Original Article

INVESTIGATION OF CROSS-CONTAMINATION IN A MYCOBACTERIUM TUBERCULOSIS LABORATORY USING EPIDEMIOLOGICAL DATA AND SPOLIGOTYPING

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ABSTRACT

Objective: To investigate possible pseudo-outbreak of Mycobacterium tuberculosis (MTB) during a two week period July 2-15th 2005, as suggested by an increase in incidence of newly diagnosed patients with tuberculosis. Many of them had negative – smears for AFB and only one positive-MTB culture.

Methodology: Retrorespective surveys of all medical and laboratory data using standard epidemiological tools and DNA fingerprinting in King Khalid University Hospital, Riyadh, Saudi Arabia.

Results: A total of 22 samples representing 20 individual patients were examined. Epidemiological and laboratory analysis of these samples revealed that 14 were identified as presumed false-positive reports due to laboratory cross-contamination. In a further 5 cases, patients were diagnosed clinically as having tuberculosis and were defined as cases of possible cross-contamination. The source was a patient with both clinical and radiographic evidence of TB and was the only one who was smear and culture positive for TB. Molecular analysis and epidemiological studies revealed that specimen patching and multi-use vials of buffer solution were the source of cross-contamination.

Conclusions: This investigation strongly supports the idea that *M.tuberculosis* grown from smear-negative specimens from patients without clinical evidence of tuberculosis should be analyzed by rapid and reliable strain differentiation techniques, such as spoligotyping, to help rule-out laboratory contamination.

KEY WORDS: Cross contamination, Mycobacterium tuberculosis, Spoligotyping.

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INTRODUCTION

The global increase in tuberculosis has resulted in a substantial rise in the number of specimens cultured in diagnostic mycobacteriology laboratories.[1] This increase and the use of molecular tools to subtype or fingerprint *Mycobacterium tuberculosis* have resulted in increased recognition of specimen cross-contamination events linked either to laboratory procedures or, in rare cases, to contaminated bronchoscopes.[2,3] False-positive cultures were identified in 13(93%) of 14 large studies that evaluated more than 100 patients,

with a median false-positive rate of 2.1%.[4] However, a much higher rate (33%) has been reported from a hospital laboratory that used a sensitive broth culture technique Mycobacterial Growth Indicator Tube (MGIT) system, (Becton-Dickinson).[5]

Here we report the use of spoligotyping and epidemiological information to confirm our suspicion that cross-contamination had occurred in our mycobacteriology during a two week period in the year 2005 and the use of simple procedures and changes in laboratory practices to control and prevent similar future instances. To the best of our knowledge, no such events have been reported from our Middle East region.

METHODOLOGY

Patient Specimens: King Khalid University Hospital, is a 800 bed tertiary care teaching hospital that serves a population of ≤1.5 million inhabitants. Approximately an average of ten to twelve patients are diagnosed monthly with culture positive MTB. After initial screening, we found a total of 47 isolates that we suspected to be from laboratory cross-contamination. These included isolates obtained between 15 June and 29 July 2005. Further analysis of laboratory and patient records revealed that one cluster of cases representing 22 specimens from 20 different patients (Table-I) were identified as suspected cases of cross-contamination. The other clusters were believed to be genuine cases based on the criterion of smear positivity, multiple cultures from the same patient, or clinical findings.

Epidemiological Data Collection: A retrospective epidemiological investigation was undertaken. First, we reviewed patient medical records and communicated with patients physicians. Data were recorded regarding clinical history, symptoms, reason for admission, clinical course, date of specimen collection, ward location, potential shared procedure, radiological findings, human immunodeficiency virus (HIV) status and whether anti-TB treatment was started and continued once sputum culture results were reported. Secondly, we reviewed specimen logs for simultaneous processing of smear-negative and smear-positive cases. In addition, we conducted an investigation of the laboratory environment, specimen processing techniques, and technologist rotation logo. All possible sources of contamination were investigated including stains, reagents and equipments used during the suspected period of contamination. None of the MGIT growth supplements or antibiotics mixtures (PANTA) were available during the period of investigation.

Table-I: Specimens cultures positive

Specimen	*No. of Samples*
Sputum	7
Bronchial alveolar lavage	1
Endobronchial biopsy	1
Gastric lavage	1
Urine	1
Lymph node	5
Ascitic fluid	1
Sacroiliac joint aspirate	1
Cerebrospinal Fluid (CSF)	1
Thyroid cyst aspirate	1
Knee Aspirate	1
Fine Needle Aspirate (FNA)	1
Total	22

Specimen Processing: On receipt by the laboratory, specimens from sites that are normally sterile were concentrated-by centrifugation (4,000 x g for 10 min) and were inoculated onto a slant of Lowenstein-Jensen medium (LJ) (Baltimore Biological Laboratory) and into a MGIT- (BBL Becton Dickinson Microbiology systems, Sparks, MD). Specimens from non-sterile sites were digested with N-acetyl-L-Cystine and decontaminated with sodium hydroxide (final concentration 2%). The contents were then mixed by inversion for 15 min. and diluted with phosphate buffer saline. The phosphate buffer was prepared in 1-L bottles and divided into smaller, multi-use containers to process eight samples at a time. The mixture was centrifuged and the sediment twice is inoculated into MGIT tubes and a Lowenstein-Jensen slant. All specimens were examined microscopically using auramine-stained and acid fast smears.

Molecular Epidemiological Technique: To conduct a retrorespective investigation examining the

possibility of specimens contamination, all 47 MTB isolates obtained during the suspected period were sent to King Faisal Specialist Hospital and Research Center (KFSH & RC) for characterization by spoligotyping. Spoligotyping was performed with a commercially available kit (Isogen Bioscience).[6,7]

RESULTS

Laboratory Investigation: Over a two week period in July 2005, 20 patients had positive culture for MTB from different type of specimens. With the exception of one patient (index patient), all had negative smear for Acid-fast bacilli (AFB) and only one positive *M. tuberculosis* culture. Three specimens, a post-bronchoscopy sputum, bronchalveolar lavage & endobronchial biopsy were obtained from the index patient. Each of these specimens were heavily positive (+++) on an AFB smear and were processed during 5 consecutive days by a new technologist in the same batch as one or more smear negative and culture positive isolates. All suspected cultures were positive only in MIGT tubes and yielded no growth on the accompanying LJ slants. Susceptibility testing performed on MGIT system for all isolates showed the same pattern: Sensitive to isoniazid, rifampin, ethambutol and streptomycin. Culturing of phosphate buffer in MGIT tube was positive for MTB after seven-day inoculation.

Ten different spoligotypes were identified among the 47 MTB isolates. The suspected twenty isolates had the same spoligotype pattern designated as "S3" (Figure-1). These isolates were cultured from specimens processed at the same time with the only smear positive index case specimen between July 2 and July 15, 2005. Isolates grown from other smear positive specimens had different spoligotypes.

Retrospective Patient Investigation and Follow-up: Medical records for all 20 patients with the possibly contaminated specimens were reviewed. The possible source was a Saudi male admitted to the medical ward with clinical features, radiological evidence and TST results consistent with tuberculosis. For the remaining 19 patients; 14 were identified as having presumed false-positive reports due to laboratory cross-contamination. Isolates were presumed to be cross-contaminant if the patient's clinical conditions and epidemiological data were not consistent with tuberculosis. The detailed assessment of these patients is summarized in Table-II. When positive cultures were reported, physicians were made aware that isolation of MTB from each of the 14 patients believed to have a false-positive culture could be the result of cross-contamination. In six of these patients, the positive results were early dismissed as there was no clinical evidence of tuberculosis. However, the existence of unrelated pulmonary diseases complicated the differential diagnosis in four patients in whom the possibility of tuberculosis could not be clinically excluded, and one of them was started on anti-tuberculosis therapy and suffered drug toxicity requiring alteration in his treatment regiment. Two patients with suspected osteoarticular tuberculosis received therapy for tuberculosis. In one of these patients, treatment was discontinued after two month when spoligotyping results became available. However, the other patient completed one year of anti-TB therapy. Case notes were unobtainable for two patients. In a further five cases tuberculosis was highly suggestive. As contamination could not be absolutely excluded, they were defined as cases of possible cross contamination. (Table-III)

DISCUSSION

Contamination in the mycobacteriology laboratory has been described with both conventional and automated methods.[8,9] Although specimens should be completely separated from one another, most laboratories process AFB cultures in batches. Preparing non-sterile specimens for mycobacterial culture involves a multi-step process of digestion, decontamination, neutralization, and centrifugation, in an attempt to concentrate and recover the mycobacteria. Carry-over from one specimen to another account for most contamination episodes in conventional processing and it

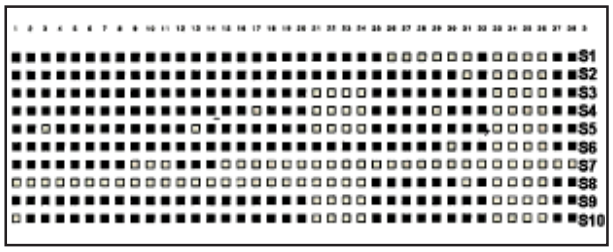

Fig-1: Spoligotype patterns identified among M.tuberculosis isolates. Solid squres, hybridization with designated spacer probe; open squares, lack of hybridization.

appears to occur most often during the mycolytic, or decontamination, step of processing. In addition the use of highly sensitive culture systems and prolonged incubation periods to grow MTB may detect relatively small inocula of MTB resulting from cross-contamination creating opportunities for introducing mycobacteria to cause false-positive results. Other factors contributing to cross-contamination may include new and inexperienced personnel, an increase in the number of specimens processed, contaminated clinical devices (bronchoscopes) and clerical errors.[10,11] In addition, several large out-breaks of false-positive culture have been attributed to defect in the exhaust system of the biological safety cabinets used for specimen processing.[12]

Our epidemiological and molecular studies revealed that cross-contamination was due to faulty laboratory technique. Review of our lab procedure revealed that a single and large bottle of phosphate buffer was routinely used to process multiple samples prior to being replenished. The same bottle was likely used for all non-sterile specimens and was the only factor shared by these cases. Culture of the buffer container grew MTB one week after inoculation. This documented that our pseudo-outbreak has resulted from point-source reagent contamination, as it was limited to the specimens processed with that single contaminated bottle of phosphate buffer. However, the cross-contamination during the addition of antibi-

Table-II: Presumed Cases of Laboratory Cross-Contamination Detailing Symptoms, Histopathology & Final Diagnosis

Case	*Reason Investigated*	*Skin Test*	*Histopathology Diagnosis*	*Assessment Conclusion*
A	Left knee pain, fever CXR[a]-miliary pattern	- ve	Synovial tissue: not suggestive of TB,	Left knee septic arthritis
B	Elderly, BA, fever, cough	ND	-	Acute exacerbation of BA[b]
C	Elderly, Bronchiectasis, Fever, Cough, hemoptysis, CXR Cystic changes	ND	-	Acute exacerbation of Bronchiectasis
D	Elderly, Medline neck swelling	- ve	Benign thyroid cyst	Thyroid cyst
E	BA, DM[c], productive cough, Fever, CXR – Rt. Middle lobe consolidation	- ve	-	Ashmatic exacerbation + Rt .side pneumonia
F	BA, DM, persistent productive Cough, CXR, bilateral infiltrate	- ve	-	BA, Broncho pneumonia
G	9 years old with iliopsoas Abscess, Lt, ascroilitis (fever, Lt. hip pain)	ND	Sacroiliac joint aspirate: no evidence of Osteomyelitis	Left hip septic arthritis
H	MS[d], fever, headache, Photo-Phobia, neck stiffness	- ve	-	Relapsing MS
I	4 years old child with chronic cough and hemoptysis CXR – Rt. lower lobe collapse	ND	-	Foreign body inhalation
J	42 years male, Breast lump	Not available	-	Unknown
K	Abdominal distention, Abdominal pain, fever	ND	-	Carcinoma of colon; Bacterial peritonitis
L	Sterile pyuria	ND	-	UTI
M	DM, Hemoptysis, CXR-Normal	ND	-	Allergy
N	Unknown	ND	-	Case Notes Unavailable

[a]CXR = Chest X-ray, DM[c]= Diabetes Mellitus, BA[b] = Bronchial Asthma, MS[d] = Multiple Sclerosis

Table-III: Possible cases of Laboratory Cross-contamination detecting Symptoms, Therapy-TST and Final Diagnosis

Case		*TST*	*Histopath*	*Rx*	*ATT*	*Ass. Conclusion*
L	Pleural effusion: Cervical lymphadenitis & SLECXR – Reticulonodular shadowing both lungs, effusion	15 mm	LN Biopsy: granulomatous inflammation	—	Full course	Treated as case of TB lymphodenitis
M	Elderly, persistant cough and hemoptysis (3 years) CXR – cystic changes is left lung lobe	15 mm	—	Cefuroxime for 10 days	None	Presumed case of TB; symptoms and signs improved post antimicrobial treatment
N	Left cervical neck swelling for 1 year, cough, fever, CXR – Rt. Upper lobe; nodular calcification	15 mm	LN biopsy: casceating granuloma,	—	Full course	Presumed case of TB lymphadenitis symptoms and signs decreased postreatment.
O	Lt. submandibular swelling, fever	20 mm	FNA – granulomatous lymphadenitis	—	Full course	(TB lymphadenitis) responded well to ATT treatment.
P	Cervical lymphadenopathy	ND	L.N. biopsy – granulomatous	— lymphadenitis	Full course	Treated as TB lymphadenitis

TST[a] = Tubercllin skin test, ATT[b] = Antituberculosis

otic mixtures (PANTA) and other MGIT supplements cannot be excluded. None of the solutions was available for culture at the time of investigation. This might explain the contamination of sterile specimen, which was cultivated directly without decontamination step. Importantly, we found that at the time of cross contamination episode, processing of specimens was done by two technicians. One of them had no prior experience in the mycobacteriology lab. Several studies found that 1-2 technicians appeared to be responsible for most episodes of cross-contamination.[13,14] This suggests that training and experience in mycobacteriology laboratory techniques are crucial in decreasing cross-contamination.

Recognition of this cross-contamination and its probable cause has resulted in the revision of our lab procedures. Changes in laboratory techniques were designed to minimize the possibility of cross contamination. Reagents such as phosphate buffer which are used in volumes are divided daily into smaller aliquots. Extrapulmonary specimen's especially cerebrospinal fluids are always processed first. In addition two full-time, thoroughly trained and experienced, Tuberculosis technicians are dedicated to mycobacteriology laboratory. We also designated one safety cabinet for dealing exclusively with sputum samples and all positive cultures.

In a recent paper by Caroll, et al (2002), found that the majority of cross-contamination events were associated with processing of culture-negative specimens in the same batch as smear-positive samples obtained within the first 3 days of treatment.[15]

In this study the authors have suggested a set of measures that resulted in the reduction of the cross-contamination rate for processing of sputum for the culture of MTB from 7.3 to 2.1%. One of the important modifications in laboratory procedures was to ensure that the order of sputum processing went from negative to positive specimens.

A false-positive culture may have profound consequences on the clinical management of the patient. Patients with false-positive cultures were subjected to additional physician consultations, radiographic studies and unnecessary administration of antimicrobial therapy resulting in adverse drug reactions. In our experience, false-positive cultures resulted in preliminary treatment of 7 patients, and one patient had drug toxicity. Burman and Reves (2000) suggested specific strategies to decrease

inappropriate treatment with false-positive culture.[16]

Although IS6110 analysis is current standard method for the differentiation of MTB strains, this technique requires a mature culture, is time-consuming, and is available only in specialized laboratories. Our findings suggested that spoligotyping can be used as an initial screening method to rapidly identify potential episodes of laboratory cross contamination. The result of the spoligotype analysis suggested that the 20 strains with pattern S3 were related. However, MTB isolates grouped together on the basis of spoligotyping sometimes can be differentiated by IS6110 fingerprinting. Unfortunately RFLP is not available in our institution.

In conclusion, our report demonstrated a lack of awareness among clinicians and laboratory personnel of the possibility of false-positive culture. Therefore, laboratories and tuberculosis control programs should develop procedures to identify patients having only one positive culture. Such patients should be further evaluated for the possibility of false-positive cultures.

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