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Tuberculosis - A multisystemic disease and antimicrobial resistance in *Mycobacterium tuberculosis*

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Abstract

Tuberculosis (TB) is the leading global cause of infectious disease death. Though all age groups are at risk but it highly effects adults in their most productive years. Tuberculosis is preventable and curable. There are several drugs that work against tuberculosis disease which target various aspects of *Mycobacterium tuberculosis* (causative agent of TB). Though some drugs (rifampicin, pyrazinamide) are much more effective but the situation gets worse by the presence of multi drug-resistant strain of Mycobacterium. Antimicrobial resistance occurs as a result of biological variations in drug uptake or substandard drugs. In the last two decades, multidrug-resistant strain of *Mycobacterium tuberculosis* has emerged as a threat to public health, stressing the need to develop new tuberculosis prevention and treatment strategies. Advances in many technologies such as Whole Genome Sequencing (WGS) technology use as a tool for rapid diagnosis and clinical management of TB. The introduction of new database such as Relational sequencing TB data platform may be helpful. In this review article, provide an update on advances in our understanding to new, existing drugs and repurposed agents, it will help devise better molecular diagnostics for more effective drug resistance tuberculosis (DR-TB) management enabling personalized treatment and will facilitate the development of new drugs.

Keywords: Antimicrobial resistance, Molecular diagnostics, Mycobacterium tuberculosis, Multi Drug-resistance, Tuberculosis, Whole Genome Sequencing.

1. Introduction

Tuberculosis is a serious health problem worldwide. It is a multisystemic disease caused by *Mycobacterium tuberculosis* bacteria that usually affect lungs and many other parts of the body such as the kidneys, the brain and the spine. According to World Health Organization (WHO), around 8 million people develop active tuberculosis and nearly 2 million people die from the disease. Tuberculosis caused by *Mycobacterium tuberculosis* remains a major public health concern. Despite having an effective treatment regimen, 10.4 million new cases were reported in 2021, of which 0.48 million were caused by *Mycobacterium tuberculosis* strains classified as multidrug resistant. Although the TB incidence has declined over the past decades, the

threat of TB on public health has further worsened with the emergence of drug resistant TB, in particular multidrug- resistant TB(MDR-TB) and extensively drug resistant TB(XDR-TB). The prevalence of drug resistant TB is becoming a global challenge to tuberculosis control programs. Causative agent of tuberculosis resistant to at least one first line drug only known as Mono- resistant. In addition, tuberculosis is resistant to at least two or more anti-tuberculosis drug other than both isoniazid and rifampicin is called poly resistance. Multidrug resistance tuberculosis (MDR-TB) is resistant to at least both isoniazid and rifampicin. Pre extensively drug-resistant tuberculosis (pre-XDR-TB) is MDR-TB plus resistant to any one of the second-line injectable drugs. Finally, extensively drug resistant tuberculosis (XDR-TB) is resistant to isoniazid, rifampicin, and fluoroquinolone antibiotic as well as bedaquiline, linezolid or one of the three second line injectable drugs.

1.1 Mechanism of drug resistance

Mycobacterium tuberculosis, causative agent of TB has natural defenses against some drugs and can acquire resistance through genetic mutations. The traditional mechanism by which bacteria achieve antimicrobial resistance are – (1) *Barrier mechanisms* - the complex lipid molecules in the cell wall of *Mycobacterium tuberculosis* act as a barrier to stop drugs from entering the cell, (2) *Degrading and inactivating enzymes* - the target gene encodes for enzymes (phosphorylated, adenylated) that deactivate drug molecules, (3) *Drug efflux systems* – the cell wall of bacteria contains molecular systems that actively expel the drug molecules out of the cell, (4) *Mutation* - spontaneous mutations in the genome can make to change the drug resistant proteins, making drug resistant strain.

1.2 Resistance to first line TB drugs

Streptomycin, isoniazid, rifampicin, pyrazinamide, ethambutanol are some first line drug that use to treat TB.

Mechanism and action of streptomycin drug

S12 ribosomal protein and 16S rRNA the main components of the 30s subunit of the mycobacterial ribosome. Streptomycin is an active drug that acts by irreversibly attaching to the S12 ribosomal protein and 16S rRNA. Streptomycin inhibits protein synthesis by blocking translation process. Mutation in *rrs* and *rpsL* genes that encodes for 16S rRNA and S12 ribosomal protein are the major mechanisms of resistance to streptomycin (Gillespie et al., 2002). Recently mutations in *gidB* gene, encoding a 7- methylguanosine methyltransferase specific for methylation of G527 in loop of the 16S rRNA have been implicated in low level streptomycin resistance. Mutations in the *gidB* gene, that encode a 7-methylguanosine methyltransferase which is specific for methylation of the G27 in loop of the 16SrRNA have been implicated in low level streptomycin resistance. Whole genome sequencing (WGS) analysis has also demonstrated a deletion of 130bp within *gidB* gene, possibly mediating streptomycin drug resistance (Ioerger et al., 2009).

Mechanism and action of isoniazid drug

Isoniazid is usually present in an inactive form but during metabolism they are activated by catalase/peroxidase enzyme by *katG* gene. The mode of action of isoniazid resistance is complex. Active form of isoniazid inhibits mycolic acid synthesis via the NADH-dependent enoyacyl carrier protein reductase encoded by *inhA* gene (Palomino et al., 2014). Mutation in *inhA* gene or its promoter region and *katG* gene are two main molecular mechanism of drug resistance of isoniazid. A recent study revealed that mutations in the *inhA* regulatory region and coding region resulted in high level isoniazid resistance and cross resistance to ethionamide. Mutations in the *drfA* gene have recently been implicated in resistance to isoniazid. A recent systematic review found that mutations in *katG* and *inhA* accounted for 64.2% and 19.2% of isoniazid resistance, respectively (Gegia et al., 2017).

M*echanism and action of rifampicin drug*

Rifampicin is one of the most effective drugs against TB treatment. Rifampicin diffuses freely through the cell wall of *Mycobacterium tuberculosis* and inside the cell it binds to the ß subunit of the RNA polymerase enzyme and inhibit gene transcription thus prevents the elongation of mRNA. Resistance to rifampicin is mediated by mutations clustered in codons 507-533 of the gene coding for the RNA polymerase β subunit, *rpoB*, this is called rifampicin resistance determining region (RRDR). Rifampicin resistance is rare type which occurs in conjunction with resistance to other drugs that makes rifampicin targets a surrogate marker of the multi drug resistant (MDR) phenotype (Traore et al., 2000). Whole genome sequencing (WGS) analysis demonstrated mutations in the *rpoA* and *rpoC* genes, which encode alpha and β' subunit of RNA di polymerase as compensatory mechanism in isolates that bear mutations in the *rpoB* gene (Fonseca et al., 2015).

Mechanism and action of pyrazinamide drug

Pyrazinamide is a nicotinamide analogue and vital characteristic of pyrazinamide is its ability to inhibit semi dormant bacilli located in acidic environments such as that of TB lesions. Like isoniazid, pyrazinamide drug is a prodrug that activated by enzyme nicotinamidase, encoded by the *pncA* gene (Konno et al., 1967). Pyrazinamide drug enters into mycobacterial cell by passive diffusion and converted into pyrazinoic acid. The pyrazinoic acid is protonated in an acidic environment condition and allows cell reabsorption. Thus lead to cellular damage. Mutations in the gene *pncA* and its promoter region remain the common uttermost mechanism mediating pyrazinamide drug resistance. Recently *panD* mutations have been associated with pyrazinamide resistance. Whole genome sequence analysis the presence of *panD* mutations in pyrazinamide resistant isolates and the inclusion of these in screening has been recommended to enhance the detection of pyrazinamide resistance.

Mechanism and action of ethambutanol drug

Ethambutanol is one of the most effective first line drug that interacts with the arabinosyl transferases which usually involves in the biosynthesis of arabinogalactan (AG) and lipoarbinomannan (LAM). It specifically inhibits the polymerization of cell wall arabian, which leads to the accumulation of b-D-arabinofuranosyl-1-monophosphoryldecaprenol (DPA). Alteration in codon 306 of the *embB* gene is most common resistance mechanism to date (Takayama and Kilburn,1989, Sreevatsan et al., 1997). Recent studies have revealed that mutations in the decaprenylphohoryl- β-D-arabinose biosynthetic and utilization pathway genes (*Rv3806c and Rv379*), which occur simultaneously with mutations in embB and embC, result in variable MIC range for ethambutanol. Allelic exchange experiments concluded that only certain amino acid substitutions led to ethambutanol resistance (Safi et al., 2008).

1.3 Resistance of second line TB drugs

Mechanism of quinolone resistance

Quinolones are potent bactericidal antibiotics currently used as second-line treatment for drug resistance tuberculosis (DR-TB). The action of this drug is inhibited by the activity of DNA gyrase and topoisomerase IV. Both targets are type II topoisomerase, that have distinctive functions within mycobacterial cell. The main mechanism of developing fluoroquinolone resistance in *Mycobacterium tuberculosis* is chromosomal mutations within quinolone resistance determining region (QRDR) of *gyrA* or *gyrB* genes. Mutations in codon 74,88,90,91 and 94 of *gyrA* have been associated with fluoroquinolone resistance. In addition, novel mutations at codons, such as Leu109Pro, Met81Thr have been detected in fluoroquinolone drug resistant isolates within the *gyrA* gene. Mutations in *gyrB* genes are rare. Recent study revealed that efflux mechanisms have also been associated with fluroquinolone resistance (Escribano et al.,2007).

Mechanism of second-line injectable drugs resistance

Second-line injectable agents

Aminoglycosides (amikacin, kanamycin) and cyclic peptides (capreomycin) referred to as second-line injectable agents currently applied to the treatment of drug resistant TB. This agents inhibit protein synthesis through modification of ribosomal structures at 16srRNA and formation of 30s ribosomal subunit respectively. High level resistance has been associated with mutations in the 1400bp of the *rrs* gene and additional resistance to capreomycin has been associated with polymorphisms of *tlyA* gene.

Mechanism of ethionamide drug resistance

Ethionamide is prodrug that usually present in an inactive form and activated by the mono-oxygenase enzyme, encoded by the *eth A* gene. It is a derivative of isonicotinic acid and a structural analogue of isoniazid. After the activation, ethionamide inhibits mycolic acid synthesis during cell wall biosynthesis by inhibiting the enoyl-acyl carrier protein reductase enzyme. Resistance to the ethionamide has been linked to the mutations that occurs in the *eta*A/*eth*A, *eth*R and *inh*A genes. study has recently demonstrated the role of the *msh A* gene encoding an enzyme essential to mycothiol biosynthesis as a target for ethionamide resistance using spontaneous isoniazid and ethionamide resistant mutant (Vilcheze et al., 2008).

Mechanism of para-amino salicylic acid

Para amino salicylic acid was one of the first antibiotics to treat tuberculosis together with isoniazid and streptomycin and it is now a part of second line treatment regimens applied to treat drug resistant tuberculosis. It is the analogue of para- amino benzoic acid. The main mechanism mediating para -amino salicylic acid resistance has been identified as mutations occurring in the *thy*A gene, accounting for 40% para- amino salicylic

acid resistance (Rengarajan et al., 2004). A study has recently demonstrated that mutations in *folc* (Zhao et al., 2014), which encodes dihydrofolate synthase, conferred resistance in clinical isolates.

1.4 Mechanism of resistance to new and repurposed drugs

Bedaquiline Bedaquiline acts by targeting mycobacterial ATP synthase, inhibiting bacterial respiration. In combination with pyrazinamide, bedaquiline has acted with remarkable sterilizing activity in a mouse model. Target based mutations in the *atpE* gene in strains selected in vitro have been associated with high level resistance to bedaquiline, with up to a 4 fold increase in minimal inhibitory concentration (MIC). The gene encodes the mycobacterial F1F0 proton ATP synthase, a key enzyme in ATP synthase and membrane potential generation.

Mechanism of delamanid and pretomanid resistance

Delamanid acts by inhibiting the production of methoxy and keto mycolic acid through the mycobacteria F_{420} system generating nitrous oxide. Delamanid with the help of isoniazid prevent the synthesis of mycolic acid which play a crucial role in the mycobacterial growth survival. Mutations in enzymes and one of four coenzymes *F420* genes. *fgd, RV3547, fbiA, fbiB* and *fbiC* have been associated to the mechanisms of resistance to delamanid drug [Manjunatha et al.,2006]. Upon activation, the redical intermediate formed between delamanid drug and desnitro imidazooxazole derivative is mediated anti-mycobacterial actions through the inhibition of methoxy mycolic acid and keto mycolic acid synthesis, leading to the dwindling of mycobacterial cell wall components and destruction of the mycobacterial cell. Pretomanid is a prodrug that requires activation by deazaflavin-dependent nitro-reductase, which is encoded by *ddn*. *ddn* converts the product into three metabolites, which include des-nitro-imidazole and two unstable byproducts. Des-nitro-imidazole compounds promote the anaerobic activity of these compounds by generating reactive nitrogen species, including nitric oxide which then boost the host-macrophage killing of *Mycobacterium tuberculosis* (MTB) (Manjunatha et al., 2006, Singh et al., 2008). Resistance to the pretomanid drug has been linked to mutations in the genes related to drug activation or genes associated with F420 biosynthesis pathway.

Mechanism of linezolid resistance

Linezolid is an oxazolidinone and the first antibiotic in its class to be approved for the treatment of TB. Linezolid acts by binding to the V domain of the 50S ribosomal subunit, thereby inhibiting an early step in protein synthesis. Resistance to linezolid has been linked with mutations in 23S rRNA gene. The mutations at codons G2061T and G2572T in the *rrl* gene were associated with higher level resistance in the range of 16-32mg/L, and mutants bearing lower- level resistance of 4-8mg/L had no alteration in the *rrl* gene. *Bloemberg et al.* detected the *rrl* G2576T and A572C mutations in a patient with corresponding phenotypic linezolid resistance (Singh et al., 2008). They sequenced sequential isolates represented a single Beijing strain that evolved over time.

Conclusion

Drug resistant tuberculosis remains a critical public health challenge of modern times. This review highlights the complexity of drug resistance in MTB, captured by the advances of sequencing technology. Large scale analysis conducted on whole genome sequences have assisted in cataloging various causative to compensatory or adaptive mutations and their varying roles in mediating drug resistance in the organism. Recent studies have also revealed harbinger mutations, capable of predicting resistance at the earliest possibility. The dynamics of resistance development and the factors that facilitate resistance development within a patient are still severely understood and require further elucidation. Resistance conferring mutations in mycobacteria can develop dynamically over time under drug pressure in patients. Rapid WGS is the most promising utility for the personalized case of patients with DR-TB.

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