Original Article

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Comparison of Bact/Alert -3D Automated Culture System and Conventional Culture Method for *Mycobacterium tuberculosis* from Extrapulmonary Specimens of the Patients Attending SKNMC and GH

Microbiology Section

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ABSTRACT

Introduction: In extrapulmonary samples, very low yield of mycobacteria results in a low sensitivity of acid fast bacilli (AFB) smear and culture. *M.tuberculosis*, the slow grower takes 4 to 6 weeks on solid based medium. Thus various automated systems using liquid culture methods are useful for reducing detection time (between 9 to 16 days).

Aim: To compare the liquid culture method by using Bact/Alert -3D automated culture system and conventional culture method from extrapulmonary specimens.

Materials and Methods: The study was carried out in Department of Microbiology. Clinically suspected 66 samples were included in the study. Microscopy was done by Z-N staining .The samples were processed by standard guidelines. LJ medium were inoculated and incubated at 37°C. Sample was also inoculated in MP Bottle containing 7H9 Middlebrooks liquid Medium. LJ slopes were examined weekly for appearance of colony till 10 wks. MP bottles with positive results by Bact/Alert system were processed for identification and no growth up to 42 days was declared negative.

Results: From 31.81% positive results for *mycobacteria*, 95.23% were detected positive by Bact/Alert while 28.57 % were positive by culture. Mean detection time of microscopy positive samples were detected in second week by Bactalert and in 4th week on LJ medium. Samples negative for microscopy, detected positive in fifth week by Bact/Alert-3D system while by seventh week on LJ medium.

Conclusion: The sensitivity of liquid culture by automation is higher and earlier than culture on LJ Medium for detection of AFB which will help the patient for early clinical management. But cost is the main barrier in low economic setting.

Keywords: Acid fast bacill, Automation system, Methods of TB culture, Tuberculosis

INTRODUCTION

Tuberculosis (TB) remains the major cause of death from a single infectious agent worldwide. Extra pulmonary tuberculosis (EPTB) constitutes about 15 to 20 % all cases of TB [1]. In extrapulmonary samples, very low yield of mycobacteria results in a low sensitivity of acid fast bacilli (AFB) smear and culture. *M.tuberculosis*, the slow grower takes 4 to 6 weeks on solid based medium.Thus various automated systems using liquid culture methods are useful for reducing detection time (between 9 to 16 days) [2].

AIM

To compare the liquid culture method by using Bact / Alert -3D automated culture system and conventional culture method from extrapulmonary specimens.

MATERIALS AND METHODS

The comparative observational study was carried out from August 2011 to July 2012 in Department of Microbiology, Smt. Kashibai Navale Medical College and General Hospital, Pune.The permission from institutional ethical committee was taken. The samples from the patients clinically suspected extrapulmonary tuberculosis submitted in the laboratory were included in study. Specimens included urine, body fluids like pleural, pericardial, synovial, ascetic and cerebrospinal fluids, lymph node aspirates, and pus. The blood specimens were excluded. The patients who were taking anti tubercular drugs were excluded. Z-N stain of all the samples was done to reveal acid fast bacilli.

Z-N smear of the entire clinical specimen was performed and examined to reveal acid fast bacilli. The body fluids, CSF, lymph node aspirates were centrifuged at 3500xg for 20 minutes and the deposit was inoculated in MP bottle containing 10 ml of modified Middle brook 7H9 broth enriched with casein, bovine serum albumin, and catalase. Before inoculation MP bottles were supplemented with 0.5 ml of MB/BacT antibiotic supplement (amphotericin B, azlocillin, nalidixic acid, polymyxin B, trimethoprim, and vancomycin),

which was reconstituted with 10 ml of MB reconstituting fluid [1]. Reconstitution fluid is Tween 80, glycerol, amaranth, and purified water. The centrifuged deposit was inoculated on two LJ medium. The heavily contaminated tissue specimens were decontaminated by N-Acetyl L-Cysteine (NALC)–NaOH method. Equal amount of samples NALC and 3% NAOH is added in the specimen. It was incubated for 15 minutes at room temperature. Phosphate buffer saline (0.067M,PH 6.8) was used to neutralise the specimens and was centrifuged at 3500 xg for 15 minutes for pellet formation. The pellet was dissolved in 1ml of phosphate buffer and inoculated in MP Bottle containing 10 ml of modified Middlebrook 7H9 broth enriched with casein, bovine serum albumin, and catalase. Before inoculation MP bottles were supplemented with 0.5 ml of MB/BacT antibiotic supplement (amphotericin B, azlocillin, nalidixic acid, polymyxin B, trimethoprim, and vancomycin), which was reconstituted with 10 ml of MB reconstituting fluid [1]. Simultaneously they were also inoculated on sets of two LJ medium . The slants were incubated at 37°C for 10 weeks. LJ slopes were examined weekly for appearance of colony till 10 weeks. MP bottles with positive results by Bact/Alert system were processed for identification and no growth up to 42 days was declared negative. Growth of *Mycobacterium* was verified by microscopy after Z-N staining.

RESULTS

Total 66 clinical samples were collected. Twenty one isolates grew from 66 specimens. Of these isolates, 4/66 (6.06%) were smear positive and 62/66 (93.9%) were smear negative. Positive results for mycobacteria were 21/66(31.81%). 20/21(95.23%) were detected positive by Bact/Alert while 6/21(9.09 %) were positive on solid culture. Four were positive on microscopy amongst which three could grow on solid medium and detected positive by automated system [Table/Fig-1] .Mean detection time of microscopy positive samples is second week by Bactalert and in 4th week on LJ medium. Samples negative for microscopy, detected positive in fifth week by Bact/Alert-3D system while by seventh week on LJ medium. Twelve samples were positive .Sample positive by Bact/alert are cultured on LJ for confirmation and identified by phenotypic methods. Three samples were positive by all the three methods by microscopy and by culture on solid and liquid medium. Amongst smear negative 17 were positive by Bact/alert 3D culture system and three by conventional culture method on solid LJ medium as showen in [Table/Fig-2].

Sr. No	Specimen	Speci-men	Positive speci-men	Smear positi-vity	Detected positive by culture on LJ medium	Detected positive by Bact/ Alert/ 3D system
1	Pus	25	10	2	5	9
2	Fluid	20	4	0	0	4
3	CSF	8	1	0	0	1
4	Lymph node biopsy/ lymphatic aspirate	5	3	2	1	3
5	Urine	5	1	0	0	1
6	Miscella-neous	3	2	0	0	2
Total		66	21	4	6	20

[Table/Fig-1]: Various specimens showing positive results by the ZN and culture method.

Isolate (No.of specimen)	No of isolate detected	
	MB/BACT ALERT	LJ Medium
All smear positive samples (4)	3	3
All smear negative specimen (62)	17	3

[Table/Fig-2]: Detection of Mycobacterium from clinical specimens according to Initial smear results.

DISCUSSION

Tuberculosis (TB) remains the major cause of death from a single infectious agent worldwide. There are many tests available to shorten the time for the diagnosis of the tuberculosis still culture remains the gold standard for definitive diagnosis of clinically significant *Mycobacterium tuberculosis*. Though newer techniques like nucleic acid amplification assays are available diagnosis of tuberculosis continues to rely on cultivation of *M.tuberculosis*. Clinical information is often inconclusive for making a diagnosis of EPTB. Rapid and sensitive detection of *Mycobacterium tuberculosis* is required for the treatment, control, and prevention of tuberculosis. In the present study amongst the positive, 95.23% samples were detected positive only by BacT/ALERT automated system as liquid culture are more sensitive for detection of acid fast bacilli than solid culture.

One sample inoculated in the MP bottle was contaminated as the Middle Brookes medium is highly enriched medium. There is possibility of contamination if the precautions are not taken during sample collection.19.04% samples were positive by microscopy, 62% were culture positive on LJ. The results were consistent with previous study by Brunello et al., Extrapulmonary paucibacillary samples takes more time for growth on LJ medium (4-8 week) [2]. The BacT/Alert 3D system allows the growth and early detection of mycobacteria [3].

Piersimoni et al., in the study observed the average number of days required for the detection of *M. tuberculosis* complex strains (14.3 days for smear-positive specimens and 17.4 days for smear-negative specimens), as seen in our study [1]. At present Culture on both solid and liquid media always remains Gold standard for isolation of Mycobacteria and for confirmation of tuberculosis. It takes 21 to 30 days after collecting specimen for detection and identification of *M.tuberculosis* [4-6].

In the present study amongst the positive 95.23% samples were detected positive only by Bact/alert automated system where the liquid medium Middle brookes 7H9 medium is used. Liquid culture are more sensitive for detection of acid fast bacilli than solid culture [4].

Amongst the positive only 19.04% samples were positive by microscopy 62% were culture positive on LJ medium. Very low yield of mycobacteria in extrapulmonary samples results in a low sensitivity of Acid Fast Bacilli (AFB) smear and culture on LJ medium. Direct visualisation through Ziel Neelsen (ZN) stain is commonly missed in single or paucibacillary specimen. One sample of lymph node aspirates revealed acid fast bacilli in Z-N stain but could not grow it. The patient was taking AKT since 3 months. The samples positive by Bactalert were precious sample and have the vital role in definitive diagnosis and management.

One sample inoculated in the MP bottle was contaminated. The Middle Brookes medium is highly enriched medium. There is possibility of contamination if the precautions are not taken during sample collection. As the scanty amount of organisms is present at extrapulmonary sites, microscopy comes negative and it is difficult to isolate *M.tuberculosis* due to small amount. So the gold standard is culture of the suspected specimen. Conventional culture method takes 4-8 weeks depending on the bacterial load. Though it takes time it can be shortened significantly by using automated liquid culture system The BacT/Alert-3D Conventional culture method takes 4-8 week system allows the growth and early detection of Mycobacteria.

Laboratory aid is commonly required to come to a conclusion. The definitive diagnosis therefore remains solely in the hand of a culture growth [4]. Rapid automated non-radiometric continuous mycobacterial liquid culture systems will be helpful in addition to solid culture .Early detection of the tuberculosis ensures the early management of the patients. The average number of period required for detection of *M.tuberculosis* complex is 14.3 days for the smear positive specimen while 17.4 days for smear negative specimens. Similar study was conducted by Martinez et al., 2014 and concluded that BacT/ALERT 3D system is a suitable method for recovering tuberculous and non tuberculous mycobacteria from clinical samples [7].

CONCLUSION

The sensitivity of liquid culture by automation is higher and earlier than culture on LJ Medium for detection of AFB which will help the patient for early clinical management. But cost is the main barrier in low economic setting. It will be extended and will be helpful if the samples were processed by molecular method. The study proposes that BacT/Alert 3D system is a rapid and sensitive method for isolation of mycobacteria from various clinical extrapulmonary samples.

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