SHORT COMMUNICATION

Use of blood agar along with Lowenstein-Jensen media for rapid isolation of *Mycobacterium tuberculosis* for early drug susceptibility testing

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ABSTRACT

Aims: Tuberculosis diagnosis is still a challenge in resource-limited settings due to poor sensitivity of available tests, long time to reporting and costs. Lowenstein–Jensen (LJ) medium is the most commonly used semisolid medium for culture of *Mycobacterium* species. However, the growth is very slow which delays the reporting. Hence, there is a need of other better medium for obtaining rapid growth of *Mycobacterium* species, where advanced automated systems are not available. The aim of the present study was to assess the use of blood agar for primary isolation of *Mycobacterium* as well as to compare the time required for growth on blood agar and LJ medium. **Materials and Methods:** All clinical specimens were processed by N-acetyl-L-cysteine-sodium hydroxide decontamination method and inoculated on blood agar slants and LJ medium. These slants were observed daily for any growth. Ziehl-Neelsen stain and auramine phenol stain were used to identify acid-fast bacilli. Time taken for any visible growth on both media were recorded and compared. **Results:** Totally 94 samples were examined, 13.8% showed acid-fast bacilli in smears. The combined culture positivity with blood agar and LJ media was 12.7%. Mean time to detect macroscopic colonies of *Mycobacterium* species on blood agar was 20 ± 6 days when compared to 30 ± 5 days on LJ medium. **Conclusion:** Blood agar slants can be used as a good supportive media to LJ medium for rapid growth of *Mycobacterium* species, especially in small laboratories of developing countries where automated systems are not available.

Key words: Blood agar, Lowenstein–Jensen medium, *Mycobacterium tuberculosis*

INTRODUCTION

Mycobacterium tuberculosis is the oldest infectious agent known, but still presents a large burden in developing countries. Rapid and efficient methods are needed for isolation, drug susceptibility and control of tuberculosis.[1,2] Conventional solid media, such as the egg-based Lowenstein-Jensen (LJ) and agar-based Middlebrook 7H10 media (BD BBL™ MGIT, Becton, Dickinson and Company, Sparks, MD 21152 USA), are recommended as the "gold standard" for isolation, culture, and definite diagnosis of *M. tuberculosis*.[3,4] However, slower growth rate (3-8 weeks) of *Mycobacterium* species on LJ media delay the reporting. To overcome this problem, various semi-automated and automated systems have been developed such automation is inaccessible in developing countries.

Blood agar is an enriched media routinely used, simple to prepare and the majority of bacteria easily cultivated on it.[5,6] The aim of the present study was to evaluate the utility of blood agar for rapid isolation of *Mycobacterium* species, which is a basic medium widely used in laboratory.

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MATERIALS AND METHODS

This comparative study was conducted at Department of Microbiology, Government Medical College, Surat for the duration of July 2012 to December 2012. All clinical specimens (sputum, pleural fluid, pus, lymph node [LN] aspirate, cerebrospinal fluid [CSF], ascitic fluid, and cyst fluid) with suspected mycobacterial infection received in laboratory were included in the study. All specimens (except CSF) were homogenized and decontaminated with the N-acetyl-L-cysteine–sodium hydroxide (NaOH) method using 1% NaOH. Slides were prepared and screened for acid-fast bacilli by Ziehl-Neelsen (ZN) stain and auramine phenol stain,[7,8] both before and after the decontamination procedure. Simultaneously, sediment was inoculated on LJ slants as well as on blood agar slants. These slants were incubated at 37°C in a nonCO_2 atmosphere for 12 weeks and observed daily for appearance of macroscopic growth.

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Time taken for growth on LJ slants as well as on blood agar slants was recorded. Smears were prepared when any visible growth appeared on slants. ZN stain and fluorescent stain were done to identify acid-fast bacilli and gram stain was done to rule out contamination.

Blood agar slants were prepared by adding 7 ml of sheep blood in 100 ml of autoclaved nutrient agar (HiMedia Laboratories Pvt. Ltd., Mumbai, India). To avoid contamination, PANTA (Polymyxin B, Amphotericin B, Nalidixic acid, Trimethoprim, and Azlocillin) procured from BD Diagnostic; Becton, Dickinson and Company, Sparks, MD 21152 USA, were added to the agar before pouring into McCartney bottles. These bottles were kept obliquely and allowed to cool until solidify. Sterility check was done by incubating at 37°C for 24 h. LJ medium was prepared as per WHO guidelines.[4]

RESULTS

Totally 94 different clinical samples were inoculated on blood agar as well as on LJ medium. Samples included were sputum (13), pleural fluid (58), pus (10), CSF (4), blood (6), ascitic fluid (1), cyst fluid (1), and LN aspirate (1). Sample-wise distribution of culture positive isolates is shown below in Chart 1. In a culture, *Mycobacterium* species showed light grey, glistening colonies on blood agar, whereas rough, cream colored colonies were observed on LJ medium. The growth reach maximum in about 4-6 weeks. Mean time for growth of *Mycobacterium* species on blood agar was 20 ± 6 days as compared to 30 ± 5 days on LJ medium. Contamination rate on LJ media and blood agar was 3.2% and 2.8%, respectively.

Among these 94 samples, 13 showed acid-fast bacilli in smears (13.8%). Culture positivity on blood agar was 7.4% while that on LJ media was 8.5%. Combined culture positivity with blood agar and LJ media was 12.7%. Based on microscopic finding, samples with ZN stain positivity had culture positivity of 30.7% on blood agar and 50% on LJ media, while combined culture positivity was 58.3%. Among auramine phenol stain positive samples, culture positivity was 27.3% on blood agar and 36.4% on LJ media, while combined culture positivity was 45.5% when compared in Chart 2.

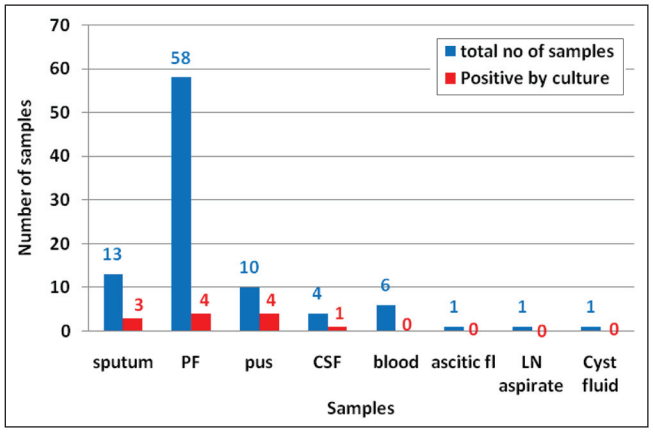

Chart 1: Sample wise distribution of culture positive isolates

DISCUSSION

Mycobacterial infections are highly prevalent in developing countries; hence use of blood agar is a good addition for rapid growth in developing countries.[8] Drancourt *et al*.[3] were the first to report the incidental growth of *M. tuberculosis* colonies on blood agar and termed this finding the "end of dogma." This report led to further research on blood agar for cultivation of *M. tuberculosis*.[9,10]

The present study showed that time for detection of *Mycobacterium species* on blood agar slants (20 ± 6 days) was significantly less than LJ medium (30 ± 5 days). Previous studies had also reported *M. tuberculosis* isolation on standard blood agar.[7,11] Josephine *et al*.[12] reported that mean time for detection of colonies on blood agar was 15 days as compared to 26 days on LJ medium. Similarly, Mathur *et al*.[11] also observed colonies on blood agar in 13.6 ± 5.2 days when compared to 20.4 ± 5.1 days on LJ medium. However, Drancourt *et al*.[3,10] noted no significant difference in time between eggs based Coletsos medium and blood agar. But afterwards, he found that median time to detect positive culture on blood agar was only 19 ± 5 days when compared to 26 ± 6 days on BACTEC 9000 MB broth (BD Diagnostic; Becton, Dickinson and Company, Sparks, MD 21152 USA). Furthermore, Coban *et al*.[11,14] reported that drug susceptibility could be obtained on blood agar in 2 weeks as compared to 3 weeks on 7H10 Middle brook agar without attempting primary isolation of *M. tuberculosis* on blood agar.

Studies had compared detection time of *M. tuberculosis* from smear positive sputum samples on automated culture

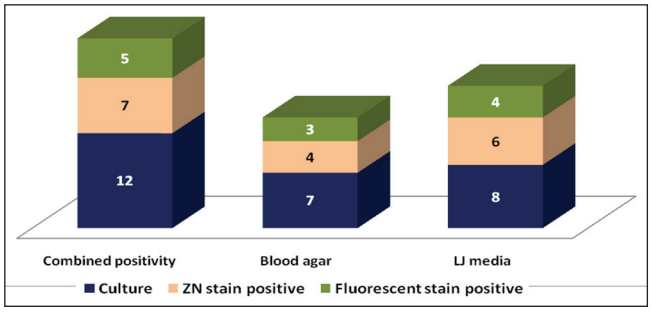

Chart 2: Comparison of individual and combined use of blood agar and Lowenstein-Jensen media for isolation of *Mycobacterium tuberculosis*

system and on LJ medium.[14,15] Due to limited resources of such automation in developing countries, blood agar slants appears to be fairly simple and reliable as far as time to detect *Mycobacterium* species is concerned. Lu *et al*.[5] reported that approximately 10% of clinically significant Mycobacterium species would be missed by the use of BACTEC 960 Mycobacterial Growth Indicator Tube (MGIT) (BD Diagnostic; Becton, Dickinson and Company, Sparks, MD 21152 USA) alone. So, reliability on automation can underdiagnosed *Mycobacterial* infections.

In the present study, 1% NaOH was used for decontamination method instead of 4% NaOH. Data obtained in the present study showed that culture positivity rate was increased to 6.6% after using 1% NaOH as compared to 2.4% while using 4% NaOH. This indicates that lowering the concentration of NaOH can give better results. It was also noted that contamination rate was less. The reason might be antibiotics supplementation that decreases other bacterial and fungal growth.

In the present study, both fluorescent stain and ZN stain were done to screen for acid fast bacilli. Fluorescent stain is considered more sensitive than ZN stain for identification of *Mycobacterium* species. It was observed that 5 (41.6%) culture positive isolates were smear negative in both stains. Hence, it is concluded that both stains should be used to minimize the errors while reporting. The discrepancy seen in smear positivity and culture negativity may be due to harsh decontamination technique or dead bacilli in sample due to treatment taken by patients. In the same way, culture positive and smear negative result can be due to lower bacillary load in the sample making them undetectable by microscopy or improper part of the sample used for making smears.

Recently, liquid culture in a mycobacterium growth indicator tube was found to be more rapid and sensitive method than culture in solid media but were more costly, even more susceptible to contamination.[16] only disadvantage of blood agar is that species level identification of *Mycobacteria* cannot be differentiated according to colony morphologies on blood agar.[5]

The present study has a limitation that species level identification of *Mycobacteria* and drug susceptibility was not done. Furthermore, the isolation rate on blood agar was not compared with MGIT, BACTEC or other sophisticated systems that detect *Mycobacterial* growth early. Blood agar cannot be more sensitive than other automated systems but can be used reliably along with LJ medium in resource-poor settings where such systems are not readily available. Therefore, the present study suggests a simple approach for rapid detection of *Mycobacterium* species by using blood agar.

CONCLUSION

Blood agar slants are easily prepared and inexpensive media, showed rapid growth of *Mycobacterium* species as compared to LJ media. Hence, use of blood agar along with LJ media is a good approach for developing countries where prevalence of mycobacterial infections is extremely high, and early drug susceptibility report can drastically change the scenario of Mycobacterial infections.

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