False-Positive Cultures of *Mycobacterium tuberculosis*

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During a single week in April 1982, cultures for *Mycobacterium tuberculosis* were reported positive from nine patients who did not appear clinically to have active infection. Each of the patients had only one positive culture out of multiple specimens cultured. At the time of investigation, five specimens were available and were found to be all of the same phage type which strongly suggested cross-contamination. Four patients received antituberculosis chemotherapy. In one year of follow-up of the five who did not receive chemotherapy, none developed clinical disease. The contamination was probably due to faulty laboratory technique, but the source of the contaminant is uncertain. This investigation suggests that patients without clinical evidence of active infection and with isolated positive cultures for *Mycobacterium tuberculosis* should be carefully evaluated before they are subjected to a prolonged, potentially toxic, and expensive course of chemotherapy.

A positive culture of *Mycobacterium tuberculosis* from any type of specimen is considered by most physicians as diagnostic of active infection. Frequently, the report of a single positive culture, even with several other negative cultures, is routinely used as the basis for beginning a prolonged course of multidrug chemotherapy.

Though the possibility of false-positive smears for acid-fast bacilli (AFB) is well recognized, rarely does the physician consider that a positive culture might be due to laboratory error, contamination, or inaccurate reporting. Reports of false-positive cultures are rare; however, the absence of well defined markers in *M tuberculosis* cultures has hindered the investigation of suspected laboratory cross-contamination incidents and epidemics in past studies. The development of practical methods to phage type *M tuberculosis* cultures has produced a sensitive tool for use as an adjunct in epidemiologic investigations. We recently uncovered a series of false-positive cultures that probably resulted from a faulty laboratory technique. The index case that raised our suspicion was admitted to the pulmonary ward with a four-day history of cough, sputum production, and right-sided pleuritic chest pain. Chest roentgenography revealed a right-middle-lobe infiltrate. A regimen of penicillin was instituted for a presumed pneumococcal pneumonia. Despite the acute history, sputum specimens were submitted for AFB smear and culture. Several days after admission, the patient underwent fiberoptic bronchoscopy to rule out bronchial obstruction as the cause of the pneumonia. No obstruction was found; wash specimens were submitted for AFB studies. Six sputum specimens and one wash specimen were cultured. All specimens were smear negative. The patient's infiltrate cleared, and he improved clinically after receiving penicillin therapy. Subsequently, only the culture of the admission sputum was positive for *M tuberculosis*.

Within the next two weeks, it was noted by tuberculosis control personnel that eight other patients in whom active tuberculosis was an unlikely diagnosis also had positive cultures. This prompted an investigation of the culturing techniques in our laboratory and a review of the patients involved.

**Materials and Methods**

**Clinical Review**

We reviewed the charts of all patients who had had cultures reported positive for *M tuberculosis* between July 1981 and July 1982. In addition, to evaluate the possibility of an ongoing technical error, we began to monitor positive cultures which were inoculated between August 1982 and January 1983. The monitoring involved review of the clinical status, roentgenograms, and charts of all patients who had positive *M tuberculosis* cultures during this six-month period.

We evaluated in detail all patients who, between July 1981 and July 1982, had had one of multiple specimens culture positive for *M tuberculosis*. Culture-positive specimens included urine, sputum, and bronchial wash. The clinical status, history, relevant roentgenographic findings, and results of tuberculin tests with five tuberculin units of purified protein derivative (PPD) were reviewed for each patient. Patients with single positive cultures from a pulmonary specimen were followed in our medical chest clinic by culturing additional sputum specimens, repeating chest roentgenograms, and monitoring clinical status. Patients with positive urine cultures were followed-up in the urology clinic.

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Laboratory Review

Specimens collected for culture of AFB are processed in a special section of the microbiology laboratory at the Bronx Veterans Administration Medical Center. During the period July 1, 1981, to April 28, 1982, an adequate venting hood was not available in the new hospital, so laboratory personnel transported all specimens and culturing materials several miles downtown to another medical center where appropriate venting equipment was available for digestion and inoculation of samples. Before this time and after discovery of the false-positive cultures, reagents were dispensed from small containers; however, to facilitate the transportation of materials to the location, reagents were carried in and dispensed from single large containers (see Discussion).

Culturing was done according to the following method as outlined in the 1969 Public Health Service Manual, "Procedures for Isolation and Identification of Mycobacteria."[5]

Ten milliliters of sputum or urine sediment was poured into a 50-ml plastic centrifuge tube, and the edge of the tube flamed. Ten milliliters of a solution made up of equal amounts of 4 percent sodium hydroxide and 2.9 percent sodium citrate and 1 g N-acetylcysteine was added. This mixture was vortex-mixed until digested (up to three minutes) and allowed to stand 15 minutes at room temperature to decontaminate. A quantity of phosphate buffer (pH 6.8) equal to twice the volume of digested specimen was added.

The resulting sample was centrifuged for 15 minutes at approximately 2,000 × g, the supernatant fluid was poured into a splashproof container under a hood, and the lip of the centrifuge tube was flamed. One to 2 ml of 0.2 percent bovine albumin was added to the sediment which was resuspended with a pipette. After 15 minutes, one slant each of American Thoracic Society and Lowenstein-Jensen media was inoculated with a pipette or swab, and a smear was made for staining by the Ziehl-Neelsen procedure.

 Cultures from five of the patients who had had single positive cultures and who were not felt to have tuberculosis on the basis of clinical findings were available at the time this investigation was undertaken. Phage typing of these isolates was performed using the soft-agar overlay methods described earlier. The phage type designations of the M tuberculosis cultures were as outlined previously.[6]

RESULTS

Between July 1981 and July 1982, 33 patients in our hospital had cultures positive for M tuberculosis. These patients could be subdivided into three groups. The first group included 14 patients who had multiple positive cultures; the second group included five patients who had only one positive culture out of multiple specimens examined but had clinical and roentgenographic findings compatible with active tuberculous infection. The final group of 14 patients also had only one of multiple specimens positive for M tuberculosis, but all appeared clinically well and none had roentgenographic evidence of active tuberculosis.

Our attention was directed to the third group. The culture-positive specimens from nine patients in this group were inoculated between April 2, 1982, and April 7, 1982. Four of the specimens were urine, four were sputum, and one was bronchial wash material (Table 1). The positive urine specimens represented two-thirds of all positive urine cultures for M tuberculosis reported from our laboratory in the year that was reviewed. No known positive specimen was cultured in proximity to these cultures except for positive control cultures inoculated March 29, 1982, and April 5, 1982. Further characteristics of these nine patients are summarized in Table 1. In 12 months of follow-up, none developed clinical or laboratory evi-

Table 1—Characteristics of a Cluster of Patients with Single Positive Cultures of M Tuberculosis

<table>
<thead>
<tr>
<th>Patient</th>
<th>Specimen</th>
<th>Date Cultured</th>
<th>Negative Cultures</th>
<th>PPD</th>
<th>Chest Roentgenogram</th>
<th>Reason Specimen Sent</th>
<th>History—Tuberculosis</th>
<th>Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sputum</td>
<td>4-2-82</td>
<td>6*</td>
<td>+</td>
<td>Lobar infiltrates§</td>
<td>Abnormal chest roentgenogram</td>
<td>No</td>
<td>Penicillin</td>
</tr>
<tr>
<td>2</td>
<td>Bronchial wash</td>
<td>4-2-82</td>
<td>7†</td>
<td>+</td>
<td>Solitary nodule (carcinoma)</td>
<td>Routine bronchoscopy specimen</td>
<td>No</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>Sputum</td>
<td>4-2-82</td>
<td>4</td>
<td>+</td>
<td>Normal chest roentgenogram</td>
<td>(?) Hemaoptysis</td>
<td>No</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
<td>Sputum</td>
<td>4-5-82</td>
<td>3</td>
<td>+</td>
<td>Lobar infiltrate§</td>
<td>Abnormal chest roentgenogram</td>
<td>Hemaoptysis</td>
<td>Penicillin</td>
</tr>
<tr>
<td>5</td>
<td>Sputum</td>
<td>4-6-82</td>
<td>5*</td>
<td>Not recorded</td>
<td>(?) Upper lobe scar</td>
<td>Hemaoptysis</td>
<td>No</td>
<td>None</td>
</tr>
<tr>
<td>6</td>
<td>Urine</td>
<td>4-6-82</td>
<td>25</td>
<td>+</td>
<td>Intravenous pyelogram abnormal—stable since 4-81</td>
<td>Under therapy for renal tuberculosis</td>
<td>Yes: renal, 1957 Yes, for one year at time of culture</td>
<td>Penicillin; isoniazid, rifampin, ethambutol 6-5-82</td>
</tr>
<tr>
<td>7</td>
<td>Urine</td>
<td>4-7-82</td>
<td>12†</td>
<td>+</td>
<td>Lobar infiltrate§ Intravenous pyelogram normal</td>
<td>Abnormal chest roentgenogram</td>
<td>No</td>
<td>Isoniazid, rifampin, ethambutol 5-27-82</td>
</tr>
<tr>
<td>8</td>
<td>Urine</td>
<td>4-7-82</td>
<td>8</td>
<td>+</td>
<td>Normal chest roentgenogram &amp; intravenous pyelogram</td>
<td>Microhematuria</td>
<td>No</td>
<td>Isoniazid, rifampin, ethambutol 4-82; ceftazolin</td>
</tr>
<tr>
<td>9</td>
<td>Urine</td>
<td>4-7-82</td>
<td>24</td>
<td>-</td>
<td>Normal chest roentgenogram; intravenous pyelogram; small left kidney</td>
<td>Scrotal abscess; Also grew Escherichia coli</td>
<td>No</td>
<td>None</td>
</tr>
</tbody>
</table>

*Includes specimens from bronchoscopy.
† Includes surgical specimen from lobectomy for carcinoma.
§Includes bone marrow, cerebral spinal fluid and sputum.
‡All lobar infiltrates cleared with penicillin.

440

False-Positive Cultures of M tuberculosis (Mauer et al)
dence of active disease; however, only the patients with positive sputum or bronchial wash received no antituberculosis chemotherapy. After collection of additional urine specimens, all urine-positive patients were placed on isoniazid, ethambutol, and rifampin.

The remaining five cultures in the third group were inoculated December 11, 1981, January 11, 1982, February 2, 1982, March 4, 1982, and April 26, 1982. All of these cultures were plated within three days of a known positive specimen. Three of the five patients had a history of tuberculosis, but all were considered to be either completely treated or had been under adequate therapy for greater than six months. Thus, the significance of these cultures is subject to question since they may have been due to cross-contamination or sporadic shedding of the Mycobacterium (see Discussion). Unfortunately, neither the cultures nor known positive controls were available for phage typing. Four patients who had positive sputum received no chemotherapy; the fifth, who had a positive urine culture, received three-drug chemotherapy for 15 months. With one year of follow-up, none of the patients developed active disease.

Five cultures from the group of single positives clustered between April 2, 1982, and April 7, 1982, were still available at the time that possible contamination was suspected. These isolates, which included three urine and one sputum specimen, and one bronchial wash, were found to be phage type 8 (7, 9, 12, 13, 14, 15) as noted in Table 2. Unfortunately, the known positive control cultures that had been plated March 29, 1982, and April 5, 1982, were not available for phage typing.

In the six months following our discovery of a possible problem with false-positive cultures and following some modification of the laboratory technique used in preparing and inoculating specimens (see Discussion), specimens from 19 patients were positive for M tuberculosis. Fourteen of these had multiple positive cultures, and five had single cultures positive. One of the patients with a single positive result had only one specimen submitted and was lost to follow-up; three had several negatives and one positive result, but had clinical findings suggestive of active tuberculosis infection, and improved on antituberculosis chemotherapy. The fifth patient had a pulmonary infiltrate that responded to penicillin. Later, when his positive culture was reported, he was readmitted and underwent bronchoscopy. All specimens were negative, and he was followed-up without therapy for nine months and remains well.

**Discussion**

In our laboratory, if nine separate patients had not had single positive cultures reported in a one-week period, the question of false positivity would not have been raised, and the possibility of cross-contamination would not have been investigated. In reviewing the histories of the nine patients, nearly all had had sufficient findings to justify the submission of specimens for acid-fast bacillus culture. The PPD skin tests were positive in six patients, five had abnormalities detected on chest x-ray film that could have been compatible with tuberculosis, and one had hemoptysis with negative findings on chest x-ray film. Of the patients with positive urines, one had a history of renal tuberculosis but had been receiving appropriate therapy for more than one year; one had a bacterial abscess of the scrotum; and one had microscopic hematuria.

Despite the clustering of the isolated positive urine cultures and from seven to 24 negative cultures from each patient, our urologists felt they could not be certain the patients did not have active disease. Therefore, patients with positive urine specimens were placed on three chemotherapeutic agents. Therapy would certainly have been initiated in the patients with positive sputum and bronchial wash as well had not this cluster of single positive specimens been recognized early. Even then, considerable discussion ensued among the staff of the Pulmonary Disease Section before the decision was made to only follow and not treat the patients with positive pulmonary specimens in the cluster group.

A more perplexing problem was what to do with the patients who had single positive specimens, no evidence of active disease, and fell outside of the cluster period in April. As was previously noted, phage typing was impossible because cultures were not available. However, none of the patients appeared clinically ill, though three of the five had histories of tuberculosis. It has been suggested that patients with old disease may occasionally shed organisms, presumably via breakdown of old granulomatous lesions. Such patients in this situation who produce isolated positive cultures probably do not need to be treated in the absence of clinical disease. Since close follow-up was possible, we did not place the four patients who had positive sputa on therapy; the one patient with positive urine was placed on chemotherapy. In the follow-up of the chest patients for up to one year, none developed clinical disease.

The step at which contamination might have occurred was carefully sought. Since patient specimens came from various locations, it was unlikely that the

**Table 2—Results of Phage Typing**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Culture Date</th>
<th>Phage Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>4-2-82</td>
<td>8 (7, 9, 12, 13, 14, 15)</td>
</tr>
<tr>
<td>3</td>
<td>4-2-82</td>
<td>8 (7, 9, 12, 13, 14, 15)</td>
</tr>
<tr>
<td>6</td>
<td>4-6-82</td>
<td>8 (7, 9, 12, 13, 14, 15)</td>
</tr>
<tr>
<td>7</td>
<td>4-7-82</td>
<td>8 (7, 9, 12, 13, 14, 15)</td>
</tr>
<tr>
<td>9</td>
<td>4-7-82</td>
<td>8 (7, 9, 12, 13, 14, 15)</td>
</tr>
</tbody>
</table>
contamination occurred on the wards or in transport to the laboratory. Since no known laboratory accident had been noted and because of the slow rate of growth of M tuberculosis, the discovery of clustered single-positive cultures was made six weeks after the date of inoculation. This made the task of specifically identifying the source of laboratory contamination nearly impossible.

Our approach was to review the process of digestion and inoculation to identify any aspects of the procedure where contamination might have occurred. During the period between July 1, 1981, and April 28, 1982, in which all plating was done at the other medical center, reagents were carried in large containers and dispensed from these containers. In particular, the phosphate buffer was placed in liter flasks and poured into specimen tubes with only flaming of the flask lip between specimens. We concluded that this was the most likely step at which contamination could have occurred.

After identifying this as the probable contamination step, we modified the technique by placing the buffer in small bottles and dispensing to individual specimens from these. The exact contamination source has not been clearly established. Positive control cultures were set up in the laboratory as previously noted on March 29 and April 5, but in addition, control cultures were used during the culture period for niacin and catalase testing.

The problem of false positivity, though uncommon, has been reported. MacGregor et al.,4 alerted to the possibility of cross-contamination by an isolated positive culture in a well patient, reviewed all positive M tuberculosis cultures reported from their laboratory over an 18-month period. Among the 239 positive specimens reported, five or fewer colonies were observed on 36 cultures (involving 31 patients). Reviewing the status of these 31 patients with "low colony isolates," the investigators found that 12 had no evidence of active tuberculosis. They were able to follow 10 of the 12 patients for an average of 2.7 months. Each of the ten had two to four additional negative sputum cultures; chest roentgenograms after two to four months of follow-up either improved or remained unchanged. Phage typing was not done.

Contamination in their laboratory was felt due to a piece of broken equipment that was makeshift-repaired. To test this possibility, bacilli were deliberately planted on the equipment which was then touched to sterile sputum specimen containers. One of 24 containers grew small numbers of M tuberculosis providing some circumstantial evidence that this was a potential reservoir of organism.

Others have considered positive cultures in the absence of signs of roentgenographic disease as adequate reasons for therapy. Schmidek and Hardy,13 in assessing a tuberculosis outbreak on board a ship, found among 25 persons with positive cultures (sputum and/or gastric wash), six were completely asymptomatic and had not only negative routine chest films, but also full lung tomograms and apical and lordotic roentgenograms. Two of the six had more than one culture positive. All received chemotherapy. Huser et al.,14 identified 40 patients in a one-year period who had positive cultures of sputum or gastric wash in the face of chest roentgenograms which were judged negative for tuberculosis. Some of these patients, however, had symptoms or other signs of tuberculosis, and all were treated.

Probably the most detailed work that has been done to evaluate laboratory cross-contamination was by Aber et al.,6 and Mitchison et al.6 These authors, from the tuberculosis laboratory of the British Medical Research Council, designed a system of quality control to evaluate possible culture cross-contamination in the East African Laboratories with which they were working. These laboratories were inoculating sputum specimens from patients enrolled in short course chemotherapy trials, and it was crucial to know if isolated positive cultures reported by these laboratories represented treatment failure or laboratory error. Sputum-like specimens made from emulsified egg and methyl cellulose and containing a specially prepared rifampin-resistant strain of M tuberculosis were sent for culturing to three African laboratories. These specimens were inserted in a known sequence among sterile specimens. Culture results showed that 45 of 5,798 specimens which should have been sterile grew the rifampin-resistant marker. The false-positive rate varied among laboratories from 0.4 to 1.3 percent and seemed to be related more to individual technicians than the laboratory procedures.

Phage typing of M tuberculosis cultures is a sensitive tool for use as an adjunct in the investigations of epidemics and possible laboratory mix-ups.9 In the present investigation, all five cultures were phage type 8 (7,9,12,13,14,15). Using the data from an earlier publication,8 the frequency of this phage type in the United States is .0874. The probability of finding five cultures with this phage type in the present study would be 0.0000055. The typing data confirm that, for the five cultures tested, there was laboratory cross-contamination.

It is important to recognize that false positivity in the culturing of M tuberculosis is possible, and single positive cultures in clinically-well patients without other evidence of disease should not be sufficient reason to begin therapy. This is of critical importance since prolonged chemotherapy for tuberculosis is attended by potentially serious side effects, particularly in older persons, and is expensive. And even today, labeling a person with a diagnosis of tuberculosis is to give him a stigma which can disrupt social, family, and work life.
ACKNOWLEDGMENT: The authors wish to thank T. Aronoff for her assistance.

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