CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

Determination of Multiple Drug Resistance in Mycobacterium Tuberculosis

by

Almas Zahra

A thesis submitted in partial fulfillment for the degree of Master of Science

in the

Faculty of Health and Life Sciences
Department of Bioinformatics and Biosciences

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I Dedicate my thesis to Allah Almighty, Prophet Muhammad (SAWW), His Descendants and to my Family members

CERTIFICATE OF APPROVAL

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[MBS171009]

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Abstract

TB is a main cause of sickness and demise than any other infectious disease worldwide, mainly, due to its higher prevalence in developing countries. The eradication of the disease is tremendously complex due to dearth, overpopulation and human immunodeficiency virus (HIV) infection. *Mycobacterium Tuberculosis* can remain active several years in the tissues of a strong person. When M. tb causes disease it turns into tireless and extended progression giving sufficient time for transmission to vulnerable hosts. Tubercular disease can create infection in an individual following quite a while of torpidity. Consequently, the disease winds up across the board when an extensive extent of populace is infected. It can deliver episode or pestilence, when brought into a populace, of which, just a little bit of people are immunologically secured. In Europe and North America, the history of tuberculosis is better known for 150 years. Nonetheless, there is lack of noteworthy data of the study of disease transmission of tuberculosis in different parts of the world. An estimated number of 11 million prevalent cases (ranges from 11-13 million) of TB cases, equivalent to 159 per 100,000 individuals were observed for the year 2013. The causative agent of TB in humans was given its name *Mycobacterium Tuberculosis* in 1886. In this study, Samples were taken from TB suspects along with data i.e. location, gender, age, treatment history, sample type, HIV status, Disease Type from their guardian or next care taker at Provincial Tuberculosis Reference Lab Khyber Pakhtunkhwa (KPK) Pakistan. All the samples were subjected to further processing in wet lab CUST Processing and Culturing of Samples TB suspects samples were digested and decontaminated to recover the M. tuberculosis. A total of 385 suspected tuberculosis patients were involved in the examination study. Among the 385 cases, 53.24% (n=205) were males and 47.01% (n=181) were females in the age group from 1 year to 90 years. The study shows that the maximum numbers of patients were found to be in the age group 16-30. Among 385 M. Tuberculosis isolates, 66.23% were sensitive and 33.76% were resistant to INH; 72.98% were sensitive and 27.01% were resistant to RIF; 92.46% were sensitive and 7.79% were resistant to EMB; and 82.59% were sensitive and 17.40% were resistant to OFX. Multiple drug resistance was found to be 4.72% in untreated

TB patients and 6.80% in treated patients whereas remaining subjects showed resistance against two or more drugs. This scientific study confirmed that sex and age do not have any relation with drug resistance.

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Chapter 1

Introduction

Tuberculosis (TB) is a main reason of sickness and demise worldwide than other contagious diseases because of its higher incidence rates in the developing nations. Its elimination is quite difficult because of death, increase in population and infection through human immunodeficiency virus (HIV). *Mycobacterium Tuberculosis* has potential to retain its activity in the tissues of a strong person for a large period of time. The infections produced by *Mycobacterium Tuberculosis* changes into tireless and extended progression and transmit into host. Tubercular disease can create infection in an individual following quite a while of torpidity. Consequently, the disease winds up across the board when an extensive extent of populace is infected. It can deliver episode or pestilence, when brought into a populace, of which, just a little bit of people are immunologically secured. It was first emerged in North America and Europe about 150 years ago. Nonetheless, there is lack of noteworthy data of the study of disease transmission of tuberculosis in several countries worldwide [1].

After HIV, TB is characterized as the second important infectious disease leading to mortality [2]. According to surveys conducted in the year 2015-16, 10.4 million people are affected with TB, among them 6 lac showed resistance against the Refampicin drug, 4 lac 90 thousand people showed multidrug resistance [3]. However, a large portion of men represented TB cases and mortality, yet the heap of tubercular infection was also found to be elevated in females. In 2013, a rough amount

of 3.3 million cases and 5 lac 10 thousand mortality of women due to TB happened. Alongside this, evaluated number of 5 lac 50 thousand cases of TB among which 80,000 mortality cases were accounted for youngsters. World Health Organization (WHO)has declared in 1993 that TB is an overall general emergency. Since 1990, TB demise rates have reduced to 45% and in developed countries, its occurrence rates are increasing gradually. The surveyed cases of TB in 2013 in Asia were 56% and Africa were 29%, independently. Among the six severely affected countries in 2013, the India is at the top having 2.0-2.3 million, trailed by China with 0.9-1.1 million, then Nigeria having 0.34-0.88 million, Pakistan contains 0.37-0.65 million, Indonesia about 0.41-0.52 million and South Africa were about 0.41-0.52 million (Fig. 1.1).

The 2017 report demonstrates that TB death rate is decreasing at around 3% for every year and TB rate is also lessened at around 2% for each year however these figures are still quite large, what is required to meet the primary achievements to overcome TB in 2020. Out of the blue, the report has shown more deep analysis of TB, underlining the requirement for a wide-going methodology that comprehends and addresses HIV disease patterns, under nutrition and smoking [2].

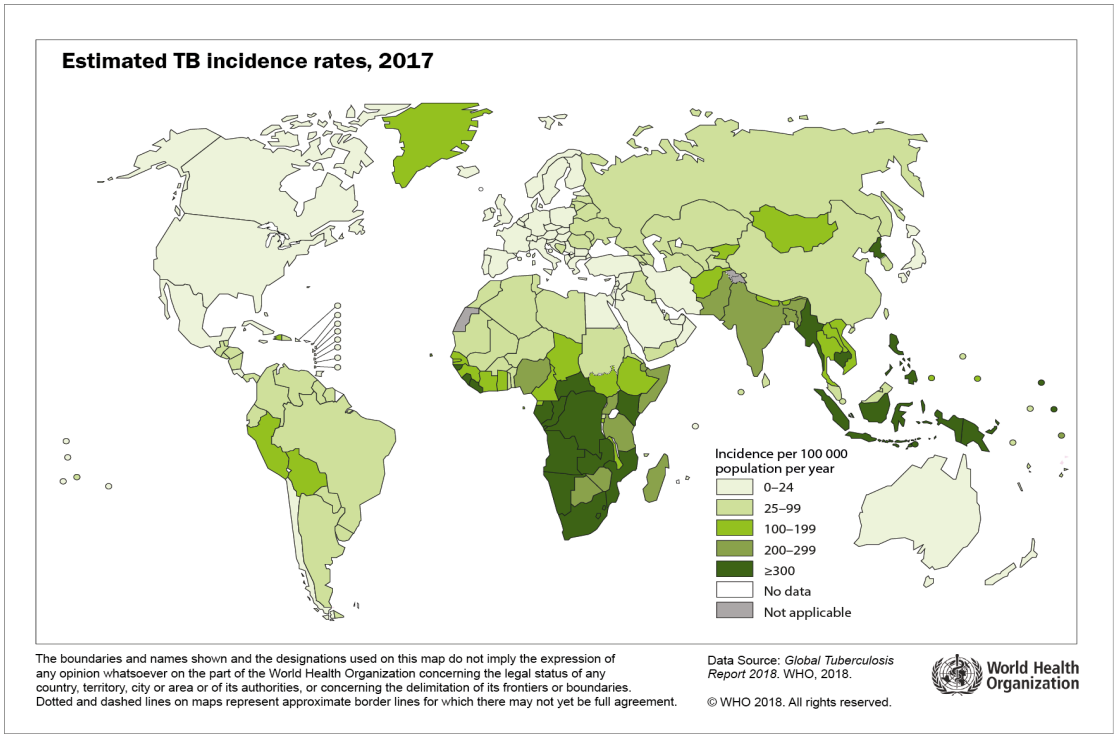

FIGURE 1.1: Estimated TB Incidence Rates in 2017 (WHO, 2017)

Tuberculosis (TB) is a main source of death around the world. Pakistan positions 6th comprehensively among the 22 high-TB trouble nations and contributes an expected 43 percent of the infection towards the Eastern Mediterranean district. Yearly around 430,000 individuals including 15,000 youngsters contract tuberculosis in Pakistan, and consistently no under 70,000 passings can be ascribed to the disease in the nation. Pakistan is likewise assessed to have the fourth most astounding pervasiveness of multi drug resistance tuberculosis (MDR-TB) all inclusive. More than 95 percent of tuberculosis deaths happen in low and middle income nations. Several reports on TB have present among different regions consisting different human advancements. In Vedas, Yakshma (wasting disease) is a name given to TB. In ancient literature of China and Arab, several TB symptoms are reported [3] Later on, in English literature, consumption word is also given to TB to depict the ailment. The name Tuberculosis was gotten from latin word tubercula (which means a little protuberance) [4]. Several names in the last 10 years have also be given to it, for example, Consumption, *Lupus vulgaris*, *King's Evil*, and *Phthisis* were assign to various types of tuberculosis.

TB happened among people for a huge number of years along with graphical discoveries from large amount of Neolithic destinations in Egypt, Europe, Roman, and Greek realms, which have appeared of an illness steady with present day TB [5]. The skeletal survives from ancient human go back to 8 thousand B.C., in Germany, indicates clear proof of the ailment. 25 thousand to 1 thousand B.C. old Egyptian skeletons have likewise uncovered the confirmations of Pott's infection of the spine. Possibly, the best proof of TB originated from an Inca mummy of a 8 year old kid lived around 700 A.D. Radiographic photo of the lumbar spine demonstrated sign of the Pott's sickness and smears of the sores uncovered corrosive quick bacilli, undoubtedly *Mycobacterium Tuberculosis* [6]. *Mycobacterium Tuberculosis*, is the responsible microbe for tuberculosis, wand first distinguished in 1882 by Robert Koch. It is an old irresistible illness with tremendous worldwide effect.

The causative agent of TB in humans was given its name *Mycobacterium Tuberculosis* in 1886 [7]. *M. tuberculosis* is sometimes referred to as the tubercle bacillus,

as the bacterium found in round nodules known as tubercles in the lungs of TB patients. *Mycobacteria* can be partitioned into a few noteworthy gatherings for motivation behind analysis and treatment: The *M. tuberculosis* complex (MTC) that can cause TB, *M. leprae* that causes infection; and *nontuberculous mycobacteria* (NTM) incorporates the various mycobacteria. *M. tuberculosis* is a non-motile bar molded bacterium with an especially moderate age time: 18-24 hours [8].

Mycobacteria have cell wall, which is thicker than most other microorganisms. It comprises of a peptidoglycan and polysaccharide layer, and contains complex unsaturated fats, for example, mycolic acids, which cause the waxy appearance and impermeability of the envelope. The three components alone make up 60% of the dry cell weight [9], whereof fatty acids comprise up to 30% [10]. The cell wall of mycobacteria impedes classical Gram staining [11], but instead these organisms are classified as acid-fast bacilli (AFB). Two noteworthy types of tuberculosis have been perceived according the infection sites, named as Pulmonary Tuberculosis (PTB) and Extra Pulmonary Tuberculosis (EPTB). Pneumonic TB is the major well-known ailment, occurs around 85 percent of all the cases reported to have TB [12].

Counting treatment disappointment and mortality, The administration of DR-TB is additionally intensified by the surprising expense, long term and debili Resistance to TB drugs is a raising worldwide wellbeing emergency. Second-line drugs that are currently available are toxic in nature. [13] Occurrence of TB is still quite large worldwide, with 10.4 million cases of treatment rules demonstrate institutionalized settled portion half year reglion occurrence cases and 1.5 million passings revealed by the WHO omens for new treatment strategies and existing one for TB in 2015. [14] *Mycobacterium Tuberculosis* (MTB) strains displaying(DS-TB). On account of DR-TB, the traditional 18– 24 month treat-in vitro protection from isoniazid and rifampicin represented 480000 episodic cases and 250000 mortalities in 2015. [14] XDR-TB ment routine has been updated and now extends somewhere in the range of 9 and 24months dependent on individual patient qualification, for example, pre-strains show extra protection from both the fluoroquinolones vious TB history and medication introduction. Notwithstanding span, mind boggling

and second-line injectable drugs have been accounted for to make sickness in 106 nations date.[14, 15] With high mortality multidrug regimens and ideal drug adherence[13, 14] are reported, XDR-TB represents a desperate danger to general wellbeing, exacerbated by required for successful treatment of TB contamination.

Challenges its dangerous association with the HIV/AIDS scourge of adherence are connected to complex dosing systems, genuine and Additional obresistance beyond XDR has been portrayed as absolutely frequently hazardous medication reactions, and drug– tranquilize cooperations. Medication resistant TB, which shows further protection from medications utilized.

Improvements in sequencing propose that present treatment choices for XDR-TB neglect to fix innovations and bioinformatics that empower customized treatment 30%– 75% of patients with XDR-TB, adds to a developing for DR-TB. General wellbeing emergency. [16, 17] New medications, for example, bedaquiline and delamanid, and repurposed medications, for example, linezolid, have been brought Implications for customized treatment into medication resistant TB (DR-TB) treatment regimens. In spite of the for DR-TB accessibility of new medications, restricted access to these operators or potentially the failure to develop a compelling routine containing no less than four The determination of DR-TB remains a test. Presently, the frontactive medications, add to progressing poor results in DR-TB, line atomic demonstrative test for the identification of Capheid (GeneXPERT) resistant medication, which distinguishes the nearness of MTB bacilli and all the while recognizes rifampicin resistance. [18] Whilst quickly distinguishing patients qualified for MDR-TB treatment, the test is constrained to the discovery of rifampicin restriction. Notwithstanding the GeneXpert case, the WHO supported the utilization of the tests used for Hain line [19].

Latest adaptations incorporate the MTBDRplus and second line injectable operators and enotype and MTBDRsl-v2.0 Genotype, which on the whole identify resistance from rifampicin,, isoniazid, fluoroquinolones [20, 21]. Whereas, investigation of distributed execution information of tests indicates problematic sensitivities [22, 23]. Thus, the most attractive stage to accomplish WGS pursue on

examining rifampicin-safe TB, which can quicken the inception of compelling handling. These components obviously underscore the dire need to distinguish quickly medication resistance and start customized treatment for each patient giving DR-TB to avert progressing of transmission of DR-TB and adequately control TB internationally.

1.1 Aims and Objectives

Multiple drug resistance is the major issue in treating tuberculosis. Aim of this study is to examine and describe the epidemiological risk factors for suspects harboring latent tuberculosis infection through sample-based screening. The objective of the study is

1. To detect risk factors for development of acquired multidrug resistant tuberculosis.
2. To determine the antibiotic resistance in samples of Human *Mycobacterium Tuberculosis*from the sputum.

Chapter 2

Literature Review

Tuberculosis (TB) is a main reason of sickness and demise worldwide than other contagious diseases because of its higher incidence rates in the developing nations. Its elimination is quite difficult due to dearth, increase in population and infection through human immunodeficiency virus (HIV). *Mycobacterium Tuberculosis* has potential to retain its activity in the tissues of a strong person for a large period of time. The infections produced by *Mycobacterium Tuberculosis* changes into tireless and extended progression and transmit into host. Tubercular disease can create infection in an individual following quite a while of torpidity. Consequently, the disease winds up across the board when an extensive extent of populace is infected. It can deliver episode or pestilence, when brought into a populace, of which, just a little bit of people are immunologically secured. It was first emerged in North America and Europe about 150 years ago. Nonetheless, there is lack of noteworthy data of the study of disease transmission of tuberculosis in several countries worldwide [1].

2.1 Tuberclosis

Tuberculosis (TB) is a main source of death around the world. Pakistan positions 6th comprehensively among the 22 high-TB trouble nations and contributes an 7

expected 43 percent of the infection towards the Eastern Mediterranean district. Yearly around 430,000 individuals including 15,000 youngsters contract tuberculosis in Pakistan, and consistently no under 70,000 passings can be ascribed to the disease in the nation. Pakistan is likewise assessed to have the fourth most astounding pervasiveness of multiple drug resistance tuberculosis (MDR-TB) all inclusive. More than 95 percent of tuberculosis deaths happen in low and middle income nations.

Inactive TB isn't dynamic, does not demonstrate any side effects and isn't irresistible, while, TB that is active in nature makes an individual ill and is profoundly irresistible. The contaminated individuals becomes immuno compromised for some, reasons incorporating lack of healthy sustenance or disease with HIV and the patients creates active TB ailment. In 90% of the cases, M. tuberculosis mostly taint the lungs and causes pneumonic tuberculosis. The patient shows various indications, for example, for 2-3 weeks cough, blood recolored sputum, chest torment, weight reduction, difficulty in breath, loss of hunger, nervousness, fever notwithstanding night perspiring [24].

2.2 Extra-Pulmonary Tuberculosis

Additional aspiratory tuberculosis happens in confinement or alongside pneumonic TB if there should arise an occurrence of scattered TB. Co-infection with HIV additionally changed the study of disease transmission and the EPTB in concentrate once more. EPTB constitutes about fifteen to twenty percent of all the cases in insusceptible people and comprises for over 50 percent of the cases in people having HIV contamination. Additional aspiratory tuberculosis basically, incorporates TB of lymph nodes, tuberculosis in cutaneous membranes, TB in genitourinary tract, pericardial tuberculosis, tuberculosis in joint and bones, radiation in pleural cavity, tuberculosis in larynx and tuberculosis of meningitis [3].

The host components demonstrate a noteworthy part in the movement of lively TB ailment. People, who sleep in open air, malnourished, ordinarily have weakness,

broad utilization of medications and liquor, are at more serious danger of catching disease. Immuno-smothered (tainted with HIV) and invulnerable patients, similar to patients with perpetual renal contamination, neoplastic illness and the people who are getting immunosuppressive treatment are moreover defenseless against TB. In human, tuberculosis is essentially brought about by *Mycobacterium tuberculosis*, yet different species, for example, *Mycobacterium Tuberculosis*, *M. microti*, *M. bovis*, *M. caprae*, *M. canettii*, and *M. pinnipedii* can likewise produce TB. These all things considered structure the *M. tuberculosis* complex (MTBC) [25].

The study of disease transmission of the illness has been more affected because of the presence of co-contamination with HIV and nearness of MDR-TB (multi-medicate safe tuberculosis) [26]. This all required the sub-atomic epidemiological examination of Mycobacterium tuberculosis segregates from various patients to separate among exogenous and endogenous diseases and furthermore to identify research center cross defilements. Besides, these facts were likewise utilized to decide the transmission connection and control procedures with the assistance of traditional the study of disease transmission [27].

2.3 Transmission

M. tuberculosis is entered through particles present in air, bead cores, of one to five microns in width and is diffused when an individual with dynamic TB of the throat or lungs hacks/sniffles. Individuals close-by might take in these microorganisms and wind up being infected (Fig. 2.1). The respiratory tract of individuals are contaminated and then the tubercle bacilli spread through the lymphatic framework and then through circulation system to various organs. The advancement of tubercular sickness is partitioned into essential aspiratory TB, inactive TB contamination and dynamic TB illness, comprising tuberculo-meningitis, scrofulous TB, skin TB, cordis TB, pleurisy TB, urinary fundamental TB, stomach related foundational TB, skeletal TB, and so on. Patients with dynamic TB infection can be dealt with and restored by chance that they seek after medicinal help. however,

people with inactive TB can take prescription so they won't suffer from dynamic TB illness [1].

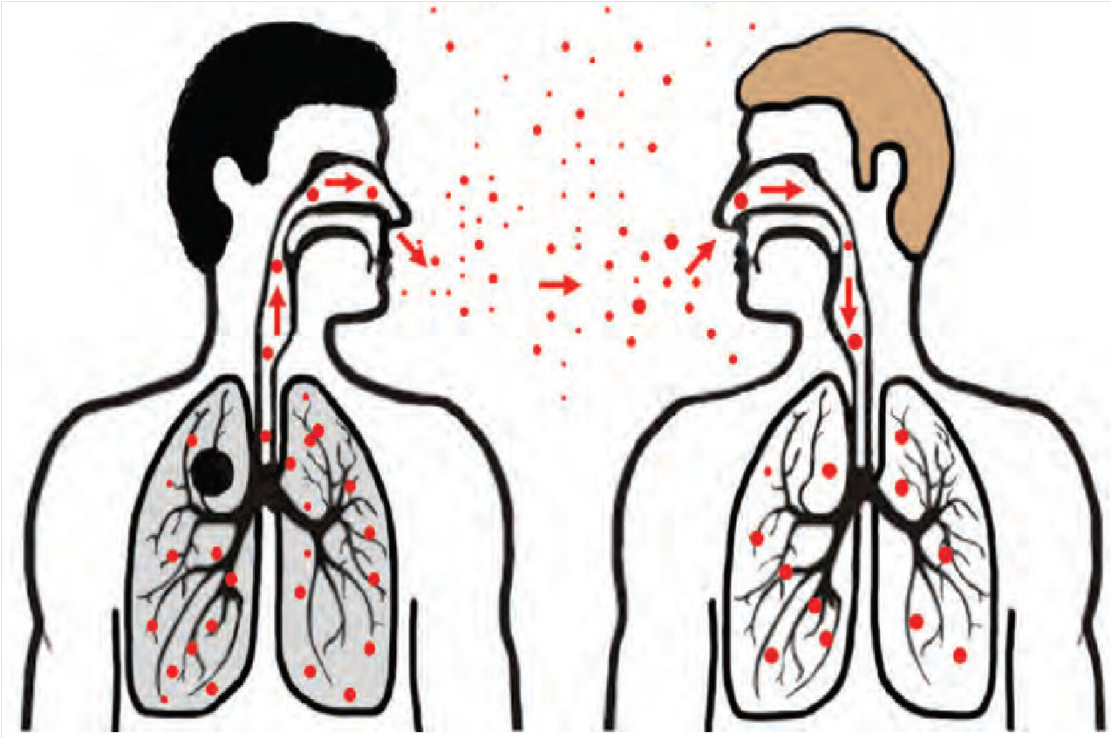

FIGURE 2.1: Transmission Procedure of *Tubercle Bacillus* (Paulson, 2013)

2.4 Mode of Transmission

Tuberculosis mode of transmission in human is based on 4 stages, (i) transmission procedure of bacilli, (ii) reaction of immune system, (iii) reaction time and (iv) disease initiation [28] (Fig. 2.1).

2.5 Transmission

People having tuberculosis malady suffer from several symptoms, similar to coughing, which pushes the microorganisms outside body, where these microbes can be breathed in by others. In this manner, TB ought to be analyzed and treated as quickly as time permits to gather the individual noninfectious and stay away from the scattering of the ailment.

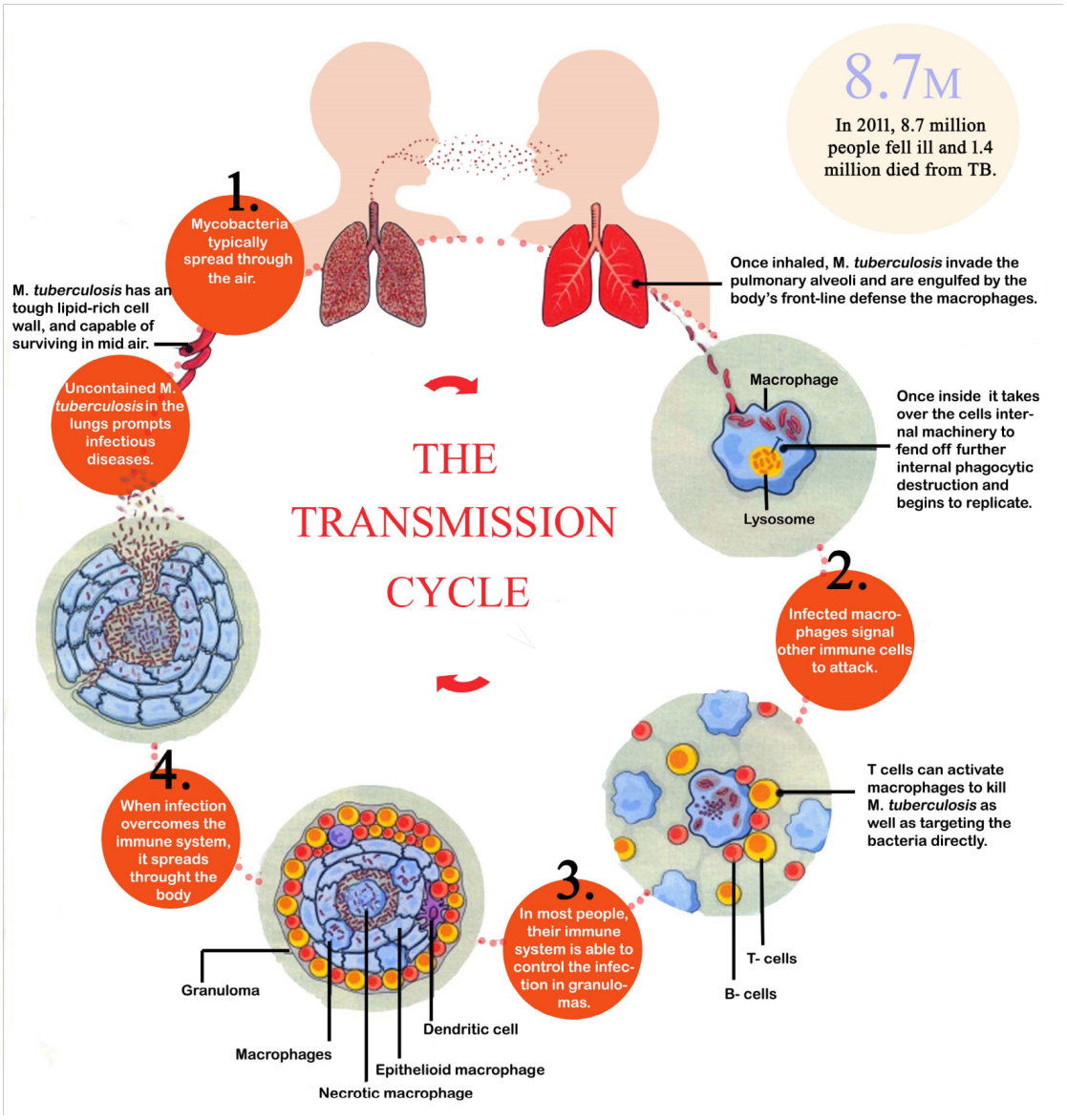

FIGURE 2.2: Transmission Cycle of*Mycobacterium Tuberculosis* (Paulson, 2013)

2.6 Response of Immune System

The BCG (Bacillus Calmette Guerin) antibody is commonly used vaccine over the world, yet is about 80 percent successful to shield youthful sound youngsters from extreme type of TB meningitis (which influences the cerebrum) and miliary tuberculosis (a deliberate contamination). BCG immunization likewise offers slight insurance to adolescents and youthful grown-ups. The cure of tuberculous illness

won't be conceivable without an enhanced antibody. Director of South African Tuberculosis Vaccine Initiative (SATVI), Willem Hanekom, said, on account of the confusion of TB treatment methods and advancement of medication resistance, it is certain that by a long shot the most appropriate intervention would be that you have an immunization that keeps the disease away [29].

2.7 Latency

A natural chemist, David Sherman, a scientist at Biomedical Research Institute, Seattle, Washington, he declares that latency is anything but a positive organic issue. Inactive TB is where tests of individuals are positive for TB and have no clinical indications. It is thought that inactivity may contain a range of states, people free from the malady, to the untreated one, sub-clinical illness. *M. tuberculosis* can be contained inside granuloma for a considerable length of time. This capacity of *M. tuberculosis* to lie lethargic might be a transformative technique [30].

2.8 Activation

The replication of microorganisms beat the insusceptible framework and granuloma separates, discharge *M. tuberculosis* into the lungs and produce an ailment, if the invulnerable ndividual debilitates HIV disease happens [28].

Different elements, which decide the *M. tuberculosis* chance of transmission are: 1) vulnerability of the individual 2) irresistibleness of an individual with TB due to the quantity of *tubercle bacilli* that the person expose into the air) 3) condition (natural facts that influence the grouping of *M. tuberculosis*, similar to grouping of irresistible bead cores, space, ventilation, air course, and so forth.) and 4) introduction (nearness, recurrence, and span of presentation) [1].

Aspiratory TB is the main disease with *M. tuberculosis*, generally, when emerges in more established newborn children and kids, hints at no manifestations. Indeed, x-rays of chest additionally does not hint at any disease. There might be development of lymph nodes and industrious hack. In such cases a test known as tuberculin skin test (TST) might be certain and shows the disease in youngster. All the regularly, the essential contamination settle at its own when a kid creates resistance over a 6 to 10-week duration however in few cases spread to lungs (called dynamic TB) or to different organs. This produces manifestations, similar to fever, hack, weight reduction, exhaustion and loss of craving [31]. Second kind of contamination is called reactivation TB. The essential disease has been settled, however microscopic organisms are in inert or torpid stage. As the immunity of patient decreases, *M. tuberculosis* is again ends up being active, it causes illness and structure depressions in lungs. Most recognizable side effects of reactivation TB, incorporates relentless fever, night sweats, weariness, weight reduction, creation of bodily fluid, coughing, spit, or mucus, which may contain blood [31].

2.9 Pathologic Process of Tuberculosis

The pathologic process of TB incorporates a number of fights between the pathogen-host. They have their very own systems to utilize against one another. Both the pathogen and host have destinations for weakness. The host system enacted macrophages to execute or restrain the microbe that are gulped and capacity of diminishing the bacillus replication intracellularly in non-incited macrophages by devastating the macrophages and transmuting a beneficial intracellular condition into the inhibitory state of a solid caseous tissue. The essential thought of bacilli is to copy logarithmically inside non-started macrophages (inside monocytes that movement from circulatory system into tissues at the site of disease) just as to multiply extracellular, regularly coming to striking sums in melted significant, for the most part inside the cavities [32].

The alveolar macrophages are diverse in their ability to kill *tubercle bacilli* [33]. A portion of the alveolar macrophages have abundant of chemicals and microbicidins, whereas remaining ones are deficient in both. This proportion is dictated by hereditary restriction of every individual just as phenotypic components. In people, aspiratory disease begins just when the solid competent bacilli are gulped by powerless alveolar macrophages. The normal number of breathed in tubercle bacilli required to build up an essential pneumonic infection is as yet not known, however most likely, may extend from 5 to 200 bacilli [32]. Approximately, pulmonary tuberculosis has five stages [34].

Stage I: It consists of no bacillary improvement. They are commonly smothered by alveolar macrophages. In any case, if the bacillus isn't executed, it expands its number and alveolar macrophages permitting such increment are at long last slaughtered.

Stage II: It is additionally called advantageous stage because the bacilli create or copy inside non started macrophages making damage, known as tubercle shown in Fig. 1.3. These non-enacted macrophages at that point enter the tubercle from principle circulation system and are refer to as monocytes. It is known as harmonious, in light of the fact that *M. tuberculosis* can develop without making hurt the two macrophages and host can store [33].

Stage III: Here, caseous putrefaction forms and the amount of bacilli turns non motile. The improvement of *M. tuberculosis* is quelled by safe response to tuberculin, like bacilli released antigens [35]. At this stage, the safe reaction is primarily tissue hurting, conceded sort extraordinary delicateness, which butchers the bacilli stacked macrophages of amicable stage. At the sore, which constitutes a soild caseous focus, bacilli don't duplicate in view of being encompassed by both nonactivated macrophages (which give intracellular enlargement of *M. tuberculosis*) similarly as fairly incited macrophages and adolescent epitheloid cells made by cell mediated invulnerability as shown in Fig. 2.3.

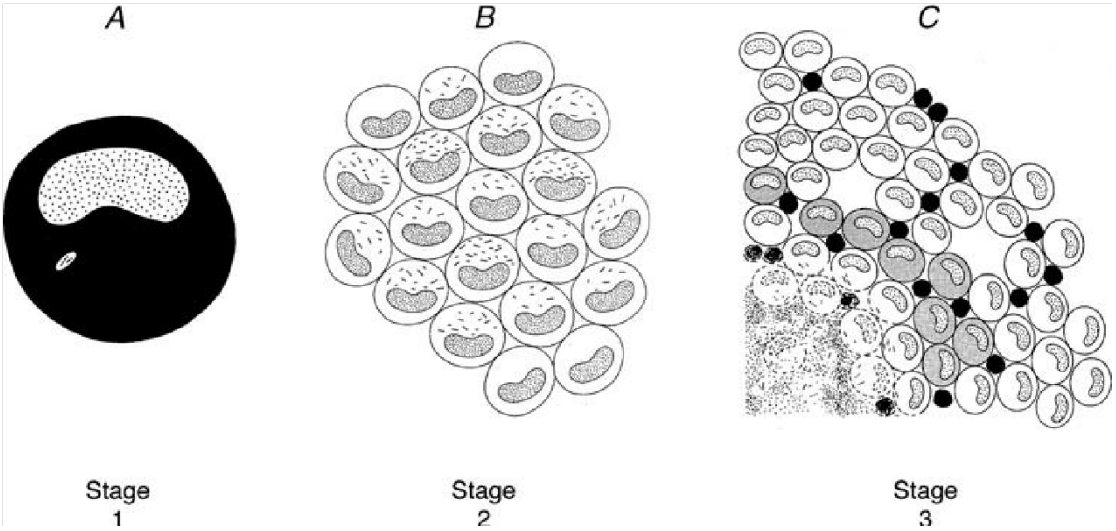

FIGURE 2.3: Pathological Process of Tuberculosis, A, Stage 1: An alveolar Macrophage. B, Stage 2: Symbiotic Stage. C, Stage 3: A tubercle of 3 Weeks Age (Source: Manabe & Dannenberg, 2006)

Stage IV: Here the cell mediated immunity performs crucial function to know regardless the infection turns out to be clinically self-evident. On the off chance that the cell intervened insusceptibility is inadequately created, *M. tuberculosis* could not identified at the caseous putrefaction, duplicate again in non-actuated macrophages and somewhat enacted macrophages. The cytotoxic DTH resistant reaction again destroy these macrophages, causing development of the caseous focus and movement of disease (Fig. 1.4 A). On the off chance that a decent cell interceded invulnerable reaction is produced, a mantle of exceptionally enacted macrophages encompasses the caseous corruption (Fig. 1.4 B). These enacted macrophages ingest and pulverize/restrain the development of *M. tuberculosis*, frequently by capturing the advancement of sore at a sub clinical stage.

Stage V: It is phase change stage, in which the bacilli elude the host's defense mechanisms shown in Fig. 1.5. At the point when phase change of the caseous area happens, the bacilli increase extracellularly to a colossal number. Indeed, even all around created cell interceded immune reaction is absolutely ineffectual to operate at this stage. The high grouping of tuberculin like items discharged by bacilli itself produces a DTH reaction that damages the tissues, consumes the bronchial divider and structures a pit. The bacilli at that point enter the bronchial tree, spread to

different areas of the lungs and furthermore to the outside condition, for the most part during cough. Capture of the infection at this stage relies upon whether the antigenic heap of both the bacilli and their items stays little enough to control by the host.

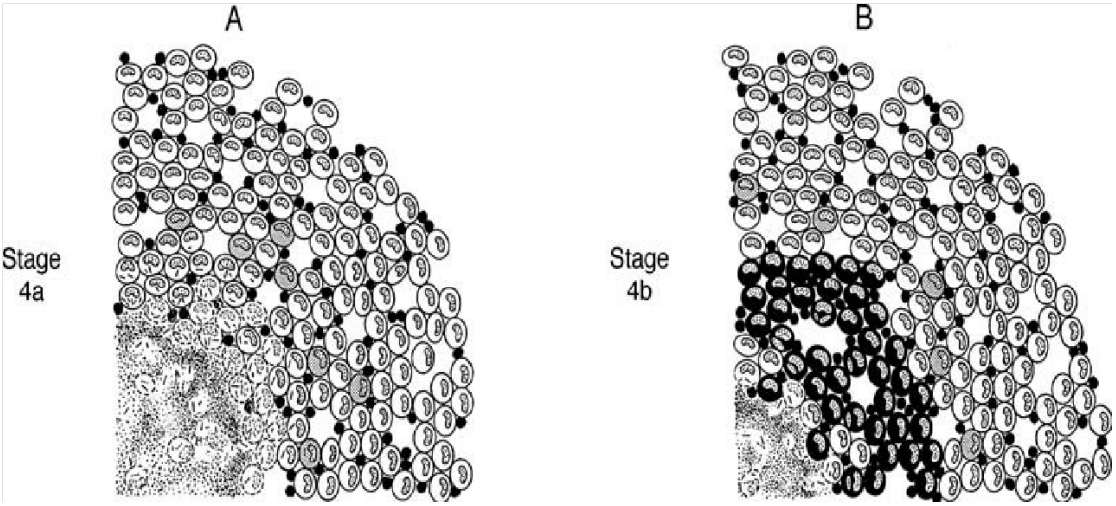

FIGURE 2.4: Pathological Process of Tuberculosis, A, Stage 4a and Stage 4b by (Manabe & Dannenberg, 2006)

The bacterial replication conquer the resistant framework and granuloma separates, discharge *M. tuberculosis* into lungs and cause sickness, if the insusceptible arrangement of an individual debilitates as an individual ages or HIV contamination happens [28].

Different elements, which decide the chance of transmittance of *M. tuberculosis* are: 1) vulnerability (immune status) of the individual, 2) (irresistibleness of an individual with TB is straight route connected to the quantity of tubercle bacilli that the person expose into the air) 3) condition (natural factors that influence the grouping of *M. tuberculosis*, similar to grouping of irresistible bead cores, space, ventilation, air course, and so forth.) and 4) introduction (nearness, recurrence, and span of presentation) [1].

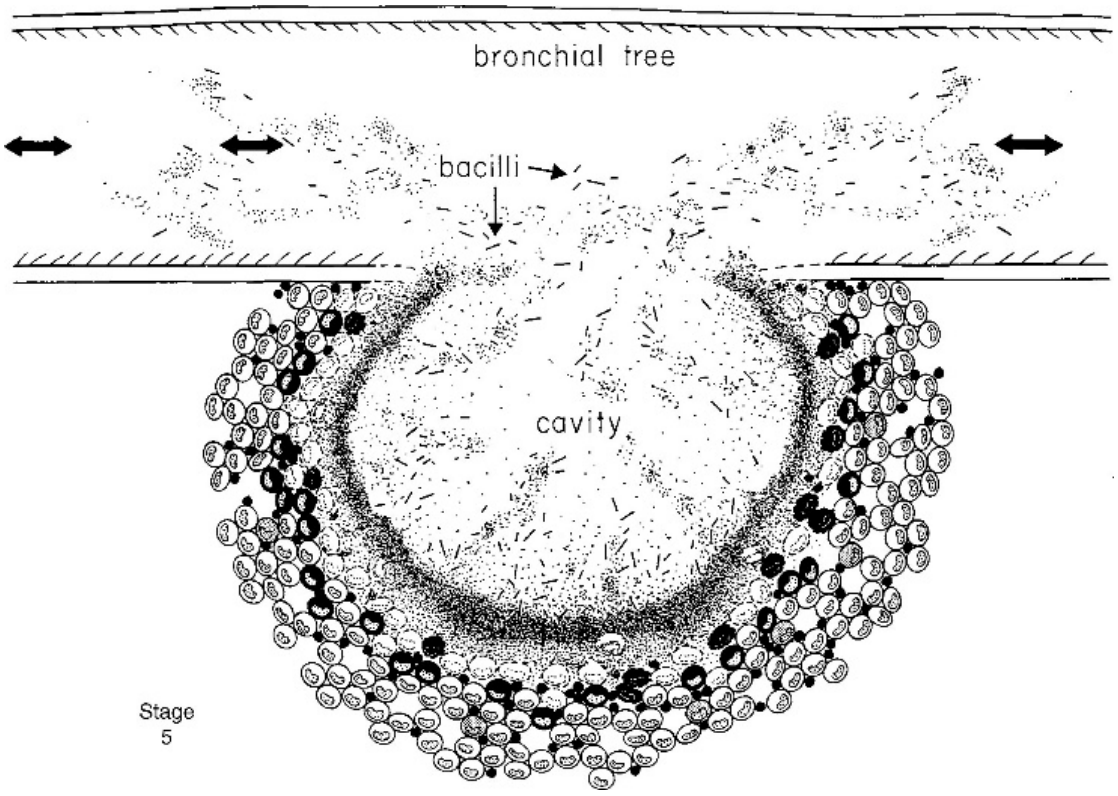

FIGURE 2.5: Pathological Process of Tuberculosis, Stage 5: Phase Change Stage (Manabe & Dannenberg, 2006)

2.10 Latent Tuberculosis

Inactive TB contamination happens when *M. tuberculosis* flee from the insusceptible framework. Inert TB disease is commonly not transmittable and creates no side effects, yet the bacilli might be available in the body. individuals who have inactive disease can't transmit contamination to other people. In this contamination, a large portion of the general population don't create dynamic sickness, yet the issue happens when dormant disease winds up dynamic. Roughly TB infection create in 10% of the general population effectively . Some of them create dynamic malady not long after the contamination, while, other individuals grow later, when their resistant framework ends up frail for either reason. People with dynamic sickness can spread contamination to other individuals. The brooding time frame (time taken for an individual to move toward becoming tainted subsequent to being uncovered) changes from weeks to months to quite a while. Alongside the aspiratory

contamination, TB may influence other organ frameworks likewise, for example, larynges, regenerative organ tract,central sensory system, musculoskeletal framework, gastric and intestinal tract, and skin (Fig. 1.6)[1].

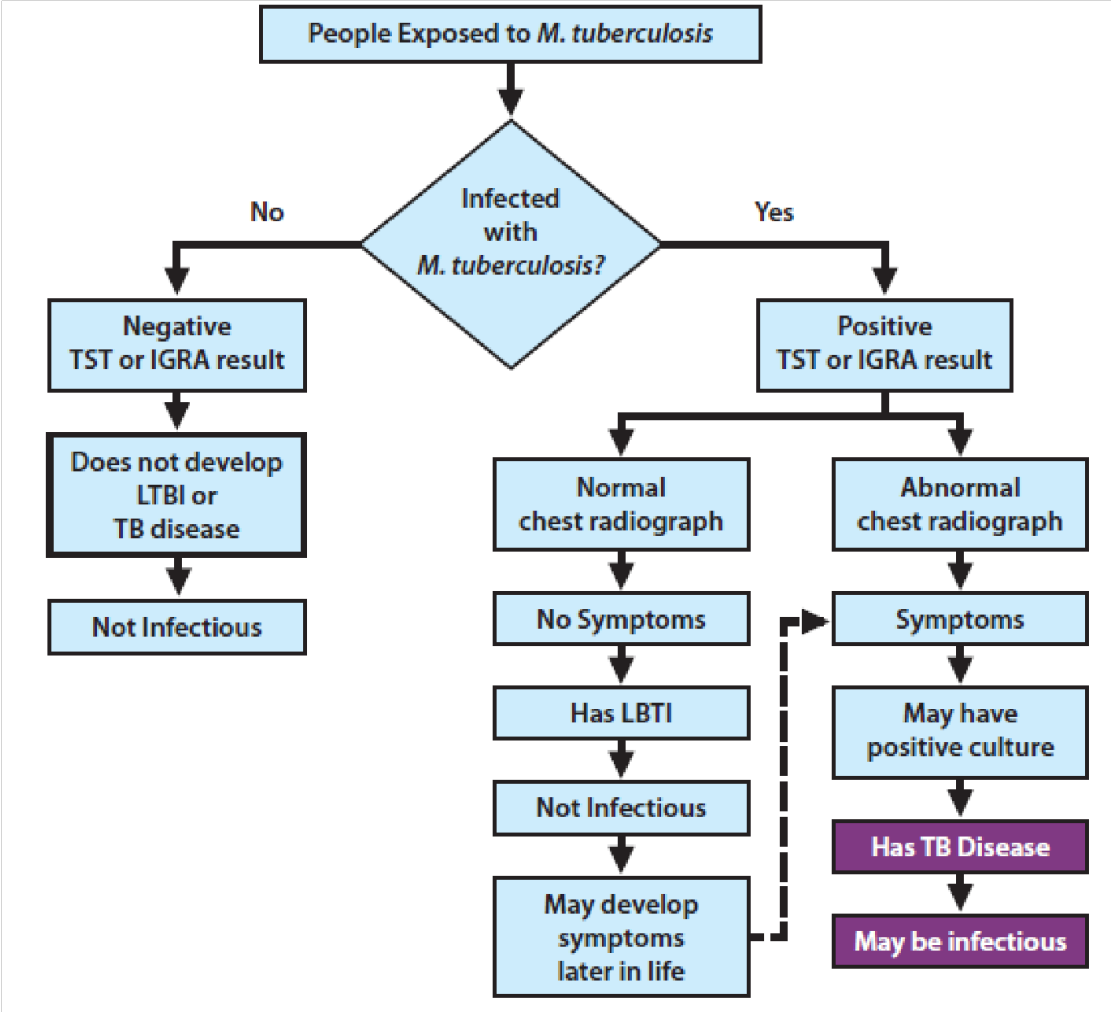

FIGURE 2.6: Movement of TB (People Who are Presented to *M. tuberculosis* might possibly create dormant TB disease. Individuals with Inactive TB Contamination might possibly create TB

Different elements, which decide the chance of transmittance of *M. tuberculosis* are: 1) vulnerability (immune status) of the individual 2) (irresistibleness of an individual with TB is straight route connected to the quantity of tubercle bacilli that the person expose into the air) 3) condition (natural factors that influence the grouping of *M. tuberculosis*, similar to grouping of irresistible bead cores, space, ventilation, air course, and so forth.) and 4) introduction (nearness, recurrence, and span of presentation) [1].

2.11 First-Line Drugs Resistance Mechanisms

2.11.1 Isoniazid

A prodrug Isoniazid enacted by the protein catalase and the gene called katG encodes it. When initiated, the isoniazid drug prevents corrosive blend of mycolic through a NADH-subordinate enoyl-acyl bearer reductase protein, and inhA gene encodes it. [36] The premise of isoniazid resistance regarding their molecular structures, are refereed as the transformations that occurs in inhA gene, katG gene or inside the locale of inhA gene. The most well-known mechanism of resistant has been distinguished as a katG S315T change, which prompts a wasteful isoniazid– NAD item restraining the antimicrobial activity of isoniazid. However, this mechanism is related with the abnormal state of isoniazid resistance in the MDR disconnects. [37 -40].

Mutations present in the inhA gene area, among them the most widely recognized mutations was obtained at the fifteen position, which results in inhA gene over expression. This component is related with the least resistance going on in the isoniazid mono-safe disconnects and also engaged with cross protection from a very basic and simple, ethionamide. Transformations taking place inside the dynamic locale of inhA gene, presents the outcome in the form of diminished partiality of an isoniazid– NAD item. Such transformations are much less continuous. [40, 41] An ongoing report revealed that transformations in the inhA administrative area and coding district brought about abnormal state isoniazid resitance and cross-protection from ethionamide. [42] dfrA gene mutations have as of late been related in resistance from the isoniazid drug. The isoniazid 4R isomer– NADH item represses the di-hydro folate reductase, which is produced by the gene dfrA. In any case, examines have neglected to set up a connection between changes in the dfrA gene and resistance from isoniazid. (43) Mutations in the ahpC gene were anticipated to be intermediary isoniazid resistant marker.

Alkyl hydroperoxidase reductase is also produced by ahpC, chemical in charge of protection from a responsive nitrogen and oxygen subordinates. Further investigation of these such changes uncovered that this is compensatory component for the decrease or loss of movement in the framework of catalase– peroxidase and it does not present any isoniazid resistance. [44] Studies have likewise revealed changes in the oxyR-ahpC, kasA, and furA-katG in the isoniazid-resistant separates of MTB. Notwithstanding, but their definite job in intervening the resistance to isoniazid is still needed to illustrate. [45, 46] More as of late, a quiet change in the gene inhA bringing about the up regulation of inhA brought about isoniazid obstruction. [47] An ongoing methodical audit found out that transformations in the inhA gene and katG gene represented the 19.2% and 64.2% of isoniazid resistance, separately. These two transformations, in a mix with the usually happening changes in the inhA gene and the ahpC-oxyR gene, represents the 84% of worldwide phenotypic resistance to isoniazid [48].

Recent WGS (World genome Sequencing) investigation indicated overpowering proof of that the isoniazid resistance goes long before the rifampicin resistance, and it is related with the transformations of katG S315T. These revelations makes this transformation a perfect marker for the phenotype of the pre-MDR. [28] Globally, the event frequency of the isoniazid mono-resistance is assessed to be around 2%–15%, and this is adapted by and refreshed from Zhang et al.; every single other source referenced in content. Related with more awful results. [49 - 57] This underscores the need to identify resistance in its most punctual structure.

2.11.2 Rifampicin

Rifampicin is a standout amongst the best enemy of TB drugs since it is viable against effectively processing and moderate utilizing bacilli, assembling the medication, a key segment of presently available first-line treatment routine connected to the DS-TB treatment. [58, 59] Resistance to rifampicin occurs due to rpoB gene , which is the objective of current mlecular tests and records for 96% of rifampicin

resistacne. Codons 526 and 531 harbor the most well-known transformations related with rifampicin resistance. [35, 36, 60, 63] However, the mutations outside the rifampicin deciding district requires to be accounted for in rifampicinresistant detaches [64].

Studies have additionally exhibited an absence of modification in the gene rpoB in a small amount of rifampicin-safe confines, proposing different instruments of rifampicin resistacne. [64] Rifampicin mono-resistance is uncommon because a rifampicin resistance happens related to protection from different medications, most ordinarily isoniazid, designing rifampicin to focuses on a phenotype of MDR markers. [65] Nonetheless, rpoB mutations have been related with the cross-protection from all the rifamycin anti-infection agents. Fundamentally, a cross-resistance among the drugs like rifabutin and rifampicin has been accounted for and they are credited to the transformations occurring inside the hotspot district, early areas of rpoB gene and twofold changes in the codons 516 and 529. But the WGS examination showed changes in the gene rpoA and gene rpoC, which encrypts the subunits of b0 RNAdi polymerase as necessary instruments in disconnects that bear transformations in the gene rpoB. These transformations relates to the expanded wellness and transmissibility of strains that are resistant. [66] Recently, a marvel of the rifampicin-subordinate/ - improved strains has been developed. Such strains have been accounted for to develop ineffectively in a typical media culture that needs the rifampicin. [67, 68].

2.11.3 Ethambutol

In 1966, the drug ethambutol was first presented as the enemy of TB medicate and still remains to be a piece of the present first-line routine. Drug ethambutol is much dynamic against the effectively increasing bacilli, by upsetting the arabinogalactan biosynthesis in divider area of cell. The mycobacterial arabinosyl transferase catalyst is encoded by embCAB operon. Resistant from ethambutol is intervened by means of transformations in the gene embB [69, 70]. Modification in the 306 codon of embB gene is widely recognized resistant instrument answered

up till now [71, 72]. It was additionally detailed that this change inclines the isolate to create resistance from different medications and isn't really engaged with ethambutol resistance. [73] Allelic trade tests have exhibited that just certain amino corrosive substitutions prompted ethambutol resistance [74].

The studies have demonstrated that changes in decaprenylphosphoryl-b-D-arabinose biosynthetic of Rv3806c and Rv379 gene and their pathway, which happen at the same time with transformations in embB and C, resulting in a varying MIC go for ethambutol. This depends upon the sort of progress that is available. Also, this recommends the embB306 change achieves moving degrees of ethambutol opposition in any case, does not start anomalous state ethambutol obstruction in solitude [75]. Whereas around 30% of ethambutol-safe separates need adjustment in embB, suggesting a substitute arrangement of opposition. Included substance changes occurring in ubiA quality have been represented to cause unusual state ethambutol opposition while they occur with the embB changes. The quality ubiA encodes for the decaprenyl-phosphate 5-phosphoribosyltransferase synthase, which is related with the cell divider component. Change in quality ubiA is represented to be lineage express, and is pervasive in the African disengages [75, 76].

2.11.4 Streptomycin

In 1942, a drug called Streptomycin, which is an aminocyclitol anti-toxin, was used as the main medication to be connected to the treatment of TB. Attributable to underlying usage of the medication as a TB monotherapy, but its resistance quickly emerged.89 Streptomycin is aggressively dynamic against the moderate developing bacilli and proceeds by ir-reversibly official to S12 and 16S ribosomal rRNA proteins; segments of the subunit bacterial 30s ribosomal subunit. Using this communication, streptomycin squares interpretation along these lines repressing protein mixture [77, 78].

The principle instrument of resistance from streptomycin is accepted to be interceded by means of transformations in the two genes; rpsL and rrs, S12 protein

of ribosome and the 16S rRNA, are produced by rpsl and rrs genes separately, representing 60%– 70% of streptomycin resistance [79]. Lately, the transformations in gidB gene, synthesize a 7methylguanosine methyltransferase explicit for the methylation of G527 in the circle of 16S rRNA, are engaged in the low dimension of streptomycin resistance. [80-82] Whole-genome investigation has likewisely exhibited 130bp cancellation inside the gidB perhaps interceding streptomycin resistance [83].

2.12 Second-Line Drugs Resistance Mechanisms

2.12.1 Second-line Injectable Agents

The cyclic polypeptide capreomycin, kanamycin, aminoglycosides, and amikacin are known as second-line injectable specialists as of now connected to the treatment of medication resistant TB. In spite of the fact that these have a place with various classes of anti-infection agents, they all apply their impact by means of a similar targets. [23, 24]. All of these are protein blend inhibitor molecules that give demonstration as authoritative to the bacterial ribosome, bringing about an adjustment of the structure of 16S rRNA. Resistance of abnormal state is related with transformations in the 1400bp district of the rrs gene and extra resistnace from capreomycin is known to be related with the tlyA gene polymorphisms.

This gene encodes rRNA methyltransferase which is necessary for 20-O-methylation of ribose in rRNA. [80, 84] The A– G polymorphism occured at position 1401 of the rrs is the most well-known system of resistance from every one of the three medications and is related with 70 - 80 percent of resistance caused by capreomycin and amikacin and 60% of kanamycin resistnace, all around. [85] An ongoing report detailed expanded wellness in clinical secludes holding the rrs A1401G change. This was exhibited by the distinction in MIC between the research facility built strains and clinical detaches with a similar transformation. This expanded wellness is

thought to happen because of the nearness of compensatory transformations that reestablish bacterial wellness [86].

Cross-resistance among amikacin, kanamycin, and capreomycin has additionally been accounted for. Every one of the medications demonstrations by hindering interpretation and in this way cross-resistance between them is probably going to happen. Full cross-resistnace among several medicines was at first accepted; be that as it may, different investigations have shown dissonant resitance designs between these two operators. [87] It has likewise been accounted for that resistacne against capreomycin changes as per the dimension of protection from kanamycin, and abnormal state protection from kanamycin was related with cross-protection from capreomycin [88].

More as of late, transformations in the advertiser locale of these genes have been accounted for to result in low-level resitacne from kanamycin. Aminoglycoside acetyltransferase protein is produced bt the same gene. At 10 and 35 positions of this gene polymorphisms brought about protein over expression item and reduced resistance of kanamycin. An examination detailed that 80% of the clinical detaches with low-level protection from kanamycin had hereditary changes in this gene [89, 90].

2.12.2 Fluoroquinolones

Fluoroquinolones are intense bactericidal anti-toxins as of now utilized as a treatment option as second-line drug resistant TB. ofloxacin and Ciprofloxacin speak to a more established age of anti-infection agents that are subordinates of nalidixic acid [91]. New age fluoroquinolones, for example, moxifloxacin and gatifloxacin, are presently used in for DR-TB. 3 These anti-toxins focuses on the DNA gyrase catalyst, in this way anticipating translation amid cell replication. The gyrA and gyrB gene encodes for DNA gyrases.

The fluoroquinolones resistance is connected to transformations happening in a preserved locale refer to as the resistance against quinolone in the gyrA/B gene

[92 to 94]. Fluoroquinolones are anti-toxins that are generally used to treat an assortment of ailments, or example, respiratory and urinary tract contaminations. counting T b yet motivations resstnace. Obstruction in TB to fluoroquinolones may happen immediately or might be procured, particularly when these specialists are utilized improperly. Cross-opposition among the fluoroquinolones has been appeared in TB.

The fluoroquinolones offer an ideal pharmacokinetic profile for the treatment of TB. Most show astounding oral bioavailability and accomplish greatest (top) serum focuses well over the MIC. They are additionally appropriated broadly, including intracellularly. The fluoroquinolones are cleared renally and additionally hepatically, with fluctuating serum half-lives. Pulmonary MDR-TB in Rawalpindi, Pakistan, is normal in youthful guys, destitution related conditions, and has poor result. DST indicates high protection from first-line hostile to tuberculosis specialists and quinolones. Rise and spread of multidrug-safe (MDR) and broadly medicate safe (XDR) tuberculosis (TB) are encouraged by lacking discovery and treatment (1).

TB identification and treatment are progressively troublesome in nations, similar to Pakistan, that are confronting complex crises, including philanthropic emergencies and clashes. MTB strains resistant to Fluoroquinolone most as often as possible presentation transformations in codons 90, 91 and 94 of the gyrA. Changes in codons 74, 88 and 91 have additionally been related with fluoroquinolone obstruction. [95-99]It is accounted for that clinically critical resistance from ciprofloxacin and ofloxacin (MIC of 2mg/L) is presented by a solitary gyrase change, though twofold transformations in the gyrA or accompanying gyrA and gyrB changes result in high MICs. [97] A change recognized in codon 95 of gyrA is a characteristic polymorphism that has no job in intervening fluoroquinolone resistance [98].

The unpredictability of fluoroquinolone resistance in MTB has been shown by the hypersusceptibility instigated by the nearness of changes in codon 80 of the gyrA gene, especially while happening with other resistances giving transformations. [99] Efflux components have additionally been accounted for to intercede

fluoroquinolone resistacne. (100) Mutations in the gyrB are uncommon. An ongoing multi-nation investigation uncovered low-level resistacne from new age fluoroquinolones. This might be represented by the broad utilization of this medication class. Besides, the projected rule of 2.0mg/L for this medication class has been accounted for to be excessively high, along these lines, speaking to an approximation of the weight of resistacnefrom new age fluoroquinolones.

2.12.3 Compensatory Evolution

It is conjectured that obstruction changes bear a health cost to the bacterium. This thought radiates from the recognition that isoniazid-safe confines indicated decreased ruinous tendency in the guinea pig illustrate. The new WHO proposition for the treatment of isoniazid-safe, rifampicin-exposed TB rely upon a study of evidence from patients treated with such regimens by a Guideline Development Group in congruity with WHO necessities for confirmation based techniques [101].

However, mulls over have since displayed the proximity of co-occasion of discretionary changes that go about as compensatory segments for the debilitated wellbeing of the pathogen. These compensatory changes are acknowledged to occur in qualities encoding a comparative protein or qualities related with equivalent metabolic pathways.64 Sherman and Mdluli showed this wonder in isoniazid-safe segregates of MTB with an inactivated katG quality [102]. The nonattendance of katG catalase– peroxidase development achieved changes in the authoritative district of the ahpC (alkyl hydroperoxidase reductase), inciting overexpression of this quality.

Changes of the ahpC are acknowledged to be compensatory for the loss of katG activity. [102] More starting late, whole genome examination showed that changes occurring in RNA polymerases rpoA and rpoC were compensatory for the loss of wellbeing mediated by changes in the rpoB quality in rifampicin-resistat detaches. [103-105] Reports on the changing components of capreomycin opposition among

A1401G inquire about office monstrosities and clinical separates bearing a comparative change, propose a possible cooperation of a compensatory framework. [99, 106] Similarly, changes in gyrB may speak to opposition introducing changes found in the gyrA quality [107].

2.12.4 Efflux-mediated Resistance

Efflux siphon frameworks are engaged with ousting drugs from the bacterial cell, empowering obtaining of resistance changes inthe bacterial genome. MTB presents with one of the biggest number of putative efflux siphons with 148 gene coding for film transport proteins inside its 4.4Mb genome. The commitment of these efflux frameworks in obtaining multidrug resistance in MTB has been exhibited by various investigations [108, 109]. The overexpression of efflux siphons is accepted to intercede the development of resistant changes, which presents abnormal state medicate resistacne permitting MTB to endure and hold on at clinically pertinent medication focuses.

The capacity of the efflux siphons to expel a decent variety of mixes enables them to remove different medications prompting the MDR phenotype [108, 109]. Efflux siphon inhibitors are mixes equipped for reestablishing the action of anti-toxins free of the dimension of resistance. The inhibitor– anti-infection mix diminishes the centralization of anti-microbials removed by efflux siphons, along these lines diminishing the MIC of the anti-microbial. The utilization of efflux siphon inhibitors is taken into account in the form of adjuvant for the treatment of TB and can possibly decrease the term of TB treatment [109 - 111].

2.12.5 Deficient Mechanisms of DNA Repair

Changes happening in DNA repair frameworks modify the capacity of such frameworks to fix effectively the harmed DNA, in this way expanding transformation rates. This gives a specific preferred standpoint to microbes that bear resistance giving changes. [112, 113] Missense changes happening in putative anti mutator

(mut) genes are distinguished in the family of Beijing strain , related with expanded transformation rates. [114, 115] Whilst extra examinations are required to clarify this instrument completely, WGS contemplates have shown expanded fluctuation in the genes encoding DNA fix proteins in Beijing strains [116].

Another system related with an expansion in transformation rates is the presentation to problematic medication focuses. Imperfect fluoroquinolone fixations have exhibited the capacity to prompt transcriptional changes in qualities in charge of DNA fix systems, for example, the SOS component. [117] Further examinations tending to DNA fix components are justified and will enhance our comprehension of the versatile development in the living being [66].

2.13 Developments in Genomics

WGS is present as alluring testing stage for resistance with the possibility to distinguish resistacne from all medications in a solitary investigation and at the same time track episodes with high goals. [118, 119] However, its use in clinics is reliant on the ability to examine things straightforwardly from sputum tests. Nonetheless, WGS needs a larger convergence of MTB DNA than is accessible in sputum examples and is in this manner used on refined disconnects. An extra test is the pollution by the DNA of host in clinical examples. [120] Latest methodologies, for example, the particular evacuation of host DNA and improvement of societies to expand the extent of MTB to have DNA, dodge the requirement for the extraction of DNA on besis of culture. Votintseva et al. [121] built up an adjusted Nextera XT convention to separate and decontaminate mycobacterial DNA inside hours to 3days from an initial positive growth of Bactec 960 Mycobacteria in Indicator Tube culture (middle culture time of 4 days).

Utilizing that method, they effectively sequenced 98 percent of the clinical examples and the reference MTB H37Rv genome mapping with .90% of grouping inclusion. [121] lately, a similar gathering detailed anti-microbial helplessness and

observation information in 8 h utilizing the Oxford Nanopore Technologies MinION sequencer. Results were accounted for to be completely concordant with genotypic information. They report this innovation to be profitable in fighting the issue related with low amounts of DNA in tests as the sequencer can keep sequencing until adequate inclusion of the genome is acquired. [122] Brown et al. [123]sequenced the entire genome of MTB straightforwardly from uncultured sputa utilizing biotinylated RNA snares planned explicitly for MTB DNA. The entire procedure can be cultivated inside 96h, even from poor quality smear-positive sputa [123].

Skill in omputational and bioinformatics examination is indispensable to the translation of extensive scale WGS information. Parallel to headways in sequencing advances, novel bioinformatics calculations have likewise been created for fast investigation and clinical understanding of MTB succession information. [124] The presentation of huge scale information sharing stages, for example, the Relational Sequencing TB Data Platform, has permitted the gathering of entire genome groupings from different investigations. This enables clients to accomplish approved, clinically significant hereditary information connected to related MTB metadata. As of now, the TB profiling device is the most precise in foreseeing the connection between identified change and its relationship with obstruction 125.

Chapter 3

Material and Methods

3.1 Ethical Statement

The study was evaluated and approved by the Institutional Ethics Committee of CUST Islamabad and Provincial Tuberculosis Reference Laboratory KPK.

3.2 Sample Collection

Samples were taken from TB suspects along with data i.e. location, gender, age, treatment history, sample type, HIV status, Disease Type from their guardian or next care taker at Provincial Tuberculosis Reference Lab Khyber Pakhtunkhwa (KPK) Pakistan. All the samples were subjected to further processing in the wet lab of CUST.

3.3 Culture and Sample Preparation

Processing and Culturing of Samples TB suspects samples were digested and decontaminated to recover the *M. tuberculosis*. This process liquefies the mucus

by applying NaOH/N-acetyl-L-cystein 37 Material and Methods 38 (NALC) to recover the *M. tuberculosis* and kill the normal flora.

3.3.1 Method 1

Samples were processed using NALC-NaOH concentration method [125] by transferring to falcon tube with equal volume of the NaOH/N-acetyl-Lcystein (NALC), vortexed and incubated at room temperature for 15 minutes for decontamination. 2. About 50 mL Phosphate buffer transferred to tubes and centrifuged at 3000 rpm for 15 minutes. 3. The supernatant was discarded in a container containing 5 percent phenol while the pellet was mixed with phosphate buffer and cultured on LJ media and MGIT tubes containing 7H9 media.

3.3.2 Culturing and Identification of MTB

About 800 micro-liter MGIT growth supplement and BBL MGIT PANTA was added to the MGIT tube. A sample of 500 micro-liter from processed decontaminated specimen was also added to this tube. The MGIT tube was then kept in MGIT 960 machine which automatically sense the growths in the tube with in recommended 42 days. The instrument was checked for positive signal every day.

3.4 Analysis of Prepared Samples

In case of sample's positive indication, the tubes were analyzed under light which on shaking showed as small clumps, or cords like snowfall moving down towards the bottom of the tube. To confirm whether the growth is MTB, BD MGIT MTBc identification test (TBc ID, Ref: 245159, Becton, Dickinson) was performed which is a rapid chromatographic immunoassay that detects *M. tuberculosis* complex antigen. Approximately 100 micro-liter of sample is taken from MGIT positive tube and added to TBc ID device well. Within 15 minutes MTB positive were

indicated because of the change in color from pink to red in the Test 'T' and the control 'C' location which showed the recognition of MPT64 antigen in the samples from MGIT positive tubes. Whole confirmed tubes of MTB were moved to pyrazinamide drug susceptibility testing (DST).

3.5 Drugs Susceptibility Testing (DST)

The BACTEC MGIT-960 method is a fast reliable system to measure the EMB drug susceptibility [126]. A sample was considered resistant if 1 percent or more of the MTB population remained to grow in the presence of critical drug concentration (100 micro-gram per milliliter).

3.6 Preparation of MGIT EMB Drug

EMB drug was reconstituted with 2.5 ml distilled water. Preparation of EMB Drug susceptibility test media: Accurately transfer 800 micro-liter EMB supplement to each MGIT tube. About 100 micro-liter EMB drug solution was transferred to its correspondingly labeled MGIT EMB medium tube.

3.7 Inoculum's Preparation and Inoculation of MGIT DST

All the TB suspect samples incubated in BACTEC MGIT-960 system were observed for growth indication. A positive MTB tube indicated by MGIT-960 on first day was considered Day 0. These tubes were further left for 24 hours in MGIT. For the preparation of test inoculum, a positive MGIT tube was used the day after it first becomes positive on the BACTEC MGIT 960 instrument (Day 1). A tube which has been positive longer than five days was sub cultured to a fresh 7 ml MGIT tube. Following is the procedure described below. All the MTB

positive tubes were mixed by inversion two to four times. A 1:100 dilution of the MTB positive tubes were prepared using distilled water.

A 500 micro-liter Inoculum taken from a MGIT tube of 1:100 diluted specimen (supplemented with BACTEC MGIT 96 Growth Supplemented without PANTA) and inoculated in a MGIT tubes. These tubes were entered into instrument until positive indication. About 500 micro-liter of inoculum was transferred to MGIT EMB tubes containing drug media and MGIT EMB tubes labeled as controls. The tubes were mixed gently by inversion three to four times. All MGIT tubes were placed into the BACTEC MGIT 960 and carefully observed the DST tubes on daily basis. The EMB resistant samples were further subjected to phenotypic DST of INH, RIF, MOX, EMB, AMK, SM, CAP, OFX, and KM through BACTEC MGIT 960 system according to the policy guidelines of the WHO.

3.8 Interpretation of Drug Susceptibility Testing

Test completion signals are generated when the Growth Control (GC) indicated the growth unit (GU) value of 400 or above. Susceptibility set of tubes were taken out from MGIT after scanning, and report of DST was printed. Susceptibility results were recorded as 'S' for susceptible 400 or more and drug tube was and 'R' for resistance when drug tube GU value was 100 or more.

Chapter 4

Results and Discussions

About 385 suspected patients of tuberculosis were involved in this examination. Their samples of saliva were obtained and used for culture development too analyze drug susceptibility for the samples that appear to be positive culture isolates.

Among the 385 cases, 53.24% (n=205) were males and 47.01% (n=181) were females having age ranging from 1 to 90 years. The maximum numbers of patients were found to be in the age group 16-30. Among 385 isolates of *M. tuberculosis*, 66.23% (n=255) were sensitive and 33.76% (n=130) were resistant to INH; 72.98% (n=281) were sensitive and 27.01% (n=104) were resistant to RIF; 92.46% (n=356) were sensitive and 7.79% (n=30) were resistant to EMB; and 82.59% (n=318) were sensitive and 17.40% (n=67) were resistant to OFX (Table 4.1) (Figure 4.1).

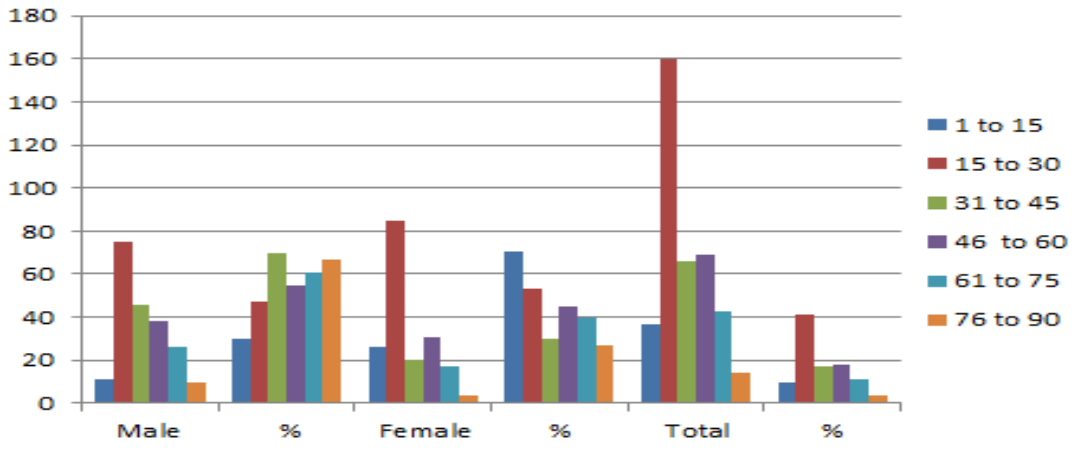

FIGURE 4.1: The Affected Male to Female ratio according to Age Groups

TABLE 4.1: Age and Sex wise Distribution of Patients

Age group	Male	%	Female	%	Total	%
1-15	11	29.72	26	70.27	37	9.61
16-30	75	47.17	85	53.46	160	41.55
31-45	46	69.69	20	30.30	66	17.14
46-60	38	55.07	31	44.92	69	17.92
61-75	26	60.46	17	39.53	43	11.16
76-90	10	66.66	4	26.66	14	3.63

Out of 385 isolates, 112 isolates were obtained from previously treated patients, 146 isolates from treated patients, and 127 isolates obtained from the untreated patients.

Multiple drug resistance was found to be 4.72% (n=6) in untreated TB patients and 6.80% (n=10) in treated patients whereas remaining subjects showed resistance against two or more drugs. The resistance to a single drug was 13.68%, to dual drugs was 9.21%, to 3 drugs was 8.42% and resistance to 4 drugs was 23.89% of the isolates.

The majority of *Mycobacterium Tuberculosis* was observed to be present in age group above 16 and below 30 i.e. (n=159) followed by (n=69) in 46-60 years of age, (n=66) 31-45 year age, (n=43) in 61- 75 year age, (n=37) in 1-15 years of age, (n=15) in 76-90 age group Among 219 isolates of sensitive strains from TB patients 13.69% (n=30) were previously treated patients and 41.09% (n=90) were untreated patients, have no previous history. The antibiotic sensitivity is given in table 4.2.

TABLE 4.2: Resistance and antibiotic sensitivity patterns of isolates

Drug Susceptibility Testing Result	Untreated TB Patients	No %	Treated TB Patients	No %	Total	Total in Percentage
Total tested	127	32.98	46	11.94	173	44.93%
Sensitive to all 4 Drugs	90	23.37	28	7.27	118	30.64%
			Resistant to 1 Drug			
INH	22	5.71	16	4.15	38	9.87
RIF	6	1.55	14	3.63	20	5.19
EMB	3	0.77	5	1.29	8	2.07
OFX	14	3.63	10	2.59	24	6.23
			Resistant to 2 Drugs			
RIF+NIH	6	1.55	12	3.11	18	4.67
EMB+RIF	1	0.35	5	1.75	6	2.10
OFX+EMB	1	0.35	3	1.05	4	1.40
			Resistant to 3 Drugs			
RIF+NIH+EMB	1	0.35	3	1.05	4	1.40

EMB+RIF+OFX	1	0.35	3	1.05	4	1.40
OFX+EMB+INH	1	0.35	3	1.05	4	1.40
Resistant to All	1	0.35	3	1.05	4	1.40
Multidrug Resistance	6	1.55	10	0.25	16	4.1

In case of male and female patients the maximum number was observed in patients with 16-30 years of age, 47.17% (n=75) male and 53.46% (n=85) female patients followed by age group 46-60, 55.07% (n=38) male and 44.92% (n=31) female, in age group 1-15 29.72% (n=11) were male and 70.27% (n=26) were female, lying in the age between 31-45, 69.69% (n=46) were male and 30.30% (n=20) were female having 61-75 years of age, 60.46% (n=26) were male and 39.53% (n=17) were female and less than 30% male and female were affected in age group 76-90. This scientific study confirmed that sex and age do not have any relation with resistance to drugs.

As statitical techniques associated with doing an investigation incorporate arranging, structuring, gathering information, dissecting, drawing significant understanding and announcing of the exploration discoveries. The statistical or factual investigation offers importance to the good for nothing numbers, in this manner reviving a dead information. The outcomes and deductions are exact just if appropriate factual tests are utilized. Variable is a trademark that shifts starting with one individual from populace then onto the next individual. Variables, for example, stature and weight are estimated by some sort of scale, pass on quantitative data and are called as quantitative factors. Sex and eye shading give subjective data and are called as qualitative factors, therefore we applied statistical measures on our data using SPSS tool.

Tuberculosis has turned out to be the most commonly occuring disease of the world in view of ongoing resurgence of TB. WHO has identified the reasons of this resurgence principally because of pandemic nature of HIV, less wellbeing is required to the illness and necessary increment of multi-drug resistance in tubercle bacilli because of deficient treatment. The causative agent of TB was found over hundreds of years prior and exceedingly compelling medications and immunizations are accessible making the TB as a preventable and reparable ailment. It stays as the important reason for mortality and morbidities because of a solitary irresistible infection on the planet.

Tuberculosis is a conceivably genuine irresistible sickness that predominantly influences the lungs. incompletely in light of the increase in HIV, AIDS causing infection. HIV debilitates an individual's immune system so it can not resist against the TB germs.

The overexpression of efflux siphons is accepted to intercede the development of resistant changes, which presents abnormal state medicate resistacne permitting MTB to endure and hold on at clinically pertinent medication focuses. The capacity of the efflux siphons to expel a decent variety of mixes enables them to remove different medications prompting the MDR phenotype. [108,109] Efflux siphon inhibitors are mixes equipped for reestablishing the action of anti-toxins free of the dimension of resistance. The inhibitor– anti-infection mix diminishes the centralization of anti-microbials removed by efflux siphons, along these lines diminishing the MIC of the anti-microbial. Table 4.3 and Figure 4.2 shows both sensitivity and resistance.

Tuberculosis is one of the real general medical issues in the underdeveloped nations with around evaluated 60% of the grown-up populace being affected with TB. In Nepal, about 45% of the populace isaffected with TB. Consistently 40,000 individuals create dynamic TB among them 20,000 have irresistible aspiratory infection. Nepal, by evaluated cases number, is positioned at 27 internationally. In

spite of the development and execution of a much enhanced National Tuberculosis Program (NTP) through DOTS Strategy all through the country,60007000 individuals still receive death from TB every year in Nepal.

TABLE 4.3: Antibiotic Sensitivity and Resistance Patterns of Isolates

Antibiotics	No of Isolates	Sensitive	No %	Resistant	No %
INH	385	255	66.23	130	33.76
RIF	385	281	72.98	104	27.01
EMB	385	356	92.46	30	7.79
OFX	385	318	82.59	67	17.40

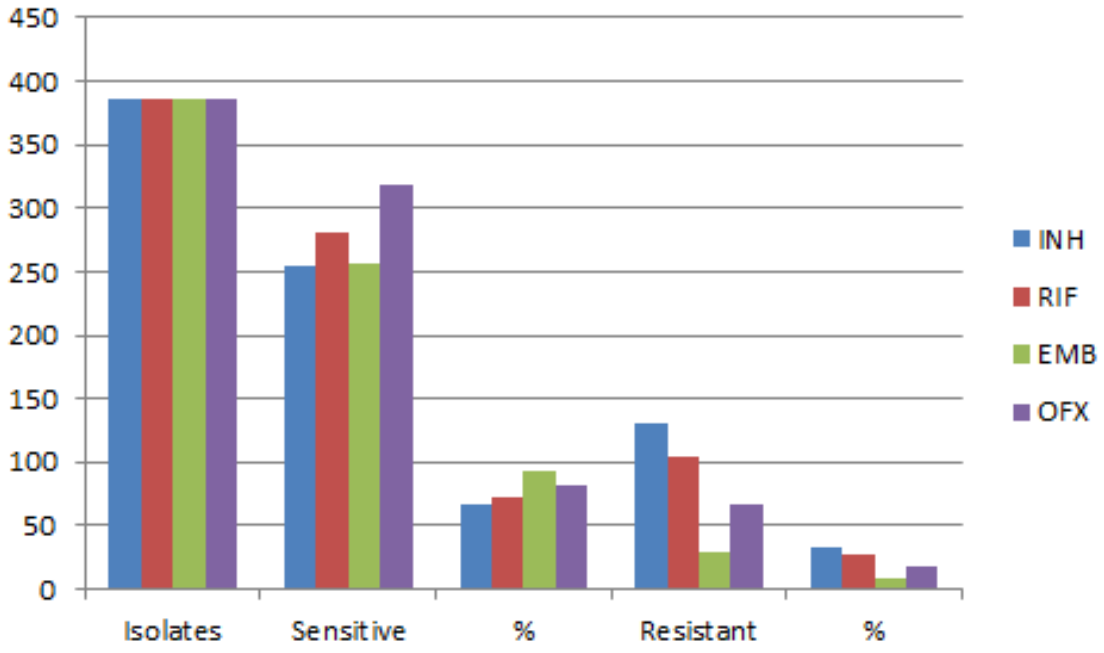

FIGURE 4.2: The ratio of Patterns of Sensitivity and Resistance in isolates

The principle objective of this examination is to realize the patterns of resistance in anti-TB medicines in TB patients. About more than 385 cases were incorporated into this investigation from September 2018 to Jan 2019. Amid this examination, among the 385 instances of TB, 225 were sensitive and 130 showed resistance

against INH.This nding was concordant with comparative investigations in different nations. In a same report given by Ponticiella et al. (1997) of Italy, declared 82.2% guys and of 17.8% of the females among 90 dynamic TB cases.

Another report was given in Atlanta USA by Blumberg et al (1991-1997), showed nitty gritty 26%of the female and 74% of the male patients having TB cases among 1536 cases. In like way, Toungoussova et al in Korea and Archangeh of Russia, itemized 66.49 to 34.31% of the male to female ration suffering from TB cases. Kuban et al of Cameroon, Yaunde, gave point by point 65.76 to 34.25% male female cases of TB among 111 cases; in Thailand Riantawan et al uncovered 77 percent of males and 23 percent of the females suffering from TB in 1441 patients.

Tuberculosis Control Program, Nepal declared 66.77% male and 33.23% female of TB cases among 14,384 as of late broke down TB cases amid the years 2002/2003.All above ndings are solid with this examination. Shrestha et al declared 47% and 3.05% of both male and female TB cases in histopathological precedents at Tribhuvan University Teaching Hospital separately. Smith uncovered that as in numerous countries of the world, in Nepal, the nitty gritty recurrence of TB is higher in man than women. Thus in all reports the number of male patiens was more than that of females.

Accordant to the signicance test, the power of TB in male and female was seen to be quantifiably signicant. These examinations illuminate the distinctions of sexual orientation, the most by and large recognized being that women are less introduced to disease than men. The second might be the common qualification, for instance, an extended lack of protection in male. Finally, influenced women may propel a significant part of the opportunity to sickness and bite the dust all the more rapidly, leaving a partner with a low inescapability of contamination.

The most elevated rates of medication resistances were found for streptmoycin drug as well as isoniazid medicine.the strains segregated were impervious to both the drugs accounting 13-14 percent of resistance individually; while 6.66% and 6.40% of the strains detached from new and recently treated patients were impervious to

streptomycin separately. Patterns of sensitivity and resistance are given in table 4.4.

TABLE 4.4: Antibiotic Sensitivity Patterns and Resistance Patterns of Isolates

SType	Extra Pulmonary	%	Pulmonary	%	Total	%
Ascitic Fluid	38	[1.2]	2	[0.1]	40	[1.3]
Bone Marrow	1	[0.0]	0	[0.0]	1	[0.0]
Bronchncial Aspirate	0	[0.0]	1	[0.0]	1	[0.0]
Bronchoalveolar Lavage/Washing	8	[0.3]	42	[1.4]	50	[1.6]
CSF	49	[1.6]	1	[0.0]	50	[1.6]
Culture	0	[0.0]	1	[0.0]	1	[0.0]
Gastric Aspirate	1	[0.0]	15	[0.5]	16	[0.5]
Gastric Lavage/Washing	1	[0.0]	0	[0.0]	1	[0.0]
Lymph Node	2	[0.1]	0	[0.0]	2	[0.1]
Pericardial Fluid	19	[0.6]	0	[0.0]	19	[0.6]
Pleual Fluid	78	[2.6]	8	[0.3]	86	[2.8]
Pus	39	[1.3]	3	[0.1]	42	[1.4]

Synovial Fluid	4	[0.1]	3	[0.1]	7	[0.2]
Tissue Biopsy	53	[1.7]	3	[0.1]	56	[1.8]
Urine	22	[0.7]	3	[0.1]	25	[0.8]

The nding of this investigation is in concurrence with different examinations directed at better areas. Al Marri detailed that 85% cases showed sensitiveness to TB drugs and 15% of the cases showed resistance to at least single TB drug.

Additionally, Rijal et al. (2003) discovered essential patients (2.63%) having MDR.The disturbing addition in MDR-TB cases might owe late identication of suspected MDRTB cases. Identication of all instances of MDR-TB needs culture and defenselessness testing of tuberculosis suspects. Tuberculosis Venture (GENETUP). Different purposes behind increment in MDR-TB might have negative smear for TB and consequently may also stay undiscovered with tuberculosis. Indeed, even among having sickness from positive smear, introductory reaction to therapies might be better, and Multi drug resistant TB may not be identified at times.

Our examination uncovered that there were larger numbers of drug resisntance against INH and RIF in both the treated and untreated cases. This might be because of minimal effort and across the board to cure TB. The most signicant nding of our examination was the low recurrence of essential resistance from OFX which is a decent pointer for achievement of DOTS. resistance from OFX was not seen in any case. Resistance to INH, OFX, and RIF anticipated resistance acts as marker of MDR.

New cases demonstrated large number of resistance to drugs which are circling and moved from one patient to another in Pakistan. Transmission of effectively resistant strains as a significant issue and danger, as it is extraordinary to treat

patients contaminated with medication resistance, it is essential for a TB control program to have solid research center offices for powerlessness testing of*M. tuberculosis* secludes.

Increased instances of MDR-TB are a worldwide issue. MDR-TB can be relieved by the powerful usage of DOTS procedure. Customary checking of MDR-TB and strategy as per the operational research nding empowers the controls and medication quality appraisal is useful for MDR-TB.

The new WHO proposals for the treatment of isoniazid-safe, rifampicin-defenseless TB depend on a survey of proof from patients treated with such regimens by a Guideline Development Group in congruity with WHO necessities for proof based strategies. The information created in this report were fortified in an as of late distributed second report. the treatment fixes essentially the patients with TB susceptible to medication and resistant to other medication by using the short-course of chemotherapy with rst line drugs.

Be that as it may, patients with MDR tuberculosis toseveral drugs are usually fail to short-course chemotherapy. Lately there is empowering proof that MDR TB patients can be relieved by proper administration dependent on second line drugs, that are tragically poisonous and least successful than rst and dependable evaluation of medication resistance is a basic essential for suitable use. Treatment is drawn out and increasingly costly. Exact lab drugs shows helplessness testing (DST) information to second-line medications but support clinical decicions of drugs aproval for patients with MDR TB. So as to address the difficulties presented by MDR TB, the WHO set up the DOTS-Plus activity to survey the achievability and cost-viability of utilizing second-line medications to oversee patients with MDR TB fundamentally in center and underdeveloped nations.

Spots Plus is required in zones where MDR-TB has risen because of past deficient TB control programs. Hence, DOTS-Plus pilot ventures are just prescribed to secure patients against the formation of further resistance produced by medication. Specks Plus is intended to fix MDR-TB. These medications ought to be put away and apportioned at specific wellbeing focuses with suitable offices and all around

prepared staff. It is fundamental that DOTS-Plus pilot ventures pursue WHO proposals so as to limit the danger of making drug resistance from second line TB drugs.

Fluoroquinolones are anti-toxins that are generally used to treat an assortment of ailments, for example, respiratory and urinary tract contaminations. counting T b yet motivations resstnace. Obstruction in TB to fluoroquinolones may happen immediately or might be procured, particularly when these specialists are utilized improperly. Cross-opposition among the fluoroquinolones has been appeared in TB. The fluoroquinolones offer an ideal pharmacokinetic profile for the treatment of TB. Most show astounding oral bioavailability and accomplish greatest (top) serum focuses well over the MIC. They are additionally appropriated broadly, including intracellularly.

The fluoroquinolones are cleared renally and additionally hepatically, with fluctuating serum half-lives.. pulmonary MDR-TB in Rawalpindi, Pakistan, is normal in youthful guys, destitution related conditions, and has poor result. DST indicates high protection from first-line hostile to tuberculosis specialists and quinolones. Rise and spread of multidrug-safe (MDR) and broadly medicate safe (XDR) tuberculosis (TB) are encouraged by lacking discovery and treatment (1).

In the event that patients falling DOTS are ventured to have MDR-TB, and if drug vulnerability testing is constrained, they may be set on an observational treatment routine comprising several medicines which should persevere through an extra 2 years of day by day, combination treatment, comprising injectable anti-infection agents, which can create horrendous reactions. Starting at July 2002, the Green Light Committee (GLC) had affirmed seven pilot undertakings to actualize the DOTS-Plus procedure, and is as of now looking into ve further applications. Fundamental outcomes from those projects officially under way show rates of culture negativization to be somewhere in the range of 46 and 79 percent. Proceeded with help for these ventures – together with the execution of new projects in different nations – will add to the working of a sound approach for the control of MDR-TB.

This examination showed no connection of sex and age, with medication resistance. Fortifying medicines will lessen dreariness, mortality and transmission occured because of MDR-TB. By guiding MDR-TB patients to compelling treatment conventions now, we are sparing direct expenses. What's more, by controlling the essential cycle of MDR-TB transmission now, we are sparing future assets and aberrant costs that would somehow or another must be redirected into the treatment for debilitated people and those who are infected because of it.

MDR-TB caused an expected 600,000 new TB cases and 240,000 passings in 2016 and MDR-TB represents 4.1 percent of all new TB cases and 19 percent of recently treated cases in the whole world.[12] Globally, most MDR-TB cases happen in South America, Southern Africa, India, China, and the previous Soviet Union. The TB microscopic organisms has regular barriers against a few medications, and can get tranquilize resistance through hereditary changes. The microbes does not be able to exchange genes for obstruction between life forms through plasmids. One precedent is a transformation in the rpoB gene, which encodes the beta subunit of the microorganisms' RNA polymerase.

In non-resistant TB, rifampin ties the beta subunit of RNA polymerase and disturb translation lengthening. Transformation in the rpoB changes the arrangement of amino acids and inevitable adaptation of the beta subunit. For this situation rifampin can never again tie or anticipate translation, and the microbes is resistant in nature. However the resistant TB is also reported in Pakistan in last few years, keeping this thing in find we tried to identify the patterns of resistance and antibiotic sensitive patterns in TB patients for 4 different drugs. out of 385 suspected patients of tuberculosis 53.24% (n=205) were the male patients and 47.01% (n=181) were female patients having age ranging from 1 to 90 years. The maximum numbers of patients were found to be in the age group 16-30.

Among 385 isolates of *M. tuberculosis*, 66.23% (n=255) were sensitive and 33.76% (n=130) were resistant to INH; 72.98% (n=281) were sensitive and 27.01% (n=104) were resistant to RIF; 92.46% (n=356) were sensitive and 7.79% (n=30) were resistant to EMB; and 82.59% (n=318) were sensitive and 17.40% (n=67) were

resistant to OFX . This study confirms that the resistance was more predominently found in the patients then sensitiveness to the drugs. Multiple drug resistance was found to be 4.72% (n=6) in untreated TB patients and 6.80% (n=10) in treated patients whereas remaining subjects showed resistance against two or more drugs. The resistance to a single drug was 13.68%, to dual drugs was 9.21%, to 3 drugs was 8.42% and resistance to 4 drugs was 23.89% of the isolates.

Strains in our examination were hereditarily assorted contends against spread of 1 specific genogroup in charge of medication ressitance and backings the idea that TB in Pakistanis is probably going to be an outcome of insufficient treatment of TB. The testing sociopolitical circumstance in Pakistan is probably going to compound this general medical issue. Crisis measures are required to stay away from an exponential ascent in medication safe TB in the nation and the area. We prescribe that expanded TB rates here be considered of national worry as well as be perceived as a territorial general medical problem needing presentation of agreeable and help measures went for constraining the spread of medication safe TB inside southern Asia.

Chapter 5

Conclusion and Future Recommendations

Tuberculosis (TB) is a main reason of sickness and demise worldwide than other contagious diseases because of its higher incidence rates in the developing nations. Its elimination is quite difficult because of dearth, increase in population and human immunodeficiency virus (HIV) infection. *Mycobacterium Tuberculosis* has potential to retain its activity in the tissues of a strong person for a large period of time. The infections produced by *Mycobacterium Tuberculosis* changes into tireless and extended progression and transmit into host. Tubercular disease can create infection in an individual following quite a while of torpidity. Consequently, the disease winds up across the board when an extensive extent of vulnurable population. It can deliver episode or pestilence, when brought into a populace, of which, just a little bit of people are immunologically secured. Pakistan positions 6th comprehensively among the 22 high-TB trouble nations and contributes an expected 43 percent of the infection towards the Eastern Mediterranean district. Yearly around 430,000 individuals including 15,000 youngsters contact tuberculosis in Pakistan, and consistently no less than 70,000 passings can be ascribed to the disease in the nation. Pakistan is likewise assessed to have the fourth most astounding pervasiveness of multidrug resistance tuberculosis (MDR-TB) all inclusive. More than 95 percent of tuberculosis deaths happen in low and middle income

nations. In this study Samples were taken from TB suspects along with data i.e. location, gender, age, treatment history, sample type, HIV status, Disease Type from their guardian or next care taker at Provincial Tuberculosis Reference Lab Khyber Pakhtunkhwa (KPK) Pakistan to determine the resistance level. Among 385 *M. tuberculosis* isolates, 66.23% (n=255) were sensitive and 33.76% were resistant to INH; 72.98% were sensitive and 27.01% were resistant to RIF; 92.46% were sensitive and 7.79% were resistant to EMB; and 82.59% (n=318) were sensitive and 17.40% were resistant to OFX. It was also observed that genders do not pla any role in developing resistance against drugs.

Chapter 6

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