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SHORT COMMUNICATION

Evaluation of thin-layer agar 7H11 for the isolation of *Mycobacterium tuberculosis* complex

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SUMMARY

We evaluated the thin-layer agar (TLA) method for the recovery of *Mycobacterium tuberculosis* complex and compared the results with the BACTEC Mycobacteria Growth Indicator Tube (MGIT) 960 system. A total of 53 mycobacterial isolates were isolated on both media. The recovery rates of mycobacteria on TLA and BACTEC MGIT 960 system were respectively 90.6% and 96.2%. Mean time to detection of mycobacteria on TLA was 12.5 compared to 11.2 days on BACTEC MGIT 960. TLA is a simple technique and can be used as an alternative to the Löwenstein-Jensen medium and BACTEC MGIT 960 for the isolation of mycobacteria in resource-poor settings.

KEY WORDS: BACTEC; culture; *Mycobacterium tuberculosis* complex; tuberculosis

PAKISTAN ranks sixth among the high tuberculosis (TB) burden countries, with an incidence rate of 181 per 100 000 population and a prevalence of 359/100 000.[1] Direct microscopy and Löwenstein-Jensen (LJ) medium are still considered the gold standard for the diagnosis of TB in low-income countries. However, these methods have either low sensitivity, especially in paucibacillary pulmonary and extra-pulmonary TB,[2] or take a longer time (3–4 weeks) for mycobacterial culture. The BACTEC 460 system and the BACTEC Mycobacteria Growth Indicator Tube (MGIT) 960 system (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD, USA) have been studied and evaluated in various contexts.[3,4] Thin-layer agar (TLA) and MODS (microscopic observation broth drug susceptibility) are two promising modalities that detect the presence of mycobacteria by microscopic observation of colonies. Both of these methods can detect the colonies microscopically within 1–2 weeks and can also give a presumptive identification of mycobacteria based on their characteristic colony morphology.[5,6]

The objective of the present study was to evaluate the performance of TLA medium in terms of recovery rate, time to detection and contamination rate, comparing it with the BACTEC MGIT 960 system in our settings.

MATERIALS AND METHODS

Place and duration of study

The study was carried out at the Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi, Pakistan, from July to October 2009. A total of 247 consecutive clinical samples (sputum 106, bronchoalveolar lavage 76, pus 8, tissue 8, pleural fluid 20, lymph node 4, peritoneal fluid 11, urine 7, synovial fluid 7) were treated during the 4-month study period. All the specimens (except sterile body fluids) were subjected to the standard N-acetyl-L-cysteine sodium hydroxide digestion-decontamination method as described by Kent and Kubica.[7] Processed specimens were stained with Ziehl-Neelsen (ZN) acid-fast staining and inoculated in both media.

TLA medium

The TLA technique uses Middlebrook 7H11 agar for the detection of microcolonies of various mycobacteria. About 5 ml of the autoclaved 7H11 medium was poured into a 60 mm × 15 mm plastic petri dish. Before pouring the medium, 10% OADC (oleic acid, albumin, dextrose, catalase) and antibiotics, including piperacillin, trimethoprim and amphotericin, were added at concentrations of respectively 0.05, 0.02 and 0.02 μg/ml. A second set of 7H11 medium containing 500 μg/ml of paranitrobenzoic acid (PNB) was prepared to differentiate between *Mycobacterium tuberculosis* complex and non-tuberculous mycobacteria (NTM); 0.1 ml of the concentrated specimen was inoculated onto both plain and PNB TLA. All the plates were sealed with adhesive tape, leaving an area of about 2 cm for ventilation, and incubated at 37°C in a 5–8% CO_2 incubator for 6 weeks. The plates were examined for appearance of microcolonies (cording) twice weekly by optical microscopy (Figure).

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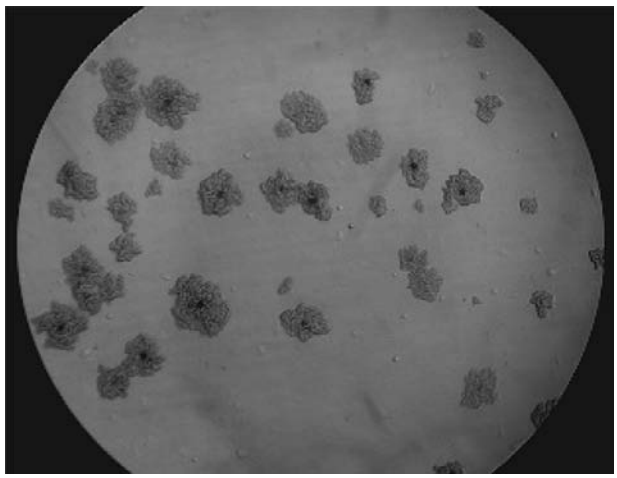

Figure Colonies of ATCC 25177 of *Mycobacterium tuberculosis* examined at 10× magnification at 10 days. ATCC = American Type Culture Collection.

BACTEC MGIT 960

Inoculation into a BACTEC MGIT 960 tube was carried out according to the manufacturer's instructions (Becton Dickinson, USA). Each positive tube was subjected to ZN staining and subcultured on Columbia agar (Oxoid, Basingstoke, UK) containing 5% sheep's blood to check for any contaminant growth. The day when the tubes were placed in the system for incubation was taken as day zero, while the day at which a positive culture tube showed presence of acid-fast bacilli (AFB) was taken as the last day for time to detection. A positive culture in MGIT was further confirmed as *M. tuberculosis* complex by PNB test.[6]

Statistics

Statistical analysis was performed using SPSS version 11 (Statistical Package for the Social Sciences, Chicago, IL, USA). Independent sample *t*-test was used to compare the differences in the mean time to detection of mycobacteria and the recovery rate between MGIT 960 and TLA medium. A P of < 0.05 was considered statistically significant.

Controls

H37Ra ATCC (American Type Culture Collection) 25177 was used as a positive control. An uninoculated MGIT tube was used as a negative control. An institutional control strain of *M. chelonae* was used to check MGIT PNB and PNB used in TLA.

RESULTS

Of a total of 247 clinical samples, 14 (5.6%) were contaminated in the MGIT 960 system and 16 (6.4%) in TLA. None of the contaminated cultures yielded growth of *M. tuberculosis* on any medium. A total of 54 (24.4%) mycobacterial isolates (*M. tuberculosis* complex, $n = 53$; NTM $n = 1$) were recovered from the remaining 217 specimens. Due to very low numbers, NTM was not included in the statistical analysis; 51 (23.5%) were isolated on MGIT 960 and 48 (22.1%) on TLA medium (Table). Rates of recovery of mycobacteria on MGIT 960 system and TLA were respectively 96.2% and 90.6%. Among the 53 positive isolates, 49 (92.5%) were recovered from pulmonary specimens, while only four (7.5%) were isolated from extra-pulmonary specimens. A total of 47 isolates were recovered from pulmonary specimens on MGIT 960 and 44 isolates on TLA (Table). No isolate was recovered from urine, pleural fluid, peritoneal fluid or synovial fluid. Surprisingly, there was not much difference in mean time to detection between MGIT 960 and TLA. The mean time to detection of mycobacteria on MGIT 960 and TLA for smear-positive cases was respectively 9.6 (3–17) days and 10.1 (4–22) days, while for smear-negative cases it was 14.3 (11–21) days on MGIT 960 and 15.8 (12–28) days on TLA. TLA detected 20.9% of the total mycobacterial isolates in the first week, 46.7% in the second week and 18.7% in the third week. Only one isolate was detected in the fourth week. The only NTM was detected in respectively 3 days and 2 days on MGIT 960 and TLA. The P value was 0.69 for recovery of mycobacteria between MGIT 960 and TLA; the difference is non-significant. Colonies of *M. tuberculosis* complex showed characteristic cording, while the NTM recovered had smooth colonies with rounded margins.

DISCUSSION

Early diagnosis and prompt treatment have become a priority in recent years due to the rapid increase in the spread of TB worldwide. Various studies have

Table Relationship of detection time of mycobacteria on individual system/medium with specimen type

		MGIT 960				TLA			
		Smear-positive		Smear-negative		Smear-positive		Smear-negative	
Specimen type	Specimens *n*	Isolates *n*	TTD days	Isolates *n*	TTD days	Isolates *n*	TTD days	Isolates *n*	TTD days
Respiratory	163	30	9.3	17	14.3	30	10.2	14	15.8
Body fluids	35	0	—	0	—	0	—	0	—
Pus, abscess	8	1	11	0	—	1	13	0	—
Tissue	11	3	13.2	0	—	3	12.1	0	—
Total	217	34	—	17	—	34	—	14	—

MGIT = Mycobacteria Growth Indicator Tube; TLA = thin-layer agar; TTD = mean time to detection.

been carried out in various contexts to evaluate the performance of TLA.[5,8–10] This is the second reported study on TLA and its comparison with BACTEC MGIT 960. The majority of the isolates (46.7%) were detected in the second week on TLA, and thus TLA has an edge over the conventional LJ medium for early diagnosis of mycobacteria.

Two smear-negative isolates from pulmonary specimens were recovered only on TLA but not on MGIT 960, and five smear-negative isolates from respiratory specimens were recovered only on MGIT 960. All the smear-positive samples yielded mycobacterial growth on both TLA and MGIT 960.

The mean time to detection of mycobacteria on TLA and MGIT 960 was nearly identical, i.e., respectively 12.5 and 11.2 days. In another study, the mean time to detection of mycobacteria for smear-positive cases on TLA and MGIT 960 was respectively 10.6 days and 9.6 days.[10] Another study reported an average time to detection of mycobacteria using TLA of 11.5 days.[5]

Colonies of *M. tuberculosis* exhibit a characteristic cording with irregular wavy margins. Initially, the colonies appear like small spirals, but as they mature, these small colonies acquire cording with distinct irregular margins and ultimately spread over the entire area when viewed through the microscope. The average cost per test, i.e., AFB culture and sensitivity, on TLA is US$5.8 (500 PKR) and US$9.3 (800 PKR) on MGIT 960.

TLA is rapid, easy to perform and can be implemented as a reliable, economical alternative to conventional LJ medium and even to the automated MGIT 960 system for the detection of mycobacteria in low- to medium-volume laboratories, especially in resource-limited countries. Some constraints when using the MGIT 960 should be taken into consideration before starting this method, such as stable electricity, the time to obtain consumables and maintenance issues. Further improvement of this technique can help in reducing the time to detection of mycobacteria and breaking the chain of TB transmission.

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RÉSUMÉ

Nous avons évalué la méthode sur agar en couche fine (TLA) pour la mise en évidence du complexe de *Mycobacterium tuberculosis* et nous avons comparé ses résultats avec le système BACTEC Mycobacteria Growth Indicator Tube (MGIT) 960. On a isolé au total 53 cultures mycobactériennes sur les deux milieux. Les taux de mise en évidence des mycobactéries ont été respectivement de 90,6% sur TLA et de 96,2% avec le système BACTEC MGIT 960. La durée moyenne avant détection des mycobactéries a été de 12,5 jours avec TLA et de 11,2 jours avec le système BACTEC MGIT 960. La TLA est une technique simple qui peut être utilisée comme alternative au milieu de Löwenstein-Jensen et au BACTEC MGIT 960 pour l'isolement des mycobactéries dans les contextes à faibles ressources.

RESUMEN

En el presente estudio se evaluó el método con cultivo en agar de capa delgada (TLA) para recuperar el complejo *Mycobacterium tuberculosis* y se compararon sus resultados con los del sistema BACTEC Mycobacteria Growth Indicator Tube (MGIT) 960. Se aislaron 53 cepas de micobacterias en ambos medios de cultivo. La tasa de recuperación en agar fue 90,6% y en el sistema BACTEC MGIT 960 fue 96,2%. El tiempo necesario para la detección de micobacterias fue en promedio 12,5 días con el cultivo en agar y 11,2 días con BACTEC MGIT 960. El método TLA constituye una técnica sencilla que se puede usar como alternativa al cultivo en medio Löwenstein Jensen en el sistema BACTEC MGIT 960, con el fin de aislar las micobacterias en los entornos con recursos limitados.