CONTENTS

1. Introduction 1-9
   1.2. Objective 10

2. Review of Literature 11
   2.1. Mycobacterium tuberculosis complex
   2.2. Morphology of Mycobacterium tuberculosis
      2.2.1 Microscopic morphology 11-13
      2.2.2 Culture characteristics 13
   2.3. Etiological characteristics 14-15
   2.4. Epidemiological Characteristic
      2.4.1 General epidemiology 15-16
      2.4.2 Epidemiological scenario in India 16-17
   2.5. Anti-Tuberculosis Drugs 17
      2.5.1 Drug resistance and sensitivity 17-18
      2.5.2 Types of resistance 18-20
   2.6. Diagnosis and detection
      2.6.1 Acid Fast staining (Ziehl-Neelsen) 21
      2.6.2 Biochemical Tests 21-24
   2.7. Drug Susceptibility Tests 24
      2.7.1. Critical concentrations of drugs 24-25
      2.7.2. Culture methods 25-29
      2.7.3. Nucleic acid amplification techniques 29-32
   2.8. Strain typing and identification of Mycobacterial Species
      2.8.1. Amplified Ribosomal DNA Restriction Analysis (ARDRA) 33-34
      2.8.2. DNA Sequencing 34-35

3. Materials and methods
   3.1. Sample collections 36
   3.2. Digestion and Decontamination process 36
      3.2.1 Acetyl-L-cysteine–sodium hydroxide (NALC-NaOH) method 36-37
3.3 Detection tests
  3.3.1 Acid fast Staining 37-39

3.4 Biochemical Tests
  3.4.1 Niacin accumulation 39-41
  3.4.2 Catalase test 41-42

3.5 Drug Susceptibility testing
  3.5.1 Critical concentration of antibiotics in Drug Susceptibility Testing 43
  3.5.2 Antibiotic Preparation for DST 43-44
  3.5.3 Proportion method 45-47
  3.5.4 Nitrate reductase test 48-51
  3.5.5 Microscopic observation drug susceptibility assay (MODS) 51-53
  3.5.6 BacT/Alert 3D Drug susceptibility testing 53-55

3.6 DNA Extraction 55-57

3.7 DNA purification 57-58

3.8 Line Probe assays (Genotype MTBDRplus and Genotype MTBDRsl) 58
  3.8.1 Drug Susceptibility/Resistance Test by Genotype Strip Methods 58-62

3.9 Strain typing 62
  3.9.1 Amplified Ribosomal DNA Restriction Analysis 62-65
  3.9.2 DNA sequencing and phylogenetic analysis 66
  3.10 Statistical Analysis 66-67

4. Results
  4.1 Demographic characteristics 68-70
  4.2 Detection tests of MTB 70-71
  4.3 DST patterns 71
    4.3.1 Proportion method 71-73
    4.3.2 Drug susceptibility pattern by MODS method 73-76
    4.3.3 Comparison of DST Pattern of Proportion Method and Mods 76-78
    4.3.4 Drug susceptibility pattern by NRA method 78-79
    4.3.5 Comparison of DST pattern of Proportion method and NRA 80-81
    4.3.6 DST PATTERN BY BacT/Alert 3D 81-82
    4.3.7 DST Pattern by Genotype MTBDRplus 82-83
    4.3.8 Comparison of Genotype Hains Test and BacT/Alert 3D 83-85
4.3.9 Pattern of gene mutations associated with INH, RMP and EMB as detected by MTBDRplus and MTBDRsl

4.4 Strain typing and phylogenetic analysis of selected mycobacterial species representatives using ARDRA and 16srRNA sequences

4.4.1 ARDRA

5. Discussion

Abstract

Conclusion

Future Prospect

References

Appendix A: 16S rRNA sequence

Appendix B: Publication