A Prospective, Multicenter Study of Laboratory Cross-Contamination of Mycobacterium tuberculosis Cultures

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A prospective study of false-positive cultures of Mycobacterium tuberculosis that resulted from laboratory cross-contamination was conducted at three laboratories in California. Laboratory cross-contamination accounted for 2% of the positive cultures. Cross-contamination should be a concern when an isolate matches the genotype of another sample processed during the same period.

Cultures remain the reference standard for diagnosis of disease caused by Mycobacterium tuberculosis. However, false-positive results can be caused by cross-contamination of cultures in the laboratory, e.g., when M. tuberculosis bacilli are transferred from one specimen to another specimen that does not contain viable bacilli (1–10). Historically, determining whether false-positive results are caused by laboratory cross-contamination has been difficult because of the lack of specific strain identification and nonsystematic criteria. False-positive cultures for M. tuberculosis have important implications for clinical management of patients. Many patients are treated on the basis of the results; therefore, patients can be exposed to unnecessary, potentially toxic, and costly treatment. Genotyping of M. tuberculosis strains has become the standard method for determining whether isolates are clonal (11–14). This technique, in combination with a review of clinical and radiographic data, allows a determination of the incidence of laboratory cross-contamination of M. tuberculosis cultures. In this study, we used predefined criteria to investigate possible laboratory cross-contamination of M. tuberculosis cultures and prospectively determine its incidence in an effort to find methods to decrease the occurrence of cross-contamination.

Methods

Study Laboratories and Patients

This study was conducted by staff of the Microbial Diseases Laboratory, California Department of Health Services, Berkeley, California, which is one of seven participants in the National Tuberculosis Genotyping and Surveillance Network of the Centers for Disease Control and Prevention. Participating California laboratories included those at the San Francisco General Hospital, Santa Clara Valley Medical Center (San Jose), and Solano County Public Health Laboratory (Vallejo). The study was conducted from January 1, 1998, to June 30, 1999.

Laboratory Methods

M. tuberculosis isolates from all sources underwent IS6110-based DNA restriction fragment length polymorphism (RFLP) analysis (11) if they were 1) the patient’s first M. tuberculosis–positive culture derived from a sample cultured in the participating laboratory; 2) cultured from a specimen collected >30 days after an M. tuberculosis culture–negative specimen was obtained; or 3) cultured from a specimen collected >90 days after the start of appropriate anti-tuberculosis (TB) therapy. When five or fewer bands were present, the isolates underwent secondary genotyping with a RFLP analysis based on a polymorphic GC-rich sequence (12–14).

RFLP pattern images were entered into a database and compared to identify isolates with matching genotypes. Any of the following cultures were considered potentially cross-contaminated and underwent further investigation: 1) the first M. tuberculosis–positive culture for a patient whose isolate had a genotype that matched that of another isolate cultured or used in the participating laboratory 2 days before or after the potentially cross-contaminated culture; 2) an M. tuberculosis culture from a specimen obtained >30 days after the collection of an M. tuberculosis culture–negative specimen was obtained; or 3) cultured from a specimen collected >90 days after the start of appropriate anti-tuberculosis (TB) therapy. When five or fewer bands were present, the isolates underwent secondary genotyping with a RFLP analysis based on a polymorphic GC-rich sequence (12–14).

RFLP pattern images were entered into a database and compared to identify isolates with matching genotypes. Any of the following cultures were considered potentially cross-contaminated and underwent further investigation: 1) the first M. tuberculosis–positive culture for a patient whose isolate had a genotype that matched that of another isolate cultured or used in the participating laboratory 2 days before or after the potentially cross-contaminated culture; 2) an M. tuberculosis culture from a specimen obtained >30 days after the collection of an M. tuberculosis culture–negative specimen that had an isolate with a genotype different from that of any previous isolate from the same patient; or 3) an M. tuberculosis culture, from a specimen collected >90 days after the start of appropriate anti-TB therapy, in which the isolate had a genotype different from
that of any previous isolate from the same patient.

Patients with specimens meeting the above criteria and for whom potential source isolates were identified (i.e., their isolates had a genotype that matched that of a potential source isolate) underwent further investigation. The investigation included a review of all clinical data and radiologic studies, if applicable, and possible epidemiologic connections between patients with potential source and contaminated specimens. Clinical data included prior history of TB, results of tuberculin skin tests, treatment of latent TB infection, symptoms of present illness, results of diagnostic evaluations for TB, and alternative diagnoses. Personnel in laboratories where the present illness, results of diagnostic evaluations for TB, and applicable, and possible epidemiologic connections between patients (involving Patient 4) probably resulted from a malfunctioning broth-culturing system (BACTEC 460) (Becton Dickinson Microbiology Systems, Sparks MD). Patient 4’s specimen was in the BACTEC instrument immediately after another patient’s specimen (not processed on the same day), and genotypes of the two isolates matched. Cultures from two patients (Patients 7 and 9) were cross-contaminated from the same source patient during sample processing. All six of the laboratory cross-contamination incidents occurred with the initial rather than follow-up specimens for mycobacterial culture. Five of these six patients were treated for TB. Of the remaining four patients whose isolates were suspected of being cross-contaminated in the laboratory, one had a false-positive culture attributed to specimen mislabeling by a healthcare provider; one had either a mislabeled specimen or mixed infection; one had active TB; and one had either a mislabeled specimen or a cross-contaminated specimen (Table). Having only a single positive culture was highly associated with laboratory cross-contamination ($p<0.001$, Fisher exact test).

**Discussion**

Laboratory cross-contamination was the cause for a positive culture result in 2% of all patients with *M. tuberculosis*–positive cultures. Cross-contamination accounted for one fifth (22%) of patients having only one culture positive for *M. tuberculosis*. Of the six patients who had cross-contaminated cultures, five were treated unnecessarily with multiple anti-TB medications.

The rate of cross-contaminated cultures in our study is similar to the rates in previous studies of *M. tuberculosis* cultures. Most population-based studies found rates of 0.9% to 3.5% (1–8). However, such studies were retrospective and did not assess the extent of the problem in different types of clinical mycobacteriology laboratories. In this study, we used predefined criteria, which were based largely on DNA genotyping, to identify suspected cases of laboratory cross-contamination prospectively in an effort to correct factors associated with its occurrence. Our study included all clinical specimens submitted during a 1.5-year period to one county public health laboratory and two county hospital laboratories. This study was possible because of the large databank of RFLP results conducted as part of being a member of the genotyping network.

Multiple factors can cause false-positive cultures, including contaminated clinical equipment (e.g., bronchoscope), clerical errors, and cross-contamination that occurs in the laboratory. The last category can be caused by batch processing, transfer of viable bacilli from the sample needle of a broth-culturing system, e.g., BACTEC (15), a faulty exhaust hood (4), and contamination from species identification procedures such as the niacin production test (6).

Five of the six cross-contamination incidents were in a single laboratory. In four of these five cases, contamination probably occurred when reagents were dispensed with a common flask. Previous studies have reported that the step of adding the phosphate buffer was likely to have been the source of the cross-contamination (1,6,9). This procedure was later discontinued on the basis of the results of this study. None of the laboratories used positive control cultures.
As in other studies, we found that a single positive culture for *M. tuberculosis* was a sensitive but nonspecific marker for detection of a false-positive culture, since most patients (78%) with a single positive culture had TB. In a New York study (7), 12 (44.4%) of 27 patients with a single positive culture had a false-positive culture. These findings suggest that clinicians and laboratorians should be increasingly suspicious of a single false-positive culture. Additional specimens should be collected in cases of a single false-positive culture and the patient evaluated carefully for TB and other illnesses; the laboratory
should also retain the isolate and others processed that day for genotyping. Because all specimens that met the inclusion criteria in our study were from the respiratory tract, we cannot draw any conclusions about the rate of cross-contamination of nonrespiratory specimens (e.g., cerebrospinal or pleural fluid). Nor can we draw any conclusions about a single positive culture when only a single specimen is submitted to the laboratory, as is often the case with nonrespiratory specimens.

Clinical judgment is also important in raising suspicion about cross-contamination. TB classically is accompanied by symptoms of prolonged cough, fever, weight loss, and night sweats, but other diseases such as bacterial pneumonia can cause these symptoms. Therefore, no specific clinical criterion alone can be used to definitively state that TB is present. However, an inconsistent clinical course or absence of symptoms should certainly raise suspicion that cross-contamination may have occurred (3,8,16). A determination regarding the presence of cross-contamination requires a thorough evaluation of a patient’s symptoms and clinical course as well as laboratory evaluation, with additional specimens obtained if only a single culture is positive as described above. Genotyping should be performed if cross-contamination is suspected on the basis of an inconsistent clinical course or the presence of only one positive culture (8).

Our assessment of the rate of cross-contamination did not include private laboratories; thus, our results may not be underestimated the true rate of laboratory cross-contamination. In addition, our methods depended on identifying, obtaining, and genotyping an isolate from a positive source culture; thus, we may have overestimated the rate of laboratory cross-contamination.

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