REVIEW OF LITERATURE
Review of Literature

Background

Tuberculosis, MTB or TB (short for tubercle bacillus) is a common and in some cases deadly infectious disease caused by various strains of *mycobacterium*, usually *Mycobacterium tuberculosis* in humans (196). Since Robert Koch’s initial cultivation of the acid fast tubercular bacillus more than a century ago, tremendous research has been done about tuberculosis and its causative agent *Mycobacterium tuberculosis*. The killer disease came into light with discovery of anti tuberculosis drugs. However, it still remains one of the biggest public health concerns today in the 21st century. Spread of drug resistant strains and synergy of TB with HIV is a roadblock in the control of the disease.

In the past tuberculosis was referred to as the “white plague” and by John Bunyan as “the captain of all these men of death.” Accounts of the disease appeared in the Vedas and other ancient Hindu texts, in which it was termed “rajyakshma” - the king of disease. Hippocrates (460-370 BC) called this disease as pthisis sylvias meaning wasting away and Herdatus (484-425 BC) described the exclusion of those afflicted with leprous or scrofulous lesions from general population (33).

Laemaec and Bayle (1789-1826) performed hundreds of post-mortems and found nodules in the lung which they called tubercles and thus decided to call the disease *Tuberculosis* (34). The transmissible nature of tuberculosis was established by Villemin (1868) by inoculating rabbits with tubercular material from humans and cattle. Villemin also established that scrofula and pulmonary tuberculosis were manifestations of the same disease processes.

In 1882, Robert Koch succeeded in cultivating it on inspissated serum. By a large series of inoculations with pure cultures of bacillus, Koch transmitted the disease to many animals of different species. His classical study established without doubt that the bacillus he had isolated was the cause of tuberculosis (35).

Smith (1898) differentiated human tubercle bacilli from bovine type. As research progressed, certain cases of pulmonary tuberculosis turned out to be caused by
organisms which were mycobacteria like but appeared to be different and distinct from *Mycobacterium tuberculosis* or other well characterized mycobacterial species. Pinner (1935) labelled these organisms as “atypical mycobacteria.” Later Runyon (1959) called these organisms as “anonymous” and American Thoracic Society (1963) proposed the name – unclassified mycobacteria.

**Tuberculosis in early civilization**

In 2008, evidence for tuberculosis infection has been discovered in human remains from the Neolithic era dating from 9,000 years ago, in a settlement in the eastern Mediterranean. This finding was confirmed by morphological and molecular methods; to date it is the oldest evidence of tuberculosis infection in humans.

Evidence of the infection in humans was also found in a cemetery near Heidelberg, in the Neolithic bone remains that show evidence of the type of angulation often seen with spinal tuberculosis. Some authors call tuberculosis the first disease known to mankind. Signs of the disease have also been found in Egyptian mummies dated between 3000 and 2400 BCE (36). The most convincing case was found in the mummy of priest Nesperehen, discovered by Grebart in 1881, which featured evidence of spinal tuberculosis with the characteristic psoas (disambiguation needed) abscesses. Similar features were discovered on other mummies like that of the priest Philoc and throughout the cemeteries of Thebes. It appears likely that Akhenaten and his wife Nefertiti both died from tuberculosis, and evidence indicates that hospitals for tuberculosis existed in Egypt as early as 1500 BCE (33). The Ebers papyrus, an important Egyptian medical treatise from around 1550 BCE, describes a pulmonary consumption associated with the cervical lymph nodes. It recommended that it be treated with the surgical lancing of the cyst and the application of a ground mixture of acacia seyal, peas, fruits, animal blood, insect blood, honey and salt.

The Old Testament mentions a consumptive illness that would affect the Jewish people if they stray from God. It is listed in the section of curses given before they enter the land of Palestine.
Nineteenth century

A romantic disease

It was during this century that tuberculosis was dubbed the White Plague, mal de vivir, and mal du siècle. It was seen as a "romantic disease." Suffering from tuberculosis was thought to bestow upon the sufferer heightened sensitivity. The slow progress of the disease allowed for a "good death" as sufferers could arrange their affairs. The disease began to represent spiritual purity and temporal wealth, leading many young, upper-class women to purposefully pale their skin to achieve the consumptive appearance. British poet Lord Byron wrote, "I should like to die from consumption," helping to popularize the disease as the disease of artists. George Sand doted on her phtitic lover, Frédéric Chopin, calling him her "poor melancholy angel." In France, at least five novels were published expressing the ideals of tuberculosis: Dumas's La Dame aux camélia, Murger's Scènes de la vie de Bohème, Hugo's Les Misérables, the Goncourt brothers' Madame Gervaisais and Germinie Lacerteux, and Rostand's L'Aiglon. Even after medical knowledge of the disease had accumulated, the redemptive-spiritual perspective of the disease continued in literature. (More recently the 2001 film Moulin Rouge is based in part on La traviata, which itself is based on La Dame aux camélia).

Taxonomy of Mycobacterium tuberculosis

Scientific classification
Kingdom: Bacteria
Phylum: Actinobacteria
Order: Actinomycetales
Suborder: Corynebacterineae
Family: Mycobacteriaceae
Genus: Mycobacterium
Species: tuberculosis

Mycobacterium tuberculosis (M. tuberculosis) is a pathogenic bacterial species in the genus Mycobacterium and the causative agent of most cases of tuberculosis. M. tuberculosis has an unusual, waxy coating on the cell surface (primarily mycolic acid), which makes the
cells impervious to Gram staining so acid-fast detection techniques are used instead. The physiology of *M. tuberculosis* is highly aerobic and requires high levels of oxygen. Primarily a pathogen of the mammalian respiratory system, MTB infects the lungs, causing tuberculosis (37).

**Physiology**

*Mycobacterium tuberculosis* looks slender, straight or slightly curved with beaded or barred appearance. They are aerobic non motile with a size range of 2-3 × 0.2 × 0.4 µm. *Mycobacterium tuberculosis* are acid fast due to the presence of mycolic acid (38).

**Optimum temperature and pH**

*M. tuberculosis* normally grows at 37°C; however growth stops below 25°C and above 40°C. The optimum pH range is 6.4-7.0. *M. tuberculosis* is more susceptible to acid pH than *M. smegmatis* is, as *M. smegmatis* has more efficient internal pH homeostasis (39).

**Cell wall structure** - The cell wall of *Mycobacterium tuberculosis* has several unique features, which distinguishes it from all other prokaryotes. Robert Koch first pointed out the unusual cell wall of *Mycobacterium tuberculosis* and its importance in mycobacterium physiology. The cell wall consists of a plasma membrane surrounded by a lipid and carbohydrate rich wall, which in term is enriched by a capsule of polysaccharides, proteins and lipid. The cell wall complex contains peptidoglycan, but otherwise it is composed of complex lipids. Over 60% of the mycobacterium cell wall is lipids. The lipid fraction of *Mycobacterium tuberculosis* cell wall consists of three major components.

(i) *Mycolic acids* - Mycolic acids are unique β-branched present in *Mycobacterium* & *Corynebacterium* and make up 50% of dry weight of mycobacterial cell envelope. Mycolic acids are strong hydrophobic molecule that form lipid shell around the organism and affect permeability properties at the cell surface. Mycolic acid are thought to be a significant determinant of virulence in *Mycobacterium tuberculosis* they prevent *Mycobacterium tuberculosis* by other cationic proteins lysozyme and oxygen radicals in the phagocytic granule. They also protect extra cellular mycobacterium from complement deposition in serum.
(ii)  **Cord factor**- It is responsible for the serpentine cording, cord factor is toxic to mammalian cells and is also an inhibitor of PMN migration. Cord factor is most abundantly produced in virulent strain of *Mycobacterium tuberculosis*. The virulence of *Mycobacterium tuberculosis* culture has been correlated with the formation of long cord like structure on agar or in liquid medium, due to side to side aggregation and inter winding of long chain of bacteria (fig 1). Growth in cord reflects the presence on the cell surface of a characteristic lipid, the cord factor (trehalose 6, 6 dimycolate) that is a glycolipid (40).

![Structure of cord factor in M. tuberculosis (40)](image-url)

Lipoarabinomannan (LAM) is a heat shook protein (65 kDa) and is highly immunogenic, the another virulent factor that present in cell wall .It inhibit macrophage activation by TNF-α and induces macrophages to secrete TNF-α which causes fever, weight loss and tissue damage.

*iii) Wax D*- Wax-D presents in the cell envelope and is the major component of Freund’s complete adjuvant (CFA).

This high concentration of lipids in cell wall of *M. tuberculosis* has been associated with these properties of bacteria (fig 2):

- Impermeability to strains and dyes.
Resistances to many antibiotics.
- Resistance to killing by acidic and alkaline compounds.
- Resistance to osmotic lyses via complement deposition.
- Resistance to lethal oxidation and survival inside of macrophage.

![Mycobacterium cell wall diagram](image)

**Fig 2:** A simple concept of the Mycobacterium cell wall

**Microscopic examination**

*M. tuberculosis* is characterized by caseating granulomas containing Langhans giant cells, which have a "horseshoe" pattern of nuclei (fig 3). Organisms are identified by their red color on acid-fast staining.

![Mycobacterium tuberculosi stained in tissue](image)

**Fig 3:** *Mycobacterium tuberculosis* (stained red) in tissue (blue) by acid fast staining
Cultural Isolation characteristics

- Tubercle bacilli are slow grower with generation time in vitro being 14-15 hours and visible colony appears within 2–8 weeks.
- *M. tuberculosis* is an obligate aerobe but growth is stimulated by 5-10 % CO₂.
- This is a facultative intracellular parasite usually infecting mononuclear phagocytes (e.g. macrophages).
- Optimum temperature for growth is 37°C and growth does not occur below 25 °C or above 40°C
- Optimum pH is 6.4-7.0.
- Colonies generally appears in about 2 weeks and may sometime take up to 8 weeks. Figure 4 shows the *M. tuberculosis* colonies on LJ media.

![Image of M. tuberculosis colonies on LJ media](image)

Fig 4: *M. tuberculosis* bacterial colonies on Lowenstein-Jensen media

Genome of *M. tuberculosis*

Sequence of *M. tuberculosis H₃₇Rv* genome was annotated by Cole *et al* (41). It is the second largest genome sequence currently available after *E. coli*. The genome of *M. tuberculosis* is shown in figure 5. The main features of *M. tuberculosis* genome are:

- The whole genome of *M. tuberculosis* (H₃₇Rv) has been sequenced and contains 4,411,532 base pair.
- It contains around 4000 genes out of which 50 genes codes for functional RNA molecule.
16 copies of IS6110 and 6 copies of more stable element IS1081 are found within the genome of H$_{37}$Rv stain.

- It has been 65% of G+C content (41).
- Genome is rich in repetitive DNA, particularly insertion sequences and in new multigene families and duplicated housekeeping genes.

![Diagram of Mycobacterium tuberculosis genome](image)

**Fig 5: M. tuberculosis H$_{37}$Rv genome**

- Over 51% genes of *M. tuberculosis* appear to have arisen as a result of gene duplication or domain Shuffling events.
- 3.4% of the genome is composed of insertion sequences (IS) and prophage (phi Rv 1, phi Rv 2). There are 56 copies of IS elements belonging to the well known IS3, IS5, IS21, IS30, IS110, IS256 and IS1535.

### Causes

The main cause of TB, *Mycobacterium tuberculosis* (MTB), is a small aerobic non-motile bacillus. High lipid content of this pathogen accounts for many of its unique clinical characteristics. It divides every 16-20 hours, an extremely slow rate compared with other bacteria, which usually divide in less than an hour. (For example, one of the fastest-growing bacteria is a strain of *E. coli* that can divide roughly every 20 min.) Since
MTB has a cell wall but lacks a phospholipid outer membrane, it is classified as a Gram-positive bacterium. However, if a Gram stain is performed, MTB either stains very weakly Gram-positive or does not retain dye as a result of the high lipid & mycolic acid content of its cell wall. MTB can withstand weak disinfectants and survive in a dry state for weeks. In nature, the bacterium can grow only within the cells of host, but *M. tuberculosis* can be cultured *in-vitro*.

Using histological stains on expectorate samples from phlegm (also called sputum), scientists can identify MTB under a regular microscope. Since MTB retains certain stains after being treated with acidic solution, it is classified as an acid-fast bacillus (AFB). The most common acid-fast staining technique, the Ziehl-Neelsen stain, dyes AFBs a bright red that stands out clearly against a blue background. Other ways to visualize AFBs include anauramine-rhodamine stain and fluorescent microscopy (fig 6).

The *M. tuberculosis* complex includes four other TB-causing mycobacteria: *M. bovis*, *M. africanum*, *M. canetti* and *M. microti*. *M. africanum* is not widespread, but in parts of Africa it is a significant cause of tuberculosis. *M. bovis* was once a common cause of tuberculosis, but the introduction of pasteurized milk has largely eliminated this as a public health problem in developed countries. *M. canetti* is rare and seems to be limited to Africa, although a few cases have been seen in African emigrants. *M. microti* is mostly seen in immunodeficient people, although it is possible that the prevalence of this pathogen has been underestimated.

**Signs and symptoms**

Main symptoms of variants and stages of tuberculosis, overlap with other variants, while others are more (but not entirely) specific for certain variants. Multiple variants may be present simultaneously.
Tuberculosis usually attacks the lungs but can also affect other parts of the body. It is spread through the air when people who have an active MTB infection cough, sneeze, or otherwise transmit their saliva through the air. Most infections in humans result in an asymptomatic, latent infection, and about one in ten latent infections eventually progresses to active disease, which, if left untreated, kills more than 50% of its victims.

The classic symptoms are a chronic cough with blood-tinged sputum, fever, night sweats, and weight loss (the last giving rise to the formerly prevalent colloquial term "consumption"). Infection of other organs causes a wide range of symptoms. Diagnosis relies on radiology (commonly chest X-rays), a tuberculin skin test, blood tests, as well as microscopic examination and microbiological culture of bodily fluids. Treatment is difficult and requires long courses of multiple antibiotics. Social contacts are also screened and treated if necessary. Antibiotic resistance is a growing problem in (extensively) multi-drug-resistant tuberculosis. Prevention relies on screening programs and vaccination, usually with Bacillus Calmette Guérin vaccine.

One third of the world’s population is thought to be infected with M. tuberculosis, and new infections occur at a rate of about one per second. The proportion of people who become sick with tuberculosis each year is stable or falling worldwide but, because of population growth, the absolute number of new cases is still increasing. In 2007, there were an estimated 13.7 million chronic active cases, 9.3 million new cases, and 1.8 million deaths, mostly in developing countries. In addition, more people in the deve-
Developed world contract tuberculosis because their immune systems are more likely to be compromised due to higher exposure to immunosuppressive drugs, substance abuse, or AIDS. The distribution of tuberculosis is not uniform across the globe; about 80% of the population in many Asian and African countries test positive in tuberculin tests, while only 5–10% of the US population test positive.

When the disease becomes active, 75% of the cases involve infection in the lungs (pulmonary TB). Symptoms include chest pain, coughing up blood, and a productive, prolonged cough for more than three weeks. Systemic symptoms include fever, chills, night sweats, appetite loss, weight loss, pallor, and fatigue.

In the other 25% of active cases, the infection moves from the lungs, causing other kinds of TB, collectively denoted extrapulmonary tuberculosis. This occurs more commonly in immunosuppressed persons and young children. Extrapulmonary infection sites include the pleura in tuberculosis pleurisy, the central nervous system in meningitis, the lymphatic system in scrofula of the neck, the genitourinary system in urogenital tuberculosis, and bones and joints in Pott's disease of the spine. An especially serious form is disseminated TB, more commonly known as miliary tuberculosis. Extrapulmonary TB may co-exist with pulmonary TB as well.

**Transmission**

When people suffering from active pulmonary TB cough, sneeze, speak, kiss, or spit, they expel infectious aerosol droplets 0.5 to 5 μm in diameter. A single sneeze, for instance, can release up to 40,000 droplets (41). Each one of these droplets may transmit the disease, since the infectious dose of tuberculosis is very low and the inhalation of just a single bacterium can cause a new infection (180,181). The probability of transmission depends upon infectiousness of the person with TB (quantity expelled), environment of exposure, duration of exposure and virulence of the organism.

Therefore, successful passage of the TB bacillus from one person to the next requires an infectious source case, a virulent microorganism, a vulnerable host, and favorable
environmental conditions. Upon closer inspection, there are complex factors that contribute to this process, including factors that determine the strength and infectivity of the source, the integrity of the host defences of the exposed individual, intrinsic properties of the bacillus itself, including viability, vulnerability or resistance to environmental stressors, virulence factors, genetic mutations, drug resistance, and virulence for a particular host.

**Epidemiology**

Tuberculosis remains a worldwide public health problem despite the fact that the causative organism was discovered more than hundred years ago. Tuberculosis infects one-third of world’s population (42). There are approximately nine million new cases of all forms of tuberculosis occurring annually and three million die from it each year (43). Out of these 95% of tuberculosis cases and 98% of tuberculosis deaths are in developing countries.

Incidence of disease and mortality is most common (75%) in adult age group. To make global situation worse, tuberculosis has formed a lethal combination with HIV. At the same time, drug resistant tuberculosis is a growing threat worldwide (44).
TB remains one of the world’s major causes of illness and death and in 1993, the World Health Organization (WHO) declared TB as a global health emergency. One-third of the world’s population carry the TB bacteria, more than 9 million of whom become sick each year with “active” TB which can be spread to others. TB disproportionately affects people in resource-poor settings, particularly those in Asia and Africa (1). More than 90% of new TB cases and deaths occur in developing countries, posing significant challenges to the livelihoods of individuals and developing economies as TB primarily affects people during their most productive years. Poor health systems, limited laboratory capacity for case detection, treatment barriers and complications (unreliable drug supply, patients not completing treatment, or prescribing errors), TB and HIV co-infection, and the emergence of drug-resistant TB pose serious threats to global TB control. Figure 7 shows the Global trends in estimated rates of TB incidence, prevalence and mortality.

Fig 7: **Global trends in estimated rates of TB incidence, prevalence and mortality.**

Current global snapshot

TB is found in every country in the world, but the majority of TB cases are concentrated in developing countries, particularly those in Asia and Africa. Figure 8 shows the estimated TB incident rates, by country in 2010. In 2010, there were 8.8 million (range, 8.5–9.2 million) incident cases of TB, 1.1 million (range, 0.9–1.2 million) deaths from TB among HIV-negative people and an additional 0.35 million (range, 0.32–0.39 million) deaths from HIV-associated TB (1).

Figure 9 shows the estimated HIV prevalence in new TB cases in the year 2010. Twenty-two countries are considered “high-burden countries (HBCs),” accounting for approximately 80% of new TB cases each year; most HBCs are in Africa and Asia. India, China, South Africa, Nigeria, and Indonesia have the highest number of new TB cases in the world. In 2010, there were 5.7 million notifications of new and recurrent cases of TB, equivalent to 65% (range 63–68%) of the estimated number of incident cases in 2010. India and China accounted for 40% of the world’s notified cases of TB in 2010, Africa for a further 24% and the 22 high-TB burden countries (HBCs) for 82%.

At global level, the treatment success rate among new cases of smear positive pulmonary TB was 87% in 2009. Between 1995 and 2010, 55 million TB patients were treated in programmes that had adopted the DOTS/Stop TB Strategy, and 46 million were successfully treated. These treatments saved almost 7 million lives. Alongside these achievements, diagnosis and appropriate treatment of multidrug-resistant TB (MDR-TB) remain major challenges. Less than 5% of new and previously treated TB patients were tested for MDR-TB in most countries in 2010. The reported number of patients enrolled on treatment has increased, reaching 46 000 in 2010. However, this was equivalent to only 16% of the 290 000 cases of MDR-TB estimated to exist among notified TB patients in 2010 (1).
Prevalence

Prevalence is a robust indicator of the burden of disease caused by TB when it is directly measured in a nationwide survey. There were an estimated 14 million prevalent cases (12–16 million) of TB in 2009, equivalent to 200 cases per 100 000 population.
Mortality
In 2010, an estimated 1.1 million deaths (range, 0.9 million–1.2 million) occurred among HIV-negative cases of TB, including 0.32 million deaths (range, 0.20 million–0.44 million) among women (fig 10). This was equivalent to 15 deaths per 100 000 population. In addition, there were an estimated 0.35 million deaths (range, 0.32 million–0.39 million) among incident TB cases that were HIV-positive (data not shown); these deaths are classified as HIV deaths in ICD-10.1 Thus in total, approximately 1.4 million people (range, 1.2 million-1.5 million) died of TB in 2010. This estimate is considerably lower than the estimates of 1.3 million TB deaths among HIV-negative people and 0.4 million deaths from TB among HIV-positive people that were published in 2010, following a major revision of estimates of the numbers of TB cases and deaths in African countries. The number of TB deaths per 1,00,000 population among HIV-negative people plus the estimated number of TB deaths among HIV-positive people equates to a best estimate of 20 deaths per 100 000 population. Globally, mortality rates (excluding deaths among HIV-positive people) have fallen by more than one-third since 1990, and the current forecast suggests that the Stop TB Partnership’s target of a 50% reduction by 2015 compared with a baseline of 1990 will be achieved. Mortality rates are also declining in all of WHO’s six regions. The Region of the Americas and the Western Pacific Region halved the 1990 level of mortality by 2000 and 2003 respectively, well in advance of the target year of 2015. The Eastern Mediterranean and European regions appear to have halved the 1990 level of mortality by 2010, and the South-East Asia Region is on track to reach the target by 2015. It is only in the African Region that the target of halving mortality rates by 2015 looks out of reach.

Among the 22 HBCs, mortality rates appear to be falling with the possible exception of Afghanistan. Even allowing for uncertainty in the estimates, five countries have reached the target of halving the 1990 mortality rate by 2010 (Brazil, Cambodia, China, Uganda and the United Republic of Tanzania), and several other countries have a good chance of achieving the target by 2015 (1).
MDR-TB and XDR-TB

There were an estimated 440,000 cases of multi-drug resistant TB (MDR-TB) in 2008 (range, 3,90,000–5,10,000). The 27 countries (15 in the European Region) that account for 86% of all such cases have been termed the 27 high MDR-TB burden countries. The four countries that had the largest number of estimated cases of MDR-TB in absolute terms in 2008 were China (100,000; range, 79,000–120,000), India (99,000; range, 79,000–120,000), the Russian Federation (38,000; range, 30,000–45,000) and South Africa (13,000; range 10,000–16,000). By July 2010, 58 countries and territories had reported at least one case of extensively drug-resistant TB (XDR-TB).

TB burden in India

Though India is the second-most populous country in the world, India has more new TB cases annually than any other country (45). In 2008, out of the estimated global annual incidence of 9.4 million TB cases, 1.98 million were estimated to have occurred in India, of whom 0.87 million were infectious cases, thus catering to a fifth of the global burden of TB. About 40% of Indian population is infected with TB bacillus. The incidence of TB in India is estimated based on findings of the nationwide annual risk of tuberculosis infection (ARTI) study conducted in 2000-2003. The national ARTI being 1.5%, the incidence on smear positive TB cases in the country is estimated as 75 new smear positive cases per 100,000 populations. The prevalence of TB has been estimated at 3.8 million bacillary cases for the year 2000, by an expert group of Govt. of India. However the recent estimate by WHO gives a prevalence of 2.186 million.

The five countries that rank first to fifth in terms of number of incident cases in 2008 are India (1.98 million), China (1.3 million), South Africa (0.47 million), Nigeria (0.45 million) and Indonesia (0.43 million). India and China alone account for an estimated 35% of TB cases worldwide. There were an estimated 11.1 million prevalent cases of TB in 2008 equivalent to 168 cases per 100,000 populations. The South East Asia region accounts for 34% of the global TB burden.
On a national scale, the high burden of TB in India is illustrated by the estimate that TB accounts for 17.6% of deaths from communicable disease and for 3.5% of all causes of mortality (46). More than 80% of the burden of tuberculosis is due to premature death, as measured in terms of disability-adjusted life years (DALYs) lost. WHO estimated TB mortality in India as 276,000 (24/100,000 population) in 2008. With RNTCP implementation, there is 43% decline in death due to TB in India by 2008 is compared to 1990. It was estimated that the TB mortality was over 500,000 annually at the beginning of the revised national TB control programme (RNTCP). Data from specific surveys, however, suggest that case fatality rates prior to RNTCP were generally greater than 25%. In RNTCP era, case fatality has remained less than 5% for new cases registered under the programme.

Drug resistance

Drug resistant tuberculosis is transmitted in the same way as regular TB. Primary resistance occurs in persons who are infected with a resistant strain of TB. A patient with fully susceptible TB develops secondary resistance (acquired resistance) during TB therapy because of inadequate treatment, not taking the prescribed regimen appropriately, or using low quality medication. Drug-resistant TB is a public health issue in many developing countries, as treatment is longer and requires more expensive drugs.

MDR-TB

Multi-drug-resistant tuberculosis (MDR-TB) is defined as resistance to the two most effective first-line TB drugs: rifampin and isoniazid. Control of tuberculosis is threatened by widespread emergence of drug resistance in *Mycobacterium tuberculosis*. Understanding the molecular basis of resistance might lead to development of novel rapid methods for diagnosing drug resistance. MDR-TB is developed due to resistance to anti-TB drugs rifampin and isoniazid.
Mechanism of action of Rifampicin and Isoniazid

*Rifampicin* is a first line tuberculosis medication. The highly effective bactericidal action of this drug against *M. tuberculosis* has made it a key component of the initial anti-tuberculosis regimen. Analysis of approximately 500 Rifampicin strains from global sources has found that 96% of Rifampicin resistant clinical isolates of *Mycobacterium tuberculosis* have mutations in the 81-bp core region of *rpoB* gene, which encodes the β subunit of RNA polymerase enzyme. These mutations are absent in susceptible organisms. Although minor discrepancies have been reported, in general there has been a strong correlation of a specific amino acid substitutions and MIC. Mis-sense mutations in codons 513, 526, or 531 result in high level Rifampicin resistance, whereas amino acid changes at position 514 or 533 usually result in low levels of Rifampicin resistance. The molecular mechanism of resistance in 4% of Rifampicin resistant tuberculosis isolates that lack RRDR changes is unknown. It is estimated that 90% of Rifampicin-resistant isolates in some areas are also resistant to Isoniazid, making Rifampicin resistance a useful surrogate marker for multidrug resistance and indicating that second and third line drugs to which these isolates are susceptible are urgently required.

Rifampicin inhibits DNA-dependent RNA polymerase in bacterial cells by binding its β-subunit, thus preventing transcription to RNA and subsequent translation to proteins. Its lipophilic nature makes it a good candidate to treat the meningitis form of tuberculosis, which requires distribution to the central nervous system and penetration through the blood-brain barrier. Rifampicin acts directly on messenger RNA synthesis. It inhibits only prokaryotic DNA-primed RNA polymerase, especially those that are Gram-stain-positive and *Mycobacterium tuberculosis*. Much of this acid-fast positive bacteria’s membrane is mycolic acid complexed with peptidoglycan, which allows easy movement of the drug into the cell. Evidence shows that in vitro DNA treated with concentrations 5000 times higher than normal dosage remained unaffected; in vivo eukaryotic specimens’ RNA and DNA polymerases suffered few problems as well. Rifampicin interacts with the β subunit of RNA polymerase when it
is in a α2β trimer. This halts mRNA transcription, therefore preventing translation of polypeptides. It should be made clear, however, that it cannot stop the elongation of mRNA once binding to the template-strand of DNA has been initiated. The Rifampicin-RNA polymerase complex is extremely stable and yet experiments have shown that this is not due to any form of covalent linkage. It is hypothesized that hydrogen bonds and also the π-π bonds interaction between naphthoquinone and the aromatic amino acids are the major stabilizers, though this requires the oxidation of naphthohydroquinone which is found most commonly in Rifampicin. Mutations in rpoB gene replace phenylalanine, tryptophan, and tyrosine with non-aromatic amino acids result in poor bonding between rifampicin and the RNA polymerase. Rifampicin-resistant bacteria produce RNA Polymerases with subtly different β subunit structures which are not readily inhibited by the drug.

Isoniazid (INH- Isonicotinic Acid Hydrazide) is a synthetic, bactericidal agent, used as a first line Tuberculosis drug. Despite its widespread application to tuberculosis therapy and prophylaxis and intensive laboratory investigation, there is much that is not yet understood about the bacteria targets and mode of action of Isoniazid. Investigators on several continents have reported that many (50-60%) Isoniazid resistant patient isolates have mutations, small deletions or insertions that are not represented among Isoniazid sensitive control strains. Mutations leading to Isoniazid resistance have been identified in different gene targets including katG, inhA, ahpC and other genes that remain to be established. Isoniazid is a pro drug and must be activated by a bacterial catalase-peroxidase enzyme that in M. tuberculosis is called katG. KatG couples the isonicotinic acyl with NADH to form isonicotinic acyl-NADH complex. This complex binds tightly to the enoyl-acyl carrier protein reductase known as inhA, thereby blocking the natural enoyl-AcpM substrate and the action of fatty acid synthase. This process inhibits the synthesis of mycolic acid, required for themycobacterial cell wall. Isoniazid inhibits the synthesis of saturated fatty acids greater than C26 and of unsaturated fatty acids greater than C24. The variable-exposure experiment at low Isoniazid concentration showed that the syntheses of mycolic acids and long-chain
fatty acid fractions II and III were inhibited to the same extent. These fatty acids may thus be precursors of mycolic acids.

\textit{katG gene}

\textit{Mycobacterium tuberculosis} catalase-peroxidase (KatG) is a bifunctional hemoprotein that has been shown to activate Isoniazid (INH), a pro-drug that is integral to frontline anti-tuberculosis treatments. The activated species, presumed to be an isonicotinoyl radical, couples to NAD (+)/NADH forming an Isoniazid-NADH adduct that ultimately confers anti-tubercular activity. To better understand the mechanisms of Isoniazid activation as well as the origins of \textit{katG}-derived INH-resistance, catalytic properties were compared (including the ability to form the INH-NADH adduct) of the wild-type enzyme to 23 \textit{katG} mutants which have been associated with Isoniazid resistance in clinical \textit{M. tuberculosis} isolates. Neither catalase nor peroxidase activities, the two inherent enzymatic functions of \textit{katG}, were found to correlate with Isoniazid resistance. Furthermore, catalase function was lost in mutants which lacked the Met-Tyr-Trp crosslink, the biogenic cofactor in \textit{katG} which has been previously shown to be integral to this activity. The presence or absence of the crosslink itself, however, was also found to not correlate with INH resistance. The \textit{katG} resistance-conferring mutants were then assayed for their ability to generate the INH-NADH adduct in the presence of peroxide, superoxide, and no exogenous oxidant. The results demonstrate that residue location plays a critical role in determining INH-resistance mechanisms associated with INH activation; however, different mutations at the same location can produce vastly different reactivities that are oxidant-specific.

\textit{XDR-TB}

Extensively drug-resistant TB, is a form of TB which is resistant to at least four of the core anti-TB drugs. XDR-TB involves resistance to the two most powerful anti-TB drugs, isoniazid and rifampicin, also known as multidrug-resistance (MDR-TB), in addition to resistance to any of the fluoroquinolones (such as ofloxacin or moxifloxacin) and to at least one of three injectable second-line drugs (amikacin, capreomycin or kanamycin)
**MDR-TB and XDR-TB in India**

The emergence of drug resistant TB, and particularly MDR-TB, has become a significant public health problem in a number of countries and an obstacle to effective TB control. Globally Multi drug resistant TB is emerging as a major challenge to programme managers. Multi drug resistance occurring primarily as a consequence of poor treatment services could lead to emergence of XDR TB if MDR TB is not managed properly. There were an estimated 0.5 million cases of MDR-TB in 2007. The countries that ranked first to fifth in terms of total numbers of MDR-TB cases in 2007 were India (131,000), China (112,000), the Russian Federation (43,000), South Africa (16,000) and Bangladesh (15,000). By November 2009, 57 countries had reported at least one case of XDR-TB.

A large scale population based survey in the state of Gujarat and Maharashtra has indicated multi drug resistance levels of <3% among new TB cases and 14-17% among previously treated TB patients. Though the rate of MDR-TB is relatively low in India, this translates into a large absolute number of cases, with an estimated annual incidence of 131,000 cases of MDR TB in the country. XDR-TB has been reported in India by isolated studies with non-representative and highly selected clinical samples. The magnitude of the problem remains to be determined due to the absence of laboratories capable of conducting quality assured second line DST. However, what is frightening is the potential threat of XDR-TB in India with unregulated availability and injudicious use of the second line drugs along with non-existence of systems to ensure standardized regimens and treatment adherence for MDR-TB outside the national programme. The problem of MDR and XDR TB in India and across the world raises the possibility that the current TB epidemic of mostly drug susceptible TB will be replaced with a form of TB with severely restricted treatment options.

**DOTS**

The DOTS (Directly Observed Treatment Short-course) strategy of tuberculosis treatment recommended by WHO was based on clinical trials done in the 1970s by Tuberculosis Research Centre, Chennai, India. The country in which a person with TB
lives can determine what treatment they receive. This is because multidrug-resistant tuberculosis is resistant to most first-line medications, the use second-line anti-tuberculosis medications is necessary to cure the patient. However, the price of these medications is high; thus poor people in the developing world have no or limited access to these treatments.

**Pathogenesis of tuberculosis infection**

About 90% of those infected with *Mycobacterium tuberculosis* have asymptomatic, latent TB infection (sometimes called LTBI), with only a 10% lifetime chance that a latent infection will progress to TB disease. However, if untreated, the death rate for these active TB cases is more than 50% (47). People infected with tubercle bacillus will not necessarily become sick with the disease. The immune system “walls off” the TB bacillus which, protected by a thick waxy coat, can lie dormant for years. When someone’s immune system is weakened the chances of becoming sick are greater. TB infection begins when the mycobacteria reach the pulmonary alveoli, where they invade and replicate within alveolar macrophages (48). The primary site of infection in the lungs is called the Ghon focus. Bacteria are picked up by dendritic cells, which do not allow replication, although these cells can transport the bacilli to local (mediastinal) lymph nodes. Further spread is through the bloodstream to the more distant tissues and organs where secondary TB lesions can develop.

The interaction between the macrophage and *Mycobacterium tuberculosis* is mediated by a variety of macrophage membrane-associated proteins. Surprisingly little is known about the capacity of macrophages from patients with tuberculosis (TB) compared to that of healthy controls to adhere and/or ingest mycobacteria and how the process could relate with the pathogenesis of the disease (fig 10). Complement receptors have been implicated in the adherence of *M. tuberculosis* to macrophages. The adherence and/or ingestion is enhanced by fresh serum and inhibited by heat inactivation, EDTA treatment, and anti-CR1 and anti-CR3 antibodies (49).
Tuberculosis is classified as one of the granulomatous inflammatory conditions. Macrophages, T lymphocytes, B lymphocytes and fibroblasts are among the cells that aggregate to form a granuloma, with lymphocytes surrounding the infected macrophages. The granuloma functions not only to prevent dissemination of the mycobacteria, but also provides a local environment for communication of cells of the immune system. Within the granuloma, T lymphocytes (CD4+) secrete cytokines such as interferon gamma, which activates macrophages to destroy the bacteria with which they are infected (50). A number of cytokines and chemokines involve in the development of granuloma in tuberculosis (51).

**Description of infection**

1. **Primary Infection**
   a. Alveolar implantation and rapid multiplication of bacilli.
   b. Ingestion by phagocytes with slow intracellular replication.
   c. Enters lymph nodes; tissue necrosis and calcification; spread through blood.
2. **Granulomas**
   
a. Immune response at 1-3 months post infection.
   
b. Formation of caseating granulomas containing slowly replicating organisms.
   
c. Reactivation through liquefaction of granulomas, spread 8-9% in 1-2 year.

**Diagnosis**

Tuberculosis is diagnosed definitively by identifying the causative organism (*Mycobacterium tuberculosis*) in a clinical sample (for example, sputum or pus). When this is not possible, a probable (although sometimes inconclusive) diagnosis may be made using imaging (X-rays or scans), a tuberculin skin test (Mantoux test) and/or an Interferon Gamma Release Assay (IGRA).

The main problem with tuberculosis diagnosis is the difficulty in culturing this slow-growing organism in the laboratory (it may take 4 to 12 weeks for blood or sputum culture). A complete medical evaluation for TB must include a medical history, a physical examination, a chest X-ray, microbiological smears, and cultures (197). It may also include a tuberculin skin test, a serological test. The interpretation of the tuberculin skin test depends upon the person’s risk factors for infection and progression to TB disease, such as exposure to other cases of TB or immunosuppression.

New TB tests have been developed that are fast and accurate. These include polymerase chain reaction assays for the detection of bacterial DNA. One such molecular diagnostics test gives results in 100 minutes and is being currently offered to 116 low and middle-income countries at a discount with support from WHO and the Bill and Melinda Gates foundation.

Another such test, which was approved by the FDA in 1996, is the amplified mycobacterium tuberculosis direct test (MTD, Gen-Probe). This test yields results in 2.5 to 3.5 hours, and it is highly sensitive and specific when used to test smears positive for acid-fast bacilli (AFB).
Prevention

TB prevention and control takes two parallel approaches. In the first, people with TB and their contacts are identified and then treated. Identification of infections often involves testing high-risk groups for TB. In the second approach, children are vaccinated to protect them from TB. No vaccine is available that provides reliable protection for adults. However, in tropical areas where the levels of other species of mycobacterium are high, exposure to non-tuberculous mycobacterium gives some protection against TB.

The World Health Organization (WHO) declared TB a global health emergency in 1993, and the Stop TB Partnership developed a Global Plan to Stop Tuberculosis that aims to save 14 million lives between 2006 and 2015. Since humans are the only host of Mycobacterium tuberculosis, eradication would be possible. This goal would be helped greatly by an effective vaccine.

Treatment

Treatment for TB uses antibiotics to kill the bacteria. Effective TB treatment is difficult, due to the unusual structure and chemical composition of the mycobacterial cell wall, which makes many antibiotics ineffective and hinders the entry of drugs. The two antibiotics most commonly used are isoniazid and rifampicin. However, instead of the short course of antibiotics typically used to cure other bacterial infections, TB requires much longer periods of treatment (around 6 to 24 months) to entirely eliminate mycobacteria from the body. Latent TB treatment usually uses a single antibiotic, while active TB disease is best treated with combinations of several antibiotics, to reduce the risk of the bacteria developing antibiotic resistance. People with latent infections are treated to prevent them from progressing to active TB disease later in life.

Drug-resistant tuberculosis is transmitted in the same way as regular TB. Primary resistance occurs in persons infected with a resistant strain of TB. A person with fully susceptible TB develops secondary resistance (acquired resistance) during TB therapy.
because of inadequate treatment, not taking the prescribed regimen appropriately, or using low-quality medication. Drug-resistant TB is a public health issue in many developing countries, as treatment is longer and requires more expensive drugs. Multi-drug-resistant tuberculosis (MDR-TB) is defined as resistance to the two most effective first-line TB drugs: rifampicin and isoniazid. Extensively drug-resistant TB (XDR-TB) is also resistant to three or more of the six classes of second-line drugs.

Table 2: Drugs used for the treatment of tuberculosis

<table>
<thead>
<tr>
<th>First line drugs</th>
<th>Second line drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Essentials</strong></td>
<td><strong>Old</strong></td>
</tr>
<tr>
<td>Isoniazid</td>
<td>Ethionamide</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>Cycloserine</td>
</tr>
<tr>
<td></td>
<td>Capreomycin</td>
</tr>
<tr>
<td></td>
<td>Kanamycin</td>
</tr>
<tr>
<td></td>
<td>PAS</td>
</tr>
<tr>
<td></td>
<td>Thiocetazone</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td></td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td></td>
</tr>
<tr>
<td>Ethambutol</td>
<td></td>
</tr>
<tr>
<td>Streptomycin</td>
<td></td>
</tr>
</tbody>
</table>

The DOTS (Directly Observed Treatment Short-course) strategy of tuberculosis treatment recommended by WHO was based on clinical trials done in the 1970s by Tuberculosis Research Centre, Chennai, India. The country in which a person with TB lives can determine what treatment they receive. This is because multidrug-resistant tuberculosis is resistant to most first-line medications, the use of second-line anti-tuberculosis medications is necessary to cure the person. However, the price of these medications is high; thus poor people in the developing world have no or limited access to these treatments.
Vaccines

Many countries use Bacillus Calmette-Guérin (BCG) vaccine as part of their TB control programmes, especially for infants. The protective efficacy of BCG for preventing serious forms of TB (e.g. meningitis) in children is greater than 80%; its protective efficacy for preventing pulmonary TB in adolescents and adults is variable, ranging from 0 to 80%.

BCG provides some protection against severe forms of pediatric TB, but has been shown to be unreliable against adult pulmonary TB, which accounts for most of the disease burden worldwide. Several new vaccines to prevent TB infection are being developed, among others by areas and TBVI and the first recombinant tuberculosis vaccine rBCG30.

Prognosis

Progression from TB infection to TB disease occurs when the TB bacilli overcome the immune system defences and begin to multiply. In primary TB disease—1–5% of cases—this occurs soon after infection. However, in the majority of cases, a latent infection occurs that has no obvious symptoms. These dormant bacilli can produce tuberculosis in 2–23% of these latent cases, often many years after infection. The risk of reactivation increases with immunosuppression, such as that caused by infection with HIV. In people co-infected with M. tuberculosis and HIV, the risk of reactivation increases to 10% per year.

Studies utilizing DNA fingerprinting of M. tuberculosis strains have shown that re-infection contributes more substantially to recurrent TB than previously thought, with between 12% and 77% of cases attributable to re-infection (instead of reactivation).

Female genital tuberculosis

Female genital tuberculosis, though known to have existed for centuries, was first described by Morgagni in 1744 during autopsy on a 20-year old girl known to have died of tuberculosis peritonitis (52). A century later, Hager in 1886 conducted a deta-
iled study of the course and treatment of the disease. It presents with various manifestations. It is almost always secondary to a focus elsewhere in the body. A number of patients may remain asymptomatic and the disease may be discovered incidentally.

**Epidemiology**

Twentieth century has witnessed dramatic reduction of female genital tuberculosis cases in developed countries. But this is not so in developing countries. More recently, most reports of genital tuberculosis are reported from India, South Africa, Russia and Turkey (195). Incidence of genital tuberculosis varies depending upon geographical location ranging from being less than 1 percent in United States to 10.3 percent in India. Schaefer estimated the worldwide incidence of genital tuberculosis in infertile women to be 5 to 10 percent (11).

Incidence of genital tuberculosis has been estimated to range from 1 to 19 percent in various studies from India. Malkani and Rajani based on endometrial biopsies from infertile women reported the incidence of genital tuberculosis to be 9.3 percent (53). Deshmukh et al reported a similar incidence at laparoscopy in 500 infertile women (54).

Schaefer reported that 80 to 90 percent of his patients were between 20 and 40 years of age (11). A changing trend in the age diagnosis has been highlighted by Sutherland in a large series of 704 patients with female genital tuberculosis seen between 1951 and 1980 (55). The mean age was 28.2 years in the initial 10 years compared to 38.9 years observed in the last decade of study. A series from Turkey reported 29.2 percent cases of female genital tuberculosis to be older than 40 years of age. However, in most large series from India 68 to 89 percent cases of genital tuberculosis were between 20 to 30 years of age (182,183,184).

90%–95% of female genital tuberculosis is caused by *Mycobacterium tuberculosis*. 5%–10% of cases are caused by *Mycobacterium bovis* especially when acquired from gastrointestinal tract.
Table 3: Table showing age distribution among TB patients in various studies

<table>
<thead>
<tr>
<th>Study</th>
<th>No. of patients</th>
<th>Age group (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&lt; 20</td>
</tr>
<tr>
<td>Gupta (1956)</td>
<td>47</td>
<td>13.0</td>
</tr>
<tr>
<td>Devi (1962)</td>
<td>144</td>
<td>12.0</td>
</tr>
<tr>
<td>Hafeez and Tandon (1966)</td>
<td>120</td>
<td>3.3</td>
</tr>
<tr>
<td>Chhabra (1995)</td>
<td>58</td>
<td>1.7</td>
</tr>
</tbody>
</table>

Pathogenesis

Genital tract tuberculosis is almost always secondary to tuberculosis infection elsewhere in the body. Although pulmonary involvement is most common, extra pulmonary organs such as kidneys, gastrointestinal tract, bones or joints may also be the primary source of infection. Primary genital tuberculosis although extremely rare, has been described in female partners of males affected by active genitourinary tuberculosis. In females of primary genital tuberculosis, cervix or vulva may be the site of involvement.

Haematogenous or lymphatic route is the usual mode of spread. However, direct contiguous spread from other intraperitoneal organs may occur in a minority of patients. Simultaneous occurrence of peritoneal tuberculosis in patients with genital tuberculosis increases the possibility of ruptured caseous lymph nodes or involvement of genital organs during the haematogenous phase.

Fallopian tube is usually the initial site with subsequent spread to other genital organs, being affected 90% to 100% cases. Other affected sites include endometrium in 50% to 60%, ovaries in 20% to 30%, cervix in 5% to 15% and vagina in 1% (13).
Fallopian tube tuberculosis

Fallopian tubes are involved in almost all patients with genital tuberculosis. Most common site of disease is ampullary portion of fallopian tube. Generally the involvement is bilateral. Gross appearance of the tube may vary depending upon the severity of the disease and the stage at which it is encountered. In early cases, congestion of tube and other pelvic organs with filmsy adhesions and fine miliary tubercles on the surface of them can be seen. In severe cases, dense plastic adhesions between the fallopian tubes and surrounding organs are seen. In old healed cases, hydrosalpinx or pyosalpinx may be present. In 25% to 50% cases, the fallopian tubes remain patent with everted fimbriae giving rise to so called “tobacco pouch appearance”.

Endometrial tuberculosis

Endometrial involvement in genital tuberculosis is secondary to tubal involvement. Various texts reported endometrial involvement in 50% to 80% cases. In a large series of 1436 cases of genital tuberculosis, Norgales-Ortiz et al reported endometrial involvement in 79% cases (56). Oosthuizen et al, in a study of 109 patients with infertility, found evidence of genital tuberculosis in the form of positive culture in menstrual blood in 16 and positive endometrial tissue for Mycobacterium tuberculosis in four patients (57).

Gross appearance of endometrium is mostly unremarkable. However, in advanced cases, ulcerative or atrophic endometrium or an obliterated endometrial cavity due to extensive intrauterine adhesions may be seen. Total destruction of endometrium by the disease process with resultant secondary amenorrhea has been reported in a few cases.

Tuberculous endometritis

Endometrial tuberculosis is now a relatively infrequent finding in the western world, but is still a common problem in the developing world, where it occurs in 50-60% of women with genital tuberculosis (11). Infection occurs by direct transluminal spread
from the fallopian tube to the superficial zone of the functionalis. The infected endometrium is shed monthly, allowing only 22-23 days for the establishment of the infection. Therefore, it is uncommon to see well established tuberculoid granulomas which typically take a minimum of 15 days to form (58). Therefore, caseation is rarely observed except after the menopause or in the unusual event that the severity of the systemic disease impairs ovulation. Nogales-Otriz et al (1979) have shown that basalis was affected in 40% of cases. Infection may therefore take place from the basalis or by direct haematogenous spread (56,185).

Advanced endometrial tuberculosis presents with gross endometrium of the uterine wall, the endometrium is severely ulcerated and the patient presents with caseous and purulent discharge and sometimes with bleeding. The glandular structure of the endometrium is completely destroyed and the curetting reveal typical caseating epithelioid and giant cell granulomas.

However, early cases of tubercular endometritis are seen where one may find only an occasional tubercle or clusters of tubercles with the characteristic epithelioid and giant cells. In these cases, the glandular structure may almost be intact and one may find marked chronic inflammatory infiltration (186). In regularly menstruating women, the lesions are small. May not contain giant cells and rarely show caseation (59). Therefore, the extent of the inflammatory involvement in TB Endometritis varies from a focal process with very few granulomas to a diffuse process of ulceration of the mucosa and extensive caseous necrosis (60).

Granulomas in different stages of development frequently co-exist suggesting that complete shedding of functionalis does not always occur; and some granulomas being retained until the next cycle. These mature granulomas may be more typical of tuberculosis. Biopsies taken earlier in the cycle may reveal a non-specific picture of intra stromal plasma cells and lymphocytes or intraglandular polymorphonuclear leucocytes (59).

The histological reporting (61) of endometrial tuberculosis is categorized as:
A. Tuberculous Endometritis
i) Epitheliod and giant cells granulomas with or without caseation.
ii) Non reactive granulomas characterized by foci of necrosis but with poor cellular reaction consisting of scant epitheliod cells and lymphocytes but not giant cells.

B. Doubtful cases of tuberculous endometritis where lesions are composed mainly of lymphocytes and polymorphs with occasional macrophages and minimal epithelial necrosis (61).

Prevalence of genital tuberculosis

The actual incidence of genital tuberculosis in the general population cannot be determined accurately because in a large number of patients, the disease is symptomless and discovered incidentally or may remain undiscovered (11). At least 11% of the patients are symptomatic and the disease is discovered incidentally (62).

The reported frequency of GTB has been based on postmortem examination, surgical specimens and endometrial biopsies from sterility studies. The incidence also varies greatly according to the socioeconomic and public health conditions; therefore, there is wide variation in figures published from various countries. In USA, Australia and Western European countries the incidence of genital tuberculosis is less than 1%; but the incidence in some African countries and India is 15-19% (63,64). Moreover, in less developed areas of the world, there is inadequacy of diagnostic procedure for diagnosis of genital tuberculosis (65). The incidence is also influenced by the lack of highly sensitive and specific tests to diagnose the condition (15).

Schaefer (1976) estimated that 5-10% of infertile females around the world have genital tuberculosis (11). Pelvic tuberculosis is an uncommon gynecological problem in countries like Malaysia and Thailand and is seen in 0.03% to 0.05% of gynecological cases (66, 67). Studies from African countries have shown a high incidence of genital tuberculosis in infertile women. In Western Cape, prevalence of genital tuberculosis was 7.98% (68).
Over the last decade, in developed countries, there has been a steady decline in the incidence of pulmonary and extra-pulmonary tuberculosis. However, in developing countries, there has been an increase in the incidence of pulmonary and extra-pulmonary tuberculosis including the drug-resistant forms due to emergence of HIV infection (69). The proportion of extra-pulmonary tuberculosis is increasing in South India and currently stands slightly higher than smear pulmonary forms (70).

Genital tuberculosis is responsible for a significant proportion of females presenting with infertility (71). Genital tuberculosis as a cause of infertility is 10-15 times more common in developing countries (72).

High prevalence of genital tuberculosis has been reported by various authors from African countries. From South Africa, Margolis (1992) have reported a 6.15% prevalence of genital tuberculosis in infertile women (73). A bacteriological study of 114 infertile patients in the Northern Nigeria revealed a prevalence of 16.7% of genital tuberculosis (74). Oosthuizen et al (1990) have given an incidence of 21% from Africa (57). In a report from Pakistan, out of 534 infertile women, genital tuberculosis was diagnosed in 2.43% by histopathology and culture in Lowenstein-Jensen (LJ) media (43).

In Chandigarh, India, by using a simplified tuberculosis algorithm genital tuberculosis was diagnosed in 7.2% of women with infertility (75). Recently, by using PCR technique along with AFB smear microscopy, HPE and culture, Rozati et al, (1996) reported 50% incidence of genital tuberculosis in 65 infertile women suspected of suffering from female genital tuberculosis (13).

Studies have shown that the prevalence of genital tuberculosis is higher in tubal factor infertility; a study from Cuttack, India (2002) showed that the incidence of GTB was 3% of infertility cases and 41% of the tubal factor infertility (76). Similar findings were shown by Sharman (1952) and Halbrecht (1959). When the tubes were occluded the prevalence of tuberculosis reported was between 25% and 44% (77,78).
Ovarian tuberculosis

Ovarian involvement occurs in 15% to 25% cases and most often results from direct extension of the disease from fallopian tuberculosis. In such cases, ovary may be surrounded by adhesions or may be the site of tubo-ovarian cyst formation or tubo-ovarian mass with adhesions surrounding them.

Tuberculosis of cervix

Tuberculosis of cervix may be seen a 5% to 15% case of genital tuberculosis. Cervix mostly gets affected by downward spread of disease from the endometrium. However, rarely cervical disease may occur secondary to deposition of infected semen by the male partner. Mostly, cervical lesions tend to be hypertrophic resembling cervical carcinoma and less often an ulcerative lesion may be seen. The endocervical involvement is common and may explain increased mucus production. Cytological diagnosis of genital tuberculosis in association with carcinoma in situ and Trichomonas vaginalis has been described.

Tuberculosis of vulva, vagina and Bartholin's gland

Tuberculosis of vulva and vagina occurs in 1% of cases. Tuberculosis infection of Bartholin gland and vesicovaginal fistula due to genital tuberculosis has been described. Involvement of vagina or vulva is usually secondary to the involvement of other parts of genital tract. However, transmission of the disease by a male partner due to involvement of epididymis or seminal vesicle has been reported. Lesions on vulva and vagina may present as hypertrophic lesions resembling malignancy, ulcers in the vulva may be seen.

Clinical presentation

The most frequent presenting symptoms in patients with female genital tuberculosis include infertility, menstrual disturbances, pelvic pain, vaginal discharge and poor general conditions (188). Table 4 shows the various symptoms in female genital tuberculosis reported in previous studies.
Table 4: Symptoms in female genital tuberculosis in previous studies.

<table>
<thead>
<tr>
<th>Study (year)</th>
<th>No. of patients</th>
<th>Infertility (%)</th>
<th>Amenorrhea (%)</th>
<th>Menorrhagia/ Oligomenorrhea (%)</th>
<th>Chronic pelvic pain (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sutherland (1949)</td>
<td>250</td>
<td>40</td>
<td>10</td>
<td>18</td>
<td>NA</td>
</tr>
<tr>
<td>Malkani and Rajani (1954)</td>
<td>106</td>
<td>NA</td>
<td>43.4</td>
<td>43.3</td>
<td>NA</td>
</tr>
<tr>
<td>Mukherjee et al (1970)</td>
<td>138</td>
<td>100</td>
<td>60</td>
<td>19.7</td>
<td>NA</td>
</tr>
<tr>
<td>Munjal et al (1970)</td>
<td>140</td>
<td>37.1</td>
<td>42.8</td>
<td>41.4</td>
<td>NA</td>
</tr>
<tr>
<td>Klein et al (1976)</td>
<td>20</td>
<td>70</td>
<td>20</td>
<td>NA</td>
<td>30</td>
</tr>
<tr>
<td>Falk et al (1980)</td>
<td>187</td>
<td>12.8</td>
<td>41.2</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Bazaz-Mallik et al (1983)</td>
<td>1000</td>
<td>47</td>
<td>26</td>
<td>15</td>
<td>2.4</td>
</tr>
<tr>
<td>Bobhate et al (1986)</td>
<td>337</td>
<td>58.6</td>
<td>26.4</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Chhabra (1990)</td>
<td>58</td>
<td>29.3</td>
<td>18.9</td>
<td>15.5</td>
<td>43.1</td>
</tr>
<tr>
<td>Sfar et al (1990)</td>
<td>118</td>
<td>81</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

**Infertility**

Primary or secondary infertility is the most common presentation in patients with female genital tuberculosis. Reported incidence of infertility in patients with female genital tuberculosis has varied between 40-80%.

**Chronic lower abdominal or pelvic pain**

Chronic lower abdominal or pelvic pain is the second most common symptom in patients with female genital tuberculosis. Reported incidence of chronic pelvic pain in these cases varies between 20-50%. Pain is non-characteristic and usually localized in the lower abdomen or pelvis. Pain tends to be chronic and dull aching. Episodes of acute pain, as a result of superadded bacterial infection, can occur and require administration of antibiotics. Acute episodes of pain may occur after diagnostic procedures such as endometrial biopsy, dilation and curettage or hysterosalpingography. Patients with chronic pain are more likely to have abnormal findings on pelvic examination.
Alteration in menstrual pattern

Alterations in menstrual pattern may be seen in 10-60% of cases. All types of menstrual irregularities such as amenorrhoea, menorrhagia, oligomenorrhoea or even postmenopausal bleeding can occur.

*Amenorrhoea:* Various Indian series have reported the occurrence of primary and secondary amenorrhoea to be 18.9-60% of the cases.

*Menorrhagia or oligomenorrhoea:* Menstrual symptoms such as heavy periods and prolonged menstrual cycles are almost equally common as the symptom of amenorrhoea in patients with genital tuberculosis. Various Indian series have reported menorrhagia and oligomenorrhoea in 15%-43.3% of cases.

Postmenopausal bleeding

Uterine bleeding after menopause has been noted as one of the presentations of female genital tuberculosis. In series from India and abroad, postmenopausal bleeding as the presenting symptom has been reported in 1% to 20% of patients with female genital tuberculosis.

Persistent/ abnormal vaginal discharge

Occasionally, patients with persistent vaginal discharge may be found to have genital tuberculosis affecting cervix or vagina. Such a symptom is more likely to occur in women with endocervical tuberculosis or in patients with tuberculosis of cervix or vagina.

Unusual symptoms

Several unusual presentations of female genital tuberculosis have been described from time to time. These include vulval lesions, Bartholin gland swelling, vesicovaginal fistula, pelvic masses, uterocutaneous fistula, and retention of urine due to pelvic masses of tuberculosis origin.
Physical signs

No physical sign on abdominal or pelvic examination is characteristic of genital tuberculosis. A high index of suspicion is therefore, required to make an early diagnosis.

Minimal induration in adnexal mass on both sides is the most commonly noted physical finding during pelvic examination in these patients. However, it is not specific for female genital tuberculosis. Bilateral tubo-ovarian masses, especially in nullipara or unmarried girls in the absence of fever should raise a strong suspicion of tuberculosis. Enlargement of uterus due to pyometra especially in a post menopausal woman may be due to pelvic tuberculosis.

Sutherland (55), in a large series of patients over a 30-year period, found a decreasing incidence of palpable adnexal masses. Falk et al (12), in a series of 187 patients from 47 Swedish hospitals, found tubo-ovarian masses in 46 patients. Lack of tenderness during palpation may be an indication of tuberculosis mass. Physical examination may be entirely normal in 31.6 to 50 percent cases.

Diagnosis

There are some non specific tests like haemogram, ESR, Mantoux test, chest X-ray which helps in the diagnosis of genital tuberculosis. One has to rely on imaging and histopathology. Laparoscopy, hysterosalpingography, ultrasonography of pelvic organs, computed tomographic (CT) scan and magnetic resonance imaging (MRI) are the imaging modalities available for the diagnosis. Most definitive diagnosis of female genital tuberculosis is mycobacterial isolation in tissue. But as genital tuberculosis is a paucibacillary disease, it is not possible to demonstrate *Mycobacterium tuberculosis* in every case. ELISA helps in indicating the status of the infection and the use of ELISA has been banned by WHO in December 2010. Now PCR has revolutionized the microbiological diagnosis.
Endometrial biopsy

Specimen of endometrium obtained by endometrial biopsy curette or by endometrial aspiration or by dilatation of cervix and curettage of the endometrium is the most easily obtained tissue for the diagnosis of genital tuberculosis. Best time to perform such a procedure is shortly before the menstruation as lesions are likely to be close to the surface of endometrium during the phase of the menstrual cycle.

Gross appearance of endometrium is mostly unremarkable. However, in advanced cases, ulcerative or atrophic endometrium or an obliterated endometrial cavity due to extensive intrauterine adhesions may be seen.

Microscopically, diagnosis is based upon the presence of chronic inflammatory cells with or without caseation, granulomas with lymphocytes, Langhans giant cells and epithelioid cells. Such lesions may be focal or localized.

However, typical granulations may not be seen in all cases. Bazaz-Malik et al in a series of 1000 cases of tuberculosis endometritis noted discrete granulomas and caseation in 60% only. They suggested presence of dilated glands, destruction of epithelium, and inflammatory exudate in the lumen as additional criteria for diagnosis of tuberculosis endometritis. Bourno and Williams suggested that focal collection of lymphocytes in the endometrium should be considered to be tuberculosis origin unless proven otherwise. Malkani and Rajani suggested that focal collection of chronic inflammatory cells or presence of proliferative endometrium in the premenstrual week in a patient with past history of tuberculosis would favor a diagnosis of female genital tuberculosis.

A negative endometrial biopsy does not rule out the pelvic involvement since sampling errors are common and the disease may have involved other pelvic organs without associated tuberculosis endometritis.

Ultrasonography

Ultrasonography, being non-invasive with no radiation hazard, has been increasingly used in evaluating pelvic and other abdominal masses. Lee et al described sonographic
features of tuberculosis endometritis in a 59 year old female. Demonstration of bilateral, predominantly solid, adnexal masses containing scattered small calcifications is highly indicative of tuberculosis involvement.

*Laparoscopy*

Laparoscopy is now a well recognized procedure in the diagnostic work up of patients with infertility. Laparoscopy provides direct visualization of the pelvic organs and peritoneal surfaces and at the same time helps in establishing tubal patency. Endoscopy has a dual advantage of pelvic organ visualization and sample collection from inaccessible sites for laboratory diagnosis (79).

Laparoscopy has been used as an additional tool to evaluate women with high suspicion of genital tuberculosis. Endoscopy helps to obtain microbiological samples, evaluate the condition of the organ and the extent of the damage and provides an opportunity for therapeutic intervention (75). Laparoscopy has been found to be a superior method of bacteriological sampling, since the laparoscopic collection is done under direct vision (80-82).

Three clinical forms of tuberculosis of the uterine appendages are distinguished: latent or minor inflammation, marked inflammation with tubo-ovarian lesion and tuberculomas. Early/latent tuberculosis does not produce tubal or peritoneal changes. Evidence of acute infection by laparoscopy is small military tubercles, T-O mass, peritoneal congestion, swollen and reddened serosa of uterus and tubes. Chronic infection manifests as thickened tubes, terminal hydrosalpinx with retort shaped tubes, flimsy adhesions in the POD and intravasation and extravasation on chromopertubation (83).

Confirmation of diagnosis alone with laparoscopy is insufficient (191). Certain conditions like tubo-ovarian masses of gonococcal/pyogenic origin, pelvic endometriosis, small ovarian cyst and old pelvic haematocoele may closely mimic a T-O mass of tuberculous origin. Rarely, the whole appearance may be difficult to distinguish from that of ovarian malignancy (84).
Therefore, definitive diagnosis can only be made by positive histology of tissue or by positive culture of tissue or POD aspirate.

Semenovski et al (1999) showed that laparoscopy may detect pathognomonic signs of rashes on the visceral peritoneum and enlarged mesenteric lymph nodes. This study showed that laparoscopy increases the diagnostic potentiality by 19.7% in diagnosing abdominal and genital tuberculosis (85).

Avan et al 2001 compared the clinical and laparoscopic features which may help to differentiate between infertility in females due to genital tuberculosis from pelvic inflammatory disease (PID) and endometriosis. This study reported that tortuous, bilaterally blocked and thickly adherent tubes are common in genital tuberculosis when compared to other groups. The primary infertility patients with chronic malnutrition and masses and adhesive fallopian tubes on laparoscopic examination should be evaluated for genital tuberculosis (86).

The following laparoscopic findings were reported in patients with proven genital tuberculosis by Amarnath et al: pelvic adhesions in 46-48%, tubercles in 33.8% and adnexal masses in 32.3% of cases and encysted effusion in 8.45% cases and diagnostic laparoscopy in clinically suspected cases of genital tuberculosis. Their observation showed that bacteriology could detect only 2.8% of cases, histology 21.71%, HSG 51.11% of cases and laparoscopy was suggested of tuberculosis in 74% of cases. Their study concluded that laparoscopic visualization of genital tract is more effective as compared to bacteriology and histological methods and laparoscopy helps in diagnosing genital tuberculosis at an early stage (87).

In early and latent cases, there may not be evidence of pelvic tuberculosis in laparoscopy. In a study by Vynck et al (1990) from South Africa, in cases that were positive by microbiological studies in menstrual fluid, there was laparoscopical evidence of tuberculosis only 55.5% of cases and remaining 44.5% cases, the pelvis was considered normal (68). Similar findings were also noted in Deshmukh et al study, where out of 45 cases with histologically proven genital tuberculosis, 3 cases did not show evidence of tuberculosis at laparoscopy (54).
Moreover, laparoscopy is an invasive procedure and should be done carefully to avoid injury to an adherent bowel. Matted tuberculous adhesive did not always give rise to a “doughy” abdomen and in fact they can be remarkably silent and prone for dangerous injury to the bowel (55,88).

Genital tuberculosis presents unique diagnostic challenges including subtle clinical manifestations that may be overlooked at laparoscopy during early stages of infection (15).

A number of conditions may be discovered during laparoscopy in these cases. These include endometriosis, pelvic inflammatory disease or fibroids.

Studies from India and elsewhere have reported 5% to 33.8% incidence of genital tuberculosis at laparoscopy in patients with infertility.

Based upon various laparoscopic finding and guide biopsy, Palmer and Olivera have described a subacute and chronic stage in the natural history of pelvic tuberculosis.

*Subacute stage*

The subacute stage of female genital tuberculosis is characterized by the presence of miliary granulations, whitish-yellow and opaque plaques surrounded by hyperaemic areas over the fallopian tubes and uterus. Pelvic organs may be red and oedematous with adhesions.

*Tuberculosis and HIV infection*

The incidence of HIV-associated tuberculosis is increasing worldwide especially in developing countries (76). The immunocompromised state due to HIV infection causes reactivation of endogenous tuberculosis infection to development of tuberculosis disease (89). HIV infected patients rapidly clinically significant disease and respond poorly to treatment, it is also known that in HIV-positive patients extra pulmonary tuberculosis occurs more often (90, 91).
Although a relative increase in GTB would be expected, this has been reported. Probably tuberculosis systemic disease is diagnosed earlier before extra-pulmonary manifestation occurs (56). However, diagnosis of GTB should be considered more often and more carefully in HIV-infected women and all patients with tuberculosis should be screened for HIV infection (92).


*Tuberculin test*

Tuberculin reactivity was described by Koch (1891) during a search for a remedy for tuberculosis but the characteristic dermal reaction induced by old tuberculin was utilized by Von Pirquet (1907) in epidemiological studies as an indicator of past infection by tubercle bacillus (193).

There are three main tests currently in use: the Mantoux intradermal test, the Heaf and the Tine multiple puncture tests. The Heaf test is usually preferred for testing large groups of people because it is quick, easy to perform, cheap and reliable. The Mantoux test is preferred when a more precise measurement of tuberculin sensitivity is required. The Tine test is considered by some authorities as unreliable and therefore not recommended.

*Types of tuberculin*

1. *Old tuberculin (OT)*: It was originally described by Robert Koch. It is prepared by autoclaving or boiling a culture of tubercle bacilli, concentrating it 10 fold on a steam bath, filtering off the debris and glycerol as a preservative. It is a crude product and different batches vary in their activity.

2. *Purified protein derivative (PPD)*: A slightly more refined tuberculin called PPD was prepared by Seibert in 1939 by growing *Mycobacterium tuberculosis* in a semi-synthetic medium, autoclaving , removing debris by filtration, concentrating the filtrate by ultra filtration and precipitating several times with 50% saturated ammonium sulphate. The
product is mostly a mixture of small proteins. It is stable but not specific. It cross reacts with other members of the slow growing mycobacteria subgenus.

One large batch of PPD made by Seibert in 1939 (PPD-S) was recognized by the WHO as the international standard PPD-tuberculin and arbitrarily designated to contain 50,000 TU/mg. 1 TU is equal to 0.01 ml of OT or 0.00002 mg of PPD-S. The WHO advocates a PPD tuberculin known as PPD-RT-23 with Tween80. It is obtained by culturing Mycobacterium tuberculosis H37Ra strain with Tween 80 on a synthetic protein free medium Quinosol.

3. New tuberculin: Stanford and his colleagues in 1983 disrupted mycobacteria by ultrasonication and prepared new tuberculin. Though not entirely free from shared antigens, these are richer in species specific antigens.

Uses of tuberculin test

- To diagnose active infection in infants and young children. If a child below two years is found to be tuberculin positive, it is indirect evidence of an active tubercular lesion in the body even if it is not manifested.
- To measure prevalence of infection in a community.
- To select susceptible for BCG vaccination.
- Indication of successful BCG vaccination.

False negatives

Snider et al found that approximately 10% of adults with culture documented tuberculosis do not react initially to PPD, a similar phenomenon is also seen among children (189). Age, nutrition, immunosuppression, viral infections, disseminated tuberculosis; corticosteroid therapy can all diminish response to tuberculin. A host of factors related to wrong test techniques may lead to false negatives.

False positives

- Recent exposure to environmental non-tuberculous mycobacteria (NTM).
- Immunization with Bacillus-Calmette –Guerin (BCG) vaccine.
Koch’s isolation of the tubercle bacillus in 1882 was followed by many attempts to prepare a vaccine against tuberculosis, mostly by the various techniques established by Pasteur. Calmette and Guerin, two French scientists began attenuating a virulent strain of *Mycobacterium bovis* in 1906 with a view to develop a vaccine against tuberculosis. After 239 subcultures over a period of 13 years, they were able to evolve a strain known as Bacillus-Calmette-Guerin or BCG– which was avirulent for man while retaining its capacity to induce an immune response.

The duration of protection is from 15 to 20 years. The first prospective control trial of BCG showed it to be 80% effective over an observation period of 20 years.

Studies have shown that the range of protection offered by BCG varied from 0 to 80% in different parts of the world. In spite of this, there is a large body of evidence which supports the conclusion that BCG gives an appreciable degree of protection against miliary tuberculosis when given in children younger than two months of age. The WHO, on the basis an extended review of BCG including the South India trial, holds that it would be unreasonable to stop current BCG vaccination programmes and recommends that the use of BCG should be continued as an anti–tubercular measure.

It offers a protective effect (approximately 64%) against tubercular meningitis. Centers for Disease Control (CDC) reported incidence of TBM reduced to 52%–100% lower in vaccinated children (94). Several studies from Brazil, estimated vaccine efficacy at 80 to 85%.

**BCG vaccination and Mantoux test**

BCG vaccination can lead to a reactive tuberculin test because of cross-reactivity of antigens. Nevertheless, the induration response to tuberculin testing after BCG is usually less than 10 mm in diameter and declines over time. Karalliede et al in a study reported that approximately 50% of children vaccinated with BCG in infancy (before 2 months of age) have a positive tuberculin test at 12 months of age, <40% will be positive at 3 years of age and only 5% will have a positive tuberculin test at 5 years of age.
Despite much confusion concerning the use of the Mantoux skin test after previous BCG vaccination, BCG vaccination is not a contradiction to tuberculin testing.

*Mantoux test in diagnosis of genital tuberculosis in women*

The Mantoux test had a sensitivity of 55% and specificity of 80% in women with laparoscopically diagnosed tuberculosis in a study by Raut V S et al (95). Pelvic focal reaction was absent in all groups including infertile women with positive Mantoux test. They concluded that Mantoux test has a limited utility in diagnosing active genital tuberculosis during child bearing age. However, in infertile women with positive Mantoux test, laparoscopy is advocated early.

*Microscopy*

Mycobacteria are characterized by acid-fastness, which depends upon the composition and integrity of its cell wall i.e. killed or fragmented mycobacteria may not be acid-fast. The acid-fast smear is an essential adjunct to the diagnosis of tuberculosis though less sensitive than culture.

*Acid fast stains*

Mycobacteria possess cell walls that contain mycolic acids, which are long chain, multiple cross-linked fatty acids. These long chain mycolic acids probably serve to complex basic dyes, contributing to the characteristic acid fastness that distinguishes them from other bacteria. Acid-fastness is affected by the age of the colonies and the medium on which growth occurs. Rapidly growing species appear to be acid-fast variable.

Robert Koch (1882) used hot alkaline methylene blue as the primary stain and vesuvin (Bismarck brown) as decolouriser and counterstain. Ehrlich (1882) used fuchsin as the primary stain, aniline as the mordant and a mineral acid as the decolourizer. Ziehl (1882) used phenol as mordant. Also in 1882 Rindfleisch heated the slide instead of putting it in hot water. Finally Neelsen (1883) combined Ehrlich’s fuchsin stain with
Ziehl’s mordant. Thus the Ziehl-Neelsen stain as we know today should properly be called Ehrlich-Ziehl-Neelsen stain.

**Culture**

Mostly specimen submitted for culture of mycobacteria contains many other organisms which grow in one or two days and, within a week they would overgrow the entire surface of the medium and probably digest it before the mycobacteria start to grow. Such material must be treated in an attempt to destroy them but to preserve the mycobacteria. Specimens such as endometrial biopsy (EB) material, endometrial aspirate (EA) fluid, pouch of Douglas (POD) fluid, CSF and certain pleural fluids are likely to be free from contaminating bacteria, should not be treated by any chemical agents before culture. They can be placed directly onto the culture media but for specimens like EB material, sensitivity is increased if they are centrifuged and the sediment is inoculated onto the culture media.

The probability of finding bacilli is greater by culture than by microscopy when specimens contain only small number of mycobacteria. Many different culture media have been devised for growing the tubercle bacillus.

These may be divided into three groups:

1. Egg based media
2. Agar based media
3. Liquid media

**Egg based media**

Historically, the egg based media are among the best known solid media used for isolation of *Mycobacterium tuberculosis*. The various egg-based media for the growing tubercle bacilli are shown in table 5.
Characteristics

- These media are solidified by heating to 85ºC-90ºC for 30-45 minutes (inspissation) for three days. These are very rich media and contain phospholipid and proteins that tend to bind or neutralize toxic products in clinical specimens.
- Tend to yield high number of positives from direct clinical specimens because it is less inhibitory to the mycobacteria.
- Not useful for research purposes because of being very complex, not reproducible, variation in quality of ingredients and effects of heat.
- Colonies are rough and beige to brown color and show up well on the green background.
- Chromogenic studies and biochemical tests are more accurate when performed on subculture from LJ medium.

Table 5: Various egg-based media and their constituents devised for growing tubercle bacillus

<table>
<thead>
<tr>
<th>Medium</th>
<th>Components</th>
<th>Inhibitory agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lowenstein- Jensen medium</td>
<td>Coagulated whole eggs, defined</td>
<td>Malachite green</td>
</tr>
<tr>
<td></td>
<td>salts, glycerol, potato flour</td>
<td></td>
</tr>
<tr>
<td>Petragnini medium</td>
<td>Coagulated whole eggs, glycerol,</td>
<td>Malachite green</td>
</tr>
<tr>
<td></td>
<td>milk, potato flour</td>
<td>0.052 gm/100ml</td>
</tr>
<tr>
<td>American thoracic society</td>
<td>Coagulated egg yolk, glycerol,</td>
<td>Malachite green</td>
</tr>
<tr>
<td>medium</td>
<td>potato flour</td>
<td>0.02 gm/100ml</td>
</tr>
<tr>
<td>IJ- GRUFT modification</td>
<td>Same as LJ medium</td>
<td>Penicillin, Nalidixic acid,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Malachite green</td>
</tr>
<tr>
<td>Mycobactosel (IJ medium)</td>
<td>Same as LJ medium</td>
<td>Lincomycin, Cycloheximide,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nalidixic acid, Malachite</td>
</tr>
<tr>
<td></td>
<td></td>
<td>green</td>
</tr>
<tr>
<td>IUAT version of LJensen</td>
<td>Defined salts, whole eggs, glycerol,</td>
<td>Malachite green</td>
</tr>
<tr>
<td>medium</td>
<td>asparagines</td>
<td>0.025 gm/100ml</td>
</tr>
<tr>
<td>Dorset’s egg medium</td>
<td>Beated eggs, sterile broth</td>
<td>Malachite green</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.025 gm/100ml</td>
</tr>
</tbody>
</table>
Agar based media

The various agar-based media for the growing tubercle bacilli are shown in table 6.

These media are usually prepared from semisynthetic basal media enriched with supplements.

Characteristics

- These are transparent media and offer better opportunity to study colonial morphology microscopically after just 10 days of inoculation.
- These offer better defined components than egg based media.
- These allow a more rapid recovery of growth (within 2-4 weeks).
- Addition of 0.1% casein hydrolysate to Middle brook 7H11 medium improves the recovery rate and enhances the growth of INH resistant mycobacteria.
- Usually reserved for Identification, sensitivity tests and research purposes.

Table 6: Various agar-based media and their constituents devised for growing the tubercle bacillus

<table>
<thead>
<tr>
<th>Medium</th>
<th>Components</th>
<th>Inhibitory agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Middle brook 7H10</td>
<td>Defined salts, glycerol, dextrose, albumin, vitamins, cofactors, oleic acid, catalase</td>
<td>Malachite green0.0025gm/100ml</td>
</tr>
<tr>
<td>Middle brook 7H11</td>
<td>Same as above+ casein hydrolysate0.1%</td>
<td>Malachite green0.0025gm/100ml</td>
</tr>
<tr>
<td>Middle brook 7H11 (selective)</td>
<td>Same as above</td>
<td>Carbenicillin, PolymixinB, trimethoprim lactate, AmphotericinB, Malachite green</td>
</tr>
<tr>
<td>Middle brook 7H12</td>
<td>7H9 broth, casein hydrolysate, bovine serum albumin, catalase, C14 palmitic acid</td>
<td>Polymixin, Azlocillin, Nalidixic acid, Trimethoprim, AmphotericinB (PANTA)</td>
</tr>
</tbody>
</table>
**Liquid media**

Liquid media are useful when the specimens contain very small numbers of bacilli, as positive cultures could be more often obtained in these media using a large inoculum.

A list of liquid media used commonly is given below:

- Kirchner-Herman medium
- Dubo’s medium
- Sula’s medium
- Youmen and Karlsons
- Proskauer and Beck medium
- 7H9 synthetic medium
- 7H9 broth + PANTA → Biphasic medium → Septi-check AFB system

Middlebrook 7H9 broth medium is most commonly used for subculturing mycobacteria and preparing inocula for antimicrobial susceptibility and biochemical testing.

**Disadvantages**

- The growth of tubercle bacilli in the liquid media is difficult to follow, since it can’t be measured optically.
- Sample plating is not accurate because of severe clumping of the bacilli.

Mycobacterial growth observed on culture media should be quantified in some way. The following is the widely used scale which is recommended by the American Thoracic Society:

<table>
<thead>
<tr>
<th>Number of colonies</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>No colonies</td>
<td>-</td>
</tr>
<tr>
<td>Fewer than 50 colonies</td>
<td>-</td>
</tr>
<tr>
<td>50-100 colonies</td>
<td>-</td>
</tr>
<tr>
<td>100-200 colonies</td>
<td>-</td>
</tr>
<tr>
<td>200-500 colonies (almost confluent)</td>
<td>-</td>
</tr>
<tr>
<td>&gt;500 colonies (confluent)</td>
<td>-</td>
</tr>
</tbody>
</table>
**BACTEC culture**

In 1969, Deland and Wanger developed a technique for automated detection of the metabolism of bacteria by measuring the $^{14}$CO$_2$ liberated during decarboxylation of $^{14}$C-labelled substrates present in the medium. This technique has been applied successfully to blood culturing, detection of antibiotic effect on bacterial growth, *Nesseria spp.*, differentiation by substrate metabolism and serum assay of aminoglycoside antibiotics. Cummings and co-workers in 1975 carried out preliminary work that showed the same principle could be applied to detect growth of *Mycobacterium tuberculosis*. Middlebrook further developed the technique and introduced 7H12 liquid medium containing a $^{14}$C-labelled substrate specific for mycobacterial growth. He reported a significant time saving in the primary isolation of mycobacteria from clinical specimens using the new radiometric medium.

**Principle**

The BACTEC TB medium (12B) is an enriched Middlebrook 7H9 broth base. Mycobacteria utilize a $^{14}$C labelled substrate (fatty acid) present in the medium and release $^{14}$CO$_2$ into the atmosphere above the medium. When the vials are tested on the BACTEC 460 TB System instrument, the gas is aspirated from the vial and the 14CO$_2$ radioactivity is determined quantitatively in terms of numbers on a scale from 0 to 999. These numbers are designated as the Growth Index (GI). The GI numbers are displayed by the BACTEC 460TB System instrument and are also printed along with the identifying rack and bottle numbers (100 GI units are approximately equal to 0.025 μCi). The daily increase in the GI is directly proportional to the rate and amount of growth in the medium.

The BACTEC instrument also introduces fresh 5%-10% CO$_2$ into the medium head space every time a vial is tested. This enhances the growth of mycobacteria. The instrument automatically tests 60 vials at the rate of approximately one vial every 82 seconds and stops at the end of the run.
If an inhibitory agent is introduced into the medium, inhibition of metabolism is indicated by reduced production of $^{14}$CO$_2$ when compared to a control having no inhibitory agent. This basic principle is applied for drug susceptibility testing and in differentiating TB from other mycobacteria.

The BACTEC 460 TB System instrument must be used with a special TB hood when employed for mycobacteriology. The TB hood provides HEPA filtered exhaust air and negative pressure in the test area. In addition, the TB hood is equipped with an ultraviolet light source in the test area. The unit is designed for automatic testing of vials and must not be used for inoculation or sub culturing in place of biological cabinet.

*The development of BACTEC 460 TB system*

The development of 7H12 medium (BACTEC12A) led to several studies which reported excellent recovery of mycobacteria from sputum as well as extra-pulmonary specimens. These studies used an inoculum volume of 0.1 ml per 2.0 ml of medium. Subsequent studies showed that improved recovery occurred if 0.4 ml of medium per vial was inoculated with 0.5 ml of inoculums. A modified 7H12 was then introduced and designated as BACTEC 12B medium.

Since the BACTEC system utilizes a liquid medium, it is important to add an antimicrobial supplement to suppress growth of contaminating microorganisms which may survive decontamination process. Initially, a modification of Mitchison’s antimicrobial mixture which contained polymixin B, amphotericin B, carbenicillin and trimethoprim (PACT) was added to 12A medium. With increased inoculum size in 12B medium, it was found that the contamination rate higher. Siddiqi et al reported another antimicrobial mixture (PANTA) which suppressed contamination significantly better than PACT. PANTA contains polymixin B, amphotericin B, nalidixic acid, trimethoprim and Azlocillin (96).

Although BACTEC TB medium supports rapid growth of most mycobacteria, occasional strains of *Mycobacterium tuberculosis*, such as isolates from treated chronic
cases, may grow poorly. Recently, a growth promoting substance, polyoxyethylene stearate has been reported by Siddiqi et al when added to the BACTEC TB medium, this substance enhanced growth of those strains which grew slowly or poorly. This growth-promoting substance has been incorporated in the PANTA reconstituting fluid (RF), which should be used to reconstitute lyophilized PANTA supplement.

The increased volume of BACTEC TB medium (12B) with PANTA supplement was evaluated at three different sites. The following are the data comparing 12A, 12B, LJ and 7H10 or 7H11 media. A total of 2736 specimens were inoculated into each of the four media and the results have been analyzed as follows:

Total specimens : 2736
Total AFB culture positive : 219

Table 7: Tuberculosis positivity by different media used for growing tubercle bacilli

<table>
<thead>
<tr>
<th>Medium</th>
<th>Total isolates</th>
<th>% positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>12B</td>
<td>195</td>
<td>89</td>
</tr>
<tr>
<td>12A</td>
<td>178</td>
<td>81</td>
</tr>
<tr>
<td>7H10 or 7H11</td>
<td>151</td>
<td>69</td>
</tr>
<tr>
<td>LJ</td>
<td>134</td>
<td>61</td>
</tr>
</tbody>
</table>

The data indicate that 12B medium offer an improvement over 12A medium (Table 7). When 12B medium is used, the addition of LJ tube or a plate of 7H10 or 7H11 medium contributes 2%-3% in the total recovery of mycobacteria.

The BACTEC 460 TB system offers a simple automated technique with significant convenience and time saving. It also offers the opportunity to bring much needed standardization into TB bacteriology, allowing laboratory results to be compared throughout the country. The BACTEC 460 TB system offers techniques and media which are well established through numerous clinical trials and comparative studies.
Enzyme-linked immunosorbent assay (ELISA)

ELISA is assay based on the measurement of enzyme labeled antigen, hapten or antibody. Enzyme-labeled conjugates were first introduced in 1966 for localization of antigens in tissues, as an alternative to fluorescent conjugates. In 1971, enzyme labeled antigens and antibodies were developed as serological reagents for the assay of antibodies and antigens. Their versatility, sensitivity, simplicity, economy and absence of radiation hazard have made them the most widely used procedure in clinical serology. The availability of test kits and facility for automation has added to their popularity.

Anti mycobacterial antibodies are absent in healthy individuals. However, non-apparent or abortive infections due to mycobacteria are much more frequent than usually suspected. In particular, IgM antibodies are frequently observed after a contact inherent to professional occupations (e.g hospital personnel and social workers) or to adverse social conditions. A positive IgM test observed in the serum is most useful in latent pulmonary and extra pulmonary tuberculosis primary infection and prognosis of relapses.

Large amount of work has been carried out to establish the clinical validity of IgG test allowed the following conclusions to be drawn:

1. Healthy people are negative; even if they have a positive intradermal reaction and even if they live in a country with severe endemcity.

2. In patients suffering from a tuberculous infection, the test shows the presence of IgG antibodies if the patient has undergone an antigenic booster stimulus. The test will be positive mostly in cases of patent active infection.

3. In patients affected by extra pulmonary tuberculosis, the test will be effective according to the organ infected.

4. In 10% to 20% of the patients, the humoral immunologic activity is weak. Patients showing such anergy may appear negative.
The presence of IgG antibodies indicates a good immunological response of the patient to the infection.

The production of IgA antibodies is largely independent from the production of IgG antibodies. IgA antibodies easily form complexes with antigen and are at the origin of inflammatory processes in various organs. IgA antibodies are readily detected in serum of some apparently healthy individuals at risk, in the sputum of some patients suffering from pulmonary tuberculosis infection and in biological fluids of patients suffering from extra pulmonary tuberculosis.

**Polymerase chain reaction (PCR)**

The polymerase chain reaction (PCR) is a method for amplifying specific nucleic acid sequences by use of repeated cycles DNA synthesis (fig 11). The principle of the PCR is simple, requiring a three step process:

1. Denaturation of double stranded DNA
2. Annealing of primers
3. Primer extension

![Fig 11: Principle of Polymerase Chain Reaction](image-url)
This powerful technique offers several advantages over existing DNA technologies:

1. PCR can amplify specific DNA sequences from as few as 25 base pairs up to 10,000 bp in length using only the primer specified target sequence rather than the entire genome.

2. It is more sensitive than direct hybridization and requires only a single target DNA molecule that need not be highly purified.

3. It is fast, copying a single DNA sequence over a billion times within 3 hours.

The limitations of PCR should not be underestimated. False negative reactions can result from an inadequate number of primers and false positive reactions can result from the amplification of contaminating DNA.

Potentially, PCR represents a direct application of biomolecular research techniques from the bench to bedside and in the recognition of the impact and promise of this technique, its developer, Kary Mullis, was awarded the Nobel Prize in Chemistry in 1993 (97).

**PCR in tuberculosis**

More than a century ago, Robert Koch identified the etiologic agent of tuberculosis by staining and culturing it from clinical specimens. Today, the diagnosis of tuberculosis is usually established using staining and culturing techniques that do not differ substantially from those that Koch used. Microscopic and cultural techniques have been shown to be inadequate for diagnosing extrapulmonary tuberculosis in adults and all forms of tubercular infections in children. The most rapid method of identifying *Mycobacterium tuberculosis* to the species level in clinical specimens is PCR.

It has several advantages over the existing diagnostic techniques for mycobacterial infections. It is more sensitive than the direct smear examination (can detect 10 bacilli as against 10,000) and more rapid than culture (results are available in 3 days rather than 6 weeks). Samples from partially treated patients which were culture negative
could be detected by PCR. Its sensitivity is especially useful for samples containing very few mycobacteria such as the CSF. Its power can however, be its greatest weakness as even the smallest amount of contaminating DNA can be amplified, resulting in misleading results.

One early sequence identified in mycobacterium tuberculosis was the gene for the 65 kilo Dalton (kDa) antigen, a heat shock protein believed to be distinct from other bacterial shock proteins (14). However, subsequent work demonstrated that this gene although distinct from corresponding gene in other bacterial pathogens, is identical among all species of mycobacteria and is therefore unsuitable for detecting Mycobacterium tuberculosis particularly in areas in which other species such as Mycobacterium avium and Mycobacterium kansassi are prevalent. A more useful genetic marker (IS6110) has since been identified and is now in wide use, serving as basis not only for a great deal of research in diagnostic applications of PCR as it relates to Mycobacterium tuberculosis; but also as a marker to be used in molecular epidemiological investigations using restriction fragment length polymorphism (RFLP) analysis. This DNA sequence is called IS6110, after the insertion sequence it represents. However, there have been recent reports that isolates from some geographical areas like the Indian subcontinent contain less copies of this insertion sequence as compared with the eight to fifteen copies usually found in strains from most developed countries. Of 124 strains of Mycobacterium tuberculosis from South India, 53 (42.7%) showed single to no copies of IS6110. As the number of copies of the target sequence is an important determinant of PCR sensitivity, it would be lower for strains having only a few copies of IS6110. As India accounts for a large proportion of tuberculosis cases, it has become necessary to evaluate PCR protocols based on other genes of Mycobacterium tuberculosis in developing countries like ours.

In this study, we have amplified a portion of DNA which codes for a specific protein, MPB64 and is present only in members of the Mycobacterium tuberculosis complex i.e. Mycobacterium tuberculosis, Mycobacterium bovis and same strains of BCG. MPB64 is an immunogenic protein that has been cloned and characterized from Mycobacterium bovis.
A PCR assay has been reported for diagnosing *Mycobacterium tuberculosis* by targeting *hupB* gene. This gene codes for a histone-like protein which allows differentiation of two closely related species, namely *M. tuberculosis* and *M. bovis* of the MTB complex. The N and S primer-generated PCR amplicons differed in *M. tuberculosis* and *M. bovis*; these amplicons were determined to be 645 bp and 615 bp respectively.

**Reverse Transcription PCR (RT-PCR)**

The starting template for a PCR reaction can be DNA or RNA. DNA is usually the appropriate template for studying the genome of the cell or tissue (as in inherited genetic diseases, somatic mutation in a tumor, or somatic rearrangement in lymphocytes) and for the detection of DNA viruses. For information on gene expression in a cell or tissue or the presence of genomic RNA in a retrovirus such as HIV, RNA is the appropriate template. RNA can be better than genomic DNA for detecting structural changes in long genes, since amplifying the spliced RNA transcript instead of the genomic sequence greatly reduces the length of DNA to be handled without losing any of the coding regions where clinically significant deletions may be expected. RT-PCR combines cDNA synthesis from RNA templates with PCR to provide a rapid, sensitive method for analyzing gene expression. RT-PCR is used to detect or quantify the expression of mRNA, often from a small concentration of target RNA. The template for RT-PCR can be total RNA or poly (A) + selected RNA. RT reactions can be primed with random primers, oligo (dT), or a gene-specific primer (GSP) using a reverse transcriptase. RT-PCR can be carried out either in two-step or one-step formats. In two-step RT-PCR, each step is performed under optimal conditions. cDNA synthesis is performed first in RT buffer and one tenth of the reaction is removed for PCR. In one-step RT-PCR, reverse transcription and PCR take place sequentially in a single tube under conditions optimized for both RT and PCR.
Real-time PCR (qPCR) mRNA RT-PCR assay

Principle

A method for the detection and quantitation of an amplified PCR product based on incorporation of a fluorescent reporter dye; the fluorescent signal increases in direct proportion to the amount of PCR product produced and is monitored at each cycle (fig 12). Real time chemistries allow for the detection of PCR amplification during the early phases of the reaction i.e. measuring the kinetics of the reaction in the early phases of PCR. Real-time PCR has the ability to monitor the progress of the PCR as it occurs (i.e. in real time). Data is therefore collected throughout the PCR process, rather than at the end of the PCR. This completely revolutionizes the way one approaches PCR-based quantitation of DNA and RNA. In real-time PCR, reactions are characterized by the point in time during cycling when amplification of a target is first detected rather than the amount of target accumulated after a fixed number of cycles. The higher the starting copy number of the nucleic acid target, the sooner a significant increase in fluorescence is observed. In contrast, an endpoint assay (also called a “plate-read assay”) measures the amount of accumulated PCR product at the end of the PCR cycle.

Advantages over traditional PCR:

• Traditional PCR is measured at End-Point (plateau), while Real time PCR collects data in the exponential growth phase
• An increase in Reporter fluorescent signal is directly proportional to the number of amplicons generated
• Increase dynamic range of detection
• No-post PCR processing
• Detection is capable down to a 2-fold change
TaqMan chemistry (also known as ‘fluorogenic 5’ nuclease chemistry’):

Background

It uses a fluorogenic probe to enable the detection of a specific PCR product as it accumulates during PCR cycles. Initially, intercalator dyes were used to measure real-time PCR products. The primary disadvantage to these dyes is that they detect accumulation of both specific and nonspecific PCR products.

Development of TaqMan chemistry

Real-time systems for PCR were improved by the introduction of fluorogenic labelled probes that use the 5’ nuclease activity of Taq DNA polymerase. The availability of these fluorogenic probes enabled the development of a real-time method for detecting only specific amplification products. The development of fluorogenic labelled probes also made it possible to eliminate post-PCR processing for the analysis of probe degradation.

How TaqMan sequence detection chemistry works

The TaqMan chemistry uses a fluorogenic probe to enable the detection of a specific PCR product as it accumulates during PCR. Here’s how it works:

Step process

- An oligonucleotide probe is constructed containing a reporter fluorescent dye on the 5’ end and a quencher dye on the 3’ end. While the probe is intact, the proximity of the quencher dye greatly reduces the fluorescence emitted by the reporter dye by fluorescence resonance energy transfer (FRET) through space.
- If the target sequence is present, the probe anneals downstream from one of the primer sites and is cleaved by the 5’ nuclease activity of Taq DNA polymerase as this primer is extended.
- This cleavage of the probe:
• Separates the reporter dye from the quencher dye, increasing the reporter dye signal.
• Removes the probe from the target strand, allowing primer extension to continue to the end of the template strand. Thus, inclusion of the probe does not inhibit the overall PCR process.
• Additional reporter dye molecules are cleaved from their respective probes with each cycle, resulting in an increase in fluorescence intensity proportional to the amount of amplicon produced.

**Two types of TaqMan probes**

Applied Biosystems offers two types of TaqMan probes:

• TaqMan probes (with TAMRA dye as the quencher dye)
• TaqMan MGB probes

**TaqMan MGB probes contain:**

• A non-fluorescent quencher at the 3' end - The SDS instruments can measure the reporter dye contributions more precisely because the quencher does not fluoresce.
• A minor groove binder at the 3' end - The minor groove binder increases the melting temperature (Tm) of probes, allowing the use of shorter probes.

Consequently, the TaqMan MGB probes exhibit greater differences in Tm values between matched and mismatched probes, which provide more accurate allelic discrimination.

![Fig 12: Principle of Real-Time PCR assay](image)
*Advantages of TaqMan Chemistry*

- Specific hybridization between probe and target is required to generate fluorescent signal
- Probes can be labeled with different, distinguishable reporter dyes, which allows amplification of two distinct sequences in one reaction tube
- Post-PCR processing is eliminated, which reduces assay labor and material costs.

*Disadvantage of TaqMan Chemistry*

The primary disadvantage of the TaqMan chemistry is that the synthesis of different probes is required for different sequences.

*Studies about female genital tuberculosis*

Until now, traditional methods like direct smear microscopy and culture in Lowenstein- Jensen medium were used for diagnosis of female genital tuberculosis like pulmonary tuberculosis. But unlike pulmonary tuberculosis where the bacterial load in sputum is high, the bacterial load in endometrial tissue in cases of female genital tuberculosis is very less. So it is difficult to diagnose female genital tuberculosis by these conventional methods. Also, it is difficult to visualize acid fast bacilli in tissue biopsy samples. So, histopathology is not effective to diagnose this condition.

Studies are being done to evaluate newer methods like BACTEC TB system, PCR in diagnosing female genital tuberculosis. Any new method must be compared with previous standard method to evaluate its effectiveness.

Gupta *et al* in New Delhi analyzed clinical and laparoscopic findings of 40 infertile patients with genital tuberculosis (98). The findings are presented in table 8.

Bhanu *et al* studied 25 women aged 20-40 years presenting with infertility. They collected endometrial biopsy samples (EB), endometrial aspirate samples (EA) and pouch of Douglas fluid (15). Laparoscopy was performed on all patients. Patients were
classified according to laparoscopic findings as suggestive diagnosis of tuberculosis, probable diagnosis of tuberculosis, incidental findings and normal findings.

Results of various microbiological methods were as follows: positivity in direct smear 1.6%, growth on Lowenstein-Jensen medium 3.2%. Positivity in PCR were found to be 53.3%. All patients having suggestive diagnosis of tuberculosis on laparoscopic findings were found to be positive by PCR (100%). 60% of patients having probable diagnosis and 33.3% of patients showing incidental findings were positive by PCR. However one patient having normal findings was also positive indicating false positivity.

Abebe et al collected biopsy or curettage samples from 25 women suspected to have genital tuberculosis in Ethiopia. They found direct smear positivity in 1 patient, 3 were culture positive, 7 were histology positive and 12 were PCR positive. They concluded that combination of PCR with other available methods was found to be the best alternative to achieve sufficient sensitivity and specificity for the diagnosis of genital tuberculosis in women.

Weerakiet S et al in a study from Thailand showed incidence of genital tuberculosis in women to be 0.01% in outpatients and 0.05% in inpatients (66). A rising trend of incidence was observed. Chest x-ray in all patients was not suggestive. Sensitivity of direct smear microscopy was found to be 40% and that of PCR was 50%.

Table 8: Clinical and laparoscopic findings among infertile patients.

<table>
<thead>
<tr>
<th>Findings</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary infertility</td>
<td>30</td>
</tr>
<tr>
<td>Secondary infertility</td>
<td>10</td>
</tr>
<tr>
<td>Menorrhagia</td>
<td>9</td>
</tr>
<tr>
<td>Amenorrhea</td>
<td>7</td>
</tr>
<tr>
<td>H/O pulmonary tuberculosis</td>
<td>9</td>
</tr>
<tr>
<td>H/O abdominal tuberculosis</td>
<td>6</td>
</tr>
<tr>
<td>Positive Mantoux test</td>
<td>2</td>
</tr>
<tr>
<td>Peritubal and periovarian adhesions</td>
<td>18</td>
</tr>
<tr>
<td>Bowel adhesions</td>
<td>15</td>
</tr>
<tr>
<td>Positive culture on Lowenstein-Jensen medium</td>
<td>1</td>
</tr>
<tr>
<td>PCR positivity</td>
<td>9</td>
</tr>
</tbody>
</table>
Misra et al, in a study done on 40 women suspected to have genital tuberculosis in New Delhi reported evidence of tuberculosis on chest X-ray in 12.5% (99). Histopathology was negative in all patients. Five percent of patients had positive endometrial culture for *Mycobacterium tuberculosis*. ELISA was done for the detection of antibodies against A-60 antigen of *Mycobacterium tuberculosis*. IgM antibody was positive in 72.5% and IgG antibody was positive in 35% of patients. The results of the study emphasize the value of ELISA as a corroborative investigation for diagnosis of pelvic tuberculosis, especially in cases where culture is negative and there is evidence of tuberculosis on laparoscopy.

A study was conducted correlating conventional methods (direct smear microscopy, culture on Lowenstein-Jensen medium), BACTEC 460TB system and PCR (14). Each PCR test was found to have a much higher positivity than conventional methods and BACTEC ($p< 0.05$). Cheng V C et al found sensitivity of PCR to be 78.3% taking culture as the gold standard.

Some studies have also been carried out on RT-PCR-based detection of active genital tuberculosis. Rana et al conducted a study evaluating DNA and RNA as methods to differentiate between active and past infection (100). They found that RT-PCR could detect the same number of samples as culture. Thus, they found no superiority of RT-PCR over the conventional methods in the diagnosis of active genital tuberculosis.

Limited studies have been carried out on drug resistance pattern in case of genital tuberculosis. We thus carried out sequencing to check for the presence of any mutations associated with Rifampicin and Isoniazid, the drugs responsible for multidrug resistance in case of tuberculosis.