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Conventional and Advanced Diagnostic and Therapeutic Methods for Multi Drug Resistant Tuberculosis.

Shuchi Kaushik, Priyanka, Sharmistha Banerjee, and Rajesh Singh Tomar *.

Amity Institute of Biotechnology, Amity University Madhya Pradesh, Gwalior [M.P.], INDIA

ABSTRACT

Infectious diseases are the most important health concern in developing and underdeveloped countries. The poor or impoverished population of developing countries does not have proper access to fundamental medicines. Tuberculosis [TB] is one among such diseases, caused by Mycobacterium tuberculosis [MTB] and is widely dispersed and known to humans since long back. Although mass vaccination with Mycobacterium bovis BCG has been done and several anti-tubercular drugs [ATD] are developed, the clinical management of drug-resistant TB remains as a major challenge. The rapid identification of new phenotypic and genotypic traits, the execution of effective antimicrobial therapy and infection control programs, and the advancement of alternative treatment processes are required to combat the spread of drug resistance. Nanotechnology based drugs may prove to be an effective treatment in this direction.

Keywords: Drug resistance, anti-tubercular drugs, clinical management, nanotechnology,

Corresponding author

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INTRODUCTION

The capacity of bacteria or other microbes to tolerate the effects of an antibiotic or when an antibiotic is not able to inhibit bacterial growth then it is called antibiotic resistance [1]. In other terms, the multiplication of bacteria carries on in the presence of an antibiotic and the bacteria are termed to be antibiotic tolerant or resistant [2]. In bacteria various types of mechanisms have evolved which may be the reason of an antibiotic resistance, such as antibiotic is inherently resistant, change in pre existing genetic material of microbe, or by adding genetic material which is not originally present, from other sources via vertical or Horizontal gene transfer. One or more mechanisms may play role in drug resistance and more than one antibiotic can be resisted by the bacteria [1]. Tuberculosis is one of the major challenges faced by world especially developing countries. The prevalence of multidrug resistant tuberculosis bacteria demands the use of advance tools and techniques such as nanotechnology based methods to combat against this dreadful bacteria.

ANTIBIOTICS: MODE OF ACTION & MECHANISM OF DRUG RESISTANCE

Antibiotics

An antibiotic is a compound produced by microorganism which ceases the growth of other microorganisms. Each antibiotic may be effective against a particular bacterium or may show broad spectrum activity. Antibiotics may kill microorganisms or stop them from regenerating [1].

Table 1: Classification of Antibiotics and their mode of action

S. No	Group	Example	Mode of action
1	Beta-lactams	Penicillins, Cephalosporins, Carbapenems, Monobactams	Restrain Cell Wall Synthesis
2	Glycopeptides	Vancomycin, Teicoplanin, Telavancin	Restrain Cell Wall Synthesis
3	Macrolides and Ketolides	Azithromycin, Telithromycin Erythromycin, Clarithromycin	Restrain Protein Synthesis
4	Aminoglycosides	Gentamicin, Amikacin Tobramycin, Netilmicin Streptomycin	Restrain Protein Synthesis
5	Tetracyclines and Glycylcyclines	Tetracycline, Tigecycline, Doxycycline, Minocycline	Restrain Protein Synthesis
6	Quinolones	Ciprofloxacin, Norfloxacin, Levofloxacin, Moxifloxacin	Restrain DNA Gyrase
7	Lincosamides	Clindamycin	Restrain Protein Synthesis
8	Streptogramins	Quinupristin/ Dalfopristin	Restrain Protein Synthesis
9	Oxalidinones	Linezolid	Restrain Protein Synthesis
10	Lipopeptides	Daptomycin	Destroy Cell Membrane Structure
11	Polymixins	Colistin, Polymixin B	Destroy Cell Membrane Structure
12	Ansamycins	Rifampicin	Restrain Protein Synthesis
13	Sulfa drugs	Sulfamethoxazole-trimehoprim	Restrain DNA Synthesis

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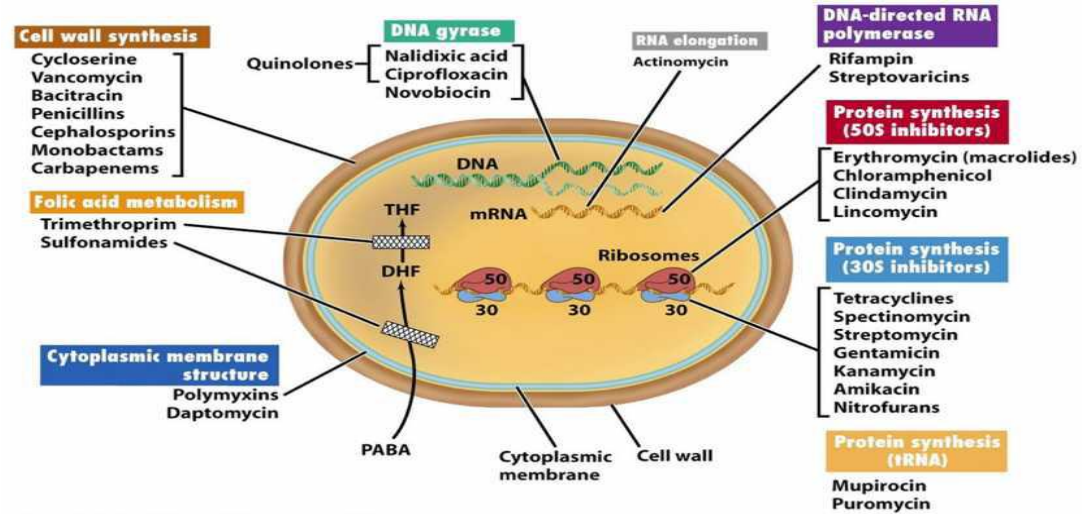

Fig. 1 Mechanisms of action of antibiotic in bacteria
(Source: Brock Biology of Microorganism 11/e, Pearson Prentice Hall, Inc.)

Antibiotic resistance among bacteria

The ability of an organism to tolerate or resist an antibiotic is called antibiotic resistance. An organism may remain alive or multiplies continuously in the presence of an antibiotic. Bacteria may be described as resistant when an antibiotic is not able to inhibit the bacterial growth and when the bacterial colonies are unsusceptible to the concentration of antibacterial agent used in practice [3]. Antibiotic tolerance generates when antibiotic is used excessively or misused. Resistance is generated when harmful bacteria are developed over time. Their first function is to regenerate, flourish and to disseminate fast and efficiently. Thus bacteria accommodate to their environment and modify in ways that helps them for their existence [1].

Bacteria Show Resistance in numerous ways

- **Intrinsic Resistance:**

  Intrinsic Resistance to an antibiotic agent characterizes resistance that is an inborn attribute of a specific species; the proper susceptibility targets of antibiotic may be limited or natural barriers are contained by these bacteria which hinder the agents from achieving the target; for instances the resistance of vancomycin drug in bacterial strain which is gram negative is due to incapability of vancomycin to enter the outer membrane, or the penicillin resistance is also intrinsic resistance of bacteria [4,5].

- **Circumstantial Resistance**:

  The antibiotic that seem to be responsive *in vitro* may be effectless *in vivo* due to incapability to reach the particular infection site is called Circumstantial Resistance, as, for example, the inability of first generation cephalosporins to cross the blood-brain barrier, *in vivo* antagonistic activity of trimethoprim-sulfamethoxazole can be overcome by enterococci via their inability to take up and internalize environmental folate [6,7].

- **Acquired Resistance:**

  Acquired Resistance displaces the true change in the bacterial genetic composition through which the drug that once was effective *in vivo*, no longer remains effective [6]. The major mechanisms that bacteria employ so that they may be able to evade the antimicrobials comprise following strategies: bacteria may limit the cellular concentration of the drug by decreased entry rate or increased outflow of the drug, neutralization of the drug by biocatalysts that reversibly or irreversibly inactivate the

drug, modification of the target so that the agents no longer will obstruct it, and removal of the target by creating new metabolic pathways [8, 9, 10].

Mechanism of antibiotic resistance in bacteria

Many different mechanisms are evolved in the bacteria which are responsible for drug resistance. Microbes may employ single or merge several mechanisms against a single drug or class of drugs or a single alteration may cause development of resistance to various agents [6, 8, 9, 10]

Genetics of antibiotic resistance

A recognizable genetic plasticity is found in bacteria which permit them to respond to a numerous set of environmental challenges. Bacteria sharing the same ecological niche as that of antimicrobial generating organisms may have developed classic mechanism to oppose the effect of dangerous antibiotic agent and as a result intrinsic resistance allow them to flourish in its presence [11].

Mutational Resistance

In this context, a group of bacterial cells obtained from a susceptible population create gene mutation through which the drug activity is affected; consequently cell survival is preserved even in the presence of the antibiotic agent. When a resistant mutant is emerged, the susceptible population of bacteria is eliminated by the antibiotic and the resistant bacteria predominate.

In general, mutation creates antibiotic resistance which alters the action of antibiotic through different types of mechanism such as [11]

1. Modification in the target of antibiotic agent [affinity of the drug is decreased], drug uptake is decreased,
2. Efflux mechanisms are activated for throwing out the pernicious molecule,
3. Global disparity in crucial metabolic pathways through modulation in regulatory networks.

Horizontal Gene Transfer

HGT [Horizontal gene transfer] is described as the foreign genes acquisition by organisms. Bacterial antibiotic resistance is mostly acquired by HGT [11]. Three major processes are involved in HGT between bacteria:

1- Transformation- DNA which is freely available in the environment is taken up by the competent bacteria. This phenomenon is defined as transformation [12, 13, 14].
2- Transduction- DNA [Non viral] may be transferred through virus from bacterium which is infected to a new host by DNA acquisition mechanism [15].
3- Conjugation - In conjugation a DNA molecule [conjugative transposon or plasmid] is received by recipient cell from donor which is attached physically through the conjugal tube apparatus [16].

TUBERCULOSIS: TYPES, DIAGNOSIS AND TREATMENT

Tuberculosis is a disease caused by *Mycobacterium tuberculosis* complex. Tuberculosis is spread through air so it is also called as airborne disease. The causative agent of tuberculosis was discovered by Robert Koch in 1882 [36]. Almost any part of the body can be infected by *M. tuberculosis;* thus it can cause tuberculosis in almost any part of the body.

Tuberculosis can be classified according to the site of infection as Pulmonary and Extra-pulmonary. When the lungs are infected by the *Mycobacterium tuberculosis* then is called pulmonary tuberculosis and when *Mycobacterium tuberculosis* infect other parts of the body like lymph node, bone, joints etc then it is called extra pulmonary tuberculosis.[37].

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Table 2. Burden of tuberculosis globally and on India

	Incidence	Prevalence	Mortality
Global	**9.6 million [176/lakh/year]**	**13 million [227/lakh/year]**	**1.1 million [21/lakh/year**
India	**2.2 million [167/lakh/year]**	**2.5 million [195/lakh/year]**	**0.22 million [17/lakh/year]**

Source: WHO Report [38]

India has the highest burden of tuberculosis as most of the adults are killed by tuberculosis than any other disease in India [37].

Types of Tuberculosis according to drug resistance:

According to drug resistance tuberculosis can be defined as;

1-**Multi drug resistant [MDR]** - When the causative agent of tuberculosis [*Mycobacterium tuberculosis*] show resistance against rifampicin and isoniazid with or without other anti-tubercular drug then it is called as multi drug resistant tuberculosis.
2- **Extensively drug-resistant [XDR]-** When the causative agent of tuberculosis show resistance against rifampicin, isoniazid, any floroquinolone and any second line injectible drugs [amikacin, capreomycin,and kanamycin] then it is called Extensively drug-resistant [17].

Diagnosis of tuberculosis:

To diagnose tuberculosis many different types of techniques are used:

1. Chest X-Ray [CXR]- It is an imaging technique that used to detect tuberculosis by detecting the abnormalities of lungs [18]. Chest X-ray is highly sensitive for and especially used to detect pulmonary tuberculosis. However, specificity of X-ray is poor; although it may show specificity for some CXR pulmonary abnormalities but such abnormalities are also seen in many other lung pathologies [19].
2. Microscopy- Sputum smear microscopy is the primary method which is used to diagnose the pulmonary tuberculosis. Microscopy is cheapest, simplest and rapid technique to diagnose tuberculosis. Microscopy is highly specific in area which has high tuberculosis prevalence, that's way it is used as an important tool of the global strategy for TB control [20]. There are two types of microscopy technique used, florescence microscopy and Ziehl-Neelsen microscopy. Ziehl-Neelsen staining has less sensitivity than fluorescence microscopy [auramine-rhodamine staining]. However microscopy has also some limitation like when the bacterial load is not sufficient [less than 10,000 bacilli/ml] in sputum sample then microscopy is not able to detect them. It is not able to track extra pulmonary tuberculosis and TB-HIV cases [21].
3. Culture- Culture technique is also used to diagnose tuberculosis. Cultures are standard diagnosis method for detection of Mtb complex. There are two types of culture used to diagnose tuberculosis, solid culture and liquid culture. Solid media [Löwenstein-Jensen] is a sensitive method than microscopy. The limitation of this method is that, it is more expensive and takes more time to give result (4-8 weeks). Liquid culture is automated culture technique. It is more sensitive than solid culture and takes less time to give result (2-4 weeks) [22].
4. Molecular Method- Line probe assay [LPA] was approved by WHO in 2010. This technique is used to identify *M. tuberculosis* complex with sensitivities of 95% for smear-positive cases and 55% for smear-negative cases [23]. Another technique for diagnosing tuberculosis is CBNAAT. CBNAAT is specific and automated assay to diagnose Mtb. It is cartridge based nucleic acid amplification test which gives results within 100 minutes [24].

Drug susceptibility testing- There are many methods used to detect drug resistance in tuberculosis for both first line and second line drugs. The most commonly used conventional method is LJ DST method but it takes more time to give result. In order to shorten the time of result now-a-days, liquid Bactec 960 is used for first line as well as second line DST of tuberculosis. Molecular based DST methods are also used to detect the resistance against first line drug [LPA, CBNAAT] as well as second line drug [LPA] [25-26].

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Treatment of tuberculosis

Different drugs are used to treat tuberculosis which has a specific mode of action by which they kill the bacteria.

Table 3: Drugs used in the treatment of Tuberculosis:

Grouping	Drugs
First- Oral anti-tuberculosis drugs [first line]	INH [Isoniazid], RIF [Rifampicin], Pyrazinamide, ETM [Ethambutol]
Second- Anti-Tuberculosis drugs [Injectible]	Amikacin, Capreomycin, Streptomycin, Kanamycin
Third- Oral second line anti tuberculosis drugs	Ethionamide, Cycloserine PAS
Group 4- Fluoroquinolones	Ciprofloxacin, Ofloxacin, Levofloxacin, Moxifloxacin

Source –RNTCP, guideline on PMDT Tb India, 2012

Table 4: Drugs and their mode of action against tuberculosis:

Drug name	Discovery year	MIC µg/ml	Gene[s] involved in resistance	Gene function	Role	Mechanism of action
Isoniazid	1952	0.02-0.2	*kat*G *inh*A	Catalase-peroxidase Enoyl ACP reductase	Pro-drug conversion Drug target	Inhibition of mycolic acid biosynthesis and other multiple effects
Rifampicin	1966	0.05–1	*rpo*B	β subunit of RNA polymerase	Drug target	Inhibition of RNA synthesis
Pyrazinamide	1952	16–50 [pH 5.5]	*pnc*A	Nicotinamidase /pyrazinamidase	Pro-drug Conversion	Depletion of membrane energy
Ethambutol	1961	1–5	*emb*B	arabinosyl transferase	Drug target	nhibition of arabinogalactan synthesis
Streptomycine	1944	2–8	*rps*L *rrs* *gid*B	S12 ribosomal protein 16S rRNA rRNA methyltransferase [G527 in 530 loop]	Drug target	Inhibition of protein synthesis
Amikacin/ kanamicine	1957	2–4	*Rrs*	16S rRNA 16S rRNA	Drug target	Inhibition of protein synthesis
Capreomycin	1960		*tly*A	2'-O-methyltransferase	Drug target	
Floroquinolones[Ofloxacin,ciprofloxacin,moxifloxacin].	1963	0.5–2.5	*gyr*A *gyr*B	DNA gyrase subunit A DNA gyrase subunit B	Drug target	Inhibition of DNA gyrase
Ethionamide	1956	2.5–10	*eta*A/*eth*A	Flavin monooxygenase	Pro-drug conversion	nhibition of mycolic acid synthesis
PAS	1946	1–8	*nh*A *thy*A	Thymidylate synthase	Drug activation	nhibition of folic acid and iron metabolism

Source- TB[PMDT] in India 2015 [27]

Use of Nanobiotechnology in diagnosis and treatment

Major problem with already existing therapy against tuberculosis is the non specific entry of drugs which result in side effects to the body as the drugs are administrated intravenously or orally which limits its outreach to the specific targets. Due to their rapid clearance from the body, drugs have got less time to act and thus have limited effect. Tuberculosis can be diagnosed, prevented or may even cured by using nanotechnology based tools. Nanotechnology paved a promising path for the development of more effective treatment method for Tb which includes drug delivery system and development of next generation Tb vaccine. It has the ability to increase the retention time of drug in the body and thus bioavailability. Nanodrugs of size 150 to 200nm are reported to be effective by inhalation route [28].

Several nano based diagnostic kits for tuberculosis are under trial and are showing potential nature because of their cost effectiveness. The several other advantages of nanoparticle-based drug delivery system for treatment of tuberculosis are: high constancy/longer time period, high carrier ability; that is, multiple drugs can be encapsulated in the matrix, less side effects compared to conventional drugs, increased bioavailability [slow, sustained, and controlled drug release], viability of various routes of administration like oral delivery and inhalation, minimal side effects and improved compliance [29-31].

CONCLUSION

Tuberculosis is a high prevalence disease with high morbidity and mortality all over the word as well as in India. There are limited diagnosis techniques and drugs available to treat tuberculosis. The problem of multidrug resistant tuberculosis is also a major challenge. To eliminate tuberculosis many research works are in pipeline some of which mainly focus on the use of nanotechnology or nanoparticle for diagnosis and treatment of tuberculosis. In future for early detection and treatment of tuberculosis more sophisticated techniques and effective drugs should be identified which should be easily accessible in all conditions.

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