Investigation of Laboratory Cross-Contamination of Mycobacterium tuberculosis Cultures

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Many clinicians and laboratory personnel are unaware that a culture positive for Mycobacterium tuberculosis may represent contamination. Laboratory cross-contamination with the M. tuberculosis laboratory control strain (H37Ra) occurs infrequently and therefore demands heightened awareness and recognition. We report 3 occurrences of laboratory cross-contamination from the same laboratory. These occurrences illustrate the importance of interpreting laboratory results in conjunction with the patient’s clinical presentation. Failure to recognize laboratory cross-contamination with M. tuberculosis leads to both erroneous administration of unnecessary medications and expenditure of resources required to conduct contact investigations.

In July 1998, a hospital in Maryland contacted the Maryland Department of Health, who contacted the Centers for Disease Control and Prevention (CDC; Atlanta) Division of Tuberculosis Elimination (DTBE) to report suspected laboratory cross-contamination with Mycobacterium tuberculosis cultures involving 3 specimens. The specimens included a breast biopsy specimen, a skin biopsy specimen, and a sputum sample. Clinical findings for the 3 patients from whom the specimens were obtained were not consistent with active tuberculosis (TB). DNA fingerprinting by IS6110 restriction fragment–length polymorphism (RFLP) analysis revealed identical fingerprint patterns in all 3 specimens. The strain of M. tuberculosis found in these 3 specimens matched the laboratory’s avirulent positive control strain, H37Ra. To date, there are no known instances in which H37Ra has been reported to cause TB disease in humans.

**Methods.** All patient clinical records were reviewed and telephone interviews were conducted with 2 of the 3 patients from whom the M. tuberculosis isolates were obtained. The remaining patient was not available for interview.

The mycobacteriology laboratory was assessed and laboratory technicians were interviewed to obtain information about specimen handling and processing. Records and logs for the mycobacteriology laboratory were reviewed. Specimen processing was observed in the mycobacteriology laboratory.

**Results.** Patient 1, a 54-year-old woman with infiltrating ductal carcinoma of the breast that had been diagnosed in October 1997, underwent excision of a recurring breast cyst in April 1998. One of 3 specimens from this lesion was acid-fast bacillus (AFB) smear 4+ positive and culture positive for M. tuberculosis. The patient had no signs or symptoms of TB or wound infection and had no known contact with an active case of TB. Her tuberculin skin test (TST) result was negative, and her chest radiograph was normal. Antituberculous therapy was initiated on the basis of the positive smear and culture results and was discontinued after 2 months.

Patient 2, a 50-year-old man with an erythematous, macular rash on a lower extremity, underwent 2 punch biopsies of this skin lesion in April 1998. One of the biopsy specimens was AFB smear–negative and culture-positive for M. tuberculosis, although the findings of histologic examination were not consistent with TB. The patient’s lesion resolved spontaneously without further medical therapy. No antituberculous medications were given, and the patient had no symptoms suggestive of TB.

Patient 3, a 38-year-old South American woman who was employed at the hospital’s day care center, experienced a sudden onset of fever, rigors, and productive cough in April 1998. Findings of a clinical examination were consistent with community-acquired pneumonia, and she was admitted for intravenous antibiotic therapy. Bacterial cultures were not performed. The patient denied any recent exposure to a case of active TB but reported taking isoniazid for 6 months after a positive TST result (induration unknown) in 1986. Three sputum specimens were AFB smear negative; the patient experienced clinical improvement while receiving antibacterial ther-
apy and was discharged. Three weeks later, the patient was informed that 1 of 3 cultures was positive for *M. tuberculosis*.

The patient had no clinical signs of TB, but 3 additional sputum specimens were obtained and all were AFB smear–negative and culture-negative for *M. tuberculosis*. A clinical decision was made to treat the patient with 6 months of antituberculous therapy. In addition, a screening for latent TB infection was conducted at the nursery where this patient worked and, subsequently, isoniazid prophylaxis was initiated for 60 day care workers and 103 infants and children attending the center. Only 6 persons (3 foreign-born staff and 3 children) had positive TST results.

The hospital mycobacteriology laboratory processed 0–4 specimens per day and identified an average of 4 cases of *M. tuberculosis* per year. Laboratory staff used the control strain, H37Ra, at least once per week during biochemical and susceptibility testing of isolates from culture. A conventional laminar air-flow safety cabinet where all mycobacterial specimens were processed was evaluated and certified for use in February 1998. There was no routine sanitizing schedule for the cabinet. Inside the small cabinet there were numerous items used for specimen processing, producing a crowded work space. The safety cabinet was sanitized prior to the investigation and, consequently, environmental samples for culture were not obtained. The techniques used to culture *M. tuberculosis* involved use of individual aliquots of reagents for each specimen.

Culture-positive specimens from patients 1, 2, and 3 were processed in the mycobacteriology laboratory on different days by 3 different laboratory technicians during a period of 17 days. A total of 99 other specimens from patients were processed during this 17-day period, and none of these was culture-positive for *M. tuberculosis*. The control strain, H37Ra, was not handled on any of the days that specimens from patients 1, 2, and 3 were processed.

**Discussion.** This investigation highlights the need for heightened awareness among health care providers and laboratory personnel of the potential for laboratory cross-contamination with *M. tuberculosis*. This phenomenon has been well described in the literature and in several studies was found to occur in ∼4% of reported cases from laboratories that test specimens for the presence of mycobacterial species [1–4]. In addition, H37Ra has been implicated in laboratory cross-contamination in a recent study involving 13 reported cases of TB [5].

Laboratory cross-contamination should be suspected when an inordinately high number of *M. tuberculosis*–positive cultures are observed, relative to previous time periods [3]. The laboratory at this hospital generally identified 4 cases of TB per calendar year. Because 3 specimens were positive for *M. tuberculosis* within 2 months, suspicion of cross-contamination was heightened.

Laboratory cross-contamination should also be suspected when clinical scenarios are inconsistent with the diagnosis of TB. Two of the 3 patients in this investigation (patients 1 and 2) had no evidence of clinical TB. For patient 3, the validity of the positive culture result for 1 sputum specimen remained somewhat unclear; she had a respiratory illness that was consistent with community-acquired pneumonia but also consistent with an atypical presentation of TB. The match between a single *M. tuberculosis* isolate and the control strain, in conjunction with the lack of deterioration in the patients’ clinical condition during the 6-week interval between her illness and initiation of TB treatment, suggest that she did not have TB. The results of the TST screening at the day care center did not clarify whether patient 3 had active TB, because TST results in the 2 US-born children may have represented false-positive reactions to the PPD used for testing.

The usual mechanisms of cross-contamination with *M. tuberculosis* include technician error, reagent contamination, or equipment failure, such as malfunction of a Bactec machine, as has been described elsewhere [6, 7]. Interviews with personnel in the mycobacteriology laboratory and observation of the techniques for specimen processing did not yield an obvious mode of *M. tuberculosis* cross-contamination. Since the positive control was handled in the same area as patient isolates, an inadvertent spillage inside the safety cabinet could have provided an opportunity for cross-contamination. The cabinet was not routinely cleaned, and it is probable that crowding of equipment inside the cabinet made it impossible for germicidal UV radiation to thoroughly sanitize the area. If contamination of the cabinet work area had occurred at some point in time, aerosolization of organisms inside the safety cabinet could have produced cross-contamination of specimens. This hypothesis could not be explored further in this investigation, because the safety cabinet was sanitized before environmental samples could be obtained for culture.

The use of molecular testing techniques, such as DNA fingerprinting or RFLP, was invaluable in this investigation. Laboratories that infrequently process or identify cultures positive for *M. tuberculosis* may experience difficulty maintaining proficiency in testing for this organism. Given this, the utilization of RFLP techniques can be useful in the timely identification of false-positive cultures, and thereby can prevent the unnecessary expenditure of financial and personnel resources [4].

This report illustrates the extensive expenditure of hospital and public health resources associated with cross-contamination of *M. tuberculosis* cultures. Patients and other persons considered exposed to *M. tuberculosis* received unnecessary tests and treatment, and hospital staff and public health workers conducted extensive investigations. Recognition of the potential for laboratory cross-contamination with *M. tuberculosis* by health professionals and laboratory personnel helps prevent...
erroneous TB diagnoses and unneeded treatment for active TB. In addition, futile contact investigations and unnecessary administration of treatment for latent TB infection can be avoided.

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References


