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CULTURE ISOLATION OF MYCOBACTERIUM TUBERCULOSIS FROM SPUTUM SAMPLES USING MODIFIED LOWENSTEIN-JENSEN (LJ) MEDIA COMPARED WITH THE CONVENTIONAL LJ MEDIA

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ABSTRACT

Tuberculosis (TB) is the most common cause of death due to a single infectious agent worldwide in adults. India alone accounts for 30% of the global tuberculosis burden. There is a need for a method of cultivation of mycobacteria that is reliable and economical and has a short turnaround time. The present study was attempted to assess the feasibility of using modified Lowenstein-Jensen (LJ) media, the isolation media for mycobacteria. They were compared with the conventional LJ medium, which was the gold standard. Sputum specimens from a total of 1812 clinically suspected cases of TB were studied. All the samples were decontaminated by using the modified Petroff's method. Each sample was subjected to ZN staining and it was simultaneously inoculated onto the modified LJ medium and the conventional LJ medium. The growth from the cultures were confirmed by ZN staining and biochemical reactions. In DE-LJ the Mycobacterial growth was seen on the second week for +++ samples and on the third week for the ++ and + samples. . Mean number of weeks taken on three experimental slants was compared with mean number of weeks on control slants using using paired't' test. The difference in mean number of weeks taken on supplemented medium was significantly less than that on unsupplemented medium. Modified Lowenstein-Jensen (LJ) media is a better medium as compared to the conventional LJ medium, both in terms of the number of isolates and the isolation rate. It was proved to be a very speedy method and it could isolate Mycobacterium tuberculosis 5- 10 days earlier as compared to the conventional L J medium.

KEYWORDS: Mycobacterium tuberculosis, Lowenstein-Jensen (LJ) media, Sputum sample, Acid fast staining, Modified LJ.

INTRODUTION

Tuberculosis has been for many centuries the most important of the human infections, in its global prevalence, it's devastating morbidity and its massive mortality. Despite many advances in its diagnosis and treatment, the problem of tuberculosis is on its rise, both globally and in India. At present, the global incidence of this disease is increasing at the rate of 0.4 % per year.[1]

It has been estimated that a third of the world's population, about 2 billion people, are infected with the tubercle bacilli. Every year, between 8 and 9 million new cases of tuberculosis appear and 3 million persons die from the disease.[2]

A large majority of the cases and deaths are reported from the poor nations. India is one of the worst affected countries. More than 40 % of the population is infected and some 15 million suffer from tuberculosis in our country, of which over three million are highly infectious open cases. In 2009, out of the estimated global incidence of 9.4 million TB cases, 2 million were estimated to occur in India.[3]

With the progress of the AIDS pandemic, tuberculosis has become a problem for the rich nations also. A close relationship has emerged between tuberculosis and HIV.

The worldwide spread of Multi Drug Resistant Tuberculosis (MDRTB) has added new troubles to the already existing problem. At present, 3.2 % of the world's new cases of TB are multi drug resistant. [4] In India, the incidence of MDRTB ranges from 1.3 to 3%.[5]

The most effective control measure for checking the spread of TB is to detect it early and to treat it optimally at the earliest. Although ZN staining smear microscopy is most commonly employed for an early detection, it is rather insensitive and it fails to detect a large number of cases.[6]

Under these circumstances, the cultivation of Mycobacterium tuberculosis provides a sensitive and a

specific means for the diagnosis of TB. The conventional culture methods such as the use of Lowenstein Jensen medium requires 3 to 6 weeks for its isolation, plus an additional 1 to 2 weeks for its speciation. Such a prolonged turnaround time in the diagnosis is unacceptable, as rapid detection and identification of MTB is essential both for medical and epidemiological purposes.[7]

Clinical mycobacteriology laboratories play an important role in the control of the spread of tuberculosis (TB) through the timely detection, isolation, identification, and drug susceptibility testing of Mycobacterium tuberculosis. In the past, the mycobacteriology laboratory has had to rely on diagnostic methods that often do not rapidly give a definitive diagnosis of tuberculosis.[8] Conventional methods include the acid-fast stain, culture, and biochemical tests for detecting and identifying members of the M. tuberculosis complex (MTBC). Even with concentrated samples, the sensitivity of microscopy is not great, and M. tuberculosis cannot be reliably differentiated from other mycobacteria in acid-fast-stained smears. Although the use of fluorescent (auramine-rhodamine) stains increases the sensitivity and shortens the time required for reading of smears, microscopy, as a direct method, can provide at best only a preliminary diagnosis. Culturalmethods, properlyapplied, detect M.tuberculosisin clinical samples with reasonable sensitivity and provide accurate identification of the isolates.[9]

Since culture detection of M. tuberculosis using conventional methods is time consuming, the aim of the study was to compare the efficacy of modified Duck Egg Lowenstein-Jensen (DE-LJ) culture media in supporting the growth of M. tuberculosis with the conventional Lowenstein-Jensen (LJ) medium.

MATERIALS AND METHODS

This study was conducted in the Department of Microbiology, R.V.S Dental college and hospital over a period of 2 years from July 2014 to June 2016. A total of 1812 smear Positive sputum samples were included in this study.

Processing of specimens

All sputum samples were decontaminated by the N-acetylL-cysteine (NALC)-NaOH method. Two volumes of NALC-NaOH solution (4% NaOH, 1.45% Na-citrate, 0.5% NALC) were mixed with the specimen on a test tube mixer for digestion, and the mixtures were allowed to stand for 15 min at room temperature. Ten volumes of 6.7 mM phosphate buffer (pH 7.4) were added for dilution, and the mixtures were centrifuged at 3,000 3 g for 15 min. The sediment was resuspended in approximately 1.5 ml of the same phosphate buffer containing 0.5% Tween 80. Tween 80 was added to achieve better homogenization of the sediment and a more even distribution of the bacteria within the suspension. A 100-ml aliquot of the suspension was directly inoculated onto culture media and remaining was used for acid fast staining.

Smear Examination

The smears were prepared and stained with the Ziehl-Neelsen technique and examined as positive scores of 1+ (10-99/100 oil immersion fields) or more were evaluated in this study. Some scanty positive (1-9 bacilli/100 oil immersion fields) specimens were also included to check the sensitivity of the test.

Media preparation

The conventional LJ media was prepared based on RNTCP 2009 manual,[1] the following ingredients are aseptically pooled in a large, sterile flask and mixed well: Mineral salt solution with malachite green 600ml and Homogenized hen's eggs (25-30 eggs, depending on size) 1000ml.The complete egg medium is distributed in 6-8ml volumes in sterile universal containers and the caps tightly closed and inspissated without delay to prevent sedimentation of heavier ingredients. DE-LJ: The duck egg solution is added instead of Country Hen egg in the Conventional LJ medium.

Inoculation, Incubation and Identification

The sputum samples were inoculated on the modified and conventional Medias. (Two slopes of medium are inoculated per specimen) All cultures are incubated at 35°-37°C until growth is observed or discarded as negative after eight weeks.

The following notations were used to indicate the degree of growth

++++ = Confluent growth on the medium and growth in condensed water.
+++ = Confluent growth.
++ = More than 20 colonies without confluent growth.
+ = 1 to 20 colonies.
Negative = No growth.

The Smears prepared from colonies were stained by the Ziehl-Neelsen method and the growth was identified by using standard biochemical test. The results obtained in the modified LJ Media are compared with the conventional LJ media.

RESULTS AND DISCUSSION

A total of 1812 sputum specimens were cultured on the modified Lowenstein-Jensen medias and the control. Mean number of weeks taken on three experimental slants was compared with mean number of weeks on control slants using using paired't' test. The difference in mean number of weeks taken on supplemented medium was significantly less than that on unsupplemented medium. In DE-LJ the Mycobacterial growth was seen on the second week for +++ samples and on the third week for the ++ and + samples the details of the growth was described in the table 1. The growth obtained in the DE-LJ was compared with the control and seen that it was much earlier than the control and the P value

(<0.001) obtained was significant.Growth on the DE-LJ was shown in the fig.1.

The definitive diagnosis of Mycobacterial disease still requires the recovery of the causative agent on culture media. The standardization of any material which cannot be clearly defined in physical and chemical terms is difficult. In Biology as the composition of biological materials is very variable, standardisation is still harder to achieve.[10] If the vehicle of measurement (e.g., a nutrient medium containing indistinctly defined constituents such as different kinds of proteins, fatty substances, enzymes, and vitamins) and the material to be measured (e.g., Mycobacteria, whether in pure cultures or in pathological specimens, which vary in their nutritional requirements) are of uncertain quality and composition, it is obvious that standardisation almost impossible.[11]

For low inoculum specimens (1+to +2 smears) the DE-LJ medium was more efficient in the recovery of Mycobacteria as compared to control. The low rates of contamination obtained with the DE-LJ medium. The growth in DEB-LJ is better, the colony morphology is well appreciated compared to other modified LJ. In DET-LJ the growth of the Mycobacteria cultures were noticed without mineral salt solution.[12]

As regards the rates of recovery of *M. tuberculosis* by eachmethod, were better than theconventional media, In our study, DE-LJ yielded the highest rate of recovery of *M. tuberculosis*. The differences between DE-LJ and the solid media were statistically significant. No statistical difference was found between the DEB-LJ and conventional standards in terms of the rates of recovery of *M. tuberculosis.*[13]

In duck egg media the nutritional difference may be the reason for efficient recovery of Mycobacteria from clinical specimens.[14]

It will be observed that the preparations with a high sensitivity contributing to the significant variation between the media are the L-J media prepared at the state Bacteriological Laboratory.

Table 1: Average number of weeks to positive cultures.

ZN Smear	Conventional LJ	DE-LJ
Scanty	5	3
+	5	3
++	4	3
+++	4	2
++++	3	2
Negative	5	3

LJ - Lowenstein-Jensen media; DE- Duck Egg LJ.

Figure 1: Growth on DE-LJ and Conventional LJ Media.

In order to investigate the possible factors causing variability in the sensitivity of the media as prepared and despatched from various laboratories, another experiment was carried out with L-J medium prepared at the Statens Seruminstitut, Copenhagen, and at the Tuberculosis Research Institute, Prague, using different glass tubes.[15]

The L-J medium prepared at the Statens Seruminstitut, Copenhagen, and kept in Danish tubes was the most sensitive. That prepared in Prague and kept in Danish tubes ranked second; and that prepared in Prague and kept in Czechoslovak tubes appeared to be the least sensitive.[16]

To conclude the study, the results with the conventional egg based culture medium for the recovery of mycobacteria, Improved isolation rate is possible if duck egg culture techniques are used. Further studies are necessary to evaluate the accurate compound responsible for the growth promoter activity in duck egg.

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