination, the clinical, radiological, laboratory, DNA fingerprint, and epidemiological data were reviewed by three investigators (J.H.B., W.W.S., and C.R.B.). Evidence in favor of cross-contamination included the following observations: the patient from whom the specimen was obtained had a clinical course inconsistent with tuberculosis; another culture-positive specimen (a potential source of contamination) was processed concurrently in the same laboratory; the isolate from the potential source specimen had a matching DNA fingerprint; and there were no known epidemiological connections among the patients involved. In some cases, conflicting information required further investigation.

Additional evidence in favor of cross-contamination of a suspicious positive culture included the observation that the specimen in question closely followed a potential source of contamination in the order of processing, that a culture yielded few (five or less) *M. tuberculosis* colonies on solid medium, and that the number of bands in matching DNA fingerprints was greater than five. A DNA fingerprint band count of less than five was not considered to discriminate all *M. tuberculosis* strains [13]. Unique fingerprints were considered to strongly suggest no cross-contamination between the cultures in question. After review of the above evidence for or against cross-contamination in each case, the positive cultures for these patients were judged as “unlikely,” “possibly,” or “likely” due to cross-contamination.

**Results**

During 1992 and 1993 in Arkansas, there were 361 cases of culture-positive tuberculosis. In 259 (72%) of these cases, isolates were available for DNA fingerprinting. Isolates from 149 patients had DNA fingerprint patterns that matched those of one or more other isolates, and 35 clusters of patients were formed. AFB smear-negative specimens and either one *M. tuberculosis* culture-positive specimen (20 patients) or two culture-positive specimens processed on the same day (two patients) were obtained from 22 (14.8%) of those 149 patients.

Isolates from 13 of the 22 patients whose cultures were identified as being potentially cross-contaminated were considered unlikely to be due to cross-contamination after review of clinical, radiological, laboratory, DNA fingerprint, and epidemiological information (i.e., the patients were considered to have had tuberculosis). The three reviewers were in agreement concerning the classification of these patients. All 13 patients had symptoms and/or radiographic findings consistent with tuberculosis. No potential source isolates with matching DNA fingerprints were identified for 11 of the 13 patients.

For the remaining two patients, a potential source isolate for cross-contamination was identified. In both cases, DNA fingerprints of the suspect isolate and the potential source isolate matched, and there were no epidemiological links identified between the patients. However, in one case, the potential source isolate had a differing drug resistance pattern (resistant to isoniazid and rifampin vs. fully susceptible to all drugs tested). This finding led us to believe that the matched fingerprint was due to some error such as mislabeling of specimens. The other patient had radiological findings consistent with cavitory tuberculosis. The DNA fingerprint of the isolate from this patient and that of the potential source isolate had only two hybridizing fragments, a pattern that does not allow accurate discrimination between strains [15].

Of the remaining nine patients (41% of 22; 3.5% of 259), all reviewers considered one and five positive cultures as possibly and likely, respectively, due to laboratory cross-contamination. Positive cultures for three patients were considered as possibly due to cross-contamination by one reviewer but likely due to cross-contamination by the other two reviewers. The clinical features of and outcomes for these patients are listed in table 1. Antituberculosis therapy for 6–12 months was prescribed for six of the nine patients. Therapy for one patient was stopped because of hepatotoxicity. Two patients died of other causes before the results of the positive cultures were known; these patients did not receive therapy for tuberculosis. In each of the nine cases, a potential source isolate for contami-
Table 1. Clinical and laboratory presentation of patients whose Mycobacterium tuberculosis cultures were considered as “possibly” or “likely” due to laboratory cross-contamination.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>P</th>
<th>L</th>
<th>Age (y)/race, sex</th>
<th>Signs and/or symptom(s)</th>
<th>Chest radiograph finding(s)</th>
<th>Treatment</th>
<th>Underlying condition(s)</th>
<th>Outcome</th>
<th>Specimens obtained*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>3</td>
<td>23/black, M</td>
<td>Fever, cough</td>
<td>Normal</td>
<td>Empirical anti-TB therapy that was stopped at 2 mo because of hepatotoxicity</td>
<td>AIDS</td>
<td>No evidence of TB after 2 y</td>
<td>3 sputa</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>3</td>
<td>61/white, M</td>
<td>1-mo history of cough, congestion</td>
<td>Stable calcified scarring</td>
<td>12-mo course of anti-TB therapy after culture result obtained</td>
<td>Prior genitourinary TB</td>
<td>Alive</td>
<td>6 sputa</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>3</td>
<td>66/white, F</td>
<td>Cough</td>
<td>Infiltrate</td>
<td>Treatment for acute bacterial pneumonia and then 9-mo course of anti-TB treatment after culture result obtained</td>
<td>TST positive in 1972</td>
<td>Condition improved after therapy for acute bacterial pneumonia</td>
<td>14 sputa</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>3</td>
<td>29/Hispanic, M</td>
<td>Wheezing, cough</td>
<td>Infiltrate</td>
<td>9-mo course of anti-TB therapy delayed by 4 mo in which time symptoms had abated</td>
<td>Prior TB in 1990</td>
<td>Initially lost to follow-up, which delayed therapy; alive</td>
<td>3 sputa</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>3</td>
<td>72/white, M</td>
<td>Fever, cough, chest pain for 3 mo</td>
<td>Pleural effusion, pleural thickening</td>
<td>6-mo course of anti-TB therapy started after 3 mo at the time of positive culture result obtained</td>
<td>None</td>
<td>Fibrothorax requiring decortication; alive</td>
<td>1 bronchial wash, 1 pleural biopsy, 6 sputa</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>2</td>
<td>77/white, M</td>
<td>Cough, fever, dyspnea for 6 w</td>
<td>Progressive infiltrates, ARDS</td>
<td>No response to broad-spectrum antibiotics, steroids</td>
<td>COPD and Mycobacterium avium complex infection in 1977</td>
<td>Died 3 w after admission; no autopsy</td>
<td>1 transbronchial biopsy, 1 bronchial wash</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>2</td>
<td>76/white, M</td>
<td>Cough for 2 mo, episode of hemoptysis</td>
<td>Left hilar fullness, peripheral nodule with surrounding infiltration</td>
<td>None</td>
<td>Prior TB in 1985, lung cancer</td>
<td>Died 3 d after admission; no autopsy</td>
<td>1 bronchial wash</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>2</td>
<td>44/black, M</td>
<td>Asymptomatic</td>
<td>Normal</td>
<td>6-mo course of anti-TB therapy started after culture result obtained</td>
<td>Conversion of TST to positive; patient was a school bus driver</td>
<td>Alive</td>
<td>8 sputa</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>0</td>
<td>44/white, F</td>
<td>Asymptomatic</td>
<td>Left lower lobe infiltrate that was present 6 mo earlier</td>
<td>Treated 6 mo before with erythromycin for bacterial pneumonia; 6-mo course of anti-TB therapy started after culture result obtained</td>
<td>Conversion of TST to positive; patient contact with a household member with TB</td>
<td>Alive</td>
<td>6 sputa</td>
</tr>
</tbody>
</table>

NOTE. ARDS = adult respiratory distress syndrome; COPD = chronic obstructive pulmonary disease; L = no. of reviewers indicating that the positive culture was likely due to cross-contamination; P = no. of reviewers indicating that the positive culture was possibly due to cross-contamination; TB = tuberculosis; TST = tuberculin skin test.

* Does not include specimens obtained after 2 months of anti-TB therapy.

nation was identified, which had a matching DNA fingerprint pattern.

Patients 1–7 had no epidemiological link with the patient from whom the potential source specimen was obtained. In contrast, for patients 8 and 9, an epidemiological link was identified. Patient 8 was asymptomatic after conversion of the tuberculin skin test (TST) to positive, and the chest radiograph for this patient was unremarkable. The indication for obtaining sputum samples is unclear from the history, although this patient was a school bus driver and the care provider may have been aggressive in the evaluation for tuberculosis because of the potential exposure of children. The patient lived in the same town as the patient from whom the potential source specimen was obtained, and the two coached Little League together. Thus, there is epidemiological evidence of contact and possibly M. tuberculosis transmission between them. However, the care provider questioned the diagnosis of active tuberculosis, and the patient’s only positive culture occurred when specimens from both patients were processed together.

Conversion of the TST to positive (from a negative result 2 years previously to a 20-mm induration) for patient 9 was identified during the contact investigation concerning a household member for whom smear-positive and culture-positive tuberculosis had been diagnosed. At the time of clinical evaluation for TST conversion, patient 9 was asymptomatic, but a small left lower lobe infiltrate was evident by chest radiograph.
Smears of all of the six sputum specimens obtained within 2 months of the start of antituberculosis therapy were negative, and culture of only one of these specimens was positive for M. tuberculosis; this specimen was processed in the laboratory on the same day that an AFB smear-positive and culture-positive specimen from the household member with tuberculosis was processed.

Review of her prior chest radiographs and clinical history revealed an episode of acute pneumonia 6 months earlier in which a similar infiltrate in the same location on the chest radiograph occurred; she was treated with macrolide antibiotics and recovered clinically. No follow-up radiograph was obtained at that time. Follow-up chest radiographs after the beginning of treatment for tuberculosis showed a decreased but not resolved infiltrate by the end of treatment. Of the nine cases, this was the only one in which all reviewers considered laboratory cross-contamination as possible rather than likely (table 1).

Information regarding the number of colonies of M. tuberculosis observed in cultures with solid media was available for 10 of the 13 patients whose cultures were considered unlikely to be due to cross-contamination (1 colony, 3 patients; 4 colonies, 3 patients; and 6–17 colonies, 4 patients). Similar information was available for five of the nine patients whose cultures were considered as possibly or likely due to cross-contamination (1 colony, 3 patients; 2 colonies, 1 patient; and 4 colonies, 1 patient). Thus, cultures of specimens that yielded five or more colonies fell into the group considered as unlikely due to cross-contamination, but cultures with fewer than five colonies fell into the group considered unlikely or the group considered possibly or likely due to cross-contamination.

Discussion

DNA fingerprinting can provide strong evidence for the identification of cross-contaminated M. tuberculosis cultures [11]. Small and colleagues [16], who used criteria similar to ours, found that M. tuberculosis cultures for 1.8% of patients in their study were false-positive. Alland and colleagues [17], who used DNA fingerprinting to study isolates from a New York City hospital, concluded that cultures for 2.6% of patients were false-positive. Aber et al. [18] intermixed autoclaved sputum samples with simulated specimens containing a strain of M. tuberculosis with an unusual drug resistance pattern. Of cultures of the autoclaved specimens, 0.4%–1.3% yielded M. tuberculosis with the unusual drug resistance pattern characteristic of the isolates with which they were processed. Clerical errors accounted for an additional 1% of culture-negative specimens that were reported as positive.

In our study, we identified 22 patients (8.6%) whose M. tuberculosis cultures were potentially cross-contaminated; cultures for nine patients (3.5%) were judged as false-positive. This percent is a minimum estimate of the proportion of patients whose culture-positive specimens were due to laboratory cross-contamination. The subset of patients included were those from whom M. tuberculosis isolates with DNA fingerprint patterns that matched those of one or more other patients’ isolates. It was assumed that the recovery of M. tuberculosis isolates with unique DNA fingerprints would not be the result of laboratory cross-contamination. Clinical and laboratory information was not obtained for these patients. However, 28% of isolates from patients whose cultures were positive were not available for DNA fingerprinting; it is possible that some of the isolates for which DNA fingerprinting was done and deemed unique could have matched other isolates not available for DNA fingerprinting, and these isolates may have been the result of cross-contamination.

In fact, for five cases in which cultures were deemed as unlikely due to cross-contamination in this study, there were other culture-positive specimens processed concurrently that did not undergo DNA fingerprinting (either the culture was not available or the fingerprint could not be produced because of technical reasons). It is possible that if a potential source of contamination was identified for these cases, the judgment concerning the likelihood of cross-contamination may have been different.

Several investigators maintain that positive cultures due to cross-contamination are more likely to have few colonies (less than five) when solid medium is used [9, 19, 20]. In this study, although a colony number of five or more was discriminatory for those cultures considered as unlikely due to cross-contamination compared with those considered as likely or possibly due to cross-contamination, a lower colony number (less than five) was not discriminatory for the two groups.

The primary prevention of laboratory cross-contamination depends on laboratories adopting standardized procedures that minimize its occurrence, as has been thoroughly outlined in the study by Small and colleagues [11]. Our study included cultures from many laboratories that were processed as much as 3 years before the time when the contamination was first suspected. Thus, we were not able to determine the specific causes for the cross-contamination in any laboratory. The three laboratories where these episodes of contamination occurred had not established surveillance for or methods to assess potentially cross-contaminated cultures. However, they were certified by the College of American Pathologists and/or met the standards of the Clinical Laboratory Improvement Act of 1988. All three laboratories followed the methodology for sputum processing set out by the Centers for Disease Control and Prevention [21].

This study demonstrates a significant rate of false-positive M. tuberculosis cultures that may be generalized to clinical mycobacteriology laboratories in other areas of the United States. Health care providers should consider the possibility of a false-positive culture when caring for patients whose clinical course is inconsistent with or questionable for tuberculosis. The findings in this study suggest that clinical situations associated with false-positive M. tuberculosis cultures include the following: the evaluation of other diagnoses (including the
evaluation of adult respiratory distress syndrome or lung mass by culture of bronchoscopy specimens; the evaluation of asymptomatic patients whose abnormal chest radiographs are identified in tuberculosis contact investigations; the evaluation of pulmonary symptoms in patients with AIDS; and the evaluation of patients who present with signs or symptoms consistent with tuberculosis but whose conditions improve with therapy for other bacterial illnesses or fail to improve with appropriate antituberculous therapy.

Laboratory findings include a single culture positive for *M. tuberculosis* and no smears of specimens positive for AFB, even when only one specimen is obtained. Additional evidence of cross-contamination of a single *M. tuberculosis*—positive culture may be obtained by reviewing the laboratory processing log to identify another culture-positive specimen (a potential source of contamination) processed on the same day and preceding the culture in question. DNA fingerprint analysis of isolates from both the potential source and contaminated cultures can determine if the cultures share the same *M. tuberculosis* strain.

DNA fingerprint analysis of *M. tuberculosis* isolates is generally available and may be arranged through tuberculosis control programs or mycobacteriology laboratories at state departments of health. Regional *M. tuberculosis* DNA fingerprinting laboratories of the Centers for Disease Control and Prevention can usually provide results and interpretation of DNA fingerprint analysis within 4 weeks. If a potential source isolate is identified, investigation is required to determine that the patients were not part of a chain of *M. tuberculosis* transmission (including nosocomial transmission), in which case the patients would be expected to share the same strain.

Mycobacteriology laboratories and tuberculosis control programs should also develop surveillance for patients for whom smears of specimens are negative for AFB and a single culture is positive and for unusual clustering of positive cultures. Identification of suspect cultures should trigger an investigation of possible laboratory cross-contamination on the basis of the evidence outlined above. Other laboratory evidence may be helpful in the investigation, such as antimicrobial drug resistance patterns, the amount and rapidity of growth in cultures, and possibly colony counts. If the laboratory methods include parallel culturing by two techniques (i.e., those using solid and liquid media), then it can be determined if the results from the two culture methods are discordant.

With the advent of DNA fingerprinting, health care providers, state tuberculosis control programs, and mycobacteriology laboratories should work in concert to identify patients whose *M. tuberculosis* cultures are cross-contaminated. Specifically, laboratories should report suspicious positive cultures or clusters of positive cultures to the local health department and the responsible physician(s). In addition, physicians should notify the laboratory and health department when caring for a patient whose clinical course is questionable for tuberculosis but for whom a culture is reported positive for *M. tuberculosis*.

The identification of cross-contaminated cultures has several important consequences. Most importantly, an erroneous diagnosis and needless therapy can be avoided. In addition, the laboratories may discover equipment or procedural errors leading to false-positive cultures, and if corrected, the same problem may be minimized in the future. As a result of such efforts, tuberculosis prevention and control programs can avoid unnecessary source and contact investigations, can save the cost of incentives that aid in patient adherence with treatment and the cost of directly observed therapy, and can reduce or eliminate the use of unnecessary drugs. Finally, patients for whom tuberculosis is erroneously diagnosed due to a cross-contaminated culture would not be counted in local and national surveillance for tuberculosis.

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