4. SCOPE AND PLAN OF WORK

Scope:

Culture of all clinical samples should be included in the routine diagnosis irrespective of smear results to increase the sensitivity of TB diagnosis. Culture of blood and stool in all the patients irrespective of the site of pathology may provide better sensitivity in TB diagnosis.

The previous studies of drug resistance, very clearly indicated that the drug resistance is increasing in incidence. So in the present study, all the clinical samples of TB patients (both smear positive and smear negative) were subjected to culture and sensitivity testing which helped in the early diagnosis and treatment of drug resistant TB. This also helped in choosing a proper regimen for treatment and in preventing the spread of drug resistant TB in the community.

Knowledge of Candida speciation and DST provides proper selection of the drug in the therapy.

Plan of work:

Study period: August 2010 to June 2013

First 6 months (Aug-Dec 2010) - to standardize protocol for AFB blood culture 28 months (Aug 2010 - Dec 2013): for sample collection, processing, culture and speciation for both tuberculosis and candidiasis.

2 months (Jan 2013 – Mar 2013): for drug susceptibility testing of both Mycobacteria and Candida isolates.

2 months (Apr 2013-Jun 2013): Data compilation, statistics, and for preparation of manuscript.
Group A:

Clinical suspicion of tuberculosis

Sample collection

Sputum  Stool  Blood  Body fluids  Biopsy/FNAC

Processed as per standard protocol

Inoculated onto Lowenstein Jensen slants

Colony morphology recorded

Smears prepared and stained by ZN technique to confirm as AFB

Isolates subjected to p-nitrobenzoic acid test for speciation

Isolates tested for drug susceptibility by proportion method and then confirmed by GenoType MTBDR plus assay (Hain’s Lifescience).
Group B:

Clinical suspicious of candidiasis

↓

Sample collections

↓

Oral swab       Esophageal swab

↓

Inoculated on Sabouraud dextrose agar with chloramphenicol medium

↓

Colony smeared, gram stained and observed for yeast cells

↓

Germ tube test

↓

24 hrs cultures were tested for speciation

↓

Morphology       Biochemical tests

On Cornmeal agar

↓

Sugar assimilation       Sugar fermentation       Urease test

↓

Antifungal susceptibility

by Vitek 2 automated system.
MATERIALS

Equipments:

1. Autoclave                  Alpha Scientific Co
2. Biosafety cabinet        Alpha Scientific Co
3. Centrifuge                REMI R-8C
5. Digital balance           Alpha Scientific Co.
6. Hot air oven              Alpha Scientific Co.
7. Incubator                 Alpha Scientific Co.
8. Lumbar puncture needle    Tarsons Product Pvt. Ltd.
9. Micropipette              Eppendorf
10. Microscope               Olympus
11. Refrigerator             Godreg 2SK92
12. RT PCR                   Corbett Research, Rotergene RG 3000.
13. Twincubator              Hain Lifescience GmbH
14. VITEK 2 automated system Biomerieux
15. Vortex mixer             REMI
16. Water bath               KEMI
17. Wintrobe’s tube (10ml)   AQRA

Miscellaneous:

1. Bunsen burner
2. Coarse glass beads
3. Conical Centrifuge tube (15ml)   Tarsons Product Pvt. Ltd.
5. Culture swab                Himedia Laboratories P. Ltd.
6. EDTA Vacutainer tubes       BD Diagnostics
Materials and Methods

7. Eppendorf tube  Tarsons Products Pvt. Ltd.
8. GenoType MTBDR plus assay  Hain Lifescience
9. Inoculation loop  Himedia Laboratories P. Ltd.
10. Microtips (10 µl, 100 µl, 1000µl)  Eppendorf
11. Non-absorbent Cotton roll
12. pH strips  Glaxosmithkline Pvt. Ltd
15. Syringes (2ml, 5ml)  Dispo Van.
16. Test tube stands
17. Tweezers (Forceps)
18. YST card  Biomeriux

Glasswares: (Borosil)

1. Beakers
2. Conical flasks
3. Coverslips
4. Durham’s tubes
5. Glass slides
6. Graduated Measuring cylinder
7. Petriplates
8. Test tubes

Chemicals: (Himedia)

1. Acid Alcohol
2. Agar agar
3. Andrades indicator
4. Barium chloride
5. Carbol fuchsin
Materials and Methods

6. Christensen’s urea agar
7. Hichrome *Candida* differential Agar base
8. Cornmeal agar
9. Crystal violet
10. Glucose
11. Glycerol
12. Hydrochloric acid
13. Iodine
14. Lactose
15. Leishmann stain
16. Lowstein Jensen slants
17. Maltose
18. Methylene blue
19. Middlebrook 7H9
20. P- nitrobenzoic acid slants
21. Peptone
22. Phenol
23. Saffranin
24. Saponin
25. Sodium chloride
26. Sodium citrate
27. Sodium hydroxide
28. Sucrose
29. Sugar discs:
   (Glucose, Maltose, Sucrose, Lactose, Galactose, Mellibiose, Cellobiose, Inositol, Xylose, Raffinose, Trehalose, Dulcitol.)
30. Sulphuric acid
31. Tween 80
32. Yeast nitrogen base.