Estimation of the Rate of Unrecognized Cross-Contamination with *Mycobacterium tuberculosis* in London Microbiology Laboratories

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Isolates from patients with confirmed tuberculosis from London were collected over 2.5 years between 1995 and 1997. Restriction fragment length polymorphism (RFLP) analysis was performed by the international standard technique as part of a multicenter epidemiological study. A total of 2,779 samples representing 2,500 individual patients from 56 laboratories were examined. Analysis of these samples revealed a laboratory cross-contamination rate of between 0.54%, when only presumed cases of cross-contamination were considered, and 0.93%, when presumed and possible cases were counted. Previous studies suggest an extremely wide range of laboratory cross-contamination rates of between 0.1 and 65%. These data indicate that laboratory cross-contamination has not been a common problem in routine practice in the London area, but in several incidents patients did receive full courses of therapy that were probably unnecessary.

Isolation of *Mycobacterium tuberculosis* is the definitive method for confirming the diagnosis of tuberculosis. Cross-contamination is an inherent problem in culturing mycobacteria due to both the sensitive recovery systems in use and the ability of the bacilli to survive outside the host for extended periods. Viable tubercle bacilli have been recovered from heat-fixed sputum smears and from 0.9% sodium chloride decontamination solutions up to 3 weeks after inoculation (1). False-positive culture results can occur as a result of contamination at many stages, including patient sampling, microscopy, and specimen inoculation (7, 17).

A suspected diagnosis of tuberculosis has significant implications for patients and their contacts and for health care resources. For the patient, it entails a course of potentially toxic chemotherapy. For the contacts, it involves worry and time taken to attend screening. For health services, it further dilutes already stretched resources and personnel.

Our research team has recently completed a multidisciplinary collaborative study to investigate the epidemiology of tuberculosis within the London area using the international standard IS*6110* restriction fragment length polymorphism (RFLP) method and secondary typing techniques (12). An important aspect of any analysis of tuberculosis epidemiology is the identification and quantification of laboratory cross-contamination that may falsely increase the number of cases included in clusters. In a recent review, most large studies were shown to have clusters containing strains that were likely to be present through cross-contamination (8). In order to ensure that the clusters produced by the study were as reliable as possible, a thorough review of the data was performed to elucidate which samples could have represented cross-contamination in the laboratory. Laboratories have different procedures to identify laboratory cross-contamination. In this paper, we report on the frequency of cross-contamination where it has not been recognized by laboratories through routine procedures.

MATERIALS AND METHODS

Patient samples. A total of 2,779 isolates of *M. tuberculosis* were isolated in London laboratories between 1 July 1995 and 31 December 1997. After multiple isolates from the same patient were eliminated, isolates from 2,500 individual patients were included in the study; 448 isolates had one to four copies of IS*6110*, and 2,042 isolates had five or more copies. The methods and overall results of the study have been described in detail elsewhere (12).

Molecular epidemiological techniques. All isolates that had been identified as *M. tuberculosis* were typed by IS*6110* RFLP analysis using the international standard technique (18). All patterns were entered by one researcher into a database using GelCompar software (version 4.0; Applied Maths, Koutrai, Belgium) and then analyzed independently. The isolates were compared using the Dice coefficient with the parameter settings at 1.2% band position tolerance with optimization. A molecular cluster was defined as a series of isolates that had identical banding patterns (100% identity), and this computer similarity was subject to visual verification. Strains that differed by one band were regarded as not belonging to the same molecular cluster.

Epidemiological data collection. Epidemiological information was gathered in the first instance by a structured pro forma questionnaire, which was used during record review to collect information about cases of culture-confirmed tuberculosis. Two health authorities (in east and southeast London) were able to provide data that were stored in local databases. Additional microbiological data from the PHLS United Kingdom antimicrobial resistance surveillance network (MY-COBNET) database was obtained. Potential links between members of the clusters were determined by reference to these pro forma questionnaires after molecular cluster results were known.

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Defining cross-contaminants. All clustered isolates with greater than four bands on IS*6110* RFLP typing sent from the same source laboratory were considered for possible laboratory cross-contaminants and investigated further. Those received at the PHLS Mycobacterial Reference Unit within 12 weeks of each other were also identified for further study as possible cases of cross-contamination. If samples were found to be smear positive or patients had multiple positive cultures, laboratory cross-contamination was excluded. Any isolate obtained from a sample that had been shown to be smear negative and that constituted the only positive sample from that individual was defined as "negative smear one positive" and was checked for the date of processing within the source laboratory. If the sample was processed within 1 week of another with the same RFLP fingerprint, the possibility of laboratory cross-contamination was suspected. One week was chosen because laboratories processing smaller numbers of mycobacterial samples might batch them over two or more days, and hence, samples booked into the laboratory on different dates could be processed on the same day, exposing them to the risk of cross-contamination. More detailed information was collected to determine the likelihood of these being cases of cross-contamination. This included the original study pro forma questionnaire completed for each isolate, the microbiology records obtained by visiting the source laboratory, inspection of the clinical case notes for each patient, consultation with the tuberculosis liaison nurse, and the opinion of the physician in charge of the patient. Strains were defined as presumed cases of cross-contamination if the patient's clinical condition was not consistent with tuberculosis and an alternative diagnosis had been identified. Alternatively, they were defined as presumed cases of cross-contamination if the clinical and epidemiological data were inconsistent with tuberculosis. Possible cases of cross-contamination were defined as those where the clinical data were consistent but the epidemiological data were inconsistent with transmission. Wherever the final diagnosis could not be accurately determined, it was considered to be a possible case of cross-contamination. This diagnostic process is illustrated as a decision tree in Fig. 1.

RESULTS

Our first action was to detect and remove double entries. We identified three previously unrecognized cases of double entry of the same patient's details, one that was entered under an anonymous genitourinary medicine number and then reentered under a chest clinic number and two that were entered twice with different dates of birth.

After this initial screening, we found a total of 74 isolates (each from a single patient) that might be suspected to be from laboratory cross-contamination. These included isolates from 35 clusters obtained from 19 separate laboratories. The other members of the clusters were believed to be genuine cases based on the criterion of smear positivity, multiple cultures from the same patient, or clinical opinion.

After analysis of the patient records, 11 (0.54%) were identified as presumed false-positive reports due to laboratory cross-contamination. The detailed assessment of these cases is summarized in Table 1. One case (I) involved a refugee hospitalized after detention at an airport on the day of arrival in the United Kingdom for the first time. The patient was diagnosed clinically as having a case of tuberculosis, although the initial specimen was smear negative. The isolate apparently grown from this specimen was of a type identical to a strain cluster from the local community, consisting of patients who had no links to the country of origin of the refugee. This was defined as a case of presumed laboratory cross-contamination in a patient with presumed tuberculosis.

In a further eight cases (0.32%), it was not possible to state categorically whether clustering was due to cross-contamination, as some of the patients had died or been lost to follow-up or the final diagnosis remained unclear. An example of this is the case (N) of a patient who was given a full course of therapy but whose symptoms persisted posttherapy. Also, there were two cases (M and P) of clinically defined tuberculosis that were smear negative and had isolates that were identical to others processed in the laboratory in parallel. Since there was no clear link between the patients but more distant transmission could not be excluded, these cases were defined as possible cross-contamination. As transmission could not be absolutely excluded, this was defined as a case of possible cross-contamination. The clinical interpretation of these cases is summarized in Table 2. The remaining 55 isolates were considered to represent genuine cases of tuberculosis.

The overall previously unrecognized laboratory cross-contamination rate was 11 cases out of 2,042 patients (0.54%) when only the presumed cases of cross-contamination were considered. When the possible cases of cross-contamination were also included, 19 out of 2,042 (0.93%) were identified. Cross-contamination incidents were recorded for 11 (19%) of the 56 laboratories involved in the London-wide study. Cross-contamination strains were found in 14 (8.77%) of the clusters identified during the study (12).

DISCUSSION

This study was initiated to determine the significance of cross-contamination where the primary laboratory had not identified it. The problem of cross-contamination has always been recognized, but it is less clear how common this problem is. Review of the literature revealed a large variation in reported rates of cross-contamination, ranging from 0.1 to >65% (Table 3). However, the highest rates reported were found in studies that were initiated because of suspicion that incidents of cross-contamination were occurring. This was usually due to isolation rates that were above those normally expected. It is not surprising, therefore, that such studies produce high rates of cross-contamination. If the comparison is limited to large unselected population-based studies, the rates (with one exception) are below 3%. A recent review (7) found a mean false-positive rate of 3.1% from 14 studies of >100 patients. However, it is extremely difficult to compare these studies because they are not standardized in any way. In some cases, the denominator is described as total samples submitted for analysis, while in other cases, the denominator consists of positive samples only. Furthermore, some laboratories will try to identify cases of cross-contamination. Thus, retrospective studies of all isolates will not give a true picture of the extent of cross-contamination. Our study provides an estimate of the risk of cross-contamination that is unrecognized by the reporting laboratory and that is likely to result in inappropriate or unnecessary therapy in our catchment area. Lack of recognition may have occurred because there was no system for identifying cross-contaminants in the host laboratory or because the system failed to detect it.

Several factors contribute to laboratory cross-contamination, ranging from simple mislabeling of specimens to laboratory protocols that are not adapted for left-handedness (4, 17). Initial sample processing can result in the transfer of bacilli by aerosol, splash, loop, or pipette, which invariably involves consecutive sample numbers. While it might be better to process samples individually, the widespread practice of alkaline decontamination inevitably means having several samples in a batch in the safety cabinet at the same time, since it is too
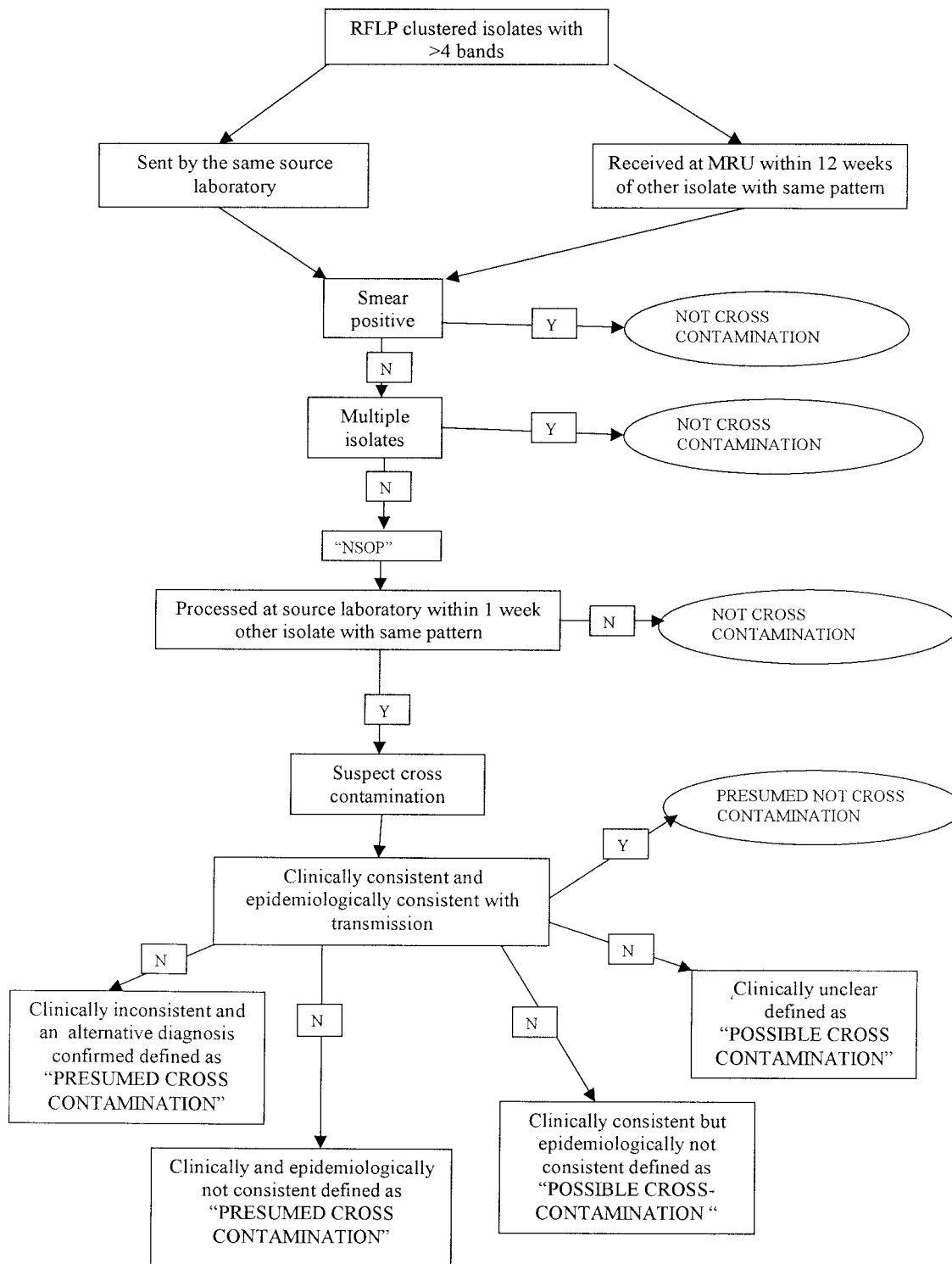
FIG. 1. Flowchart illustrating the decision pathway used to identify cases of cross-contamination and to assess their clinical significance. MRU, PHLS Mycobacterium Reference Unit; NSOP, negative smear one positive.

time-consuming to wait for decontamination of each one separately. Ensuring adequate airflow through the cabinet is of course essential for the safety of the staff, but it also helps to minimize aerosol spread. Subsequent processing of samples with contaminated equipment has been a well-documented source of cross-contamination in several instances (3, 8, 17), particularly involving needle carryover from Bactec460 radiometric analyzers (Becton Dickinson, Sparks, Md.). This appears to be less of a problem recently, which may reflect adjustments in the temperature and maintenance programs for

TABLE 1. Presumed cases of laboratory cross-contamination detailing symptoms, therapy, and final diagnosis

Case	Reason investigated[a]	Antituberculous drugs given	Assessment conclusion
A	CXR—prominent hilum	None	Left atrial myxoma
B	Persistent cough	None	Lung carcinoma
C	Cough, fever	None	Presumed pneumonia
D	CXR—parenchymal shadowing, cough	None	*Pneumocystis carinii* pneumonia
F	Persistent cough, breathlessness	None	Laryngeal tumor
G	HIV positive with skin lesions	None	Kaposi's sarcoma
H	Elevated inflammatory markers	Chemoprophylaxis	Polymyalgia
I	Port of entry screening; symptomatic; CXR—abnormal	Full treatment course	Clinically considered case of pulmonary tuberculosis; no possible epidemiological link to cluster
J	Port of entry screening; asymptomatic	None	Not clinically or epidemiologically consistent
K	Port of entry screening; asymptomatic	Chemoprophylaxis	Not clinically or epidemiologically consistent
L	Sterile pyuria	None	Bacterial urinary tract infection

[a] CXR, chest X ray; HIV, human immunodeficiency virus.

the needles and more widespread use of liquid culture systems using noninvasive growth detection. Another frequently cited problem has been the contamination of common delivery tubes or containers for additives. The effects of such contamination can produce startling cross-contamination levels of up to 60% (9). Another factor is the number of positive samples being processed within the laboratory—the more positive samples, the more opportunities for cross-contamination (13).

While good laboratory practice can minimize the risk, cross-contamination remains a possibility in any center, and maintaining levels of suspicion is important to allow prompt detection. In particular, attention should be given to any negative smear one positive sample. Additionally, laboratories using both liquid culture systems and solid slopes (e.g., Bactec and Lowenstein-Jensen) may be alerted by growth in one system only or if <6 colonies are isolated on the slope. Investigation typically shows that cross-contaminated samples were processed concurrently with a consecutively numbered true-positive sample. However, cross-contamination has been discov-

ered from samples processed 15 specimens apart due to needle carryover (3).

Our data suggest the importance of identifying incidents of cross-contamination quickly. Among the cases where cross-contamination was defined as presumed, 3 of the 11 patients had some form of antituberculosis therapy: prophylaxis in two cases and a full treatment course in one. However, among the eight cases of possible cross-contamination, the therapeutic consequences were identified in five cases and all were given a full course of antituberculosis chemotherapy. It is possible that these patients received unnecessary antituberculosis drugs, with all the possibilities of adverse events, for no benefit. Also, while an incorrect diagnosis of tuberculosis is being entertained, the correct diagnosis may not be made. In three cases, we detected presumed or possible cross-contamination when the patient was thought to have tuberculosis. This too can have significant adverse outcome for the patient if the strains differ in their antibiotic susceptibility test results.

The data presented in this report argue strongly for the need

TABLE 2. Possible cases of laboratory cross-contamination detailing symptoms, therapy, and final diagnosis

Case	Reason investigated[a]	Anti-TB drugs	Assessment conclusion
M	CXR—upper zone shadowing, cavitation; clinically consistent	Full course	Presumed case of TB; no obvious epidemiological link to clustered cases possible laboratory contamination between two known cases
N	CXR—upper zone shadowing and effusion; pleuritic chest pain	Full course	Treated as case of TB; symptoms and signs persisted posttreatment
O	Fine-needle aspirate of persisting lymphadenopathy	Unknown	Unknown—case notes lost
P	CXR—upper zone shadowing; clinically consistent	Full course	Presumed case of TB; no obvious epidemiological link to clustered cases: possible laboratory contamination between two known cases
Q	CXR—effusion, pneumothorax; breathless, cachexia, persistent cough	Unknown	Known congestive cardiac failure and carcinoma of bronchus; diagnosis of TB uncertain; already transferred abroad for terminal care before culture result
R	Unknown	Unknown	Case notes unobtainable
S	HIV positive, low CD4 counts, febrile, cough	Full course	Multiple other samples isolated MAIC; probably not TB but possibility of dual infection, hence not denotified
T	CXR—pleural effusion; immunocompromised post-renal transplant; past history of TB	Full course	Current diagnosis uncertain; fully treated in view of past history and immunocompromise

[a] CXR, chest X ray; HIV, human immunodeficiency virus; MAIC, *Mycobacterium avium-M. intracellulare* complex; TB, tuberculosis.

TABLE 3. Reported cross-contamination rates for *M. tuberculosis*

Rate (%)	No. of cross-contaminants	Sample size	Total or positives only	All samples or incident only	Date	Place (reference)
0.1	1	907	Total	All	1980	Africa (1)
0.1	3	2,305	Positive	All	1997	Chicago (5)
0.2	3	1,500	Positive	All	1992	Denmark (4)
0.2	8	3,600	Positive	All	1993	San Francisco (17)
0.3	12	4,075	Positive	All	1996	Chicago (19)
0.8	45	5,798	Total	All	1980	Africa (13)
1.5	Not reported	Not reported	Positive	All	1995	Holland (4)
1.8	9	496	Positive	All	1994	San Francisco (16)
2.29	49	1,439	Positive	All	1994	Denmark (4)
2.6	3	117	Positive	All	1994	New York City (2)
3.5	9	259	Positive	Incident	1992	Arkansas (6)
4	8	199	Positive	Incident	1997	Denver (8)
7.8	24	306	Total	All	1995	France (10)
8	60	750	Positive	Incident	1995	New York City (15)
9	11	114	Positive	Incident	1997	Wisconsin (3)
12	5	42	Positive	Incident	1997	Denver (8)
13	9	70	Positive	Incident	1993	Los Angeles (14)
65.9	60	91	Positive	Incident	1995	Brazil (9)

to perform some routine test to determine whether isolates from the same laboratory are possible cross-contaminants as soon as possible to prevent unnecessary therapy. Rapid PCR-based methods, such as variable number tandem repeat and spoligotyping, have been proposed for this purpose (11). Isolates with the same profile but no epidemiological link would suggest laboratory cross-contamination, and the clinicians could be advised appropriately.

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