

# Study of in Vivo and in Vitro Growth of Mycobacterium Tuberculosis From the Intra-operative Samples of Patients of Osteoarticular Tuberculosis

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**Abstract:** Background: “Study of in vivo and in vitro growth of mycobacterium tuberculosis from the intra-operative samples of patients of osteoarticular tuberculosis”.

Methods: Study was carried out in patients volunteers of osteoarticular tuberculosis. Experimental animal was Swiss albino mice. Pus samples for the study was obtained from osteoarticular kochs patients intraoperatively. One part was sent for BACTEC 460 TB for culture and the other was used for the inoculation in study animal.

Comparison of the final reports of Z-N staining, L-J medium for culture, HPE, BACTEC 460 TB was done between the patient sample and the mice peritoneal lavage sample.

Conclusion: The Z-N staining and LJ medium though cheaper and freely available are not a good method for diagnosis of tuberculosis. The study reflected an outcome of 30% growth by TB - BACTEC method, there is more possibility for isolating the bacilli by TB - BACTEC method from pus sample than that of granulation tissue. As the TB - BACTEC was negative from all mice samples the use of murine model is not suitable for isolation and culture of Mycobacterium tuberculosis.

Keywords: Osteoarticular tuberculosis, murine model.

## THESIS SUMMARY

### Aims and Objective

The principle aim and objectives of this study are as follows:

1. To grow bacilli in the peritoneum of mice from intra operative pus samples of Koch's patients.
2. To isolate and identify the bacilli and do culture sensitivity against 1st & 2nd line anti tubercular drugs.

To correlate these with clinicoradiological follow up of patients.

### Methods

Institutional Ethics committee permission was obtained prior to start of the study.

A) Volunteer selection:

Study was carried out in patients (male / female) volunteers of osteoarticular tuberculosis. After taking written informed consent, all patients with suspected clinicoradiological tuberculosis were included in the study.

B) Inclusion criteria:

- Volunteers of all age group

- Willing to give written informed consent

C) Study procedure:

The study was done to fulfill above-mentioned objectives. The study methodology is given below.

D) Experimental animal:

- 1) Animal: Mice
- 2) Strain: Swiss albino mice of either sex
- 3) Randomization: Randomly selected at the time of delivery.
- 4) Animal Identification: By cage number and individual marking on tail
- 5) Weight at the start of study: 20-25 gm.

Animals were handled according to the CPCSEA guidelines for laboratory animal facility<sup>15</sup>

E) Husbandry conditions:

1) Environment:

Air conditioned with 12-15 filtered fresh air changes per hour, temperature: 22-30°C, relative humidity: 30-70%. The

temperature in the experimental room was recorded once daily and the humidity in the experimental room was calculated daily from the dry and wet bulb temperature recordings.

#### 2) Accommodation:

A mouse (1 per cage) was housed in separate cages during acclimatization and study (approximate size of cage: 1.290 x W220 x H140 mm). The cage will be of stainless steel top grill having facilities for food and drinking water in glass bottles with stainless steel sipper tube.

#### 3) Diet and water:

Rodent food of Chakan Oil Mills Ltd. Maharashtra given ad libitum. Aqua guard pure water in glass bottle ad libitum.

#### 4) Acclimatization:

Seven days prior to initiation of the treatment for adult mice.

#### F) Collection of pus sample:

Pus samples for the study was be obtained from osteoarticular kochs patients intraoperatively at Orthopedic Surgery Department. The sample was collected in sterile air tight containers. This pus samples was divided in two parts. One part was send for BACTEC 460 TB for culture and the other was used for the inoculation in study animal at Central Animal house. Sample was also send for Z-N staining, L-J medium for culture and HPE

All the samples were sent immediately for testing to respective laboratories. In case of delay they were refrigerated in OT refrigerator (2-80 C) as advised by concerned microbiologist.

#### G) Procedure of BACTEC 460 TB16:

Semi automated radiometric BACTEC 460 TB (Becton Dickinson, Sparks, MD, USA) liquid media is used. The detection of mycobacterium growth in BACTEC 12B medium is carried out quantitatively by measuring of the  $^{14}\text{CO}_2$  liberated by the metabolism of  $^{14}\text{C}$  – labelled substrate present in the medium. Specimen is first decontaminated from normal bacterial flora by using standard N-acetyl-L-cysteine-NaOH method.

All inoculated 12B vials will be tested twice for first three weeks and then once a week for remaining three weeks. Positive vials will be subjected to smear microscopy. Final identification of M. tuberculosis complex (MTB) will be done by the BACTEC NAP (r-nitro- $\alpha$ -acetyl amino- $\beta$ - hydroxy propiophenone) differentiation test.

#### H) Inoculation in study animals:

The study animal of either sex was included in the study and injected in two divided doses with pus specimen intraperitoneally (40 ml/kg). At the end of 28 days, all the animals were sacrificed by euthanasia, laparotomy was performed, the viscera was irrigated gently with saline and washings was collected. This irrigated sample was sent for identification and isolation MTB by BACTEC 460 TB. Sample was also send for Z-N staining, L-J medium for culture and HPE.

Comparison of the final reports of Z-N staining, L-J medium for culture, HPE, BACTEC 460 TB was done between the patient sample and the mice peritoneal lavage sample.

#### I) Parameters of assessment:

1. Activity level, feeding (average 10 to 15 gram/week).
2. Serial weekly weight monitoring.
3. Fur coat-luster and appearance.

#### J) Disposal of animals:

All the sacrificed animals were disposed taking standard precautions.

K) Implication of the study: The peritoneal lavage collected during the study was used for culture and sensitivity testing against 1st line and 2nd line AKT. Depending upon the sensitivity pattern the therapy for tuberculosis on the patients can be modified. Many times we come across situations where clinicoradiologically patient does not improve to the expectation, laboratory culture might not be positive for bacteria but if it grows in vitro then sensitivity testing can help us change the drug regime accordingly.

## Result

1) Total Patients: 22

2) Ziehl Nelson staining positivity—Human : None

Ziehl Nelson staining positivity—Mice : None

3) AFB Culture in L-J Medium—Human : None

AFB Culture in L-J Medium—Mice : None

4) AFB Culture with Tb-Bactec Method—Human : Six

AFB Culture with Tb-Bactec Method—Mice : None

5) Scab: Staph. aureus in three specimen in both Human & Mice

6) HPE—Human : Seven S/O of TB

One of NHL

HPE—Mice : Three S/O of TB

7) MDR Cases: None

8) HIV Positive: None

9) Clinicoradiological improvement : ALL

10) No. of Death : Two (Died of unrelated causes)

11) TB BACTEC Positive results from tissue : One

TB BACTEC Positive results from pus : Five.

## Conclusion

The conclusions of the study came out to be from the 22 samples of osteoarticular tuberculosis are as follows. The Z-N staining and LJ medium though cheaper and freely available are not a good method for diagnosis of tuberculosis.

Out of 22 cases 3 cases turned out to be Staph. aureus which was confirmed with the help SCAB and experimental model of mice with patients responded to routine antibiotics. One patient was diagnosed as NHL. Rest of the patients responded to 1st line AKT with clinicoradiological improvement. 6 patients showed growth on TB – BACTEC culture which was found sensitive to 1st line AKT confirming the clinicoradiological picture.

The study reflected an outcome of 30% growth by TB- BACTEC method. Out of the six samples 5 were pus sample and only 1 sample was granulation tissue sample.

So there is more possibility for isolating the bacilli by TB – BACTEC method from pus sample than that of granulation tissue.

Histopathological examination came out positive in 7 human samples and three mice samples supporting the diagnosis of tuberculosis.

As the TB – BACTEC was negative from all mice samples the use of murine model is not suitable for isolation and culture of Mycobacterium tuberculosis.

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