World Journal of Medical Sciences 6 (3): 121-125, 2011
ISSN 1817-3055

Specificity of Sputum Smear Compared to Culture in Diagnosis of *Pulmonary tuberculosis*

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Abstract: The specificity of sputum smear in comparison with culture in diagnosis of pulmonary tuberculosis was determined. Three hundred and twenty nine smear-positive sputum samples were collected from 3 tuberculosis clinics in Khartoum State, Sudan, decontaminated and cultured onto Loöwenstein-Jensen medium. Growth was monitored daily during the first week for the presence of rapidly growing mycobacteria, nocardiae or other acid-fast organisms and then weekly for up to 8 weeks for the presence of *Mycobacterium tuberculosis* complex (MTC). The isolates with microscopic and cultural properties consistent with MTC were confirmed by biochemical testing and IS6110-PCR. The percentage of agreement between the sputum smear and culture was 89.4%. The rest of the samples (10.6%) revealed either *Nocardia* species, rapidly growing mycobacteria, found to be contaminated or showed no growth. This study indicated that acid-fast bacilli such as *Nocardia* spp. and rapidly growing mycobacteria should be considered when smear method is used to diagnose pulmonary tuberculosis and a confirmatory diagnostic method such as sputum culture or PCR is necessary to avoid the implications of mistreatment.

Key words: Specificity · Sputum smear · *Mycobacterium tuberculosis* · Rapidly growing mycobacteria · Nocardia

INTRODUCTION

Diagnosis of tuberculosis in the developing countries, where over 90% of TB cases occur [1], is based primarily on symptoms, microscopic examination of smears for acid-fast bacilli (AFB) and, occasionally on chest x-ray [2]. Slide microscopy is a convenient, rapid and economical test used for determination of *M. tuberculosis* infection. However, microscopic errors are likely to misclassify or misdiagnose cases as non cases and the vice versa and therefore compromise the national efforts to control tuberculosis. Mycobacteria other than tuberculosis bacilli (MOTT) are found in the environment, but they can also colonize man [3]. In immuno-compromised patients, MOTT such as *M. avium* and *M. intracellulare*, have become important in clinical practice [4]. Organisms other than mycobacteria may demonstrate various degrees of acid-fastness. Such organisms include *Rhodococcus* spp., *Nocardia* spp., *Legionella* spp. and the cysts of *Cryptosporidium* and *Isospora* spp. [5]. Clinical symptoms and x-ray may not be specific too, especially in immuno-compromised patients. Pulmonary nocardiosis mimics pulmonary tuberculosis both clinically and radiologically and many a time is wrongly treated with anti-tuberculosis drugs [6]. Mistreatment with TB drugs takes a very long time, costly and it may lead to suffering and ultimate death [7]. So, the accurate detection of *M. tuberculosis* is of paramount importance in the effective treatment and control of TB [4].

Bacteriological culture can identify the *M. tuberculosis* organism in over 80% of TB cases with a specificity of over 98% [8]. Being more specific than the sputum smear, it can be used to confirm the results of microscopic examination [9]. Genotypic methods such as PCR were also claimed to be more specific than smear method [6].

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In Sudan, the studies that have attempted to evaluate the specificity of microscopy diagnosis of pulmonary tuberculosis in comparison to culture are very scarce. Accordingly, this study was undertaken to more enrich the literature about the topic.

MATERIALS AND METHODS

Samples: Three hundreds and twenty nine smear-positive sputum samples were obtained from 3 TB clinics in Khartoum State, Sudan, during the period from October 2005 to December 2006. The sputum samples were collected from patients with symptoms of pulmonary TB according to WHO [10] criteria and tested for presence of acid-fast bacilli by technicians at the laboratories of these clinics. Samples were cultured and their isolates were identified in the Tuberculosis Laboratory, National Health Laboratory, Federal Ministry of Health, Khartoum. The patients were aged between 5 and 70 years, 238 (72.3%) and 91 (27.7%) were males and females, respectively. The majority of them came to the clinics from remote poor areas in the Sudan.

Culture Method: Each sputum sample was aseptically transferred into sterile 50 ml conical tube and decontaminated at room temperature with equal volume of 3% NaOH. The mixture was vortexed and kept at room temperature for 15 minutes and then it was neutralized with 1N HCl which was added drop by drop until the color changed [11]. Then, it was concentrated by centrifugation at 3000 *g* for 15 min. Two tubes of LJ medium, one contained glycerol and the other contained pyruvic acid [12], were each inoculated with 4 Pasteur pipette drops from the pellet. The inoculated tubes were incubated aerobically at 37 °C for up to 8 weeks before being discarded. Growth was monitored daily during the first week to observe the presence of rapidly growing mycobacteria, nocardiae or other organisms and then it was observed weekly. Characteristics of different colony types were recorded and smears for Ziehl-Neelsen (ZN) staining were made. Purification was ensured by repeated sub-culturing and pure isolates were maintained in slants of LJ medium at 4°C.

Biochemical Testing: Biochemical tests included catalase at 68°C, nitrate reduction [13] and sensitivity to para-nitrobenzoic Acid (PNB) [12]. The tests were conducted for the *M. tuberculosis*-like colonies.

PCR Assay: To confirm the isolation and biochemical results, 100 isolates consistent with *M. tuberculosis* characteristics were randomly taken and tested by a polymerase chain reaction method targeting the insertion element IS6110 [14]. The DNA was extracted according to the method of Kirsi *et al.* [15]. Amplification of the insertion sequence IS6110 was performed with a set of primers having the following sequence: CCTGCGAGCGTAGGCGTCGG and CTCGTCCAGCGCCG CTTCGG in a programmable thermal cycler (TGradient, Biometra, Göttingen, Germany). A master mix of reagents of 25 μl was used, which contained: 2.5 μl PCR buffer (QIAGEN), 2.0 μl dNTP mixture (10mM) (Roche), 1.0 μl each primer (25 μM) (Biotech), 0.12 μl Taq polymerase (5 U/μl) (Fermentas), 16.38 μl distilled water and 2.0 μl template DNA. The chromosomal DNA of *M. tuberculosis* strain H37Rv (provided by the National Reference Laboratory, Khartoum) was used as a positive control. PCR conditions were the same as described by Eisenach *et al.* [14]. Amplicons were detected by agarose gel electrophoresis following staining with ethidium bromide and subsequent visualization under ultraviolet light.

RESULTS

Isolation: Of 329 sputum samples, 294 (89.4%) gave growth that was compatible with *M. tuberculosis*, 10 (3.0%) revealed growth consistent with *Nocardia* spp., 6 (1.8%) were considered rapidly growing mycobateria, 6 (1.8%) were found contaminated and 13 (4%) showed no growth. The growth rate of *M. tuberculosis* isolates ranged between 2 to 5 weeks. Smears from all colony types were found to be positive for AFB. Cultural properties of all isolates of *M. tuberculosis* were almost the same and all colonies were dry, rough and cream (buff) colored.

Biochemical Testing: Out of 294 of *M. tuberculosis*-like isolates, 214 (72.8%) were positive for para-nitrobenzoic acid (PNB); 223 (75.8%) were positive for nitrate reduction and 278 (94.5%) were catalase negative at 68°C.

PCR: All tested isolates showed a band that was typical in size (123bp) to the target gene (1S6110) as indicated by the standard DNA marker (Fig. 1).

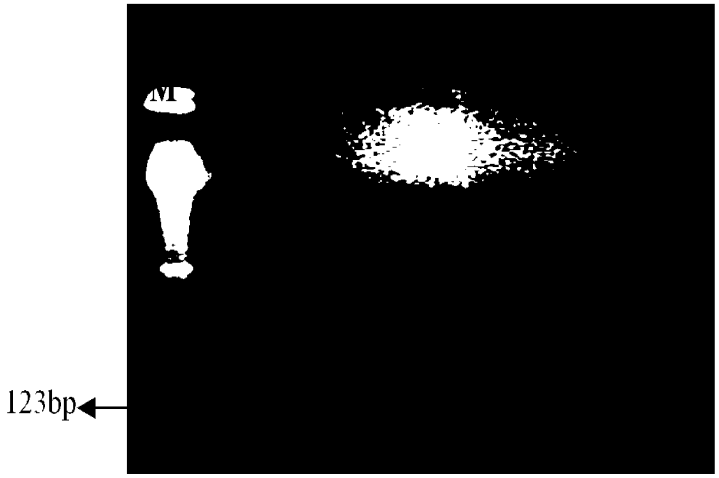

Fig. 1: A photograph of agarose gel electrophoresis of the PCR product: Lane M: DNA marker, Lane P: positive control, lane N: negative control and lanes 4-11: clinical isolates.

Table 1: Results of growth types, biochemical tests and PCR

			Biochemical reactions (% of positive isolates)			
Growth type	% of samples positive	Duration of growth	NO_3 red.	PNB	Catalase at 68 °C	PCR
M. tuberculosis	89.4	2-5 wks	75.8	72.8	5.4	+
Nocardia	3.0	≤ 7 d	ND	ND		
Rapidly growing mycobacteria	1.8	≤ 3 d	ND	ND		
Contaminated samples	1.8	1-3 d				
No growth	4.0	8 weeks				

+: positive, ND: Not Done

DISCUSSION

Tuberculosis poses a serious health problem in developing countries such as the Sudan. Besides clinical symptoms and chest x-ray, the diagnosis of pulmonary tuberculosis is mainly based on microscopic examination of smears for acid-fast bacilli [2]. However, the positive result may not be always specific. There are acid-fast organisms other than tubercle bacilli and some can cause pulmonary infections or co-existing with other infections [16]. So, more specific method of diagnosis or confirmation of microscopy examination is necessary to avoid the implications of mistreatment.

In the present study, using the conventional LJ medium culture method, only 89.4% (294/329) of the investigated sputum samples were confirmed to contain *M. tuberculosis* and 10.6% of the samples showed either *Nocardia*spp., rapidly growing mycobacteria or revealed no or contaminated growth. The *M. tuberculosis* isolates were confirmed with biochemical testing and PCR. This percentage of disagreement (10.6%) could be true and is considered very high if the implications of mistreatment are measured. The concentration of the decontaminating agent (3% NaOH) used may be responsible for the presence of the contaminated samples, especially if they contained a high number of contaminants. It is recommended to use 4% NaOH to get rid of non-tubercle bacilli [17]. However, the used concentration may be still high and could exert a killing effect on acid-fast bacilli including tubercle bacilli, especially if they exist in low numbers [17]. Also, there is a possibility that some of the AFB which appeared in the smears has failed to grow in the growth medium and conditions of incubation used in this study [18]. A few studies [6, 19] were carried out in the Sudan to differentiate clinical isolates of *M. tuberculosis* complex species and none was able to confirm the presence of any species other than *M. tuberculosis*.

In this study, the IS6110 target gene is a feature of all species of MTC and therefore it cannot differentiate them. However, the cultural and the biochemical properties of the isolates were more consistent with *M. tuberculosis*

species than with any other MTC species. So, the clinical MTC isolates in the present study are most probably *M. tuberculosis* species. The presence of *Nocardia* spp. and rapidly growing mycobacteria and their level of detection in the current study are compatible with previous works in the Sudan [6] and abroad [7].

In this study, the microscope specificity compared to culture was 89.4%, which is similar to some previous findings. El-Dawi *et al.* [6] have reported 90.5% in the Sudan and Mfinanga *et al.* [7] have reported 88.9% in Tanzania. Slide microscopy is unable to distinguish between tuberculous and non-tuberculous mycobacteria as well as other AFB. The non-tubercle AFB are especially important in immuno-compromised patients [4]. *Nocardia* was found with *M. tuberculosis* causing a mixed pulmonary infection [16] and found alone causing pulmonary nocardiosis which is similar to pulmonary tuberculosis [6]. Nocardiosis is wrongly diagnosed as TB and often wrongly treated with anti-tuberculosis drugs [6].

Culture is considered the diagnostic gold standard; it can identify *M. tuberculosis* with a specificity of over 98% [8]. However, it takes at least 2 weeks to allow for sufficient growth for biochemical or genotypic confirmation; and a small percentage of cultures may be contaminated by other non-fastidious microorganisms, making accurate diagnosis difficult and compromising the biochemical identification. But, it can be used to confirm the results of microscopic examination [9]. PCR has also been used for detection of *M. tuberculosis* complex, directly in the sputum samples [6, 9] or after isolation [20] within a few hours and with much higher degree of sensitivity and specificity compared to sputum microscopic examination.

This study is an addition to the previous findings that clinical symptoms, x-ray and sputum smear are of not enough specificity to diagnose pulmonary TB and introduction of sputum culture or PCR is crucial to prevent the mistreatment with TB drugs which may take several months, costly and it may lead to suffering and ultimate death.

REFERENCES

1. Foulds, J. and R. O'Brien, 1998. New tools for the diagnosis of tuberculosis: the perspective of developing countries. Int. J. Tuberc. Lung. Dis., 2: 778-783.
2. Kumar, V., A.K. Abbas, N. Fausto and R.N. Mitchell, 2007. Robbins Basic Pathology 8th Edition. Saunders Elsevier, pp: 516-522.
3. Watt, B., A. Rayner and G. Harris, 1993. Modern methods in mycobacteriology. Rev. Med. Microbiol., 4: 97-105.
4. Zheng, X., M. Pang, H.D. Engler, S. Tanaka and T. Reppun, 2001. Rapid detection of *Mycobacterium tuberculosis* in contaminated BACTEC 12B broth cultures by testing with amplified *Mycobacterium tuberculosis* direct test. J. Clin. Microbiol., 39: 3718-3720.
5. Echeverri, C., J. Matherne, J.H. Jorgensen and L.J. Fowler, 2001. Fite stain positivity in Rhodococcus equi: Yet another acid-fast organism in respiratory cytology-a case report. Diagn. Cytopathol., 24: 244-246.
6. El-Dawi, T.G., N.S. Saeed and M.E. Hamid, 2004. Evaluation of a PCR-amplified IS6110 insertion element in the rapid diagnosis of pulmonary tuberculosis in comparison to microscopic methods in Sudan. Saudi Med. J., 25: 1644-1647.
7. Mfinanga, G.S., E. Ngadaya, R. Mtandu, B. Mutayoba, D. Basra, G. Kimaro, *et al.*, 2007. The quality of sputum smear microscopy diagnosis of pulmonary tuberculosis in Dar es Salaam, Tanzania. Tanzania Health Res. Bull., 9: 164-168.
8. Lee, J.J., J. Suo, C.B. Lin, J.D. Wang, T.Y. Lin and Y.C. Tsai, 2003. Comparative evaluation of the BACTEC MGIT 960 system with solid medium for isolation of mycobacteria. Int. J. Tuberc. Lung. Dis., 7: 569-574.
9. Al Zahrani, K., H. Al Jahdali, L. Poirier, P. René and D. Menzies, 2001. Yield of smear, culture and amplification tests from repeated sputum induction for the diagnosis of pulmonary tuberculosis. Int. J. Tuberc. Lung. Dis., 5: 855-860.
10. WHO, 1996. Report of the tuberculosis epidemic. World Health Organization, Geneva, Switzerland.
11. Petroff, S.A., 1915. A new and rapid method for the isolation and cultivation of tubercle bacilli directly from the sputum and feces. J. Exp. Med., 21: 38-42.
12. Collee, J.G., A.G. Fraser, B.P. Marmion and A. Simmons, 1996. Mackie and McCartney Practical Medical Microbiology 14th Edition. Churchle Living Stone, London, pp: 329-337.
13. Betty A.F., F.S. Daniel and S.W. Alice, 1998. Diagnostic Microbiology 10th Edition. Mosby, London, pp: 715-781.
14. Eisenach, K.D., M.D. Cave, J.H. Bates and J.T. Crawford, 1990. Polymerase chain reaction amplification of a repetitive DNA sequence specific for *Mycobacterium tuberculosis*. J. Infec. Dis., 161: 977-981.

15. Kirsi, P., M. Merja, R. Nalin, S. Christophe, F. Ingrid, R. Petri, H. Pekka, K.V. Matti and S. Hanna, 2003. Characterization of Finnish *Mycobacterium tuberculosis* isolates by spoligotyping. J. Clin. Microbiol., 41: 1525-1538.
16. Inthraburan, K. and A. Wongsa, 2009. Empyema thoracis due to nocardiosis and *Mycobacterium tuberculosis* mixed infections in an AIDS patient. Southeast Asian J. Trop. Med. Public Health, 40: 776-780.
17. Tamara, V.N., J.W. Burdza and K. Amin, 2003. Evaluation of sputum decontamination methods for *Mycobacterium tuberculosis* using viable colony counts and flow cytometry. Diagn. Microbiol. Infect. Dis., 47: 503-509.
18. Flournoy, D. and J. Twilley, 2001. Modified Middlebrook 7H9 broth for the rapid detection of mycobacteria. Clin. Lab. Sci. Spring, 14: 85-88.
19. Abass, N.A., K.M. Suleiman and I.M. El Jalii, 2010. Differentiation of clinical *Mycobacterium tuberculosis* complex isolates by their *GyrB* polymorphism. Ind. J. Med. Microbiol., 28: 26-29.
20. Shinnick, T. and R. Good, 1995. Diagnostic mycobacteriology laboratory practices. Clin. Infect. Dis., 21: 291-299.