Original Article

Utility of MPT 64 antigen detection to differentiate between mycobacterium tuberculosis complex and other mycobacteria species

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Abstract

Background: India has the highest burden of tuberculosis accounting for one fifth of the global incidence. The clinical presentation of pulmonary disease due to nontuberculous mycobacteria (NTM) is similar to that caused by *M. tuberculosis* complex (MTBC).Accurate diagnosis of tuberculosis is crucial to facilitate early treatment of the patients, and to reduce its spread. Conventional methods are cumbersome and genotypic methods need expertise. A MPT64 TB Ag rapid test has been reported to identify the MTBC from the NTM. The present study was conducted to check the utility of the MPT64 TB Ag test. **Material and Methods:** A total of 100 mycobacterial isolates recovered from both pulmonary (50 isolates) as well as extra pulmonary specimens (50 isolates) were screened to differentiate the growth of mycobacteria into MTBC and NTM by phenotypic characters and biochemical tests. Further they were tested by MPT64 TB Ag test and results were compared to evaluate the utility of MPT64 TB Ag test. **Results:** Among 100 mycobacterial isolates, 98 isolates were identified as MTBC and the two isolates from extra pulmonary cases were identified as NTM on the basis of biochemical tests. There was no discrepancy found in differentiation of these isolates between biochemical tests and TB Antigen MPT64 rapid test. **Conclusion:** The sensitivity and specificity of TB Antigen MPT64 rapid test was 100% as compared to biochemical methods. The MPT64 TB Ag test was found to be a simple, rapid and reliable test to differentiate MTBC from NTM.

Keywords: Tuberculosis, *M. tuberculosis* complex, Nontuberculous mycobacteria, MPT64 TB Ag test.

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INTRODUCTION

Tuberculosis (TB) is one of the major causes of morbidity and mortality worldwide. One fourth of the global incident TB cases occur in India annually. As per WHO Global TB Report, 2015, out of the estimated global annual incidence of 9.6 million TB cases, 2.2 million were estimated to have occurred in India.[1] The *Mycobacterium tuberculosis* complex (MTBC) is a known agent for infectious pulmonary TB. In recent years, disease caused by mycobacteria other than tuberculosis (MOTT), also called asnontuberculous mycobacteria (NTM), are on the rise due to parallel increase in HIV infection and other immunocompromisedstates[2]. The clinical presentation of pulmonary MTBC or NTMinfections may be similar as their signs and symptoms often resemble, and the differentiation through acid fast stain is also not possible. Identification and speciation of the mycobacterium becomes essential for the appropriate management and treatment of the affected individuals. Conventional phenotypic identification methods are laborious and time-consuming, while genotypic methods require expertise and infrastructure[3,4]. Thus a simple, rapid, and sensitive discriminatory test for rapid identification of the *M.*

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tuberculosis complex is necessary for accurate diagnosis and treatment of the disease. The Mycobacterium tuberculosis protein 64 (MPT-64) antigen is one of the major culture filtrate protein (24 kDa) encoded by the RD2 region genes and has been shown to be a specific antigen that differentiates the MOTT species[5]. An MPT64-based, simple and rapid immunochromatographic assay known as the SD Bioline TB Ag MPT64 RAPID test (SD Bioline kit) has been reported to identify the *M. tuberculosis* complex from the NTM using the mouse monoclonal anti-MPT64 antibody[6]. The present study was conducted to check the utility of the SD Bioline kit for the identification of mycobacteria in the liquid media. These findings were correlated with a conventional biochemical test and the sensitivity and specificity of this kit were also assessed.

MATERIAL AND METHODS

The study was carried out after obtaining Institutional Ethical Committee's permission. In this prospective study, a total of 100 mycobacterial isolates on Lowenstein-Jensen (L-J) medium recovered from both pulmonary (50 isolates) as well as extra pulmonary specimens(50 isolates) were included. The isolates were confirmed by Ziehl -Neelsen (ZN) staining. The positive cultures were screened to differentiate the growth of mycobacteria into MTBC and NTM by niacin accumulation test, nitrate reduction test, heat stable catalase at 68^0C/pH7 and Para- nitrobenzonic acid (PNB) susceptibility test [2].The cultures which were not identified as MTBC and suspected to be NTM were further identified by rate of growth, pigment production, Urease test, Tween – 80 hydrolysis test, Arylsulfatase test, Mac Conkey agar test and Sodium Chloride tolerance test[2]. All the cultures were also subjected to TB Antigen MPT64 rapid test for differentiation into MTBC and NTM. Briefly,[3-4] colonies from L-J were emulsified in 200 µl of extraction buffer. 100 µl of suspended solid culture in buffer was added into the sample well. The inoculated cassettes were kept undisturbed atroom temperature and were examined at the end of15 minutes for presence of pink band in "Control" and "Test" region[7]. The appearance of control band confirmed the validity of the test. If the control band was not visible in 15 minutes, the result was considered invalid and the sample was retested. The presence of only control band in the absence of test band wasconsidered a negative test. Presence of both control and test band indicated a positive test.

RESULTS

All the isolates from pulmonary cases (n=50) were positive for niacin and nitrate reduction test while negative for catalase and PNB test. In extrapulmonary isolates, 48 were positive for niacin test, negative for catalase and PNB test while 49 were positive for nitrate reduction test. The two isolates from extrapulmonary cases were positive for PNB test. Therefore, all the 98 isolates were identified as MTBC and the two isolates from extrapulmonary cases identified as NTM on the basis of biochemical tests. These two NTM isolates from extrapulmonary specimens were further identified as *Mycobacterium fortuitum* and *Mycobacterium chelonae* on the basis of biochemical tests. These isolates belong to Group IV (Rapid Growers) of Runyon's Classification. When subjected to TB Ag MPT64 Rapid test, the control band was seen in all the tested cultures (n=100), validating the test.All the 50 isolates from pulmonary cases and 48 isolates from extrapulmonary cases showed positive results (visible band for MPT64 antigen) and the two isolates from extrapulmonary cases gave negative results.

Table 1: Comparison of differentiation of MTBC and NTMby conventional biochemical methods and TB Antigen MPT64 Rapid test

MPT64 Ag Test Result	Biochemical Test Result		Total
	MTBC	NTM	
Positive	98	0	98
Negative	0	2	2
Total	**98**	**2**	**100**

There was no discrepancy found in differentiation of these isolates between biochemical tests and TB Antigen MPT64 rapid test (Table 1).The sensitivity and specificity of TB Antigen MPT64 rapid test was found to be 100% as compared to biochemical methods.

DISCUSSION

M. tuberculosis poses diagnostic and therapeutic problems due to low sensitivity of the diagnostic tools available for its identification and discrimination with NTM. In developing countries like India, more than 90% of tuberculosis infections are still caused by *M. tuberculosis*.NTM are emerging as important causative agents of pulmonary and extra pulmonary disease in immuno-compromised as well as immuno-competent hosts. Many small hospital laboratories do not discriminate between MTBC and NTM, meaning that NTM are inappropriately managed with first- line tuberculosis drugs which worsens the patient's condition and raising the risk of drug resistance. Recently, Mycobacterium tuberculosis protein 64 (MPT-64) antigen is found to be specific for MTBC which is secreted during mycobacterial growth. Cost-effective analytical studies of MPT64 TB Ag test, other rapid molecular methods and culture combined with conventional

biochemical tests have shown MPT64 TB Ag test as more economical than the other two methods[8]. Further, this test requires only 15 min of analysis as compared to other methods. Kumar *et al* (India)[8], Toihir *et al* (Madagascar)[9] and Abe *et al* (Japan)[6] showed 100% sensitivity and specificity as seen in the present study. The MPT-64 TB antigen is a M. tuberculosis complex specific antigen secreted during bacterial growth, thus making it an excellent antigen for the identification of MTBC. In conclusion, MPT64 TB antigen detection does not require any special equipment, is simple and less time consuming. It can easily discriminate between MTBC and NTM and thus can help in appropriate management of tuberculosis.

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