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Isolation of MTB Strains and Determining the Antibiotic Susceptibility Pattern via Bactec 320 from the Females of Child Bearing Age

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Abstract

Background: The BACTEC MGIT 320 is a new, lower-capacity instrument for liquid culture developed for the growth and detection of *M. tuberculosis* and antimicrobial susceptibility testing. MGIT has an improved speed and sensitivity of MTB isolation and drug susceptibility testing, irrespective of the HIV status of the patient. This study was undertaken to find the appropriate antibiotic susceptibility pattern of confirmed positive MTB strains via MGIT 320 liquid culture technique. *Objectives*: Isolation of positive MTB strains and determination of their antibiotic susceptibility pattern using BACTEC 320 from the females of child bearing age group. *Material and Methods*: A total of 217 samples were processed involving the isolation of MTB strains along with the antibiotic susceptibility pattern of positive MTB's. Both techniques were used culture and RT-PCR to find the prevalence and AST via BACTEC 320 system. Analysis of the results was done at the end of the procedure. *Results*: Out of 217 samples, there were 29 positive MTB strains by RT-PCR technique whereas via liquid culture there were 11 positive MTB strains. The Antibiotic susceptibility pattern for MTB positive strains for both the first and second line was Levofloxacin, Kanamycin and PAS were found to be much sensitive whereas Isoniazid and Ethionamide were found to be more resistant than others. *Conclusion*: RT-PCR technique detects the total count of mycobacterial bacilli whereas via liquid culture only viable bacteria are detected i.e. true positive MTB strains. There were 2 MDR and 2 XDR TB detected, out of 11 confirmed MTB strains.

Keywords: AST; BACTEC 320; MTB Strains; RT-PCR Technique; Liquid Culture.

Introduction

Tuberculosis (TB) is a serious global public health problem. The diagnosis is made by detection of acid-fast bacilli on microscopy or culture, as Polymerase chain reaction may be false positive hence alone is not sufficient to make the diagnosis. To achieve early diagnosis and effective treatment of TB, rapid and accurate drug susceptibility testing (DST) methods must be used. The World Health Organization and the Centers for Disease Control and Prevention have recommended the use of liquid culture systems for DST and for improving time to detection [1,2,3]. Recent publications demonstrate the fundamental importance of liquid culture and phenotypic drug susceptibility testing (DST) as part of a complete strategy in the ongoing global efforts to combat

tuberculosis.

The BACTEC MGIT 320 is a new, lower-capacity instrument for liquid culture developed for the growth and detection of M. tuberculosis and antimicrobial susceptibility testing. MGIT has an improved speed and sensitivity of MTB isolation and drug susceptibility testing, irrespective of the HIV status of the patient. It is now employed for the diagnosis of female genital TB, a common cause of infertility in India. DST of *Mycobacterium tuberculosis* (MTB) with the BD BACTEC MGIT 320 system produces accurate results more rapidly than the conventional agar proportion method [4,5,6]. Multidrug resistant (MDR) and extreme-drug resistant TB (XDR) are a cause of serious concern [7,8].

In olden days before rifampicin, the antituberculous therapy (ATT) was given for 18–24 months with significant side effects and poor compliance but now a day's short-course chemotherapy for 6–9 months has been found to be effective for medical treatment of FGTB [9]. Surgery is only recommended after the continuous drug treatment of 12-18 months duration.

Material and Methods

The present study was done at Santosh Medical College and Hospital (Ghaziabad, Delhi NCR) in collaboration with Oncquest Laboratories Pvt. Ltd. (03 Factory Road, Safdarjung Delhi) in which a total of 217 samples were processed using different techniques like AFB smear microscopy, AFB liquid culture via BACTEC 320 and RT-PCR was done along with the DST of culture positive MTB strains for both first line and second line of drugs.

Sample Type

- Menstrual blood
- Endometrial tissue biopsy
- Tubal tissue biopsy
- Product of conception

Inclusion Criteria

1. Females willing to participate with their consent were included.
2. Study involved infertile and Tb suspected females with any of these symptoms like-

- Irregular menstrual cycle
- Pelvic pain
- Vaginal discharge that is stained with blood or which is persistent, heavy and discoloured.
- Bleeding after intercourse
- Infertility
- Abdominal mass
- Tubo-ovarian abscess
- Pregnancy loss
- Strong clinical suspicion of TB.

Exclusion Criteria

Eligible female patients not willing to participate and patients already on ATT and HIV positive were excluded for the study.

Methodology

Specimen Collection

Clinical specimens for female genital infections including endometrial and ovarian tissues along with menstrual blood were taken. Specimens were transported to the laboratory as soon as possible after collection. In case of delay, the specimens were refrigerated to inhibit the growth of unwanted micro-organisms.

Sample Processing

1. The sample was divided into three parts. First part was subjected for ZN staining, second was used for isolating the mycobacterial species by culturing and third was being used for molecular detection via PCR.
2. Samples were smeared with Ziehl-Neelsen (ZN) staining to confirm Acid – fastness followed by Homogenization and decontamination by NAOH-NALC method.
3. Isolation of Mycobacteria was carried out by culturing on liquid media by BACTEC 320 using MGIT tubes.
4. Real time PCR was run to amplify the product using the proper gene for the detection of MTB strains. IS6110–specific primers for Mycobacterium tuberculosis complex was used.
5. Analysis of staining, molecular, and liquid culture diagnosis for MTB strains was done and the efficacy of the techniques was defined along with the prevalence rate of infected infertile females suspected to have genital tuberculosis.
6. Before doing DST of confirmed MTB strains they

were first subcultured on blood agar to check for any contamination and if it comes sterile then again they were subcultured on MGIT to get appropriate growth conditions for performing DST. (Incase, if, contamination is detected on blood agar the tube was again processed for decontamination procedure to obtain the pure growth of Mycobacterium tuberculosis)

7. The MGIT reagents which were used in this study were MGIT Mycobacteria Growth Indicator Tubes (7 mL), MGIT Growth Supplement, MGIT PANTA, MGIT 960 SIRE Susceptibility Test Kit and the MGIT PZA Susceptibility Test kit.
8. Drug Susceptibility Testing (DST) was done by using automated BACTEC for confirmed TB cases. First and Second line drugs were used for Drug Susceptibility Testing.
   - First Line Drugs
     - Rifampin
     - Pyrazinamide
     - Streptomycin
     - Ethambutol
     - Isoniazid
   - Second Line Drugs
     - Levofloxacin
     - PAS (Para-aminosalicylic Acid)
     - Ethionamide
     - Kanamycin
     - Rifabutin

Results

Out of 217, TB suspected infertile female's maximum were from the age group of 26-30 i.e. 34.10% (approximately 74 females) and the lowest were under the age group of 15-20 years i.e. 2.3% (5 females).

By liquid culture there were 11 positive MTB strains. The maximum numbers of females were from the age group of 41-45 years (16.67%) whereas the minimum number lied under the age group of 15-20 (0%) and 31-35 years (0%).

Table 1: Depicting Age Wise Distribution of Infertile females Due To MTB Infection diagnosed via Bactec 320 (liquid culture)

Age Range	Total Patients	MTB Culture Positive	Percentage
15-20	5	0	0 %
21-25	33	3	9.09 %
26-30	74	6	8.10 %
31-35	70	0	0 %
36-40	29	1	3.44 %
41-45	6	1	16.67 %
Total	217	11	5.06 %

Table 2: Depicting Age Wise Distribution of Infertile females Due To MTB Infection diagnosed via Multiplex RT-PCR

Age Range	Total Patients	MTB PCR Positive	Percentage
15-20	5	1	20 %
21-25	33	4	12.12 %
26-30	74	17	22.97 %
31-35	70	2	2.85 %
36-40	29	4	13.79 %
41-45	6	1	16.66 %
Total	217	29	13.36 %

Table 3: Depicting MTB positivity by RT-PCR and AFB Cultures considering sample type

Sample Type	MTB Positives by RT-PCR		MTB Positives by Culture	
	Total Number	Rate (%)	Total Number	Rate (%)
Endometrial Biopsy / Tissue	24	11.05 %	11	5.06 %
Menstrual Blood	4	1.84 %	0	0 %
Product of Conception	0	0 %	0	0 %
Tubal Biopsy	1	0.46 %	0	0 %
Total	29	13.36 %	11	5.06 %

By RT-PCR technique there were 29 positive MTB strains. The maximum numbers of females were from the age group of 26-30% (22.97%) whereas the minimum number lied under the age group of 15-20

(0%) and 31-35 (2.85%).

Total 217 cases of infertile TB suspected females were observed via RT-PCR technique and liquid culture. Out of those 217, Endometrial Biopsy / Tissue (195), 24 were found to be MTB positive via RT- PCR whereas 11 were found to be positive by liquid culture. Menstrual Blood (17), 4 MTB's by RT-PCR and 0 were detected by culture. POC (4), out of which there was no MTB strain detected by RT –PCR technique and culture too. Tubal Biopsy (1), which was MTB positive by RT-PCR and 0 were positive by liquid culture.

The Antibiotic susceptibility pattern for confirmed MTB culture positive strains for both the first and second line was performed via automated BACTEC 320 system. Levofloxacin (90.9%), Kanamycin (90.9%), Pyrazinamide (100%) and PAS (100%) were found to be much sensitive whereas Isoniazid (45.5%) and Ethionamide (36.36%) were found to be more resistant than others.

Table 4: Depicting the antibiotic susceptibility of MTB strains

S. No.	Antibiotics	No. Of Patients Sensitive (n = 11)		Number of Patients Resistant (n = 11)	
		Number	Rate (%)	Number	Rate (%)
1	Streptomycin	9	81.81%	2	18.18%
2	Rifampin	9	81.81%	2	18.18%
3	Pyrazinamide	11	100%	0	0%
4	Isoniazid	6	54.54%	5	45.45%
5	Ethambutol	9	81.81%	2	18.18%
6	Levofloxacin	10	90.9%	1	9.09%
7	PAS	11	100%	0	0%
8	Ethionamide	7	63.63%	4	36.36%
9	Kanamycin	10	90.9%	1	9.09%
10	Rifabutin	9	81.81%	2	18.18%

Discussion

Genitourinay tract is the frequent site for extrapulmonary tuberculosis after the pulmonary one. GTB is generally secondary to renal tuberculosis [10].

TB is still a major health problem hence it is important to predict the possibility of GTB in patients presenting with infertility [11]. Most of time it remain undiagnosed due to lack of symptoms and lack of diagnostic modalities because of which the disease is prone to false positive as well as false negative results.

In this prospective study, via RT-PCR technique, 29 positive MTB strains whereas via liquid culture 11 positive MTB strains were detected. RT-PCR test detected MTB with in 24 h, compared with average 24 days required for detection by conventional method, as supported by earlier studies [12]. But still the conventional culture and microscopy is considered as gold standard.

The RT-PCR technique is restricted by the need for an appropriate infrastructure and high cost of the test. Molecular diagnosis of TB by RT-PCR has a great potential to improve the ability of diagnosis of GTB as RT-PCR is a rapid, sensitive and specific technique that can be used for early diagnosis of GTB. Though culture is a time consuming method, early RT-PCR can enable the consultant to diagnose GTB and start early treatment. But in this study RT-PCR was negative for 2 cases which were found to be culture positive by BACTEC MGIT 320.

ZN smear examination and RT-PCR results were positive but culture was negative; this could be due to the existence of nonviable mycobacterial bacilli in the samples [13].

The only drawback is that sometimes there may be false positive results by PCR test which could be due to the ability to detect very low number and even dead bacteria in a sample which can be present in a symptomatic individual [14].

Therefore, to confirm the diagnosis of TB, either acid-fast staining or culture must be performed. Both of these tests have poor sensitivity than RT-PCR because of paucibacillary tissue samples [15,16]. RT-PCR technique detects the total count of mycobacterial bacilli whereas via liquid culture only viable bacteria are detected i.e. true positive MTB strains.

The Antibiotic susceptibility pattern for MTB culture positive strains using both the first and second line of drugs was evaluated by which Levofloxacin, Kanamycin, Pyazinamide and PAS found to be much sensitive whereas Isoniazid and Ethionamide were more resistant than others. There were 2 MDR and 2 XDR TB detected. There was no literature found, as per our knowledge, to support our results determining antibiotic susceptibility pattern of infertile females due to tubercular infections using BACTEC MGIT 320.

Conclusion

RT-PCR technique detects the total count of mycobacterial bacilli whereas via liquid culture only viable bacteria are detected i.e. true positive MTB strains. By RT-PCR technique, 29 positive MTB strains whereas via liquid culture 11 positive MTB strains were detected. The Antibiotic susceptibility pattern for MTB culture positive strains using both the first and second line of drugs was Levofloxacin, Kanamycin, Pyazinamide and PAS found to be much sensitive whereas Isoniazid and Ethionamide were more resistant than others. There were 2 MDR and 2 XDR TB detected.

Abbreviations

- MTB: Mycobacterium Tuberculosis
- FGTB: Female Genital Tuberculosis
- RT-PCR: Real Time Polymerase chain reaction
- AFB: Acid Fast Bacilli
- RIF: Rifampicin
- NTM: Non - Tuberculous Mycobacterium
- MGIT: Mycobacterium Growth Indicator Tube
- (ZN) staining: Ziehl-Neelsen staining
- AST: Antibiotic Susceptibility Testing
- ATT: Anti Tubercular Treatment

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