Original Article

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Microbiology Section

Comparative Evaluation of the Rapid Slide Culture and Microscopy with the Conventional Culture Method in the Diagnosis of Pulmonary Tuberculosis

HEMAVATHI, POOJA SARMAH, RAMESH D.H.

ABSTRACT

Introduction: Tuberculosis (TB) is a global health problem. An early diagnosis and an effective treatment are essential to prevent the spread of infection and to reduce the disease burden. Though the causative bacterium for this disease was discovered in the eighteenth century, its diagnosis in the twenty-first century is still a dogma.

Objectives: To compare of the rapid slide culture (RSC) method with the growth on Lowenstein Jensen (LJ) media and to know the sensitivity and specificity of the rapid slide culture method and the acid fast smear examination in comparison with the growth on the L J media.

Materials and Methods: One early morning sputum sample was collected from 220 clinically suspected pulmonary tuberculosis cases. All the samples were subjected to three tests: 1. Zeihl Neelsen Staining 2. Rapid slide culture and 3. Culture on Lowenstein Jensen media and the results were compared.

Results: Of the total 220 sputum samples which were tested by all the three methods, 51 samples (23.18 %) were found to be smear positive, 75 (34.09%) were found to be positive by the RSC method, 81 (36.81%) were found to be positive by the LJ culture method and 38(17.27%) samples were found to be positive by all the three methods and 93 (42.27%) samples were found to be positive by any one method. 127 (57.72%) samples were negative by all the three tests The sensitivity of RSC in comparison with the LJ culture was 88.88% with a specificity of 97.8%, whereas the smear showed a sensitivity of 49.4 % and a specificity of 92.1%.

Discussion: The RSC method is rapid, sensitive and more specific than microscopy. Hence, this method can be adopted by any simple laboratory for the diagnosis of tuberculosis.

Key Words: RSC, Microscopy, LJ Media, Pulmonary Tuberculosis

INTRODUCTION

Tuberculosis is of great concern worldwide. The WHO has estimated 9.4 million incident cases and 11.1 million prevalent cases of TB globally [1]. India accounts for nearly one-third of the TB cases worldwide and every year, around two million persons develop TB, of which 0.8 million are new-smear, highly infectious cases, with an incidence rate of 185 per one lakh population and a prevalence rate of 256 per one lakh population. About 3.5 to 4 lakh people die of TB every year, which amounts to the death of more than one thousand persons every day. The death rate is 26 per one lakh population. The case detection rate is 59% [2,3].

The situation has become worse because of HIV and drug resistance. For the diagnosis of tuberculosis, different modalities are being adopted but the confirmation is usually done by microscopy and culture or molecular methods. An early diagnosis and an effective treatment are essential to prevent the spread of infection and to reduce the disease burden. Different combinations of tests are being tried to reach an early diagnosis.

Robert Koch, in 1882, observed under microscope, that a growing culture of *Mycobacterium tuberculosis* formed serpentine cords at a very early stage [4]. Dickinson and Mitchison described a new slide method that was rapid, simple and safe, but it needed a fluorescent microscope [5]. P.R Gupta et al. modified the method and got similar results by using a bright field microscope [5]. Purohit et al compared human blood media and egg enriched sheep blood media which provided a low contamination rate and growth after seven days, as compared to the growth after six weeks on the Lowenstein Jensen (L J) media. They found this to be an advantage in the primary isolation and evaluation of drug sensitivity tests for tuberculosis [6].

OBJECTIVES

1. Comparison of the Rapid Slide Culture (RSC) method with growth on the L J media.
2. To know the sensitivity and the specificity of the rapid slide culture method and acid fast smear examination in comparison with the growth on the L J media.

MATERIALS AND METHODS

This study was done in 2007, at a medical college hospital in Bangalore, India. 220 morning sputum samples from clinically suspected cases (one sample from each patient) were collected; smears were made and they were stained by the Ziehl Neelsen (ZN) staining method and were reported.

All the samples were decontaminated and concentrated by the modified Petroff's method [7]. One smear was made at the centre of the slide (Slide-1), it was fixed and Z N staining was done. The reading was taken and it was recorded. Two smears were made at the lower 1/3rd of the glass slides which were split longitudinally. The smears were air dried dipped into the McCartney's bottle

which contained Human Blood Medium (HBM), so that the smears remained dipped in the medium. The inoculated HBM were incubated at 37^{0}C. The slides were taken out on the seventh day and were dipped in sterile distilled water to remove the excess media which were present on the slide surfaces. The slides were then placed in an oven at 80^{0}C for 30 minutes and were later stained by ZN staining. The growth was recorded as has been mentioned below: The readings of the 0 grades was taken as negative and those of the grades 1-4 were taken as positive

Grade 0- No division of AFB as compared to slide 1
Grade 1+ Small clumps –up to 4 bacilli were present but absent in the control
Grade 2+ large clumps of bacilli
Grade 3+ large clumps with some cord formation
Grade 4+ Micro colonies with good cord formation

Human Blood Media for the Slide Culture Method

Citrated human blood from the blood bank, which was not more than four weeks old, was mixed with an equal volume of sterile distilled water. The mixture was mixed well till the blood got haemolyzed. The medium was made selective by the addition of chemotherapeutic agents like-polymixin B-200000 units/liter, carbenicillin-100mg/liter, trimethoprim-10mg/liter and amphotericin B-10 mg/liter. The pH of the medium was adjusted to 6.5-7.5. Ten ml of the medium was transferred to each of the Mc Cartney's bottles and these were stored at 2-8^{0}c.

After the decontamination of the sputum, the deposit was inoculated on the LJ media slants and these were incubated at 37^{o}C. The readings were taken weekly and the growth was identified by assessing the colony morphology, rapidity of the growth and the ZN staining.

RESULTS

Of the total 220 sputum samples which were tested by all the three methods, 51 samples (23.18 %) were smear positive, 75 (34.09%) were found to be positive by the RSC method, 81 (36.81%) were found to be positive by the LJ culture, 38 (17.27%) were found to be positive by all the three methods and 93 (42.27%) were found to be positive by any one method. 127 (57.72%) samples were negative in all the three tests [Table/Fig-1].

When we compared the growth of two culture methods, the results were as follows: The total 72 (32.72 %) samples were found to be culture positive by both the culture methods, whereas 75 (34.09%) samples were found to be positive by the RSC method and 81(36.81%) samples were found to be positive by the LJ media [Table/Fig-2]. The sensitivity of RSC in comparison with the LJ culture was 88.88%, and the specificity was 97.8%.

Test	Positive	Percentage
AFB smear	51	23.18
RSC	75	34.09
L J media growth	81	36.81
Positive by all three tests	38	17.27
Positive by any one of the tests	93	42.27
Negative by all three tests	127	57.72

[Table/Fig-1]: Distribution of results according to tests used

Rsc Growth	L J Media Growth		
	Positive	Negative	Total
Positive	72 (32.72%)	3 (1.36%)	75 (34.09%)
Negative	9 (4.09%)	136 (61.81%)	145 (65.90%)
Total	81 (36.81%)	139 (63.18%)	220 (100%)

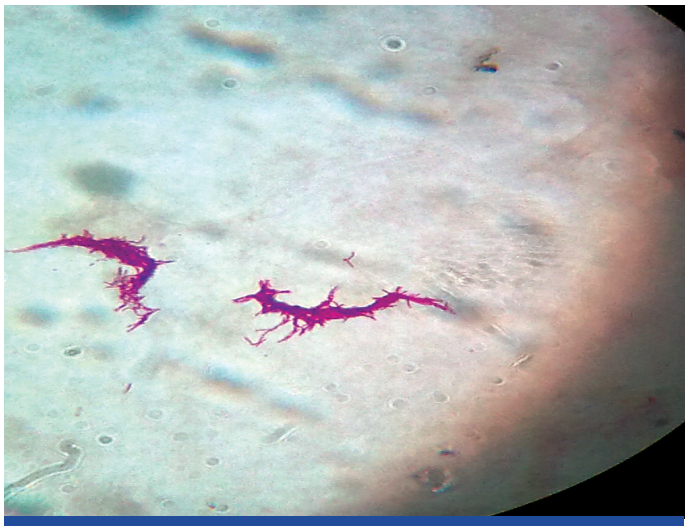
[Table/Fig-2]: Comparison of Rapid Slide Culture method with L J media growth

AFB Sputum smear	L J media growth		
	Positive	Negative	Total
Positive	40	11	51
Negative	41	128	169
Total	81 (36.81%)	139 (63.18%)	220 (100%)

[Table/Fig-3]: Comparison of Sputum smears for AFB with L J media growth

RSC Growth	L J media growth		
	Positive	Negative	Total
Positive	34 (20.11%)	1 (0.59%)	35 (20.71%)
Negative	7 (4.14%)	127 (75.14%)	134 (79.28%)
Total	41 (24.26%)	128 (75.73%)	169 (100%)

[Table/Fig-4]: Correlation between RSC and L J culture results in smear negative cases.



[Table/Fig-5]: Cord formation of M. tuberculosis in RSC Method–ZN Stain

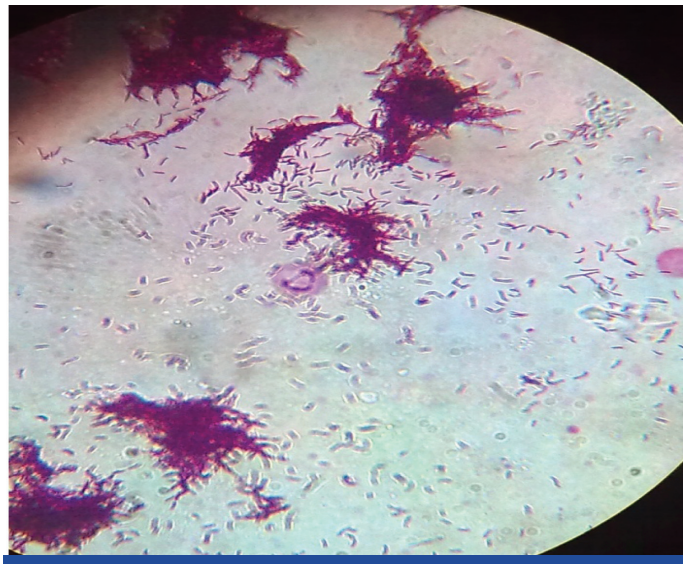
The comparison of the smear positivity with the growth on the LJ media showed a sensitivity of (40*100/81) 49.4 % and a specificity of (128*100/139) 92.1% [Table/Fig-3].

Out of 169 smear negative samples, 35(20.71%) samples were RSC positive and 41(24.26%) were LJ culture positive. A total of 127(75.14%) samples were found to be negative by both the methods [Table/Fig-4].

DISCUSSION

This study included a total of 220 clinically suspected pulmonary tuberculosis cases. The sputum samples were studied by all the three methods, of which 51 samples (23.18 %) were found to be positive by the AFB staining, 75(34.09%) showed growth by the slide culture method and 81(36.81%) showed growth on the LJ media.

A total of 72(32.72%) samples were found to be positive by both the culture methods, whereas 51 (23.18 %) samples were found to be positive by AFB staining. Both the culture techniques were found to be more sensitive as compared to the smear examination. Concentration of the sputum samples before the inoculation also would have added to the sensitivity, but it is a concern, as the



[Table/Fig-6]: Microcolony formation of M. tuberculosis in RSC Method–ZN Stain

[Table/Fig-7]: L J media showing rough and buff colored colonies typical of M. tuberculosis

National Programme incorporates only smear examination for the screening of the TB cases without concentration.

Out of the 169 smear negative samples, 35(20.71%) samples were RSC positive and 41(24.26%) were LJ culture positive. The RSC showed a sensitivity of 92.59%, and a specificity of 97.8%, whereas the smears showed a sensitivity of (40*100/81) 49.4%, (62.96) and a specificity of (128*100/139) 92.1% as compared to the growth on the LJ media. This difference indicated that the culture of the TB bacteria was very important for a definite diagnosis in the paucibacillary pulmonary tuberculosis cases. Though the LJ media showed more positivity, the growth appeared on the LJ media only after four weeks, which hindered the early start of the medication, leading to the spread of infection among the patients' contacts and the patients had to bear the brunt of the nonspecific treatment.

The RSC showed less positivity because of the following reasons- (1) Wet smear inoculation, leading to the washing off of the smear into the HBM. (2) Over drying of the smear, leading to death of the bacteria and (3) Use of very cold media [8].

A similar study which was done by Jena et al [8] showed that among 336 patients of pulmonary tuberculosis, the smear was positive only in 91(27.08%) patients, that the RSC showed positivity in 105 patients (31.25%), that the LJ culture showed positivity in137(40.77%) patients and that 161(52.08%) cases showed positivity by any method. An early availability of growth was possible in 27 smear negative cases by the RSC method.

The RSC can be used for the routine cultivation of MTB in the laboratory, excluding the above said faults. The growth on RSC took only one week, which helped in initiating the treatment early. The RSC is cheap, it does not need special ingredients or equipment and it can be used for antibiotic susceptibility testing.

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AUTHOR(S):
1. Dr. Hemavathi
2. Dr. Pooja Sarmah
3. Dr. Ramesh D.H.

PARTICULARS OF CONTRIBUTORS:
1. Professor & Head, Department of Microbiology, Sapthagiri Institute of Medical Sciences, Chikkasandra, Bangalore, India.
2. Assistant Professor, Department of Microbiology, Sapthagiri Institute of Medical Sciences, Chikkasandra, Bangalore, India.
3. Post Graduate Student, Department of Microbiology, Vydehi Institute of Medical Sciences, White field, Bangalore, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr Hemavathi
No. 12 Mount View Enclave, Bettahalasur cross
Bangalore North – 562157, Karnataka, India.
Phone: 9886218454
E-mail: hemasathyanarayana@yahoo.com

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