Laboratory Cross-Contamination Of Mycobacterium Tuberculosis During Preparation Of Smears

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Citation

R Diaz, F Crespo, S Herrera, J Sevy-Court, A Marrero, D van Soolingen. *Laboratory Cross-Contamination Of Mycobacterium Tuberculosis During Preparation Of Smears*. The Internet Journal of Third World Medicine. 2004 Volume 2 Number 2.

Abstract

A possible cross-contamination of Mycobacterium tuberculosis at the Epidemiology and Hygiene Center of Havana (EHCH) occurred in 1999. Nine M. tuberculosis isolates from two acid-fast bacilli (AFB) smear-positive sputa and seven AFB smear-negative specimens were analyzed by IS6110 DNA fingerprinting. All isolates had identical fingerprints indicating a laboratory cross-contamination. No breaking rules were registered on the specimen transportation, sample labeling neither during sputum processing for culturing. Additional investigations in the peripheral laboratory (ML-HC2) showed that the recent entrance of new personnel in conjunction with severe difficulties in preparing smears for microscopy might have provoked the cross-contamination incident. The use of only one metal wire inoculation loop for smearing all series of sputa, a sand flask without alcohol and a faulty burner for loop sterilization, indicating the cross-contamination most likely occurred in ML-LC2. Multiple actions taken in the ML-LC2 have allowed that none laboratory contamination event has been reported after this.

INTRODUCTION

After several decades of decline, a resurgence of tuberculosis occurred in Cuba in the last decade (Marrero et al. 2000). This led to a two-three fold increase in the number of specimens being processed for the detection of Mycobacterium tuberculosis by microbiological laboratories, many of which have limited or diminishing resources. Increased volumes of testing may overload the capabilities of some laboratories, thereby enhancing the likelihood of procedural lapses or equipment malfunction. Laboratory cross-contamination has e.g. been detected retrospectively in patients whose cultures yielded M. tuberculosis in the absence of symptoms of tuberculosis and who had good clinical outcomes without therapy (Small et al. 1993).

In recent years, to detect cross-contaminations and to identify possible sources, IS6110 restriction fragment length polymorphism (RFLP) typing of M. tuberculosis has been used widely (Small et al. 1993, Van Duin et al. 1998, Trakas et al. 2000).

In this report, we applied IS6110 RFLP typing to confirm a cross-contamination event at the Epidemiology and Hygiene Center of Havana (EHCH), Cuba.

MATERIALS AND METHODS

This study concerns persons with respiratory symptoms (cough and expectoration for more than 14 days) and positive Mycobacterium tuberculosis cultures obtained from sputa processed on May 31, 1999.

Nine M. tuberculosis strains were isolated from seven acid-fast bacilli (AFB) smear-negative specimens and two from AFB smear-positive sputa from patient 2 (Table 1).

Figure 1

Table 1: Laboratory data of all specimens processed on May 31, 1999 in the Reference Tuberculosis Laboratory at the Epidemiology and Hygiene Center of Havana (RTL-EHCH).

Patient no.	Batch no.	Sample no.	Processed Date	Smear Results	Culture Results[c]	Notification Date	Health Centre
1		99-4878	31/05/99	-	-	23/08/99	1
2[a]	1	99-4879	31/05/99	3+	4+	05/07/99	2
		99-4880	31/05/99	3+	4+	06/06/99	
3[b]		99-4881	31/05/99	-	1	28/07/99	2
4		99-4882	31/05/99	-	-	23/08/99	1
5		99-4883	31/05/99	-	-	23/08/99	1
6		99-4884	31/05/99	-	-	23/08/99	1
7	2	99-4885	31/05/99	-	-	23/08/99	1
8		99-4886	31/05/99	-	-	23/08/99	1
9		99-4887	31/05/99	-	-	23/08/99	1
10		99-4888	31/05/99	-	-	23/08/99	1
11		99-4889	31/05/99	-	-	23/08/99	3
12	3	99-4890	31/05/99	-	-	23/08/99	3
13		99-4891	31/05/99	-	-	23/08/99	3
14		99-4892	31/05/99	-	-	23/08/99	3
15		99-4893	31/05/99	-	-	23/08/99	3
16		99-4894	31/05/99	-	-	23/08/99	3
17	4	99-4895	31/05/99	-	-	23/08/99	3
18		99-4896	31/05/99	-	-	23/08/99	3
19[b]		99-4897	31/05/99	-	6	28/06/99	2
20[b]		99-4898	31/05/99	-	12	28/06/99	2
21[b]		99-4899	31/05/99	-	9	28/06/99	2
22[b]	5	99-4900	31/05/99	-	3	19/07/99	2
23		99-4901	31/05/99	-	-	23/08/99	3
24		99-4902	31/05/99	-	-	23/08/99	3
25[b]	6	99-4903	31/05/99	-	3	12/07/99	2
26[b]		99-4904	31/05/99	-	5	05/07/99	2

[a] Source case (samples No. 99-4879 and 99-4880)

[b] False positive case

[c] Culture results: 4+, confluent; 1-12, number of isolated colonies.

Sputa were analyzed in the microbiology laboratory of a peripheral health center (ML-HC2) in Havana by acid-fast microscopy using the Ziehl-Neelsen method (Kent and Kubica 1985). Specimens were processed in the reference tuberculosis laboratory (RTL) at the EHCH by the Petroff´s NaOH method and the final sediment was inoculated onto Löwenstein-Jensen slants (Kent and Kubica 1985).. Identification of M. tuberculosis isolates was done by conventional tests (Kent and Kubica 1985).

IS6110 RFLP analysis was performed in the National Reference Laboratory on Tuberculosis and Mycobacteria, at the Pedro Kourí Institute of Tropical Medicine (IPK) in Havana, according to the standardized protocol (Van Embden et al. 1993).

The laboratory records and procedures for all false-positive cultures were reviewed to try to determine at which step the cross-contamination may have occurred.

RESULTS

Nine mycobacterial primary cultures all processed on May 31, 1999 by the same technician in the RTL-EHCH, were identified as M. tuberculosis (Table). Two out of the nine isolates were obtained from two AFB smear-positive sputa from a patient (patient 2) with typical signs and symptoms of active pulmonary tuberculosis. The remaining seven positive cultures were isolated from seven sputa of patients with AFB-negative smears (analyzed in the ML-HC2) and the respective patients did not reveal clinical and radiological evidence suggestive of active tuberculosis.

The extent of growth of isolates from smear negative specimens varied from a single colony (1) to a dozen colonies (12) per slant.

Public health officials were informed of the suspicion that M. tuberculosis cultures from these seven patients were presumably false-positive (all were single-positive culture patients) and thereby the cases were widely discussed at the National and Provincial Technical Committees of Tuberculosis. The suspected cases were subjected to additional physician consultations and chest radiographic studies but none of them were put on anti-tuberculosis therapy. Additional specimens of these patients were all negative in microscopy and culture. Epidemiological investigation found that all patients were living in the same neighborhood; however, no previous contact between them was demonstrated. All nine isolates had identical IS6110 banding patterns, suggesting a laboratory cross-contamination (Figure 1).

Figure 1: IS6110 RFLP patterns of M. tuberculosis strains isolated due to a cross- contamination episode.

Lane one, DNA from M. tuberculosis reference strain Mt 14323.

Lanes 2 and 10, isolates from AFB smear-positive sputa of a patient with a clinical and radiological evidence of pulmonary tuberculosis (source case).

Lane 3-9, culture isolates of M. tuberculosis from AFB smear-negative sputa of seven patients without typical signs of active tuberculosis.

Numbers on the left indicate the size of standard DNA fragments (in kilobase pairs).

Figure 2

FIGURE
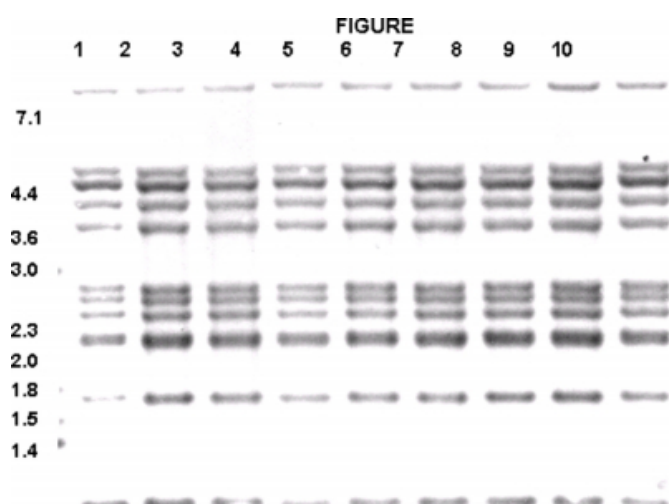
Because the specimens had been processed at the same day, and by the same technician, but in batches with other culture-negative specimens from geographically separated patients and because at the RTL-EHCH a well-trained and experienced technician had strictly followed all laboratory procedures recommended by the National Reference Laboratory at IPK, the laboratory cross-contamination was hard to imagine at this level.

A detailed investigation in the RTL-EHCH showed there was no evidence of the incorrect application of a certain technique in this laboratory. Specimens were divided in batches and processed in a properly maintained, vertical laminar down flow biosafety cabinet. Work surfaces were disinfected regularly with phenol. Each specimen was carefully manipulated to avoid accidental transfer of bacteria from one sample to another via pipettes, caps of tubes or common reservoirs of reagents or containers to discard material. A separate sterile glass pipette for each specimen or each reagent aliquot was used in all cases. Specimens were decontaminated, digested and neutralized with ready-to-use working solutions that were discarded later on. After centrifugation, the decontaminated specimen sediments were inoculated on Löwenstein-Jensen slants with a separate pipette.

On the other hand, the transportation procedure of specimens for culturing, from ML-HC2 to RTL-EHCH, was also evaluated. The personnel who transported the containers were interviewed to detect any incident of mix of samples in the sputum containers. However, no irregularity was observed in both cases.

A different investigation done in the ML-HC2, which does not have biological safety cabinet, revealed some difficulties during the preparation of smears for microscopy and the presence of new personnel in the laboratory. There was only one nichrome wire inoculation loop to pick up the sputa for smearing, which was passed through a sand flask without alcohol in between the processing of different samples.

Additionally, it was inadequately heated for a few seconds (but not sterilized) under the yellow flame of a faulty and old Bunsen burner (no blue cone was observed in the burner flame). An AFB smear-positive sputum (sample no. 99-4880) of patient 2 was smeared prior to the seven AFB smear-negative sputa, of patients from that area, analyzed that day.

DISCUSSION

In the last decade, many cross-contamination events have been adequately confirmed by the high resolution IS6110 RFLP typing (Small et al. 1993, Bauer et al.1997, Braden et al. 1997, Van Duin et al. 1998, Trakas et al. 2000, Jasmer et al. 2002). Cross-contaminations of the robust M. tuberculosis appeared to occur more frequently than previously assumed. The rate of false positive cultures in published studies in First World laboratories has ranged from 0,9% to 13% (Small et al. 1993, Bauer et al.1997, Braden et al. 1997, Van Duin et al. 1998, Trakas et al. 2000, Ruddy et al. 2002). However, it could be much higher in laboratories from developing countries. For instance, an extensive cross-contamination of M. tuberculosis with 60 false-positive cases (65,9% of false-positive rate) was recently reported in a reference laboratory in Brazil (De Ramos et al. 1999).

In Cuba, some possible cross-contamination incidents have been found earlier (Diaz et al. 1998, 2001), but these have been documented only partly.

We here describe seven "patients" whose M. tuberculosis cultures were cross-contaminated and, hence, false-positive. We hypothesize that the contamination might have occurred in the peripheral microbiology laboratory due to an insufficient decontamination and sterilization of the microbiologic loop during the sputum smear procedure. It might have facilitated the cross-contamination of bacilli from the AFB smear-positive sample of the patient 2 to the AFB smear-negative sputa through the non-sterilized inoculation loop. A month after this incident, the burner was re-tested inside of a biological safety cabinet. We found that 10 seconds of heating under the yellow flame of this burner (similar to the working procedure followed in ML-HC2 for

smear microscopy) were insufficient to entirely kill around 104 bacilli of a pure culture of M. tuberculosis (H37Ra strain) in a loop. Three weeks later, 50-100 colonies grew in LJ slants. In spite of this simple experiment did not prove the laboratory cross-contamination occurred via a non-sterilized inoculation loop, it is another evidence to reinforce our hypothesis.

Finished our investigation, some actions were taken in the ML-HC2 to implement the good laboratory practice in this setting and therefore to minimize the risk of cross-contamination. These actions included changing the Bunsen burner, adding 95% alcohol to the sand flask, supplying five new nichrome wire inoculation loops, training of laboratory technician and professional personnel, re-evaluating of general conditions of the laboratory. None laboratory contamination event has been reported for sputum samples from ML-HC2 in the last five years.

In Cuba, wire loops to prepare smears of sputa are commonly used. We are now recommending using disposable wooden sticks for smear microscopy in Cuba in stead of nichrome wire loops. Single-use wooden sticks are cheaper and avoid the transfer of mycobacteria from a sample to another during the smear process.

The episode mentioned here was an alert calling to the National Control Program for improving the quality assurance of smear microscopy in peripheral laboratories.

Although in this event none of the seven false-positive patients were treated with antituberculous drugs, false-positive cultures resulted in unnecessary physician consultations and diagnostic tests, including radiography and bronchoscophy, for the respective patients. It would also have affected their social, laboral and familiar environment as suspected cases of active tuberculosis.

To our knowledge, this is the first report of laboratory cross-contamination during the acid-fast microscopy procedure, at the primary level health services, adequately confirmed by IS6110 RFLP typing.

Because many laboratories in Third World settings still work in this way, this may disclose another pathway of laboratory cross-contaminations leading to over diagnosis of tuberculosis.

This event again shows that good laboratory practice is of the utmost importance to prevent laboratory mishaps that have serious consequences for the concerned patients.

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