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RAPID DIAGNOSIS OF MYCOBACTERIUM TUBERCULOSIS

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ABSTRACT

Early laboratory diagnosis of tuberculosis is the need of the hour, to envisage early institution of treatment and ensure convalescence of this dreadful disease. Several culture media is used to isolate the pathogen from the clinical samples including liquid and solid media. Our study the importance of middle brook media which yielded growth as early as one week in relation to bacterial load in the sample in contrast to the conventional LJ medium which required 6-8 weeks incubation which delayed the treatment and proved costly both for the institution and the diseased.

KEYWORDS: Tuberculosis, Rapid diagnosis, Middlebrook

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INTRODUCTION

The world faces an acute emergence of extremely multidrug resistant strains of *Mycobacterium tuberculosis* bacilli including developing countries like India[1]. India is the highest tuberculosis burden country in the world and accounts for 20% of Tuberculosis. The precipitating factors are overcrowding, low socio economic status and immune compromised status of the infected individuals which is commonly seen in India[2].The increase in Mycobacterial infections due to the above mentioned factor is of serious public health concern[3]. Presently one third of the world population is infected with this bacillus[4]. It is estimated that currently there are about 10 million new cases of tuberculosis every year with 3 million deaths occurring worldwide. It implies that every minute a death occurs due to this infection. Therefore the diagnosis of tuberculosis becomes very important in treatment as well as in preventing the spread of infection. Although the classical diagnostic method employs microscopic examination of sputum smear, culture is more sensitive than smear microscopy, as only 10-100 bacilli result in positive culture, a minimum of 10,000 AFB is required for detecting single bacilli in the smear[5]. Therefore culture becomes more specific than smear examination and it becomes very important diagnostic tool for tuberculosis, even though the smear examination is the commonest technique employed in almost all the labs[6]. The conventional culture media utilizes LJ medium that requires 8-12 weeks of incubation[7], while the media which was used in our study enhanced the rate of growth and early detection of tuberculosis in the form of micro colonies as early as 2 weeks[8].This is due to the inoculation of biotin and catalase to stimulate revival of damaged bacilli in clinical specimen and the addition of casein hydrolysate which will improve the rate and amount of growth of the mycobacterium with resistance to INH [8]. A further modification of this is TL7H11 which includes piperacillin, amphotercin B and trimethoprim, oleic acid, bovine albumin V, catalase. Recovery of Mycobacteria is definitely improved, particularly when 7H11 medium is used with NALC-1% NaOH decontamination procedure[9]. With this in our mind, we undertook a study of early detection of tuberculosis growth in the culture by comparing two different culture media – LJ and Middlebrookes . thereby early diagnosis of the diseases in the patient.

MATERIALS AND METHODS

The project was submitted to IEC and approval was obtained. The patients were given informed consent form and the entire protocol was explained and after getting their consent, samples were collected.Fifty sputum samples were collected from the patient who has been attending our Pulmonology and General Medicine Departments with a diagnosis of pulmonary tuberculosis. The expectorated early morning sputum was collected in a sterile, disinfectant free container and was transported to the lab immediately with a properly filled request and consent form with the details of the patients and antibiotic therapy if given[10]. If there was a delay, the samples were stored in the refrigerator. Once the sample was received in the laboratory. it was decontaminated by N-acetyl-L-cysteine sodium hydroxide procedure[10]. Then it was concentrated by centrifugation 3000 rpm for 30 minutes. The sediment was used for inoculation on to TL7H11 plates[11]Kept in a candle jar at 35°c and another 0.1ml to LJ medium, which was incubated at 37°c[12]. The plates were observed twice weekly for 4 weeks using a conventional microscope. LJ media was observed on the third day and then on weekly basis for 8 weeks. Distinctive micro colonies in the TL7H11 plates were identified by the initial morphology, taking into account the consistency, tendency of cord formation[13] The petridish was sealed with adhesive tape to avoid aerosol spills [(10)]. Further confirmations were done with niacin and catalase tests All these procedures were done in class II biological safety cabinet with the researcher using cap, mask and gloves as per standard protocol to avoid aerosol infection. Niacin test was done for confirmation of human tubercle bacilli with 10% cyanogen bromide, 40% aniline in 96% ethanol to a suspension of bacterial culture of *Mycobacterium tuberculosis*, which give a canary yellow colour. Catalase was performed with 30% H2O2 and brisk effervescence indicated positive result[11].

RESULTS

Out of 50 samples processed, 34 were found to be positive, 20 were smear positive & 14 were smear negative, giving a growth on middlebrooke thin layer agar media. The positive percentage being 68%, the remaining 16 did not have growth even after incubating the media for more than four weeks (FIGURE: 1).The growth in 34 specimens appeared as micro colonies as early as 7 days and in some instance it appeared on the 14th day .there were 12 samples which had its growth appeared on the 7th day, 2 samples on 9th day, 3 samples on 10th day, 8 samples on 11th day, 7 samples on 12th day, 2 samples on the 14th day, giving a total of 34 samples positive the remaining 16 samples showed no growth even after incubation up to 4 weeks. The samples which showed micro colony was subjected for acid fast staining, niacin test and catalase test which was positive. There were 39 males and 11 females involved in the study (Chart: 1) out of which 29 males and 5 females were positive. The remaining 16 which become negative 10 were males and 6 were females. Making the ratio of males and females were 39:11. There are more males who were positive when compared to females, due to associated risk factors like smoking. The age group ranged from 21 years to 80 years, among the positive samples the distribution in the 34 were as follows. The high incidence in the age groups around 50 years was in concordance with their waxing and waning immune status and associated risk factor like smoking ,underlying diseases etc., The inoculated plates showed growth as early as 7 days and as late as 14 days. Out of 34 samples, 7 days incubation had growth detection in 12 samples, 9 days incubation proved to be positive for 2 samples, while 10days incubation was fruitful in 3 cases. 8 samples became positive on the 11th day of incubation and 7 more specimens exhibited their micro colonies on the 12th day, 2 cases required 14 days for making the growth to appear. The growth was further confirmed by

performing Zeihl nelson stain and doing the two biochemical tests: niacin and catalase tests. A simultaneous inoculation was done on the LJ medium which had growth only after 7 weeks of incubation (FIGURE: 2). Almost 30 samples showed growth after 7 weeks incubation and the Middlebrooke's media became an early indicator and a suitable medium for facilitating the growth of the mycobacterium tuberculosis bacteria within two weeks as proved by our study. Moreover this early confirmation by laboratory method will further enhance the clinician to initiate an early therapeutic management among the positive cases and improve their compliance and reduce the development of drug resistance with a careful follow up.

Figure 1
Growth of Mycobacterium on Middle Brooke

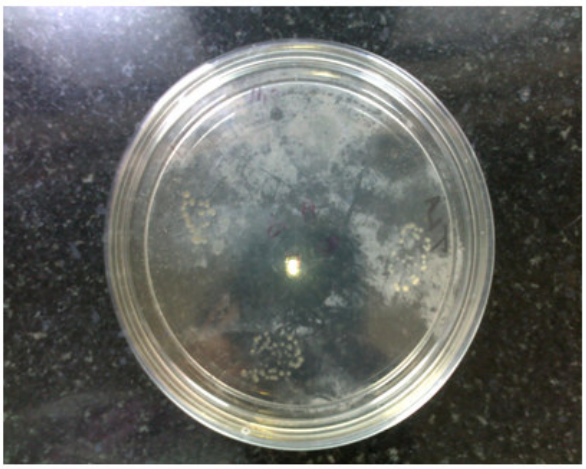
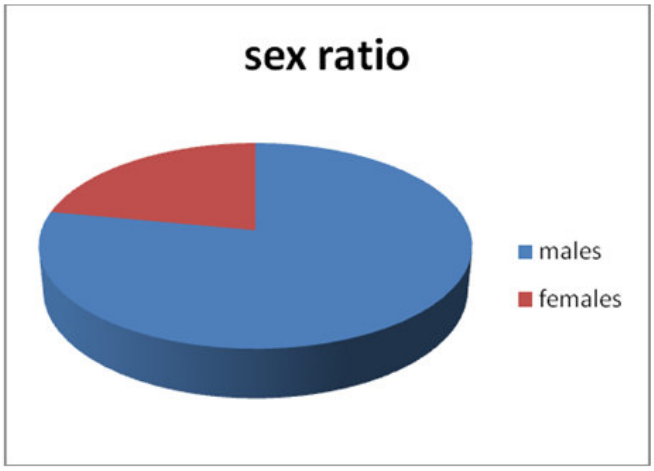


Figure 2
Growth of Mycobacterium on LJ medium

Chart 1
Sex Ratio of the patients



DISCUSSION

Our study indicates the importance of the Middle brookes thin layer 7H11 medium in laboratory diagnosis of mycobacterium tuberculosis bacteria from clinical specimens like sputum. With this media, which we can clinch the diagnosis as early as 7th day and as late as 14th day – only 2 weeks from the time of inoculation. This is the contrast to the inoculation & incubation of the conventional LJ medium, where the time required for the colony to make its appearance is as late and as long as 7-8 weeks. This is associated with undue delay of reporting. Which may give unnecessary apprehension, anxiety moments for the diseased and may also prove costly for the family? While the middle brookes media is fairly inexpensive and gives an early report as revealed by our study and easy to prepare, can be used in any laboratory. The samples which had more bacilli greater 10 AFB per oil immersion field as shown in the smear examination had its growth on the 7th day. The culture which grew on the 10th-12th day had a distribution of 1-10 AFB per oil immersion field. Those which grew beyond 12 days and around 14th day had 10-99/100 oil immersion field. Therefore from the day of appearance of the colonies in the 7H11 media, we can also judge the bacterial load in the sputum samples and thereby the severity of the clinical condition. This is yet another yard stick which will help to assess the prognosis of the disease. The earlier the colony appears the more is the bacterial load and if there is a delay in the appearance of the colonies the less is the bacterial load in per milli litre of sputum sample. As there was an early detection of growth in the middlebrookes TL7H11, it can be considered as a good substitute for the LJ media and can be used as the rapid diagnostic tool in place of the conventional media which requires prolonged incubation which is 6-8 weeks, which is three times more than the time required for TL7H11 media, used in our study.

CONCLUSION

The TL7H11 is the ideal culture medium for the diagnosis of *Mycobacterium tuberculosis* and has shorter incubation period, easy to prepare, maintain and facilitate rapid growth when compared to the conventional LJ media as proved by our study. This becomes of vital importance when the Nation is facing the emergence of MDR & XDR Tubercule bacilli.

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