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Molecular Mechanisms of Genetic Interaction (Epistasis) in the Evolution and Management of Antibiotic Resistance Tuberculosis: Current Consequence and Future Perspectives

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Authors' contributions

This work was carried out in collaboration between both authors. Author AS designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed the literature searches. Author NB managed the analyses of the study. Both authors read and approved the final manuscript.

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ABSTRACT

Tuberculosis (TB) is an infectious chronic human disease caused by *Mycobacterium tuberculosis* (MTB) bacteria. *M. tuberculosis* has a great capability of resistance with plentiful natural and acquired mechanisms in their genome that contribute to the spread of highly drug resistance strains and became major public health concern. The majority of drug resistance in *M. tuberculosis* strains has been resulted from a numbers of chromosomal mutation events most of which are due to the mechanisms of epistasis that leads to the creation of resistance genes to anti-TB drugs. Epistasis can occur when two or more mutations interact with each other to express new phenotypic traits to modify their fitness cost. Thus, the objective of this review was to assessed the molecular mechanisms of epistasis and its consequences in the evolution and managements of antibiotic resistance-TB. The epistatic interactions within and between resistance gene mutations in M. tuberculosis could be detected by co-culture competitive fitness experimental assay under optimal growth conditions that showed either significantly negative or improving deleterious positive fitness

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effect. Molecular mechanisms of epistatic interaction could have important practical consequences in the trajectory of drug resistance, evolution of antimicrobial resistance and management of antibiotic resistance-TB. Understanding the evolution of *M. tuberculosis* under antibiotic treatments is a burning issue today. Unlike the deleterious positive epistasis, the beneficial negative epistatic interaction of resistance gene mutations under multidrug therapy method and/or collateral drug sensitivity approaches based on the knowledge of drug combinations help to mitigate the spread of drug-resistant strains, reduce treatment duration, minimize adverse drug effects on evolution of MDR/XDR-TB and improve treatment outcomes of TB patients.

Keywords: M. tuberculosis; epistasis; mutation; multidrug resistance-TB; multidrug therapy.

ABBREVIATIONS

AMR	*Antimicrobial resistance*
HGT	*Horizontal gene transfer*
MDR-TB	*Multidrug-resistant tuberculosis*
MTB	*Mycobacterium tuberculosis*
SNPs	*Single nucleotide polymorphisms*
WHO	*World Health Organization*
XDR-TB	*Extensively drug- resistant tuberculosis*

1. INTRODUCTION

Tuberculosis (TB) is an airborne infectious chronic human disease in worldwide and remains a major public health concern, caused by gram-positive M. *tuberculosis*. One of the factors involved in developing disease is the genetics of the host cell *M. tuberculosis*. Beside other bacteria, *M. tuberculosis* has a remarkable capacity of resistance through the variety of extrinsic and intrinsic mechanisms contribute to the spread of highly drug resistant strains and also plays an important role in the evolution of antibiotic resistance-TB [1,2]. According to the WHO report [3], even with having an effective drug treatments using a combination of anti-TB drugs, an estimated 10.0 million people developed TB, leading to 1.5 million deaths in 2018 and also without changed drug treatments the global patients with TB have led to the emergency of multidrug resistant (MDR-TB), which is defined as *M. tuberculosis* resist at least the two more effective fist-line drugs INH and RIF. So that the treatment of these MDR-TB requires second- line-drugs (SLDs). However, patients have been again developed another extensively drug resistant (XDR-TB) [4], which is defined as in addition to first-line drugs INH and RIF, M. tuberculosis resistant to at least one of the three second-line injectable drugs (i.e. KAN, AMK and CAP) and one fluoroquinolone group. Currently, it is supreme to understand the molecular mechanisms of drug resistant-TB in order to limit the spread of drug resistant strains, reduce treatment duration, minimize adverse drug effects and improve treatment outcomes of patients [5,6].

The majority of drug resistance in clinical *M. tuberculosis* strains is attributed to chromosomal mutations [5]. The development of multidrug resistant tuberculosis (MDR-TB) is the result of a number of mutational events, most of which are due to the mechanisms of epistasis that leads to the formation of resistance genes to anti-TB drugs and accumulate its genetics changes over time [7]. Disease causing mutations do not always have the same consequences in different individuals [8], due to several risk associated factors vary among individuals and can change the effects of mutations [2]. For instance, a mutation that causes a disease in one individual may have no effect in another. According to studies have conducted in *M. tuberculosis*, drug resistance can be occurred either naturally or artificially (acquired) mechanisms of resistance [2,9,10]. In addition to the naturally (intrinsically) drug resistance mechanisms, the principal acquired mechanisms of resistance genes causing factors (in the absence of HGT) which affect the chromosomal mutation rate in *M. tuberculosis* are; (i) cellular mechanisms, such as inefficiency of mismatch repair, inadequate gene expressions, (ii) external stress factors, including absence of rapid diagnostic facilities, improper anti-TB drugs prescribing practices and also (iii) interaction of genetic background or drug resistance mutations called epistasis (10). Previously, studies have discussed on the classification and the use of epistasis as a tool for understanding of the medical biotechnology [8,11]. However, the understanding of the mechanisms of epistatic interaction among various drug resistances in *M. tuberculosis* is still quite poor. In this context, the main aim of this review was to assessed the molecular mechanisms of genetic interaction (epistasis) in the evolution and management of antibiotic

resistance tuberculosis and areas where more research will be needed.

2. DETECTION OF EPISTASIS IN *M. tuberculosis*

The concept of epistasis has been studied using experimental approaches in a high-throughput manner [12]. The meaning of epistasis was defined as in different manner but with manly related meanings. Some scientists descried that the effect of a genetic variant that masked the effect of the others or the phenomenon where the phenotypic effect of one mutation differs depending on the presence of another mutation nearby [11], while in the current context, epistasis is defining in terms of the fitness effects of the two mutations, which is a fitness difference (may be higher or lower) from the additive combination of two loci in their effects on a phenotype [13]. Fitness refers to the ability of organisms (genotypes) to survive and reproduce in a given environment or in an appropriate culture medium where they are studied *in-vitro*, and the microbe's fitness is often measured using competitive assays [14], using exponential growth rates in liquid culture or colony sizes on solid media. Formally, epistasis for fitness is defined as any situation in which the fitness of a double mutant differs from the expected fitness of the initial single mutants. The expected fitness is either the sum or the product of the single mutant fitness values in the absence of epistasis [15].

Genetic interaction (epistasis) occurs when several mutations interact with each other and then to express new organisms' phenotypic traits, which is often necessary for *M. tuberculosis* to modify their fitness cost [16]. During epistatic interactions, the effect of multiple mutations is greater or less than the effect of the individual mutation and can lead to either beneficial or deleterious phenotypes [17]. Epistatic interactions can be classified depending on whether the double mutant outcome is better than the expected outcome (termed as positive or antagonistic epistasis) or worse than expected outcome (called negative or synergistic epistasis); whether the interaction involves two (pairwise epistasis) or between three or more variants (higher-order epistasis); whether interactions between mutations/ sequence variants within same gene (intramolecular or intragenic epistasis) or between mutations/ sequence variants in different genes (intermolecular or intergenic epistasis) that can lead to either negative or positive effect [15]. The fitness value of epistasis varieties within and between gene mutations under optimal growth conditions are measured based on co-culture competitive fitness experiment [1].

A study has been reported on the role of positive epistasis in *M. tuberculosis* drug resistance using a combination of mutations in rpoB and gyrA that conferred resistance to rifampicin (RIF) and ofloxacin (OFX). This study showed that the rpoB/gyrA D94G mutation is associated with improving deleterious fitness effects and underlie a positive epistasis (i.e. double mutants increase their fitness) and it is frequently occurred within XDR-TB strains. While negative epistasis in *M. tuberculosis*, may be either interactions between double beneficial mutations provide a simple additive effect on fitness (i.e. double mutants fitness lower than expected after mutation interactions) or interactions between double deleterious mutations are lethal (kill M. tuberculosis)[15]. Similarly, an investigation has conducted by using competitive fitness experiments suggested that the streptomycin (STR) in *M. tuberculosis* resistant strains with K43R mutation showed no difference in relative fitness compared with their susceptible ancestor, while K43N, K43M, K43T, and K88E in rpsL exhibited a significantly relative fitness [18], for the ofloxacin (OFX) resistant mutants with D94A, D94Y, A90V, G88A, D94N, and D94G in gyrA all showed no difference in their relative fitness. Among *M. tuberculosis* strains in those rpsL and gyrA dual mutations significantly negative epistasis was detected (lower fitness) in the strain with K43M/D94Y, K43M/D94G, and K43T/D94G mutations. However, an investigation using competitive fitness experiments in an appropriate co-culture media showed that a positive sign epistatic effects acting on the evolution of antibiotic resistance and overall M. tuberculosis fitness in MDR/XDR-TB [1]. The *M. tuberculosis* isolates carrying particular mutations conferring resistance between allelic forms of gyrA and rpoB genes have higher fitness than individual mutations in allele which are associated with large fitness defects. That means, unexpected fitness gains from the interaction of mutations that independently reduce fitness. These contrary results confirm that studies on the molecular mechanisms of epistatic interactions between drug resistances mutations are still poor and the area will need more study and clarification. Numerous works have done on molecular mechanisms to find interacting

variants, but identifying the genetic mapping of epistasis is difficult [19].

3. MOLECULAR CAUSES OF EPISTASIS IN *M. tuberculosis*

Mostly, the effects of mutations on epistasis within and between genes have been reported as an important roles for the emergence of MDR/XDR tuberculosis [15], in which epistatic interactions have greatly influenced the molecular evolution of both genomes and proteins [13].

3.1 Molecular Mechanisms that Cause Epistasis between Genes

Chromosomal mutations can have different effects in different individuals of a species and can have different consequences for phenotype, which is because of the outcome of a mutation can depend on the individual genes or genetic backgrounds are typically differ in gene sequence variants. As a result of particular mutation effects and its epistatic interaction between genes affecting many different phenotypic traits [8]. Various epistatic interactions have been identified in the laboratory, but the molecular mechanisms that cause these epistatic interactions are in most cases mysterious which is because of multiple molecular mechanisms can underlie similar epistatic interactions and much more work will be needed in this field. Here are some of the molecular mechanisms that could cause epistatic interactions between gene mutations: (i) if variant genes or their two protein products directly interact and underlie negative/synergistic epistasis, here the double mutants have lower fitness than the expected, (ii) If two genes, for instance two ancestral duplicates (A1 and A2) perform a common function or functional genetic redundancy, then the loss of one gene can be compensated for by continued activity of the second gene. Epistatic interactions between both genes must be inactivated to lose the function resulting in strong negative epistasis [20].

3.2 Molecular Mechanisms that Cause Epistasis within a Gene

Epistasis can also occur by mutations within genes or same molecule termed as intra-molecular epistasis. These intra-molecular interactions have been studied more by researchers interested in protein engineering and evolution, that involve mutations combine to produce new functions through fitness, which means the effects of one mutation can depend on other mutations in the same macromolecule. For instance, mutations in an enzyme can have little individual effect on activity, but can have dramatic consequences in combinations [21].

Some molecular mechanisms that causes intra-molecular interactions are: (i) Stability thresholds: threshold effects in protein stability can cause negative epistatic interactions. Under co-culture competitive fitness experiment, MDR-TB strains with single mutations exhibit minimal fitness for epistasis in comparison with the wild-type ancestor [1]. If a protein has a redundancy in its stability, meaning that two mutations alone (A and B) could have little effect, but in combination (A+B) been very detrimental effects. For instance, synergistic interactions between mutations in the bacterial antibiotic-resistance enzyme b-*lactamase* has been excess or redundant stability and required to have a crucial effect on folding, (ii) Conformation change is required for a beneficial mutation to realize its effect on protein function and underlie negative/synergistic epistasis. The beneficial mutation itself has no effect until a second mutation that causes a conformation change allows the mutated residue to contact a novel substrate. For instance, change in ligand specificity resulted from a pair of mutations that one introduced a residue and allow binding of the new ligand, and the second caused a conformation change that repositioned the first residue. This epistasis is reasonable to many potentially beneficial mutations rely on conformation changes before they can alter the function of a protein, (iii) Intra-molecular pleiotropy: epistasis occurs within gene mutations can have multiple different effects on a protein called pleiotropic. This epistatic interaction could be beneficial to one function of a protein but detrimental to another, because function altering mutations can change protein stability [22].

4. MOLECULAR CONSEQUENCES of EPISTASIS IN *M. tuberculosis*

Molecular mechanisms of epistatic interaction between and within a gene mutation could have an important consequence of molecular phenomena, including the trajectory of drug resistance or determines the path of evolutionary change, persistence and evolution of antimicrobial resistance (AMR), and

management of antimicrobial resistance (AMR) tuberculosis [23,24].

4.1 Epistasis for the Trajectory of Antibiotic Resistance Tuberculosis

The trajectory of antibiotic resistance evolution could be based on a complete knowledge of epistasis (genetic interaction) and with other significantly influenced related factors including mutation rates, drug resistance associated mutations or drug resistant acquisition, fitness cost of resistance mutations, selection pressure exerted on the organism and compensatory mutations [14,15,25].

4.1.1 Mutation rate and drug resistant genes acquisition

M. tuberculosis has a drug resistance due chromosomal mutation, characterized by a low mutation rate with a rate of at least one or SNPs/genome. Mutation rate is defined as a number of mutations per nucleotide site (bp) in the case of antibiotic resistance. Despite this low mutation rate, the number of drug resistant (MDR/ XDR-TB) cases are progressively increasing worldwide due to the acquisition of new gene mutations. As the genes responsible for resistance to the various anti-TB drugs, the risk of emergence of double, triple and quadruple drug resistant mutants is theoretically become low, ranging from about 10-10 mutants (for INH and RIF) to 10-24 mutants (for INH, RIF, PZA and EMB) per population [14]. However, a study has done by Gao et al. [26] suggested that 62/1671 acquired different resistance patterns during the short course chemotherapy (by INH, RIF, STR and EMB). Among the 62 strains with acquired drug resistance, approximately 10% were resistant to four drugs, 22.6% to three drugs, 21% to two drugs and the remaining 46.8% were one drug resistant. These data underline that multiple drug resistance acquisition emerges at higher rate under strong drug selection pressure than theoretically predicted and the imperfect drug penetrance leads to a rapid evolution towards MDR-TB rather it needs the knowledge of multi-drug combination therapy.

The mutation frequency and type vary in function of the drug resistance pattern and genetic background, for instance, among the 300 mutations found in the katG gene, the prevalence of katG S315T mutation can vary between 32% to 95% in INH resistant clinical isolates [27]. Furthermore, different mutations in the same gene or in different genes can produce similar drug resistance phenotypes [28], but can be associated with similar or different drug resistance levels, some mutations in rpoB genes of S531L, H526Y, H526D and H526R, are often associated with high levels of RIF resistance, while mutations including rpoB L511P, H526L, H526N, L533P and I572F are generally low levels of RIF resistance [29].

The majority of the experimentally examined resistance mechanisms result in reduced fitness relative to a susceptible ancestor as measured by growth and survival under different conditions. The relative fitness of a drug resistant M. tuberculosis, both in the absence or presence of the drug is also the key parameter in determining its evolutionary success [30]. Some studies have reported on how the competition and interchange between resistance related mutations can lead to MDR-TB. A study has done by using seven isolates from three patients; the first patient was free from *M. tuberculosis* drug resistance, but after 19 months of treatment, four independent mutations were detected (three mutations in katG and one mutation in inhA). After 5 months, most of the mutations relapsed, and only one mutation in katG was noticed. The second patient harbored *M. tuberculosis* with a mutation in rpoB (L533P) but sensitive to RIF. After 18 months, the L533P mutation was replaced with a second mutation in rpoB (H526Y) leading to RIF resistant. The third patient was a relapsed case with two unfixed mutations of ethA (L35R and A341E) and after 11 months of treatment that showed no change in EMB resistance status [31].

4.1.2 Genetic background variability of drug resistance

Genetic background describes the genetic diversity present in a strain's genome resulting from mutations via insertions/ deletions/ rearrangements compared to other strains. Although the genetic diversity of *M. tuberculosis* is low compared to other pathogenic bacteria. The strain genetic background has been demonstrated to influence multiple aspects in the evolution of drug resistance. The rate of resistance evolution and the fitness costs of drug resistance mutations may vary as a function of the genetic background [2]. So that, genetic background not only plays an important role in determining the costs of resistance and/or persistence of antimicrobial resistance (AMR) in the absence of antibiotic pressure, but also an

important factor influencing epistatic interactions [15]. There are several possible explanations for the persistence of costly drug resistant alleles in the absence of antibiotic [14,32]. So that, epistatic interactions can have a major impact on the trajectory of antibiotic resistance and adaptive evolution based on in what order and how quickly. For instance, when bacteria from different ancestry were exposed to the same dose of rifampicin (RIF), they exhibited different fitness costs and resistance levels. These support the role of epistatic interactions between genetic background and acquired mutations that confer various levels of resistance across MTB ancestry [17]. So epistatic interactions could affect the level of resistance in minimum inhibitory concentrations (MIC), or the relative fitness of a resistant mutant [30].

4.1.3 Fitness cost of drug resistance associated mutations and selection pressure

Many chromosomal resistance mutations bear fitness costs of resistance, that causes reduced fitness in the absence of antibiotic [14,32], while some of drug resistance elements may be cost free, either universally or in specific environments [33]. A fitness cost describes the reduction in the number of offspring produced by a drug resistance mutation in a given environment and it would manifest itself as a reduction in growth rate or yield. Fitness costs caused by drug resistance mutations may be by secondary so-called compensatory mutations (a mutations do not contribute to drug resistance directly) [2]. Costs of resistance were widespread across drug classes and bacterial species. This widespread occurrence of costs of resistance points to controlling resistance. For instance, rifampicin resistance mutation typically carries a cost; the resistance fitness should be decrease if use of rifampicin were to be stopped. In some cases, a single resistance mutation (e.g., RIF) results in a ∼20% reduction in fitness under antibiotic free conditions.

Indeed, mutations associated with high biological cost of resistance (underlie negative epistasis) detected in *in-vitro* drug- resistant mutants are rarely found in clinical drug- resistant isolates. Interestingly, resistance has persisted for months or years and results in mitigating MDR-TB [34]. However, most resistance mutations with low biological cost of resistance do not reduce bacterial fitness (underlie positive epistasis) in the absence of treatment, that explaining the successful spread of these highly drug- resistant strains (MDR/XDR-TB) in the community [35,36]. In addition, the strength of selection process for resistance will vary considerably depending on whether drug concentrations are high enough to prevent pathogen growth (lethal selection if >MIC, nonlethal selection if <MIC) and to allow growth of both susceptible and resistant bacteria. A rate of enrichment is determined by the number of mutants in the population and the fitness difference between susceptible and resistant (at the specific antibiotic concentration). Importantly, the weaker the selection, which means at non- lethal antibiotic concentrations (i.e., the smaller the fitness differential between susceptible and resistant bacteria) and the stronger the enrichment for mutants with low fitness cost [37].

4.1.4 Compensatory mutations

When a drug resistance mutation is initially costly, those costs may be reduced by compensatory mutations that increase fitness without eliminating resistance costs. Compensatory mutation is a mutation that occurs after an initial resistance mutation [15] and used for compensatory evolution. Compensatory evolution has been an important mechanism for the persistence of antimicrobial resistance (AMR) strains in the absence of antibiotics or drug free environment [14], whereby resistance mutations bearing a fitness cost to the bacterium, that have shown compensatory mutations in bacterial species [38–41].

Compensatory mutations are not involved in conferring resistance, but can revolutionize the fitness cost by interacting epistatically with the resistance mutation [2]. The presence of co-occurrence of secondary mutations that act as compensatory mechanisms for the impaired fitness of the pathogen. These compensatory mutations are occurring in genes encoding the same protein or genes involved in similar metabolic pathways [42], for instance, mutations occurring in rpoA and rpoC gene encoding RNA polymerases were compensatory for the loss of fitness mediated by mutations in the rpoB gene in rifampicin resistant isolates [35,36]. The mechanisms underlying compensation have involve restoring processes that are disrupted by the initial resistance mutation [39], which implying that resistant genotypes are likely to regain fitness in a drug free environment without losing resistance or compensatory evolution can

mitigate some of the initial fitness defects, a mutation in rpoA and ropB encode RNA-polymerase proteins that confer high-levels of rifampicin resistance with an associated cost of ~15%. After 250 generations of *in-vitro* selection through mechanisms of compensation in rpoA and ropB double mutants, fitness was recovered to 90% of the wild-type with rifampicin resistance, which replaced important physiological functions [43].

Similarly, compensation for costs associated mutation also important for restoration of cellular functions (disrupted transcription and translation), a mutation in rpoB encodes the β-subunit of RNA-polymerase to cause high levels of rifampicin resistance with an associated fitness cost. Compensatory mutation in rpoA and rpoC genes that encodes the α and β' subunits of RNA polymerase, respectively, could play the role of fitness compensatory mutations in rifampicin- resistant rpoB mutant and improve deleterious effects of the rpoB mutation and then restore the disrupted functions of rpoB mutation on rates of transcription [44]. The compensatory evolution occurs in the laboratory is somewhat clear, but the clinical and epidemiological importance of compensation is still unclear. In M. tuberculosis, data on compensatory mutations are still limited and mainly focused on first line drugs especially rifampicin resistance and needs further investigation [45].

4.2 Epistasis for the Persistence and Evolution of Antibiotic Resistance-TB

From several multiple mutations resistance elements; single mutations affecting permeability can cause antibiotic resistance tuberculosis [46]. Regarding on the drug resistance, studies have showed that epistatic interactions can occur between different drug resistance mutations, between drug resistance mutations and fitness compensatory mutations, and between drug resistance mutations and the genetic background, which can have important implications for the evolution of antibiotic resistance tuberculosis [2,15].

4.2.1 Epistasis between drug-resistant mutations

According to the previous findings the epistasis between two or more drug resistance mutations in *M. tuberculosis* could play an important role in the emergence and evolution of MDR/ XDR- TB strains. The interaction between drug resistance mutations may restore or even increase the biological fitness of drug resistant mutants compared with drug susceptible strains, for instance, the double mutants of clinical *M. tuberculosis* isolates rpsL K43R/katG S315T, rpsL K43R/rpoB S531L and rpoB S531L/katG S315T are grow faster than drug susceptible strains [47]. Similar studies on the epistatic interactions between resistance conferring mutations in DNA-directed RNA polymerase subunit-β (rpoB) that cause resistance to rifampicin and mutation in DNA gyrase subunit-A (gyrA) resistance to fluoroquinolone can have a higher competitive fitness than strains carrying only one of these mutations [2,15]. Another finding has been described by Borrell et al. [1] epistatic interactions between mutations associated with resistance to ofloxacin and rifampicin among 17 *M. smegmatis* mutants as model the double resistant mutants have a significant higher fitness (35%) than at least one of the corresponding single drug resistant M. smegmatis mutants (24%) resistant to RIF (RIF gene) and OFX (gyrA gene). This result suggests that the double resistance mutants underlie positive epistasis and bearing lower biological costs that may increase the fitness of drug resistant and drive the evolution of MDR acquisition. However, other study on M. tuberculosis recognized that the double mutant epistasis between katG S315T/rpsL K43R mutations are less frequent among MDR strains than among single case MDR strains, thus suggested that the double resistance mutants bearing higher biological costs and underlie a sign of negative epistasis [48]. The above different result suggested that the result of epistasis varies according to the strain genetic background.

4.2.2 Epistasis between drug resistance mutations and compensatory mutation

The epistatic interactions between drug resistances associated mutations and compensatory mutations have an important role in the determination of drug resistant (DR) isolates without reduction in fitness, for instance, a study using *M. smegmatis* as model has done by Song et al. [49] demonstrated that higher growth rates or higher relative fitness in recombinant strains carrying both rpoB S531L and rpoC F452L mutations than in strains harboring only the rpoB S531L mutation, which means the rifampicin resistant-TB strains carrying the rpoB S531L mutation are often associated with a compensatory mutations in the

rpoA or rpoC genes are also more likely to acquire additional resistance mutations. Another epistatic interactions between drug resistance either of katG315 and rpoB531 mutations with a rifampicin resistant fitness compensatory mutation (e.g., rpoC mutation) in clinical MDR-TB isolates, suggested that these genotypes lead to the emergence of XDR-TB which seems to be transmit of XDR strains directly from person to person rather than by inadequate MDR treatment [50]. Thus, compensatory evolution and epistasis could play an important role in the emergence and spread of highly resistant strains in the community called fully drug resistance (FDR) tuberculosis.

4.2.3 Epistasis for the management of antibiotic resistance tuberculosis

Treatments of infectious diseases (MDR-TB) often fail because of the rapid evolution of drug resistance, which is a significant challenge for clinicians worldwide today. Understanding the evolution of pathogens under antibiotic treatments is a burning issue. Studies have been suggested that to assure effective treatment to influence the evolution of drug resistance, as most are forced to make multidrug therapy decisions [51], which is a combination of anti-TB drugs in the strategy [2], that was introduced by the World Health Organization [52]. However, as the MDR-TB can be developed due to the interactions of chromosomal mutations within or between more genes, there was a problem in the application of combination of anti-TB drugs as a treatment strategy.

So that, knowledge of the molecular mechanisms of epistatic interaction between resistance mutations helps to inform decisions as to which drug combinations to set up in clinical settings in order to limit the evolution and spread of drug resistant-TB strains that reduce treatment duration, minimize adverse drug effects and improve treatment outcomes of patients [5,6]. Drug combinations are favored to maximize the rate of clearance. Synergistic drug combinations that combined inhibitory effect of two drugs are stronger or preferred for their ability to clear infections via multi-drug therapy. Thus, suggested that the primary goal of multi-drug therapy should be to slow or prevent the evolution of MDR-TB quickly [53,54]. There are several ways in which epistasis is relevant in the evolution of MDR-TB, which are; (i) Negative epistasis between resistance mutations approach might be to slow the evolution of MDR-TB strains. In such cases, pairs of resistance mutations interact negatively that the double mutant has lower fitness than expected given single mutants' fitness. As such, genes resistant to both drugs have highly reduced fitness and are expected to be outcompeted by single resistant genotypes. Thus, suggested that it should employ pairs of antibiotics (multidrug therapy) under negative epistasis between resistance mutations to slow the evolution of MDR-TB [55], for instance, quinolone and streptomycin resistance conferring mutations between K43T in rpsL and D87G in gyrA mutations show strongly negative epistasis in *E. coli* has a shortage of K43T/D87G genotypes. (ii) another promising strategy is based on evolved collateral drug sensitivity approaches: the evolution of resistance against one drug (drug-A) concomitantly causes hypersensitivity (i.e., collateral sensitivity) to a second drug (drug-B), thereby preventing the emergence of multidrug resistance (i.e. in evolved collateral drug sensitivity first the strains were evolved to resist drug- A, and at the same time became more sensitive to another drug-B) [56]. Process of bacteria evolution is one of the major causes of antibiotics resistance, whereby drug resistance mediated mutations become resistant to multiple drugs and can no longer be destroyed using antibiotic treatment. However, when a drug resistant bacterium become and resulted an evolutionary trade- off known as collateral sensitivity, when evolving resistance to one drug causes to gain increased sensitivity to another drug [57].

More importantly, evolved collateral sensitivity can slow down resistance evolution during combination [58], and the sequential therapy can limit the spread of resistance genes [59], which is based on the criteria that first switch between two drugs, because the evolutionary dynamics after the first switch will reveal the ability of the bacteria to adapt to the second drug, against which they evolved sensitivity (i.e. effective evolutionary trade-offs, where evolved resistance to one antibiotic causes hypersensitivity to another one). The evolutionary trade-off could be exploited to tackle the antibiotic crisis and will prevent *M. tuberculosis* adapting to different treatments. If this evolutionary trade-off is to be used medically, it must be stable long enough for the *M. tuberculosis* strains to either become extinct, or less able to evolve multi-drug resistance. To date, the evolutionary stability and thus clinical utility of this evolutionary trade- off is still poor and will need more findings and clarification [57,60].

However, the multi-drug therapy evolutionary experiment results might be altered by factors that affected the stability of evolutionary trade-off including; the molecular structure of the M. tuberculosis strains evolved sensitivity to, the strength of the original evolutionary trade-off (i.e. how sensitive *M. tuberculosis* strains became), the drug identity and order were administrated [58], epistasis among adaptive mutations and large fitness cost (i.e. when the genetic mutations promoting resistance affect bacteria's ability to replicate and survive in normal conditions), drug combinations of PIT/STR and CAR/GEN against *Pseudomonas aeruginosa* the evolutionary response to collateral sensitivity caused more population extinct for STR/PIT switches, whereas evolutionary response was possible for the GEN/CAR pair caused multidrug resistance) [57]. This result suggested that one drug order, GEN>CAR switch affects the ability to counter collateral sensitivity and that drug identity and order can determine treatment efficiency, enhance or minimize multidrug resistance.

For proper management of the evolutionary response of MDR-TB, several identifying collateral sensitivity approaches are required and all have an intend to maximize the costs of resistance either in the absence of drugs or in the case of collateral sensitivity in the presence of specific antibiotics, such as; (i) chemical-genetic approaches by a wide range of known antibiotics against a set of AMR genotypes [61,62], (ii) Novel genetic interaction screening approaches by microbially derived compounds that are particularly effective against AMR strains [63]. For instance, if a particular AMR mutant shows negative interactions with in cell wall genes, then this suggests that drugs targeting the cell wall genes might select against that AMR mutation (e.g., β-lactams). Confidently, the success of genetic interaction screens identifying novel drug targets specific to cells provides a promise of this approach in fighting AMR [64]. These approaches have clearly an admirable goal in maximizing the costs of resistance; i.e. low-fitness (high cost) genotypes are eliminated more quickly than higher-fitness genotypes from a population [33].

5. CONCLUSION AND FUTURE PERSPECTIVES

Currently, MDR-TB is an intensifying public health challenge worldwide, which is untreatable and painful definitely in economically poor countries [2]. The main reason is that they have not captured expensive drugs and advanced molecular technologies for diagnostic and sequencing purpose. Now a days, the *M. tuberculosis* diagnostic techniques for monitoring resistance mutations are largely limited because of our current knowledge of mutation pattern complexities and their various causative to compensatory mutations, which varying roles in mediating drug resistance in MDR-TB [65]. Resistance conferring mutations can evolve dynamically over time under antibiotic pressure in patients, so that the dynamics of developing resistance and the factors that facilitate resistance development within a patient are still poorly understood and require further explanation. There is an urgent need to understand the mechanisms by which the resistance mutations in order to identify new drug targets and to design new drugs.

Scientists have been practiced the co-culture competitive fitness experimental assays for detection of epistatic interaction within and between gene mutations under optimal growth conditions [1], but still there is a challenges in using genetic mapping of epistatically interacting variants and can lead to either beneficial or deleterious phenotypes [17]. Various epistatic interactions have been identified in the laboratory worldwide, but the molecular mechanisms that cause these epitatic interactions are in most cases mysterious because of multiple molecular mechanisms can underlie similar epistatic interactions. However, epistatic interactions are still poorly understood at the molecular level and will be needed more work in the near future. And also, the molecular characterization of both positively or negatively interacted resistance mutations is still very poor and will be needed more work.

The epistatic interactions of antimicrobial resistance mutations may play an important role in the persistence of resistance via compensatory evolution and in the evolution of MDR due to positive epistasis between different resistance mutations. Moreover, an understanding of the epistatic interactions of AMR mutations would be helpful in development of strategies and therapeutic agents for mitigating the evolution of resistance. Furthermore, studies were acknowledge the importance of epistasis in the management of AMR-TB via multidrug therapy strategies for proper treatment of MDR/XDR-TB [51] which is a combination of anti-TB drugs decision. Multi-drug

strategy is the current well-known strategy to reduce the incidence of both drug susceptible and drug resistant-TB variants [2]. However, not only the knowledge of epistatic interaction with resistance mutations that helps to inform decisions for collateral drug sensitivity approaches (drug combinations) in order to limit the spread of drug resistant strains, but also the genetic mapping of genetic interactions between different mutations are still poor or unclear and broader efforts with an excellent investigations and clarifications will be needed to fully characterize the genetic interactions of AMR mutations (i.e. beneficial or deleterious genes).

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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