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Tuberculosis Drug Resistance: An Overview

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ABSTRACT

Tuberculosis (TB) is a serious public health problem worldwide. The situation is worsened by presence of multidrug resistant (MDR) strain of *Mycobacterium tuberculosis*, the causative agent of disease (2).Tuberculosis (TB) declared as a global emergency in 1993 by WHO (9). The current global concern in the treatment of tuberculosis (TB) is the emergence of resistance to the two most potent drug *viz.*, isoniazid and rifampicin (1). This review article discusses the mechanism of action of anti-tuberculosis drug and drug resistance in *M. tuberculosis.*

INTRODUCTION

Despite all the advances made in treatment and management, tuberculosis (TB) still remains as one of the main public health problem, particularly in the developing countries. Drug resistance in tuberculosis was observed early as 50 years ago, the response of patient with MDR-TB to treatment is poor and mortality rate is usually high (1). More recently, a more worrying situation has emerged with description of *M. Tuberculosis* strain that has been found resist to all antibiotics that were available for testing, a situation labeled as totally drug resistant (TDR)-TB (2,13,14). A person with active TB disease has drug resistance TB if the bacteria that the person is infected with, will not respond to, at least one of the main TB drugs (5). Multidrug-resistance TB (MDR-TB), caused by a strain of *M. tuberculosis* resistant to at least rifampicin and isoniazid, and extensively drug-resistant TB (XDR-TB), caused by strain of *M. tuberculosis* that, in addition to being MDR, are also resistant to any fluoroquinolone and to at least one of the three injectable drug *Kanamycin, Capreomycin* and *Amikacin*, again threaten adequate control of the disease (9,15,16). A better knowledge of the mechanism of action of anti –TB and development of drug resistance will allow identifying new drug target and better way to detect drug resistance (2).

DEFINITION

Drug resistance in mycobacterium is defined as a decrease in sensitivity to a sufficient degree to be reasonably certain that the strain concerned is different from a sample of a wild strain of human type that have never come in contact with the drug (1,17).

Types of resistance:

Resistance is of two types

I. **Primary resistance:**

When drug resistance is demonstrated previously in a patient who has never received anti-TB treatment previously (1). Who have never treated for TB and who were presumably infected with a resistant strain of *M. Tuberculosis* (6).

II. **Acquired resistance:**

Resistance occurs as a result of specific previous treatment (1). It is mainly caused by a spontaneous mutation in chromosomal genes, producing the selection of resistant strains sub-optimal drug therapy (4,18).

Intrinsic drug resistance of *M. tuberculosis* has traditionally been attributed to the usual structure of its mycolic acid- cell wall that give bacteria low permeability for many compounds such as antibiotics and other therapeutic agents (4,19). The world health organization (WHO) and the International Union Against Tuberculosis and Lung Disease (IUATLD), in the light of discussion in several international fora, have replaced the term primary resistance by the term "drug resistance among new cases" and acquired resistance by the term "drug resistance among previously treated cases (1,37).

Causes of drug resistance:

The drug resistance in *M. Tuberculosis* has been associated with variety of management, health provider and patient-related factor (1):

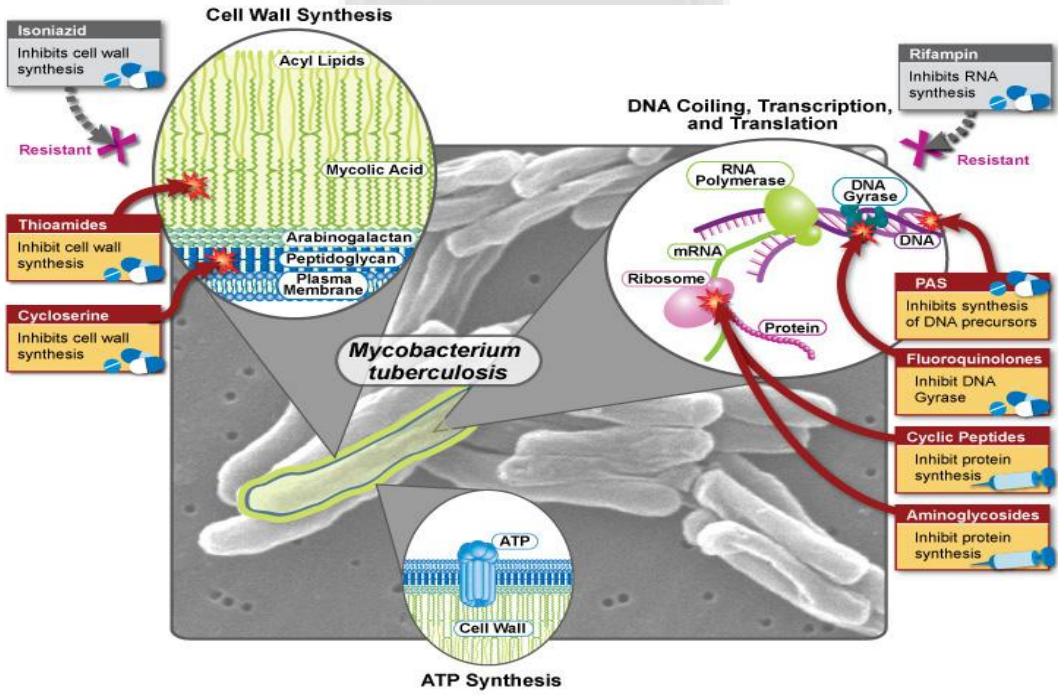
- Deficient or deteriorating TB control programmes resulting in inadequate administration of effective treatment
- Poor case holding, administration of substandard drug, inadequate or irregular drug supply and lack of supervision
- Ignorance of health care workers in epidemiology, treatment and control
- Improper prescription of regimens
- Interruption of chemotherapy due to side effect
- Non-adherence of patient to the prescribed drug therapy
- Availability of anti –TB drug across the counter, without prescription
- Massive bacillary load
- Illiteracy and low socio- economic status of the patient
- The epidemic of HIV infection
- Laboratory delays in the identification and susceptibility testing of *M. Tuberculosis* isolates

- Use of non-standardized laboratory techniques, poor quality drug powder and lack of quality control measures
- Use of anti-TB drug indications other than tuberculosis (1).

Mechanism of resistance:

Drug resistance in *M. tuberculosis* occurred by random, single step, spontaneous mutation at a low but predictable frequency, in large bacterial population. The probability of incidence of drug-resistant mutant is 10^{-8} for rifampicin, while for isoniazid and some of the other commonly used drug it is 10^{-6}. Therefore, the probability for resistance to both isoniazid and rifampicin to develop is 10^{-14} (1). The phrase "MDR state" in mycobacteriology refers to simultaneous resistance to at least RIF and INH (3,20) (with or without resistance to other drug). Genetic and molecular analysis of drug in MTB suggest that resistance is usually acquired by the bacilli either by alteration of the bacilli or by alteration of drug target through mutation (3,21) or bacilli titration of the drug through overproduction of target (3,22).

Table no: 1



(see ref no.8)

Mechanism of action and resistance in *Mycobacterium tuberculosis*:

❖ **First line anti –TB drugs**

Rifampicin

Rifampicin is one of the most effective anti-TB antibiotics and together with isoniazid constitutes bases of multi-drug treatment regimen for TB (2). Rifampicin is active against growing and non-growing (slow metabolizing) bacilli (2,23). The mode of action of rifampicin in *M. tuberculosis* is by binding to the β-subunit of the RNA polymerase, inhibit the elongation of messenger RNA (2,24). Rifampin resistance is caused by point mutation or nucleotide deletion or insertion in an 81base pair region of the *rpoB* subunit of DNA-dependant RNA polymerase (6,25). The rate of mutation is 2.25×10^{-10}, resulting in resistance of 1 in 10^8 bacilli in the drug-free environment (6). Rifampin resistance is considered a major surrogate marker for MDR-TB, since greater than 90% of isolates resistant to rifampin are also resistant to isoniazid (12).

Isoniazid

Isoniazid is an anti-TB agent and it remains together with rifampicin (2). Isoniazid act by inhibiting synthesis of mycolic acid through the NADH-dependant enoyl-acyl carrier protein (ACP)- reductase, encoded by *inhaA* (2,26). Isoniazid is a prodrug that requires activation by the mycobacterial catalase peroxidase KatG after it enters the cell by passive diffusion. The activated isoniazid targets two principle enzymes that are involved in the elongation cycle of the fatty acid molecule, an enoyl-acyl carrier protein reductase (inhA) and beta-ketoacyl-acyl carrier protein synthase, resulting in the inhibition of synthesis of the mycolic acids necessary for the mycobacterial cell wall (12,27,28). Co-resistance to isoniazid and ethionamide has been clearly demonstrated to caused by mutations in ndh in *M. smegmatis* and *M. bovis* BCG, by altering the NADH/NAD ratios inside the cell, leading to a competitive of the INH-NAD (2,29,30).

❖ **Second line Anti-TB drugs:**

Fluoroquinolones

Fluoroquinolones are currently in use as a second-line drug in the treatment of MDR-TB. Both ciprofloxacin and ofloxacin are synthetic derivatives of the parent compound nalidixic acid, discovered as a by-product of the antimalarial chloroquine (2,31). Mode of action of fluoroquinolones is by inhibiting the topoisomerase II (DNA gyrase) and topoisomerase IV, two critical enzymes for bacterial viability (2). Their proteins are encoded by the genes *gyrA, gyrB, parC* and *parE* respectively (2,32). The main mechanism of development of fluoroquinolone resistance in *M. tuberculosis* is by a chromosomal mutation in the quinolone resistance-determining region of *gryA* or *gryB* (2).

Kanamycin, Capreomycin, Amikacin, Viomycin:

Kanamycin and amikacin are aminoglycoside antibiotics while capreomycin and viomycin are cyclic antibiotics. They belonging to two different antibiotic families, all exert their activity at the level of protein translation (9). Kanamycin and amikacin inhibit protein synthesis by alteration at the level of 16S rRNA. The most common mutation found in kanamycin – resistant strains are at position 1400 and 1401 of the *rrr* gene, conferring high-level resistance to kanamycin and amikacin (2).

New Anti-TB drugs

Several new drugs are being proposed as candidates for the treatment of TB. They exert their activity by interacting with different targets, which are in many cases different from the classical target of other anti-TB drugs (4).

Bedaquiline

It is also known as TMC207 or R207910, bedaquiline is a new antibiotic belonging to the class of diarylquinolines with specific activity against *M. tuberculosis*, which has shown *in vitro* activity against other non-tuberculous mycobacteria (2,33).The mode of action of bedaquiline is

by inhibiting the ATP synthase of *M. tuberculosis*, which was a completely new target of action for an antimycobacterial drug (2).

Delamanid

Previously known as OPC-67683, is a derivative of nitro-dihydro-imidazooxazole with activity against *M. tuberculosis* that acts by inhibiting the synthesis of mycolic acid and is undergoing clinical evaluation in a phase III (2,34). Delamanid shown to have a very good *in vitro* and *in vivo* activity against drug-susceptible and drug-resistant *M. tuberculosis* (2,35).

SQ-109

The mode of action of SQ-109 is by interfering with the assembly of mycolic acids into the bacterial cell wall core, resulting in accumulation of trehalose monomycolate, a precursor of the trehalose dimycolate (2). It also shown *in vitro* and *in vivo* activity against drug-susceptible and drug resistant (2,36)

Drug–susceptibility testing method

Drug-susceptibility of *M. tuberculosis* can be determined either by observation of growth or metabolism inhibition in a medium containing the antituberculosis drug or by detection, at the molecular level of mutation in genes related to drug action (11). Drug susceptibility is determined on the basis of growth inhibition induced by the drug by mean are as follows:

1. Macroscopic observation of growth in drug-free and drug containing media
2. Detection or measurement of metabolic activity or products
3. Lysis with mycobacteriophage
4. Detection of genetic mutation using molecular techniques (11).

Many new possibilities emerge for detection of drug resistance in *M. tuberculosis* and for performing drug susceptibility test (10), They are as follows:

Phenotypic method

The original system using liquid media for DST was the BACTEC 460 TB system. Limited by an issue with handling and disposing of radioactive material, it was supplanted by the BACTEC MGIT 960 system which used fluorescent light emission for detection of TB growth (12).

Other novel rapid phenotypic method include the colorimetric method that use the colour change of chemical dye (i.e., tetrazolium bromide and resazurin) for culture DST, the microscopic observation of drug susceptibility and the nitrate reduction assay (12). In phenotypic method, following method are there,

1) Absolute concentration method

Several concentrations of each drug is tested and resistance is expressed in term of the lowest concentration of the drug that inhibit growth i.e., minimal inhibitory concentration (MIC) (10).

2) Resistance ratio method

It compares the growth of unknown strains of tubercle bacilli with that of a standard laboratory strain (H37Rv). Resistance is expressed as a ratio of MIC of test strain to the MIC of standard strain in the same set (10).

3) Proportional method

This method is currently the method of choice for estimating drug resistance and this principle is being applied to the following rapid testing method.

i. BACTEC 460 (First line and second line drug)
ii. MGIT 960
iii. MB/ Bac T system, and
iv. ESP II system (10).

Genotypic method

These are essentially required for rapid identification of multidrug resistance (MDR) TB strains. Genotypic method has the advantage of being rapid and specific. Genetic and molecular analysis

of drug resistance in MDR-TB suggest, that the bacilli usually acquire resistance either by altering the drug target by mutation or by titration of the drug through over- production of the target (10).

Important genotypic drug susceptibility testing methods are given below:

1. DNA sequencing:

Conventional DNA sequencing utilizes a "chain –termination method" to sequence DNA fragment. It first binds a primer to a denatured single strand DNA. DNA extension then begins at the primer site using a DNA polymerase (12). The DNA sequencing is used for characterization of the mutation responsible for drug resistance (10).

2. PCR SSCP:

It is based on the property of single stranded DNA to fold into a tertiary structure whose shape depends on its sequence. Single strand of DNA differing by only one or a few bases will fold into a different configuration with different conformation with different motilities on a gel, producing what is called single strand conformation polymorphism (SSCP) (10).

3. LiPA (solid phase hybridization assay):

The LiPA is based on the hybridization of amplified DNA from the cultured strain or clinical specimen to ten probes encompassing the core region of *rpo* B gen of *M. tuberculosis*, which is immobilized on nitrocellulose strip (10).

Management

The World Health Organisation now insists on the practice of directly observed therapy (DOT) i.e. supervised swallowing. This should not only cure the patient but also prevent the development of drug-resistant disease since the patient will have no opportunity to give himself or herself monotherapy (7).

CONCLUDING REMARKS

Till today, many reviews on tuberculosis drug resistance in *M. tuberculosis* were published. Here we published an updated overview of the tuberculosis drug resistance in *M. tuberculosis* to main classical and new anti-TB drugs. The new techniques are introduced for detecting drug resistance in *M. tuberculosis.*

There are many new techniques available for diagnosis of TB. By taking advantage of new genotypic and phenotypic methods we can detect and increase drug resistance to TB.

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