CHAPTER - 2
AIMS AND OBJECTIVES
2. AIMS AND OBJECTIVES

The present study is being conducted with the following aims and objectives:

1. To study the efficacy of standard Ziehl Neelsen smear and Auramine O smear for detection of acid-fast bacilli in sputum.

2. To see the role of repeat sputum smear examination in the diagnosis of pulmonary tuberculosis.

3. To study the efficacy of different concentration methods for detection of AFB in sputum.

4. To see the rate of isolation of Mycobacteria in Lowenstain Jensen medium from sputum samples of pulmonary tuberculosis patients.

5. To characterize the isolation of Mycobacteria by biochemical tests.

6. To see the incidence of non-tubercular Mycobacteria in pulmonary tuberculosis.

7. To see the prevalence of drug resistance in Mycobacteria isolated from pulmonary tuberculosis patients.
CHAPTER - 3

REVIEW OF LITERATURE
3. REVIEW OF LITERATURE

3.1 Characteristic features of Mycobacteria

The genus *Mycobacterium* has several distinctive biological characters differentiating its members from most other microorganisms. As per the existing VIIth edition of “Bergey’s Manual of Determinative Bacteriology” 1974, the systematic position of *Mycobacteria* is as described below:

- **Division**: Prokaryotes
- **Sub-division**: Protophyta
- **Class**: Schizomycetes
- **Order 6th**: Actinomycetales
- **Part 17th**: Actinomycetes and related Organism
- **Family**: *Mycobacteriaceae*
- **Genus**: *Mycobacterium*

*Mycobacteria* belong to the order Actinomycetales and thus have some resemblance with other members of *Actinomycetes, Nocardia* and *Corynebacteria*. Lehman and Neumann (1896) established the generic name *Mycobacterium* and to provide nomenclature to leprosy and tubercle bacilli. Classically, members of the genus *Mycobacterium* are aerobic to microaerophilic, range from obligate parasites, saprophytes and intermediate forms differing in their nutritional requirements. The
original description of the genus was based mainly on morphological and staining properties. There were 41 approved species of the genus Mycobacterium till 1980 (Seirman et al, 1980). Since then several new 'Taxons' have been identified. As a result, this genus has now more than 54 species (Good, 1985) of which 17 are pathogenic to man and animals. Mycobacteria other than tubercule and leprosy bacilli have been often termed as "Atypical". Earlier these were divided into four provisional groups (Runyon, 1959):

**Group I (Photochromogens)**- Slow growing cultures, which produce bright yellow pigments on exposure to light.

**Group II (Scotochromogens)**- Slow growers, which produce pigment even in the dark. The growth in culture, produce orange pigment when exposed to light.

**Group III (Non-chromogens)**- Slow growers that remain colourless. even after exposure to light.

**Group IV (Rapidgrowers)**- Include strains, whose rate of growth is fast. On primary culture, the growth should be visible i.e. produce colonies within 7 days, under optimal conditions of nutrition and temperature.
The important characteristics of *Mycobacteria* are:

### 3.1.1 MORPHOLOGY

Morphologically mycobacteria possess mycelial type of colony characters (*Mycobacterium*: Gr. n. myces – a fungus, Gr. neut, dim. n. bakterion a small rod; M.L. neut n. *Mycobacterium* a fungus rod let). These organisms rarely exhibit grossly visible aerial hyphae (Goodfellow and Wayne, 1982).

According to the growth conditions, variations in size and shape occur between species and within species, ranging between short coccobacilli (*M. avium*), curved rods (*M. microti*) and long filament forms (*M. smegmatis*) (Runyon et al., 1974). Some of these may be straight, slender, slightly curved, and stubby and rod shaped bacilli. *M. tuberculosis* was first demonstrated by Robert Koch in 1882. The size of tubercule bacilli ranges from 1 to 4 μm in length and 0.3 to 0.6μm in diameter, which may be considered as typical of the genus. Some members may have tapering, rounded or club-shaped ends measuring 1.0 to 8.0μm in length and 0.2 to 0.5μm in width (*M. leprae*). The leprosy bacillus (*M. leprae*) that was first discovered by Hansen (1874) closely resembles the tubercle bacillus. Under electron microscope, whole *M. leprae* cells appeared to be electron-transparent with dense polar ends (Koike and Takeya, 1961; Imaeda and Ogura, 1963).
3.1.2 Culture

Most of the *Mycobacterial* species tend to grow slowly. Some *Mycobacterial* species like *M. lepae* have not grown on artificial culture media (Katoch *et al*, 1989). In general, *Mycobacteria* require enriched culture media. Primary growth may be obtained preferably on a medium containing whole egg or egg yolk, malachite green usually added to inhibit contaminants and to provide contrast colour to colonies, incubated aerobically at 37°C (Cruickshank *et al*, 1974). Commonly used solid media are L-J medium (Lowenstein, 1930; Jenson, 1932), Dubos oleic acid-albumin agar (Dubos and Middlebrook, 1947), Middlebrook 7H-9 and 7H-10 or 7H-11 (Middlebrook and Cohn, 1958). Several liquid media have been tried and found useful for growing *Mycobacteria*. These include Sauton’s medium (Sauton’s, 1912), Dubos broth (Dubos and Middlebrook, 1947) and Sula’s medium (Sula, 1963). The generation time of *In-vitro* cultivable *Mycobacteria* varies from 6-12 hrs. (Cruickshank *et al*, 1974). Growth comprises of mainly 4 phases, viz. Lag, log, stationary and decline. The incubation period for primary growth to appear on a solid media generally varies from <7 days for rapid growers and >7 days for slow growers (Runyon, 1959). In liquid medium, bacteria grow on the surface as a wrinkled pellicle. A diffuse growth can be obtained by adding 0.5% Bovine serum albumin (BSA) along with 0.1-1% Tween-80 i.e. polyoxyethylene sorbitan mono-oleate (Cruickshank *et al*, 1974).
Mycobacterium leprae is often referred to in the standard texts as the "non-cultivable Mycobacterium". This inaccuracy is perpetuated because as yet others have accepted no claim for the successful cultivation of M. leprae on a laboratory medium. The cultivation of this organism has been attempted on almost all types of laboratory media and media containing cellular extracts, vitamins, and hormones and at various temperatures and under numerous ranges of atmospheric conditions. Nevertheless the numerous claims to grow M. leprae on any artificial media have not been accepted. At present, the nutritional requirement of M. leprae is not known (Stewart-Tull. 1982).

3.1.3 Staining

Mycobacteria are basically Gram-positive organisms, but do not easily take up gram stain. They are usually acid, alcohol or acid-alcohol fast organism. A special staining technique called Ziehl-Neelsen staining is commonly employed for Mycobacteria. Mycobacteria are generally stained by treatment with hot (as well as cold) carbol fuschin which allows impregnation by the dye which is retained despite attempts to remove it with acid or alcohol, possibly due to the anatomical peculiarities of cell wall and presence of lipids and mycolic acid esters (Draper, 1982) and other waxy structures in the cell envelope. Mycobacteria are thus called as "acid-fast" organism. Acid fastness has been of immense practical importance in the detection of presence of Mycobacteria, especially in pathological specimens from the cases or
suspected cases of *Mycobacterial* diseases (Draper, 1982). Leprosy and tubercle bacilli are acid fast as well as alcohol fast, and a mixture of acid and alcohol is usually used in the standard method of staining. *M. leprae* is less acid fast than *M. tuberculosis* (Jopling and McDougall, 1980) and other *Mycobacterial* species. If stained smears are treated with pyridine, *M. leprae* loses its acid-fastness, this is known as pyridine extractability, and has been used to distinguish *M. leprae* from all other acid fast *Mycobacteria* (Convit and Pinardi, 1972; Draper. 1986; Jopling and McDougall, 1988). However, this property has been found to be of limited value by others (Skinsness *et al.*, 1975; Sloscareck *et al.*, 1978; Datta *et al.*, 1983). The density of bacilli in smears from leprosy patients is popularly known as the bacteriological Index (BI). The percentage of solid staining bacilli is known as “morphological Index (MI) and it has been described to correlate with viable population (Shepard and McRae, 1965; McRae and Shepard, 1971; Katoch *et al.*, 1989a). These indices have been used to monitor the effect of chemotherapy (Jopling and McDougall, 1988).

### 3.1.4 Pathogenicity

Several species of *Mycobacteria* are pathogenic to man and other mammals, birds, reptiles and fishes (Chapman, 1977; Wolinsky, 1979; Manjula, S. and Sritharan, V. 2002). Many slow growing *Mycobacterial* species and some rapidly growing strains are pathogenic to man, birds
and poikilothermic animals (Lepper and Corner, 1983; Rastogy, N. et al, 2001).

In general *Mycobacterial* pathogenicity appears to depend on their being able to resist destruction by lysosomal enzymes when inside the phagocytes (Cruickshank et al, 1974,) and they proceed to multiple in cytoplasm of phagocytes, especially in the macrophages, which are particularly active in their ingestion (Cruickshank et al, 1974). After intracellular growth has taken place macrophage become laden with numerous bacilli, the macrophage dies and disintegrates, the liberated bacilli may continue to multiply extracellularly such as in tuberculous tissue fluids (Cruickshank et al. 1974).

### 3.1.4.1 Tuberculosis

The organism usually responsible for human tuberculosis is *M. tuberculosis*, rarely *M.bovis* and in a few instances *M.africanum*. Most cases are caused by *M.tuberculosis* for which humans constitute the only significant reservoir (WHO, 1991). The tuberculosis may involve lung, intestine, kidney, genital tract, bone joints and lymph nodes. More generalized infection may follow, leading to milia ry or bronchopneumonic tuberculosis, usually with lesions in other organs besides the lungs e.g. in brain and meninges (Tuberculous meningitis), spleen, liver and kidneys (Cruickshank et al, 1974). Primarily infection of alimentary tract usually results from ingestion of milk infected with
M. bovis but sometimes may occur from use of common eating utensils of food contaminated with *M. tuberculosis* (Cruickshank *et al.*, 1974).

**HISTORICAL ASPECTS:**


Hippocrates (460-370 BC), the father of the modern medicine and eminent and renowned epidemiologist described it “Phthisis” to waste away (Hudson and Sellors, 1963). Aristotle and Celsus also recognized and described the disease and its management. The literature of the library of “Leipzig” revealed that “Jesus Christ, an eraman, has suffered from this distorios disease (Rao, 1981).

Various forms and manifestation of this disease, such as tubercular cold abscess, bovine tuberculosis, haemoptysis, contagious nature of the disease were identified and detailed by Pliny (50 A.D.), Aretacus of Ramo, Galen (130-200 A.D.) and Vegetious (420 A.D.) respectively. The Arabian physicians of middle ages (400-1400 A.D.) namely Rhazes
(850-923 A.D.) and Aveenna (930-1037 A.D.) led the knowledge towards dark by explaining misbelieve, based on totally unscientific facts. In England, the disease was called as king's evil during the 11th and 12th century and touching of king's feet was practiced as a measure of its cure (Rao, 1981).

Jerome Fracastor in 1483 described the infectious nature of the disease. Franciscus Sylvius (1614-1672) found 'tubercles' after autopsy of lung in cases of tuberculosis. Richard Morton (1637-1668) in his famous book 'Phthisiologia' (1689) wrote on clinical features of tuberculosis and distinguished it from other forms of pulmonary disease. He further, recognized that in youngs the disease tended to be acute and in olds, it tended to be chronic (Hudson and Sellors, 1963). Morgani (1682-1771) was the first to describe the pathological condition of the lung after autopsies. Pierre Desault (1674-1740) observed that the disease spread through sputum. Bengamin Mortin (1720) hypothesized the existence of microbe in the pulmonary circulation, one hundred and twenty years before the discovery of Mycobacterium. Gaspard Lauret Bayle (1774-1816) Introduced the term tuberculosis for the first time and established a relationship between pulmonary tuberculosis and tuberculosis of other organs (Hudson and Sellors, 1963; Rao, 1981).

At the beginning of nineteenth century a french man, Rene Theodore Laennec (1781-1826), himself a patient of tuberculosis, invented stethoscope and demonstrated technique of auscultation; he showed that
various forms and types of tuberculosis were a single entity. Viillemin (1865) hypothesized that tuberculosis was due to specific causative organisms and demonstrated the transmissibility of the disease. Robert Koch (1843-1910) a myopic, short statured, great German microbe hunter described the presence of tubercle bacilli, in March 1882, in tubercular patients. Later on, Rudolf Virchow (1821-1902), the founder of cellular pathology, described the development of caseation in tuberculous tissue. In December 1890, Koch produced tubercul in and described ‘Koch’s phenomenon’. During two decades of 19th century, lot of work on bovine tuberculosis was done by Coni 1884. Magueei 1890 and Theobald Smith 1898 to find out chickens tubercle bacilli, avium bacilli and bovine bacillus respectively. Wilhelm Roentgen (1885), a Professor of physics in Germany, discovered x-ray, which helped much in the diagnosis of tuberculosis. In subsequent years, radiology and bacteriology both helped much in developing further knowledge of the disease (Hudson and Sellors, 1963; Mehrotra, 1976; Rao, 1981; Editorial, 1981; Crofton and Douglad, 1981).

THE WORLD SITUATION:

Tuberculosis, ‘a worldwide malady’ has been posing a great threat especially in the developing countries since long. There are about 20 million infectious cases in the world with a yearly addition of 4-5 million new highly contagious cases (x-ray and culture positive) only from developing countries. However, 300 million die each year leaving
the pool of infectious cases. On an average, two third deaths occur within two years of the disease and an untreated case in its life span of two years infects, on an average, 24 persons. It has been estimated that if nothing is done and population growth remains as such, 40-50 million persons will develop tuberculosis and two third of them will die of it in coming ten years (W.H.O., 1965, 1974, 1982a; Chaparas, 1982; Styblo, 1982).

The notification data on tuberculosis reveal that in countries of western hemisphere viz. Northwest territories of Canada, Guatemala, Peru, Elsalvador and in majority of the European countries viz. Italy, Rumania, Yugoslavia, Austria, France, West Germany etc. The notification rates vary within 50-100 or more per 1,00,000 population, however, in other countries viz. Canada, U.S.A., Belize, Panama, England and Wales, Sweden, Netherlands and Denmark notification rates are below 24/1,00,000 population. The highest notification rates were observed in Bolivia (436/1,00,000) in Western hemisphere and European countries respectively (Lowell, 1975; Bulla, 1977; W.H.O., 1975, 1978; Public Health Service, 1982).

A great variation has been observed in the notification rates of tuberculosis in African countries. The rates ranged from 250-300/1,00,000 in Morocco, Sahara, Mauritania and South Africa to 50-100/1,00,000 in Egypt, Nigeria, Zimbabwe and Ghana etc., however, Niger, and Cameroon had notification rates below 24/1,00,000.
population. Recently, Fourie et al., (1980) and Arabin et al (1979) in black home lands of South Africa, reported prevalence of active tuberculosis and bacillary tuberculosis to be 8,700/1,00,000 and 220/1,00,000 in Transkei and 2,700/1,00,000 and 840/1,00,000 in Kwazulu respectively which were greatly higher than the officially reported notification rates (W.H.O., 1974; Lowell, 1975; Bulla, 1977; Fourie et al, 1980; Arabin et al, 1979).

Among Asian countries, Singapore, Japan and Philippines reported notification rates to be 126, 118 and 326/1,00,000 population respectively. Pakistan and Afghanistan, Where notification rates were not available, reported prevalence of active tuberculosis to be 4,700 and 2,000/1,00,000 population respectively, however, Nepal reported prevalence of bacillary disease to be 1,000/1,00,000 population showing highest morbidity in the world. Australia, among the ocean countries, had lowest notification rate of 10.5/1,00,000 population (Lowell, 1975; Bulla, 1977; Abdulla, 1976; Nepal Medical Association, 1978).

A tremendous reduction has been observed in the mortality rate of tuberculosis during last 30 years. In many advanced countries viz. Netherlands, Australia, Denmark and Canada etc., the mortality rate has come down to 1/1,00,000 population, however, it remains still high (up to 76.1/1,00,000 in Macao, an island in Western pacific) in countries of Asia, Africa and South America (Lowell, 1975; Bulla, 1977).
Infected population varies widely in different parts of the world ranging from about 7% in U.S.A. to approximately the entire population in certain countries in Asia, Africa, and South America. In most of the developing countries, 2-5% of the population has been at risk of infection during last 25 years, which is 20-50 times higher than developed countries. The probability of the development of disease among tuberculin positive individuals varies from about 30/1,00,000 in Denmark to about 600/1,00,000 in some Eskimos’ population. According to W.H.O. reports, in developing countries every 1% of the population at risk of infection appears to correspond to 50 new cases of smear positive pulmonary tuberculosis per 1,00,000 population (W.H.O., 1982a; 1982b; Chaparas, 1982).

**The Indian Scene**

Tuberculosis continues to be one of the most important public health problems in India with 10 million total and 2.5 million sputum positive highly contagious cases. Furthermore, about 2.5 million new cases arise and 0.5 million die of this disease every year. The information on the prevalence of the disease had been meagre until late thirties and forties from this country when a few small-scale surveys were carried out in various parts of the country to ascertain the extent of the problem. These surveys revealed that the overall prevalence of tuberculosis ranged from 4.2-70.0 per 1,000 populations and that of bacillary tuberculosis, ranged from 2.4-30.0 per 1,000 populations (Lal et al, 1943; Aspin, 1945;
Frimodt Moller, 1949; Hertuberg, 1952; Frimodt Mollar et al, 1952; Bajaj, 1982).

The National Sample Survey 1955-1957, covering a population of 3,00,000, aged 5 years and over nesting in rural/urban/slum areas revealed that the overall x-ray positive and bacillary positive prevalence of tuberculosis ranged from 13.5 to 26.6 and 2.4 to 8.2 per 1,000 population respectively (ICMR, 1959.) However, Raj Narain (1963) in Tumkur, Pamra et al (1973) in Delhi and Bagga et al (1974) in Madras reported an overall x-ray positive and bacillary positive prevalence of tuberculosis to be 19.0 and 4.1, 8.8 to 17.2 and 2.1 to 4.0 (In four longitudinal surveys) and 16.0 and 6.9 per 1,000 populations respectively in their investigations. However, National Tuberculosis Institute (1974) in Bangalore, reported prevalence of bacteriologically confirmed disease ranging from 4.06 to 3.37 per 1,000 populations in these successive surveys.

Gothi et al (1976; 1979); Krishnaswamy et al (1978); Chakraborty et al (1979) and Tuberculosis prevention Trial, Madras (1979) reported prevalence of bacillary tuberculosis to be 3.2, 4.4, 8.0, 2.6 and 11.0 per 1,000 population reactively in their studies. Furthermore, Krishnaswamy et al (1978), Chakraborty et al (1979), and Gothi et al (1979) also reported an overall prevalence of x-ray positive tuberculosis to be 20.8, 4.4 and 10.0 per 1,000 populations respectively in their investigation.
Incidence of tuberculosis has been reported to be about 1.3 per 1,000 populations by Frimodt Moller (1960) in Madanepalle, Pamra et al (1973) and Goyal et al (1978) in Delhi and National Tuberculosis Institute (1974) in Bangalore, however, Tuberculosis prevention Trial (1980) in Chingleput, Madras, revealed an incidence rate of 2.5 per 1,000 populations.

Incidence of tuberculosis has been found to be higher amongst the tuberculin reactors and in persons having probably active x-ray shadows in comparison to tuberculin non-reactors and in those having inactive lesions in x-ray. Gothi et al (1978) in his study, observed an incidence of tuberculosis to be 0.41 per 1,000 amongst tuberculin non-reactors (0 to 9mm, size) in contrast to 1.7 per 1,000 populations amongst tuberculin reactors (10 mm, or more). In a follow-up study of the same population, it was revealed that the incidence rate was 3.73 and 26.0 per 1,000 populations respectively amongst those with inactive/probably active lesions in chest x-ray, having no treatments. Further in depth, study analysis revealed that 76.0% of these cases arose from previously tuberculin positive in contrast to 48.0% of these cases appearing from the population with normal chest x-ray.

National Tuberculosis Institute (1974) also reported considerable higher incidence of tuberculosis (1.9 per 1,000) amongst persons with tuberculin reaction of 20mm. Or more; the incidence was lowest (0.27 per 1,000) among persons with normal chest x-ray and tuberculin
reaction between 10 and 19 mm. Moreover, Krishnamurthy et al. (1976) correlated the attack rate of disease to the duration of infectivity and found that attack rate in recently infected persons was 7 times higher than those infected for more than 9 months. He observed that of the total new cases, 72.0% arose from the previously infected population.

Infection rate of tuberculosis has been worked-out by various workers in different parts of the country amongst general population as well as special group. Raj Narain et al. (1963) in Tumkur and Tuberculosis Institute (1974) in Bangalore and Tuberculosis Prevention Trial, Madras (1979) in Chingleput, reported overall infection rates of 38.0%, 30.0% and 50.0% respectively. Various other surveys carried out in different parts of the country revealed that prevalence of infection in the age-groups of 0-9 years and 10 years or more ranged from 5.5% to 11.0% and 21.0% to 75.0% respectively (Ukil, 1930; Benamin, 1939; Primoedt Moller, 1949; 1960; Raj Narain et al., 1963; N.T.I., 1974; Krishnaswamy, 1978; Gothi et al., 1979; T.P.T., 1979).

The mortality rates due to tuberculosis reported by Lankaster (1920), MacDougal (1949) and Benjamin (1939) have been 4.0, 2.5, and 4.62 per 1,000 populations respectively. Frimodt Moller (1960), on the basis of Madanapalle study, estimated mortality rate as 2.53 per 1,000 in 1949, however he observed later, 0.64 and 0.21 per 1,000 mortality rates in repeat surveys during 1951-1963 and 1954-1955 respectively. However, Chakarborty (1978) in a longitudinal study during 1961-1968
from National Tuberculosis Institute estimated 0.84 per 1,000, and, Goyal et al (1978) 0.4 per 1,000 fatality rate due to tuberculosis

SPECTRUM

The spectrum of tuberculosis may vary from localised hypersensitive variety to anergic extensive forms. At the lower end of spectrum there are few cases but the infection is rapidly fatal (Ridley and Waters, 1969; Rdiley, 1983).

3.1.4.2 OTHER MYCOBACTERIAL DISEASES

Other Mycobacterial species shown to be responsible for human diseases are:

a) *M. kansasi*: *M. kansasi* has been reported to cause chronic pulmonary disease, skeletal (bone, joint, tendon) and disseminated afflictions. This species occasionally cause meningitis and local lymphadenitis (Snijdner. 1965; Wolinsky, 1979).

b) *M.avium-intracellulare*: Chronic pulmonary disease resembling tuberculosis has been reported to be the most important clinical problem associated with *M.avium-intracellulare*. The most common predisposing conditions are pneumoconiosis, chronic bronchitis, chronic obstructive lung disease, bronchiectasis, and chronic aspiration from oesophageal disease. Local lymphadenitis
in children, skeletal and disseminated types are other involvements attributed to this group of organism (Kazda et al., 1967; Schonell et al., 1968; Wolinsky, 1979; Gopinathan. R., et al, 2001).

c) *M. scrofulaceum*: Cases of pulmonary disease, and lymphadenitis caused by *M. scrofulaceum* have been reported. *M. scrofulaceum* may also be occasionally associated with disseminated diseases (Greensberg et al, 1963; Gracey and Byrd, 1970; Karlson. 1973; Wolinsky; 1979: Gopinathan, R., et al. 2001; Digvijay Singh et al, 2002).


e) *M. fortuitum - chelonei* complex: Disease associated with *M. fortuitum-chelonei* consists mainly of soft tissue abscesses, pulmonary lesions and wound infections (Dreisin et al. 1976; Wolinsky, 1979). Oesophageal disease causing chronic aspiration has been reported to be associated specifically with this group of *Mycobacteria* (Burke and Ullian, 1977). Chronic bronchitis.
sternal osteomyelitis and lymphadenitis are the other lesions, which have been attributed to this organism (Wolinsky, 1979).

f) *M. marinum*: Infection of skin and subcutaneous lesions with *M. marinum* has been demonstrated (Linell and Norden, 1954; Even-paz *et al*, 1976). Cutaneous granuloma such as ‘swimming pool granuloma’, ‘fish tank granuloma’, ‘sporotichoid’ ulceration and scab formation have been associated with this organism (Wolinsky, 1979).

g) *M. xenopi*: Pulmonary disease resembling pulmonary tuberculosis has been reported to be caused by *M. xenopi* (Marks and Schwabacher, 1965; Richter *et al*, 1969; Riston and Duffy, 1973; Tellis *et al*, 1977,Dauendorffer *et al.*, 2001). This organism has also been isolated from cases of tonsilitis (Stewart *et al*, 1970).

h) *M. ulcerans*: The species name was well chosen, because the infection due to this etiologic agent of the skin and subcutaneous tissues is characterized by dermal ulceration and skin ulcers known as Buruli ulcers (Dodge, 1964; Cornor and Lunn, 1966; Wolinsky, 1979).

i) *M. simiae*: There are only a few reports of human disease due to this organism. In a case of chronic cavitary lung disease, *M. simiae* have been isolated repeatedly thus linking this organism with pulmonary disease (Krasnow and Gross, 1975).
Other *Mycobacterial* strains like *M. haemophilum* have been associated with human diseases (WHO, 1991). In addition to above isolates from human cases, several others e.g. *M. bovis, M. paratuberculosis, M. farcinogenes, M. microti, M. lepraeumurium* have been associated with animal diseases (Lepper and Corner, 1983).

### 3.2 Drugs against Mycobacterial Diseases

While considering the mechanism of action of a particular drug, the following steps are important:

a) Penetration of drug to its site of action- this steps may be very important in view of the complexity of the *Mycobacterial* cell envelope.

b) Conversion of drug into its active form by the bacterium- There are some evidences which state that an activation step may be involved in the action of INH, the same possibility may exist for some other superficially studied anti-*Mycobacterial* drugs.

c) Interference by the drug with the function of a sensitive enzyme of structure- several drugs may have a target enzymatic site.

d) Secondary effects which results from the primary structural or enzymic level caused by the drug- Rifampicin binding to RNA polymerase leads to the absence of free RNA polymerase and
promoter regions for the normal transcription and thus, obscure the RNA synthesis and, indirectly protein synthesis.

e) Bacteriostatic and/or Bactericidal action- Some drugs have exclusively bacteriostatic action, some may have bactericidal effect, while others may possess either effect depending upon the growing conditions of bacteria as well as concentration of drugs.

f) Inactivation of drug by the bacteria- the activity of drug against the bacteria may depend upon the outcome of a race between the inhibitory action of drug and its inactivation by the bacterium.

Thus, there may be two principal directions for investigating the mode of action of a particular agent, one is the identification of sensitive enzyme(s), or structure related to the biosynthetic pathway of cellular components, and then extrapolation of the mechanism at molecular level at which the drug interacts with the specific sites. The other direction is conceptual understanding as to how the drug interferes with the sensitive pathway leading to the inhibition of viability or eventual death.

3.2.1 Major groups of anti-mycobacterial drugs

The major groups of drugs shown to be active against Mycobacteria are:

1) Ansamycins: The important members of this group shown to be active against Mycobacteria are Rifampicin,

2) Aminoglycosides: Various members of this group are known to be potent inhibitors of protein synthesis (Winder, 1982). Streptomycin, Kanamycin and Amikacin are the important members of this group, which have been shown to have anti-\textit{Mycobacterial} activity (Gelber \textit{et al}, 1984).

3) Beta-lactam antibiotics: penicillins, Cephamycin (Cefoxitin) (Misiek \textit{et al}, 1973; Sanders \textit{et al}, 1980; Shepard \textit{et al}, 1987) have shown activity against \textit{Mycobacteria} and they have been shown to be inhibitors of cell wall synthesis (Sykes and Gorgopoulos, 1981).

4) Tetracyclines: Tetracyclines act on various organisms by primary inhibiting protein synthesis (Corcoran and Hahn, 1975). The important member of this group exhibiting anti-\textit{Mycobacterial} action is Minocycline (Gelber, 1986).

5) Dihydrofolate reductase inhibitors: Among various drugs of this group Dapsone, Trimethoprim, Brodimoprim have shown anti-
Mycobacterial activity (Gelber, 1986; Sydel et al, 1986). These drugs have been shown to potent inhibitors of dihydrofolate reductase.

6) Ribonucleotide reductase inhibitors: The active agents include various alpha-nonheterocyclic thiosemicarbazones and have been believed to act through inhibitors of nucleic acid synthesis (Schaper et al, 1986).

7) Thioamides: Ethionamide and Prothionamide are the two important members of this group, which have shown activity against *Mycobacteria*. The compounds have demonstrable action on mycolic acid biosynthesis (WHO, 1982; Levy et al, 1984; Quemard et al, 1992).

8) Quinolones: The important members of Quinolones, which have been reported to show antimycobacterial activity, are Ciprofloxacin, Norfloxacin, Oxfloxacin and Pefloxacin (Tsukumara, 1985; WHO, 1985, Fur et al, 1987; Leysen et al, 1989; Franzblau and White, 1990; Tuberculosis Research Centre, 2002). The primary site of action of Quinolones has been thought to be through DNA gyrase.

The other important drugs active against *Mycobacteria* are hydrazides (INH), Ethambutol, D-Cycloserine, Pyrazinamide, Clofazimine and Deoxyfructoseronotonin (McClune et al, 1956; barry et

3.2.1.1 Anti-tubercular drugs

Various other drugs have been demonstrated to be active against *M. tuberculosis* either in in-vitro experiments or experimental animals. Some of these are used occasionally in the therapy also. These compounds are Aminoglycosides-Neomycin, Amikacin. Kanamycin (Tanaka, 1975; Vazquez, 1978; Heifets and Lindholm-Levy, 1989), Thiosemicarbazones (Domagn *et al.*, 1940), Diaryl Thoureas-4, 4'-Di-isoamlyoxydiphenyl thiours-4, 41-Di-isoamlyoxythiocarba-nilide, isoxy1, Thiocarldides (Youmans *et al.*, 1958), phenazines- Clofazimine (Barry *et al.*, 1957) and Beta-lactam antibiotics- Cephalosporins: 7-Aminocepha-losporintic acid, Cefuroxine and Cephamycin (Cefoxitin) (Misiek *et al.*, 1973; Sanders *et al.*, 1980), Anilinoasposafranine (Barry *et al.*, 1957), Basic peptide antibiotics- Viomycin and Capreomycin (Sutton *et al.*, 1966; Heifets and Lindholm-Levy, 1989), D-Cycloserine (Neuhans, 1967; Gale *et al.*, 1972) and Quinolones- Ciprofloxacin, Norfloxacin, Ofloxacin and Pefloxacin (Tsukamura *et al.*, 1984; Gay *et al.*, 1984; Tsukamura, 1985; Texier-Maugein *et al.*, 1987; Fur *et al.*, 1987; Sanders *et al.*, 1987; Leysen *et al.*, 1989; Furet and Pechere, 1991; Sulochana. S. *et al.*, 1999; Tuberculosis Research Centre, 2002).

### 3.2.1.2 Drugs Effective Against Atypical Environmental Mycobacteria

The infections due to environmental *Mycobacteria* are difficult to treat and they tend to be usually resistant to conventional anti-tubercular
drugs. However susceptible strains among these non-tuberculous *Mycobacteria* have been reported-

a) *M. kansasii* strains sensitive to INH, Streptomycin, PAS, Rifampicin, Ethambutol, Erythromycin, Ethionamide and 4-4′ Di-isoamyloxythiocarbanilid Ciprofloxacin Ofloxacin, Norfloxacin and Pefloxacin have been reported. (Hedgecock and Blumenthal, 1965; Tacquet *et al.*, 1967; Tsang *et al.*, 1976; Chapman, 1977; Wolinsky, 1979; Leysen *et al.*, 1989).


c) Sensitive strains of *M. intracellulare* to INH, PAS, Streptomycin. Rifampicin, Benzyl Penicillin, Clofazimine, Capreomycin. Ciprofloxacin and Ofloxacin have been reported (Noufflard and Berteaux, 1958; Rynearson *et al.*, 1971; Tsukamura, 1972; Chapman, 1977; Leysen *et al.*, 1989).

e) Sensitive strains of *M. szulgai* to INH, Streptomycin, PAS, Rifampicin, Ethambutol and Ethionamide have been reported (Schaefer et al, 1973; Chapman, 1977; Wolinsky, 1979).


g) *M. marinum* strains sensistive to INH, PAS, Rifampicin, Ethambutol, Cycloserine, Streptomycin, Ethionamide, Pyrazinamide, Kanamycin, Ciprofloxacin and Ofloxacin have been reported by various investigators, (Walkar, et al., 1962; Adams,
et al., 1970; Flowers, 1970; Barrow and Hewitt, 1971; Silox and David, 1971; Van Dyke and Lake, 1975; Leysen et al., 1989).

h) *M. xenopi* strains sensitive to INH, Streptomycin, PAS, Viomycin, Erythromycin, Dimethylchloro-tetracycline and Penicillin G, Rifampicin, Cycloserine, Ethionamide, Ciprofloxacin, Ofloxacin, Norfloxacin and Pefloxacin have been reported (Boisvert, 1965; Engbaek, 1067; Doyle *et al*., 1968; Wolinsky, 1979; Leysen *et al*., 1989).

i) Susceptible strain of *M. ulcerans* to INH, Streptomycin, Oxytetracycline, DDS, PAS, Rifampicin, Ethionamide, Ethambutol, Viomycin, Kanamycin and Cycloserine have been reported by Clancey *et al* (1961), Wolinsky (1979) and Fingold and Martin (1982).

j) *M. simiae* has been usually found to be resistant to most of the antimycobacterial drugs except Cycloserine and Ethionamide (Wolinsky, 1979).