Investigation of suspected laboratory cross-contamination: interpretation of single smear-negative, positive cultures for Mycobacterium tuberculosis

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ABSTRACT

Restriction fragment length polymorphism (RFLP) analysis can be used to assess genetic relatedness of Mycobacterium tuberculosis isolates. This study reports a collaborative investigation of false-positive cultures for M. tuberculosis, suspected when the DNA fingerprint from an index case matched an epidemiologically improbable source case. RFLP analysis matched fingerprints in ten of 16 cases of suspected laboratory contamination to four separate smear-positive sources that were processed on the same day in the same laboratory. All single smear-negative, positive cultures processed on the same day as smear-positive specimens should be reviewed on a case-by-case basis to identify possible false-positive cultures.

Keywords Cross-contamination, DNA fingerprints, false-positive cultures, Mycobacterium tuberculosis, restriction fragment length polymorphism analysis, tuberculosis

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A clinical diagnosis of tuberculosis (TB) is, ideally, confirmed with a positive culture. Even in cases without symptoms or with a normal chest radiograph, a positive culture constitutes an active case in need of treatment, as colonisation with Mycobacterium tuberculosis is not thought to occur. Recent advances have improved the diagnosis of TB, but have been associated with a parallel rise in the number of reports of false-positive cultures [1,2]. Laboratory cross-contamination is a well-documented source of false-positive cultures, which have important clinical and public health consequences [3–10]. Diagnostic criteria for suspected laboratory cross-contamination with M. tuberculosis have been published [11,12], and the recent rise in the number of reports is probably related to the ease with which these incidents can be confirmed by molecular epidemiological studies [13,14]. The present report describes a retrospective collaborative investigation of four clusters of false-positive TB cultures caused by probable laboratory cross-contamination. The investigation was initiated when the restriction fragment length polymorphism (RFLP) cluster results for a case could not be explained by epidemiological evaluation.

Between December 2001 and January 2003, 84 M. tuberculosis isolates from 44 patients were identified by laboratory culture according to published guidelines [15,16]. Laboratory contamination was considered if patients had a single
smear-negative, culture-positive respiratory specimen, or positive cultures from an unusual site, or if specimens were processed on the same day as specimens from a patient with a positive smear for acid-fast bacilli (AFB). Laboratory contamination was considered unlikely for patients with repeatedly positive cultures (n = 24), or for those who were AFB smear-positive and culture-positive for M. tuberculosis (n = 1), or if symptomatic, tuberculin skin testing (TST) and radiology were consistent with active TB (n = 3). These patients were excluded from further investigation.

The isolates from the 16 cases in which culture contamination was suspected were processed for RFLP analysis using standard methodology for the IS6110 sequence and standard definitions for clustering [17]. Cluster analysis was performed using computer-assisted DNA pattern recognition software (BioNumerics v. 3.5; Applied Maths, Sint-Martens-Latem, Belgium). Similarity measures were calculated using the Dice coefficient, with 1.5% position tolerance, 0.75% optimisation, and 0% minimum height and surface. Complete linkage UPGMA (unweighted pair group method using arithmetic averages) was used to develop the dendrogram, with patterns also verified visually. The DNA fingerprints of suspect isolates were compared to the fingerprints used to develop the dendrogram, with patterns of isolates from potential smear-positive contaminants. RFLP analysis demonstrated matched fingerprints between the suspect contaminated cultures and four separate contamination sources in four separate pseudo-clusters on four separate dates during a 14-month period (Fig. 1). Potential source cases (A, B, C and D) had AFB smear-positive DNA fingerprint. For single band differences, confirmatory typing was performed by multiple interspersed repetitive units variable number tandem repeats (MIRU-VNTR) analysis using standard methodology [18].

Ten cases of laboratory cross-contamination were identified among the 16 patients suspected of having contaminated cultures. The clinical and laboratory features of these cases are summarised in Table 1. All patients had an initial negative smear and yielded a single positive culture. Four of the ten patients were identified as probable cases of contamination and no treatment or contact investigation was initiated. The six remaining patients were treated for TB for a duration of 35–255 days; two of these patients had medication-related adverse events. Of the 71 named contacts of these six patients, 69 presented for evaluation at TB Control for 51 TSTs (12 new positive and 39 negative) and 23 chest radiographs (11 previous positive TSTs and the 12 new positive TSTs). Four patients accepted treatment with isoniazid, three of whom received treatment for 9 months and for latent infection.

Of the 16 isolates fingerprinted, six had a discordant RFLP pattern compared with the putative source case, and were thus excluded as suspect contaminants. RFLP analysis demonstrated matched fingerprints between the suspect contaminated cultures and four separate contamination sources in four separate pseudo-clusters on four separate dates during a 14-month period (Fig. 1). Potential source cases (A, B, C and D) had AFB smear-positive

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**Table 1.** Clinical and laboratory features of suspected tuberculosis (TB) cases involving laboratory cross-contamination

<table>
<thead>
<tr>
<th>Patient</th>
<th>TB symptoms</th>
<th>Clinical scenario</th>
<th>Chest radiograph</th>
<th>TB skin test</th>
<th>TB factor</th>
<th>Initial process date</th>
<th>Initial specimen</th>
<th>Subsequent specimen</th>
<th>Subsequent smear and culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 CB Female 50 years</td>
<td>None</td>
<td>Cirrhosis</td>
<td>Bilateral effusions</td>
<td>ND</td>
<td>None</td>
<td>12/12/2001</td>
<td>Peritoneal fluid</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>2 FB Male 76 years</td>
<td>Cough/sputum fever</td>
<td>Bacterial OM</td>
<td>Consolidation</td>
<td>ND</td>
<td>DM2 CRF</td>
<td>12/12/2001</td>
<td>Spinal aspirate</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>3 CB Female 61 years</td>
<td>Cough</td>
<td>Lung cancer</td>
<td>Pleural thickening</td>
<td>N</td>
<td>None</td>
<td>12/12/2001</td>
<td>BAL</td>
<td>Sputum</td>
<td>4</td>
</tr>
<tr>
<td>4 FB Male 27 years</td>
<td>None</td>
<td>Post-MVA</td>
<td>hilar fullness</td>
<td>ND</td>
<td>None</td>
<td>12/12/2001</td>
<td>Sputum</td>
<td>Sputum</td>
<td>4</td>
</tr>
<tr>
<td>5 CB Male 87 years</td>
<td>None</td>
<td>UTI</td>
<td>Pleural thickening</td>
<td>ND</td>
<td>None</td>
<td>05/06/2002</td>
<td>Tracheal aspirate Sputum</td>
<td>Sputum</td>
<td>2</td>
</tr>
<tr>
<td>6 CB Female 59 years</td>
<td>None</td>
<td>Post-op surgery</td>
<td>Bilateral effusions</td>
<td>ND</td>
<td>Remote contact DM2</td>
<td>05/06/2002</td>
<td>Pleural fluid</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>7 CB Male 76 years</td>
<td>Cough/sputum anorexia</td>
<td>COPD Lung cancer</td>
<td>Pleural thickening</td>
<td>N</td>
<td>RA on steroid</td>
<td>18/11/2002</td>
<td>BAL</td>
<td>Sputum</td>
<td>4</td>
</tr>
<tr>
<td>8 CB Male 59 years</td>
<td>Cough</td>
<td>Lung cancer</td>
<td>Bilateral effusions</td>
<td>ND</td>
<td>None</td>
<td>18/11/2002</td>
<td>Palmar fluid</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>9 CB Female 43 years</td>
<td>None</td>
<td>Chronic arthritis</td>
<td>Bilateral effusions</td>
<td>ND</td>
<td>None</td>
<td>09/01/2003</td>
<td>Obturator fluid</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>10 CB Male 61 years</td>
<td>None</td>
<td>Post-op surgery</td>
<td>Effusion, atelectasis</td>
<td>ND</td>
<td>None</td>
<td>09/01/2003</td>
<td>Pleural fluid</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Index case.*

CB, Canadian-born; FB, foreign-born; N, negative; ND, not done; OM, osteomyelitis; DM2, diabetes mellitus type 2; CRF, chronic renal failure; BAL, bronchoalveolar lavage; MVA, motor vehicle accident; UTI, urinary tract infection; COPD, chronic obstructive pulmonary disease; RA, rheumatoid arthritis.

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respiratory specimens (with large numbers of organisms) processed on the same dates as the suspected cases of laboratory contamination. All source cases had culture-confirmed pulmonary TB with a fully sensitive organism, and chest radiographs consistent with active disease. All but two of the isolates had an identical match with the RFLP pattern of the possible contaminating source. The two remaining isolates differed from the source by one band, but were confirmed to be similar by MIRU-VNTR analysis. Despite extensive evaluation, no epidemiological link between the suspected contaminant cases and members of this cluster could be identified.

This is the first Canadian report of laboratory cross-contamination involving multiple pseudo-clusters confirmed by molecular epidemiology. Molecular typing of isolates, together with knowledge of the local molecular epidemiology, was invaluable in the recognition of false-positive TB cultures. Confirmatory typing by MIRU-VNTR analysis further validated the probability of contamination, despite the single band differences noted by RFLP analysis. The period that had elapsed between the processing of the original specimen and notification by the hospital laboratory precluded an investigation of all the factors that might have contributed to potential error. Despite extensive laboratory investigation, the exact cause of the cross-contamination events could not be determined. The four dates on which cross-contamination occurred were widely separated and random. Comparison of the RFLP patterns from the dates when cross-contamination occurred revealed distinct patterns for each of the four dates, suggesting that each cross-contamination event was unique.

Laboratories require a review process to detect unusual numbers of false-positive cultures and a mechanism to determine the possible causes. As a minimum, all smear-negative, single positive cultures, processed on the same day as a smear-positive specimen, should be reviewed on a case-by-case basis to identify possible false-positive cultures. Since this review was completed, no cases of laboratory cross-contamination have been identified during a 24-month follow-up period. Ongoing open and timely communication between clinicians and the laboratory remains critical in the interpretation of potentially unusual findings.

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REFERENCES


