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Review Article

Heterogeneity and Drug Resistance in *Mycobacterium tuberculosis* Infections: Implications for Treatment Outcomes

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Abstract

Mycobacterium tuberculosis, the cause of tuberculosis (TB), infects a third of the world's population. The global TB crisis is fueled by the spread of multidrug resistant (MDR) TB, which necessitates the use of less-effective, more expensive, and more toxic second-line drugs. Besides drug resistance, effective treatment of TB is complicated by several problems associated with it (e.g. problems of diagnosis, empirical treatment, risk factors to health care workers, delays in diagnosis, the long duration of culture for detection and drug susceptibility testing [DST], lack of resources and facilities for DST analysis, and bio-safety concerns). Most TB patients worldwide are treated applying the old-fashioned approach of empirical treatment. Empirical treatment and other factors combined contribute largely to treatment failures and further spread of all forms of TB. Most appropriately, treatment should be based on drug susceptibility tests and regular evaluations of treatment efficacy. The treatment success rates reported by the World Health Organization for DR TB are disappointingly low. The factors contributing to the success or failure of treatment include host immunity, social factors, host adherence to treatment, presence of other co-morbidities, mixed infections, re-infections, preexisting resistance, bacterial strain diversity, bacterial evolution to resistance and lack of resources and facilities for proper handling of TB patients. The intent of this short review article is not to delve into the roles of all these factors. It raises several issues (including some that may be considered challenging), argues that success in treatment of TB should not always be taken for granted, and suggests some radical paradigm shifts for better control and treatment of TB. In the following paragraphs, the latter factors and their interrelations are briefly discussed as to their effects on the outcome of treatment.

Keywords: *Heterogeneity; Drug Resistance; Mycobacterium tuberculosis*

Introduction

Mycobacterium tuberculosis, the causative agent of tuberculosis (TB), is believed to have infected a third of the world's population. In 1993, the World Health Organization (WHO) declared TB a global health emergency; and in 2012, the WHO declared multidrug-resistant (MDR) TB (defined as TB caused by strains that are resistant to at least both first-line drugs, isoniazid and rifampicin) a global health crisis. According to the WHO 2014 report [1], there were 9 million new cases of TB the previous year, of whom ~13% were human immunodeficiency virus (HIV)-positive; and 1.5 million human lives lost to TB, of whom 360,000 were HIV-positive. About 500,000 people developed MDR TB in 2013 alone. These figures are more or less replicated in each yearly report of the WHO. The WHO itself confirms that "worldwide, the proportion of new cases with MDR TB was 3.5% in 2013 and has not changed compared with pevious years" [1]. The highly social and global nature of the disease and the ease with which it can be transmitted make TB control challenging.

The global TB crisis is further fueled by the spread of MDR TB, which necessitates the use of less-effective, more expensive, and more toxic second-line drugs. During the last two decades, there have been even higher rates of drug resistant TB, especially in East Asia, Russia and some African countries [2]. Thus, recently, extensively drug-resistant (XDR) strains (MDR TB with additional resistance to a fluoroquinolone and an injectable second-line drug) have emerged, exacerbated by co-infection with the HIV [3].

Besides drug resistance, effective treatment of TB is complicated by several problems associated with it (e.g., problems of diagnosis, empirical treatment, risk factors to health care workers, delays in diagnosis, the long duration of culture for detection and drug susceptibility testing (DST), lack of resources and facilities for DST analysis, and bio-safety concerns). Where culture and DST coverage are available, these are usually reserved for only treatment failures and retreatment TB cases. Most countries adopt this approach and new TB cases are administered, by the book, empirical first-line treatment. These conditions have the capacity to fuel further transmissions, especially in congregated settings (e.g., prisons, many households and/or working places in which settings, people usually stay for periods of days to months. With one or more infectious persons with TB in those congregated settings, the level of transmissions and chains of transmissions can be high. Such chains of transmissions have been encountered in hospitals also [4,5] and can entail risk to health care workers and patients hospitalized for other ailments.

Treatment of TB is generally characterized by the requirement for long duration and multidrug nature of the therapy. It is generally true that timely treatment of an uncomplicated TB case (ideally one with fully drug susceptible infection in a compliant individual with intact immune system) will lead to cure. This may be difficult to achieve in other cases, especially in the presence of multiple drug resistance and other confounding factors within the host.

Ideally, DST should be administered in both new and retreatment TB patients before initiation of therapy. However, this is a "tall order" for most high TB-burden countries. It is suggested that the prevalence of drug susceptible TB may decrease with successful treatment, but that of drug resistant TB may not decrease [6]. The WHO reported treatment success rates of 48% and 33% for MDR and XDR TB, respectively. Moreover, only a small percentage of global MDR TB cases are reported to the WHO (e.g., about 25% of the total in 2014) (WHO, 2014). With 48% treatment success (reported by the WHO), that means ~85% of MDR TB cases were left untreated or inappropriately treated. Mortality from MDR or XDR TB can be high, especially in HIV-XDR TB-confected individuals [7,8].

The factors contributing to the success or failure of treatment include host immunity, social factors, host adherence to treatment, presence of other co-morbidities, mixed infections, re-infections, preexisting resistance, bacterial strain diversity, bacterial evolution to resistance and lack of resources and facilities for proper handling of TB patients. The intent of this short review article is not to delve into the roles of all these factors. It raises several issues (including some that may be considered challenging), argues that success in treatment of TB should not always be taken for granted, and suggests some radical paradigm shifts for better control and treatment of TB. In the following paragraphs, the latter factors and their interrelations are briefly discussed as to their effects on the outcome of treatment.

Why Treatment of TB is not a Trivial Thing

Drug Resistance

Some expectations are deeply established and are used as premise for TB treatment. For example, empirical treatment is administered to new case patients based on the assumption that they are drug susceptible. Here, expect the unexpected. There can be new cases that are drug-resistant, i.e., harboring primary drug-resistant TB. MDR and even XDR TB cases can be found in new case patients [9,10]. Success in TB treatment may be impaired due to the presence of preexisting resistant mutants and their enrichment by selective pressures imposed by inadequate or inappropriate therapy [11,12]. Likewise, retreatment cases are believed to have developed some form of drug resistance; but, a significant proportion of retreatment TB patients can be drug-susceptible. These raise serious concerns for the efficacy of treatment and TB transmission control strategies. Evolution into MDR and XDR TB can occur even when treatment adherence is observed unless supportive control and treatment optimization measures are taken simultaneously, such as enhanced active case finding, timely diagnosis and appropriate therapy, and continued evaluation of efficacy of drugs [9,13-16]. The threat that XDR TB poses had already been recognized more than two decades ago [17].

TB Drugs and Vaccines

It is also noteworthy that drug and vaccine development have by no means kept pace with that of the frequency and speed of resistance development. Streptomycin, isoniazid, pyrazinamide, ethambutol and rifampicin were introduced between 1943 and 1963. That means it has been more than 5 decades since the last major anti-TB drug was introduced, save rifapentine, which was licensed in 1998. Resistance to these drugs in mycobacterial strains or other bacteria was reported soon after their introduction (Table 1, [18-31]), as indicated by some of the earliest reports of resistance. However, the molecular mechanisms of the resistance traits for most of them were determined decades later (Table 1, [18-31]) There are some new drugs in the pipeline, but these are still in clinical trial stages [32]. Equally importantly, however, there is concern that such drug development efforts may be similarly beset by emergence of resistance [33,34]. Likewise, there are some vaccine candidates but, as with drugs, it takes years of testing and clinical trials before approval for widespread use. Of note, even after such years of trials, there can at times be setbacks as seen recently for the most advanced TB vaccine MVA85A, which was found to be not protective in children [35].

Drug	Description/Activity	Introduced	Earliest report(s) of resistance (reference)	Report of molecular mechanism of R (reference)
Streptomycin	Aminoglycoside	1948	1948 (Crofton and Mitchison) [16]	1993 (Nair., *et al*. 1993; Finken., *et al*. 1993) [17]
Isoniazid	Hydrazide	1952	1952 (Middlebrook, 1952)	1992, 1994 (Zhang., *et al*. 1992; Banerjee., *et al*. 1994) [18]
Pyrazinamide	Bacteriostatic/ bactericidal	1954	1953 (Tarshis and Weed, 1953) 1956 (Lee., *et al*. 1956)	1996 (Scorpio and Zhang,1996) [19]
Ethambutol	Bacteriostatic	1962	1987 (Bendov, and Mason, 1987)	1997 (Telenti., *et al*. 1997) [20]
Rifampicin	Broad spectrum	1963	1967 (Ezekiel and Hutchins, 1967), 1969 (Rabussay and Zillig, 1969)	1993 (Telenti., *et al*. 1993) [21]

Table 1: *Overview TB drugs.*

Strain Diversity

SStrain diversity (in terms of virulence, drug resistance, transmissibility, etc) is another major factor influencing treatment outcome. Global strains' diversity and variable host-pathogen associations may impair the development and efficacy of new tuberculosis diagnostics, drugs, and vaccines [36-38]. Lineage 2 and lineage 4 strains of *M. tuberculosis* (represented by the Beijing and Euro-American strains, respectively) are believed to be more virulent, more widespread geographically, more transmissible and more likely to cause severe disease than other lineages within the *M. tuberculosis* complex (MTBC) [39]. Resistance to INH is mediated by several mutations in different genes (*katG*, *inhA*, *ndh*, etc). Of these, the *katG* Ser315Thr mutation appears to be the most common, and most? isolates with this mutation were found to belong to the Beijing family, have tendency to cluster, develop into MDR form and have higher tendency for transmission than strains with other mutations [37,40]. These and related phenomena add to the challenge of developing diagnostics, and finding universally safe and effective new drugs and vaccines [41].

Mixed Infections

Simultaneous infections with multiple strains or clonal variants (including both susceptible and resistant) of M. tuberculosis have been reported, including in new and immune-competent individuals and retreatment cases [42-48]. Detection of mixed infections may be complicated by several factors including lack of multiple sampling (multiple colonies from a single sample or from serial samples), limitations of some typing methods to detect all the strains within the mixed infection and loss of minority populations during processes of decontamination [49]. The occurrence of mixed infections can complicate DST results, treatment outcomes and/or development of a universally effective vaccine, widely applicable vaccine. Whether the different variants result from in vivo evolution from a single isolate or not, the chances are high that differences in drug susceptibility and the presence of mixed infection could be associated.

Heterogeneity of *Bacilli* Populations

Analysis of recurrent TB, TB molecular epidemiology, drug susceptibility testing and initiation of anti-TB treatment are usually based on simple microscopic confirmation of the presence of bacilli and the analysis of one mycobacterial isolate obtained from a single sputum sample. This approach relies on the assumption that a culture of one sputum sample is homogeneous and representative of the total bacillary population in a patient. Studies have shown that there can be extensive heterogeneity with respect to both the specific strains and the resistant mutants [50-52]. These studies also demonstrated that resistance to a single drug can involve a shift from one resistant mutant type to another, more fit mutant type which will take over the bacterial population, especially when with antibiotic treatment. The occurrence of such heterogeneity also complicates distinguishing between mixed clonal infections and exogenous re-infection. Heterogeneity can also be in different resistance/susceptibility profiles of sub-clones of a single clone that may be indistinguishable by their molecular typing pattern. Thus, heterogeneity that existed within sub-clones of a single isolate would not be detected by analyzing a single isolate at a given time point. Moreover, such heterogeneity in resistance profiles can apply to several anti-TB drugs as well (e.g. ethambutol, fluoroquinolones) as shown by Kaplan., et al. [50], where parallel evolution in distinct lung compartments from a single founder strain is believed to have generated heterogeneity in resistance-associated alleles, while isolates exhibited identical IS6110 RFLP pattern. The similarities of the molecular typing patterns suggest that the different variants may have arisen due to in vivo evolution from a single isolate. In another study [53], several MDR TB patients were found to harbor strains that acquired additional drug resistant populations with different mutations or mixtures of susceptible and resistant strains. Such phenomena probably help to explain at least what some of the recurrent TB cases are due to once patients appear cured immediately following completion of treatment. Complex dynamics and heterogeneity with respect to the resistance profiles of serial isolates from TB patients, similarly to the Kaplan study, were also reported elsewhere [51,54,55]. These studies have shown that heterogeneity in the resistant bacterial populations within the host can exist. Aside from the existence of heterogeneous bacterial populations, these studies also demonstrated that resistance to a single drug can involve a shift from one resistant mutant type to another, more fit, mutant type. These phenomena highlight the importance of analyzing multiple clinical specimen from a single patient. If these phenomena are found to be not uncommon, and they do not appear to be, it is disturbing, especially for high TB-burden, resource-poor countries where even a one-time, one-sample pretreatment analysis of bacterial isolates usually proves challenging and exceedingly difficult. Some examples of studies that have analyzed heterogeneity of populations of bacilli between sputum and resected tissue samples are summarized in Table 2 [50,53,55-58].

Protection from Prior Infection

There may not be efficient protective immunity conferred by an initial infection against re-infection, or even previous infection may be a risk factor. Some studies have shown that re-infections, including with MDR TB (in previously successfully treated cases), can be more common than new infections, implying there is little if any protection afforded by a previous infection and may be age-dependent [59-62]. Previous anti-TB treatment and MDR/XDR status were risk factors for poor treatment outcomes [63]. Verver., et al [61] found higher rate of re-infection in previously successfully-treated people than new infections; however, they did not analyze the study population with re-infection by other risk factors (although they have ruled out HIV) and it needs to be ascertained if reactivation was involved, rather than re-infections.

Study	Patients/Case	Procedure/Findings	Resistance	Heterogeneity	Treatment outcome
Post., *et al*. 2003 [35]	13 HIV$^-$ MDR-TB patients with or without previous TB. All patients infected with a single strain.	Collected serial isolates from sputum and genotyped by spoligotyping, RFLP and sequencing of gene targets for first- and second-line drugs	4/13 isolates developed resistance mutations during study (either changing from wt to mutant or additional resistance mutation in a given gene. Some discrepancy between phenotypic and genotypic resistance testing observed to INH EMB, Km and O; but in some with this discrepancy, resistance mutation was discovered later.	Two types of heterogeneous populations were detected in 4/13 patients: mixtures of susceptible and resistant bacilli with different resistance mutations.	Incurable TB in 8 patients
Kaplan., *et al*. 2003 [32]	Patients with pulmonary TB or hemoptysis who had received TB treatment, most of them with caseation, cavitation and fibrosis	Cultures performed on sputum and resected lung tissue samples. RFLP Typing and sequencing of drug targets performed.	No discrepancy found for *rpoB*, *inhA* and *pncA* genes in all patient samples from sputum and different lobes of lungs (i.e., either all wild-type or mutant). The same for *katG*, except in one patient where *katG* and *embB* from caseous (mutant) and normal tissue (wild-type) differed. Another patient had wt *rpsL* in his sputum and mutant (K43R) in his left upper lobe. Discrepancies also found in *rrs* or *gyrA* between sputum and different lobes of 2 other patients. 3/6 patients culture-negative (no DST or sequencing results).	No mutation in patient 1 except K43R found in his left lobe. Patients 2 and 3 had resistance mutation in *rpoB* (S531L) for all their samples. Patients 2 and 3 had C15T and S315T mutations in *inhA* and *katG* respectively. A single founder strain underwent evolution resulting in cumulative acquisition of drug resistance mutations.	Incurable TB.
Mariam., *et al*. 2011 [37]	A retrospective study of a patient with multiple drug resistant TB	Serial sputum isolates collected over 9 years and stored were analyzed for susceptibility to 1st- and 2nd-line drugs. Multiple colony isolates tested for mutaions in *katG*, *rpoB*, *rpsL*, *embB* and *rrs*.	Initial isolate was resistant to INH. Subsequent isolates progressively developed resistance to RMP, STR EMB and 2nd-line drugs. Latter isolates w burdened with series of resistance mutations to 1st- and 2nd-line drugs.	Sequentially acquired mutations analyzed. A shift from GAC516GTC to TCG531TTG in latter isolates. *rpsL* mutation AAG→AGG also shifted from codon 87 to 42 in latter isolates.	Treatment regimen Periodically modified. Treatment was unsuccessful (patient deceased).
Kempker., *et al*. 2012	Pulmonary patients with MDR/XDR TB	Performed DST for 1st and 2nd line drugs on bacilli from both sputum and resected lung tissue	Disparate DST results for 1-2 drugs between sputum and tissue isolates observed for each patient, i.e., for all isolates with disparate DST results, all of the sputum isolates were susceptible and contrasting resistance was obtained only from the corresponding tissue isolates.	Resistance to 2nd-line drugs in samples from resected tissue, but corresponding samples were susceptible	4/7 patients were cured by adjustment of regimen based on both DST results
Hingley-Wilson., *et al*. 2013	Patients with initial mixed infection with susceptible and resistant strains	Bronchoalveolar lavage used to obtain samples. Smear- and culture-positive after 2 and 4 months of treatment. Followed patients for 6 months. MIRU-VNTR patterns of isolates at initial and after 2 months were the same but different patterns after 4 months of treatment.	Sensitive to INH initially and after 2 months. Resistant to INH and EMB at 4 months of treatment.	Mixed infection with strains susceptible and resistant to INH	Smear- and culture-negative after 4 months of treatment with a modified drug regimen.
Liu., *et al*. 2015	Pulmonary TB	CT image revealed lesions with or without cavity. Serial sputum samples collected for 6 months. Variable (low and high) bacillary load in sputum during treatment. Smear microscopy, and LJ and MGIT cultures performed for each serial sample. Deep WGS performed.	Initially MDR at week 0 and resistant to AMK, susceptible to EMB, LEV and PAS. Different drug resistant patterns resulting in disparate treatment responses	3 dominant subclones having several SNPs revealed by WGS. Branched microevolution in vivo from a single parental strain; however, didn't show the lesion distribution.	Treatment responses were heterogeneous between lesions.

Table 2: *Studies that have documented finding of heterogeneous mycobacterial populations in sputum or resected lung tissue.*

Abbreviations used: AMK: Amikacin; EMB: Ethambutol; Km: Kanamycin; LEV: Levofloxacin; O: Ofloxacin; PAS: Para Aminosalicylic Acid; SNP: Single Nucleotide Polymorphism; WGS: Whole Genome Sequencing.

Hypermutability of Strains

Research has shown that in addition to the previously known risk factors leading to MDR development (i.e., inappropriate drug regimen, patient defaulting, primary infection with MDR strains), higher MDR rates may also occur even before the initiation of therapy in lineage-dependent manner due to the existence of higher basal antibiotic resistance-conferring mutations to rifampicin that are naturally-harbored in lineage 2 strains (primarily represented by the Beijing and East Asian Indian strains) of M. tuberculosis than lineage 4 strains (e.g., H37Rv), with the mutation rates in lineage 2 being ~ 10-fold higher than in lineage 4 strains [64,65]. These mutation rates can enhance the development of MDR or of acquired resistance after therapy [64-66]. Importantly, the authors [64] suggested that such mutation rates found in vitro correlate with mutation rates in vivo in clinical isolates. Moreover, lineage 2 strains also acquire higher fitness mutations [67]. The modern sub-lineages of the Beijing strain have been the subject of intense research with respect to their predilection for hyper-mutability, drug resistance, evolution and virulence [68-71]. Beijing strain was reported to have higher mutation frequency to rifampicin resistance than the EAI strain (1.6×10-5 to 5.4×10-3 vs 6.3×10-8 to 3.8×10-4). Higher concentrations of rifampicin (~32 fold) were also required to achieve 100% killing of Beijing than were required for EAI, both at high density cultures [72]. The existence of such preexisting MDR and hyper-mutable strains and the rate of transmission are threats to tuberculosis control efforts.

Cross Resistance

Furthermore, cross resistance shared between different drugs drains available treatment options. For example, the occurrence of cross resistance between isoniazid (INH) and ethionamide (ETH) due to a mis-sense mutation in the promoter region of *inhA* (a gene whose product is involved in mycolic acid biosynthesis) necessitates testing for susceptibility in INH-resistant or MDR *M. tuberculosis* isolates and may impair second-line anti-TB treatment [23,73-76]. Similarly, mutation in *rrs* can confer cross resistance to the second-line drugs amikacin, capreomycin and kanamycin [74,77-80]. Such cross resistance to multiple drugs should severely limit treatment options in MDR or XDR TB patients [81,82].

Microevolution

Merker., *et al.* [83] demonstrated within patient microevolution of Beijing isolates by stepwise acquisition during therapy of mutations conferring resistance to the XDR state and final fixation in the bacterial population. Several isolates obtained from three body sites from one patient were indistinguishable by MIRU-VNTR typing, a result suggestive of microevolution in a clonal infection [84]. Evolution to drug-resistant form of the bacilli may occur even when treatment adherence is observed, although there are several other factors that can contribute to the outcome and need to be elucidated [13-16]. These findings argue against the belief that homogenous *M. tuberculosis* populations exist within hosts.

Requirement for DST/Mutation Detection Methods

As well as genotyping is important for strain identification and epidemiological studies, drug susceptibility testing (DST) of strains is also critically important to institute early appropriate therapy. Many cases of TB, including drug-resistant TB, remain undetected or unreported in resource-limited countries [85]. In such cases, transmission of all forms of TB continues unabated. DST by the conventional agar- or egg-based culture methods, though generally serving as gold-standard in national TB control programs, have the serious disadvantage of requiring up to eight weeks to produce a definitive confirmation of pulmonary TB, and another six weeks to produce DST results and are not directly applicable on clinical specimen. The liquid culture method MGIT-960 can significantly reduce the turnaround time to results, while also moderately increasing sensitivity. With liquid culture, confirmation of pulmonary TB can be obtained in less than two weeks, and DST results in an additional one to two weeks. There are several DST/resistance mutation detection methods. Among them, the WHO-approved molecular methods, such as the molecular line-probe assays give DST results with faster turnaround times and can be applied on smear-positive specimen. Rapid methods of DST allow for the early design of appropriate treatment regimens based on patients' drug resistance profiles using diagnostics that can be feasibly implemented in settings worldwide, resources permitting.

GeneXpert, another rapid diagnostic method, enables the diagnosis of TB and simultaneous detection of rifampicin resistance within 2 hours, and the WHO recommends the GeneXpert to diagnose HIV-associated TB and multidrug-resistant TB [86,87]. Another rapid method for analysis of drug resistance is based on sequencing, and whole genome sequencing (WGS) is becoming increasingly applicable. A prerequisite for detection of resistance mutations by most of these methods is the mutation must have been already identified and an assay system developed for its detection, i.e., the spectrum of clinically relevant mutations must be catalogued. With some 10-40% of resistance causes not yet identified (i.e., undocumented), there are no assay platforms available for these causes [88]. To fill this gap, new mutations that contribute to resistance directly, or indirectly by compensation or modification of cell wall permeability continue to be identified [88,89-91].

None of the modern semi- or fully-automated DST methods with faster turnaround times than culture-based DST methods is entirely perfect. One report [92] casted doubt whether line probe assay and sequencing can detect resistance in a mixed susceptible-resistant population when the density of the resistant population is 10% or less of the total. On the other hand, the liquid culture system MGIT DST can fail to detect certain *rpoB* mutations depending on codon position and amino acid substitution, which makes it difficult to discern without a sequencing procedure, a service unavailable in most low-income TB laboratories. Rigouts., *et al.* [93] found full agreement between LJ and MGIT for testing resistance to rifampicin caused by mutations at codons 513 (Lys or pro) and 531 (Leu, Trp). However, discordant results were found for mutations at codons 511Pro, 516Tyr, 533Pro, 572Phe, and several 526 mutations. However, these disputed mutations have clinical relevance, e.g., high transmissibility [94]. The MGIT may also not detect all levels of MICs for clinically relevant rifampicin resistance [93-95] and may create discordance between phenotypic and genotypic DST results. Phenotypic DST may also miss some RMP resistance due to mutations with low MIC values and higher fitness costs [94]. GeneXpert, one of the fast molecular diagnostic platforms, has good? sensitivity to detect RMP mutations, but does not detect INH resistance mutations. This limits its application to RMP only. However, INH mono-resistant strains are common and initially INH-mono-resistant strains may shift/switch into MDR forms [96]. This shows that not only undocumented resistance mutations but also some of the common mutations can be missed, which are exemplified by known and new INH resistance mutations [96]. Further, the Xpert assay may have lowered sensitivity to detect RMP resistance when the samples contain mixed (both resistant and susceptible) infections [97].

Bacterial Fitness and Resistance Selection

The success of the TB pathogen is dependent upon several host and pathogen factors. Most resistance development in *M. tuberculosis* occurs via mutations in the chromosome segments known to be drug targets. Mutated genes that confer drug resistance are usually essential for bacterial metabolism [97]. This implies that such mutants will be impaired to some extent in their ability to compete for resources (i.e. are less fit), with the implication that their population relative to the total remains low in the absence of drug therapy. Mutations in genes that are otherwise involved in pro-drug activation can also cause resistance without substantial loss of fitness. Compensatory mutations within the same or different genes may make-up for fitness costs of drug resistant mutations while at the same time maintaining the selectively advantageous drug resistance property [88,98,99]. Other mutations (e.g., mutations that mediate active efflux of drugs or reduce cell wall permeability to drugs) may provide selective advantages. Without a prior resistance-conferring mutation, these other mutations by themselves may contribute to resistance development [88,100,101]. Furthermore, fitness loss may be compensated by immune-suppression and drug mal-absorption within the host [102,103]. Even without acquisition of compensatory or other fitness-enhancing mutations, the resistant population(s) can become predominantly selected and fixed, especially during inadequate or inappropriate dosage of anti-TB therapy [51,104,105]. On the other hand, some resistant forms may have no loss of fitness, and strains of *M. tuberculosis* that are single or multiple drug resistant without substantial loss of fitness have been described [6,106-108], with the implication that their population may remain stable and comparable to that of the drug-susceptible counterparts. For example, the Lys43Arg streptomycin resistance mutation in *M. tuberculosis* showed nearly the same level of fitness both *in vitro* and *in vivo* compared to Lys43Asn and Lys43Thr. Moreover, the Lys43Arg mutation confers high level resistance and is the most frequent [107,109]. Similarly,

strains harboring the *katG* Ser315Thr INH resistance mutation (which appears to be the most common among INH resistance mutations) have competitive fitness and have been associated with increased transmissibility in some areas. Moreover, such strains were found to have tendency to cluster, belong to the Beijing family and develop into MDR form [37,40].

Expert Opinions

Experts warn it is just a matter of time before the TDR TB spreads around the world and TDR will eventually develop any place where there is MDR or XDR. The scientific literature is replete with warnings from experts about the challenges and danger posed by TB in general and drug-resistant TB in particular. For example, Elkington [110] wrote in a recent perspective: "Transmission of *Mycobacterium tuberculosis* continues uninterrupted, perhaps now is time to re-focus on the old strategy of actively finding and treating the aerosol super-shedders who drive the pandemic in order to break the infectious cycle". Nardell and Churchyard [111] stated: "ongoing transmission and re-infection are fundamental problems thwarting tuberculosis prevention in high-burden settings and unless active transmission can be reduced by intensified case finding and the use of new rapid diagnostics…, the benefits of chemoprophylaxis or immunization are likely to be elusive" [111]. Moreover, researchers warn the coming of possibly untreatable TB [17,112,113]. Stoffels., *et al.* [114] reported an upward trend of MDR-to-XDR ascent of isolates from TB patients. Conveyance of such warnings was not restricted to the scientific literature. For example, the London Times wrote on March 18, 2014: "Europe Failing to Tackle Drug-Resistant Tuberculosis: Data from the European Centre for Disease Prevention and Control and the WHO's regional office showed that drug-resistant TB strains affect at least 76,000 people in the region. But more than half are not properly diagnosed and only one in every three patients is successfully treated".

Elsewhere, Shah., *et al*. [112] stated that of 19 patients with XDR TB who had DST coverage for second line drugs, 13 (68%) had isolates resistant to all 8 drugs, which left no more options for treatment.

Untreated patients or defaulting patients as well as patients with resistant infection are risk factors to society as they can serve as sources of amplification of infections. Identifying such patients is useful as it can be a first step in management of such patients as well as reducing further spread of resistant infections. It is estimated one infectious case can spread the infection to over 10 uninfected individuals per year [110]. The rising levels of anti-TB drug resistance raise concern for all countries, as TB is both a social and global disease.

Conclusions

TB is regarded as a disease of poverty, in fact, it is a debilitation that is a cause of poverty itself because it affects mostly people of economically-active and productive age. Thus, fighting TB is also fighting poverty.

Some of the control measures do not cost at all, or cost only very little (e.g., ventilation, isolation, etc). Relatively simple, inexpensive measures (e.g., construction designs for good ventilation, isolation of patients with all forms of TB, use of protective respirators, etc can both reduce transmissions and TB management costs [115].

The very low level of the public's awareness about TB, widespread misconceptions and the presence of deep-rooted behavioral and attitudinal obstacles hinder curtailment of the transmission. This responsibility to educate should be assigned to dedicated professionals on a permanent basis and not to some sport or music stars (so-called "Ambassadors") who are both with no knowledge of TB and act on an on-and-off basis. To grossly present TB as a treatable disease is too simplistic and can only encourage complacency on the part of society. The public should also be made aware of the existence of latent TB, which can be activated at any later time.

The most dependable priority is prevention combined with combinations of measures including active case finding, isolation of infectious TB suspects, institution of rapid diagnostics, timely treatment with simultaneous cut in active transmissions to new hosts are needed for meaningful TB control (Calver et al., 2010). These require a real commitment on the part of policy makers and health professionals and breaking chains of transmissions [116,117].

Paradigm shift is also needed on the "classical symptom screening" system ("cough lasting 2 or more weeks") of case finding (1). This can be counterproductive; instead, anyone with cough for 3 consecutive days along with other typical symptoms of TB (e.g., night sweats, weight loss, loss of appetite…) should be referred to a health care center for immediate evaluation. Active case detection should be the main mechanism for finding and treating TB patients, and incentives should also be provided for self presentation.

Investing in rapid TB diagnostics and DST, in combination with other control measures, will be cost-effective in the long run. TB also drains budget that could be expended on other health care systems.

When and where there is urgency to initiate anti-TB treatment, it is advisable to collect and store at least 2-3 specimens, which should be used (resources permitting) immediately in the analysis of drug resistance, and depending on the results, can be used in regimen modification. Treatment records should be kept properly.

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