Available online at www.elixirpublishers.com (Elixir International Journal)

Biosciences

Elixir Biosciences 114C (2018) 49570-49578

ISSN: 2229-712X

Molecular Mechanism of Drug Action and Antibiotic Resistance on *Mycobacterium Tuberculosis*: A Review

Daskum, A. M[1].,Mustapha A[2]. and Dagona A.G[3]

[1]Department of Biological Sciences, Yobe State University, P.M.B 1144, Damaturu, Nigeria.

[2]Department of Microbiology, University of Maiduguri, P.M.B 1069, Maiduguri, Borno State, Nigeria.

[3]Department of Biological Sciences, Federal University, Gashu'a, Yobe State, Nigeria.

ARTICLE INFO

Article history:

Received: 20 October 2017;

Received in revised form:

2 January 2018;

Accepted: 12 January 2018;

Keywords

Mycobacterium Tuberclosis,

Resistance,

Drugs,

Action,

Pathogen.

ABSTRACT

Mycobacterium tuberculosis, an anaerobic, acid-fast, non-motile, non-encapsulated and non-spore forming bacillus is an obligate intracellular pathogen and the etiological agent of tuberculosis (TB). Infections with *M. tuberculosis* results in significant morbidity and mortality globally, with an estimated 14 million individuals infected in 2007 and increasing annually. The emergence of drug resistant strains such as the multidrug-resistant (MDR), extensively drug-resistant (XDR) and totally drug-resistant (TDR) strains of *M. tuberculosis* poses a significant threat to the control of TB. These variations in sensitivity and resistance to different antitubercular agents by strains of *M. tuberculosis* are attributed to their genetic diversity and distribution in different regions of the world. This however, determines their virulence, immunogenicity, and drugs resistance and/or sensitivity pattern. The use of Molecular techniques to differentiate between strains of *M. tuberculosis*, the causative agent of TB is key to the understanding and characterization of resistance strains and virulence. This review will highlight and discuss *M. tuberculosis*, its challenges to global health sector, the emergence and spread of drug resistance, and the molecular mechanisms of the drug action and drug resistance.

© 2018 Elixir All rights reserved.

Introduction

Mycobacterium tuberculosis, the causative agent of tuberculosis (TB) remains an important global public health concern; it is an obligate intracellular pathogen that can infect many animal species but human remains the principle host. *Mycobacterium tuberculosis*, is an aerobic, acid-fast, non-motile, non-encapsulated and non-spore forming bacillus (Lawn and Zumla, 2011). These characteristic features help in infectivity of the pathogen. There has been a question of whether *M. tuberculosis* is a Gram-positive or Gram-negative organism (Hadano, 2013). The pathogen is highly transmissible, and infection results in significant morbidity and mortality globally. There is an increase in the prevalence of tuberculosis, with an estimated 14 million individuals infected worldwide in 2007 (Almeida *et al*., 2011). Several reports of the World Health Organization (WHO) show that one-third of the world`s population is infected with *Mycobacterium tuberculosis* (Ginsburg *et al*., 2003; Pedrazzoli *et., al* 2012; Smith *et al.,* 2013). Health protection Agency (HPA) shows increase in the number of TB cases among UK prisoners, with 56% having pulmonary diseases associated with TB (Anderson *et al.*, 2010). However, overall TB cases have been stabilizing since 2005, with 8,963 cases in 2011, and 9287 new cases in 2016 in the USA, at an alarming rate of 14.4 cases per 100,000 and 2.9 cases per 100,000 individuals respectively (Ginsburg *et al*., 2003; Pedrazzoli *et al*., 2012, world TB day, 2017). In pre-antibiotic era, people infected with *M. tuberculosis* would have lost ray of hope from cure. But with the discovery of the "magic-bullet" this disease became curable due to the potency of the drugs (Da Silva and Palomino, 2011; Smith *et al.*, 2013).

It is not only the prevalence of tuberculosis that is of concern to the global health sector, but also the emergence of resistance by the causative agent to the prescribed TB antibiotics. The increasing emergence of multidrug-resistant strains (MDR) and extensively drug-resistant (XDR) tuberculosis are quite alarming, and pose significant threat to the control of the disease (Zhang and Yew, 2009; Smith *et al.*, 2013; Fonseca *et al.*, 2015). In addition, there is much concern that the TB situation will become worst with Human Immunodeficiency Virus (HIV) pandemic worldwide, as the virus can weaken the immune system of infected individuals and predispose them to *Mycobacterium tuberculosis* infection (Palomino and Martin, 2014). Multi-drug resistance tuberculosis (MDR-TB) is primarily due to mutations, which lead to, at least resistance to isoniazid (INH) and rifampicin (RMP), the most important "first-line" drugs against TB. Extensively drug-resistance (XDR) by *Mycobacterium tuberculosis* is due to additional resistance caused in MDR strains to any of fluoroquinolone and one of the three injectable second line drugs, capreomycin, kanamycin and amikacin. Most recently, the appearance of strains that are completely resistant to all antituberculosis drugs was noticed in several regions of India. These are however, termed the totally drug-resistant (TDR) strains (Ebrahim, 2007; Borrel and Gagneux, 2011; Fortune, 2012; Wabale and Joshi 2016). XDR-TB has been reported from Asia, North and South America, Europe and more recently, Africa. Also, patients with XDR-TB have fewer options for treatment and high risk mortalities, especially in HIV-infected persons. Around 40,000 cases of XDR-TB are estimated to emerge globally each year.

Tele:

E-mail address: daskum341@gmail.com

© 2018 Elixir All rights reserved

The countries ranked first to fifth in terms of total numbers of drug-resistant cases are India, China, the Russian Federation, South Africa and Bangladesh, which collectively make up 50% of the global burden. The global distributions of TB are directly linked to low-income and emerging economies (Mathema *et al*., 2006; Ebrahim, 2007; Zhang and Yew, 2009; Almeida *et al*., 2011; Lawn and Zumla, 2011). Recently, Pourakbari *et al.* (2016) reported increasing burden of TB in eastern Mediterranean particularly Iran. In the systematic review, the authors observed that increase resistance to the first line drugs Isoniazid and Ripampin. The burden of TB epidemic is very high in developing countries. It is estimated that 95% of TB cases and 98% of TB deaths occur in the third world countries, due susceptibility of people to TB as a result of poor living conditions, poor/late diagnosis and limited access to treatment (Hordofa and Adela, 2015).

This review discusses Mycobacterium *tuberculosis* and its challenges in global health sector, the emergence and spread of multi-drugs resistance, and the molecular mechanisms of drug resistance.

Microbiology of *Mycobacterium tuberculosis*

Mycobacterium tuberculosis was identified by Robert Koch (1843-1910) in 1882, by developing a staining method based on methylene blue combined with brown counterstaining of the host tissue that allow to visualize the bacterium (Kaufmann and Gengenbacher, 2012). The basic principle of this method is still in use years after it was introduced. Generally, Mycobacteria are divided into two groups: fast-growing and slow-growing. *Mycobacterium tuberculosis* is a member of *Mycobacterium tuberculosis* complex (MTBC), a group of closely-related slow-growing mycobacteria that includes *M. tuberculosis, M. africanum, M.bovis, M.canettii* and *M.microti*. The first three species are pathogenic to human. *M. tuberculosis* is the first cause of tuberculosis in humans, while infection with *M.bovis* is related to ingestion of unpasteurized milk and causes disease in many animal species. *Mycobacterium africanum* is commonly found in Africa (Bonfioli *et al.,* 2005; Arnold, 2006). The different *Mycobacterium* species are attributed to their genetic diversity of the strains to different region of the world, which determine their virulence, immunogenicity, and drugs resistance. Researchers have attributed the diversity in *Mycobacterium tuberculosis* to various factors. Hershber *et al.* (2008) linked the diversity in the *Mycobacterium tuberculosis* strains directly to human demographic and migratory events. Their work used DNA sequencing method of global collection of MBTC strains. Samples were collected from human-adapted strains in different geographic locations and animal-adapted strains including *M. microti, M. bovis*, *M. pinnipedii*, and *M. caprae* to develop a phylogenetic tree which classified *Mycobacterium tuberculosis* to ancient and modern forms based on the presence or absence of a genomic deletion known as TbD1. Comparatively, the diversity is more pronounced in human-adapted members of MBTC than animal-adapted strains.

The study of Roetzer *et al*. (2011) compared different methods of genotyping of *Mycobacterium tuberculosis* complex to elucidate the diversity of MTBC, the molecular methods includes; restriction fragment length polymorphism (RFLP) typing based on the IS6110 insertion sequence, Spoligotyping which relies on analyzing the polymorphism of unique DNA sequence comprising identical fragments repeated on the *Mycobacterium* genome and the 224-loci, *Mycobacterial* interspersed repetitive unit-variable number tandem repeats (MIRU-VNTR) typing which is based on analysis of repeated sequences of multiple loci that are amplified using primers . This work highlighted disadvantage of some of the methods, for instance IS6110 insertion as laborious , time consuming, and require skilled personnel; Spoligotyping has low power to differentiate the strains; and MIRU-VNTR as new procedure that is not commonly used. However, the methods are used to trace MTCB strains. The study of Kulaga *et al.* (2004) and Shamputa *et al.* (2010) used a similar approach to determine diversity of *Mycobacterium tuberculosis* strains. Generally, the strategies used to study the pattern of genetic variation in bacteria can be classified into three; (i) small local changes in nucleotide sequence of genome, for instance, single nucleotide polymorphism (SNPs); (ii) intra-genomic rearrangements of segments of genomic sequence, example recombination between repeat sequences and deletions; and (iii) acquisition of DNA sequences from other organisms (Arnold, 2006). These strategies gives knowledge of the origin, molecular evolution of *Mycobacterium tuberculosis* and pattern of spread, infectivity, resistance, pathogenicity and development of biomarkers of active tuberculosis that would help in the diagnosis, vaccine production, monitoring treatment, and assessing outcome (cure or relapse).

Host-pathogen interaction: The interaction of *Mycobacterium tuberculosis* with the host primarily starts by inhalation of aerosolized droplet nuclei containing the pathogen. The bio-aerosols are produced by infectious patient with active tuberculosis during sneeze, cough or speak. The rate of transmission is high within few years of infection (Bonfiali *et al*., 2005; Lawn and Zumla, 2011). Once the small particle (1-5 µm in diameter) are inhaled and phagocytosed by the resident macrophages, cellular immune response involving cytokines and a large number of chemokines would set up. Many researches described *M. tuberculosis* as successful pathogen on its ability to utilize macrophages for its replication. The process starts when the bacilli gain access to alveolar macrophages, and got ingested, thus resulting in its multiplication and subsequent destruction of the macrophages. The fate of infected macrophages plays a vital role in protection of *M. tuberculosis* by regulating innate and adaptive immunity. Virulent strains of *M. tuberculosis* inhibit apoptosis by a number of anti-apoptotic genes and trigger macrophage necrosis, there by evading innate immunity and delaying the start of adaptive immune responses (Crevel *et al.,* 2002). Kaufmann and Gengenbacher, (2012) described macrophages as prime defense cells against microbial intruders through phagocytosis, a process consisting of membrane invaginations resulting in phagosome formation. However, MacDonugh *et al*. (1993) demonstrated phagolysosome fusion in *M. tuberculosis* infection of macrophages *in vitro*, using three different *Mycobacterium* strains, including the virulent *M. tuberculosis* strain H37Rv, the avirulent strain H37Ra derived from H37Rv, and the attenuated *Mycobateriumbovis* BCG vaccine strain originally derived from bovine tubercle bacillus isolate. The research indicated that tubercle bacilli were directed to phagolysosomes immediately after engulfment by macrophages.The complex interaction of *M. tuberculosis* with macrophages was extensively demonstrated in two stages, namely early and late events (Smith, 2003). The early events start after alveolar

invasion, resulting inphagocytosis of the pathogen by dendritic cells and monocyte-derived macrophages.

Dendritic cells play a vital role in the early stage due to their antigen presenting nature, and subsequent activation of T cells with specific *M. tuberculosis* antigen by their expression on the surface. Surfactant protein A (Sp-A), a glycoprotein found on alveolar surfaces helps in binding and uptake of *M. tuberculosis* by up-regulating mannose receptor activity. Crevel *et al*. (2002) reported the complement receptors (CR) as CR1, CR3 and CR4. The complement receptor type 1 (CR1) is a monomeric transmembrane protein that binds C3b and C4b but not C3bi. It has complement regulatory activity and can mediate phagocytosis. CR3 and CR4 are heterodimeric proteins of the integrin superfamily (Ernst, 1998). Other host receptors include Toll-like receptors (TLRs), nucleotide-binding oligomerization domain-(NOD)-like receptors (NLRs), and C-type lectin such as mannose receptor (CD207), the dendritic cell-specific intracellular adhesion molecule grabbing non-integrin (DC-SIGN) and Dectin-1 (Ahmad, 2011). In contrast to surfactant protein A (Sp-A), surfactant protein D (Sp-D) inhibits phagocytosis of *M. tuberculosis* by blocking mannosyl oligosaccharide residues on the bacterial cell surface and thus prevents *M. tuberculosis* with mannose receptors on the macrophage surface. Normal phagosomal maturation cycles (phagosome-lysosome fusion) will create a hostile microenvironment that is characterized with acidic pH, reactive oxygen intermediates (ROI), lysosomal enzymes and toxic peptides. Although, *M. tuberculosis* bacilli are postulated to be unable to multiply in this condition, some organisms may remain dormant but alive for years. Here, the host immune status response would determine whether an infection would be arrested at this stage or will progress to the next stage. This enclosed infection is referred to as latent or persisted TB, and can persist throughout a person`s life in an asymptomatic and non-transmissible state. In a person with efficient cell-mediated immunity, the infection may be arrested at this point, which leads to the healing of granulomas, leaving small fibrous and calcified lesions (Ahmad, 2011). Animal models were used to mimic the mechanisms of *M. tuberculosis* latency (Parnish *et al*., 1998; Flynn and John, 2001; Capuano *et al*., 2003). However, the works described some strategies employed by tubercle bacilli to manipulate infected host cell in order to evade immune response, and thus persist in the host. The most important factor is use of gene; the sigma factor gene (sigF gene) which play a role in slow-growing *Mycobacteria*. Zhang *et al*. (2003) described the ability of *M. tuberculosis* to establish, maintain, and reactivate from persistent infection to the host-pathogen dynamics interaction which is regulated by gene encoding secondary metabolisms, cell surface determinants, and transcriptional factors. Also, a two-component signal transduction system, mprAB contributed to latency of *M. tuberculosis*. The two-component allows organism to adapt to environmental changes by phosphor transfer reactions between a membrane-localised histidine kinase sensor and a cytoplasmic response transcription factor. Thus, it is likely to allow intracellular growth of the pathogen. The late events start when infected macrophages in the lungs lead to the formation of granulomatous lesion by their production of chemokines that attract inactivated monocytes, lymphocytes, and neutrophils. Here, if the latently infected person`s immune system becomes low due to factors such as HIV/AIDS infection, immunosuppressive drugs, aging, malnutrition or other factors, the granulomas can become liquefied and serve as a medium to allow growth of bacteria (Ahmad, 2011).

Hence, the viable *M. tuberculosis* can escape from the granuloma and spread within the lungs and other part of the body through lymphatic system (Ahmad, 2011). Thus, infection develops and antibiotics therapy needs to be employed.

Mechanisms of Drug resistance in *Mycobacterium tuberculosis*

The scope and gravity of challenge of tuberculosis in the global health sector need to be halted, especially in the emergence of HIV/AIDS and the increase of drug resistance by this pathogen to the anti-TB agents. *Mycobacterium tuberculosis* resistance to chemotherapeutic agents have been reported long before emergence of HIV/AIDS, but HIV/AIDS allows drugs resistance TB strains to transmit due to the immune deficiency in the patients. The World Health Organization (WHO) estimated over 20 million HIV infected people, 6 million of which are co-infected with *Mycobacterium tuberculosis* (Kaufmann and Gengenbacher, 2012). The resurgence of tuberculosis is worldwide, but occurring mainly in developing countries. Similarly, Pedrazzoli *et al*. (2012) in the report of Health protection Agency (HPA) on tuberculosis in the UK showed an increase rate in urban areas, especially amongst youth. However, this recent result indicated London with the highest rate of 35%, and then followed by West midlands region with 11%. Also, the report showed similar trend in 2010, but more than 70% of these were born outside UK and mainly originated from south Asia and sub-Saharan Africa. Nair *et al*. (2010) reported more than 2 million cases annually in the region, which was attributed to low income and lack of effective TB control. An increase in tuberculosis cases had been reported among HIV/AIDS patients with 74% living in sub-saharan Africa (Corbett *et al*., 2006; WHO, 2015). The increase of tuberculosis shows similar pattern with the antibiotic resistance by the causative organism. This poses great challenge to the hope that tuberculosis will be eradicated by 2050 as set by Millennium Development Goal. Thus, details of how *Mycobacterium tuberculosis* developed resistance to the important antitubercular agents would help to develop possible rapid methods to detect resistant strains. Generally, anti-tuberculosis drugs are classified into two, the first-line drugs and second –line drugs. Resistance to the first-line drugs including isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), ethambutol (EMB), and streptomycin (SM) have been reported to be due to drug misuse, wrong diagnosis or interrupted drug supply, which eventually lead to development of multi-drugs resistance tuberculosis (MDR-TB). However, if MDR-TB fails to be treated with second-line drugs such as flouroquinilone (levofloxacin and maxifloxacin), aminoglycoside (kanamycin and amikacin), capreomycin, para-aminosalicylic acid (PAS) and cycloserine could lead to resistance that subsequently result in the development of extensively resistant-resistant tuberculosis (XDR-TB) (Ebrahim, 2007; Borrel and Gagneux, 2011;Takiff and Guerrero 2011; Daum *et at*., 2012).

General mechanisms of antibiotic resistance

Bacteria employ different mechanisms to develop resistance to antibiotics. Wade and Zhang, (2004) classified the antibiotics resistance strategies into five; (i) decreased up take (ii) increased efflux (iii) enzymatic inactivation (iv)

reduced pro-drug-activating enzymes activity, and (v) modification of antibiotics target. The organism can use one or more of these mechanisms to develop resistance. Bacteria may be intrinsically (natural) resistant or acquired resistant by *de novo* (point mutation, insertions, deletions or duplication) mutation or through acquisition of resistant genes from other organisms (bacterial recombination). Similarly, Tenover, (2006) reported the mechanisms of antibiotic resistance in these ways; first acquisition of gene encoding enzymes such as β-lactamase to disrupt antibacterial agents. Secondly, through effect of efflux pump that remove the antibiotic from the cell before reaching its main target and Thirdly, the bacteria may acquire some genes in a metabolic pathway that change their cell wall, thus many antibiotic binding site would be lost target, or bacteria to mutation that limit entry of antibiotic to intracellular target site. In recent years, advances in molecular studies have been conducted in different organisms which paved way to a better understanding of the mechanisms of resistance to drugs (Ahmad, 2011). In 1996 John S. Blanchard, demonstrated that some broad spectrum antibiotics such as streptomycin, rifampin, and flouroquinolone act on the same target in *M. tuberculosis* as they do in *E. coli*. Resistance to these agents results from single mutagenic event that leads to amino acid substitution to their target protein. In contrast, mobile genetic elements including plasmid, integrons and transposons, transmit resistance genes among bacterial population other than *M. tuberculosis*. Stephen, (2002) in his mini review described *M. tuberculosis* resistance to anti-tubercular agents through spontaneous chromosomal mutation at different rates being highest in ethambutol and low in rifampin and quinolone, which can be amplified in clinically drug-resistant TB. These include monotherapy due to non-adherence of patient to treatment, poor drug prescription and supply. However, the MDR/XDR strains are encoded with different resistance genes that allow replication of the organisms (Table 1).

Table1. Summary of the molecular mechanisms of antituberculosis drug resistance.

Drug	Associated mutated gene or mutation
Rifampin	rpoB
Isoniazid	KatG, inhA, oxyR
Streptomycin	rr, rpsL
Pyrazinamide	pncA, IS6110 insertion
Ethambutol	embB
Fluoroquinolones	gyrA,

Isoniazid (INH) resistance

Isoniazid is an important first-line, specific, and the most active anti-tubercular drug. It is a prodrug that needs activation by the endogenous *M.tuberculosis* enzyme catalase-peroxidase (KatG) to form an electrophic species before activating with the targets such as InhA. Structurally, it is similar to pyridoxine and with other drugs such as rifapentine. Isoniazid form the bases of directly observed treatment short course for the treatment of TB which reduce the long term treatment of 9 months due to its toxicity (Quemard *et al*., 1992; Coninx *et al*., 1999; Siddiqi *et al*., 2007).

Mode of action

The mode of action of drug would give a better understanding of the drug on the susceptible organism. Wade and Zhang, (2004) reported the basic mode of action of Isoniazid (INH) on its ability to decrease growth of tubercle bacilli in the presence of oxygen, which is reduced under anaerobic conditions. However, the authors demonstrated the activity of IHN at 37^{o}C, an optimum temperature for KatG activation. Thus, the biologically active IHN showed ability to disrupt the synthesis of mycolic acids by inhibiting the NADH-dependent enoyl-ACP reductase enzyme encoded by inhA, thereby exerting its lethal effect by forming covalent complexes. Zhang and Yew, (2009) described the dual antimicrobial activity of INH, bacteriocidal against actively growing *M. tuberculosis* and bacteriostatic on non-replicating organism. The minimal inhibitory concentration of IHN is 0.02-0.06 mg/L (Viveiros *et al*., 2002).

Mechanism of Resistance to INH

The resistance of INH was noticed shortly after it was introduced as an anti-tubercular agent in the 1950's (Wade and Zhang 2004), but the underlying mechanisms of resistance were fully revealed in the 1990's. Zhang and co-workers were the first to demonstrate that mutations or deletion in *Kat*G gene result to resistance of INH in clinical sample of *M. tuberculosis*. Similarly, Viverioes *et al*. (2002) described the complex mechanisms of resistance of *M. tuberculosis* to INH on mutations in genes encoding a catalase-peroxidase (KatG), an enzyme of the mycolic pathway (inhA) and β-ketoacyl-acyl carrier protein synthase (kasA). Furthermore, Zhang and Yew, (2009) demonstrated various mutations in *kat*G gene among INH isolates using polymerase chain reaction based (PCR-based) molecular approach. Clinical samples were collected from Japan (105 samples) and Poland (33 samples) from patients with pulmonary tuberculosis. Drug sensitivity of the clinical samples was conducted using Middlebrook 7H10 agar medium method used by the United state Public Health Service, the egg-based Ogawa medium method recommended by Japanese Society for Tuberculosis and rapid broth method such as BD BECTAC. DNA sequencing (two-temperature PCR) was used to amplify region of resistance gene, revealing the varied length of the gene 315 to 2,748 base pairs (bp). Out of the 138 isolates from Japan and Poland, 37 had no mutation in KatG whereas 81 isolates had single point mutations, 11 isolates had two points mutation and 1 isolate with 3 points mutation. The authors reported 10 novel point mutations, and the drug sensitivity pattern of the isolates showed more than 55 of the 138 being resistant to INH and other drugs tested. Using similar approach, Siddiqi *et al.* (2007) demonstrated that INH could also induce its own resistance by selecting pre-existing mutants in non-replicating persisters, which was demonstrated using cell culture containing INH to determine the inhibition of the isolates, and allowing it to grow after exposure. The work concluded that INH induced its own resistance, though it's only applied to INH because the resistant cultures were susceptible to other TB drugs. Furthermore, Wade and Zhang, (2004) have reported the *inhA*gene contributing to INH resistance, the mutation location in promoter region of an upstream gene mabA encoding 3-ketoacyl ACP reductase, which form an operon with *inhA*. The *mab*A causes over expression of target inhA, while mutation in *inh*A causes changes in *inh*A targets, both of which result to INH resistance. The resistance cause by *inh*A mutation is relatively low while katG mutations are associated with high levels of INH resistance. Sekiguchi *et al*. (2007) correlate drug susceptibility and mutation in *M. tuberculosis*. In the study, the authors found that in 11

mutations and CTP insertion at position 1170 in *kat*G, of which Q295P and G297V conferred INH resistance.

Rifampin (RIF) Resistance

Rifampin (RIF) is another important first-line drug for the treatment of tuberculosis. It is a semisynthetic macrocyclic antibiotic originated from *Streptomyces mediterrane.*

The discovery of rifampin became the back-bone modern of anti-TB chemotherapy, by its ability to act against *M. tuberculosis* in exponential growth state as well as being active against non-replicating persistent bacilli with MICs ranging from 0.05-1 µg/ml on solid or liquid media (Gumbo *et al*., 2007; Zhang and Yew, 2009).

Mode of Action

Rifampin (RIF) is a large lipid-soluble compound that is bactericidal against all intracellular microorganisms. RIF binds strongly to the β-subunit of bacterial DNA-dependent RNA polymerase, and thus, inhibits RNA synthesis resulting in the formation of stable drug enzymes with binding constant of 10^{o} at 37^{o}C. However, it has been reported that inhibition of bacterial RNA synthesis is not the only mode of action of this antibiotics, but also inhibition of the growth of some mammalian viruses (Wade and Zhang, 2004).

Mechanism of Resistance to RIF

Resistance to this key drug in modern tuberculosis regimes has been increasingly noticed, especially in patients with HIV-related tuberculosis (Vernon *et al*., 1999). Wade and Zhang, (2004) reported that resistance to RIF in *M. tuberculosis* results from point mutation in rpoB, an 81 base pair (bp) gene responsible for β-subunit of RNA polymerase. However, the authors described the mutation in rpoB gene as commonly associated with the codons ser531 and His526 which result to high-levels of resistance to RIF. The single point mutation in rpoB not only conferred resistance to RIF but also cause cross-resistance to many other rifamycin derivatives which account for 96% of RIF-resistant *M. tuberculosis* strains. Campel *et al.* (2011) used similar approach to detect mutations in different anti-TB drug resistance. A DNA sequencing was used, and nine loci: ropB (for resistance to RIF), katG and *inh*A (INH), *pnc*A (PZN), *emb*(EMB), *gryr*A (CIP and OFX), and rr.eis, and tlyA (KAN, AMK, and CAP) detected. Majority of the strains resistant to RIF were reported to possess different point mutations. Moreover, in the same study, a total of 26 non-synonymous single nucleotide polymorphisms (nSNP), one 3-base pairs insertion, and a silent mutations were identified. Bartfai *et al.* (2001) demonstrated the two regions of *rpo*B gene to be responsible for rifampin resistance by sequencing 29 rifampin-resistant isolates of *M. tuberculosis* from patients in Hungary. Of the 29 resistant strains, 27 had mutation in either the 81-bp region, or the N-terminal region but two strains had no mutation in either region. In contrast, the study found that the frequency of mutation in strains from East Hungary which could lead to resistance was low mutation, which could be attributed to variation in geographical distribution of the resistant strains.

Pyrazinamide (PZA) Resistance

Pyrazinamide (PZA) is another first-line drug used in combination with INH and RIF. PZA, is an analog of nicotinamide, and plays a vital role in shortening the long regime of TB treatment from 12 months to six months (Zhang *et al*., 2003).

Mode of action

The mechanism of action of PZA is poorly understood due to its unusual properties of inactivity against tubercle bacilli in normal culture near neutral pH, but active at acid pH with MICs range from 6.25-50µg/ml. PZA is a prodrug that is converted into active form pyrazinoic acid (POA) by bacterial enzyme nicotinamidase/pyrazinamidase (PZase) (Zhang *et al.*, 2003; Wade and Zhang, 2004). The target of PZA is bacterial fatty acid synthase in mycolic acid synthesis.

The POA reaches cell surface through passive diffusion. The extracellular acid condition facilitates the formation of uncharged protonated POA, which accumulates and disrupt *M. tuberculosis* membrane (Heifets *et al*., 1989).

Mechanism of Resistance to PZA

Experimental evidence showed that acquired resistance to PZA in *M. tuberculosis* is due to loss of pyrazinamidase/nicotinamidase activity which is encoded in *pnc*A gene (Somoskovi *et al*., 2003). Scorpio *et al*. (1997) cloned the PZase gene (pncA) from *M.tuberculosis* and reported mutations in this gene in five PZA- resistant *M. tuberculosis* as well as *M. bovis*, but the *M. bovis* strains were found to have a single mutation in 'C' to 'G' direction at nucleotide position 169 which resulted to changes in position 57 of the pncA sequence. Thus, the different mutation pathway in *M. bovis* serves as a useful marker for easy and rapid differentiation of bovine from human tubercle bacilli.

Ethambutol (EMB) Resistance

Ethanbutol is a first-line anti-TB drug produced in 1966. The activity of this drug is restricted on growing bacilli with an MIC of 1-5 µg/ml. The mechanism of action of EMB is inhibition of arabinogalactan, a major cell wall component of mycobacterium (Da Silva and Palomino, 2011). In addition, Wade and Zhang, (2004) described that EMB inhibits the polymerazation of arabionon in arabinogalacton and lipoarabinomannan, resulting to accumulation of decaprenylarabinose, an intermediate in the synthesis of arabinan of cell wall arabigalactan and arabinommna. They have showed that target of EMB is an enzyme, arabinosyltransferase (EmbB) involved in synthesis of arabinogalactan. Pilke *et al.* (2010) using molecular technique indicated that 69% of ethambutol-resistant *M. tuberculosis* strains carry a mutation in the embB gene, mutation in codon embB306 point mutation at high frequency. The authors also indicated relationship between embB306 point mutations and in vitro experimental resistance by allelic-exchange experiments. In contrast, they demonstrated that one-third of ethambutol-resistant clinical isolates do not carry a mutation in embB306, thus are not detected using molecular method. Other studies have found that *emb*B mutation and drug resistance are highly related. Shi *et al*. (2011) used 138 multi-resistant clinical isolates of *M. tuberculosis*, including 86 ethambutol-resistant and 52 ethambutol-susceptible strains which served as control. The strains were analyzed to determine mutation in entire coding region of embB gene. The results showed 27 *emb*B mutation type were found in 19 different codons and strongly reported high mutation in other anti-TB. Similarly, Perri and Bonora, (2004), Pilke *et al.* (2011) and Da Silva *et al*. (2011) revealed that mechanism of resistance to EMB is associated with single mutation in embCAB operon encoding various arabinosyl transferase, indicating that mutation in embB gene result to high-level resistance in *M. tuberculosis*.

Aminoglycosides
(Streptomycin,kanamycin/amikacin/capreomycin)

Streptomycin (SM) Resistance

Streptomycin (SM) is one of the oldest and most commonly second-line anti-TB drugs. SM is an amionoglycoside antibiotic that is active against many bacterial species, including *M.tuberculosis* (Zhang and Yew, 2009). SM like ethambutol kills actively growing tubercle bacilli with MICs of 2-4 µg/ml, but it is not active on non-growing or intracellular bacilli. SM act by inhibiting polypeptide synthesis through binding to the 30s ribosomal subunit, resulting in misreading of mRNA message during translation (Wade and Zhang 2004; Da Silva *et al*., 2011). In contrast to other mycobacterium such as *M. fortuitum*, resistance to SM is associated with an enzyme aminoglycoside 3"-O-phosphotransferase. In *M. tuberculosis* however, molecular studies revealed that resistance to SM is mostly due to mutation in rrs or rpsL which cause changes in the SM binding sites (Spies *et al*., 2007). Furthermore, resistance to SM due to *gid*B mutation that encodes a conserved 7-methylguanosine methyl transferase specific for rRNA had been demonstrated (Spies *et al.,* 2007; Wong *et al*., 2011). The authors associated the low-level SM resistance to Efflux system, a system that allows bacteria to survive a hostile condition such as presence of antibiotics.

Kanamycin (KM) and Capreomycin Resistance

Kanamycin (KM) and Capreomycin (CPM), like SM are protein synthesis inhibitors by modifying ribosomal structure at 16s rRNA. Mutations at *rrs* position 1400 are associated with high-level resistance to KM and CPM in *M. tuberculosis* (Zhang and Yew, 2009). Also, studies revealed that mutation in *tly*A gene, encoding a putative rRNA methyl transferase is responsible for resistance in *M. tuberculosis*. Moreover, cross-resistance has been reported between KM and CPM (Maus*et al*., 2005). This could be attributed to multiple mutations in *rrs* gene in one strain. In contrast, Zhang and Yew, (2009) demonstrated that SM resistant strains are usually susceptible to KM and CPM.

Fluoroquinolones (FQs) resistance

Fluoquinolones (FQs) are important second-line drugs used for tuberculosis treatment. FQs are very active against *M. tuberculosis* with MICs of 0.25-3µg/ml. FQs such as Ciprofloxacin, Ofloxacin, Levoflaxacin and newer drugs such as gatifloxacin and maxifloxacin have broad spectrum activity (Wade and Zhang 2004). These drugs target DNA gyrase and prevent DNA supercoilling. The drugs contain two A and two B subunits, the gyrA and gryB. Boogaard *et al.* (2011) suggested that the problem associated with resistance to FQs by *M. tuberculosis* could be attributed to high prescriptions of the drugs to treat other bacterial infections. The mechanism of FQs resistance in *M. tuberculosis* depends on the frequency of mutation in subunit A of DNA gyrase encoded by *gyr*A (Wang *et al.,* 2007; Campbell *et al.,* 2011). Recently, new mechanism of quinolone resistance mediated by MfpA was reported (Wade and Zhang 2009). MfpA is a member of the pentapeptidase repeating family of protein from *M. tuberculosis*, whose expression result in resistance to FQs.

β-Lactam Antibiotics Resistance

Members of this group have been widely used in the treatment ofmany bacterial infections, but they are ineffective on *M. tuberculosis* due to production of a β-lactamase enzyme encoded in the chromosome of *M. tuberculosis* at a particular gene *bla*C (Wang *et al.,* 2007). In addition to production of β-lactamase, cell wall permeability and variation in some peptidoglycan biosynthetic enzyme is contributing to the increase in resistance (Flores *et al*., 2005).

Conclusion

Tuberculosis remains the second leading killer of Mankind after HIV. The increase in drug-resistance strains of *Mycobacterium tuberculosis* is associated with drug misuse, poor prescription, wrong diagnosis or interrupted drug supply coupled with HIV burden globally. The understanding of the nature of this pathogen and molecular study would serve to detect resistance rapidly and, to control the threat of the epidemic of multi-drug resistance, thereby opening a new beginning for developing new effective anti-TB drug with no resistance.

References

Ahmad, S. (2011). Pathogenesis, immunology, and diagnosis of latent *Mycobacterium tuberculosis* infection. *Clinical and Developmental immunology*. **doi:10**.1155/814943.

Almeida, D., Pedro E., Groll V. A., Martin. A., and Palomino, C. J. (2011). Efflux as a mechanism for drugs resistance *in Mycobacterium tuberculosis.FEMS. Immunology and medical microbiology*. **63**, Pp.1-9.

Anderson, C., Story, A., Brown, T., Drobniewski, F., & Abubakar , I. (2010). Tuberculosis in UK prisoners: a challenge for control. *Journal of epidemiology and community health, 64*(4), 373-376.

Arnold, C. (2006). Molecular evolution of Mycobacterium tuberculosis. *Clinical Microbiology and Infection.***13**, Pp.120-128.

Bårtfai, Z., Åkos, S., Kődmon, C., Nóra, S., Puskås, E., Kosztolányi.,Farago, E., Mester, J., Parsons, M. L., and Salfinger, M. (2001). Molecular characterisation of Rifampin-Resistant isolates of Mycobacterium tuberculosis from Hungary by DNA sequencing and the Line probe Assay. *Journal of Clinical microbiology*. **39** (10), Pp. 3736-3739.

Blanchard, S. J. (1996). Molecular mechanism of Drug Resistance in *Mycobacteriumtuberculosis .Annual Review of Biochemistry*. **65,** Pp. 215-239.

Bonfiali, A. A., De-miranda S. S., Compos, R. W., and Orefice, F.(2005).*Tuberculosis. Seminars in Ophthalmology.* **20,** Pp.169-175.

Boogaard, V. D. J., Semvua, H. H., Ingen, V. J., Mwaigwisya, S., Laan, V. T., Soolingen, D. V., Kibiki, S. G., Doeree, J.M., and Aarnoute, F. R. (2011). Low rate of fluoquinolones resistant in M. tuberculosis isolated from Northen Tanzania. *Journal of Antimicrobial Chemotherapy.* **66**, Pp. 1810-1814.

Borrel S. and Gagneux S. (2011). Strain diversity, epistatis and the evolution of drug resistance in *Mycobacterium tuberculosis*. *Clinical Microbiology and Infection*. **17**, Pp. 815-820.

Campbell, P. J., Morlock, G. P., Sikes , R. D., Dalton , T. L., Metchock B, B., Starks, A. M., et al. (2011). Molecular

detection of mutations associated with first- and second-line drug resistance compared with conventional drug susceptibility testing of Mycobacterium tuberculosis. *Antimicrobial Agents and Chemotherapy*, *55*(4), 2032-2041.

Capuano, V. S., Croix, A. D., Pawar, S., Zinovik, A., Myers, A., Lin, L. J., Bissel, S., Fuhrmann, C., Klein, E., and Flynn, L. J. (2003). Experimental *Mycobacterium tuberculosis* infection of Cynomolgus of human *M. tuberculosis*. *Infection and immunity*. **71** (10), Pp. 5831-5844.

Coninx, C., Mathieu, C., Debacker, M., Mirzoev, F., Ismaelove, A., Dehacker, R., and Meddling, D. (1999). First-line tuberculosis and drug-resistant Mycobacteria in prisons. *The New England Journal of Medicine*. **348**, Pp.199-128.

Corbett, L. E., Marstan , B., Churchyand, J. G., Decock, M. K. (2006).Tuberculosis in the Sub-saharan African: Opportunities, chakkenges, and change in the era of antiretroviral treatment. *The Lancet*. **367**, Pp. 926-937.

Crevel, V. R., Ottenhoff, H. T., and Vandermeer, W. M. J. (2002). Innate immunity to *Mycobacterium tuberculosis* .*Clinical Microbiology Reviews*. **15** (2), Pp.294-309.

Da Silva P.E.A and Palomino J.C (2011) Molecular basis and mechanisms of drug resistance in Mycobacterium tuberculosis: classical and new drugs. *Journal of Antimicrobial Chemotherapy*. **66**, Pp. 1417–1430.

Daum, T. L., Rodriguez, D. J., Worthy, A. S., Ismail, A. N., Omar, V. S., Dreyer, W. A., Floune, P. B., Hoosen, A. A., Chambers, P. J., and Fisher, W. G. (2012). Next-generation ion Torrent sequencing of Drug resistance mutation in *Mycobacteriumtuberculosis* strains. *Journal of clinical Microbiology*. **50** (12), Pp. 3831-3837.

Ebrahim, J. G. (2007). Drug Resistance in Tuberculosis. *Journal of Tropical paediatrics*. **53**(3), pp. 147-149.

Ernst, D. J. (1998). Macrophages Receptors for *Mycobacterium tuberculosis*. *Infection and Immunity*. **66** (4), Pp. 1277-1281.

Flores, R. A., Parsons, M. C., and Pavelka, S. M. (2005). Genetic Analysis of the β-lactamase of *Mycobacterium tuberculosis* and *Mycobacterium smegmatis* and susceptibility to β-Lactam. *Interantional journal of Systematic and Evolutionary Microbiology.* **151**, Pp. 521-531.

Flynn, L. J., and Chen, J. (2001). Tuberculosis: Latency and Reactivation. *Infection and immunity*.**69** (7), Pp.4195-4201.

Fonseca J.D., Knight G.M., McHugha, T.D. (2015). The complex evolution of antibiotic resistance in Mycobacterium tuberculosis. *International Journal of Infectious Diseases.* **32**, Pp. 94-100.

Fortune, M. S. (2012). The surprising Diversity of Myco bacterium tuberculosis: Change you can believe. *The Journal of Infectious Diseases*. **doi: 1093.**

Ginsburg Sarah Amy, Grosset H. Jacques and Bishai R. Willam (2003). Fluoroquinolones and tuberculosis. The Lancet Infectious Disease. 3(7), Pp.432-442.

Gumbo, T., Louie, A., Dezial, R. M., Liu, W., Parsons, M. L., Salfinger, M., and Drussano, L. G. (2007). Concentrate-dependant *Mycobacterium tuberculosis* killing and prevention of Resistant by Rifampin. *Antimicrobial Agent and Chemotherapy*. **51** (11), Pp. 3781-3788.

Hadano, Y (2013). Gram-ghost cells. BMJ Case report. doi:10.1136/bcr.2012-008477.

Heifets, L.B., Flory, M. A., and Lindholm-Levy, P.J. (1989). Does pyrazionoic acid as an active moiety of pyrazinaminde have specific activity against Mycobacterium tuberculosis? *Antimicrobial agent and Chemotherapy*. **33** (8), Pp. 1252-1254.

Hershber, R., Lipatove, M., Small, P. M. , Sheffer, H., Niemann, S., Homolka, S., Roach, R. J., Kremar, K., Petrov, A. D., Feldman, W. M., and Gagneux, S. (2008). High Functional Diversity in *Mycobacterium tuberculosis* Driven by Genetic Drift and Human Demography. *PLos Biol*. **6** (12), Pp.311-317.

Hordofa M.W., and Adela, T.B. (2015). Prevalence of Rifampicin Mono Resistant Mycobacterium Tuberculosis among Suspected Cases Attending at Yirgalem Hospital. *Clinical Medicine Research.* **4** (3), Pp. 75-78.

Kaufmann, H. E. S., and Gengenbacher, M. (2012). Myco bacterium tuberculosis: Success through dormancy. *FEMS Microbiology Reviews*. **36,** Pp.514-532.

Kulaga, S., Behr, M., Nguyen, D., Brinkman, J., Westley, J., Menzies, D., Brassard, P., Tannenbachem, T., Thibert, L., Boivin, J. F., Joseph, L., and Schwartzman, K. (2004).

Diversity of *Mycobacterium tuberculosis* Isolates in an Immigrant population: Evidence Against a founder effect. *American Journal of Epidemiology*. **159** (5), Pp. 507-513.

Lawn, D. S., and Zumla, I. A. (2011). Tuberculosis. *The Lancet*. **38,** Pp.57-72.

Mathema, B., Kurepina, E. N., Bifani, J. P., and Kreiswirth, N. B. (2006). Molecular Epidemiology of tuberculosis: Current insights. *Clinical Microbiology Review*. **19** (4), Pp. 658-685.

Maus, E.C., Plikaytis, B. B., and Shinnick, M. T. (2005). Molecular analysis of Cross-Resistance to Capreomycin, Kanamycin, and Viomycin in *M. tuberculosis*. *Antimicrobial Agents and Chemotherapy*. **49** (8), Pp. 3192-3197.

McDonough, A. K., Kress, Y., and Bloom, R. B., (1993). Pathogenesis of Tuberculosis: Interaction of *Mycobacterium tuberculosis* with macrophages. *Infection and Immunity*. **61** (7), Pp. 2763-2773.

Nair, N., Wares, F., and Sahu, S. (2010). Tuberculosis in the WHO South-east Region. *Bulletin of the World Health Organisation*. **8** (3), Pp. 164.

Palomino J.C., and Martin A. (2014) Drug Resistance Mechanisms in *Mycobacterium tuberculosis. Antibiotics*. ***3,*** Pp. 317-340.

Parnish, M. N., Dick, D. J., and Bishai, R. W. (1998). The mechanism of latency in *Mycobacterium tuberculosis .Trends in Microbiology***. 6 (**3), Pp.107-112.

Pedrozzoli, D, Fulton, N., Anderson, L., Lalor, M., Abubakar I., and Zenner (2012). Tuberculosis in the UK: Annual report on tuberculosis in the UK, 2012. London: Health Protection Agency. 2012.

Perri, D.G., and Bonora, S. (2004). Which Agent should we use for the Treatment of multi-drug resistant *Mycobacterium tuberculosis*? *Journal of Antimicrobial Chemotheraphy***. 54,** Pp. 593-602.

Pilke, C., Cox, S. H., Zarku, Nana., Karimovich, A. H., Braker, K., Diel, R., Rüsch-Gerdes, S., Fuerriege, S., and Niemann, S. (2010). embCAB sequence variation among ethambutol-resistant *M. tuberculosis* isolates without emb306 mutation. *Journal of Antimicrobial Chemotherapy*. **65**, Pp. 1359-1367.

Pourakbari B, Mamishi S., Zadeh, M.M., and Mahmoudi S (2016) First-Line Anti-Tubercular Drug Resistance of *Mycobacterium tuberculosis* in IRAN: A Systematic Review. *Frontiers in Microbiology*. **7***:1139. doi: 10.3389/fmicb.2016.01139.*

Quemord, A.,Lanèelle, G.,and Lacave, C.(1992). Mycolic acid synthesis: A target for ethionamide in Mycobacteria ?.*Antimicrobial Agents and chemotherapy*. **36** (6), Pp.1316-1321.

Roetzer, A., Schuback, S., Dieal, R., Gasau, F., Ubben, T., Di-Nauta, A., Richter, E., Rüsch-Gerdes, S., and Niemann, S. (2011). Evalution of *Mycobacterium tuberculosis* Typing methods in a 4-year study in Scleswing-Holstein, Northern Germany. *Journal of Clinical Microbiology.***49(**2), Pp.4173-4178 .

Scorpio, A., Lindaholm-levy, P., Heifets, L., Siddiqi, S., Cynamon, M., nad Zhang, Y. (1997). Character azationodpncA mutation in pyrazinamide -resistant Myco bacterium tuberculosis. *Antimicrobial Agents and Chemo therapy*. **41**(3), Pp.540-543.

Sekiguchi , J., Miyoshi-Akiyama , T., Augustynowicz-Kopec, E., Zwolska, Z., Kirikae, F., Toyota , E., et al. (2007). Detection of multidrug resistance in Mycobacterium tuberculosis. *Journal of Clinical Microbiology, 45*(1), 179–192.

Shamputa, C. I., Lee, J., Allix-Bèguec, C., Cho, E. J., Lee, J. I., Rajan, V., Lee, G., Min, H, J., Caroll, W. M., Goldfeder, C. L., Lee, H., Supply, P., Cho, S., Via, E. L., and Barrylll, E. C. (2010). Genetic diversity of *Mycobacterium tuberculosis* isolates from tertiary care tuberculosis Hospital South Korea. *Journal of clinical Microbiology.* **48** (2), Pp.387-394.

Shi, D., Li, L., Zhao, Y., Jia, Q., Li, H., Coulter, C., Jin, Q., and Zhu, G. (2011). Characteristic of embB mutation in multi-resistant Mycobacterium tuberculosis isolate in Henan China. *Journal of Antimicrobial Chemotherapy*. **doi:10.** 1093/jac/dkr284

Siddiqi, S., Takhar, P., Baldeviano C., Glover, W., and Zhang, Y. (2007). Isoniazid induces its own resistance in nonreplicating *Mycobacterium tuberculosis*. *Antimicrobial Agent and Chemotherapy*. **52** (6), Pp. 2100-2104.

Smith T., Wolff K.A, and Nguyen L. (2013) Molecular Biology of Drug Resistance in *Mycobacterium tuberculosis. Current Topic Microbiology and Immunology.* **374,** Pp. 53–80

Smith, I. (2003). *Mycobacterium tuberculosis* pathogen and molecular Determinants of virulence. *Clinical Microbiology Review*. **16** (3), Pp.463-496.

Somoskovi, A., Parsons, L. M., and Salfinger, M. (2001). The molecular basis of resistance to isoniazid, rifampin and pyrazinamide in *Mycobaaterium tuberculosi. Respirotory Research*. **2,** Pp. 164-168.

Spies, S.F., Almeida, E.P.D., Ribeiro, O. M., Rosetti, L. M., and Zaha, A. (2007). Identification of mutations related to streptomycin resistant in clinical isolates of *Mycobacterium tuberculosis* and possible involvement of Efflux mechanisms. *Antimicrobial Agent and Chemotherapy*. **52** (8), Pp. 2947-2949.

Stephen , H. G. (2002). Evolution of Drug Resistance in Mycobacterium tuberculosis. *Clinical and Molecular Perspective of Antimicrobial agents and chemotherapy, 46*(2), 267–274.

Takiff, H., and Guerrero, E. (2011). Currents projects for the Fluoroquinolones as first-line tuberculosis treatment. *Antimicrobial Agents and Chemotherapy*. **55** (12), Pp. 5421-5429.

Tenover, F. (2006). Mechanism of antimicrobial Resistance in bacteria. *The American Journal of Medicine*. **6,** pp. 3-10.

Vernon, A., Burman, W., Benatov, D., Khan, A., Bozeman, I. (1999). Acquired rifamycinmonoresistance in patients with HIV-related tuberculosis treated with once-weekly rifapentine and isozianid. *The Lancet*. **353,** Pp. 1843-1847.

Viveiros, M., Portugal, Isabel. Bettencourt, R., Victor, C. T., Jordan, M. A., Leando, C., Ordway, D., and Amaral, L. (2002). Isoniazid-induced Transient High-level Resistant in *Mycobacterium tuberculosis*. *Antimicrobial Agent and Chemotherapy*. **46** (9), Pp. 2804-2810.

Wabale V.R., and Joshi A.A. (2016) Pyrazinamide Drug Resistance in *Mycobacterium Tuberculosis*: A Minireview. *Journal of Bacteriology & Mycology.* **3** (2), Pp. 1-3.

Wade, M. M., and Zhang, Y. (2004). Mechanism of Drug Resistance in *Mycobacterium tuberculosis. Frontiers in Bioscience***. 9,** Pp.975-994.

Wang, J.Y., Lee, L.N., Lai, H.C., Wang, S.K., Jan, I.S., Yu, C.J., et al. (2007). Fluoroquinolone resistance in Myco bacterium tuberculosis isolates:associated genetic mutations and relationship toantimicrobial exposure. *Journal of Antimicrobial Chemotherapy, 59*, 860– 865.

World Health Organization. (2015). *Global Tuberculosis Report.* Geneva: World Health Organization. World Health Organization (2017). World TB Day: World health organization.
Doi:http://dx.doi.otg/10.15585/mmwr.mm6611a1.

Zhang, Y and Yew, W.W. (2009) Mechanism of drug resistance of in *Mycobacterium tuberculosis*. *International Journal of Tuberculosis and Lung Disease*. 13 (11), pp. 1320-1330.

Zhang, Y., Wade, M.M., Scorpio, A., Zhang, H., and Sun, Z. (2003). Mode of action of parazinaminde: disruption of Mycobacterium tuberculosis membrane transport and energetic by pyrazinoic acid. *Journal of Antimicrobial chemotherapy.* 52 (5), Pp. 790-795.