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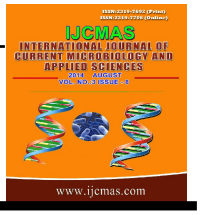

Review Article

Efflux pumps in drug resistance of *Mycobacterium tuberculosis*: A panoramic view

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K e y w o r d s

Tuberculosis, *Mycobacterium tuberculosis*, MDR, Efflux Pumps, drug resistance

A B S T R A C T

Majority of the problems in treating Tuberculosis (TB) is the appearance of drug resistant TB strains, including strains with multiple drug resistance (MDR) and more recently, strains with extensive drug resistance (XDR). From the various MDR mechanisms, main focus is on efflux pumps as they contribute to MDR of *Mycobacterium tuberculosis* (MTB) in most of the cases. Efflux pumps are transmembrane proteins which actively take part in transporting wide range of substrates including the anti-TB drugs from cytoplasm to exterior of cell, thereby nullifying the drug activity. Efflux pumps are classified in five families based on the energy source they require; that may be ATP driven transporter belonging to ATP-binding cassette (ABC) family or proton driven antiporter belonging to Major facilitator superfamily (MFS), Small multidrug resistance (SMR), Resistance-nodulation-cell division (RND) and Multidrug and toxic compounds extrusion (MATE) secondary active transporter. Efflux pumps of these families impart resistance to broad range of antibiotics like fluoroquinolone, tetracycline, ofloxacin, and isoniazid. Taken together, drug efflux pumps prove to be a major challenge for the scientific community and also the focus of this review. Thus, they require special attention to understand their functioning to combat the emerging crisis of MDR and finding a better solution for anti-TB therapy.

Introduction

Tuberculosis (TB) disease occurs in every part of the world and large numbers of cases have been noted from all over the world. About one third of the population has TB infection and millions of people are dying every year. The disease is caused by *Mycobacterium tuberculosis* (MTB) which usually attacks lungs but can infect other organs also. In the current situation, the major problem in treating this disease is emergence of Multidrug resistance (MDR-TB) and Extensive drug resistance (XDR-TB) strains, which remains a critical but largely unresolved issue. MDR-TB refers to the TB bacteria which are resistant to first line drugs such as isoniazid and rifampicin, while XDR-TB refers to the TB bacteria which are resistant to fluoroquinolone (FQ) in addition to isoniazid and rifampicin and to at least one of the second line drugs like

amikacin, kanamycin or capreomycin (Francis et al., 2014). There are various mechanisms which are involved in development of bacterial resistance (Fig.1). For instance, complex cell wall is an effective way to keep a drug from reaching its target and to prevent it from being taken up by the cell. Bacteria do this by changing the penetrability of their membranes or by lessening the number of channels available for drugs to diffuse through (Brennan et al., 1995). Another mechanism includes drug modifying & inactivating enzymes for many antibiotics which work by sticking to their target and preventing them from interacting with other molecules inside the cell. For example, some kinds of bacteria produce enzymes called as beta-lactamases that degrade penicillin (Lomovskaya et al., 2001).

Some bacteria respond by changing the structure of the target (or even replacing it with another molecule altogether) so that the antibiotic can no longer recognize or bind to it (Lambert et al., 2005). Spontaneous mutations in bacterial genome can also acquire resistance by getting a copy of a gene encoding an altered protein even from those of a different species. FQ target, DNA gyrase which is required as DNA supercoiling enzyme having subunits and substitution in these subunits results in mutation (Ramaswamy et al., 2003).

However, the most prevalent mechanism of MDR and XDR phenotype involves drug efflux system (Adams et al., 2011) which actively takes part in transporting wide range of substrates including the anti-TB drugs (Table.1) from cytoplasm to exterior of cell, thereby nullifying the drug activity (Li et al., 2004). Therefore, this review mainly focuses and summarizes various classes of drug efflux system present in bacteria and their mechanism of action with more emphasis on MTB.

Drug Efflux Pumps

Efflux pumps are transporter proteins that can extrude wide range of dissimilar substrates out of the cell which also includes anti-TB drugs, (Webber et al., 2003). Efflux pumps associated with MDR are classified in five major super-families in MTB on the basis of energy source (Paulsen et al., 1996). The major ABC (ATP binding cassette) super family utilizes ATP hydrolysis while other four families MF (major facilitator), MATE (multidrug and toxic efflux), RND (resistance-nodulation-cell division), SMR (small multidrug resistance) utilize proton motive force as a source of energy.

ABC Efflux pumps

ABC class is the largest known super-family which encodes 2.5% of the genome of MTB. They utilize the energy from ATP hydrolysis to export the substrate (Hollenstein et al., 2007). These pumps are multi subunit complexes that transport a variety of molecules such as peptides, lipids, ions, drugs etc. Proteins are classified as ABC transporters based on the sequence and organization of their ATP-binding cassette (ABC) domain(s). Usually, the common feature of all ABC transporters is consisting of two distinct domains viz. transmembrane domain and nucleotide binding domain (Fig. 2A). Till date it has been noted that importers are only found in prokaryotes, whereas exporter-type ABC transporters are expressed ubiquitously in lower to higher kingdoms (Locher et al., 2009). Significant efforts have been made to understand the mechanism of this transporter.

Structural architecture of ABC efflux pump consists of 12 transmembrane alpha-helices which forms four domains (Higgins C.F, 1986; Jones, 2004): two transmembrane domains (TMDs), also known as the permease or the transporter domains and two

nucleotide binding domains (NBDs) or ATP binding domains (Fig 2B). TMDs are ring like structures which form channel pore, sandwiched between membranes forming a pathway for translocation of substrates from interior to exterior of the cell. The sequence and architecture of TMDs are variable, reflecting the chemical diversity of substrates that can be translocated. Based on the few conserved sequences in TMDs, it is divided into at least three topologically different folds: type I ABC importers, type II ABC importers and type III ABC exporters (Locher, 2009; Rees, 2009).

Type I importers: These mediate the uptake of particles, sugars, amino acids and also other substrates, which are all caught by particular binding proteins that convey them to the transporters (Davidson et al., 2004) and the core topology holds 10 TM helices. Type II importers: Importers of type II encourage the uptake of metal chelates that are bigger than the substrates of type I ABC importer. *Type II importers* have a dissimilar TMD architecture from that of type I, with 10 helices in every TMD for a total of 20 transmembrane fragments in the gathered transporter (Locher, 2009). *Type III exporters*: Numerous life forms have various distinctive transporters with different physiological capacities, various included in multi-drug expulsion of lethal substances. Despite the fact that ABC exporters can distinguish different substrates, they all impart a typical core construction modeling that comprises of 12 transmembrane helices. The hydrophilic NBDs have many conserved sequences attached to the TMD. Basically, there are three characteristic motifs found in all ABC-ATPases. The Walker A motif consists of the sequence GXXGXGKS/T where X represents any amino acid (Walker et al, 1982). Together with the Walker B motif (ΦΦΦΦD, where Φ is any hydrophobic residue), this motif forms the nucleotide binding fold of the P-loop (phosphate binding loop) ATPase family (Vetter et al, 1999). However, unique to ABC-proteins is the C-loop (LSGGQ), also called the signature motif (Schmitt L et al, 2000). When the ATP is not present at its binding site there is a gap between the NBDs and the site which is accessed by water and in the presence of ATP, the interface at which ATP binds is sandwiched between NBDs.

Methods

Generally, both ABC importers and transporters have similar mechanisms in transporting substrates because of resemblance in their structure. To describe the mechanism of ABC superfamily transporters; essentially, transporters must cycle between high-and low-affinity states for ligand on different sides of the membrane. The ATP switch mechanism describes how these states are coupled to the ATP catalytic cycle in a way that is consistent with the structural data available.

Step: 1 Binding of substrate to TMD with high affinity bring conformational changes in NBD for binding of ATP: When the nucleotide is not bound, ABC transporters have greater affinity for the ligand. Pump is activated by the interaction of substrate to the TMD from the cytoplasmic side. When drug binds to TMD, it induces conformational changes which are then passed to NBD to initiate ATP binding (Neumann et al, 2002; Manciu et al , 2003; Linton et al, 2006) (Fig 3).

Step: 2 Binding of ATP brings NBD close to each other which bring conformational change in the TMDs: When two molecules of ATP binds to its conserved sequence, it brings both NBDs in close proximity in such a way that ATP is sandwiched between them. This ATP binding leads to

conformational changes in TMD in such a manner that TMD opens to facing outward, thereby releasing the substrate or drug.

Step 3: Hydrolysis of ATP: The momentarily dimerization of NBDs which is induced by ATP indicates that hydrolysis is an inevitable consequence of closed NBD dimer formation. This, upon hydrolysis, again brings the NBD conformation back by destabilizing this domain which is mandatory for the finishing of one transport cycle (Azzaria et al, 1989). Usually, for the completion of single cycle generally two molecules of ATP are required but in some exceptional cases like cystic fibrosis mutation, only one molecule of ATP is required.

Step 4: Release of ADP: ATP hydrolysis leads to release of ADP and Pi and because ADP has low affinity towards the NBD, so it restores the conformation of TMD facing inwards (Locher, 2009).

Association between ABC efflux pump expression and drug susceptibilities of MTB

Rv1217c-1218c : In order to find out whether or not the expression of efflux pump have affect on alteration of drug susceptibility of MTB the correlation between efflux pump and MIC was determined. It has been shown (Wang et al., 2013) that the overexpression of Rv1217c and Rv1218c led to enhanced MIC values. Overexpression of Rv1217c-Rv1218c led to the higher MICs of RIF whereas over-expression of only Rv1218c led to the higher MICs of isoniazid.

Rv2686c-Rv2687c- Rv2688c: MTB efflux pump Rv2686c-Rv2687c- Rv2688c operon resistance to FQ when overexpressed in *M. smegmatis* showed 8- fold increase in the MIC for wild type. In the presence of efflux inhibitor it shows reduce ciprofloxacin resistance to the same level as that of control which do not contain this operon and proves that the resistance was because of efflux (Pasca et al., 2004).

Rv1456c-Rv1457c-Rv1458c: Efflux pump Rv1456c-Rv1457c-Rv1458c which is similar to Rv2686c-Rv2687c- Rv2688c was quantitatively examined and found that over-expression of this operon increased MICs by 3.4, 4.6, and 5.4 fold respectively in drug-resistant strains when compared with susceptible group (Hao et al., 2011).

drrA & B: Similarly, expression of drrA & B in *E.coli* give 8-fold increase resistance to EtBr when expressed in *M. smegmatis* conferring resistance to broad range of antibiotics which was previously turned back by efflux inhibitors (Choudhuri et al., 2002).

MFS-type drug efflux transporters

About 25% of all known transporter proteins in prokaryotes belong to the major facilitator superfamily (MFS) (Saier et al, 1999), one of the largest and diverse secondary active transporters. This class of transporter is omnipresent in all the kingdoms of life and comprises of members which have clinical as well as pharmaceutical relevance. The MFS transporters are single-polypeptide carriers skilled just for transporting little solutes because of proton motive force (Pao et al, 1988). MFS holds members that serve as uniporters, symporters or antiporters. It transports a variety of substrates like inositols, metabolites of Krebs cycle , sugars, sugar phosphates, drugs, nucleosides, neurotransmitters, amino acids and peptides. All transporters of MFS category possess 12 or 14 α- helical

transmembrane protein which are interconnected by hydrophilic loop having both the NH_2 and COOH terminals in the cytoplasm. It has been hypothetically reported that genome of MTB contains 16 ORFs, encoding drug efflux protein, belonging to MFS (De Rossi et al., 2002).

Structural architecture of MF superfamily, consisting of 400-600 amino acids and 12 α-helical transmembranes, is divided into two halves of six α- helical transmembranes each first half containing NH_2 (TM-1 to TM-6) and another half (TM-7 to TM-12) containing COOH attached to it (Fig 4A). This architectural organization points out toward the fact that they have evolved from a gene duplication/fusion process (Maiden et al, 1987) where the first half corresponds to second half like TM-1 corresponds to TM-7, TM-2 corresponds to TM-8 likewise TM-3,4,5,6 corresponds to TM-9,10,11,12. Each half consists of two three helix bundles, TM-1,5,6 and TM-2,3,4 on first NH_2 half and TM-7,11,12 and TM-8,9,10 on the other COOH half because of structural similarity. In 14 α- helical transmembranes, two extra α- helices are probably present due to the insertion of cytoplasmic loop in the membrane. The loop connecting TM-1-6 to TM-7-12 is connected by long loop of 45 amino acids and it is believed that insertion occurs in this amino acid chain. From the 12 TMs, eight of them surrounds the four TMs. TM-2 and TM-11 are on front side, TM-5 and TM-8 on back side and two each on both left and right side. The other four are joined in sets and make a kind of hourglass shape that is encompassed by a wall (Law et al, 2008). These TMs form a pore which opens to the cytosolic side. Opening and closing of this pore is regulated by nine amino acid residues between TM-1 to TM-7. The pore consists of distinct arrangement that is C_i (inward facing conformation) and C_o (outward facing conformation) and so that contact between either side of the membrane is inhibited when the transporter is in former confirmation. The interior part of pore is lined with side chain of amino acid which is specific for every transporter to give a positive surface which enhances the binding of negatively charged substrate for translocation (Auer et al, 2001) which is hydrophobic in nature, so it resists the attachment of hydrophilic molecules like water and binds just below electropositive binding site.

Mechanism

As already discussed in case of ABC transporters, drug binds to the hydrophobic site of inner leaflet of cytoplasmic membrane and extrude to the outside. Mechanism of MFS transporter is same as other multidrug transporters and suggests that simultaneously many substrates can be attached to the inward substrate binding site which do not overlap each other (Saidijam et al, 2006; Fluman et al, 2009). Detailed mechanism of substrate binding is explained by the Glycerol-3-Phosphate transporter of *E.coli* taken as a model, which is similar in all MFS transporters. GlpT is a phosphate transporter which operates via alternate-access mechanism (Vidavar, 1996) of roker-switch type of movement of substrate (Huang et al, 2003; Hong H et al, 2006). Binding site had a cationic positive charge and requires an opposite anionic negative charge for binding, thus arginine is a cation present at the binding site which is accessed by anion molecule phosphate. TM-1 and TM-7 are located in close proximity to each other and both contain positively charge arginine in each TM. TM-1 contains arg-45 and TM-7 contains arg-269 at the end of internal cavity formed by TMs (Fig. 4B). Both the arginines are located at same height of TM and mutation in either of these arginine results in aborting the transporter

activity. Arginine recognizes phosphate by forming a hydrogen bond with oxygen atom of phosphate where substrate pulls these TM1 and TM7 towards each other and brings them close together (Huang et al, 2003); thereby resulting in altering the stable position of transporter, narrowing the cytoplasmic side and forcing out substrate to periplasm until the cytoplasmic side stabilize again.

Association between MFS efflux pump and drug susceptibilities of MTB

Rv2459 (jef A): It was concluded that increase in expression of jefA lead to increase in resistance to ethambutol and isoniazid in MTB which led to enhanced MIC that could be reversed by inhibitor rescuing the MIC (Gupta et al., 2010).

Rv2333c (the Stp protein): Rv2333 gene expressed in *M. bovis* was inactivated and decrease in MIC was noted (Ramón-García et al., 2007).

Tap (1258): Tap efflux pump require energy to extrude antibiotics out of the cell. CCCP (carbonyl cyanide *m*-chlorophenylhydrazone), a known inhibitor of proton motive force inhibits these pumps. It has been showen that the MICs of aminoglycosides were reduced in the presence of CCCP suggesting decrease in the efflux of aminoglycosides (Ainsa et al., 1998).

Rv1634: One of the pump, Rv1634, when expressed in *M. smegmatis* showed resistance to different FQ compounds and the data gained from the accumulation of these compounds demonstrated that this pump was responsible for efflux of FQ compounds (De Rossi et al., 2002). *Rv2846c (efpa):* Another pump of this family, Rv2846c (efpa), when overexpressed in *M. smegmatis* conferred resistance to EtBr and when the gene was deleted resulted in enhanced susceptibility to EtBr (Li et al., 2004).

RND drug efflux transporter

Bacteria have developed various efflux mechanisms which help in detoxification of cells and Resistance-Nodulation-Division (RND) is another such class. RND pump utilizes energy from proton motive force, so it is a drug/proton antiporter which transports a wide variety of substrates. It is a tripartite membrane protein that transports variety of substrates to the outside of cell. Structural architecture consists of 700-1300 amino long polypeptide chain with 12 transmembrane span and having loops between TMS 1 & 2 and TMS 7 & 8. They are widespread in gram negative bacteria but differ in gram positive bacteria with more resemblance to MFS family. However, in case of MTB these RND pumps are similar to gram-negative bacteria. Genome of MTB contains 15 genes which codes for RND transporter. This tripartite efflux systems are composed of cytoplasmic membrane associated protein, membrane fusion protein(MFP) (Paulsen et al, 1997) which form ring around the tube like outer membrane protein(OMP) (Koronakis et al, 2000), needed for the extrusion of drug out of the cell (Zgurskaya et al, 1999(a); Zgurskaya et al ,1999(b)). *E.coli* AcrAB–TolC transporter provides a model for explaining a mechanism of this class of transporter (Fig. 5). AcrA is a MFP while TolC is OMP (Zgurskaya et al, 2000). RND pump have an advantage to transport drug from periplasmic space which are not permitted to cross the cytoplasmic membrane (Murakami et al, 2002). TolC forms tunnel that spans periplasmic space and OM. The TolC opens for a short time period when the inner membrane is

energized and makes a bridge from cytosol to the external environment.

Mechanism

In RND efflux pump, there are two modes of action through which drug are effluxed: efflux through periplasm (MFP) or efflux through innermembrane of cytoplasmic side (RND). The former involves efflux through periplasm where substrate binds through membrane fused protein and gets effluxed through the outer membrane of RND pump. The efflux in later case is through inner membrane where the substrate binds through entire RND pump and efflux through the outer membrane of RND pump without losing the substrate in periplasm i.e., RND mediate both the process. In MTB, MmpL (Mycobacterial membrane proteins, large) is the efflux pump belonging to this category, when aligned with AcrAB of *E.coli* using bioinformatics tools, similarities were found and it was suggested that AcrA (MFP) is analog to MmpS and MmpL is to AcrB (Poole, K., 2004).

Association between RND efflux pump and drug susceptibilities of MTB

The hydrophobic nature of the MmpL protein recommends that they will be naturally involved in the transport of fatty acids. Indeed the MmpL7 macromolecule catalyzes the export of phthiocerol dimycocerosate (PDIM) in MTB (Camacho et al, 2001). Overexpression of MmpL in *M. smegmatis*, conferred high level of resistance to INH (Pasca et al, 2005) and to confirm whether the resistance was due to drug efflux, accumulation of INH was monitored in presence of inhibitors reserpine and CCCP.

SMR drug efflux transporter

As indicated by their name, Small multidrug resistance (SMR) is smallest known transporters. It is a α-helix transmembrane protein of approximately 100–140 amino acids in length (Paulsen et al, 1996). Four α-helices transmembrane (TM) are connected to each other by a short hydrophilic loops (Fig. 6A) (Yerushalmi et al, 1995, 1996; Winstone et al, 2002, 2005; Putman et al, 2000). It is a drug/metabolite transporter (DMT) superfamily (Saier Jr., 2000; Chung, 2001; Saier Jr., 2001). Similar to MFS superfamily proteins, SMR efflux drug via proton motive force (Littlejohn., 1992; Grinius et al, 1994; Yerushalmi., 1995; Paulsen., 1996). Apart from imparting resistance to a variety of quaternary ammonium compounds (QAC) and cationic dyes, they also transport negatively charged and neutral compounds (Jack et al, 2000).

Mechanism

There are various mechanisms for transportation in SMR and one of these pump is EmrE, a pump of *E.coli* that confers resistance to methyl viologen and EtBr (Yerushalmi et al, 1995). The transport mechanism model as proposed by Soskine et al (2004) depicts that there are highly conserved sequences present at binding site in EmrE. In addition negatively charged glutamate residue (E14 in Eco-EmrE) present on TM-1 is necessary for the transportation of drug. Before binding of substrate to its binding site a single proton is released (or deprotonation occur) from EmrE monomer and then drug bind to its binding site. Drug is transported from cytoplasmic side to periplasmic side where proton again bind to its site (protonation occur again) and it bring a conformational changes resulting in drug release (Fig. 6B).

Association between SMR efflux pump and drug susceptibilities of MTB

In MTB single transporter Mmr (Rv3065) in this category has been explained (De Rossi

et al, 1998) which is resistant to variety of substrate viz. tetraphenyl phosphonium (TPP1), EtBr, acriflavine, safranin O, erythromycin and pyronin Y (Table.1). Mmr protein when expressed in *E.coli* conferred resistance to EtBr, methyl viologen and acriflavine (De Rossi et al, 2005). Sequence analysis of mycobacteria revealed that homologues of Mmr are also present in other species such as *M. avium* , *M. smegmatis* and *M. leprae* (Doucet-Populaire et al., 2002).

Table.1 MTB efflux pumps associated with drug resistance

Family	Efflux Pump	Resistant to drug	References
ABC	Rv2686c-Rv2687c-Rv2688c	FQ	Pasca et al., 2004, Takiff et al., 1994
ABC	drrA and drrB	norfloxacin, streptomycin, chloramphenicol, tetracycline, erythromycin, ethambutol, norfloxacin, streptomycin, chloramphenico and anthracyclines	Choudhuri et al., 2002
ABC	Rv1217c-Rv1218c	biarylpiperazines, bisanilinopyrimidines novobiocins, pyrazolones, pyrroles and pyridines	Balganesh et al., 2010
ABC	Rv1456c-Rv1457c-Rv1458c	one of the first line drugs like isoniazid, rifampicin, streptomycin, ethambutol.	Hao et al., 2011
MFS	Rv1634	FQ	De Rossi et al. (2002)
MFS	Tap	aminoglycosides & tetracycline	Ainsa et al., (1998), De Rossi et al., (2002)
		INH & ethambutol	Gupta et al., (2010)
MFS	Rv2459 (JefA)		De Rossi et al., (2002)
MFS	Rv2333c (Stp)	spectinomycin & tetracycline	Ramon Garcia et al., 2007 De Rossi et al., 2002
MFS	Rv2846c (EfpA)	EtBr, acriflavine, ciprofloxacin & gentamicin	Li et al., 2004 De Rossi et al., 2002
RND	MmpL7	INH	Camacho et al., 2001 Domenech et al., 2005, Lamichhane et al, 2005 Pasca et al, 2005, Rodrigues et al, 2011; Sassetti and Rubin, 2003
RND	MmpL10	azole	Milano et al, 2009
SMR	Mmr (Rv3065)	TPP1, EtBr, erythromycin, acriflavine, safranin O and pyronin Y	De Rossi et al., 1998

ABC-ATP binding cassette; MFS- major facilitator superfamily; SMR- small multidrug resistance; RND-resistance-nodulation-cell division; INH- isoniazid; RIF- rifampicin; FQ- fluoroquinolone; TPP1-tetraphenyl phosphonium; EtBr- ethidium bromide.

Fig.1 Major mechanisms involved in MTB drug resistance

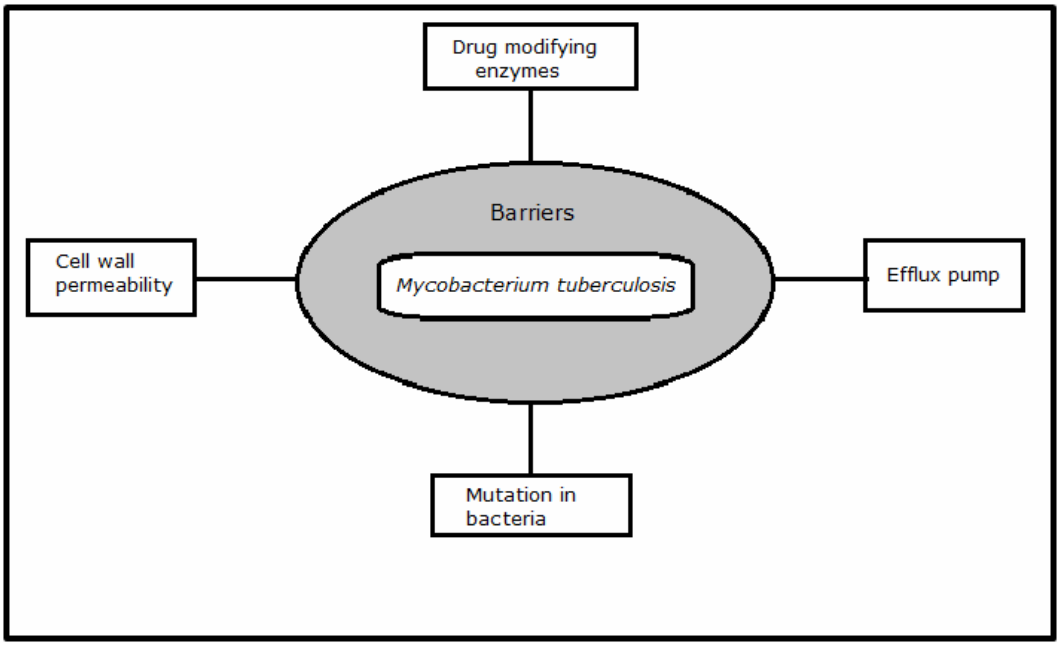


Fig.2A Arrangement 12 α helix TMDs

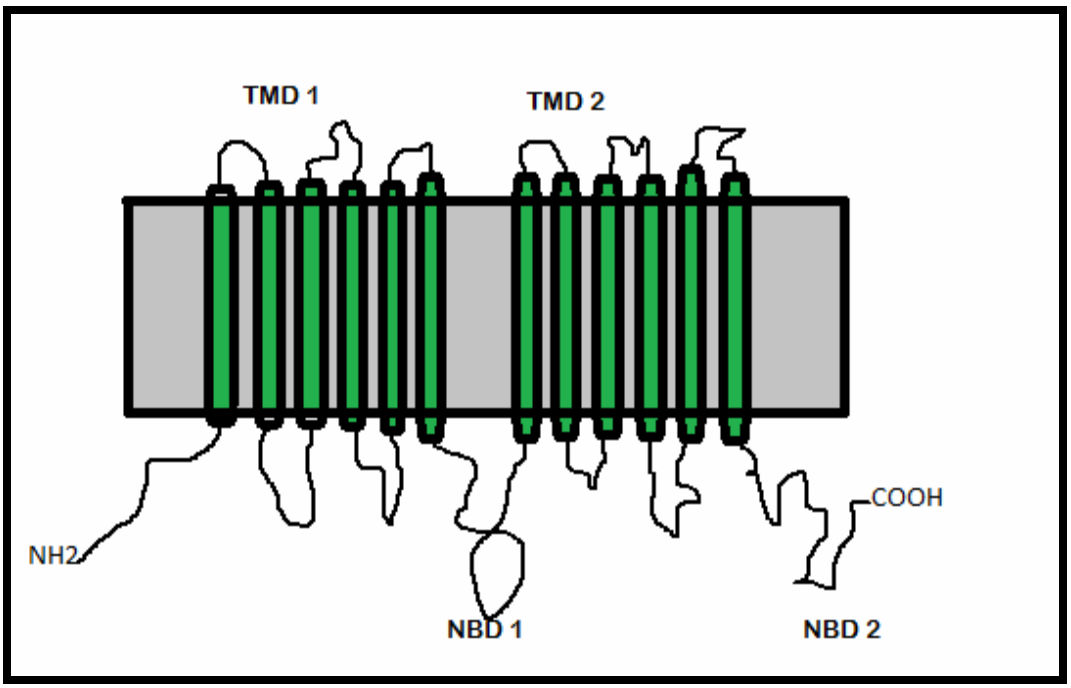


Fig.2B Organization of ABC transporter

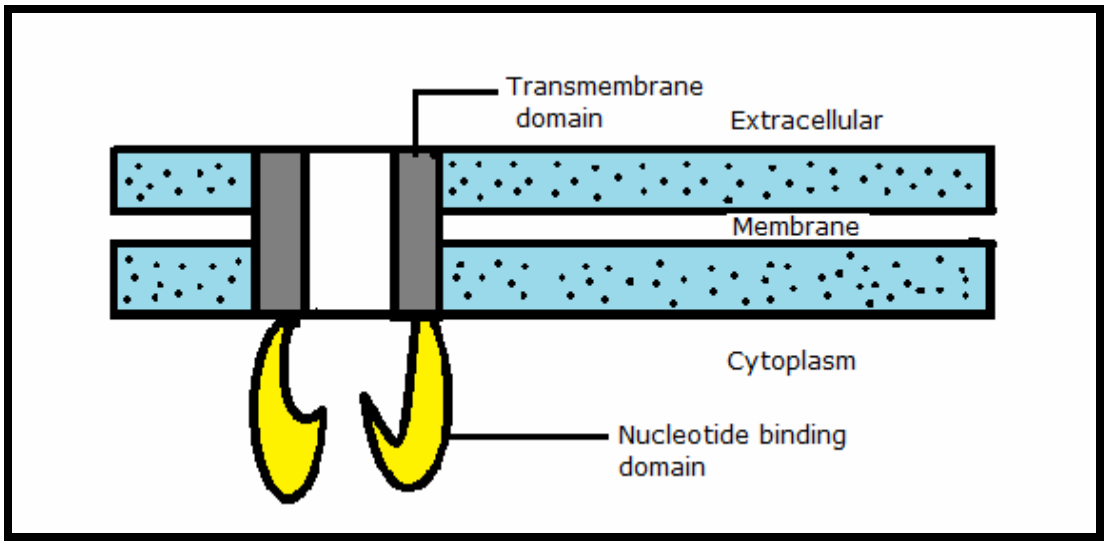


Fig.3 Mechanism of ABC transporter: Binding of substrate to TMDs with high affinity bringing conformational changes in NBDs. Binding of ATP bring NBDs in close proximity which bring conformational change in TMDs and hydrolyze to release ADP and Pi and because ADP has low affinity towards the NBDs, so it restore the conformational of TMDs facing inward.

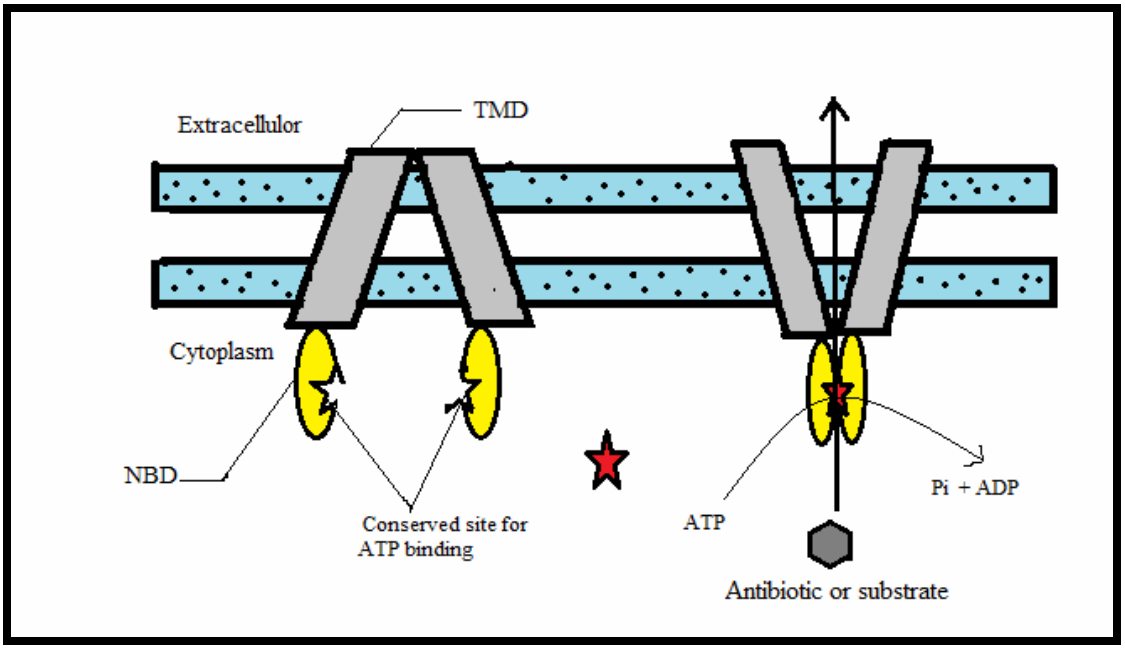


Fig.4A Arrangement of 12 α helical TMDs of MFS transporter.

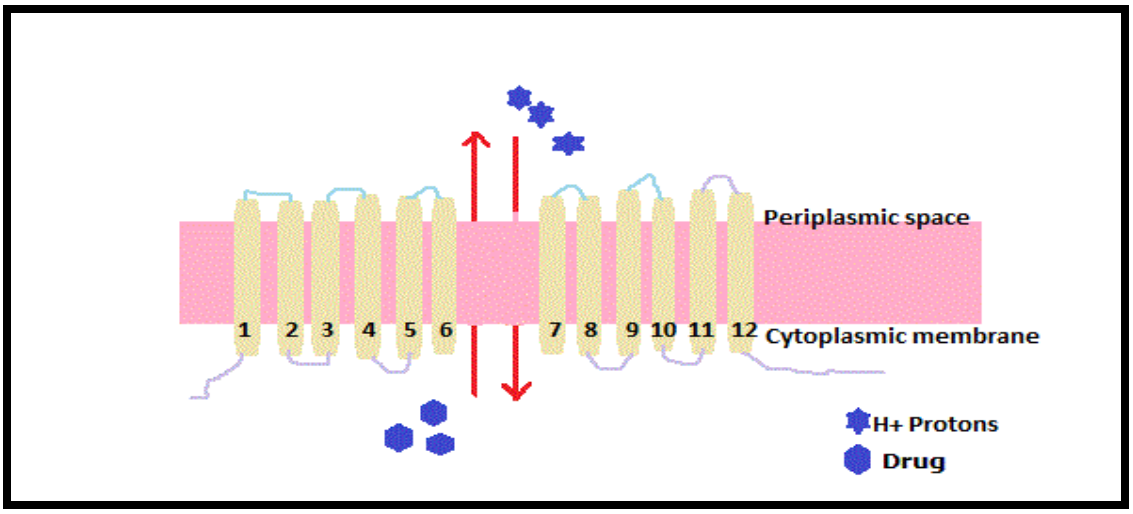


Fig.4B Mechanism of MFS transport: Substrate binds to its cationic charge binding site and pull the TM-1 and TM-7 towards each other and brings them close together resulting in altered stable position of transporter narrowing the cytoplasmic side and forcing out substrate to periplasm until the cytoplasmic side stabilizes again.

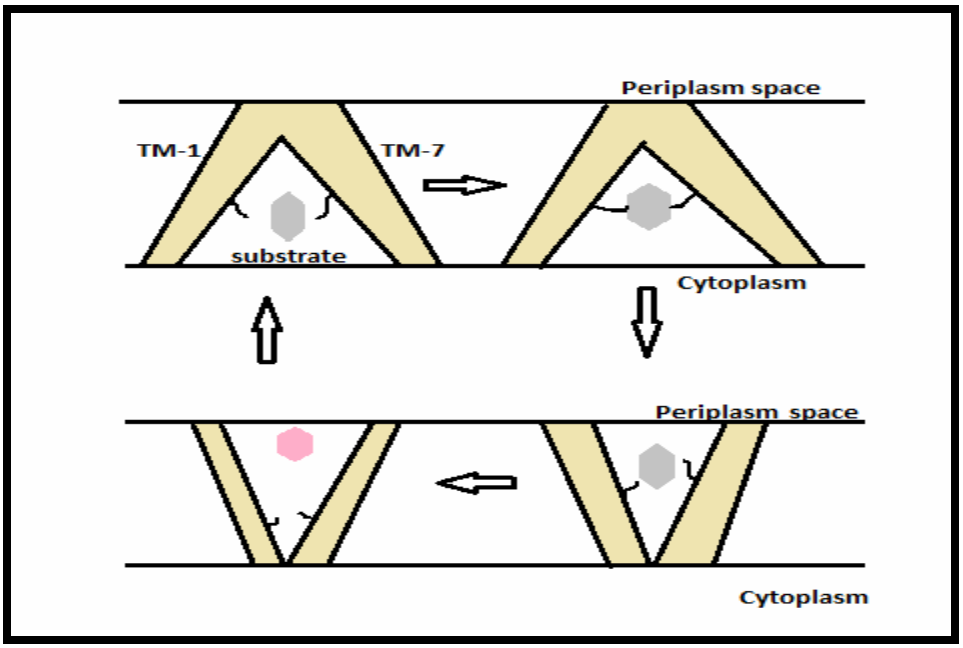


Fig.5 Framework of RND pump depicting tripartite complex structured by the internal membrane RND protein, OMF and MFP

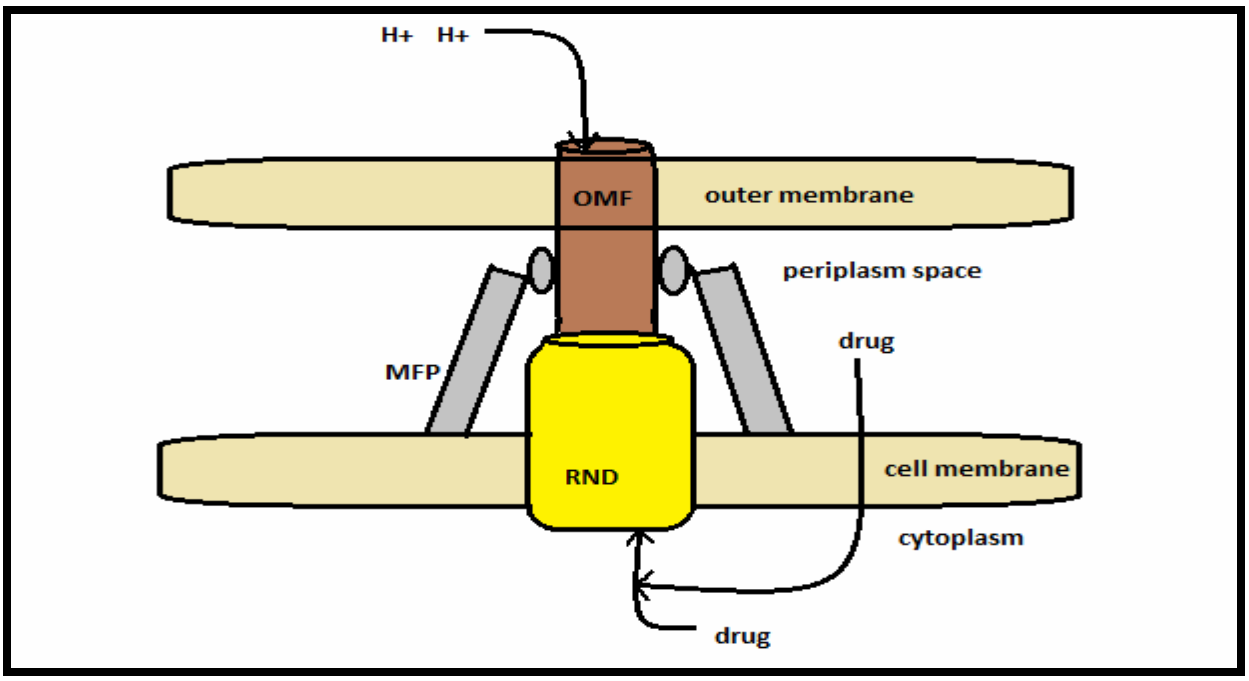


Fig.6A Arrangement of four α-helix TMDs of SMR transporter

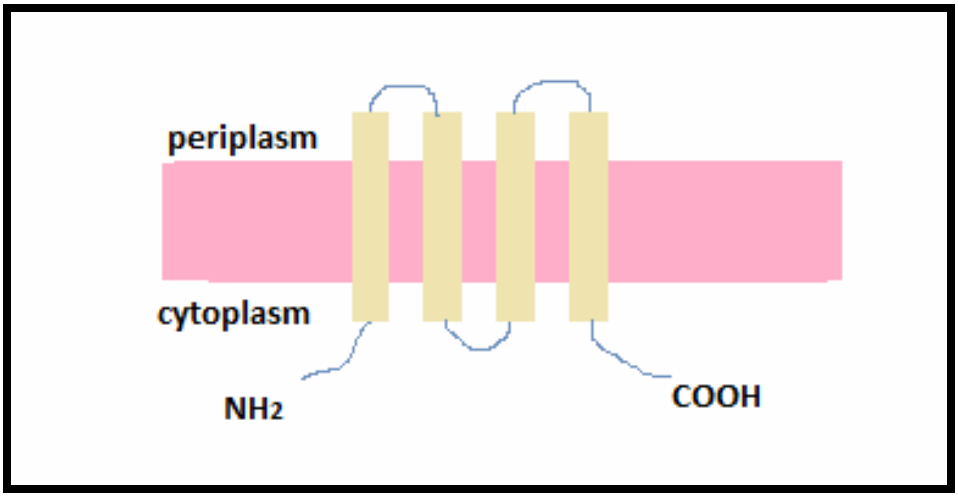


Fig.6B Mechanism of SMR transport: Blue hexagon represent proton and red star represents drug. 1) Antibiotic binds inside the pit open to the cytoplasmic of the lipid bilayer. 2) Conformational changes occur which opens the coupling chamber to the periplasm. 3) Binding of proton moves out drug into the periplasm and 4) Binding of another proton brings conformational changes reorienting the coupling site facing towards the cytoplasm.

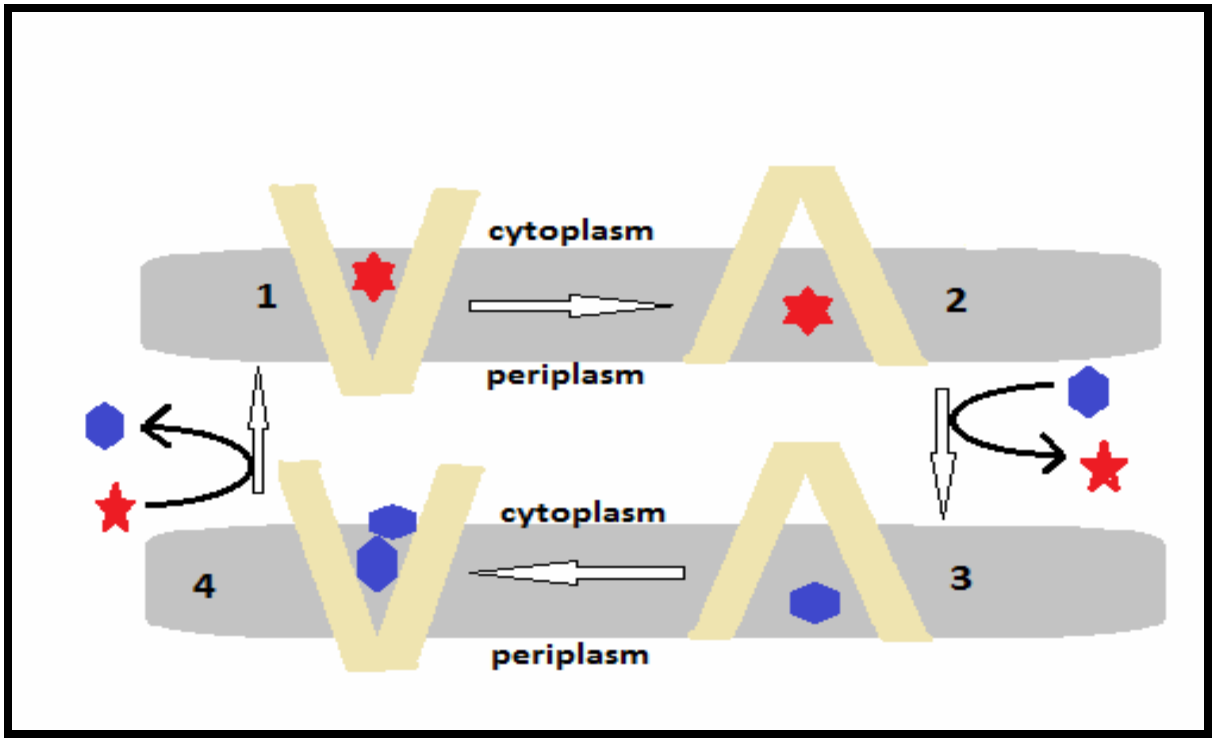
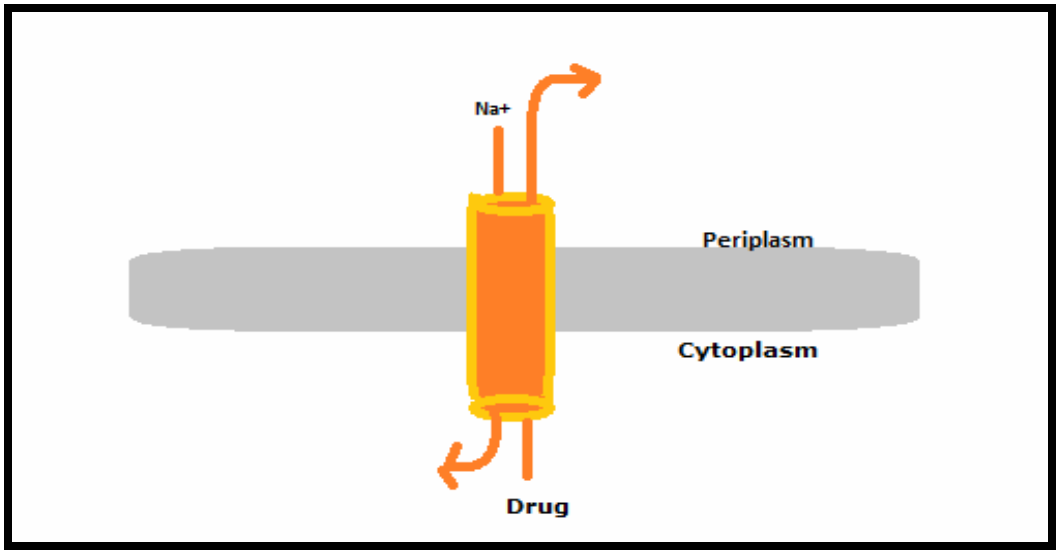


Fig.7 Na+/drug antiporter of MATE family



MATE drug efflux transporter

Multidrug and toxic compound extrusion (MATE) family transporters are found in all three domain of life (Archaea, Bacteria and Eukarya). They transport substrates via utilizing electrochemical gradient of H^+ or Na^+ (Omote, 2006; Kuroda., 2008). The MATE family is the most unexplored grouped family with just about twenty transporters having been recognized so far. NorM is a well studied MATE type efflux pump in *Neisseria gonorrheae* (NorM-NG). They are generally consist of 12 putative transmembrane domains divided into two halves (TM1-6) amino halves and (TM7-12) carboxyl halves which are connected by 11 loops denoted as L-1 to 11 among which L3-4, L6-7,L9-10 are longest loops. Major substrates for transporting are aromatic and cationic compounds.

Mechanism

In Na^+/drug antiporter (Fig.7) a substrate binds to its site in absence of cation and when the cation Na^+ is loaded, the TM-7 and TM-8 will move away from central cavity towards TM10. This induces conformational changes and substrate is disrupted from its binding site resulting in release of substrate. After release of substrate, TM7-8 moves back to its initial position. Likewise, alternatively Na^+ and substrate binds to its site instead of competing for binding site. Till date, in MTB there is no transporter discovered that belongs to MATE family and involved in MDR.

Bacteria have advanced modern components for protecting itself from effective drugs including drug efflux pumps that accommodate an extensive variety of substrates. In MTB, it plays double role and provides the leading cause of drug resistance and virulence. The initial role of these pumps is to oppose xenobiotic compounds but MTB utilize it for intracellular survival. As already discussed, overexpression is the major contributing factor for developing MDR-TB (Orme, 2011; Koul et al, 2011). Therefore, it is imperative to understand the mechanism and functioning of these efflux pumps which will help in formulating better therapeutic regimen of MDR-TB and finding an improved solution for anti-TB therapy.

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