Acta Scientific MEDICAL SCIENCES (ISSN: 2582-0931)

Volume 8 Issue 10 October 2024

Review Article

Exploring the Epidemiological, Microbial and Molecular Approaches for Improved Diagnosis and Understanding of Human Tuberculosis

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DOI: 10.31080/ASMS.2024.08.1919

Received: August 12, 2024

Published: September 06, 2024

Abstract

Human or animal tuberculosis is caused by a group of nine closely related species of mycobacteria known together as the *Mycobacterium tuberculosis* complex (MTBC). One of these nine members, *Mycobacterium tuberculosis* is responsible for the vast majority of cases of tuberculosis in humans. Although there is a limited range of species within MTBCs, distinct biological and phenotypic variations exist among the various MTBC lineages. Variable-pathogenicity MTBC species presumably evolved due to insertion/deletion processes operating on a common ancestor. MTBC persistence involves a complex web of interactions between determinants on both the host and the bacterial sides. Granuloma development by MTBC has not been conclusively linked to either host defense or survival. MTBC species not only have the ability to control the host immune response but have also evolved several methods to evade immune system attacks. ;However, current methods for diagnosing human tuberculosis have some limitations. Several studies have hinted at the potential use of host biomarkers for early TB detection, illness differentiation and monitoring treatment efficacy. Epidemiological, microbial and molecular approaches may shed light on the tangled web of interactions between MTBC species and humans. This review will not only provide new information and insights on the epidemiological, microbial and molecular approaches to the MTBC ~~but~~ will also facilitate these approaches in a significant and worthwhile manner.

Keywords: *Mycobacterium Tuberculosis* Complex (MTBC); *Tuberculosis;* Epidemiology; Microbial; Molecular; Pathogenesis

Introduction

Mycobacteriaceae family comprises a heterogeneous assemblage of bacteria that display distinct characteristics of pathogenicity in animals and humans, as well as unique host reservoirs and growth dynamics in laboratory culture [1]. This assemblage of bacteria is often characterized by their non-spore-producing nature, aerobic metabolism, lack of motility, and gram-positive staining. They

are classified as acid-fast bacilli due to their ability to retain a stain even after acid treatment [2]. These bacteria have a slightly curved morphology and may exhibit some branching, which is attributed to their mycolic acid cell wall. Mycobacteria may be divided into the fast-growing and the slow-growing subgenera depending on their doubling time. The MTBC includes several genetically related Mycobacteria species, including *M. tuberculosis*, *M. africanum*, *M. bovis*, *M. canettii*, *M. microti*, *M. pinnipedii*, and *M. caprae*. In addition to the seven species often seen, the term MTBC encompasses two more distinct species, namely *Mycobacterium orygis* and *Mycobacterium mungi* [3]. Some infections possess the ability to adapt to animals, which could allow them to affect a wide variety of mammalian species. Examples of mycobacteria include *Mycobacterium pinipedii*, which is derived from sea lions or seals; *Mycobacterium microti*, which is obtained from voles; and *Mycobacterium orygis*, which is sourced from oryxes [4].

Tuberculosis (TB) is an infectious disease caused by the bacterium *Mycobacterium tuberculosis*. It primarily affects the lungs but can also involve other parts of the body. If left untreated, TB can lead to severe health consequences such as chronic cough, weight loss, fatigue, fever, night sweats, and in some cases, can be fatal. TB is a major global health concern due to its contagious nature and the rise of drug-resistant strains, making early detection and appropriate treatment crucial in preventing its spread and managing its impact on individuals and communities. *Mycobacterium tuberculosis* and *Mycobacterium africanum* are two bacterial species belonging to the MTBC that can induce TB infection. There is a global prevalence of about 5.8 million individuals affected by MTBC infection. According to the latest research conducted by the World Health Organization (WHO), around 10 million persons globally are now afflicted with health complications associated with TB in the year 2020 [5]. According to available data from 2020, TB accounted for the mortality of around 1.1 million individuals who did not have a human immunodeficiency virus (HIV) infection, as well as 209,000 HIV-positive individuals [6]. Except Africa and the Western Pacific, four out of the six areas designated by the (WHO) conformed to the prevailing trend seen globally, which included a decline in the TB mortality rate (measured as TB deaths per 100,000 people per year) as well as an overall reduction in the absolute number of TB-related fatalities up to the year 2019 [7] (Rephrase). However, these regions saw an increase in both the mortality rate and the absolute number of TB deaths in the following year, 2020. The WHO European Region is projected to achieve the closest approximation to the 2020 milestone with a projected reduction of 26% in TB mortality between 2015 and 2020 [8]. Several factors contributed to a 10% decrease in the annual tuberculosis mortality rate in the Russian Federation from 2010 to 2020. These include improved healthcare infrastructure, enhanced public health programs, better treatment options, increased funding, improved surveillance, and collaboration with international organizations. The African Region experienced a notable 18% decrease, suggesting a relatively positive outcome [9]. Nevertheless, it is worth noting that the number of tuberculosis-related deaths in the Americas in 2020 exceeded the figures recorded in 2015 by a significant margin, with an increase of 10%. There were also decreases seen in other regions of the World Health Organization (WHO), including a fall of 13% in the Western Pacific region, a decrease of 6.2% in the Eastern Mediterranean region, and a decline of 0.1% in the Southeast Asia region compared to the data from 2015 [10].

Mycobacterium tuberculosis complex (MTBC) infection may cause primary and secondary TB, often known as reactivation TB. The initial TB infection mainly affects those with inadequate immune responses to limit MTBC granulomatous appearance [11]. This syndrome may cause meningitis, disseminated TB in AIDS patients, miliary TB, and extrapulmonary granulomas. Those without past pathogen contact or those with impaired immune systems, such as small children and elderly individuals, are more likely to get the sickness [12].

On the other hand, primary tuberculosis provides immunity to individuals with a healthy immune system by triggering a strong systemic immune reaction that efficiently controls the spread of the infection. After-many weeks, the infection has been successfully controlled, leading to the subsequent healing of the lesions [13]. The establishment of systemic immunity during the main phase of TB is often followed by the emergence of secondary TB, also known as reactivation TB. Mycobacteria can undergo replication and then disperse into the surrounding environment after the evasion and disruption of the systemic immune response in cases of post-primary tuberculosis. The predominance of paucibacillary secondary TB infections in humans may be attributed to hypersensitivity towards MTBC antigens rather than the number

of bacilli present. Individuals who are carriers of latent TB have the potential to regain their infectiousness many years after the initial infection, thereby serving as reservoirs for the transmission of the illness [14]. Individuals who have robust a responses to skin testing are more susceptible to the development of clinical disease. TB, an infectious illness that mostly affects the respiratory system, is a leading cause of death in humans. Necrotizing granulomatous inflammation of the afflicted organs is commonly cited as a defining characteristic of TB as a pathological illness. Inhalation of polluted aerosols by people with active pulmonary sickness is the primary mode of transmission of MTBC infection throughout the population [15].

Tuberculosis (TB), caused by the bacterium *Mycobacterium tuberculosis*, remains a global health challenge, with a profound impact on public health and healthcare systems. Despite significant advances in our understanding of the disease and the development of effective treatment strategies, TB continues to afflict populations around the world, posing a persistent threat to human health [16]. In Saudi Arabia, like many other regions, TB remains a critical public health concern, demanding both increased attention and innovative approaches for its diagnosis and management. Saudi Arabia, with its unique sociodemographic and geographic characteristics, presents a compelling case study for the exploration of epidemiological, microbial, and molecular aspects of TB [17]. The Kingdom's diverse population, international migration patterns, and variations in healthcare infrastructure create a complex landscape for TB transmission and control. In this context, gaining a deeper understanding of TB in Saudi Arabia cannot only inform local strategies but also contribute to the global effort to combat this ancient disease [18].

This comprehensive review aims to provide a multidimensional perspective on TB in Saudi Arabia, emphasizing three critical approaches: epidemiological, microbial, and molecular. Through analyzing these aspects, we aim to understand the complex array of factors that uphold the presence of TB in the region. Epidemiological studies will shed light on the prevalence, risk factors, and patterns of TB transmission within Saudi Arabia's diverse communities [19]. Concurrently, microbial investigations will delve into the genetic diversity and drug susceptibility profiles of *M. tuberculosis* strains circulating in the Kingdom, offering insights into the evolving landscape of TB strains. Finally, molecular approaches, including genotyping and genomic analysis, will provide a finer-grained understanding of the genetic determinants of TB virulence and drug resistance [20]. This exploration is not only timely but also essential, given the evolving challenges posed by TB, including the emergence of drug-resistant strains and the impact of the COVID-19 pandemic on TB control efforts.

Through integrating data gathered from epidemiological, microbial, and molecular research, we aspire to guide the development of enhanced approaches for diagnosing, treating, and preventing TB in Saudi Arabia. Furthermore, our findings may have broader implications for TB control efforts worldwide, as we continue the collective pursuit of a TB-free world [21].

Newly diagnosed with TB in the world

The use of quick tests is still far too restricted. Only 38% (2.5 million) of the 6.4 million patients newly diagnosed with tuberculosis in 2021 employed a WHO-recommended fast molecular test as the initial diagnostic test, up from 33% (1.9/5.8 million) in 2020 and 28% (2.0/7.1 million) in 2019 [22]. There was a significant difference between countries (Figure 1).

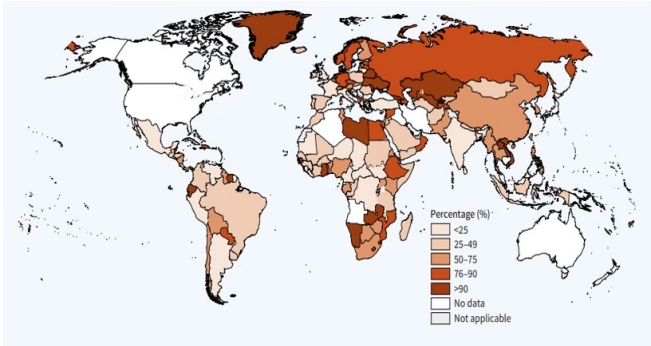
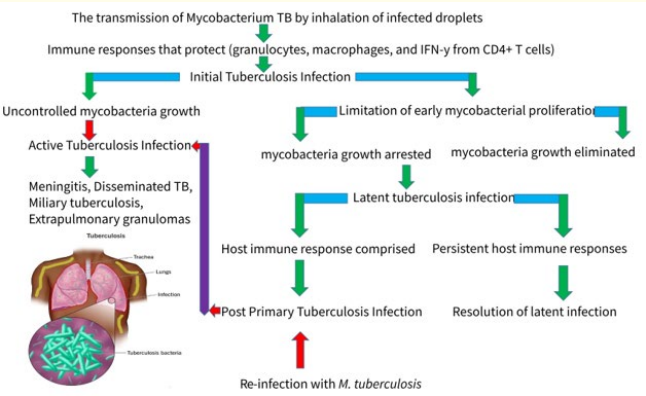


Figure 1: Age of people newly diagnosed with TB who were initially tested with a WHO-recommended rapid test at the country level in 2021 (Global Tuberculosis Report 2022).

Unraveling the origins of tuberculosis in humans

The pathogenesis of human tuberculosis is a multifaceted phenomenon characterized by intricate interactions between the host immune system and bacterial components. This disease is mostly instigated by MTBC. Numerous recent studies have shown

compelling illustrations of the impact exerted by host immunity, including both innate and adaptive immunological responses, on the modulation of the pathogenesis of human tuberculosis caused by MTBC [23]. The fundamental principle of protective immunity, sometimes referred to as the "central dogma," posits that the coordinated actions of macrophages, granulomas, and interferon (IFN) produced by CD4+ T cells during primary TB play a crucial role in safeguarding the whole organism against widespread infection. Flowchart 2 illustrates the genesis and clinical effects of human tuberculosis resulting from exposure to droplet nuclei containing (MTBC) in individuals with a fully functional immune system [24].



Flowchart 1: A comprehensive examination of the etiology of human TB, as well as the course of infection and subsequent illness consequences in individuals with a fully functioning immune system after being exposed to infectious droplet nuclei carrying MTBC.

Understanding the incidence and spread of drug-sensitive and drug-resistant tuberculosis

In the year 2021, TB claimed the lives of over 1.6 million individuals, while almost 10.6 million individuals worldwide had illnesses as a result of MTBC infection. It is noteworthy that only 4.2 million cases out of this overall total received an official diagnosis. In the year 2021, there is a greater prevalence of TB among men (57%) compared to women (33%), with children accounting for 11% of the affected population [25]. On a global scale, around 2.0 billion individuals are latently infected with MTBC, often referred to as latent tuberculosis infection (LTBI), whereby the infected individuals do not exhibit any apparent signs of the illness. The majority of individuals with latent tuberculosis infection (LTBI) tend to progress to active tuberculosis illness during the first 12 to 18 months after infection [26]. However, it is important to note that reactivation of the disease may still manifest many decades after the original infection. In the year 2021, the prevalence of worldwide multidrug-resistant/rifampicin-resistant (MDR/TB) was expected to be 3.6% among those with newly diagnosed cases, and 18% among those who had been previously treated. Additionally, it was found that around 20% of MDR-TB patients progressed to extensively drug-resistant tuberculosis (XDR-TB) [27]. The majority of drug-sensitive, multidrug-resistant (MDR), and extensively drug-resistant (XDR) cases are documented in Asian nations, namely India and China. The growth of drug-resistant strains of MTBC may be attributed to the inappropriate clinical use of anti-TB medications and suboptimal patient adherence, which are further compounded by the extended duration of multi-drug therapy. Additionally, it has been shown from molecular epidemiological data that the primary method of transmission for MDR and XDR strains of MTBC in several countries with a high burden of TB is through community-based dissemination (Table 1). Therefore, it is important to possess a comprehensive comprehension of the transmission and acquisition of new infections of MTBC to inform and implement efficient methods for TB control. Just as there is a pressing need to enhance the detection and treatment approaches for TB patients who are susceptible to drugs, there exists a critical need to address the challenges associated with multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB cases. This objective is a primary focus of the END-TB strategy created by the WHO [28].

Tuberculosis in the context of HIV and diabetes mellitus: A comprehensive analysis

The acquisition of HIV infection is considered the foremost determinant in the development of active tuberculosis, hence augmenting the vulnerability to primary infection or reinfection, as well as the likelihood of TB reactivation in individuals with latent

MTBC species	General Descriptions	Genome	Sensitivity	Year
M. tuberculosis	The species most often seen. The infection has spread to a proportion exceeding one-third of the global human population. Capable of causing infection in animals that come in contact with people.	Whole genome sequencing, which contains a total of 3,959 genes.	Antibiotic resistance (ABR)	1882
M.canettii **and** *M. africanum*	There is a close association with *Mycobacterium tuberculosis.* Additionally, it may lead to the development of TB in humans. African patients are often found to be solitary in most cases.	16S rRNA sequence	Some level of resistance to certain antibiotics, such as isoniazid and ethambutol.	1997
M. bovis	Exhibits the most extensive range of host infections. This phenomenon has an impact on both domestic and wild goats, as well as bovines and people.	Several whole-genome sequencing studies	*M. bovis* strains can exhibit resistance to antibiotics commonly used to treat tuberculosis, such as isoniazid and rifampicin.	2000s
M. bovis var BCG	A mutant strain of *M. bovis* that was specifically chosen in a laboratory setting. The only vaccination used for the prevention of tuberculosis during the early stages of infancy.	Whole Genome sequencing	sensitive to most common anti-tuberculosis drugs.	1990s
M. caprae	The samples were obtained only from goats.	Whole genome sequencing	Resistant to certain antibiotics, including isoniazid and pyrazinamide	1999
M. microti	Rodent-borne pathogens. Typically separated from voles, which are rodents belonging to the genus Microtus or other related genera, Exposure to pathogens might potentially result in the development of diseases, particularly in those with impaired immune systems.	Whole genome sequencing	Susceptible to common tuberculosis drugs, such as isoniazid and rifampicin.	1937
M. pinnipedii	Seals are susceptible to this disease.	Used various sequencing techniques, including whole genome sequencing, to analyze the genetic composition and evolution	Limited information is available regarding antibiotic susceptibility	2003
M. mungi	The pathogen responsible for the disease in the banded mongoose (Mungo Mungo).	16S rRNA sequence	Limited information is available regarding ~~the~~ antibiotic susceptibility	2010
M. orygis	Waterbucks, antelopes, oryxes, and gazelles are only few of the larger African animal species that are impacted.	whole genome sequencing	Limited data available on the susceptibility of *M. orygis* to antibiotics	2012

Table 1: A summary of the fundamental characteristics of the MTBC members.

TB [29]. Additionally, it expedites the advancement of HIV infection towards the development of Acquired Immunodeficiency Syndrome (AIDS). The progression from untreated latent TB infection to active TB infection may occur rapidly in individuals living with HIV (PLWH) due to their compromised immune system. In individuals with HIV infection, there exists an annual risk ranging from 5% to 15% for the development of active tuberculosis infection [30]. Individuals with HIV infection have a significantly elevated risk of having TB compared to those without HIV, with the former group being 26 times more likely to acquire the disease. The heightened susceptibility may be attributed to the immunosuppressive effects of HIV infection [31]. The annual incidence of TB among HIV-positive persons is estimated to range from 7% to 10%. On the contrary, those who do not have HIV infection have a lifetime risk of 10% for the development of TB. In 2018, it was predicted that around 8.6% (with a range of 7.4-10%) of the reported cases of TB were seen in individuals who were living with HIV infection [32]. The incidence rate of TB, measured as the number of new cases per 100,000 population per year, saw a 3.6% increase from 2020 to 2021. Type 2 diabetes mellitus (DM) is a substantial risk factor and an important co-morbid disease that greatly increases the mortality associated with TB on a global scale. Moreover, the presence of diabetes mellitus (DM) exacerbates the severity of TB patients and complicates their response to therapy [33]. Moreover, individuals with diabetes mellitus (DM) have an accelerated rate of disease development after (MTBC) infection, and they display a suboptimal response to therapeutic interventions. The absence of data about the coexistence of TB and diabetes mellitus (DM) in recent clinical trials for novel TB drugs is a notable gap, despite the growing body of research highlighting the significant influence of DM comorbidity on the results of TB therapy [34]. Hence, it is imperative to assign more importance to the incorporation of tuberculosis-diabetes mellitus (TB-DM) co-morbidity in future randomized clinical trials assessing the effectiveness of tuberculosis drugs. This emphasis should be coupled with a specific examination of the unique treatment results observed in patients with both tuberculosis and diabetes mellitus.

Unraveling tuberculosis: Microbiological insights and diagnostic strategies

Tuberculosis is a significant public health problem, as seen by the high number of new cases in 2020, which exceeded 10 million, and the substantial mortality toll of over 1.5 million individuals [35]. The prompt emphasizes the significance of expeditious and dependable diagnostic methods in mitigating the adverse health outcomes and death rates linked to TB, as well as in effectively managing its spread. Microbial confirmation plays a crucial role in establishing the diagnosis of TB when it is suspected based on clinical signs, epidemiological information, and radiological results [36]. Despite the advancements made in recent decades, the microbiological detection of TB remains a formidable task, especially in cases characterized by low bacterial burden. In the past, the identification of TB relied on the use of microscopy and culture techniques. The mycobacterial culture is considered the preferred approach owing to its ability to identify low quantities of organisms (< 10 organisms for liquid cultures) and its provision of access to the strain for phenotypic antibiotic susceptibility testing [37]. Nevertheless, the cultivation of (MTBC) poses significant challenges owing to its sluggish growth rate and the need to conduct experiments in biosafety level three (BSL3) facilities. Microscopy techniques that rely on the observation of acid-fast bacilli provide a rapid turnaround after smear fixation of less than 30 minutes. However, these methods exhibit a restricted sensitivity and specificity, with a limit of detection ranging from 10^3 to 10^4 bacilli per ml [38]. To enhance their sensitivity, it may be necessary to replicate these tests across many clinical specimens.

Typically, the diagnosis of pulmonary tuberculosis is considered in individuals who exhibit pertinent clinical symptoms, including a chronic and productive cough, hemoptysis, fever, weight loss, and a prior medical history of tuberculosis [39]. Chest X-ray results are often used in many tuberculosis-endemic regions to confirm the clinical evidence of pulmonary tuberculosis. The X-ray of a patient exhibiting active pulmonary tuberculosis–reveals the presence of alveolar infiltration, cavitation, lymphadenopathy, and pleural effusion. For pulmonary tuberculosis, the most common variant of the disease, the primary samples utilized for diagnosis consist of sputum samples and aspirates collected from bronchial or bronchoalveolar lavage, along with tracheal aspirate [40]. In areas where TB is prevalent, the usual way to detect (MTBC) in sputum samples is acid-fast bacilli (AFB) staining or the (Ziehl Neelsen staining – Kenyon staining) method. The sensitivity of AFB staining in the sputum samples of individuals with cavitary TB might reach up to 70%. AFB smear microscopy, although quick and cost-effective, exhibits reduced sensitivity due to false positives caused by the presence of non-tuberculous mycobacteria (NTM) in the samples [41]. The cultivation of bacteria in liquid or solid medium is often regarded as the definitive benchmark for diagnosing TB,

since this approach demonstrates superior performance compared to acid-fast bacilli (AFB) staining. Moreover, it is cost-effective and particularly suitable for nations with limited resources. The AFB smear microscopy technique requires a time frame of 12 to 24 hours, the culture procedures need a much longer period of 2 to 6 weeks to get diagnostic outcomes for tuberculosis [2]. Three diagnostic systems have been granted permission by the Food and Drug Administration (FDA) for the semi-automated, broth-based culture of mycobacteria that are commercially accessible. The systems included in this category consist of the Mycobacteria Growth Indicator Tube (MGIT) 960 system, the Versa TREK system, and the MB/BACT Alert 3D. The procedures indicated above are adapted versions of conventional culture methods used for the identification of (MTBC) [42]. Typically, these techniques need an average incubation duration of 10 days to obtain uniform bacterial multiplication. The use of these procedures has the potential to augment the overall sensitivity of culture-confirmed cases of TB. In particular, the sensitivity may be increased from 91% when using a single sputum specimen to 98% and 100% when using second and third sputum specimens, respectively. In areas with a high prevalence of TB, it is recommended to use a combination of solid and liquid culture methods; with or without-adjunct acid-fast bacilli (AFB) smear microscopy [43].

Molecular insights into the diagnosis of active tuberculosis: Cutting-Edge techniques and applications

The first use of molecular techniques to differentiate clinical isolates of M. tuberculosis occurred during the mid-1980s. While colony morphology, growth rate comparisons, medication sensitivity, and phage typing have historically been employed, their limited discriminatory ability has restricted their utility in tuberculosis epidemiology. Before the advent of molecular technologies, the comprehension of tuberculosis transmission was inaccurate and heavily dependent on observational data or anecdotal connections [44]. Nevertheless, considering the wide range of molecular tools at our disposal, it is essential to carefully choose the most suitable approach (es) for investigating a specific research inquiry, such as transmission dynamics, outbreaks, or phylogenetics. When considering an appropriate molecular technique for investigating TB epidemiology, two crucial factors are the observed rate of polymorphism, which reflects the stability of the biomarker, and the genetic variety of strains within the population. The Xpert MTB/RIF ULTRA assay is a new test that is revolutionizing TB control by contributing to the rapid diagnosis of TB disease and drug resistance. The test simultaneously detects MTBC and resistance to rifampin (RIF) in less than 2 hours Figure 2. To effectively differentiate between strains that are not epidemiologically connected and establish reliable connections between related instances, the rate of change of a biomarker must be both adequate and appropriately gradual [45]. The evaluation of this problem, in conjunction with the overall frequency of tuberculosis in the population, is crucial in the selection of molecular epidemiological methodologies or in the assessment of data.

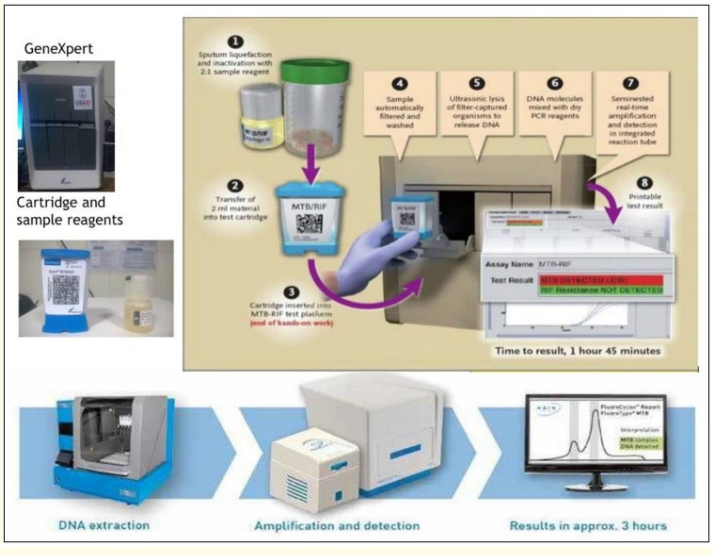


Figure 2: The flow chart shows the GeneXpert MTB/RIF ULTRA test procedure.

Genotyping of M. tuberculosis

In 1998, the fully annotated genome of M. tuberculosis laboratory strain H37Rv was published, marking the beginning of the genomic era in tuberculosis research. Since then, we have completed the genome sequences of (MTBC) CDC1551 and six closely related mycobacteria: *M. leprae, M ulcerans, M. avium, M. avium* paratuberculosis, *M. smegmatis,* and *M. bovis* [46]. Comparative sequence analysis of the 275-bp internal transcribed spacer (ITS) region, an otherwise highly polymorphic region which separates the 16S rRNA and the 23S rRNA, revealed complete conservation between members of the (MTBC). This group includes *M. tuberculosis, M. bovis, M. microti, M. africanum, M. canettii*, and, more recently, *M. pinnipedii* and *M. suricattae*. In addition, the sequencing analysis of 56 structural genes in several hundred phylogenetically and geographically varied (MTBC) isolates revealed that allelic polymorphisms are exceedingly uncommon [47]. In contrast to most bacterial pathogens, members of the (MTBC) are extremely genetically similar to one another, with an estimated rate of synonymous nucleotide polymorphisms between 0.01% and 0.03% and no significant evidence for horizontal genetic transfer between genomes. This monomorphic species, (MTBC), has polymorphism genomic regions despite its very limited (conserved) genome in comparison to other bacterial diseases [48]. Genomes of prokaryotes (like M. tuberculosis) are often marked by monomeric sequences repeated regularly (repeated units), similar to how eukaryotic genomes are interrupted by tandem repeats. Tandem repeats (TR) are direct, back-to-back repetitions, whereas interleaved repeats (IR) are–direct, front-to-back repetitions and insertion sequence-like repeats. Most bacterial genomes include several microsatellites (1- to 10-bp repeats) and minisatellites (10- to 100-bp repeats), also known as variable-number tandem repeats (VNTRs). These repeats may be found in intergenic regions, regulatory regions, or inside open reading frames [49].

Due to the lengthy turnaround time associated with culture and microscopy-based diagnostics, molecular biological technologies have developed as ~~a~~ quick diagnostic platforms for tuberculosis. Rapid clinical diagnosis of tuberculosis has been made possible by the mycobacterial nucleic acid amplification test (NAAT), which replaced the time-consuming culture and smear procedures. The NAAT has been recommended for use in diagnosing TB in clinical specimens by the US-CDC and the Association of Public Health Laboratories (APHL) [50]. Clinical laboratories frequently employ several commercially available NAAT systems, including ~~the~~ the Xpert MTB/RIF system was developed for the GeneXpert platform and is optimized for the detection of drug-resistant and drug-sensitive MTB strains in sputum samples [51]. With high sensitivity (> 90%) and results available in as little as 2 hours, this test is based on a nested real-time PCR amplifying the rpoB gene of MTB, the most prominent target for rifampicin resistance. The Xpert MTB/RIF Ultra was created to address the limitation of failing to detect MTB in paucibacillary TB infections, and it comes highly recommended by the World Health Organization. The World Health Organization recommends using the LAMP-MTBC detection kit since it focuses on the gyrB gene and IS sections of the (MTBC) genome [52].

The Genotype (MTBC) assay, developed by HAIN Life Science in Germany, is a molecular diagnostic tool specifically designed for the identification of species of (MTBC). The diagnostic utility of the Genotype (MTBC) assay lies in its ability to differentiate between various members of the (MTBC), including *M. tuberculosis, M. bovis, M. africanum, M. microti,* and others. This differentiation is crucial because these different species may exhibit variations in virulence and drug susceptibility profiles, which can impact treatment strategies [53]. This assay assists researchers and healthcare professionals in examining the genetic variability of MTBC strains, facilitating epidemiological inquiries and the monitoring of TB outbreaks. The Genotype (MTBC) assay is compatible with a wide range of clinical specimens, including sputum, culture isolates, and direct samples, making it versatile for use in different diagnostic settings [54]. Its speed, specificity, and compatibility with various specimen types make it a powerful asset in clinical and research settings, aiding in the timely and effective control of TB.

Newer kits are available for the diagnosis of MDR- and XDR-TB patients in addition to Xpert MTB.-RIF.ULTRA Resistance mutations in the MTB genome may be detected using the MTB/MDR and MTB/XDR. These include rpoB (RIF) for MTB.-RIF Ultra. katG, inhA P, fabG1and oxyR-ahpC IGE (INH) resistance (High and Low). gyrA and gyrb (fluoroquinolones). And rrs and Eis p (aminoglycosides) (AMK, KAN, CAP). InhA P FOR Ethionamide. Drug resistance to RIF (rpoB) and INH (katG and inhA) may be determined by using the MTB.-RIF Ultra kit, which has been tested and shown to be effective [55].

Another molecular method for identifying mycobacteria in samples is the Line probe assays (LiPA), which use hybridization-based probes. The LiPA uses membrane strips of nitrocellulose with genus- and species-specific probes. When using a preliminary PCR amplification, the LiPA technique has a turnaround time of roughly six hours. The Inno-LiPA Mycobacteria test, the Genotype Mycobacterium CM, and the AS assays are the three commercially available LiPA kits [56]. Mycobacteria can be detected using these assays because they focus on the 16S-23S rDNA spacer region and the 23S rDNA. With a sensitivity of 99.6 percent, the Inno-LiPA Mycobacteria is a reverse-hybridization, DNA probe assay platform developed to detect up to 17 distinct taxa concurrently. Genotype MTBC is a commercially available DNA strip test for identifying *M. bovis* BCG and distinguishing between other members of the MTB complex. Mycobacteria from primary isolations do not have to be grown on a solid medium before this procedure may be applied to them [57]. Furthermore, the use of Matrix-Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) technology in combination with mass spectrometry (MS), is an additional approach for the discrimination and characterization of mycobacteria. The technique has a specificity rate of 98.6% and requires an estimated duration of 1-2 hours for results generation from samples. Furthermore, it is regarded as a very cost-efficient approach [57].

Detecting latent tuberculosis infection: Contemporary approaches to diagnosis

In contrast to the diagnosis of active TB, the identification of latent tuberculosis infection (LTBI) is reliant on a limited number of diagnostic procedures. These people exhibit biomarker indications of being exposed to MTBC or its antigens, but do not display any symptomatic clinical manifestations of the illness [11]. The tuberculin skin test (TST) and the Interferon-gamma release assay (IGRA) are two frequently used screening assays for latent tuberculosis infection (LTBI). The traditional tuberculin skin test (TST) is the administration of pure protein derivative (PPD) through intradermal injection, followed by the assessment of the resulting induration within a timeframe of 48 to 72 hours [58]. People previously exposed to MTBC or its antigens typically show sensitization to purified protein derivative (PPD), producing cytokines at the injection site, resulting in the development of a delayed-type hypersensitivity reaction. In the conventional approach, a positive response in persons without HIV or other co-existing health issues is often defined as an induration of more than 10mm after PPD injection. Nevertheless, people who have received the BCG vaccine may also have a hypersensitive response, so rendering the tuberculin skin test (TST) as a screening test that lacks specificity [59]. Moreover, BCG-vaccinated persons and those aged 5 and above who have a low to moderate risk of having an active illness are eligible for LTBI screening with the IGRA. The QuantiFERON-TB Gold In-tube Test (QFT-GIT) and the T-SPOT.TB are two IGRAs currently on the market. While each of these assays employs MTB-specific antigens to evaluate the host immune cell response (i.e., IFNg production), they diverge in terms of methodology and endpoint. In contrast to the ELISA-based QFT-GIT test, which measures IFNg released by PBMC following stimulation with MTB antigen, the T-SPOT.TB is an ELISPOT assay that employs PBMCs isolated from whole blood. Precoated with a single cocktail of peptides from MTB proteins such ESAT-6, CFP-10, and TB7.7, the QFT-GIT tubes are ready to be used. The FDA in the United States sanctioned the QFT-GIT IGRA in 2017. For diagnosing diseases like HIV-TB that depend on CD8+ T-cells, a more advanced iteration named QFT-Plus-IGRA offers an edge. Although this test is not currently cost-efficient, additional research is essential before its implementation in TB-impacted areas.

Conclusion

In conclusion, our exploration of epidemiological, microbial, and molecular approaches has shed light on the multifaceted landscape of human TB. This comprehensive investigation has allowed us to gain deeper insights into this global health challenge, offering valuable contributions to diagnosing and understanding of the disease. Epidemiologically, our research has unveiled critical trends in TB prevalence, transmission, and risk factors. By identifying vulnerable populations and high-burden regions, we can tailor public health interventions more effectively, ultimately working towards the goal of TB elimination. On the microbial front, our examination of TB pathogens and non-tuberculous mycobacteria (NTM) has enriched our comprehension of the intricate interactions between these organisms and the human immune system. This knowledge has the potential to inform the development of novel therapies and vaccines, as well as more accurate diagnostic tools. Molecular techniques have emerged as powerful tools in TB diagnosis, enabling rapid and precise detection of active and latent infections. The advent of cutting-edge technologies has

revolutionized our ability to identify specific mycobacterial strains and drug resistance patterns, facilitating targeted treatment regimens and reducing the risk of antimicrobial resistance. In summary, the integration of epidemiological, microbial, and molecular approaches in our research endeavors has brought us closer to conquering the TB pandemic. By continuing, to advance our understanding and diagnostic capabilities, we can hope for a future where TB ceases to be a global health threat. Together, our collective efforts will drive us closer to the goal of a TB-free world, where timely diagnosis and effective treatment become accessible to all. This thorough review article offers a detailed examination of the genetic variations present in the MTBC, along with the mechanisms that drive pathogenesis and drug resistance in human tuberculosis.

Conflict of Interest

There are no conflicts of interest.

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