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# Combination therapies: Innovative strategies for enhancing treatment outcomes

**Nawal Hlal Almutairi**

KSA, National Guard Health Affairs

**Nawal Ganai M Bahari**

KSA, National Guard Health Affairs

**Alhanouf Zaid Almutairi**

KSA, National Guard Health Affairs

**Mariam Sabr Alshameri**

KSA, National Guard Health Affairs

**Hanadi Naji Alhajrasi**

KSA, National Guard Health Affairs

**Faisal Nasser Alharbi**

KSA, National Guard Health Affairs

**Manal Nasser Almasad**

KSA, National Guard Health Affairs

**Nourah Hassan Alotaibi**

KSA, National Guard Health Affairs

**Bashayer Ali Alshehri**

KSA, National Guard Health Affairs

**Mashaal Nasser Almazroa**

KSA, National Guard Health Affairs

**Zainab Ali Alqarni**

KSA, National Guard Health Affairs

**Abstract**---Background: Tuberculosis (TB) is a significant global health issue requiring prolonged treatment regimens that often involve multiple medications. Mycobacterium tuberculosis, the causative agent, is capable of residing in various tissue compartments during infection, leading to variability in drug accessibility and susceptibility.

The complexity of the infection necessitates the use of antibiotic combinations to ensure that all affected areas are effectively treated. Despite the importance of these combinations, their design has traditionally been addressed relatively late in the drug development process, leading to a limited number of evaluated drug combinations. Aim of Work: This study aims to examine the advancements made in the investigation of drug combinations for TB treatment through in vitro, in vivo, and computational approaches. It will also explore the potential integration of these methodologies with recent successful clinical trials of innovative medication combinations to enhance combination treatments for TB. Methods: We conducted thorough literature searches to identify and analyze the methodologies and experimental models utilized in the assessment of medication combinations for TB. This involved examining the capabilities of various in vitro, in vivo, and computational techniques that have been applied to study the interactions and effects of different antibiotic combinations. Results: The findings highlight substantial progress in utilizing diverse experimental models and computational methods to evaluate the efficacy and interactions of antibiotic combinations against *Mycobacterium tuberculosis*. Recent clinical trials have shown promising results with innovative drug combinations, indicating potential pathways for improved treatment regimens. Conclusion: The integration of advanced methodologies, including in vitro, in vivo, and computational techniques, provides a promising avenue for the rational design and evaluation of combination therapies for TB. This approach can lead to more effective treatment options and better outcomes for patients suffering from this infectious disease.

**Keywords**---Tuberculosis, Combination treatment, Multidrug therapy, Antibiotics, Computer modeling.

## Introduction

Ever before the advent of medicines, the treatment of tuberculosis (TB) has required many months or even years to achieve successful resolution of the condition. The number of cultivable *Mycobacterium tuberculosis* (Mtb), the microorganism responsible for causing tuberculosis (TB), in patients, drops fast during the first weeks of therapy [1]. Historically, short-duration therapies (e.g. up to a few months) have led to patients still having the disease at the end of treatment and a high rate of disease relapse [1, 2]. This suggests that there are Mtb (*Mycobacterium tuberculosis*) that can survive drug treatment, regrow, and cause disease again once the treatment is stopped. Certain individuals respond well to abbreviated treatment durations, but others may not see the same positive outcomes. Currently, there are no diagnostic tests available to determine the optimal amount of treatment required for a relapse-free cure in an individual patient. Therefore, it is necessary to have long treatment durations for all patients in order to guarantee the optimal result for the majority of patients. What is the reason for the need of lengthy treatment regimens in order to establish a long-lasting cure for tuberculosis? Extensive research spanning many decades

indicates that the answer to this issue is influenced by two main factors. Firstly, the immunological response to TB infection and the subsequent disease pathology play a role. Secondly, the capacity of Mtb to adapt its metabolism and physiology to survive in response to changing environmental signals also contributes to the answer [3].

Empirical evidence from clinical studies has shown that the treatment of tuberculosis necessitates the administration of many medications over an extended period of time in order to achieve a successful and long-lasting recovery. The current treatment protocol for drug-sensitive (DS) tuberculosis (TB) is a 6-month regimen consisting of two phases of drug therapy. The first phase lasts for 2 months and includes the administration of four medications: isoniazid (H), rifampicin (R), pyrazinamide (Z), and ethambutol (E). This is followed by a second phase lasting for 4 months, during which only two drugs, isoniazid (H) and rifampicin (R), are given. This therapeutic approach has been used for more than four decades, with an estimated efficacy rate of 85% [2, 4, 5]. Moreover, the yearly estimate of tuberculosis cases that are resistant to presently used medications, including drug-resistant (DR) and multidrug-resistant (MDR) cases, remains substantial, surpassing 500,000 [4]. Historically, the treatment for drug-resistant tuberculosis (DR TB) has included administering more than four medications for a duration of up to 24 months. This was necessary since the treatments used had limited ability to eliminate the bacteria and were very toxic. Furthermore, there was a lack of consistency in the treatment approach, resulting in less than 50% of patients being successfully cured. [6] With the emergence of innovative antibiotics, advancements in clinical trial designs, and encouraging findings from clinical trials [7, 8], there is a potential to create more potent treatments for tuberculosis. Current treatment recommendations advocate for a standardized and abbreviated therapy (BPaL) for the majority of patients with drug-resistant tuberculosis (DR TB). Hence, there is justification to be hopeful about the exploration of alternative therapeutic protocols for tuberculosis using novel medications and innovative design ideas.

### **Aim of Work**

This study will analyze the knowledge gained from previous experiences, the present status of drug treatment plans largely based on empirical evidence, and advancements in the organized and logical research of combinations of drugs to discover the next generation of tuberculosis multidrug treatment plans. In this article, we will provide a concise overview of the process by which the current standard of tuberculosis (TB) drug treatment was determined through empirical means (Section 2). We will also explore the necessity of multidrug therapies in effectively targeting *Mycobacterium tuberculosis* (Mtb) during treatment (Section 3). Additionally, we will discuss the present and future strategies for identifying the most promising new candidates that can potentially shorten treatment duration and enhance treatment effectiveness (Section 4).

### **Empirical validation of combination treatment for *Mycobacterium tuberculosis* (Mtb)**

#### **The treatment of tuberculosis has transitioned from palliative care to combined antimicrobial therapy**

In the past, the treatment of tuberculosis relied on empirical and iterative methods. Prior to the mid-twentieth century, the treatment for tuberculosis provided relief for symptoms and isolating affected patients in order to reduce the transmission of the illness [1, 2, 9]. The efficacy of drug therapy for tuberculosis had been a longstanding pursuit until the discovery of streptomycin (S) and para-aminosalicylic acid (PAS) in 1944, which were demonstrated to be successful [2, 9]. The effectiveness of these medicines in treating TB was promptly established in clinical settings [2]. Within a short span of time following initial implementation, a clinical investigation revealed that the use of both S and PAS in therapy exhibited enhanced effectiveness in combination therapies, while also reducing the emergence of drug resistance often seen in single-drug treatments [10]. In 1952, the identification of isonicotinic acid hydrazide (H, isoniazid) as a compound that may combat Mtb led to the development of a three-drug combination treatment H+S+PAS. This therapy has shown to be very effective, with a cure rate of over 90% and a low disease recurrence rate of as little as 4% [2, 10].

#### **Contemporary abbreviated treatment regimen**

These therapy advancements were substantial, but they need ongoing antibiotic medications for a duration of 24 months. During the 1960s, the introduction of ethambutol (E) resulted in the substitution of PAS in the three-drug therapy (HSE). This substitution was due to the fact that ethambutol decreased the treatment duration to 18 months and was also better tolerated compared to PAS. Several antibiotics that are presently used as second-line therapies for drug-resistant tuberculosis were also developed in the 1960s [2]. Rifamycins, antibiotics synthesized by soil bacteria, were first identified in 1957 and subsequently subjected to chemical alterations in the 1960s to enhance their effectiveness in living organisms [2, 9, 11]. Rifampicin (R), a semisynthetic antibiotic, was synthesized in the 1960s [2] and subsequently included into the TB combination regimen (HRSE) due to its significant reduction in treatment duration from 18 to 9 months [1]. Pyrazinamide (Z) was identified in 1972. Clinical studies demonstrated its sterilizing effect in the first months of therapy, resulting in a reduction of the overall treatment period to 6 months when coupled with isoniazid and rifampicin (HRZ) [1]. Enhancement in treatment result was accomplished by gradually introducing or replacing medications. The short-course therapy and treatment regimen was developed in the late 1970s, drawing upon extensive clinical studies conducted over many decades. Ethambutol was introduced soon after the creation of the short-course treatment regimen as a means to address the resistance to isoniazid. This addition has become the current standard of care, known as HRZE. A recent breakthrough in treatment improvement was achieved after over four decades of efforts. The medication therapy duration was reduced to four months by swapping isoniazid and rifampicin with moxifloxacin (M) and rifapentine (P) [7]. The development of our most successful tuberculosis (TB) treatment choices has mostly been guided by

the iterative process of adding or substituting medications in clinically proven combinations. In order to comprehend the process of developing more efficient combinations of drugs for tuberculosis (TB), it is beneficial to examine the outcomes of multidrug therapy and identify the particular population of *Mycobacterium tuberculosis* (Mtb) that need prolonged treatment. This understanding will enable the development of tailored medicines with shorter durations.

### **Combination treatment is used to treat tuberculosis (TB) in various physical compartments**

The pathophysiology of TB illness provides insight into the difficulties of treating Mtb infections, which need prolonged multiple therapy. The pathogenesis of pulmonary tuberculosis (TB) entails a synchronized immune response that encompasses several cell types that undergo changes during the course and management of the illness. The intricate and ever-changing characteristics of the infectious environment indicate that Mtb must endure a range of stresses and possess the physiological adaptability to acclimate to the shifting circumstances. The pulmonary lesions of tuberculosis (TB) are complex formations formed by many kinds of cells around a central core of either intracellular or extracellular *Mycobacterium tuberculosis* (Mtb) [12, 13]. Multiple forms of lesions (granulomas) have been identified, each exhibiting distinct immune cell composition, structure, levels of host cell necrosis, and eventual formation of cavities [14, 15]. High levels of extracellular Mtb are linked to the presence of necrotic and cavitating lesions, which pose challenges in the treatment of both people and animals [16] [17].

### **Pharmacokinetics**

There is data in humans suggesting that various kinds of lesions respond differently to pharmacological therapy, which may be affected by the quantity of medicine that reaches Mtb. A recent study using positron emission tomography/computed tomography (PET/CT) found that within individual patients, some lesions shrank while others enlarged during a 14-day drug treatment. This indicates that there is variation in how lesions respond to drugs within individual patients. Research on medication distribution in patients with multi-drug resistant tuberculosis (MDR TB) has shown that linezolid (L) equally permeates caseous lesions. Unlike clofazimine (C), which has been demonstrated in animal models (Figure 1) to not reach the necrotic core of lesions, other medications are able to do so. This confirms previous findings [19–23]. Collectively, these investigations provide evidence for the notion that *Mycobacterium tuberculosis* (Mtb) in various lesions exhibit distinct responses to medications, mostly due to the varying drug concentrations at the specific locations of Mtb inside the lesions. These findings provide a compelling rationale for the extended duration of treatment and the superior efficacy of therapy using numerous medications and/or larger dosages. Moreover, the design of novel medication combination therapy should guarantee the presence of many drugs in each kind of lesion, at concentrations that are lethal to Mtb.

### **Dormancy and persistence in Mtb**

The conditions inside the lesion, such as the presence of lipid carbon sources and iron shortage, as well as external stresses like acidic pH and oxygen levels, may trigger a non-replicative or dormant state. Mountain bikes (Mtb) that are exposed to these environments exhibit alterations in their transcriptomic, proteomic, and metabolic activities [28, 29]. Several antibiotics specifically target cells that are actively replicating, which means they may have limited effectiveness in treating infections caused by non-replicating Mtb. Evidence supporting this idea is derived from in vitro experiments, specifically those that have shown the increased efficacy of certain drugs in the standard treatment regimen against actively growing Mtb. These drugs target the growth of new mycolic acid cell wall components (H and E) [1, 2, 32–37]. Additional support for this idea can be found in other studies [28, 30, 31]. The additional medications in the treatment plan (R and Z) have the ability to eliminate both actively multiplying and non-replicating Mtb, which could partly account for the shortened treatment duration observed when these two drugs were included in the development of the current standard of care regimen [1, 2, 36, 37]. Recently, medications like bedaquiline and pretomanid, which have shown effectiveness against both replicating and non-replicating Mtb, have been successfully utilized to treat Mtb in laboratory settings [38, 39] and in real-world medical practice [8, 40, 41]. In addition, there are antibiotics available that have the ability to specifically target and eliminate non-replicating Mtb bacteria [30, 31, 34, 42–47].

Therefore, there is optimism that the combination of medications that target both actively dividing and dormant forms of Mtb may lead to the discovery of treatment regimens that can be completed in a shorter period of time. Tolerance, as described in the Consensus Statement by Balaban et al., refers to the ability of cells to endure exposure to fatal or inhibitory levels of a medication without developing genetic resistance [48]. Persistence, or the ability of a subpopulation to tolerate a certain condition, enables a subset of cells to survive for prolonged durations without altering the minimum inhibitory concentration (MIC) required for the whole population [48]. This is especially applicable to mycobacterial cells since, as a group, they naturally vary in their sensitivity to drugs. At the individual cell level, there is variation in how drugs affect the susceptibility of Mtb in laboratory settings, depending on the specific drug [49]. This indicates that there is always a group of Mtb cells that can withstand treatment with a single drug, which poses a challenge in effectively eliminating Mtb using antibiotics. Persister cells may function as an intermediate stage towards developing resistance, as has been shown in several types of bacteria. Furthermore, in these investigations, the capacity to endure the effects of one treatment occurred before the development of resistance to a second agent. The presence of Mtb persisters in TB patients may provide a potential threat for the development of resistance to various medications in the current treatment protocol. It is important to develop pharmacological combinations in a way that minimizes the risk of cross-resistance [50]. The research of novel drugs for tuberculosis is now focused on targeting resistant and dormant cells [30].

### **Combining several drugs in a therapy regimen decreases the duration of treatment and the likelihood of developing drug resistance**

Drug resistance is a significant challenge in the treatment of illnesses including bacterial infections and cancer. The probability of acquiring resistance is positively correlated with the length of time a medicine is used. Hence, the extended duration of many months for tuberculosis therapy is plenty for the development of resistance to take place [53, 54]. Drug resistance can arise when cells acquire a specific characteristic, such as a mutation in the drug target or a mutation that enhances the expression of an efflux pump. This characteristic enables the cells to survive in the presence of drug concentrations that would normally inhibit or kill cells lacking this characteristic [48, 55, 56]. These variables are genetically inheritable, and once resistance develops, a medicine becomes ineffective at doses that are therapeutically beneficial; the population of bacteria that are resistant may live and proliferate in the area that was previously inhabited by the now-eliminated susceptible bacteria.

Employing a combination of medications that act on several cellular mechanisms is a strategic approach that may effectively hinder the development of resistance both in experimental and clinical settings. The probability of a single cell developing resistance to several medications is minimal due to its ability to target many crucial cell functions. In the past, the use of many drugs to treat tuberculosis (TB) has been shown to be more effective in treating the disease and reducing the number of patients who have a relapse with drug-resistant *Mycobacterium tuberculosis* (Mtb) compared to using a single treatment [1]. Based on the established spontaneous streptomycin resistance rate of one in 105, it may be inferred that one in 1015 Mtb bacilli would exhibit triple drug resistance [57]. Therefore, it is expected that multidrug treatments including more than three medications would be successful. This is because seriously infected patients with cavitary illness are likely to have less than one spontaneously triple drug-resistant Mtb cell, based on the reference of bacillary load in severe cases [2, 58]. According to these estimates, the probability of achieving success with multidrug therapy is backed by the effectiveness of treatment regimens using three or four drugs for tuberculosis, as indicated by references [1, 7, 8].

The early experiments utilizing S and PAS clinically revealed a reduction in resistance development. According to a preliminary investigation, the use of S as the only treatment led to resistance in 20% of patients [2]. Furthermore, randomized control trials conducted by the Medical Research Council (MRC) in the United Kingdom revealed that streptomycin resistance occurred in 70% of instances [2, 59, 60]. On the other hand, the MRC research discovered that 41% of patients who received both S and PAS showed streptomycin resistance. However, when PAS was given every 3 days, the occurrence of streptomycin resistance dropped to as low as 9% [2, 59, 60]. These first trials revealed the essentiality of using combination treatment to hinder the development of resistance. Hence, it is unsurprising that the advancement of short-course therapy for tuberculosis treatment involves the use of a minimum of three medications. Empirical research has shown that a combined therapy is necessary to effectively counteract the variability in both access to and susceptibility to Mtb (*Mycobacterium tuberculosis*) that exists in various lesions. Combination therapy

should therefore include medications that work together to promptly decrease the amount of bacteria and subsequently eliminate persister cells.

### **Emergence of novel approaches in the creation of combination treatment**

The utilization of animal and in vitro models of tuberculosis has played a crucial role in the advancement and formulation of medication combinations, as well as in the enhancement of combination therapy. In the next sections, we will examine the models, their applications, and computational methods that will assist in discovering novel combinations and formulating future therapy regimens for tuberculosis.

### **Conclusion**

The effort to systematically create therapy for tuberculosis in the 20th century was successful and introduced the use of randomized control trial design, which is today considered the most reliable method for conducting clinical trials [59, 60]. We are now entering a new phase when we can take advantage of the remarkable progress in drug discovery for TB. This progress has resulted in the identification of several novel antibiotics and targets. Our emphasis is on designing optimum combination medicines. Thus far, a methodical and evidence-based strategy to identifying potent medication combinations has resulted in the development of many combination frameworks that have shown promising results in clinical trials. However, there are still a considerable amount of untested combinations, particularly those involving recently developed antibiotics. Recently, significant progress has been achieved in the area via the development of animal models, in vitro models, and outcome measures. These models were specifically intended to more accurately replicate the microenvironments where bacteria reside, particularly those that are most resistant to antibiotics and difficult to eliminate. Computational models are anticipated to be used in the next phase of TB medication combination optimization. These models will incorporate diverse data kinds to enhance technique and our comprehension of how to systematically develop optimum combination medicines.

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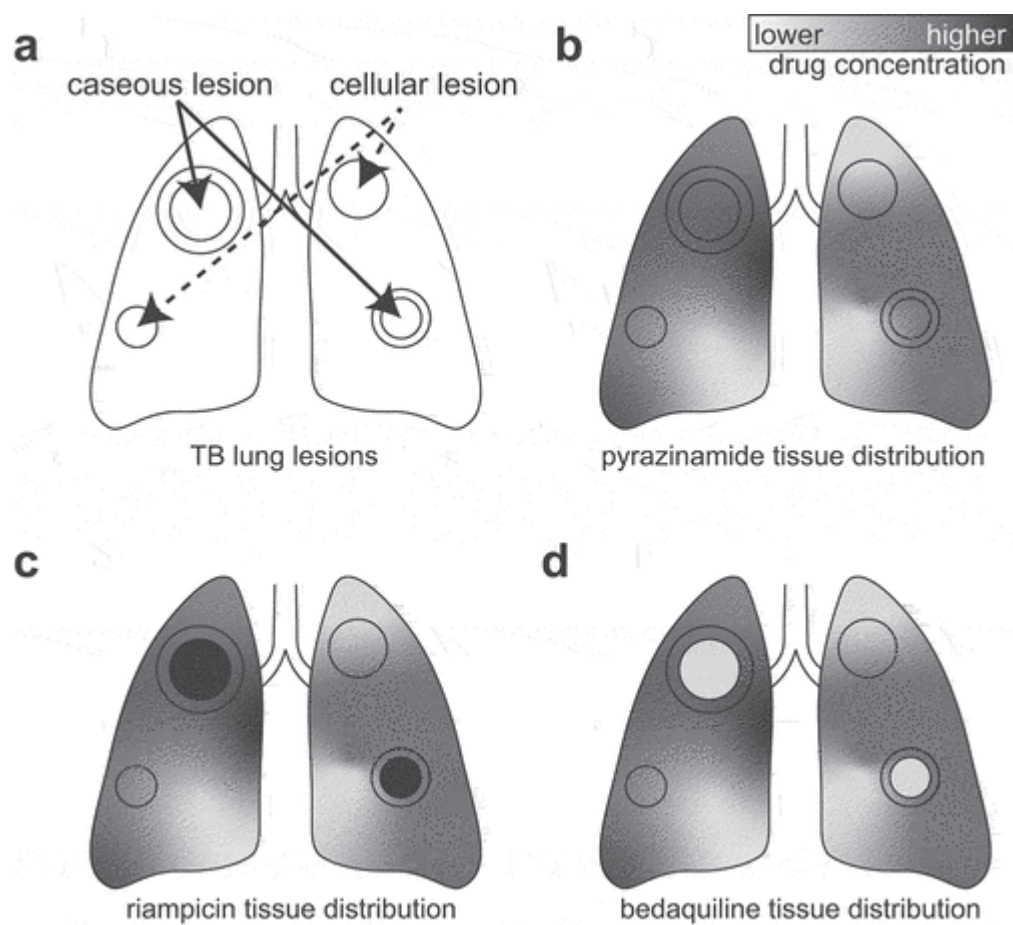


Figure 1. Illustration depicting the dispersion of drugs throughout lung tissues infected with *Mycobacterium tuberculosis* (Mtb)