Abstract

The burden and spread of drug-resistant tuberculosis disease is a major public health problem worldwide. The causative agent, Mycobacterium tuberculosis uses several mechanisms to counteract therapy through drug-resistance. A major and most common mechanism of drug-resistance is mediated through target mutations. Efflux pumps are emerging as potential agents of drug-resistance and treatment failure. In this review we explore the origin and principles of efflux pump-mediated resistance and determine their impact on second-line drugs used against extensively drug resistant tuberculosis. Inhibition of efflux pumps as a therapeutic intervention is also discussed.

Keywords: Efflux pumps; Second-line drugs; Mutations; Efflux pump inhibitors; Minimal inhibitory concentration

Introduction

Tuberculosis (TB) caused by Mycobacterium tuberculosis is a serious problem worldwide, with about 9.6 and 13 million incident and prevalent cases reported in 2014 respectively [1]. The burden of TB continues to be a global threat and has shown to be difficult to control in regions with high prevalence to human immune virus (HIV) infection [2]. There were 1.5 million TB deaths (1.1 million among HIV-negative people and 0.4 million among HIV-positive people), of which approximately 890 000 were men, 480 000 were women and 140 000 were children [1]. The global resurgence of TB due to progression of antibiotic resistance M. tuberculosis from mono-resistant through multi-drug resistant (MDR), extensively drug-resistant (XDR) and now totally drug-resistant (TDR) forms is worrisome [3,4]. Resistance to at least rifampicin (RIF) and isoniazid (INH) the two most effective TB drugs used in first line treatment is defined as MDR-TB [5]. Further resistance of MDR-TB strains to fluoroquinolone (FLQ) and at least one of the three following injectable drugs namely capreomycin (CAP), kanamycin (KAN), and amikacin (AMK) leads to XDR-TB [6].

Status of XDR-TB Treatment

As early as 2006, XDR-TB has been reported in 17 countries and now found throughout the world is difficult to treat [6]. Primary XDR-TB patients are treated with second-line drugs (SLDs) and the duration of the intensive phase of treatment with an injectable drug (i.e., CAP) is at least 6 months while the continuation phase (without the injectable drug) should last until 18 months after culture conversion [7]. According to treatment guidelines, registered chronic TB patients are started on a standard, Green Light Project-approved, category IV regimen, which consist of an intensive phase of 6 months with pyrazinamide, ofloxacin (OFL), KAN, ethionamide and cycloserine, followed by a 15-month continuation phase with OFL, ethionamide and cycloserine [8]. In South Africa, CAP has replaced AMK/KAN, while p-aminosalicylic acid replaced pyrazinamide and moxifloxacin replaced OFL for the treatment of XDR-TB [9].

Classification of Second-Line Drugs

Most of the treatment regimens against XDR-TB require the use of SLDs which are less effective and toxic. Several factors are considered when choosing the appropriate drug, including availability, rationale, the cost of the drug, and the possibility of toxic adverse events [7]. Drugs used for treatment of drug-resistant TB are classified into five groups; the first group (RIF and INH) is reserved for TB treatment while the last four groups are used for MDR and XDR-TB treatment. The second group of drugs is also mainstay in the treatment of MDR and XDR-TB and includes the aminoglycosides (streptomycin (STR), KAN, and AMK) and polypeptides (CAP) [7]. The FLQ drugs are classified under group three and deliver better clinical outcomes than drugs in the other groups [7]. Main drugs that belong to FLQ group (i.e., OFL, levofloxacin, moxifloxacin and gatifloxacin) are the most effective SLDs recommended for treatment of MDR and XDR-TB patients [10]. However, moxifloxacin a new-generation drug, has been recommended by the World Health Organization (WHO) for the treatment of XDR-TB [11]. The p-aminosalicylic acid, cycloserine and ethionamide are bacteriostatic agents and classified under group four drugs [7]. Linezolid and clofazimine classified under group five are considered as third-line may be an important option for the treatment of XDR-TB; however are associated with adverse events [12,13]. Other drugs in these groups include bedaquiline (BDQ), a diarylquinoline, approved for the treatment of MDR and XDR-TB in combination therapy with at least three other active drugs [14].

Newer drugs under this group include delamanid (a nitroimidazole) that received accelerated approvals based on small trials showing spumut culture conversion [15]. A new and more potent ethylenediamine derivative (SQ109) is active against ethambutol (EMB)-resistant M. tuberculosis strains and targets MmpL3, a membrane transporter involved in mycolic acid synthesis and cell wall assembly [16,17].

References

[1] World Health Organization. Global tuberculosis report 2015.
[2] World Health Organization. Global tuberculosis report 2014.
[3] World Health Organization. Global tuberculosis report 2013.
[4] World Health Organization. Global tuberculosis report 2012.
[5] World Health Organization. Global tuberculosis report 2011.
[6] World Health Organization. Global tuberculosis report 2010.
[7] World Health Organization. Global tuberculosis report 2009.
[8] World Health Organization. Global tuberculosis report 2008.
[9] World Health Organization. Global tuberculosis report 2007.
[10] World Health Organization. Global tuberculosis report 2006.
[11] World Health Organization. Global tuberculosis report 2005.
[12] World Health Organization. Global tuberculosis report 2004.
[13] World Health Organization. Global tuberculosis report 2003.
[14] World Health Organization. Global tuberculosis report 2002.
[15] World Health Organization. Global tuberculosis report 2001.
[16] World Health Organization. Global tuberculosis report 2000.
[17] World Health Organization. Global tuberculosis report 1999.

Copyright © 2016 Malinga et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Malinga et al., Mycobact Dis 2016, 6:3
DOI: 10.4172/2161-1068.1000222

Mycobacterium Diseases

Mycopact Dis, an open access journal
ISSN: 2161-1068

Volume 6 • Issue 3 • 1000222
Drug Resistant Mechanisms of Second-Line Drugs

The main mechanism in development of FLQ resistance in *M. tuberculosis* is by chromosomal mutations within the quinolone resistance-determining region (QDRD) of gyrA or gyrB genes [18]. However, only 60-70% of *M. tuberculosis* strains with FLQ resistance can be accounted for by these mutations within QDRD [19]. Most of the mutations are mostly found in positions 90, 91 and 94 in the gyrA gene [19]. In addition, novel mutations within gyrA such as Met81Thr, Leu109Pro, and Gln113Leu substitutions have been detected in FLQ resistant strains [20]. Other factors such as active efflux mechanisms could contribute to the rest of FLQ resistance [21].

Aminoglycosides (i.e., AMK, KAN) and cyclic peptides (i.e., CAP) group referred to as injectables inhibit protein synthesis through modification of ribosomal structures at 16S rRNA and formation of 30S ribosomal subunit respectively [22]. Appropriate use of “second-line” injectable drugs of AMK, KAN and/or CAP is critical for the treatment of MDR-TB and prevention of XDR-TB [23]. Point mutations of A1401G and G1484T within 16S rRNA gene (rrs) are responsible for high level resistance to AMK and KAN drugs [24]. Resistance to KAN drug is further caused by C14T, G37T and G10A mutations found within the promoter region of enhanced intracellular survival (cis) gene [25]. While CAP resistance is mainly caused by rrs C1402T and tlyA gene mutations, however they have been detected with low frequency [26]. Therefore, only 70-80% of global *M. tuberculosis* strains with injectable resistance harbour rrs, cis and tlyA mutations [23]. The remaining resistance cannot be explained on the basis of these target site mutations and other mechanism could be involved.

While major resistance (60-95%) is caused by drug target mutations, mechanisms to SLD drugs are not as well understood as that of first line drugs [23,27,28]. Efflux pumps (EPs) play a role in drug resistance however their influence in the presence and absence target mutations is not well understood. Our lack of complete understanding of the resistance mechanisms to SLD makes it difficult to diagnose and treat XDR-TB timely. Missed XDR-TB cases are mostly fatal and can lead to amplification of resistance potentially resulting in TDR-TB which is resistant to all known drugs with high mortality in the cases reported [2].

In this review we provide an overview on current knowledge on efflux mediated drug-resistant mechanisms to SLDs.

The Evolution of Drug Resistance in *Mycobacterium tuberculosis*

Drug resistance in *M. tuberculosis*, as in any other bacterium, is an outcome of multiple mechanisms operating simultaneously within the bacteria [29]. Changes within cell permeability, target gene mutations and drug efflux contribute to acquired and intrinsic drug resistance in *M. tuberculosis* [30,31]. Mutations in target genes are major contributors, but when in combination with efflux pump system they can make an organism hard to treat. Efflux pumps (EPs) are membrane transporters that can extrude a broad range of small molecules from the bacterial cytoplasm including drugs to the external environment [32]. They could be activated by immune or drug pressures when *M. tuberculosis* bacterium enters the human macrophage [33]. Once activated they decrease accumulation of antimycobacterial drugs and reduce their cytoplasmic concentration to sub-inhibitory levels [34]. The EPs systems can confer resistance to single drug or multiple classes of drugs leading to multidrug resistant phenotype [35].

Apart from chromosomal mutations, *M. tuberculosis* can increase its drug resistance by preventing the drug from entering through the cell wall and reaching site of action [4]. Activation EPs is a simple process but it does entail multiple coordinated processes which requires the binding of substrate, provision of energy, translocation of the substrate across the membrane and resetting of the transporter [36]. Multidrug resistant EPs actively extrude drugs out of the cell with a remarkably broad range of substrate specificity, and are typically most effective when combined with other resistance mechanisms such as mutations [2]. The action of EPs could prevent drugs from reaching concentrations lethal to the bug leading to intrinsic resistance [37]. A population of mycobacteria within the lung can contain members with different susceptibilities and drug concentrations fluctuate during therapy thus may favour the induction of EPs [34]. The over-expression of EPs through physiological induction and spontaneous mutation formation can significantly lower the intracellular concentration of drugs causing an impact on their clinical efficacy [38]. During patient treatment sub-inhibitory drug exposures normally reach the site of infection and the bacterial EPs can be activated within few bacillary replication cycles leading to low level resistance [39,40]. The development drug resistance through activation of EPs is an important mechanism in *M. tuberculosis*. Below we discuss activation of EPs that could impact on the development of resistance.

Activation of Efflux Pumps and Accumulation of Mutations

There is substantial variability in the response to TB therapy, even in those patients infected with fully drug-sensitive strains [41]. Given the pharmacokinetic (e.g. time course of drug levels in body fluids) and pharmacodynamics (e.g. rate and extent of bactericidal action) variability of drug concentrations at the site of infection, optimal microbial killing may not be achieved and resistance may ensues [42]. Schmalstieg et al. proposed that the induction of EPs is not due to the immune system but is specifically in response to sub therapeutic drug stress within the bacteria [39]. It is proposed that induction of EPs which transports two or more drugs is the first step to the emergence of resistance [43]. During INH treatment, greater than 99% of the initial spumic bacillary load is killed during the first 2 days of treatment, after which the rate of killing drops off markedly [44]. The residual bacteria are a phenotypically resistant, “drug-tolerant” population; and their minimum inhibitory concentrations (MICs) remain unchanged throughout treatment [44]. Empirical studies have shown that it takes months of therapy to eradicate drug-tolerant bacteria and produce a stable cure [45]. Adams et al. proposed that EPs induced by macrophages lead to drug-tolerance, an important barrier *in vivo* to shorten TB treatment [46]. Drug-tolerant bacteria originate in macrophages and their survival is dependent on the activation of bacterial EPs used to transport drugs out of the cytoplasm [46].

The drug-tolerant bacteria continue to replicate under the protection of EPs and can generate chromosomal mutations associated with high level resistance [47]. Once mutations within drug target genes are acquired high level drug resistance in mycobacteria is initiated. Schmalstieg et al. also hypothesized that this pathway could lead to the development of drug resistance in mycobacteria [39]. Development of drug resistance leading to MDR and XDR-TB is a stepwise process and evolution from susceptible to resistant strain occurs [48].
Metabolic Changes and Efflux Pumps Activation

During infection, the *M. tuberculosis* bacilli reside in different micro environmental conditions that include lung cavities or host macrophages that are characterized by nutrient starvation, oxidative stress, and acidic pH all of which affect their metabolic statuses [49]. Such varied conditions constitute the basis for producing heterogeneous bacterial populations, including nonreplicating persisters and growing bacteria with different capacities for persister formation [49]. Persisters may lead to a slower metabolism and transcription rate in cells without specific drug resistant mutation [50]. In the presence of drugs, the average transcription rate decreases and efflux pump activity increases [51]. Moreover persistent bacteria with slow growth rate had decreased replication rate that reduced the expression of ribosomal proteins and FLQ target genes (gyrA and gyrB) [52]. Transcription studies on *M. tuberculosis* strains that lacked mutations in drug target genes had activated drug efflux pumps (i.e., DrrA) that may promote the persistence [53]. During treatment of XDR-TB patient, iniBAC operon coded by EPs was over-expressed on the fifth month of treatment and despite lobectomy procedure the infection still persisted [48]. Therefore, it is reasonable to assume that reduced cell growth and over-expression of EPs could lead to high levels of resistance [51]. Thus EP mediated resistance might be an important mechanism in persistent bacteria of patients on treatment [21].

### Drug Efflux Pumps in *Mycobacterium tuberculosis*

Drug efflux pump genes code for transporter proteins involved in the natural removal of toxic substances from the interior of the cell to the exterior environment requires energy [54-56]. In terms of energy requirements and structural criteria EPs are divided into primary and secondary transporters. Bacterial EPs, including *M. tuberculosis* have been classified into five superfamilies: ATP-binding cassette (ABC), major facilitator super-family (MFS), resistance nodulation division (RND), small multidrug resistance (SMR) (Table 1) and the multidrug and toxic compound extrusion (MATE) family [57,58]. The efflux pump proteins of MFS, SMR, RND and MATE families uses proton motive force (H+ or Na+) provided by trans-membrane electrochemical gradient of proton or sodium ion to drive the extrusion of drugs from the cell [54]. The primary ABC superfamily uses ATP as an energy source to pump drugs out of the cell and cause multidrug efflux [54,59]. Secondary transporters (i.e., MFS, RND, SMR, MATE) are the most dominant while ABC transporters are mainly substrate specific [35]. In *M. tuberculosis* genome, genes encoding mainly for drug efflux transporters belong to ABC, MFS, SMR and RND families. Multidrug efflux systems are present on bacterial cell walls and limit the access of antimicrobial agents to their targets. Moreover EPs appear to be one of the most widespread antibiotic resistance mechanisms among most microorganisms [60].

### Efflux Pump Family

| Efflux Pump Family | Efflux pump Genes | Resistance to drugs | Inhibitors of Efflux pumps | Phenotypes | Reference(s) |
|--------------------|-------------------|--------------------|---------------------------|------------|--------------|
| **ABC**            | Rv2936 (ddrA)     | MDR-TB             | CCCP, Piperine, VER       | MDR-TB     | [53]         |
|                    | Rv1687            | MDR-TB             |                           |            | [53]         |
|                    | Rv1686            | MDR-TB             |                           |            | [53]         |
|                    | Rv2937 (ddrB)     | RIF, INH, STR, EMB | CCCP, TDZ, VER           | MDR-TB     | [68, 2, 21, 62, 98, 99] |
|                    | Rv1456/57/58      | FLQ                | CCCP, TDZ, VER           | MDR-TB XDR-TB | [62]         |
|                    | Rv2686/87/88      | OFL                |                           |            | [82]         |
|                    | Rv2477            | OFL, STR           |                           |            | [79]         |
|                    | Rv2938            | STR                | RES                       | [59, 79, 80] |
|                    | Rv0194            | STR                | VER, RES, CCCP           | [61, 62]   |
|                    | Rv1217/18         | OFL                |                           | [82]       |
|                    | Rv2209            |                    |                           | [82]       |
| **MFS**            | Rv1258            | STR, RIF, INH, FLQ | CCCP, Piperine, VER      | MDR-TB     | [2, 60, 65, 76, 86] |
|                    | Rv1410c           |                    |                           | MDR-TB     | [100]        |
|                    | Rv3728            | CFZ                | CCCP                       | MDR-TB     | [59, 80]   |
| **SMR**            | Rv0783c           | INH, RIF           |                           | [101]      |
|                    | Rv2459            | INH, EMB, STR      |                           | [55, 60]   |
|                    | Rv2994            | STR, FLQ, CIP      |                           | [82]       |
|                    | Rv3065 (mmr)      | INH, EMB, STR      |                           | [53, 74, 77, 80, 85] |

Citation: Malinga LA, Stoltz A, Van der Walt M (2016) Efflux Pump Mediated Second-Line Tuberculosis Drug Resistance. Mycobact Dis 6: 222. doi:10.4172/2161-1068.1000222
Fluoroquinolone Efflux Pump Mediated Resistance and Inhibition

Over-expression of Rv1634 and Rv2686c-Rv2687c-Rv2688c EPs conferred resistance to FLQ by increasing MICs values by eight fold when expressed in M. smegmatis [2,21]. One of these putative pumps, Rv1634 decreases susceptibility to FLQs of the same class namely norfloxacin and ciprofloxacin [60]. Several mycobacterial EPs associated with FLQ resistance have been described, including pumps of the MFS family (lfrA and Rv1258c) and ABC transporters (DrrAB, PstB and Rv2686c-2687c-2688c) [60]. The FLQ resistance due to absence of DNA gyrase mutations were found to have over-expressed EPs leading to increased MICs [2]. Sparfloxacin is a more hydrophobic FLQ and strong interaction with MfpA protein coded by Rv3661 occurs, leading to intrinsic resistance [69]. Using gene expression based analysis a tenfold increase was revealed in the Rv1258c transcript level in the presence of RIF and a sixfold increase in the presence of OFL drug [70]. Moreover OFL stress altered the expression of two more EPs namely, Rv2477 and Rv2209 of ABC family [62]. Apart from mutations within target genes, EPs are recognized as causes of resistance to FLQ group, however it remains to be seen if newer drugs of moxifloxacin and gatifloxacin are similarly affected. To counteract the effects of resistance by EPs, an efflux pump inhibitor (EPI) is used.

Other FLQ drugs such as ciprofloxacin had MICs decreased in 30% of resistant strains that over-expressed EPs [71]. While the MIC levels of linezolid (a protein synthesis inhibitor) were slightly reduced in the presence of reserpine (RES) [72]. Efflux pump inhibitors such reserpine, verapamil (VER), carbonyl cyanide m-chlorophenylhydrazone (CCCP) and thioridazine (TDZ) have been shown to inhibit overexpression of Rv2686c-Rv2687c-Rv2688c operon involved in drug resistance [21]. The ABC transporters were inhibited by VER in the development of OFL resistance in M. tuberculosis isolates [73]. Over-expression of Rv1634, used by the pathogen as a potential mechanism to resist drug activity is inhibited by TDZ [74]. Moreover FLQ resistant isolates without mutation in the DNA gyrase region, had their MICs reduced by reserpine, VER and CCCP indicating the expression of efflux mediated resistance [75].

Aminoglycosides and Cyclic Peptides Efflux Pump Mediated Resistance and Inhibition

The Rv1258c EP was shown to confer low-level resistance to aminoglycosides when expressed in M. smegmatis [76]. For instance an increase in whiB7 (Rv3197) expression leads to upregulation of at least two different antibiotic resistance genes in the whiB7 regulon namely eis and Rv1258c [25]. Furthermore a decrease in the MIC of KAN drug was observed in the knockout mutant of Rv3065 gene of the SMR family [77]. Transport proteins of the ABC and MFS families, i.e., Rv2688, Rv2938, and Rv2994 have been observed to be over-expressed causing STR drug extrusion in M. tuberculosis [57,62].

The aminoglycoside spectinomycin (a STR analog) was effluxed by Rv233c EP when expressed in M. bovis [61]. However a new class of spectinomycin analog, spectinamide evaded Rv1258c over-expression through structural modification and restored MICs to susceptible levels by binding to 16S bacterial ribosomal subunit [78]. Over-expression of ABC transporter Rv0194 lead to an increased resistance of both M. smegmatis and M. bovis to multiple drugs including ampicillin, chloramphenicol, tetracyclin, vancomycin (VAC), erythromycin, novobiocin and STR [79]. The cyclic peptide, VAC was also effluxed by Rv1258c, Rv0849, Rv1218c and Rv3065 genes [80].
It has been noted that some isolates of *M. tuberculosis* with a low level resistance to aminoglycosides did not present with any mutations in the rpsL, rrs, and gidB gene sequences but show decreased MICs in the presence of an EPI [81]. A knockout mutant of Rv1410c was studied and the levels of resistance to bacitracin, clofazimine (CFZ), econazole, novobiocin, PA-824, rifampin, valinomycin, and VAC returned to the wild-type suggesting that it contributes to the intrinsic resistance to these drugs [82].

In essence Rv1410c provides low level intrinsic resistance to a range of different antimicrobial compounds in *M. bovis* BCG [83]. The Rv1410c mutant was shown to have reduced resistance to rifampicin, amikacin, moxifloxacin, linezolid, and rifabutin [84]. Clearly this demonstrates that Rv1410c of *M. tuberculosis* is involved in multiple drug resistance.

The p-aminoalicyclic acid drug (a second-line oral anti-TB agent) together with spectinomycin, and tetracycline were identified as specific Rv1258c substrates in *M. bovis* since a four-fold change in sensitivity was observed in resistant strains as compared to the wild-type in the presence of EPI [65]. More recently the MIC levels of recently approved BDQ drug were increased four-fold together with the control strain [85].

Moreover BDQ was shown to inactivate EPs through the inhibition of ATP energy source [32]. Cross-resistance in second-line injectable drugs is a common phenomenon and this could lead to poor patient treatment outcomes. Newer drugs such as BDQ that could inhibit EPs are crucial and should be considered in new treatment regimens.

Aminoglycosides MIC levels were reduced in the presence of CCCP, suggesting that a decrease in their extrusion was predominant [76]. Since aminoglycosides are known to enter cells by an energy-dependent mechanism; CCCP can affect the levels of resistance to this group by decreasing both the uptake and extrusion through Rv1258c [79]. Furthermore piperine, an EPI was shown to play a significant role in the inhibition of Rv1258c [86]. While VER reduces drug tolerance by inhibiting Rv1258c induced upon entry of the drug into intracellular matrix [46].

On the contrary it was found that VER had an antagonistic effect when ribosome-targeting drugs such as STR, KAN, and CAP, as well as the cell wall agent cyclocerosine were used in macrophages [87]. The Rv1258c EP is well characterized and may play a significant role in aminoglycoside resistance and the effects can be reversed by EPI.

### Mutations within Drug Efflux Pump Genes

The introduction of whole genome sequencing (WGS) in *M. tuberculosis* genome analysis is revealing novel mutations within EPs. Earlier work has indicated that mutation causing an over-expression of EPs are a potential threat to overcoming drug resistance [63]. Liu and Xie identified 20 known or putative EPs with non-synonymous mutations in MDR, pre-XDR and XDR-TB *M. tuberculosis* isolates but none in H37Rv strain [88]. Non-synonymous mutations of M74T, R426H and I948V belonging to Rv0194, Rv0507 and Rv0676c respectively of the transporter families have been found in clinical isolates [88].

Detection of XDR-TB strains with non-synonymous mutations of P1098L, G198A and C213A within Rv0194, Rv1634, and Rv2688c EPs respectively in contrast to MDR-TB strains has been reported [89]. Resistant FLQ strains lacking DNA gyrase genetic changes showed various mutations in five EPs genes of Rv1217c, Rv0783c, Rv0849, Rv1877 and Rv2459 [90]. Moreover mutations were identified in transport proteins in 11% of samples that underwent WGS [91]. Using WGS we detected number of mutations within Rv0987, Rv2039, Rv0402 in an OFX resistant isolate without gyrA mutation [92].

Cross-resistance to STR and KAN due to over-expression of Rv1258c has been associated with a G133C mutation [93]. Rv3226 and Rv0849 had R26G and T403I mutations respectively in XDR-TB as compared to sensitive strains [94]. Several EPs, including the ABC transporters of Rv0194 and Rv1463, were affected by a larger number of independent mutations in resistant strains relative to sensitive strains during WGS bioinformatic analysis [95].

Genetic mutations in the form of insertions or deletions within Rv0678 caused MmpL5 protein over-expression [96]. Other mutations within 5′ untranslated region of whiB7 that regulates Rv1258c, lead to resistance [97]. Recent WGS we detected number of mutations within Rv0987, Rv2039, Rv0402 in an OFX resistant isolate without gyrA mutation [92].

As previously stated a non-synonymous caused by single nucleotide polymorphism was observed in Rv0678 gene (A202G leading to S68G) causing an increased MIC in BDQ [85]. Mutations within EPs mediating resistance over-expressed Rv1258c and treatment with inhibitors lead to reverse of resistance [46]. Furthermore drug candidate, SQ109 was inhibited by non-synonymous mutation Q40R of Rv0206c gene (MmpL) [97]. A summary of mutations in EPs is found in (Table 2).

| Efflux Pump Family | Efflux pump gene | Mutation | Drugs | Reference(s) |
|--------------------|------------------|----------|-------|--------------|
| ABC                | Rv0194           | M74T, P1098L, A277V, V398M, G431R, L486M, F705I | [88], [89, 95, 102] |
|                    | Rv2688c          | C213A    |       | [89]         |
|                    | Rv1217c          | V463C    |       | [90]         |
|                    | Rv1463           | 198E     |       | [95]         |
|                    | Rv1704           | 93L      | Fluoroquinolones | [90] |
|                    | Rv1272c          | H613N    |       | [102]        |
|                    | Rv1273           | G416V, C142K |       | [102]        |
Summary and Conclusions

Drug resistance in *M. tuberculosis* is a complicated phenomenon involving both intrinsic and acquired mechanisms. Intrinsic mechanisms in the form of EPs activation is regarded as the first step towards higher levels of resistance. Once drugs enter the organisms, over-expression and mutations in either EPs or target regions may lead to high level resistance. To counteract the effects of EPs activation, EPI in combination with drug regimens can be used in chemotherapy. Ideally they will be combined with less effective SLDS to improve treatment outcomes. Clinically approved inhibitor, VER has therapeutic effects against most of the EPs that use ATP as an energy source used in combination with BDQ it is highly effective since they both block ATP energy source. Moreover, SQ109 (Sequella) that blocks EPs currently undergoing phase II clinical trials is showing great promise. Indeed, EPs are becoming an attractive area of research in terms of drug development and diagnosis through mutations.

Acknowledgement

This work was funded by the University of Pretoria, National Research Foundation (NRF) and South African Medical Research Council.

References

1. WHO (2015) Global Tuberculosis Report. World Health Organization, Geneva, Switzerland.

2. Louw GE, Warren RM, Gey van Pittius NC, Leon R, Jimenez A, et al. (2011) Rifampicin reduces susceptibility to oloxacin in rifampicin-resistant Mycobacterium tuberculosis through efflux. Am J Respir Crit Care Med 184: 269-276.

3. Amaral L, Viveiros M (2012) Why thioridazine in combination with antibiotics cures extensively drug-resistant Mycobacterium tuberculosis infections. Int J Antimicrob Agents 39: 376-380.

4. Amaral L, Martins A, Spengler G, Molnar J (2014) Efflux pumps of Gram-negative bacteria: what they do, how they do it, with what and how to deal with them. Front Pharmacol 4: 168.

5. LoBue PA, Enarson DA, Thoen CO (2010) Tuberculosis in humans and animals: an overview. Int J Tuberc Lung Dis 9: 1075-1078.

6. Raviglione MC, Smith IM (2007) XDR tuberculosis—implications for global public health. N Engl J Med 356: 656-659.

7. Caminero JA, Sotgiu G, Zumla A, Migliori GB (2010) Best drug treatment for multidrug-resistant and extensively drug-resistant tuberculosis. Lancet Infect Dis 10: 621-629.

8. Badoum G, Saleri N, Dembele MS, Ouedraogo M, Pinsi G, et al. (2011) Failing a re-treatment regimen does not predict MDR/XDR tuberculosis: is “blind” treatment dangerous? Eur Respir J 37: 1283-1285.

9. Streicher EM, Muller B, Chihota V, Mlambo C, Tait M, et al. (2012) Emergence and treatment of multidrug resistant (MDR) and extensively drug-resistant (XDR) tuberculosis in South Africa. Infect Genet Evol 12: 686-694.

10. Laurenzo D, Mousa SA (2011) A Mechanisms of drug resistance in Mycobacterium tuberculosis and current status of rapid molecular diagnostic testing. Acta Trop 119: 5-10.

11. Dookie N, Sturm AW, Moodley P (2014) Moxifloxacin resistance in the F15/LAM4/KZN extensively drug-resistant strain of Mycobacterium tuberculosis. Infect Drug Resist 7: 223-228.

12. Anger HA, Dworkin F, Sharma S, Munsiff SS, Nilsen DM, et al. (2010) Linezolid use for treatment of multidrug-resistant and extensively drug-resistant tuberculosis, New York City, 2000-06. J Antimicrob Chemother 65: 775-783.

13. Lee M, Lee J, Carroll MW, Choi H, Min S, et al. (2012) Linezolid for treatment of chronic extensively drug-resistant tuberculosis. N Engl J Med 367: 1508-1518.

14. Wilson JW, Tsukayama DT (2016) Extensively drug-resistant tuberculosis: principles of resistance, diagnosis, and management. Mayo Clin Proc 91: 482-495.
Page 7 of 9

Citation: Malinga LA, Stoltz A, Van der Walt M (2016) Efflux Pump Mediated Second-Line Tuberculosis Drug Resistance. Mycobact Dis 6: 222.
doi:10.4172/2161-1068.1000222

15. Wallis RS, Maeder M, Mwaba P, Chakaya J, Rustomjee R, et al. (2016) Tuberculosis advances in development of new drugs, treatment regimens, host-directed therapies, and biomarkers. Lancet Infect Dis 16: e34-e46.

16. Cole ST, Riccardi G (2011) New tuberculosis drugs on the horizon. Curr Opin Microbiol 14: 570-576.

17. Ahmad S, Mokaddas E (2014) Current status and future trends in the diagnosis and treatment of drug-susceptible and multidrug-resistant tuberculosis. J Infect Public Health 7: 75-91.

18. Palomino J, Martin A (2014) Drug Resistance Mechanisms in Mycobacterium tuberculosis. Antibiotics 3: 317-340.

19. van Doorn HR, An DD, de Jong MD, Lan NT, Hoa DV, et al. (2008) Fluoroquinolone resistance detection in Mycobacterium tuberculosis with locked nucleic acid probe real-time PCR. Int J Tuberc Lung Dis 12: 736-742.

20. Devasia R, Blackman A, Eden S, Li H, Maruri F, et al. (2012) High proportion of fluoroquinolone-resistant Mycobacterium tuberculosis isolates with novel gyrA polymorphisms and agyr region associated with fluoroquinolone susceptibility. J Clin Microbiol 50: 1390-1396.

21. De Rossi E, Ainsa JA, Riccardi G (2006) Role of mycobacterial efflux transporters in drug resistance: an unresolved question. FEMS Microbiol Rev 30: 36-52.

22. Liou YF, Tanaka N (1976) Dual actions of vimycin on the ribosomal functions. Biochem Biophys Res Commun 71: 477-483.

23. Georgiou SB, Magana M, Garfein RS, Catanzaro DG, Catanzaro A, et al. (2012) Evaluation of genetic mutations associated with Mycobacterium tuberculosis resistance to amikacin, kanamycin and capreomycin: a systematic review. PLoS One 7: e33275.

24. Suzuki Y, Katsukawa C, Tamaru A, Abe C, Makino M, et al. (1998) Detection of kanamycin-resistant Mycobacterium tuberculosis by whole genome sequencing analysis. J Clin Microbiol 36: 1220-1225.

25. Zaunbrecher MA, Sikes RD, Metchock B, Shinnick TM, Posey JE (2009) Overexpression of the chromosomally encoded aminoglycoside acetyltransferase rmtA confers kanamycin resistance in Mycobacterium tuberculosis. Proc Natl Acad Sci U S A 106: 2004-2009.

26. Engstrom A, Perskvist N, Werngren J, Hoffner SE, Jureen P (2011) Comparison of clinical isolates and in vitro selected mutants reveals that tlyA is not a sensitive genetic marker for capreomycin resistance in Mycobacterium tuberculosis. J Antimicrob Chemother 66: 1247-1254.

27. Avalos E, Catanzaro D, Catanzaro A, Ganiats T, Brodine S, et al. (2015) Frequency and geographic distribution of gyrA and gyrB mutations associated with fluoroquinolone resistance in clinical Mycobacterium tuberculosis isolates: a systematic review. PLoS One 10: e0120470.

28. Seifert M, Catanzaro D, Catanzaro A, Rodwell TC (2015) Genetic mutations associated with isoniazid resistance in Mycobacterium tuberculosis: a systematic review. PLoS One 10: e0119628.

29. Sosa A, Amable-Cuevas C, Hsieh P, Kariuki S, Okeke IN (2010) Antimicrobial resistance in developing countries. Springer Science.

30. Drlica K, Malik M (2003) Fluoroquinolones: action and resistance. Curr Top Med Chem 3: 249-282.

31. Smith T, Wolf K, Nguyen L (2013) Molecular biology of drug resistance in Mycobacterium tuberculosis. Curr Top Microbiol Immunol 374: 53-80.

32. Lu P, Villalec C, Koul A, Andries K, Lill H, et al. (2014) The ATP synthase inhibitor bedaquiline interferes with small-molecule efflux in Mycobacterium smegmatis. J Antimicrob Chemother (Tokyo) 67: 835-837.

33. Gandea R, Marquez G, Chabi M, Aranda P, Espinosa R, et al. (2012) Fluoroquinolone-resistant Mycobacterium tuberculosis strains with elevated chromosomal DNA gyrase A and B gene copy numbers. Antimicrob Agents Chemother 56: 1117-1124.

34. Callaghan R (2010) Multidrug efflux pumps: the big issues. FEBS J 277: 529.

35. Daniels C, Ramos JL (2009) Adaptive drug resistance mediated by root-nodulation-cell division efflux pumps. Clin Microbiol Infect 1: 32-36.

36. Mirza ZM, Kumar A, Kalia NP, Zargar A, Khan IA (2011) Piperine as an inhibitor of the MdeA efflux pump of Staphylococcus aureus. J Med Microbiol 60: 1472-1478.

37. Schmalstieg AM, Srivastava S, Belkaya S, Deshpande D, Meek C, et al. (2012) The antibiotic resistance arrow of time: efflux pump induction is a general first step in the evolution of mycobacterial drug resistance. Antimicrob Agents Chemother 56: 4806-4815.

38. Gillespie SH, Bausi S, Dickens AL, O’Sullivan DM, McHugh TD (2005) Effect of subinhibitory concentrations of ciprofloxacin on Mycobacterium fortuitum mutation rates. J Antimicrob Chemother 56: 344-348.

39. Willis PA, Ratil S, Cheon SH, Edmonds K, Phillips M, et al. (1999) Drug tolerance in Mycobacterium tuberculosis. Antimicrob Agents Chemother 43: 2600-2606.

40. Levison ME, Levison JH (2009) Pharmacokinetics and pharmacodynamics of antibacterial agents. Infect Dis Clin North Am 23: 791-815.

41. Machado D, Couto I, Perdigao J, Rodrigues L, Portugal I, et al. (2012) Contribution of efflux to the emergence of isoniazid and multidrug resistance in Mycobacterium tuberculosis. PLoS One 7: e35358.

42. Szumowski JD, Adams KN, Edelstein PH, Ramakrishnan L (2013) Antimicrobial efflux pumps and Mycobacterium tuberculosis drug tolerance: evolutionary considerations. Curr Top Microbiol Immunol 374: 81-108.

43. Mitchison D, Davies G (2012) The chemotherapy of tuberculosis past, present and future. Int J Tuberc Lung Dis 16: 724-732.

44. Adams KN, Takaki K, Connolly LE, Wiedenhoft H, Winglee K, et al. (2011) Drug tolerance in replicating mycobacteria mediated by a macrophage-induced efflux mechanism. Cell 145: 39-53.

45. Pasipanodya JG, Gumbo T (2011) A new evolutionary and pharmacokinetic-pharmacodynamic scenario for rapid emergence of resistance to single and multiple anti-tuberculosis drugs. Curr Opin Pharmacol 11: 457-463.

46. Ekdholm VNG, von der Lippe B, Kinander W, Dahle U, Caugant D, et al. (2014) Evolution of extensively drug-resistant Mycobacterium tuberculosis from a susceptible ancestor in a single patient. Genome Biology 15: 1-11.

47. Zhang Y, Yew WW, Barer MR (2012) Targeting persisters for tuberculosis control. Antimicrob Agents Chemother 56: 2223-2230.

48. Dhar N, McKinney JD (2010) Mycobacterium tuberculosis persistence mutants identified by screening in isoniazid-treated mice. Proc Natl Acad Sci U S A 107: 12275-12280.

49. Motta SS, Chzel P, Aldana M (2015) Adaptive resistance in bacteria requires epigenetic inheritance, genetic noise, and cost of efflux pumps. PLoS One 10: e0118464.

50. Walter ND, Garcia BJ, Worondria AA, Musisi E, Ayakaka I, et al. (2015) Transcriptional adaptation of drug-tolerant Mycobacterium tuberculosis during treatment of human tuberculosis. Journal of Infectious Diseases 212: 990-998.

51. Chatterjee A, Saranath D, Bhattar P, Mistry N (2013) Global transcriptional profiling of longitudinal clinical isolates of Mycobacterium tuberculosis exhibiting rapid accumulation of drug resistance. PLoS One 8: e54717.

52. Gupta AK, Reddy VP, Lavania M, Chauhan DS, Venkatesan K, et al. (2010) JfE (Rv2459), a drug efflux gene in Mycobacterium tuberculosis confers resistance to isoniazid & ethambutol. Indian J Med Res 132: 176-188.

53. da Silva PE, Von Groll A, Martin A, Palomino JC (2011) Efflux as a mechanism for drug resistance in Mycobacterium tuberculosis. FEMS Immunol Med Microbiol 63: 1-9.

54. Kumar A, Schweizer HP (2005) Bacterial resistance to antibiotics: active efflux and reduced uptake. Adv Drug Deliv Rev 57: 1486-1513.

55. Gupta AK, Chauhan DS, Srivastava K, Das R, Batra S, et al. (2006) Estimation of efflux mediated multi-drug resistance and its correlation...
with expression levels of two major efflux pumps in mycobacteria. J Commun Dis 38: 246-254.

Lynch AS (2006) Efflux systems in bacterial pathogens: an opportunity for therapeutic intervention? An industry view. Biochem Pharmacol 71: 949-956.

Li XZ, Nikaide H (2009) Efflux-mediated drug resistance in bacteria: an update. Drugs 69: 1555-1623.

De Rossi E, Arigo P, Bellinzoni M, Silva PA, Martin C, et al. (2002) The multidrug transporters belonging to major facilitator superfamily in Mycobacterium tuberculosis. Mol Med 8: 714-724.

Ramon-Garcia S, Martin C, De Rossi E, Ainsa JA (2007) Contribution of the Rv2333c efflux pump (the Stp protein) from Mycobacterium tuberculosis to intrinsic antibiotic resistance in Mycobacterium bovis BCG. J Antimicrob Chemother 59: 544-547.

Gupta AK, Katoch VM, Chauhan DS, Sharma R, Singh M, et al. (2010) Microarray analysis of efflux pump genes in multidrug-resistant Mycobacterium tuberculosis during stress induced by common antibiotic-tuberculosis drugs. Microbial drug resistance 16: 21-28.

Webber MA (2002) The importance of efflux pumps in bacterial antibiotic resistance. Journal of Antimicrobial Chemotherapy 51: 9-11.

Vieireos M, Martins M, Couto I, Rodrigues L, Spangler G, et al. (2008) New methods for the identification of efflux mediated MDR bacteria, genetic assessment of regulators and efflux pump constituents, characterization of efflux systems and screening for inhibitors of efflux pumps. Curr Drug Targets 9: 760-778.

Ramon-Garcia S, Mick V, Dainese E, Martin C, Thompson CJ, et al. (2012) Functional and genetic characterization of the tap efflux pump in Mycobacterium bovis BCG. Antimicrob Agents Chemother 56: 2074-2083.

Wang K, Pei H, Huang B, Zhu X, Zhang J, et al. (2013) The expression of ABC efflux pump, Rv1217c-Rv1218c, and its association with multidrug resistance of Mycobacterium tuberculosis in China. Curr Microbiol 66: 222-226.

Balganesi M, Dinesh N, Sharma S, Kuruppath S, Nair AV, et al. (2012) Efflux pumps of Mycobacterium tuberculosis play a significant role in antibacterial activity of potential drug candidates. Antimicrob Agents Chemother 56: 2643-2651.

Hao P, Shi-Liang Z, Ju L, Ya-Xin D, Biao H, et al. (2011) The role of ABC efflux pump, Rv1456c-Rv1457c-Rv1458c, from Mycobacterium tuberculosis clinical isolates in China. Folia Microbiol (Praha) 56: 549-553.

Munster C, Mateu G, Rodriguez R, Takiff H (2001) Intrinsic resistance of Mycobacterium smegmatis to a tap-like multidrug efflux pump MfpA. Antimicrob Agents Chemother 45: 3387-3392.

Siddiqi N, Das R, Pathak N, Banerjee S, Ahmed N, et al. (2004) Mycobacterium tuberculosis isolate with a distinct genomic identity overexpresses a tap-like efflux pump. Infection 32: 109-111.

Huang TS, Kunin CM, Wang HM, Yan BS, Huang SP, et al. (2013) Inhibition of the Mycobacterium tuberculosis reserpine-sensitive efflux pump increases intracellular concentrations of ciprofloxacin and enhances susceptibility of some clinical isolates. J Formos Med Assoc 112: 789-794.

Richter E, Rusch-Gerdes S, Hillemann D (2007) First linezolid-resistant Mycobacterium tuberculosis complex. Infect Genet Evol 12: 695-700.

Lee RE, Hurdele JG, Liu J, Bruhn DF, Matt T, et al. (2014) Spectinomides: a new class of semisynthetic antibacterial agents that overcome native drug efflux. Nature medicine 20: 152-158.

Danilchanka O, Mailaender C, Niederweis M (2008) Identification of a novel multidrug efflux pump of Mycobacterium tuberculosis. Antimicrob Agents Chemother 52: 2503-2511.

Dinesh N, Sharma S, Balganesh M (2013) Involvement of efflux pumps in the resistance to peptidepolyglycan synthesis inhibitors in Mycobacterium tuberculosis. Antimicrobial agents and chemotherapy 57: 1941-1943.

Spies FS, da Silva PE, Ribeiro MO, Rossetti ML, Zaha A (2008) Identification of mutations related to streptomycin resistance in clinical isolates of Mycobacterium tuberculosis and possible involvement of efflux mechanism. Antimicrob Agents Chemother 52: 2947-2949.

Ramon-Garcia S, Martin C, Thompson CJ, Ainsa JA (2009) Role of the Mycobacterium tuberculosis P55 efflux pump in intrinsic drug resistance, oxidative stress responses, and growth. Antimicrob Agents Chemother 53: 3675-3682.

Zhang Y, Yew WW (2009) Mechanisms of drug resistance in Mycobacterium tuberculosis. Int J Tuberc Lung Dis 13: 1320-1330.

Viale MN, Park KT, Imperiale B, Gioeffre AK, Colombatti Olivieri MA, et al. (2014) Characterization of a Mycobacterium avium subsp. avium operon associated with virulence and drug detoxification. Biomed Res Int 2014: 809585.

Andries K, Villegas C, Coeck N, Thys K, Gevers T, et al. (2014) Acquired resistance of Mycobacterium tuberculosis to Bedaquiline. PLoS One 9: e102135.

Sharma S, Kumar M, Sharma S, Nargotra A, Koul S, et al. (2010) Piperine as an inhibitor of Rv1258c, a putative multidrug efflux pump of Mycobacterium tuberculosis. J Antimicrob Chemother 65: 1694-1701.

Adams KN, Szmowski JD, Ramakrishnan L (2014) Verapamil, and its metabolite norverapamil, inhibit macrophage-induced, bacterial efflux pump-mediated tolerance to multiple anti-tubercular drugs. J Infect Dis 210: 456-466.

Liu H, Xie J (2014) Comparative genomics of Mycobacterium tuberculosis drug efflux pumps and their transcriptional regulators. Critical reviews in eukaryotic gene expression 24: 163-180.

Ilina EN, Shitikov EA, Ilyankinova LN, Alekseev DG, Kamshas DE (2013) Comparative genomic analysis of Mycobacterium tuberculosis drug resistant strains from Russia. PLoS One 8: e56577.

Elertson B, Blackman A, Shaffer C, Mawhinney C, Peterson M, et al. (2012) Mutations in genes for efflux pumps and pentapeptide repeat proteins are associated with fluoroquinolone resistance in mycobacterium tuberculosis.

Colangeli R, Arcus VL, Curtisons RT, Ruthe A, Karalus N, et al. (2014) Whole genome sequencing of Mycobacterium tuberculosis reveals slow growth and low mutation rates during latent infections in humans. PLoS One 9: e91024.

Malinga LA, Abeel T, Desjardins CA, Dlaminz TC, Cassell G, et al. (2016) Draft Genome sequences of two extensively drug-resistant strains of mycobacterium tuberculosis belonging to the euro-american 5 lineage. Genome Announc 4: e01773-15.

Reeves AZ, Campbell PJ, Sultana R, Malik S, Murray M, et al. (2013) Aminoglycoside cross-resistance in Mycobacterium tuberculosis due to mutations in the 5′ untranslated region of whiB7. Antimicrob Agents Chemother 57: 1857-1865.
94. Ioerger TR, Koo S, No EG, Chen X, Larsen MH, et al. (2009) Genome analysis of multi- and extensively-drug-resistant tuberculosis from KwaZulu-Natal, South Africa. PLoS One 4: e7778.

95. Farhat MR, Shapiro BJ, Kieser KJ, Sultana R, Jacobson KR, et al. (2013) Genomic analysis identifies targets of convergent positive selection in drug-resistant Mycobacterium tuberculosis. Nature genetics 45: 1183-1189.

96. Milano A, Pasca MR, Provvedi R, Lucarelli AP, Manina G, et al. (2009) Azole resistance in Mycobacterium tuberculosis is mediated by the MnpS5-MmpL5 efflux system. Tuberculosis (Edinb) 89: 84-90.

97. Poce G, Bates RH, Alfonso S, Cocozza M, Porretta GC, et al. (2013) Improved BM212 MmpL3 inhibitor analogue shows efficacy in acute murine model of tuberculosis infection. PLoS One 8: e56980.

98. Srivastava S, Musuka S, Sherman C, Meek C, Leff R, et al. (2010) Efflux-pump-derived multiple drug resistance to ethambutol monotherapy in Mycobacterium tuberculosis and the pharmacokinetics and pharmacodynamics of ethambutol. J Infect Dis 201: 1225-1231.

99. Pasca MR, Guglierame P, Arcesi F, Bellinzi M, De Rossi E, et al. (2004) Rv2686c-Rv2687c-Rv2688c, an ABC fluoroquinolone efflux pump in Mycobacterium tuberculosis. Antimicrob Agents Chemother 48: 3175-3178.

100. Jiang X, Zhang W, Zhang Y, Gao F, Lu C, et al. (2008) Assessment of efflux pump gene expression in a clinical isolate Mycobacterium tuberculosis by real-time reverse transcription PCR. Microb Drug Resist 14: 7-11.

101. Khanna A, Raj VS, Tarai B, Sood R, Pareek PK, et al. (2010) Emergence and molecular characterization of extensively drug-resistant Mycobacterium tuberculosis clinical isolates from the Delhi Region in India. Antimicrob Agents Chemother 54: 4789-4793.

102. Clark TG, Mallard K, Coll F, Preston M, Assefa S, et al. (2013) Elucidating emergence and transmission of multidrug-resistant tuberculosis in treatment experienced patients by whole genome sequencing. PLoS One 8: e83012.

103. Ioerger TR, O'Malley T, Liao R, Guinn KM, Hickey MJ, et al. (2013) Identification of new drug targets and resistance mechanisms in Mycobacterium tuberculosis. PLoS One 8: e75243.