Molecular strain typing of *Mycobacterium tuberculosis* isolates to detect cross-contamination events

Proposed modifications to prevent its recurrence

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ABSTRACT

الأهداف: فحص احتمالية حدوث تلوث مخبري بالمتفطرة السلية. وتسليط الضوء ايضاً على مشكلة التلوث المخبري في مختبرات الدرن لدينا.

الطريقة: أجريت الدراسة بوحدة أبحاث الدرن – مركز الأبحاث قسم الطب المقارن – مستشفى الملك فيصل التخصصي و مركز الأبحاث – الرياض – المملكة العربية السعودية. تم إثبات 22 حالة تلوث نتيجة لمعالجة جميع هذه العينات بمحلول ملوث من مختبر محلي بالرياض 1 يوليو 2005. تم إيجاد البصمة الوراثية ل 22 معزولة بإستخدام تقنية السبلقوتايب والتي تعتمد على التفاعل التسلسلي البلمري. تمت مراجعة المعطيات السريرية والوبائية.

النتائج: أثبتت النتائج تلوث 22 حالة نتيجة لمعالجة جميع هذه العينات بمحلول ملوث. جميع المرضى لم تظهر عليهم أعراض متوافقة مع أعراض مرض الدرن. إن عدم موافقة الأعراض المرضية لأعراض المرض وكذلك نتائج البصمة الوراثية للمعزولات وعدم اشتراك المرضى بعلاقة وبائية يوحي بأننا نتعامل هنا مع حالة تلوث مخبري.

خاتمة: استخدام تقنية البصمة الوراثية لمعزولات جرثومة الدرن أصبح ضرورة ملحة لتحديد التلوث المخبري حتى نتمكن من منع الآثار المدمرة لعلاج الدرن الذي قد يعطى لمرضى غير مصابين بالمرض.

Objectives: To investigate possible cross-contamination events of *Mycobacterium tuberculosis* cultures, and also to shed light on cross-contamination problems in our laboratories.

Methods: At the TB Research Unit in the Department of Comparative Medicine Research Centre of King Faisal Specialist Hospital and Research Centre, Riyadh, Kingdom of Saudi Arabia, we received 22 TB isolates sub-cultured on Lowenstein-Jensen media from a local laboratory in Riyadh on 1st July 2005. We finger printed all 22 isolates in question using a polymerase chain reaction-based spoligotype molecular technique. The epidemiological and clinical data were reviewed.

Results: All 22 cases had been proven to be cross-contaminated as a result of processing all specimens using a contaminated buffer. All of these patients had no clinical course consistent with tuberculosis. The discordant clinical pictures, and a deoxyribonucleic acid fingerprint that matches those of other culture-positive specimens processed concurrently, in addition to a lack of an epidemiological link between the patients suggest cross-contamination events.

Conclusion: Using molecular techniques has become an absolute necessity to detect cross-contamination events in our laboratory, to prevent the deleterious consequences of cross-contamination in patients.

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Cross-contamination is the inoculation of a negative sample by mistake from a positive sample. A cross-contamination takes place during the culture of mycobacteria in the laboratory.[1] Cross-contamination has serious and deleterious consequences on patients, as patients receive unnecessary treatment. Cross-contamination may take place under certain conditions including particles of aerosol production after the

processing of smear-positive specimens, cultures positive for *Mycobacterium tuberculosis* (*M. tuberculosis*), or positive control strains during the processing of other samples, or within the same day. False-positive should be suspected if *M. tuberculosis* is cultured from a sample processed together with a smear-positive specimen, if *M. tuberculosis* is cultured from only one of the cultures in the set (usually with a low yield of bacteria), and if the clinician is considering an alternative diagnosis, that is, a diagnosis other than tuberculosis (TB).[2] Suspicion of false positivity should be considered when more than one condition mentioned above is met. False-positive culture can also occur due to procedures occurring outside the laboratories; these include patient sampling, microscopy, and specimen collection.[3] The use of inadequately cleaned instruments, such as fiber optic bronchoscopes, are also sources of cross-contamination. These types of instrument have resulted in both false-positive culture and transmission of the disease to healthy individuals. In laboratories where the definitive diagnosis takes place, cross-contamination may occur due to both sensitive recovery systems in use, and the ability of the bacilli to survive outside the host for extended periods. Live tubercle bacilli can be isolated even after 3 weeks of processing from heat-fixed sputum smears, and from 0.9% sodium chloride decontamination solutions. Cross-contamination may falsely increase the number of cases. Also, it has a consequences on patients and their relatives (as a contact), and for health care resources. For the patients, when they are administered with potential toxic antibiotics unnecessarily.[4] Contacts are also bothered by requiring them to come to screening clinics, which involves a minimum of 2 visits to have their skin tested, and read. In case of skin test positivity, the clinician might ask them to visit the x-ray department in order to have their chest x-ray taken. Another visit in 6 months time may be also requested. For health services, it is time consuming and increases the pressure on the budget, as these services may not have been budgeted for. In addition, such screening may also put additional pressure on limited personnel.[5] False-positive cultures for *M. tuberculosis* have important implications for clinical management of patients, as it may also cause drug resistance upon administering the antibiotic course unnecessarily. It is also worth mentioning in addition to all of the above, unnecessary treatment may increase spending to buy antibiotics, causing further stretches to the budget. Most important of all, is the side effects of potentially toxic drugs. Genotyping of *M. tuberculosis* strains has become available with tools to help investigators to determine whether isolates are clonal.[6-8] Genotyping techniques, in combination with a review of clinical and radiographic data, allows a determination of the incidence of laboratory cross-contamination of *M. tuberculosis* cultures. We report in this paper cross-contamination events involving 22 patients, that took place in one local TB laboratory in the country, and also, we aim to shed some light on cross-contamination in our laboratories in general, and the proposed means to deal with this problem.

Methods. At the TB Research Unit in the Department of Comparative Medicine Research Centre of King Faisal Specialist Hospital and Research Centre (TBRU-KFSHRC), Riyadh, Kingdom of Saudi Arabia (KSA), we received 22 TB isolates sub-cultured on Lowenstein-Jensen (LJ) media from a local laboratory in Riyadh on 1st July 2005, suspecting cross-contamination as a result of sudden increase in the positivity of samples at a particular time of the year. The records of this laboratory had never shown such an increase over many years. It has been noticed that all isolates treated in that interval became positive on culture. This was an unusual circumstance, and happened for the first time. It is worth mentioning that not all specimens were received on the same day. However, they were processed using a common phosphate buffer. This buffer is used to neutralize the specimen after decontamination with sodium hydroxide. Upon receiving the isolates, the spoligotype technique was applied, in brief, deoxyribonucleic acids (DNAs) were extracted, then the whole direct repeat region was amplified with 2 primers (DRa and DRb), then the amplified DNA was hybridized to a set of spacer oligonucleotides covalently bound to a membrane in parallel lines (Isogen Life Science, Lagedijk, The Netherlands). Hybridized DNA was detected, after washing with the Enhanced Chemoluminescence System (ECL) (Amersham, Buckinghamshire, United Kingdom), and by exposing ECL-Hyper film (Amersham, Buckinghamshire, United Kingdom) to the membrane.[9] A final determination of laboratory cross-contamination was made, after all the data and clinical findings where reviewed. This study was approved by both Ethics and Basic Committees of the Office of Research Affairs at King Faisal Specialist Hospital and Research Centre, Riyadh, KSA.

Analysis of the data. The films were scanned, and then transferred into Bionumerics software (Applied Maths Inc., Kortrijk, Belgium), and analyzed. A dendrogram was drawn based on the data supplied.

Patient's data. The medical records for all 22 patients with the possibly contaminated specimens were reviewed.

Results. The possible source of the contamination was a Saudi male admitted to the medical ward with TB clinical picture, positive skin test, and x-ray results indicating abnormalities in the lung. Fourteen out of these 22 patients were ruled out of having TB, as the

clinical pictures were not consistent with TB features, and therefore considered having presumed false-positive reports due to a laboratory cross-contamination event. In another 6 patients, cross-contamination could not be ruled out as the existence of unrelated pulmonary diseases made the diagnosis very difficult. A total of 4 patients received anti-TB drugs. One patient out of those 4 patients suffered drug toxicity. In one of these patients, treatment was discontinued after 2 months when spoligotyping results became available.[10] The patient's clinical and epidemiological data is discussed elsewhere,[10] as the focus of this study is to highlight the concept of the cross-contamination events as a possible problem in our laboratories.

Genotype results. All isolates are identical as shown from the dendrogram in **Figure 1**. The obtained patterns were compared with an updated SpolDB4 database (http://www.pasteur-guadeloupe.fr:8081/SITVITDemo),[11] and found to belong to the Manila clade. The Manila clade, as the name suggests was identified the first time in Manila, the capital city of the Philippines. As any other TB strains, this strain (Manila) spread across the whole world, as it exist now in many countries including Saudi Arabia as a result of the existence of the large population of Filipinos from the Philippines in KSA. Previously, this strain had been identified in KSA in a study attempting to genotype and identify all strains existing in KSA. This study showed that Manila is not a predominant strain in KSA compared to Beijing, and Indian strains.[12]

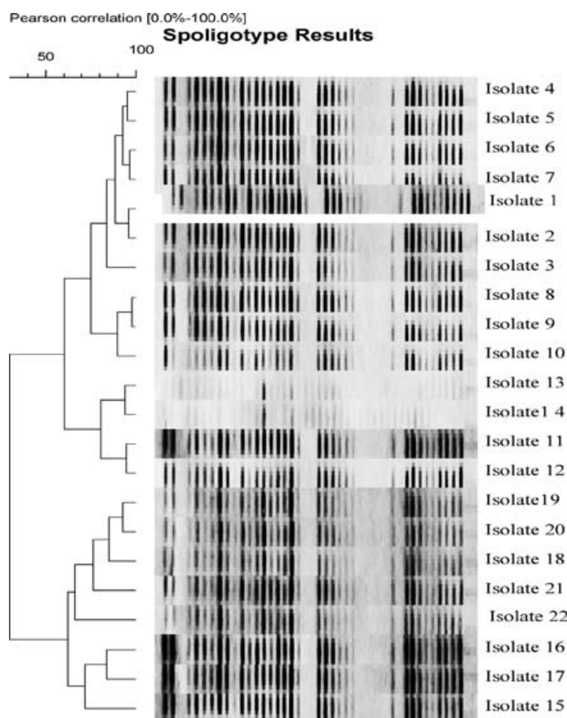

Figure 1 - Genotype of the strains using spoligotype techniques. The technique is based on the amplification of the direct repeat region. The polymorphisms in the direct repeat region were determined by either presence or absence of spacers, which separates between the direct repeats. Binumerics software and the unweighted pair group method using arithmetic averages were used to find the similarities between the strains.

Discussion. We report here a possible cross-contamination event, which took place in one of the optimal TB laboratories in KSA. The genotype results in addition to the clinical and epidemiological data (which showed no links between the patients) suggests a possible cross-contamination event. The cross-contamination that took place in a local laboratory is a worrying sign, and strong attention should be drawn to what might be happening elsewhere in other laboratories. On personal visit to different TB centers around the country, we noticed that little attention is paid to cross-contamination. As a matter of fact, many individuals confuse the cross-contamination with fungus and bacterial contamination.[1,2] Also most of these centers are not optimal in terms of setting, and have high turnover of specimens. These 2 conditions make cross-contamination possible. Through personal communication with some physicians, we noticed that cross-contamination events occurred even at the most optimal TB laboratories in the country.

Cross-contamination can take place in the best laboratories in the world. However, the difference between our laboratories and others is that international laboratories do recognize the problem, and deal with it accordingly.[13] As we mentioned earlier, the inability to spot cross-contamination events leads to unnecessary treatment, which may in turn leads to toxicity of the patients, increase the chance of antibiotic resistance, and increase pressure on the health budget. Also, false reporting due to cross-contamination may cause pressures on health personnel in case of trace contact investigation.[14-16] The estimated cost was calculated for 3 patients treated unnecessarily with anti-TB drugs in Massachusetts in 1998 and 1999 in the United States of America. The cost was estimated to be a total of US$32,618. Of the total, 97% was attributed to the public sector (local and state health departments, public health hospital and laboratory, and county and state correctional facilities), 3% to the private sector (physicians, hospitals, and laboratories), and <1% to the patient. The average cost per patient was US$10,873 based on the calculation of hospitalization, drugs, and testing procedures.[17] In comparison, little is known on the cost of cross-contamination in KSA. Indeed, there is no study in the literature showing the cost of cross-contamination events in Saudi Arabia. We are hoping that through this article, special attention will be paid to cross-contamination problems in our laboratories.

The limitations and problems that we encountered, during the current study include the technical aspects, and gathering information on some of the patients, and keeping good records on the turn-over of the specimen to a particular laboratory. Applying genotyping techniques is not always easy, as it might encounter contamination with polymerase chain reaction. Also, analysis of the data needs special training as it requires dealing with sophisticated software. For these reasons, it might be good to keep the study of cross-contamination events restricted to highly specialized centers in the country.

Detection and recognizing cross-contamination events in the laboratories can eliminate intervention with unnecessary treatment, (and therefore, prevent both toxicity), and to a large extent reduce strains on budget. To minimize the cross-contamination events, we recommend the following: isolate each specimen completely so there are no opportunities to transfer an inoculum from one sample to another by pipetting, caps of the tubes, splashes, or common reservoirs of reagents, or containers used for discarded materials. Reagents such as phosphate buffer, which are used in volumes that are difficult to transfer via a pipette should be aliquated into individual sterile tubes. Separate tubes can be used to dispense reagents to each sample. Using a common container using one dispenser is wrong practice, as it may lead to cross-contamination. Open one tube at a time to add reagents. This will reduce carry-over, and also reduce the chance of interchanging the caps of the tubes. Opening several tubes of specimens at one time, and adding reagents to them at the same time should be avoided. Do not discard the supernatant after centrifugation into a common container. In the contrary, supernatant for each tube should be poured into a separate container, preferably with a cap. After centrifugation, pour off the supernatant from each specimen into a separate disposable discard tube instead of using a common discard container. Internal policy procedures should be written clearly for the processing of cultures. The procedures should contain details of possible mistakes, and details of how to handle these mistakes if it ever happen. All the TB laboratory staff should receive proper training on how to handle cultures, processing samples, and how to spot and solve possible cross-contamination. All the procedures should be reviewed and updated from time to time by the head of the laboratory. Track positivist rates prospectively, and establish thresholds that provoke investigation, when these thresholds are exceeded.[18-19] Communicate with the physicians, if the epidemiologic information demonstrates that it is unlikely that patients could be infected by the same strain, and suspected false-positive must be finger printed. Routine check-out should be carried out in particular in those laboratories, which receive high number of specimens to be processed on a daily basis. As a matter of fact, such laboratories are more prone to cross-contamination events than any other laboratories.[19-21] The concept of cross-contamination is lacking in many laboratories. Many individuals confuse cross-contamination with the concept of the contamination of isolates with fungus, or bacteria. Therefore, special emphasis on such concepts should be exerted, and proper training for professionals should be carried out. Molecular techniques should be applied to investigate cross-contamination suspected events.[14,16,22]

In conclusion, cross-contamination can take place in the best laboratory in the world, therefore, there must be an available well-built strategy to detect, and deal with cross-contamination. We believe this study is just a highlight of what could be undetected ongoing cross-contaminations. Therefore, we would like to bring the authorities' attention to the cross-contamination problem.

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