Innovative Journal of Medical and Health Science
Received 16 Aug 2020 | Accepted 20 Sep 2020 | Published Online 9 Oct 2020

DOI: https://doi.org/10.15520/ijmhs.v10i10.3115
I Jour Med Health Science 10 (10), 1306–1319 (2020)
ISSN (O) 2589-9341 | (P) 2589-9341 IF:1.6

ORIGINAL ARTICLE

EPIs and Immunotherapy – An answer to drug-resistant tuberculosis

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Abstract

Tuberculosis (**TB**), primarily caused by *Mycobacterium tuberculosis* (Mtb) remains one of the fatal contagious diseases accountable for millions of deaths yearly across the globe – droplet or aerosol being the mode of transmission. Apart from the peculiar microbial physiology and drug efflux pumps with drug modifying enzymes that make Mtb impervious to the existing drug regimen recommended by the **WHO** via **DOTS**, lack of social awareness and misuse or mismanagement of drugs make it even more deadlier transforming Mtb to **MDR-TB** & **XDR-TB**, thus increasing the number of TB cases globally per annum. Amidst the dire need to combat the rising cases of drug-resistant TB, this article discusses the potential use of phytochemical derived efflux pump inhibitors (**EPIs**) and immunotherapy to fight TB with a brief insight into the molecular mechanism and structure of drug-efflux pumps found in Mtb.

Keywords: Mycobacterium tuberculosis, efflux pumps, DOTS, efflux pump inhibitors (EPIs), immunotherapy

1 | INTRODUCTION

Mycobacterium tuberculosis is a pathogenic bacterial species in the family *Mycobacteriaceae* and the causative agent of tuberculosis. First discovered in 1882 by Robert Koch, *M. tuberculosis* has an unusual, waxy coating on its cell surface (primarily due to the presence of mycolic acid), which makes the cells impervious to gram staining. The **Ziehl-Neelsen** stain, or **acid-fast** stain, is used instead. The physiology of *M. tuberculosis* is highly aerobic and requires high levels of oxygen. Primarily a pathogen of the mammalian respiratory system infects the lungs. The most frequently used diagnostic methods for tuberculosis are the **tuberculin skin test, acid-fast stain**, and **chest radiographs**. Types of Mycobacteria – i) *M. tuberculosis* causes most TB cases, ii) Other Mycobacteria that cause TB or TB-like disease are – (a) *M. bovis* (b) *M. africanum* (c)

Supplementary information The online version of this article (https://doi.org/10.15520/ijmhs.v10i10.3115) contains supplementary material, which is available to authorized users.

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M. microti (d) *M. canetti* (iii) Mycobacteria that do not cause TB – e.g., *M. avium* complex. TB is spread person to person through the air via droplet nuclei. *M. tuberculosis* may be expelled when an infectious person coughs, sneezes, speaks or sings. Transmission occurs when a healthy individual inhales droplet nuclei.

FIGURE 1: Global statistics

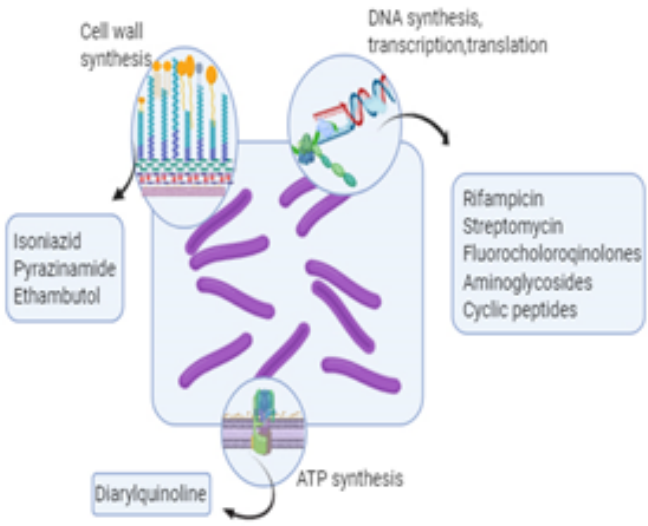

FIGURE 2: Target of first, & second line drugs

Since using a single drug for therapy often leads to the development of resistance to that drug, effective drug regimens for TB treatment contains multiple drugs to which the pathogen is susceptible. Hence, tuberculosis is usually treated with a combination of four or five different antimicrobial agents. The duration of drug therapy is from 6-9 months. The drugs that are used more often are rifampin (**RIF**), isoniazid (**INH**), pyrazinamide (**PZA**) and ethambutol (**EMB**) or streptomycin(**SM**). INH and RIF are most effective and are considered as first-line drugs. The drug regimen also includes second-line drugs (for cases of MDR-TB and XDR-TB) such as fluoroquinolones, e.g., ciprofloxacin, norfloxacin, and ofloxacin and injectables, viz., amikacin, kanamycin (aminoglycosides), and capreomycin (cyclic peptide).

As administration of a single drug often leads to the development of a bacterial population resistant to that drug and effective regimens for the treatment of TB must contain multiple drugs to which the organisms are susceptible. Hence, tuberculosis is usually treated with four different antimicrobial agents. The course of drug therapy usually lasts from 6-9 months. The most commonly used drugs are ***rifampin (RIF),isoniazid (INH), pyrazinamide (PZA***) and ***ethambutol (EMB***) or ***streptomycin(SM***). INH and RIF are most effective and are considered as first-line drugs. The drug regimen also includes second-line drugs (for cases of **MDR-TB** and **XDR-TB**) such as fluoroquinolones, e.g., ciprofloxacin, norfloxacin, and ofloxacin and injectables, viz., amikacin, kanamycin (aminoglycosides) and capreomycin (cyclic peptide). Their mode of action is briefed in ***Table 1.***

Directly observed treatment, short-course **(DOTS)** is the tuberculosis control strategy recommended by the World Health Organization (WHO), which is implemented via the government's national health policy scheme in a country. The five elements of DOTS includes – (i) Political commitment with increased & sustained financing, (ii) Case detection through quality-assured bacteriology, (iii) Standardized treatment, with supervision and patient support, (iv) An effective drug supply & management system, and (v) Monitoring and evaluation system, and impact measurement. Another strategy, **DOTS Plus** refers to a DOTS program that adds components for MDR-TB diagnosis, management, and treatment, which

was started by the WHO in 2000 with the aim of strengthening the basic DOTS strategy.

Resistance to anti-TB drugs can occur when these drugs are misused or mismanaged. Multidrug-resistant tuberculosis **(MDR-TB)** is TB that is resistant to at least two of the best anti-TB drugs, isoniazid and rifampin. Extensively drug resistant TB **(XDR-TB)** is a relatively rare type of MDR-TB. It is defined as, TB which is resistant to isoniazid and rifampin, also to any fluoroquinolone and at least one of the three injectable second-line drugs. Some of the ways the *tubercle bacillus* acquires drug resistance are – (i) Cell wall: The cell wall of *M. tuberculosis* consists of complex lipids, and it acts as a permeability barrier from drugs, (ii) Drug modifying & inactivating enzymes: The *M. tuberculosis* genome codes for certain enzymes that makes it drug resistant - the enzymes usually phosphorylate, acetylate, or adenylate the drug compounds, (iii) Drug efflux pumps: These systems actively pump the drugs out of bacterial cell and (iv) Mutations: Spontaneous mutations in the Mtb genome can give rise to proteins that makes the bacterium drug resistant, depending on the drug action.

DRUG EFFLUX PUMPS- Types, structures and mechanism of action:

The drug efflux pumps can be defined as the transmembrane proteins that actively participate in the transport of various substrates, in this case, anti-TB drugs from the cytoplasm to periplasm, thus neutralizing the drug activity. Having known that the drug resistance is due to the gene mutations on specific loci in the MTB (*mycobacterium tuberculosis*) genomes, the accountability of several other mechanisms like efflux pumps have been reported. This mechanism is omnipresent and is responsible for the innate and acquired drug resistance in both prokaryotes as well as eukaryotes. Depending on the source of energy, the Multi-Drug Resistant (MDR) TB has been classified into five super-families: [1] ATP binding cassette (**ABC**) transporter; [2] Major facilitator super-family (**MFS**); [3] Resistance nodulation cell division (**RND**) super-family; [4] Small multi-drug resistant (**SMR**) super-family; [5] Multi-drug and toxic efflux (**MATE**) super-family.

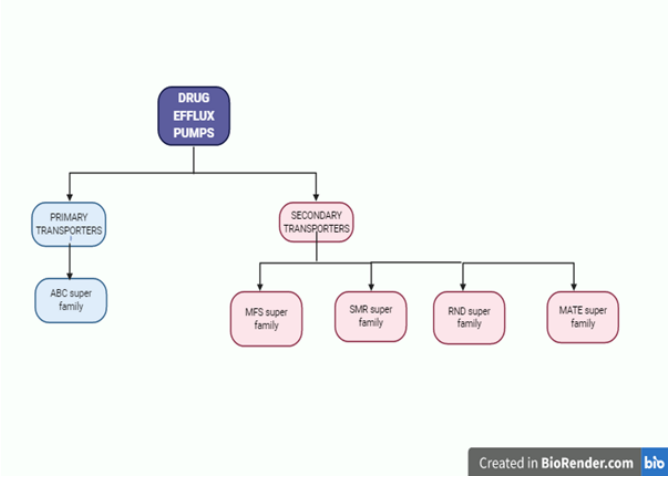

FIGURE 3: Classification of efflux pumps

Further these five super-families were divided into two mechanistically distinct types: primary and secondary transporters. The former ones exhibit an integrated function of drug extrusion from the cell and ATP hydrolysis simultaneously, while the latter ones draw energy from the transmembrane electrochemical gradients like protons or sodium ions. These transporters in turn can be categorized into single and multi component pumps. The substrate transport in single component pumps is across the cytoplasmic membrane, while the multi component pumps found in certain gram-negative bacteria work in consociation with the periplasmic membrane fusion protein (**MFP**), outer membrane protein (OMP) components and efflux substrates throughout the cell wall.ABC transporters comes under the single component pumps, whereas the MFS, SMR, RND and MATE falls under multi component pump system.

Coming to the basic idea behind the five super families, one needs to know the energy source and the mechanism of action of each for a better therapeutic regimen of MDR-TB. The ABC transporters derive their energy from ATP hydrolysis, possessing a multi subunit complex, acting both as an importer and an exporter using ATP switch mechanism. Single polypeptide carriers that obtain energy from proton motive force, acting as symporters, uniporters as well as antiporters with a single component pumps belong to the MFS super family. The phosphate transporter of MFS operates through the alternate access mechanism of roker switch type. Another super family called RND having the same energy source

TABLE 1: First and second line drugs-mode of action

DRUGS NAME		GENE MUTATION RESPONSIBLE	MODE OF ACTION	MECHANISM OF RESISTANCE
FIRST LINE DRUGS	RIFAMPICIN	rpoB	Inhibits transcription by inhibiting bacterial DNA using RNA polymerase (β- subunit).	Mutation in the rpoB gene (β-subunit) of RNA polymerase alters the amino acid sequence preventing the drug to bind to it.
	ISONIAZID	katG; inhA; ahpC; kasA	Inhibits mycolic acid synthesis.	Mutation of S315T in the KatG gene inhibits the formation of isoniazid-NAD adduct that blocks the mycolic acid synthesis.
	PYRAZINAMIDE	pncA	Inhibits fatty acid synthesis and thus, fatty acid biosynthesis.	Mutations in the pncA gene blocking the conversion of pro-drug inhibit its active form.
	ETHAMBUTOL	embCAB	Bacteriostatic obstructs the formation of cell wall.	Mutation in the embB306 of embB gene and the decaprenyl phosphoryl biosynthetic β-D- arabinose pathway renders ethambutol ineffective.
	STREPTOMYCIN	rspL; rrs	Protein synthesis is hampered as it binds to 16s rRNA of 30s ribosomal subunit, blocking fmet- tRNA.	Mutation in the rpsL and rrs genes prevent the drug from binding to 16s rRNA of 30s ribosomal subunit.
SECOND-LINE DRUGS	FLUOROQUINOLONES	gyrA; gyrB	Inhibits DNA gyrase (typeII topoisomerase) and blocks DNA replication.	Mutation in gyrA or gyrB gene blocks the drug from binding to the DNA- gyrase (protein) intermediate.
	KANAMY	rrs	Inhibition of protein synthesis on binding to 30s ribosomal subunit.	Mutation in the rrs gene does not allow the drug to bind to the 16s rRNA of 30s subunit.
	CAPREOMYCIN	rrs; tlyA	Inhibits mRna-tRNA translocation and protein synthesis.	Mutation in the tlyA gene (rRNA methyl transferase) blocks the drug from binding to the ribosome.
	AMIKAC	rrs	Inhibits 30s ribosomal subunit and thus blocking translation.	Mutation in the rrs gene does not allow the drug to bind to the 16s rRNA of 30s subunit.

as MFS has been identified in the MTB that acts as a drug antiporter with a multi subunit complex. The smallest known transporters of SMR super family acts a drug/metabolite transporter also work utilizing proton motive force, while the MATE super family utilize electrochemical gradient of protons or sodium ions and serve as Na+/drug antiporters.

Putative drug efflux pumps in the genus Mycobacterium:

The ABC superfamily consisting of 52 sub families has efflux system for a wide range of substrates including drugs, sugar, amino acids, carboxylates, metal ions and peptides. ABC transporters are supposed to be associated with the acquisition of drug resistance in *mycobacterium*. The genes coding for ABC transporters are found to represent 2.5% of the entire genome and at least 12 putative EP genes have been identified: *Rv1456c-*

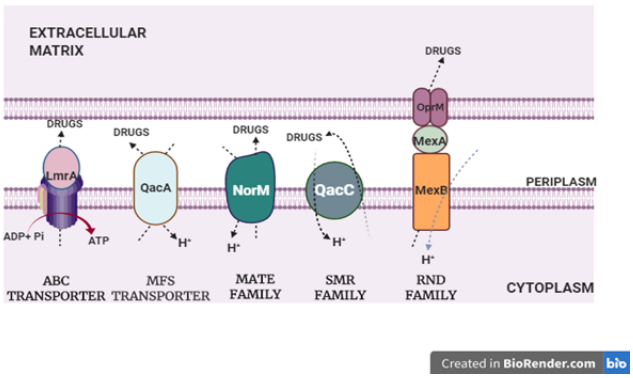

FIGURE 4: Mechanism of action of the five super-families

Rv1457c-Rv1458c, Rv1686c-Rv1687c, Rv1348-Rv1349, Rv1473, Rv1218c-Rv1217c, Rv1273c-Rv1272c, Rv0194, Rv1819, Rv2477, Rv1686c-Rv1687c, Rv2688c-Rv2687c-Rv2686c and drrA-drrB-drrC..

The multidrug EP gene Rv0194 was identified in *M. tuberculosis* during the investigation of molecular mechanism of beta-lactams in *M. tuberculosis*. Another new ABC transporter was characterized in M. tuberculosis responsible for efflux of various substrates including novobiocins, pyrazolones, biarylpiperazines pyrroles and pyridones.

20 potential EPs belonging to the MFS drug transporter in *M. tuberculosis* genome have been sequenced and identified. At least 16 putative EP genes of this family were studied :*Rv0037c, Rv0191, Rv0783c, Rv0849, Rv1250, Rv1258c, Rv1410c, Rv1634, Rv1877, Rv2333c, Rv2456c, Rv2459, Rv2846c (efpA), Rv28994, Rv3239c and Rv3728,* which mostly confers resistance to streptomycin and tetracyclin. A particular protein from the SMR family, the Mmr protein was found to confer resistance to acriflavine and erythromycin. In case of the RND family , the *M. tuberculosis* genome revealed the presence of about 15 putative transmembrane protein which showed increased econazole efflux and resistance to isoniazid.

ABC transporters-:

Being the largest known super family, these are found in all the living organisms ranging from microbes to humans. Comprising of 2.5% of the genome of MTB, the ABC transporters play a key role in moving a wide range of molecules like peptides, lipids, ions, drugs, etc by deriving their energy from ATP hydrolysis. They are classified depending upon of the sequence and organization of ATP binding cassette (ABC) domain(s). Utilizing the ATP hydrolysis, they can also regulate the permeability of certain multi protein channel complexes. There were some remarkable efforts to get to know and understand the structure and mechanism of these primarily active transporters. The main focal point of this article is to review a particular ABC efflux pump pertaining to MTB.

Typically, any ABC transporter consists of two pairs of distinct domains viz. the **TMD** (transmembrane domain) and **NBD** (nucleotide binding domain). These four domains might be present within a single polypeptide chain or two separate proteins known as full and half transporters respectively. The ABC transporters tend to become competent after specific dimerization in the latter case. The structural backbone of the ABC transporters is formed by the membrane–spanning domains (MSD). Architectonics of the ABC efflux pump reveals that, it consists of 12 transmembrane alpha-helices that form the four domains i.e.; two transmembrane /permease/transporter domains and two nucleotide binding domains or ATP binding domains. TMDs are ringed structures that form a channel pore sandwiched between the membranes allowing the translocation of substrates. The hydrophilic NBDs consist of many sustained sequences attached to the TMDs. In the absence of ATP there is a gap between the NBDs and water site, but in its presence the ATP binding interface is sandwiched between the NBDs.

Treatment of MDR and XDR strains of TB has always been a formidable global challenge. With time, the patients suffering and succumbing to this epidemic are numerous. Among those 90% of them suffer from MDR-TB while 10% of them with XDR-TB. Premature identification of the resistance and maneuvering of a properly designed regimen can aid in finding a possible cure for the disease. The root of the XDR-TB has remained mysterious and partially understood. There were several theories explaining the same. Next generation sequencing technologies (NGS) play a pivoting role in this are to decipher the

principle behind these 'extreme' strains. The MDR and XDR- TB can be detected with both phenotypic as well as genotypic means. The latter ones usually perform **PCR** (polymerase chain reaction) techniques to see if any genetic mutations are found as they confer to their drug resistance.

Contemporary microarray studies interpreting the mode of action of several experimental drugs revealed the existence of putative ABC transporters. Among these, Rv1218c has been taken for the physiological relevance in performing the efflux in the M. tuberculosis. Fascinatingly, the plasmids were not reported in this bacterium which led to the zero feasibility of horizontal gene transfer. A *Δ*Rv1218c mutant of M.tuberculosis e xhibited a 4-8 fold increase in the inhibitory as well as bactericidal capacity. The resistance of these is found to be due to the inducing expression of these genes of efflux pumps. In this article, we have characterized a single transporter Rv1218c to determine its pivoting role for the gene product in mediating the efflux pumps.*

Amplification of the DNA and screening of the single-crossover (SCO), double-crossover (DCO) of the recombinants in M.tuberculosis was performed by PCR technique using ***Taq DNA polymerase*** *. The recombinant substrate that is used to cause a deletion in the Rv1218c has a mutant gene with flanking sequences cloned into the suicide vector* pAZI0290. This is done by overlapping PCR in such a way that the deleted region was flanked by both 5' and 3' ends of deletion. A gene was amplified from the genomic DNA of *M.tuberculosis* and cloned into **PuvII** and **HindIII** sites of the expression vector pMV261 to obtain a complementary construct pBAN0192. The cloned fragments were thoroughly checked by DNA sequencing and the recombinant plasmids are transformed into *M.tuberculosis*. To understand the mode of action of pyrazolones a microarray data was generated that resulted in a 4-6 fold hyper expression of certain arbitrary ABC transporters besides a number of other genes.

Efflux Pump Inhibitors (EPI) for *M.tuberculosis*:

The efflux pump plays a key role in the regulation of antibiotic resistance. Taking that into account, it is appropriate to expect that outmaneuvering these cognitive factors can enhance the activity of substrate antibiotics. These efflux pumps could be terminated by various methods like: [1] Suppressing the expression of efflux pump genes by impeding in genetic regulation, [2] Renovation of antibiotics that are not identified as substrates anymore, [3] Hindering the assembly of actively functioning efflux pumps, [4] Obstruction of the pump in order to evade the substrate binding to the active site, [5] Crumbling the energy system that boosts the mechanism of these pumps. The chemical entities or molecules that inhibit the functioning of efflux pumps using various mechanisms, languishing the drug transport. This leads to antibiotic build-up in the cell and together with EPIs they can potentiate their activity against bacteria expressing efflux pumps.

Types of EPIs in *M.tuberculosis*:

The EPIs have proved to be great therapeutic appurtenants with a mickle of EPIs exhibiting different modes of action. They mainly show two kinds of mechanisms. One is the dissipation of energy and the other is inhibition by direct binding. In the first method, the EPIs don't have to be in contact with the substrate to turn off the efflux pump. Instead, they can just uncouple the energy and efflux process. Since the efflux pumps depend upon the proton gradient for proper functioning, this method would be advantageous to tackle their over expression. A noted EPI, Carbonyl cyanide m-chlorophenyl hydrazone (**CCCP**) which is an ionophore, deranges the proton gradient and thus affecting both the membrane potential and its pH. This EPI is said to make the bacterial cells metabolically inactive causing a synergetic effect.

In the second kind of mechanism, the EPIs necessarily bind to the substrates leading to the ineptness of pump-substrate interaction. This interaction could either be competitive or non-competitive where the EPIs vie with its substrates for a single active site and the EPI simply binds to the substrate abating the inclination of the pump to its substrate respectively. Nevertheless, the bacteria are capable of causing a few mutations in their efflux pumps, altering the target sites of inhibitors and thus evincing them nugatory. A probe in *M.tuberculosis*, unveils the role of verapamil, an ion channel blocker in improving the functioning of bedaquiline and ofloxacin. Also

displaying a reducted toxicity towards the bacterial cells not manifesting the MATE efflux pumps exhibiting a competitive mode of inhibition.

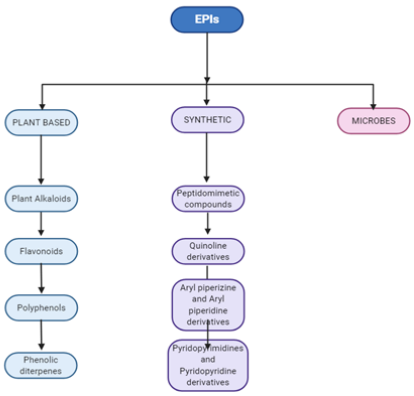

FIGURE 5: Classification of EPIs based on source

A wide range of molecules has shown their capability as EPIs, while the mechanism of action for most of them remains unknown. As a result, it is very challenging to classify them based on their mode of action. In order to billet such EPIs, they have been classified depending on their source. Plant-based, synthetic products and microbes are the three categories. Plant-derived phytochemicals consists of a broad array of chemical adjuncts that can harmoniously augment the capability of the antibiotics upto several folds proving to be efficacious. Many efficient EPIs have been identified on screening the new-fangled semi-synthetic or multi-faceted synthetic chemical libraries. These inputs have shown success at different ranges. The class of EPIs originating from the microbes is of a limited assortment. The detailed classification is given in ***Figure 4***.

Plant-based Secondary metabolites-A source of EPIs:

The rate of therapeutic failure has been observed, owing to the hike in multi-drug resistant (MDR) bacteria that can mitigate the efficacy of the repository antibiotics to a remarkable extent. Annihilation of the resistance episode in bacteria by hampering the efflux-pump can be done as they are the major facets of MDR. The idea of using phytochemicals as resistance altering means has been outstanding. A myriad of flora exhibit some exceptional efflux inhibitory activities. Plant families that are a great source of EPIs are *Apocynaceae, Berberidaceae, Convolvulaceae, Cucurbitaceae, Fabaceae, Lamiaceae and Zingiberaceae* as mentioned in ***Table 2***. Conventionally, antibiotics operate on the bacterial cellular components like cell membrane, cell wall, genes, protein synthesis and metabolism at varying degrees. This explains their mode of action in which the active agents' marked man; the bacterial cell wall requires alternative receptors for attachment and pertinent activity.

The main objective of any living organism is to sustain and so do microorganisms'. For this reason, bacteria developed several mechanisms to thrive under adverse conditions. Eventually, these mechanisms increased the intrinsic potential of bacteria so much that the antibiotic therapy has been phantomed. Although a multitude of bacterial phenotypes are found, studies show many brand new strategies through which resistance genes are amassed. Localization of these genes that code for antibacterial resistance either on the bacterial chromosome or on the genetic elements like plasmids and transposons are then transmitted vertically or horizontally. Phylogenetically, resistance is of two types: intrinsic or natural as a result of functional mechanisms, acquired or clinical obtained due to mutation of genes enciphering the target framework.

Common strategies of antibiotic resistance include (1) reduction of cell membrane permeability; (2) drug suppression; (3) drug inactivation by hydrolytic enzymes; (4) drug modification; (5) cellular target mutation allowing the antibiotic efficiency to bind to its main target; (6) active drug extrusion by membrane-spanning efflux pumps. Among all the five basic strategies, the mechanism of extrusion of drugs by an active efflux pump plays a key role in the occurrence of MDR-Tb. In this case, the resistance relies on energy and transportation systems where the pumps furnish it with recognition, fixation and transport of efflux substrates. A single pump might be particular to a provided antibiotic or might develop many types of therapeutic molecules having different structural attributes that may result in the MDR-tb phenotypes.

Quorum sensing (QS) is interlinked with the active efflux mechanism. It is a process in which, a certain communication is established between two adjacent cells during a cellular process by auto induction. The main objectives of quorum sensing are to regulate the manifestation of certain genes, thereby checking the potential to cope up with their environment and also regulating the size of the bacterial population without jeopardizing the nutrient availability. Autoinducers might cause changes in the expression of these efflux pumps resulting in the MDR phenotypes. These phenotypes may undermine the effectiveness of QS signaling which at times result in the predisposition of these pumps to several classes of antibiotics. Sometimes, certain genes might not be expressed due to the ineptness to synthesize some autoinducers. A few studies show that, efflux pumps and QS receptors share some transcription regulators. Hence, efflux pumps play a major role in QS signaling of the bacterial species.

Governance of the biofilm-associated diseases has also been a great challenge in the plight of MDR. The presence of a distinct phenotype in the biofilms due to the non-susceptibility of a batch of bacteria against high doses of antibiotics often leads to MDR mechanisms. The obliteration of biofilms has been a clinical challenge as they are recurrent. A biofilm is produced by a passive resistance mechanism that has a specific feature of the formation of a polysaccharide matrix urrounding the bacterium which can gradually result in the blocking of antibiotic transit. This explains the resistance of a bacterium to these molecules. Besides blocking the transit, they also foster dormancy of the bacterium that can null their inhibitory effect of therapeutic agents. Hence, knockdown of the efflux pumps by the EPIs can totally exterminate the biofilm formation.

Cogent EPIs from medicinal plants:

By a dint of the vast pharmacological characteristics of chemically and structurally distinct secondary metabolites, the medicinal plants have been a natural source and propitious. The extracts of these medicinal plants are known to have some putatives that are capable of eclipsing the efflux pumps in gram-positive as well as the gram-negative bacteria. Thus, rejuvenating the efficiency of antibiotics, enabling them to flow into the bacteria in adequate concentration to cause a bactericide. Majority of the species from various plant families with a lot of molecules exhibit efflux pump inhibitory effect. Since edible plants are a part of our diet, they are of growing interest. Some excellent sources of EPIs have been discovered on scrutinizing several fruits (grapefruits, grapes and pomegranate), vegetables (tea leaves, condiments and lemongrass), seeds (coffee and cocoa) and spices (pepper).

In both gram-negative and gram-positive bacteria, a sundry of dietetic phytochemicals like farnesol and geraniol; carvacrol, ellagic acid and thymol, piperine, theobromine, resveratrol, p- coumaric acid and derivatives are known to act against the active efflux pump chain. These phytochemicals are also common compounds of some essential oils extracted from the aromatic plants implying that essential oils can also be a great source of EPIs. Since these dietary elements are either less or non-toxic on eukaryotic cells, they can foster the manipulation of the development of drugs and their clinical usage. A few chemicals that are potent EPIs obtained from edible plants are terpenes, phenolic compounds and alkaloids. Typically, all plant-based EPIs deal with gram-positive bacteria. Although, its arduous to find potential EPIs for the gram-negative bacteria owing to their structural sophistication of tripartite efflux systems, otherwise have noted to act against some strongly resistant gram-negative bacteria of clinical concerns.

A few examples of compounds acting on gram-negative bacteria are oleanolic acid (from *Carpobrotus edulis*), karvavilagenin (from *Momordica balsamnia*), gallotannin (from *Terminalia chebula*) against *E.coli* ArcAB-TolC efflux pumps. Some compounds that deal with *P.aeruginosa* are osthol (from *Cnidii monnieri*), conessine (from *Holarrhena antidysenterica*), falcarcindiol (from *Levisticum officinale*), catheranthine (from *Catharanthus roseus*), resveratrol (from *Nauclea pobeuinii*), berberine and palmatine (from *Berberis vulgaris*), curcumin (from *Circuma longa*), p-coumaric acid and derivatives (from different edible plants). Tannic acids and ellagic acids (extracted from fruits and vegetables) act against *A.baumannii* pumps, whereas, theobromine (from *Theobroma cacao*) act against two efflux

TABLE 2: Plant based EPIs

PLANT FAMILY	BIOACTIVE COMPOUNDS	PLANT SOURCES	PHARMACOLOGICAL ACTIVITY ASSOCIATED WTH BACTERIAL EPI
Apocynaceae	Falcarinol	Levisticum officinale	Gram-negative bacteria efflux pumps
	Cumin		S. aureus LmrS efflux pump
	Reserpine	Cuminum cyminum Rauwolfia vomitoria Rauwolfia serpentina	S. aureus and S. pneumoniae, NorA and TetK-mediated MDR in MRSA, and LmrA of Lactococcus lactis, PmrA efflux protein in S.pneumoniae
Berberidaceae	Berberine	Berberis spp.	NorA activity in S. aureus and MexABOprM of P.aeruginosa
	Palmatine	Berberis aetnensis	MexAB-OprM of P.aeruginosa
	Porphyrin pheophorbide A		S. aureus NorA efflux pumps
	Flavonolignan 50 - methoxyhydnocarpin (50 -MHC) Pheophorbide		S. aureus efflux pumps
Cucurbitac	Balsaminol A	Momordica balsamina L	S. aureus and E. faecalis efflux pumps;
	Balsaminagenin B		NorA and AcrAB-TolC efflux pumps
	Karavilagenin C		Efflux pumps of MRSA COLOXA;
			E. faecalis efflux systems;
			Fluoroquinolone transporters in E. coli
Fabaceae	Liquiritin	Glycyrrhiza uralensis	Transporters of fluoroquinolone in E. coli
	Arylbenzofuran aldehyde (Spinosan A)	Dalea spinosa	NorA over-expressing S. aureus
	Juliflorine	Prosopis juliflora	NorA over-expressing S. aureus
	Flavanoid/phenolic compounds	Dalea versicolor	NorA of S. aureus and B. cereus
Lamiacea	Carnosic acid	Rosmarinus officinalis	S. aureus NorA efflux pumps, potentiates tetracycline against S. aureus strains possessing MsrA efflux pump
Zingiberaceae	Phenylpropanoids (1'-S-1' -acetoxyeugenol acetate)	Alpinia galangal	Mycobacterium smegmatis efflux pumps
	Trans,trans-1,7-diphenylhepta-4,6-dien-3-one	Alpinia katsumadai	Mycobacterium smegmatis efflux pumps
	Curcumin	Curcuma longa	P. aeruginosa efflux pump.

pumps – AcrAB-TolC and MexAB-OprM of gram-negative bacteria.

A legion of efflux systems infer their energy from and electrochemical gradients. For this very reason, most of the efflux pump inhibitors target the proton motive force either by competitive or non-competitive inhibition method. Several other mechanisms are also found to be operative (i) reduced control of the transcription pathways of genes encoding the efflux pumps, (ii) depravity of the ionic gradient through cell membrane, (iii) variations in the conformation of the efflux pumps or undermining the assembly of multi-component pumps like tripartite RND pumps, (iv) encumbering with the hydrolysis of ATP that imperils the revitalization of efflux pumps thereby inducing the membrane permeability in gram-negative bacteria.

Is TB immunotherapeutics a way to future?

the studies conducted on immunotherapy till now have solely focused on either finding a precise treatment or improving the management of MDR/XDR-TB. The current prophylactic treatment regimens for latent TB infection takes up to 3 to 9 months while new cases requires a minimum of 6 months treatment with multiple drugs. Recently research on immunotherapy for TB is intensified as it can ideally modulate the immune system in patients enabling better control or elimination of *M. tuberculosis*. Whole mycobacteria, cytokines, mycobacterial products and drugs are being studied extensively as possible immunomodulators for the treatment of TB in humans. Below is the summarized list of immunomodulating host-directed therapies for treatment of TB in humans

Antibodies as immunodulators

TB infection has known to induce both humoral and cell mediated responses. Recent studies on monoclonal antibodies against *M. tuberculosis* antigens have shown some conflicting results. Therefore a new line of treatment using sera from bacillus Calmette-Guerin (BCG) vaccinated individuals had shown an enhancement in antibodies mediated phagocytosis of myobacteria by phagocytic cells. Moreover a recent study reveals that high level of anti-*Mtb* IgG3 antibodies can prevent the reactivation of TB in high risk individual while in another study it was determined that intranasal administration of human gamma globulin have decreased the bacterial load in the lungs of *Mtb* infected mice. However further detailed studies are required to prove such research.

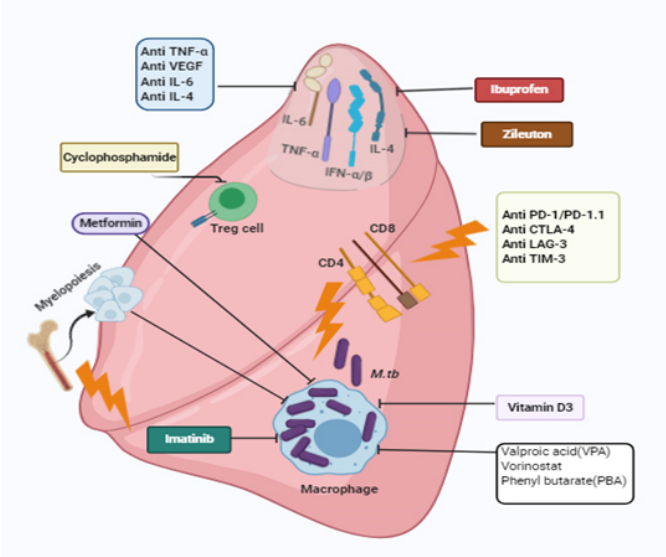

FIGURE 6: Immunotherapy target for M.tuberculosis

Cytokines and inhibitors as immunomodulators

The use of cytokines for therapeutic purposes is limited by their high toxicity and cost but nevertheless they have gained momentum as new therapy due to emergence of extensively drug-resistant tuberculosis (XDR TB) strains for which traditional chemotherapy is deemed ineffective. *M.tuberculosis*-specific T-cells produce cytokines and effector molecules such as perforin, granzymes, and granulysin which when enhanced can modulate the activation/differentiation of antigen presenting cells thereby controlling the infections. Till now cytokines such as IL-2, IFN-Gamma, IL-12 and TNF-Alpha are in clinical trials and are showing promising results. In a recent clinical study, adjunct supplementation of recombinant human IL-2 for 7 months in 50 MDR-TB patients shows increase sputum smear conversion and improved immunity status. These observations highlight the complex roles of cytokines in regulating immune cell functions and show them as a great potential for treating tuberculosis in near future.

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How to cite this article: Roy A., Hussain A., Sinha K., Praharaju M., Khatun S. **EPIs and Immunotherapy – An answer to drug-resistant tuberculosis**. Innovative Journal of Medical and Health Science. 2020;1306−1319. https://doi.org/10.15520/ijmhs.v10i10.3115