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Intitulé

A systematic review on associated risk factors for the development of multidrug resistant tuberculosis

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Abstract

Background: Drug resistant tuberculosis continues to be a public health threat. According to the most recent data published by the World Health Organization in 2019, there were about half million new cases of rifampicin resistant tuberculosis in 2018, of which 78% had multidrug resistant tuberculosis (MDR-TB). Globally, 3.4% of new tuberculosis (TB) cases and 18% of previously treated cases had MDR-TB or rifampicin resistant TB. MDR-TB is defined as disease caused by *Mycobacterium tuberculosis* with resistance to at least two anti-tuberculosis isoniazid and rifampicin. The mechanism of drug resistance can be caused by genetic factors, factors related to previous treatment and other factors such as comorbidity with diabetes mellitus. Several studies revealed that history of treatment of TB is the most powerful predictor of the presence of MDR-TB. The aim of this review is to identify and summarize, through included studies from different regions, the associated risk factors for the development of MDR-TB.

Methods: The review was conducted on associated risk factors for MDR-TB, PubMed database was searched from 1993 to July 17th, 2020 using combination of specific key terms, and without using any filters or language restrictions. Studies were assessed and selected according to a defined inclusion criteria and data from this studies were collected.

Result: Twenty two studies met the inclusion criteria among which several risk factors were identified, previous tuberculosis treatment was the most significant risk factor associated with multidrug resistant tuberculosis. Some studies were discordant about the association of MDR-TB with some chronic diseases such as diabetes mellitus and HIV, or with some social determinants such as age, gender or quality of life.

Conclusion: The discordance between studies from different regions, regarding the association of different risk factors with MDR-TB, indicates that this epidemic is associated with region-specific risk factors and the study of this association should be undertaken regionally. Drug susceptibility tests for patients with high risk of MDR-TB could be the appropriate alternative for an optimal treatment of TB positive cases.

Keywords: Tuberculosis, Multidrug resistance, risk factor.

Résumé :

Contexte et objectif : La tuberculose résistante aux médicaments continue de constituer une menace pour la santé publique, Selon les données les plus récentes publiées par l'Organisation mondiale de la santé en 2019, il y a eu environ un demi-million de nouveaux cas de tuberculose résistante à la rifampicine en 2018, dont 78% avaient une tuberculose multirésistante (MDR-TB). Dans le monde, 3,4% des nouveaux cas de tuberculose (TB) et 18% des cas précédemment traités avaient une MDR-TB ou une TB résistante à la rifampicine. MDR-TB est définie comme une maladie causée par *Mycobacterium tuberculosis* avec une résistance à au moins deux antituberculeux isoniazide et rifampicine. Le mécanisme de résistance aux médicaments peut être causé par des facteurs génétiques, des facteurs liés à un traitement antérieur et d'autres facteurs tels que la comorbidité avec le diabète sucré. Plusieurs études ont révélé que les antécédents de traitement de la TB sont le prédicteur le plus puissant de la présence de la MDR-TB. Le but de cette revue est d'identifier et de résumer, à travers à des études incluses de différentes régions, les facteurs de risque associés au développement de la MDR-TB.

Méthodes : Cette revue a été menée sur les facteurs de risque associés à la TB-MDR, la base de données PubMed a été recherchée de 1993 au 17 juillet 2020 en utilisant une combinaison de termes clés spécifiques et sans utiliser de filtres ni de restrictions linguistiques. Les études ont été évaluées et sélectionnées selon des critères d'inclusion définis et les données de ces études ont été collectées.

Résultat : Vingt-deux études répondaient aux critères d'inclusion parmi lesquels plusieurs facteurs de risque ont été identifiés. Un traitement antituberculeux antérieur était le facteur de risque le plus important associé à la tuberculose multirésistante. Certaines études étaient discordantes quant à l'association de la MDR-TB avec certaines maladies chroniques telles que le diabète sucré et le VIH, ou avec certains déterminants sociaux tels que l'âge, le sexe ou la qualité de vie.

Conclusion : La discordance entre les études de différentes régions concernant l'association de différents facteurs de risque avec la MDR-TB indique que cette épidémie est associée à des facteurs de risque spécifiques à la région, les études de cette association devraient être entreprises au niveau régional. Les tests de sensibilité aux médicaments pour les patients à haut risque de MDR-TB pourraient être la solution favorable pour un traitement optimal des cas de TB positifs.

Mots-clés : Tuberculose, Facteurs de risque, multirésistance au médicament.

ملخص

المقدمة: لا يزال السل المقاوم للأدوية يشكل تهديدا للصحة العامة. وفقًا لأحدث البيانات التي نشرتها منظمة الصحة العالمية في عام 2019 ، كان هناك حوالي نصف مليون حالة جديدة من السل المقاوم للريفامبيسين في عام 2018 ، منها 78٪ مصاب بالسل المقاوم للأدوية المتعددة. على الصعيد العالمي ، 3.4٪ من حالات السل الجديدة و 18٪ من الحالات التي سبق علاجها مصابة بالسل المقاوم للأدوية المتعددة أو السل المقاوم للريفامبيسين. يُعرَّف السل المقاوم للأدوية المتعددة بأنه مرض تسببه بكتيريا السل المقاومة لنوعين من مضادات السل الحيوية أيزونيازيد والريفامبيسين يمكن أن تكون آلية مقاومة الأدوية ناتجة عن عوامل وراثية وعوامل مرتبطة بالعلاج السابق وعوامل أخرى مثل الاعتلال المشترك مع داء كشفت العديد من الدراسات أن علاج سابق للسل هو أقوى مؤشر على الإصابة بالسل المقاوم للأدوية المتعددة الهدف من هذه المراجعة هو تحديد وتلخيص عوامل الخطر المرتبطة بتطور السل المقاوم للأدوية المتعددة من خلال الدراسات المشمولة من مناطق مختلفة.

الطريقة: أجريت هذه المراجعة على عوامل الخطر المرتبطة بالسل المقاوم للأدوية المتعددة ، وتم البحث في قاعدة بيانات PubMedمن 1993 إلى 17 يوليو 2020 باستخدام مجموعة من المصطلحات الأساسية المحددة ، ودون استخدام أي عوامل تصفية أو قيود لغوية. تم تقييم الدراسات واختيارها وفقًا لمعايير تضمين محددة وتم جمع البيانات من هذه الدراسات.

النتيجة: حققت اثنتان وعشرون دراسة معايير الاشتمال التي تم من بينها تحديد العديد من عوامل الخطر ، وكان علاج السل السابق هو العامل الأكثر خطورة المرتبط بالسل المقاوم للأدوية المتعددة. كانت بعض الدراسات متضاربة حول ارتباط السل المقاوم للأدوية المتعددة ببعض الأمراض المزمنة مثل داء السكري وفيروس نقص المناعة البشرية ، أو مع بعض المحددات الاجتماعية مثل العمر أو الجنس أو نوعية الحياة.

الخلاصة: يشير التناقض بين الدراسات من مناطق مختلفة فيما يتعلق بربط عوامل الخطر المختلفة بالسل المقاوم للأدوية المتعددة إلى أن هذا الوباء مرتبط بعوامل الخطر الخاصة بالمنطقة ، وينبغي إجراء دراسات حول هذا الارتباط على المستوى الإقليمي. يمكن أن تكون اختبارات الحساسية للأدوية للمرضى الذين لديهم مخاطر عالية للإصابة بالسل المقاوم للأدوية المتعددة الحل الأمثل للعلاج الأمثل للحالات الإيجابية لمرض السل.

الكلمات المفتاحية: السل ، مقاومة الأدوية المتعددة ، عامل الخطر.

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TB: Tuberculosis.
WHO: World health organization.
HIV: Human immunodeficiency virus.
AIDS: Acquired ImmunoDeficiency Syndrome
MTBC: *Mycobacterium tuberculosis* complex.
RGM: Rapid growing mycobacteria.
NTM: Non-tuberculosis mycobacteria.
SSTI: Skin and soft tissue infections.
MAC: Mycobacterium avium complex.
LTBI: Latent tuberculosis infection.
TST: Tuberculosis skin test.
CDC: Centers for disease control and prevention.
DOT: Directly observed therapy.
RIF: Rifampicin.
EMB: Ethambutol.
INH: Isoniazid.
PZA: Pyrazinamide.
EPTB: Extra-pulmonary tuberculosis.
PTB: Pulmonary tuberculosis.
GT: Generation time.
IDSA: Infectious disease society of America.
IGRA: Interferon gamma release assey.
SARS: Sever acute respiratory syndrome.
DOT: Directly observed therapy.
DR-TB: Drug resistant tuberculosis.
RR: Rifampicin resistance.
XDR: Extensive drug resistant tuberculosis.
MDR: Multidrug resistant tuberculosis.
PCR: Polymerase chain reaction.
DST: Drug susceptibility test.
SLD: Second linedrugs.
DM: diabetes mellitus.

General Introduction

Tuberculosis (TB) is a communicable disease that can cause major health issues. This pathology is considered as one of the top-ten causes of death worldwide and the leading cause of death from a single infectious agent (ranking above HIV/AIDS). It is caused by the bacillus *Mycobacterium tuberculosis*, which is spread when people who are sick with TB expel bacteria into the air; for example, by coughing (WHO, 2019). The geographical distribution of multidrug resistant tuberculosis (MDR-TB) is highly variable, consisting of several hot spots in low and middle-income countries, from Eastern Europe to Asia (Lange *et al.*, 2018).

The discovery and wide use of antimicrobials effective against TB starting in the middle of the 20th century allowed dramatic reductions in TB mortality. However, despite the success of chemotherapy, the disease became the first infectious killer seven decades later (Glaziou, Floyd, and Raviglione 2018). *M. tuberculosis* is able to acquire resistance to a particular antibiotic to which it was previously susceptible (Smith, Wolff, and Nguyen, 2014).

MDR-TB is a form of drug-resistant TB in which *Mycobacterium tuberculosis* can no longer be killed by the two best antibiotics most commonly used to cure TB, isoniazid and rifampicin (Mulisa *et al.* 2015). Factors such as inadequate chemotherapy, poor drug quality, poor adherence to treatment, treatment failure, prior treatment, cavity pulmonary TB, HIV infection and diabetes accounted for the development of drug resistance in TB. Of these, the most powerful predictor for the presence of MDR-TB is a previous history of treatment of TB (Mulu *et al.* 2015).

Identification of risk factors associated to the development of MDR-TB is important for clinicians since it allows them to identify patients who are at risk of having such resistance. In this systematic review we aim to determine and summarize through included studies from different regions the associated risk factors for the development of MDR-TB.

This memoire is presented in four chapters, the first one is meant to give a clear picture of tuberculosis as an infectious disease. The second is dealing with drug resistance, particularly MDR-TB. The third and fourth chapters are for methods, result, discussion and the last for the conclusion.

Chapter 1

Introduction to Tuberculosis

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Introduction to Tuberculosis

1.1 Overview of tuberculosis

Tuberculosis (TB) is a disease of antiquity, caused by *Mycobacterium tuberculosis (MTB)* that principally affects the lungs (Harries and Dye 2006). The bacillus *Mycobacterium tuberculosis* usually multiply in the lungs and are spread by air-borne droplets from infected persons (Figure 1.1) (Martini, Besozzi, and Barberis 2018). There are four factors that determine the probability of transmission of *M. tuberculosis* as shown in Table 1.1. Furthermore, the World Health Organization reported that MTB is spread when people who are sick with TB expel bacteria into the air, for example, by coughing. This typically affects the lungs (pulmonary TB) and could affect other sites too (extrapulmonary TB) (WHO. 2019).

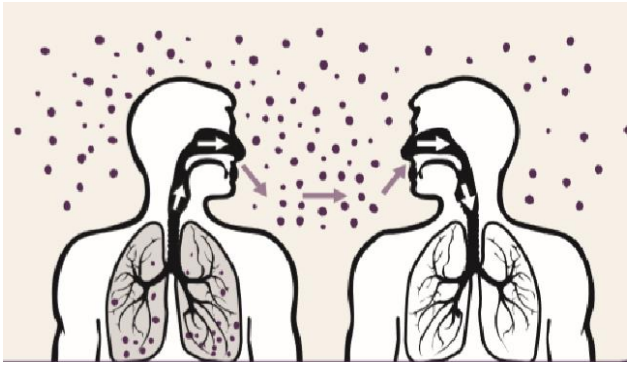

Figure 1.1 Transmission of TB: TB is spread from person to person through the air (The dots in the air represent droplet nuclei containing tubercle bacilli) (CDC 2019)

Table 1.1 Factors determining the probability of transmission of *M. tuberculosis* (CDC, 2013)

Factors	Description
Susceptibility	Susceptibility (immune status) of the exposed individual.
Infectiousness	Infectiousness of the person with TB disease is directly related to the number of tubercle bacilli that the person expels into the air. Individuals who expel several tubercle bacilli are more infectious than patients who expel less or no bacilli.
Environment	Environmental factors that affect the concentration of *M.tuberculosis* organisms.
Exposure	Proximity, frequency, and duration of exposure.

Tuberculosis is a multi-systemic disease with a protean presentation. The organ system most commonly affected include the respiratory system, the gastrointestinal (GI) system, the lymphoreticular system, the skin, the central nervous system, the musculoskeletal system, the reproductive system, and the liver (Rotimi Adigun; Rahulkumar Singh 2019).

Tuberculosis has existed for millennia and remains a major global health issue. Every year, this pathology affects millions of people**.** Since 1997 the WHO started publishing a global TB report. In 2015 TB was one of the top-ten causes of death worldwide, ranking above HIV/AIDS as one of the leading causes of death from an infectious disease (States *et al.*, 2016). TB has now become a disease mainly affecting the elderly male in developed countries. According to the WHO, TB is affecting one third of the population worldwide and one out of four adult male deaths is attributed to it (Bangwal, Joshi, and Rawat 2019).

During the 2014's Assembly, the WHO member's states adopted the end TB strategy that covers the period 2016-2035. The overall goal was to end the global TB epidemic. The most immediate milestones, set for 2020, are a 35% reduction in TB deaths and a 20% reduction in the TB incidence rate, compared with levels in 2015 (WHO. 2019). The trajectories of TB incidence and TB deaths that are required to reach these milestones and targets are shown on figure 1.2.

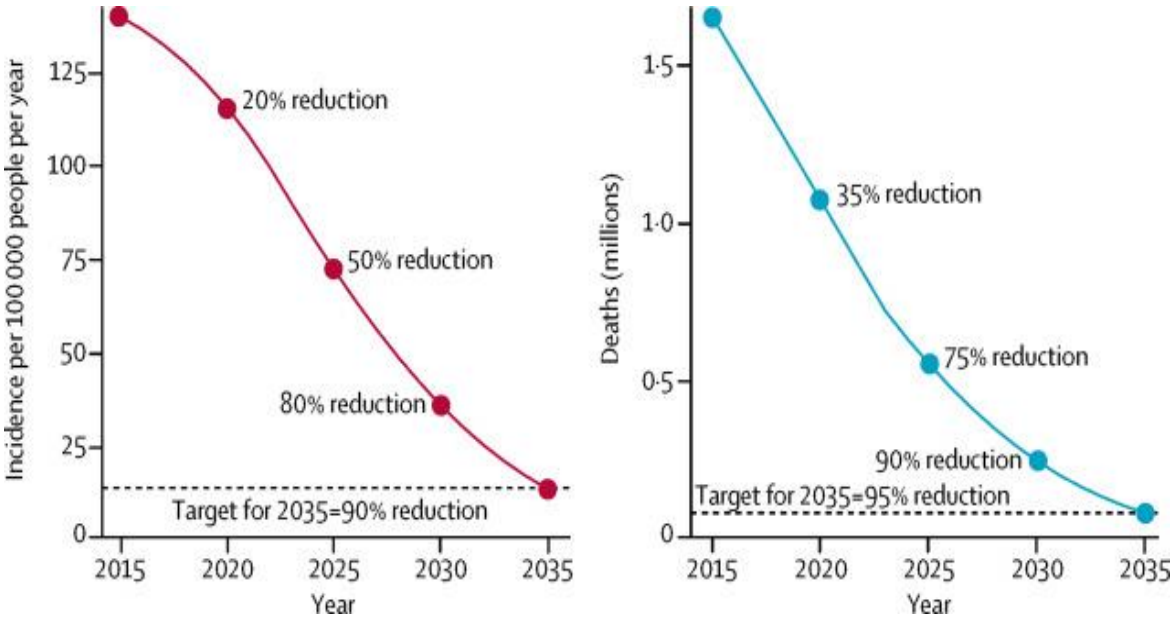

Figure 1.2: Projected incidence and mortality curves that are required to reach End TB Strategy targets and milestones, 2015–2035 (WHO, 2019)

1.2 Etiology of Tuberculosis

On 14 March 1882, Robert Koch presented his epochal paper ' Die Aetiologie der TuberKulose' to an awe-struck audience at the Berlin Physiological Society, where he convincingly demonstrated the bacterial cause of the disease responsible for one in seven deaths in Europe at that time (Gradmann, 2001).

The etiological agent of tuberculosis, termed *Mycobacterium tuberculosis* has been identified over a century ago by Hermann Heinrich Robert Koch, a physician-scientist who learned about the recent theory of infectious disease from Jacob Henle (1809-1885) a professor of anatomy and physiology (Chai, Zhang, and Liu 2018; Münch 2003). Robert Koch's studies launched a new field of scientific inquiry- the field of medical bacteriology (Blevins and Bronze 2010). Koch's work on the etiology of tuberculosis is thus connected to speculative pathology and contained a general concept of infectious diseases as bacterial activity (Gradmann 2001).

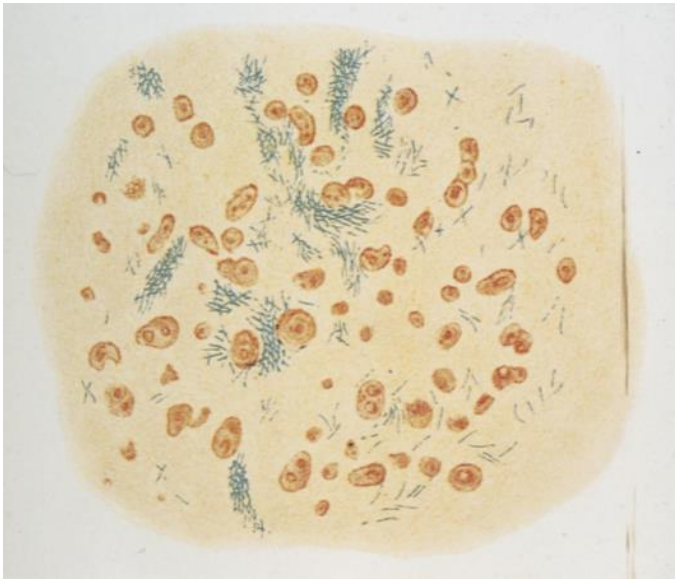

Figure 1.3: Tubercle bacilli (stained blue) in section of tissue from consumptive lung, drawn by Koch and published with the expanded 1884 version of "The Etiology of Tuberculosis." In: Koch, R., Gesammelte Werke von Robert Koch, Georg Thieme, Leipzig, 1912, Table XX (Raju 2006)

The genus Mycobacterium comprises more than 170 species, most of them are environmental organisms. Traditionally, Mycobacterium species have been divided into rapid-growers and slow-growers (Gagneux 2018) -phylogenetic analyses and phenotypic characteristics such as growth rate and pigmentation can be used to classify these species-

(Jang *et al,*. 2008), while (Rogall *et al,*. 1990) pointed out that on the basis of the mentioned criteria the genus Mycobacterium has been divided into four groups:

- Group I, consisting of the photochromogenic (pigmented) species of slow growers;
- Group II, scotochromogenic slow growers;
- Group III containing the nonchromogenic slow growers;
- and Group IV consisting of rapid growers (defined as maturing in less than 1 week).

Slow-growers are found in the environment, but most of them are potentially pathogenic, particularly in immunocompromised individuals. These include *Mycobacterium Avium*, *Mycobacterium Marinum*, *Mycobacterium Xenopi*, *Mycobacterium Gordonae* and *Mycobacterium Kansasii*. *Mycobacterium tuberculosis* differs from the other species since it has never been isolated from the environment. Instead, it has been isolated only from infected human hosts and transmitted only by infected and diseased individuals (Jang and al. 2008) .

The three major slow-growers mycobacterial pathogens of humans are (*Mycobacterium tuberculosis* complex (MTBC), *Mycobacterium leprae* and *Mycobacterium ulcerans*)(Gagneux 2018). MTBC comprised of *M. tuberculosis*, *M. africanum*, *M. canettii, M. bovis*, *M. microti, M. orygis, M. caprae, M. pinnipedii, M. suricattae* and *M. mungi* (Sinha *et al.,* 2016). MTB is the causative agent of TB in humans and also *M. africanum* but only in certain regions of Africa (Delogu, Sali, and Fadda 2013). Human-adapted *MTBC* is unique as it is an obligate pathogen of humans without any environmental or animal reservoir. The adaptation refers to the capacity of this pathogen to successfully infect, cause disease and being transmited within the primary (or maintenance) host species (Gagneux 2018). These human-adapted forms of MTBC include *Mycobacterium tuberculosis sensu stricto* and *M. africanum*. Humans are the unique known hosts where infection and transmission by *M. tuberculosis* and *M. africanum* occur efficiently and where infection cycles are known to be sustainably maintained (Brites and Gagneux 2015).

The most important human pathogen complex of rapid-growing mycobacteria (RGM) is *M. abscessus* (i.e., *M. abscessus sensu lato*) divided into three species (*M. abscessus sensu stricto*, *M. massiliense* and *M. bolletti*) (Figure 1.4) (Kozakiewicz *et al.* 2013; Laurens *et al.* 2014). These RGM belongs to non-tuberculous mycobacteria (NTM) that is susceptible to cause pulmonary disease resembling tuberculosis, skin and soft tissue infections (SSTIs), central nervous system infections, bacteremia, and ocular and other infections. It should be underlined that Mycobacteria are divided into two major groups for the purpose of

diagnosis and treatment: *Tuberculosis* mycobacteria, and nontuberculosis mycobacteria (Lee *et al.* 2015).

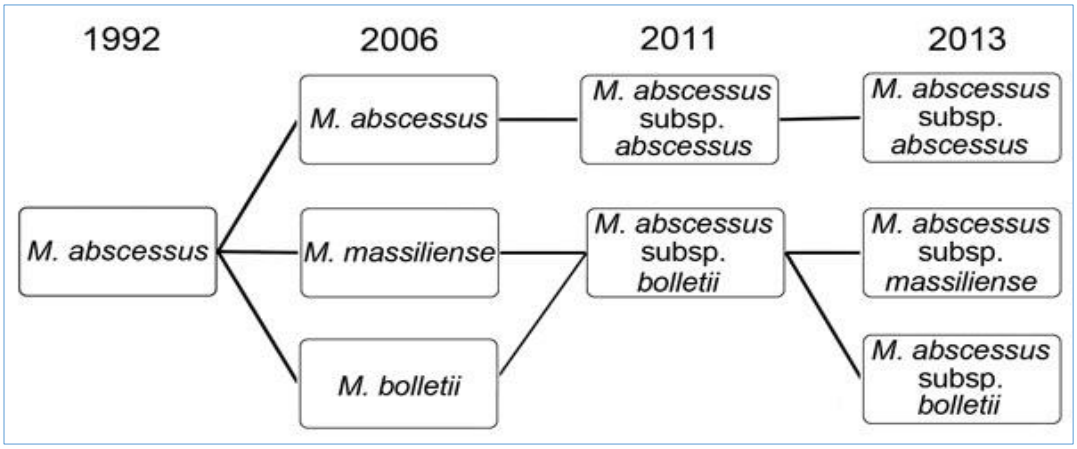

Figure 1.4 Serial changes in the nomenclature and taxonomic classification of *Mycobacterium abscessus* complex, 1992-2013 (Gagneux 2018)

1.2.1 Non-tuberculosis mycobacterium

Non-tuberculous mycobacteria represent species further to those belonging to the *Mycobacterium tuberculosis* complex (Table 1.2) and do not cause leprosy. NTM are generally free-living organisms that are ubiquitous in the environment. There have been more than 140 NTM identified species to-date (J. *et al.* 2015).

Table 1.2 Classification of non-tuberculous mycobacteria according to their growth rate (Johansen, n.d.)

Non-tuberculous mycobacteria	
Rapidly growing mycobacteria	Slowly growing mycobacteria
M. chelonae–abscessus complex : – *M. abscessus subsp. Abscessus* – *M. abscessus subsp. bolletii* – *M. abscessus subsp. massiliense* – *M. chelonae.* *M. fortuitum.* *M. smegmatis.* *M. vaccae.*	*M.marinum.* *M.ulcerans.* *M. avium complex:* – *M.avium.* – *M.intracellulare.* – *M.chimaera.* *M. haemophilum.* *M. xenopi.* *M. kansasii.* *M. simiae.* *M. terrae complex.* *M. gordonae.*

Non-tuberculosis mycobacteria are thought to be acquired only from environmental sources, such as exposure to tap water, soil, or the use of showers or cooling/heating devices. Those microorganisms can cause disease in immune-compromised individuals

with no definitive evidence of human-to-human transmission (Gagneux 2018; Bento, Gomes, and Silva 2020).

Recurrence due to relapse or re-infection is more frequent, resulting in substantial morbidity and mortality. There is a perception among several clinicians and public health tuberculosis practioners that new cases of NTM lung disease may significantly exceed case rates for TB in their communities or regions, and a long-term therapy is regularly necessary to treat NTM lung disease (Iseman and Marras 2008; Honda *et al.* 2020).

The NTM disease symptoms are non-specific and frequently occurs in patients with preexisting respiratory conditions. These latters may be difficult or impossible to distinguish from those of TB disease and make radiological images crucial for the correct diagnosis. However, unlike other lung diseases, NTM have characteristic lung presentations. The two major radiological patterns of NTM lung infection are fibro-cavity and nodular bronchiectasis pattern (Cowman and Loebinger 2018; Bethencourt Mirabal A and Ferrer G. 2019). As well, the isolation of NTM remains a clinical dilemma for clinicians. Because NTM exist naturally in the environment, their isolation from a non-sterile respiratory specimen does not mean they are causative organisms of lung disease. To overcome this factor, guidelines require the isolation of NTM from more than one sputum specimen collected on separate occasions. The diagnosis can also be performed on a single bronchoscopic sample, although this method is not invulnerable to contamination (Cowman and Loebinger 2018; Ryu, Koh, and Daley 2016).

Some of the clinical features associated with pulmonary NTM infection are similar to those of TB. It is therefore comprehensible that the first treatments used for NTM infections were antituberculous drugs, which were successful in some cases, but demonstrated lower activity in this setting than against MTB (Bento, Gomes, and Silva 2020).

In 2007, the American Thoracic Society (ATS) and the Infectious Diseases Society of America (IDSA), and more recently the British Thoracic Society (BTS), released a series of guidelines for the treatment of NTM pulmonary infections. The main consensus recommendation is the use of a macrolide based multidrug regimen that have the best correlation between in vitro susceptibility results and clinical (in vivo) response, (Bento, Gomes, and Silva 2020). Treatment recommendations for NTM (except *mycobacterium avium* complex and *M. abscessus*) are summarized in (Table 1.3).

Table 1.3 Drugs proposed in the treatment of some nontuberculous mycobacteria (Ryu, Koh, and Daley 2016)

	Antibiotic susceptibility testing	**Key drug**	**Associated drugs**	**Potential others active drugs**
Mycobacterium Xenopi	Not for a first episode No correlation	A lot of unknown data: probably rifampicin, ethambutol, clarithromycin, and/or moxifloxacin Possibility of amikacin at the beginning of treatment		
Mycobacterium kansasii	Rifampicin	Rifampicin	Ethambutol, isoniazid	Moxifloxacin, clarithromycin
Mycobacterium Szulgai	Rifampicin	Rifampicin, ethambutol with isoniazid or clarithromycin		Moxifloxacin, clarithromycin
Mycobacterium Malmoense	Not for a first episode No correlation	Isoniazid, rifampicin, ethambutol		Moxifloxacin, clarithromycin
Mycobacterium Simiae	No recommendation	Clarithromycin, amikacin, and trimethoprim–sulfamethoxazole		Moxifloxacin, clarithromycin

1.2.2 Biology of *Mycobacterium tuberculosis*

MTB is a slow growing mycobacteria with a doubling time of 12-24 h under optimal conditions. The structure of the cell envelope of these bacteria has been the subject of numerous studies because it is already clear that the powerful biological activities of known wall components contribute significantly to the disease process (Delogu, Sali, and Fadda 2013; Zuber *et al.* 2008). A major feature of MTB's peculiar cell wall structure, is that it provides an extraordinarily efficient permeability barrier to noxious compounds, contributes to the high intrinsic resistance of mycobacteria to many drugs and plays a fundamental role in virulence (Delogu, Sali, and Fadda 2013; Plitzko *et al.* 2008).

The classical view of the mycobacterial cell wall structure has been revised using the new electron microscopy technique, cryo-electron tomography on vitreous section, that preserves cell wall organization by avoiding sample dehydration. This method allows to show that mycobacteria possess an outer membrane, functionally similar to what seen in gram-negative bacteria, consisting in an asymmetric lipid bilayer made of long fatty acids in the inner leaflet (mycolic acids) and of glycolipids and waxy components on the outer layer. Indeed, lipids isolated from the cell envelope can elicit responses by the host immune

system identical to the responses generated by *M. tuberculosis* infection (Delogu, Sali, and Fadda 2013; Zuber *et al.* 2008). The outer and inner membrane form a periplasmic space, with the presence of a thin layer of peptidoglycan in the innermost side covalently linked to arabinogalactan and lipoarabinomannan which in turn are bound to mycolic acids (Delogu, Sali, and Fadda 2013).

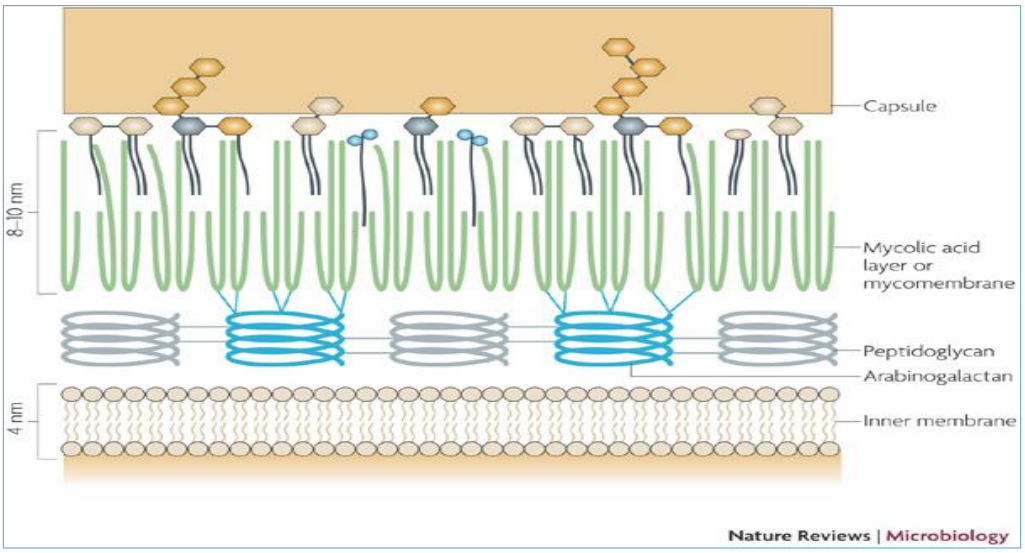

Figure 1.5: Schematic representation of the cell envelope of *Mycobacterium tuberculosis* (Abdallah *et al.* 2007)

Protein secretion systems are the main virulence factors of pathogenic bacteria. Gram-positive bacteria are generally regarded as being simpler in structure because they lack a second membrane; consequently, secretory proteins of Gram-positive bacteria only need to traverse the cytoplasmic membrane and peptidoglycan layer to enter the extracellular environment. However, recent studies have provided evidence that there is an alternative protein-secretion system in Gram-positive bacteria. Perhaps unsurprisingly, this specialized secretion system was identified in the Gram positive *Mycobacterium tuberculosis* (Delogu, Sali, and Fadda 2013; Abdallah *et al.* 2007).

In MTB, five type VII secretion systems (T7S) were identified (ESX1-5). ESX/T7S systems play a crucial role in the biology of MTB, as well as in the interactions MTB has with its host (Delogu, Sali, and Fadda 2013; Vaziri and Brosch 2019), the best characterized of these is the type VII secretion system known as ESAT-6 secretion system-1 (ESX-1) in Mycobacterium species (Figure 1.6). This secretion system is missing in the attenuated *M. bovis* vaccine strain Bacille Calmette and Guerin. ESX1 is required for the full virulence of MTB, that uses this secretion system to translocate from the phagosome into the cytosol of

infected macrophages where it may persist in a protected environment (Delogu, Sali, and Fadda 2013; Wong, n.d.).

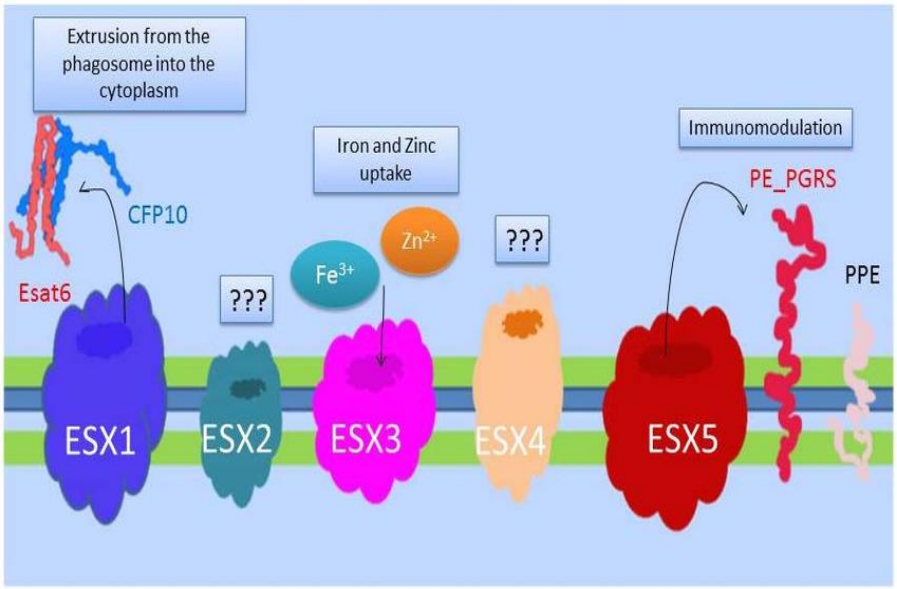

Figure 1.6: Protein Secretion systems in mycobacterium tuberculosis.(Delogu, Sali, and Fadda 2013)

In MTB, the ESX-1 type VII secretion system is essential for bacterial access to the host cytosol, the ability of MTB to perforate the phagosome is critical to virulence because that is how MTB delivers effectors to the cytosol. Several proteins secreted by ESX-1 are immunodominant and are at the forefront of infection and disease (Mittal *et al.* 2018; Tiwari *et al.* 2019).

It was suggested that necrosis is itself a means to progression of TB disease, and following intracellular replication within alveolar macrophages at the site of disease, cell death often occurs, with MTB ESX-1 promoting necrosis, not apoptosis. Indeed, MTB actively suppresses macrophage apoptosis, reducing bacterial replication with ESX-1, while ESX-1-mediated necrosis enhances bacterial replication. Furthermore, MTB mediates cellular necrosis in other host cells, such as alveolar epithelial cells in the epithelial bilayer, which is needed for infection and disease progression (Tiwari *et al.* 2019).

1.3 Types of tuberculosis

1.3.1 Latent tuberculosis

The WHO defines latent tuberculosis infection (LTBI) as a state of persistent immune response to stimulation by MTB antigens without evidence of clinically manifested active

TB. The lifetime risk of reactivation TB for a person with documented LTBI is estimated to be 5-10%. Reactivation constitutes the process by which a subclinical latent infection transits into active TB disease. (Kiazyk and Ball 2017; Ilievska-Poposka *et al.* 2018). The risk of developing TB disease following infection depends on several factors, the most important one being the immunological status of the host. Finding LTBI provides an opportunity to treat and prevent reactivation of the latent infection that leads to active disease, especially in people with compromised immune systems (Ilievska-Poposka *et al.* 2018).

LTBI is detected by measuring immune responses to MTB antigens, two tests currently measure this immune responses:

- the tuberculin skin test (TST), which is performed by intradermal injection of a purified protein derivative of tuberculin isolated from culture filtrates. In the setting of prior MTB infection, tuberculin will stimulate a delayed hypersensitivity response via T lymphocytes that is measured by induration at the injection site after 48–72 h. The test is interpreted based on the diameter of the area of induration and the clinical characteristics of the patient (Kiazyk and Ball 2017; Turetz and Ma 2016).
- The second more recent test is interferon-gamma release assay (IGRA), constiting of a whole blood–based *in vitro* assay that measures the production of interferon-gamma by immune cells in response to MTB antigen stimulation. The test does not require that the patient return to the lab and can be completed in 24 hours (Kiazyk and Ball 2017).

Four main antimicrobial regimens are currently available for LTBI treatment: isoniazid monotherapy, rifampin monotherapy, isoniazid plus rifampin in combination, and isoniazid plus rifapentine in combination (Table 1.4). Isoniazid for 6 months to 12 months has been used for decades. The efficacy in preventing progression to TB disease is approximately 90%. The overall effectiveness of this drug, however, has been hindered by low adherence and completion rates due to its prolonged duration and hepatotoxicity risk. Shorter rifamycin-based regimens have similar efficacy and are increasingly used. These regimens are associated with improved completion rates as well as reduced risk of hepatotoxicity compared with isoniazid monotherapy. Importantly, studies have not shown an increased risk of developing isoniazid or rifamycin resistant TB disease after receiving LTBI treatment regimens that contain these drugs. The CDC recommends monthly visits to assess medication adherence and signs or symptoms of drug toxicity (Huaman and Sterling 2019).

Table 1.4: Latent tuberculosis infection treatment regimens (Huaman and Sterling 2019)

Regimen	Duration	Dose	Frequency	Total doses
Isoniazid plus rifapentine	3 months	Isoniazid, 900 mg (15 mg/kg) Rifapentine, 750 mg if 32–50 kg; 900 mg if >50 kg	Once weekly	12
Rifampin	4 months	600 mg (10 mg/kg)	Daily	120
Isoniazid plus rifampin	3-4 months	Isoniazid, 300 mg (5 mg/kg) Rifampin, 600 mg (10 mg/kg)	Daily	90-120
Isoniazid	6-9 months	300 mg (5mg/kg) 900 mg (15 mg/kg)	Daily Twice weekly	180-270 52-76

1.3.2 Miliary tuberculosis

Miliary tuberculosis is a lethal form of disseminated TB that results from a massive lymphohematogenous dissemination from a Mycobacterium tuberculosis-laden focus. The term “miliary TB”, derives from the Latin word miliarius, meaning related to millet seed, that was coined to describe the similitude of gross pathological findings to that of innumerable millet seeds in size and appearance (Figure 1.7). Traditionally, the miliary pattern on a chest radiograph has been defined as “a collection of tiny discrete pulmonary opacities that are generally uniform in size and widespread in distribution, each of which measures two millimeter or less in diameter” (Sharma *et al.* 2005).

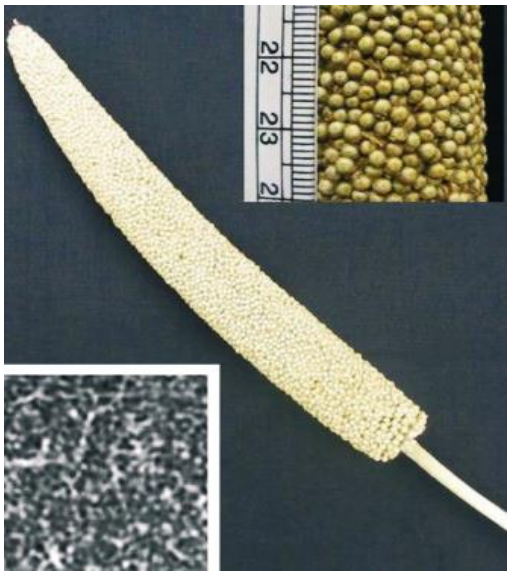

Figure 1.7 Millet seeds are small grains (average diameter <2 mm) that are consumed without their outer layer being removed. Pearl millet (Pennisetum typhoides, bajra) is shown here. These grains (inset, upper right) correspond to the approximate size of military (Baughn and Rhee 2014)

Mortality from this disease has remained high despite effective therapy being available. For a long time, miliary TB has been considered to be a childhood disease. However, during the last three decades, it is increasingly being recognized in adults as well. Several reasons are thought to be responsible for this changing epidemiological trend, such as human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) (Sharma SK, A, and A. 2012).

The clinical manifestations of miliary TB in adults are nonspecific and can be obscure till late in the disease. Classically, fever with evening rise of temperature of several weeks' duration, anorexia, weight loss, weakness and cough are evident. Occurrence of daily morning fever spikes has also been reported. Chills and rigors, similar to that described in patients with malaria and bacteremia have been frequently described in adult patients with miliary TB. Night sweats are common; sweat engraved the patient's silhouette on the bed, closely resembling a body's shadow (damp shadow sign) has been described in miliary TB (Sharma SK, A, and A. 2012).

1.3.3 Active tuberculosis

Despite advances in diagnosing latent MTB infection, a diagnostic test that differentiates LTBI from active tuberculosis or predicts the risk of progression to active disease is still in lack. One reason for the absence of such a test may be the failure of current assays to capture the dynamic complexities of the immune responses associated with various stages of TB, since these assays measure only a single parameter (release of IFN-γ) and rely on prolonged (overnight) T cell stimulation (Arrigucci *et al.* 2018).

Active tuberculosis is a multi-organ disease caused by primary infection or as a reactivation of latent tuberculosis. Accordingly, active tuberculosis could be primary tuberculosis or reactivation tuberculosis. Primary tuberculosis occurs when the immune system is unable to defend against the MTB infection. Reactivation tuberculosis, as the name suggests, is the reactivation of contained mycobacterial infection. Reactivation Tb is the most common form of active tuberculosis, representing 90% of the cases (TN *et al.* 2020).

Unfortunately, even the most accurate *ex vivo* LTBI assays, which measure IFN-γ release by antigen-stimulated peripheral T cells (Interferon gamma release assays-IGRA), do not distinguish between LTBI and active TB, nor do they provide information on the risk of reactivation and progression to the disease, new tools distinguishing LTBI from active TB based on host responses are sorely required (Arrigucci *et al.* 2018).

There are two main unmet requirements for active TB diagnosis: the first is a rapid triage

test which could rule-out active TB from the differential, secondly, a test with high specificity for all active TB cases, to be used further along the diagnostic pathway. The currently available diagnostic tools for TB infection can be separated into microbiological, radiological, and immune-based tests (Table 1.5)(Halliday *et al.* 2019).

Table 1.5 The performance of currently available diagnostic tools for active tuberculosis (Halliday *et al.* 2019)

Test	Type of test	Speed of result	Cost (high/low)	Sensitivity for active TB	Specificity for active TB	Notes
Smear microscopy	Microbiological	Fast	Low	30-80%	~97%	Lacks sensitivity in children those with EPTB and HIV+
MTB culture	Microbiological	Slow(3-6 weeks)	Low	30-85%	100% gold standard	Poor sensitivity. Poor diagnosis in children and HIV+
Gene Xpert® MTB/RIF assay	Microbiological	Fast	High	98.2% S+ 72.5% S-C+ 66% I suspected TB (pediatric)	99.20%	Poor sensitivity in EPTB and HIV+ and children
X-ray/CT scan	Radiology	Fast	Low/Med	~70%	~50-60%	Poor specificity. Cannot distinguish from other disease
Tuberculin Skin Test	Immune-based	Fast	Low	62.5%-79.5%	36.6-95.2%	Decreased specificity due to purified protein derivate cross-reacting with BCG-vaccinated individuals and those infected/exposed to NTM.
QuantiFERON Gold®TB InTube	Immune-based	Fast	Medium	67-71%	80-94%	Cannot exclude/rule-out active TB or discriminate between LTBI and active TB
T-SPOT.TB	Immune based	Fast	Medium	80-85%	86-94%	Cannot exclude/rule-out active TB or discriminate between LTBI and active TB.

(Culture positive C+, culture negative C-, smear negative S-, smear positive S+, extra-pulmonary (EPTB), non-tuberculous mycobacteria (NTM))

1.4 Pathogenesis and clinical manifestation of tuberculosis

1.4.1 Pulmonary tuberculosis

The classification of pulmonary tuberculosis is based on clinical and radiologic factors (Table 1.6). Active disease may manifest with symptoms that are only minimal initially but then develop during the course of several months. Typical symptoms of active tuberculosis include a productive cough, hemoptysis, weight loss, fatigue, malaise, fever, and night sweats. The insidious and nonspecific nature of the symptoms means that physicians caring for these patients must maintain a high index of suspicion that is based on the risk factors. Radiologists can aid in diagnosis by performing imaging examinations, sometimes even incidentally in the absence of clinical suspicion (Halliday *et al.* 2019).

Symptoms of pulmonary tuberculosis may be divided into two categories, constitutional and pulmonary. The frequency of these symptoms differs according to whether the patient has primary tuberculosis or reactivation tuberculosis. Subjects with primary tuberculosis are much more likely to be asymptomatic or minimally symptomatic. The constitutional symptom most frequently observed is fever, low grade at the onset but becoming quite marked as the disease progresses. Characteristically, the fever develops in the late afternoon and may not be accompanied by pronounced symptoms. With defervescence, usually during sleep, sweating occurs—the classic “night sweats”. Other signs of toxemia, such as malaise, irritability, weakness, unusual fatigue, headache, and weight loss, may be present (Volmink and Murphy 2007).

Pulmonary TB patients with cavitations on chest radiograph are more likely than those without cavitations to be infectious. Concerning smear-positive pulmonary TB patients with typical adult-type pulmonary, TB involves the upper lung zones with or without cavitations, but no discernible adenopathy. These patients are more likely than those without typical adult-type pulmonary TB on chest radiograph to be infectious. Howeve, for patients with laryngeal TB, the inflammation and ulceration of the vocal cords cause hoarseness. These category of patients usually have far advanced pulmonary TB upstream from the larynx and are highly infectious (Figure 1.8) (Long 2015).

Table 1.6 Classification of Tuberculosis on the Basis of Clinical and Radiologic Findings (Halliday *et al.* 2019)

Class	Definition	Clinical history	Laboratory test results	Chest Radiographic Findings
0	No exposure to tuberculosis; no infection	No history exposure	Negative results of tuberculin skin test or interferon-γ release assay	No radiographic evidence of disease
1	Exposure to tuberculosis; no infection	History of exposure	Negative results of tuberculin skin test or interferon-γ release assay (done at least 10 weeks after exposure)	No radiographic evidence of disease
2	Latent tuberculosis infection; no tuberculosis disease	No clinical evidence of disease	Positive results of tuberculin skin test or interferon-γ release assay; negative results of bacteriologic examinations (if done	No radiographic evidence of active disease
3	Active tuberculosis disease (current)	Meets criteria for active clinical case	Meets current laboratory criteria (eg, positive culture)	Radiographic evidence of active disease
4	Previous tuberculosis disease (inactive)	Medical history of tuberculosis disease; no evidence of active tuberculosis disease	Positive results of tuberculin skin test or interferon-γ release assay, negative results of bacteriologic examinations (if done)	Abnormal but stable radiographic findings; no radiographic evidence of active tuberculosis disease
5	Tuberculosis suspected; diagnosis pending	Ongoing evaluation for active tuberculosis on the basis of clinical, laboratory, and/or radiographic findings	/	/

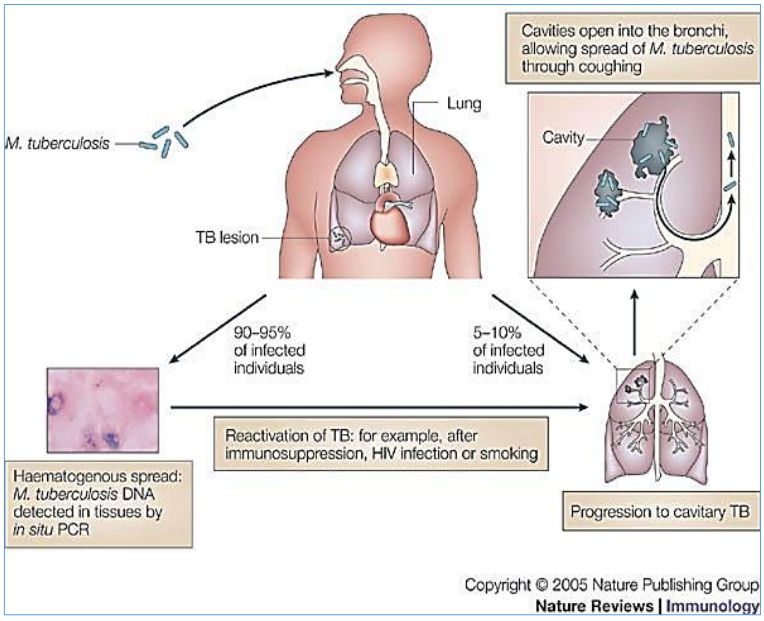

Figure 1.8 Phases of infection with *Mycobacterium tuberculosis* (Rook, Dheda, and Zumla 2005)

Standard first-line treatment of pulmonary TB consists of isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), and Ethambutol (EMB) as shown in Table1.7. With this combination, INH has the best early bactericidal activity whereas RIF is necessary to cure TB in the shortest time. PZA is effective against minimally active organisms and inclusion in the first two months facilitates cure with a 6-month course. EMB is the least active in this combination but is protective against acquired resistance when treatment is started without knowing the susceptibilities. This combination of medications is given for 2 months in the initial phase of TB treatment. With fully susceptible TB, EMB is not needed and the initial two months of treatment can be with INH, RIF, and PZA alone (Belknap 2019).

Table 1.7 treatment Drug-susceptible tuberculosis (Belknap 2019)

Drug	Common or severe Adverse effects
INH	Headache. Fatigue. Nausea/anorexia/abdominal pain. Drug-induced hepatitis. Rash. Peripheral neuropathy.
RIF	Drug-drug interactions. Red/orange discoloration of body fluids. Rash. Nausea/anorexia/abdominal pain. Flulike illness/hypersensitivity. Drug-induced hepatitis. Acute renal failure. Anemia/thrombocytopenia.
PZA	Nausea/anorexia/abdominal pain.

	Drug-induced hepatitis. Rash/acute flushing. Join pain Elvated uric acid
EMB	Visual impairment/optic neuritis.
Levofloxacin	Headache/insomnia. Nausea/anorexia/abdominal pain. Rash. Tendonitis/tendon rupture.
Moxofloxacin	Headache/insomnia. Nausea/anorexia/abdominal pain. Rash. Tendonitis/tendon rupture. Drug-induced hepatitis.

1.4.2 Extrapulmonary tuberculosis

Extrapulmonary tuberculosis (EPTB) is defined according to the WHO classification criteria as an infection by MTB that affects tissues and organs outside the pulmonary parenchyma. EPTB represents between 20 and 25% of all TB cases (Golden and Vikram 2005), and represented 15% of the 7.0 million incident cases that were notified in 2018 (Figure 1.9) (WHO. 2019).

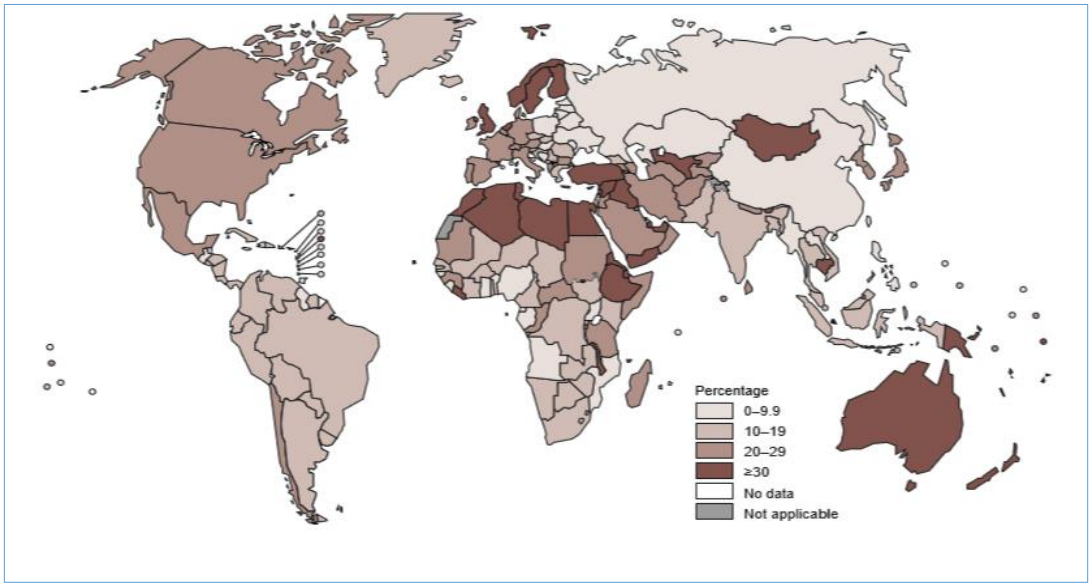

Figure 1.9 Percentage of extra-pulmonary cases among new and relapse TB cases, 2018 (WHO 2019)

Extrapulmonary tuberculosis results from hematogenous spread or direct extension from adjacent organs and may involve the larynx, lymph nodes, pleura, gastrointestinal tract,

genitourinary tract, central nervous system, or bones. Most extrapulmonary diseases are not contagious, with the exception of laryngeal tuberculosis. No evidence of tuberculosis may be seen on chest radiographs. Immunocompromised individuals and young children are at higher risk of extrapulmonary disease (Nachiappan *et al.* 2017). A patient with both pulmonary and EPTB is classified as a case of PTB. For example, miliary TB is classified as PTB because there are lesions in the lungs. On the other hand, tuberculous intrathoracic lymphadenitis (mediastinal and/or hilar) or tuberculous pleural effusion, without radiographic abnormalities in the lungs, constitutes a case of EPTB (Lee 2015).

Risk factors involved in the development of EPTB are mainly age, female gender, concurrent HIV infection and comorbidities such as chronic renal disease, diabetes mellitus or immunosuppression. The mean age of EPTB patients is higher than for pulmonary TB (Golden and Vikram 2005). Genomic analyses of samples from lung and extrapulmonary biopsies of HIV-co-infected patients have demonstrated that the dissemination of MTB from the lungs to extrapulmonary sites may occur as frequently as between lung sites (Qian *et al.* 2018).

The most common form of extrapulmonary tuberculosis is tuberculous lymphadenopathy, and its diagnosis remains a challenge since granulomatous lymphadenopathy has an extensive differential diagnosis. Several conditions, including sarcoidosis, fungal infections, and other inflammatory conditions, can present the same cytology and/or histopathology as tuberculous lymphadenopathy (Chakravorty, Sen, and Tyagi 2005).

Diagnosing EPTB remains challenging because clinical samples obtained from relatively inaccessible sites may be paucibacillary (TB disease caused by a small number of bacteria), decreasing the sensitivity of diagnostic tests. Since the limitations of the conventional smear microscopy is that it has a low sensitivity with a range of 0%–40%, negative results cannot exclude the presence of TB . The reported yields of mycobacterial culture vary from 30% up to 80%, but it usually takes 2–8 weeks to receive the results, which is too slow to help treatment decisions,.(Lee 2015; Kohli *et al.* 2018).

Pulmonary and extrapulmonary disease should be treated with the same regimens. Note that some experts recommend 9–12 months of treatment for TB meningitis given the serious risk of disability and mortality, and 9 months of treatment for TB of bones or joints because of the difficulties of assessing treatment response. Unless drug resistance is suspected, adjuvant corticosteroid treatment is recommended for TB meningitis and pericarditis. In tuberculous meningitis, Ethambutol should be replaced by streptomycin. Although

sometimes required for diagnosis, surgery plays little role in the treatment of extrapulmonary TB. Ethambutol is reserved for management of late complications of disease such as hydrocephalus, obstructive uropathy, constrictive pericarditis and neurological involvement from Pott's disease (spinal TB). For large, fluctuant lymph nodes that appear to be about to drain spontaneously, aspiration or incision and drainage appear beneficial (WHO 2010).

1.5 Transmission of TB as an infectious disease

The transmission of any infectious disease requires a source, a susceptible new potential host, and passage of the pathogen, by direct, indirect contact (eg, by fomites or an environmental medium, such as water), or via the air. Tuberculosis is the archetype of airborne-transmitted infectious diseases, with *Mycobacterium tuberculosis* serving as the causative agent. For human tuberculosis, the source of the majority of new infections is other humans with pulmonary disease (Turner *et al.* 2017).

As shown on Figure 1.10, a simple cascade for tuberculosis transmission is proposed in which a source case of tuberculosis generates infectious particles that survive in the air and are inhaled by a susceptible individual who may become infected and who then has the potential to develop tuberculosis. Interventions that target bacterial, host, or behavioral catalysts of transmission will interrupt tuberculosis transmission and accelerate the decline in tuberculosis incidence and mortality (Churchyard *et al.* 2017).

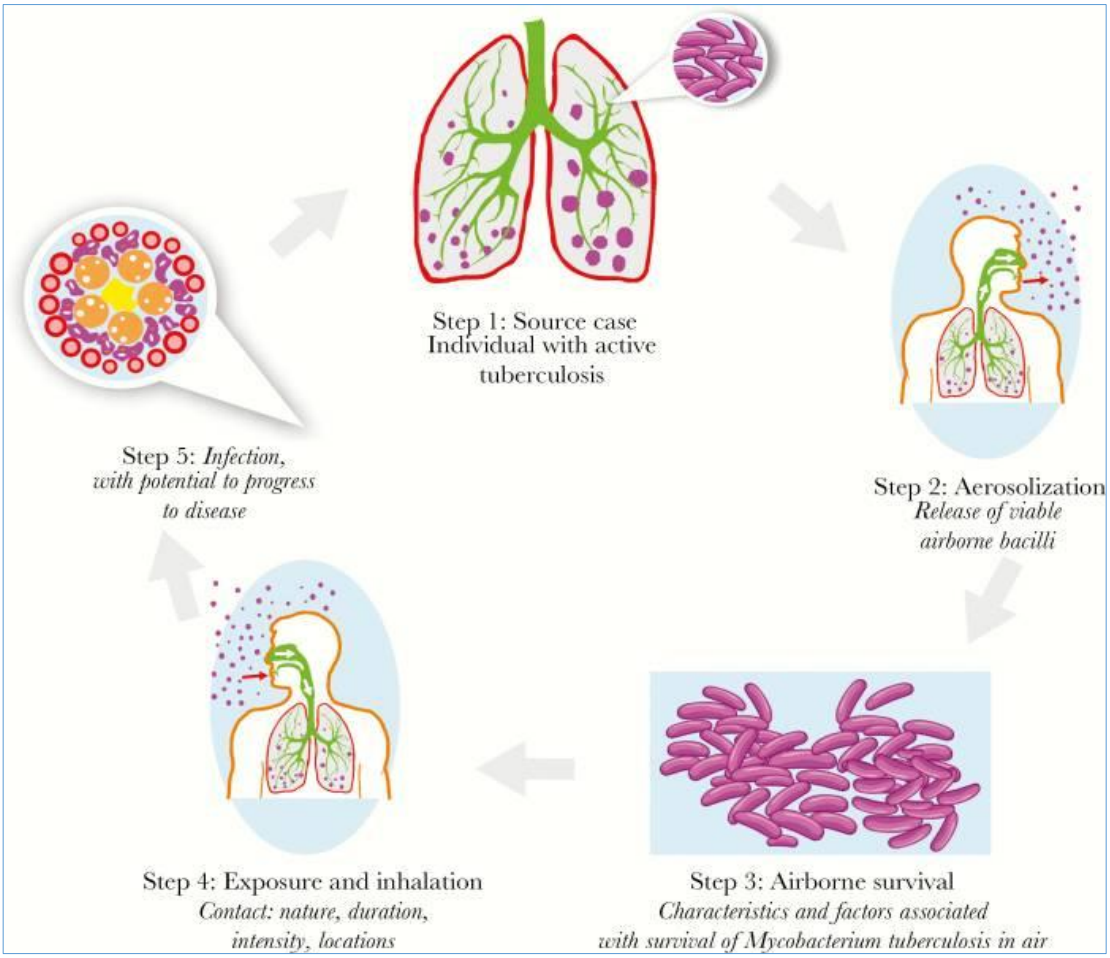

Figure 1.10 Cascade of tuberculosis transmission (Churchyard *et al.* 2017)

Four main approaches are used to measure tuberculosis transmission and identify its drivers. The first approach is case notification rates, which are used to identify countries and regions in which the risk of tuberculosis is elevated. Second, one can estimate the risk of being infected and identify risk factors for being infected. A third approach, assessing the genotypic links between presumed index tuberculosis cases and their presumed secondary cases. A fourth and indirect approach has been the characterization of source cases. Traditionally, measures of bacillary load, based on sputum smear and the presence and extent of pulmonary cavitation, have been used to estimate the infectiousness of source cases as a proxy for the expected number of infected contacts and secondary cases (Mathema *et al.* 2017).

The reproductive number and serial interval (SI) are two key quantities in describing transmission of an infectious disease (Ma *et al.* 2018).

The reproductive number is defined as the average number of secondary cases a primary infectious case will produce. A reproduction number may be calculated at any time during an outbreak, a value larger than 1 corresponding to epidemic spread of the disease. (Boëlle *et al.* 2011). Depending on the setting, the reproductive number can be expressed as a function of parameters such as infection rate, contact rate, recovery rate, making it useful in determining whether or not a disease can spread through a population (Ma *et al.* 2018).

The serial interval (SI), defined as the time between disease symptom onset of a case and that of its infector. The SI is an important quantity in the interpretation of infectious disease surveillance data, in the identification of outbreaks, and in the optimization of quarantine and contact tracing. These two quantities have been used to inform control policies during outbreaks by quantifying the transmission of infectious diseases such as influenza A (H1N1), Severe Acute Respiratory Syndrome (SARS), and Ebola, where progression to disease upon transmission occurs quickly (Ma *et al.* 2018).

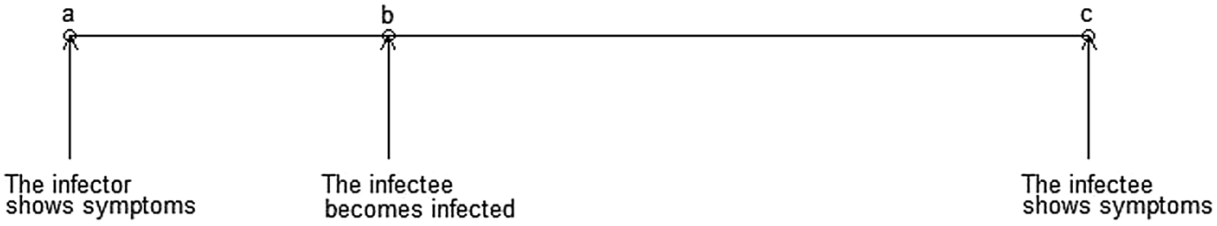

Figure 1.11 Important infectious disease intervals. The time between 'a' and 'c' is the serial interval; the time between 'b' and 'c' is the incubation period (Ma *et al.* 2018)

The early diagnosis and treatment of pulmonary TB help minimize TB transmission and are important strategy for decreasing the global TB burden (Belknap 2019), Persons who spend a lot of time in enclosed spaces with people who have infectious TB disease are the most likely to be infected with MTB. These persons, or contacts, may include family members, friends, roommates, or coworkers. The best strategy to stop transmission is to isolate infectious persons and undertake the standard TB treatment as soon as possible. The length of time required for a TB patient to become noninfectious after undergoing TB therapy varies and cannot be determined with certainty. However, once the standard TB therapy is started, and as long as the patient follows the prescribed treatment regimen, the infectiousness of the TB patient can rapidly decline (CDC 2019).

1.6 Treatment of tuberculosis

The four historical therapeutic approaches to TB started more than 100 years ago. The earliest approach included fresh air, rest, sunshine, nutrition, and isolation at sanatoria. It is of some interest that cures were achieved in approximately 50% of patients with rest therapy alone. Next was surgical treatment (collapse techniques, thoracotomy and resection surgery), and finally, the emergence of drug therapy. A fourth stage has now evolved: combined medical and adjuvant surgery, especially for drug-resistant cases. Specific TB drug treatment commenced with streptomycin in 1945, para-amino salicylic acid in 1949, Isoniazid in 1952, Ethambutol in 1961, and Rifampicin in 1996 (Figure 1.12). The early trials of chemotherapeutic agents clearly demonstrated the development of spontaneous drug resistance. As a consequence, the use of multiple drugs to which the organism is sensitive became the cardinal principle in achieving effective chemotherapy. The main causes of treatment failure include: omission of or erratic ingestion of prescribed medications, use of monotherapy or suboptimal dosing, treatment of organisms that have already developed drug resistance, and premature termination of therapy (Dewan and Pezzella 2016). Treatment of tuberculosis is focused on both curing the individual patient and minimizing the transmission of MTB to further persons, thus, successful treatment of tuberculosis has benefits both for the individual patient and the community in which the patient resides. The objectives of TB therapy are to rapidly reduce the number of actively growing bacilli in the patient, thereby decreasing severity of the disease, preventing death and halting transmission (Nahid *et al.* 2016).

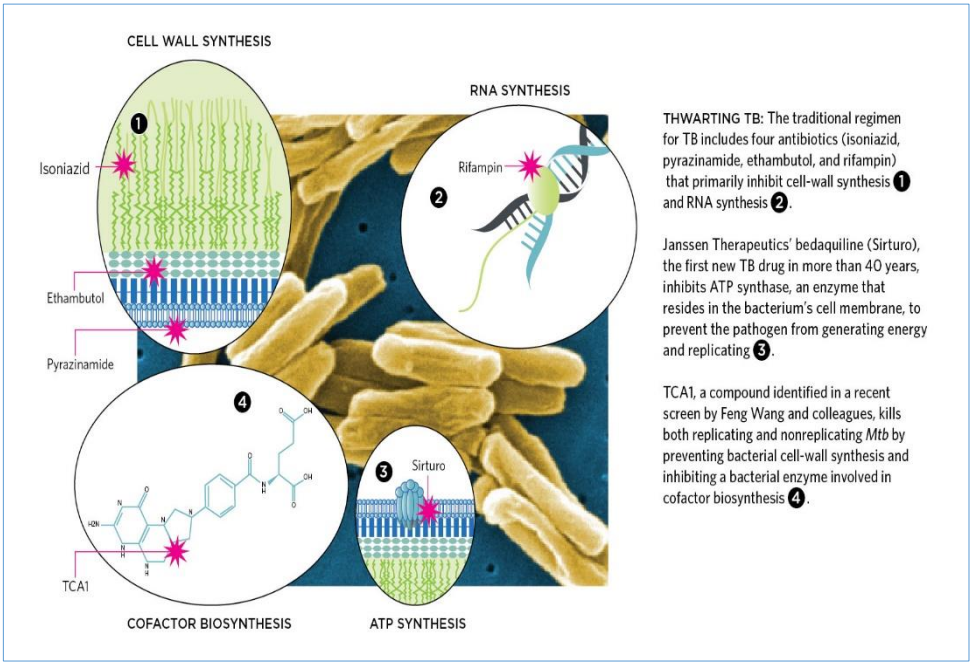

Figure 1.12 MTB with its particular cell wall, and the more used antibiotics against this bacterium (Scudellari 2013)

Improving first line treatment for drug-sensitive disease is a high priority and this is one area in which there have been significant research-led advances in recent years. Standard "short-course" chemotherapy was established in the 1980s and has changed little since then. This therapy comprises six months' treatment with a combination of drugs, each of which may cause side-effects. Adherence to long courses of treatment is challenging for several patients and poor adherence promotes the development of drug resistance. Identifying curative first line treatment regimens that are shorter in duration – preferably two months or less – is an important objective for decreasing the failure rate of treatment in a programme setting, and for reducing the pressure for development of drug-resistant TB (Paton 2018).

Treatments for patients with drug-susceptible tuberculosis last at least six months, the WHO recommends treatment for drug-susceptible tuberculosis with an initial 2-month intensive phase (isoniazid, rifampicin, pyrazinamide, and ethambutol daily) (Tiberi *et al.* 2018). With this combination, INH displays the best early bactericidal activity, whereas RIF is necessary to cure TB in the shortest time. PZA is effective against minimally active organisms and inclusion in the first two months facilitates cure with a 6-month course. EMB is the least active in this combination but is protective against acquired resistance when treatment is started without knowing the susceptibilities. This combination of medications is given for two months in the initial phase of TB treatment. With fully susceptible TB, EMB is not needed and the initial two months of treatment can be with INH, RIF, and PZA alone (Belknap 2019).

The continuation phase for drug-susceptible TB is INH and RIF. The usual duration is four months to complete six months of total treatment. There is no test to determine when a person has been cured, so the goal of treatment is to achieve a low risk for relapse (generally <5%). Factors known to be associated with higher relapse rates are cavitation, positive sputum culture after two months of treatment, and being underweight at the start and failing to gain weight during treatment (Table 1.8) (Belknap 2019). Pyridoxine (vitamin B6) is provided with INH to all persons at risk of neuropathy (eg, pregnant women; breastfeeding infants; persons infected with human immunodeficiency virus [HIV]; patients with diabetes, alcoholism, malnutrition, or chronic renal failure; or those who are of advanced age) (Nahid *et al.* 2016).

Table 1.8 Centers for Disease Control and Prevention guidelines as per WHO recommendations on basic tuberculosis treatment regimens (Dewan and Pezzella 2016)

Preferred regimen	**Alternative regimen**	**Alternative regimen**
Initial phase: Daily INH, RIF, PZA, and EMB for 56 doses (8 weeks)	Initial phase: Daily INH, RIF, PZA, and EMB for 14 doses (2 weeks), then twice weekly for 12 doses (6 weeks)	Initial phase: Thrice-weekly INH, RIF, PZA, and EMB for 24 doses (8 weeks
Continuation phase: Daily INH and RIF for 126 doses (18 weeks) or twice-weekly INH+RIF for 36 doses (18 weeks)	Continuation phase: Twice-weekly INH and RIF for 36 doses (18 weeks)	Continuation phase: Thrice-weekly INH and RIF for 54 doses (18 weeks)

Patients are usually seen monthly at in-clinic visits to monitor their response to treatment and assess for drug-related toxicity. Visual acuity and color vision should be checked for patients taking EMB. Sputum cultures should be collected each month until there are two consecutive specimens that are negative. Nucleic acid amplification tests are not recommended at this time for monitoring response to treatment or suspected failure or relapse. These tests may detect TB DNA for months or even years after effective treatment in patients who are otherwise clinically well and have negative cultures. Laboratory monitoring for toxicity is not routinely needed. Liver function tests should be checked at least monthly in patients with known liver disease or baseline abnormalities. They should also be checked immediately in anyone who develops nausea, vomiting, abdominal pain, loss of appetite, or jaundice. Further laboratory tests should be performed as required based on symptoms or further medical conditions (Belknap 2019).

Treatment success in programmatic conditions is approximately 85%. Apart from the efficacy and economic value, the regimen is lengthy, hepatotoxic, and not well tolerated by a substantial proportion of patients prescribed the medication. 4-month standard regimens are, so far, only recommended by the American Thoracic Society for patients who are sputum smear and culture negative with minimal pulmonary disease. Studies investigating the optimization of the use of approved drugs with improved formulations and pill counts are also ongoing. More palatable fixed-dose combination tablets are now available for pediatric use, simplifying dosing in children weighing less than 25 kg, while improving drug delivery and drug adherence. Efforts are also being made to render the standard quadruple regimens less toxic. One study showed that liver toxicity was reduced when methionine and vitamin B complex were added to the standard regimen (Tiberi *et al.* 2018).

Directly observed therapy' (DOT) (the practice of observing the patient swallow their

antituberculosis medications) is one component of a wider WHO strategy called 'Directly Observed Therapy Short course'. This strategy incorporates wide ranging health system improvements, political commitment to improving TB programmes, improved TB laboratory services, free TB drugs for all TB patients, and accurate documentation and monitoring of TB diagnosis and treatment outcomes. The DOT component is an attempt to improve adherence by active monitoring and recording of the consumption of each and every drug dose by an 'observer' acceptable to the patient and the health system. Decisions regarding the use of DOT must be made in concert with the patient. For example, DOT can be provided in the office, clinic, or in the "field" (patient's home, place of employment, school, or any other site that is mutually agreeable) by appropriately trained personnel (Karumbi and Garner 2015; Nahid *et al.* 2016). This is now considered a core component of TB programmes by the WHO to ensure cure and prevent the emergence of drug resistance. Proponents of DOT argue that the close monitoring has a social effect and acts as a peer pressure which leads to behavior change towards improved adherence and it has strong proponents. However, to opponents it has been seen as a coercive model which leaves the patient as a passive recipient of therapy thereby eroding the gains made in involving patients in management of their own health (Karumbi and Garner 2015).

Ensuring adherence to treatment is important to minimize the risk of failure, acquired drug resistance, and TB transmission. DOT maximizes treatment completion, allows close monitoring for drug-related side effects, and is the standard of care for patients with pulmonary TB (Belknap 2019). To reduce treatment monitoring costs and allow greater patient autonomy, mobile technology is increasingly used to improve patient care and treatment outcomes. Most mobile solutions involve patient reminders, and few actually document medication ingestion. A flexible, low burden method of providing remote DOT via smartphones called Video DOT has been developed, which involves patients video-recording themselves taking their medications and transferring the videos using a secure interface to DOT workers for review (Garfein *et al.* 2015). Studies evaluating video DOT, either real-time or recorded, have found that adherence is comparable to in person DOT with high levels of patient satisfaction (Belknap 2019).

Chapter 2

Drug resistance and Multidrug resistant tuberculosis

Chapter 2

Drug resistance and Multidrug resistant tuberculosis

2.1 Drug resistance

Drug resistance is a biological phenomenon that has been observed in Mycobacterium tuberculosis since the discovery of the first anti-TB drug, streptomycin. Many patients who were injected with streptomycin were brought from the brink of death and their sputum became temporarily clear of M. tuberculosis. But despite continuing to receive treatment, they soon began to excrete bacilli that were resistant to streptomycin in the laboratory. With the advent of new drugs—thioacetazone (also named Amithiozone is a thiosemicarbazone antimycobacterial agent with activity against isoniazid-resistant strains of Mycobacterium tuberculosis (Bethesda 2004)) and para-aminosalicylic acid (a second-line anti-TB drug) in 1948 and isoniazid in 1952—it became clear that combination chemotherapy was the key to preventing the development of resistance. The invention of rifampicin in 1957, the most powerfully sterilizing anti-TB drug, paved the way for development of the shorter and more effective isoniazid- and rifampicin-containing regimens known as short-course chemotherapy (Seung, Keshavjee, and Rich 2015).

Drug-resistant TB (DR-TB) reached alarming levels with the emergence of strains that are virtually untreatable with the existing drugs and is a serious determinant of treatment success. Therefore, to advance a treatment achievement, the Mycobacterium tuberculosis drug susceptibility patterns in community-based care should be determined. DR-TB, particularly multidrug-resistant TB and extensive drug-resistant-TB (XDR), have become the most important public health problem in many countries (Feyisa *et al.* 2019).

The first obstacle in anti-TB therapy is MTB's sturdy cell wall, which is difficult for antimicrobial agents to penetrate. It has an unusual structure, composed of three layers covered with mycolic acid and cell-adhesion molecules on the outer surface. In MTB, this complex structure naturally serves as an efficient barrier to several antimicrobial agents and chemical molecules, inhibiting the entry of anti-TB drugs into the organism's cytoplasm (Figure 2.1) (Gokulan and Varughese 2019).

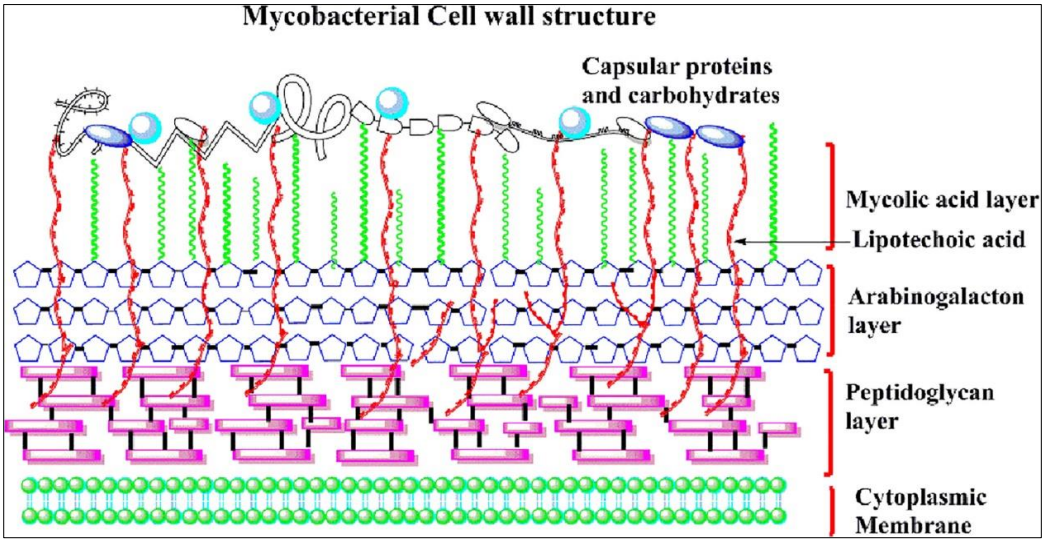

Figure 2.1: The composition and structure of the bacterial cell wall: The cell wall is made up of three layers: Peptidoglycan layer, arabinogalacton, and mycolic acid layers. The mycolic acid layer is covered with several proteins and carbohydrates. In addition, the PG layer also contains Lipotechoic acid and techoic acid (Gokulan and Varughese 2019).

Second, the efflux pump also plays an important role in shunting antimicrobial agents out of the cytoplasm (Gokulan and Varughese 2019). Efflux pumps exhibit high levels of substrate promiscuity and are able to extrude a multitude of structurally unrelated compounds. Furthermore, efflux systems have been shown to be essential in M. tuberculosis for intracellular growth in macrophages. Mycobacterial efflux pumps are able to extrude nearly all antituberculous drugs, including streptomycin, rifampicin, isoniazid, clofazimine, bedaquiline, fluoroquinolones and ethambutol (Gygli *et al.* 2017). In addition, inside the host system, to overcome antibiotic pressure, environmental stress, and immune assaults, bacteria often mutate their genomic DNA (Gokulan and Varughese 2019).

For example, isoniazid is a pro-drug, which is activated by the catalase peroxidase KatG. The activated INH forms chemical entities with $NAD(P)^+$ coenzymes which inhibit the target protein InhA, an enoyl-acyl carrier protein, which takes part in mycolic acid synthesis. Mutations in the katG gene confer high-level INH resistance (Kurz, Furin, and Bark 2016).

Another possible mechanism for resistance is the existence of β-lactamase enzyme in mycobacterium. Most of the β-lactam antibiotics bind and acylate active site of penicillin-binding protein, preventing bacterial growth by inhibiting cell-wall biosynthesis. consequently leading to cell lysis and death (Gokulan and Varughese 2019; Zeng and Lin 2013). However, mycobacterial β-lactamase enzyme inactivates β-lactam antibiotics by cleaving the lactam ring before reaching its target. Furthermore, mycobacteria also

remodels their cell walls by generating non classical linkage on peptidoglycan layer while entering into dormant state, making it harder for antibiotics to penetrate. Peptidoglycan (PG) modification has been correlated to bacterial persistence and the emergence of the drug resistance mechanism. Bacterial cell-wall enzymes are considered to be attractive candidates for drug discovery based on the structure's functional contribution toward drug resistance (Gokulan and Varughese 2019).

2.2 Multidrug resistant tuberculosis

In 2018, 78% of the half million new cases of rifampicin-resistant TB had multidrug resistant TB (WHO 2019). MDR-TB appeared after the introduction of Rifampicin in 1966 (SB 2010). The proportions of new and previously treated TB cases with MDR/RR-TB at the country level are shown in (Figure 2.2) (WHO 2019).

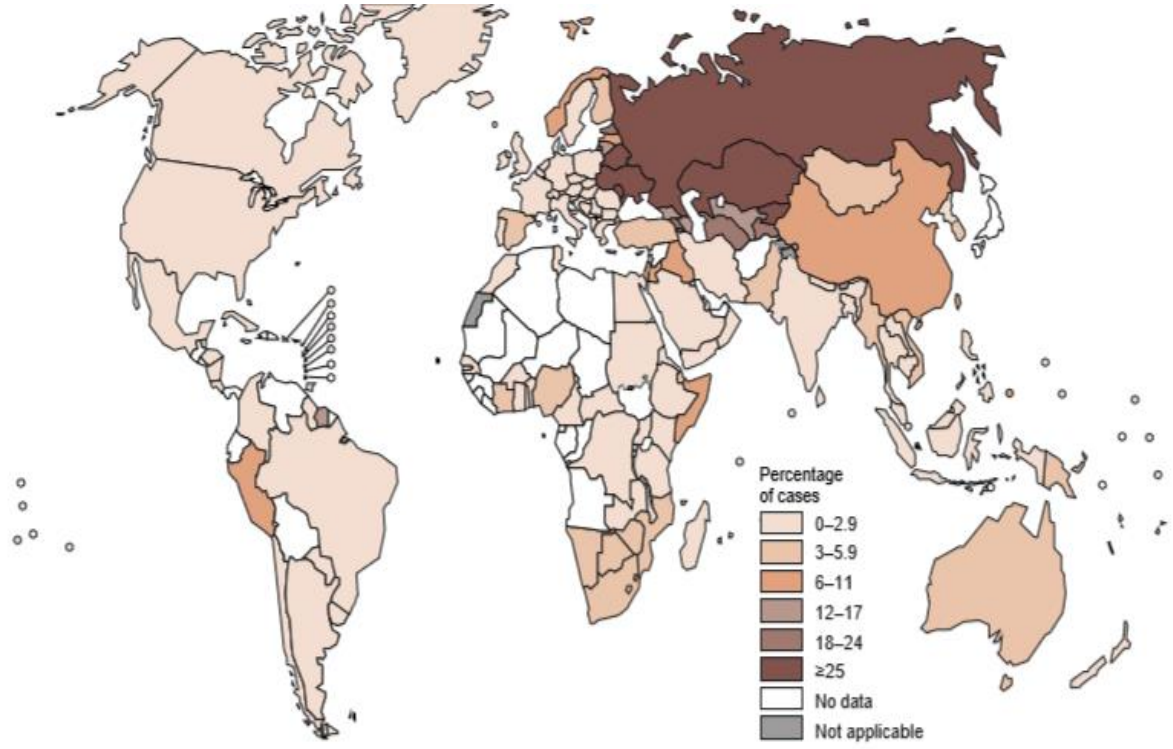

Figure 2.2: Percentage of new TB cases with MDR/RR-TB: Percentages are based on the most recent data point for countries with representative data from 2004 to 2019. Model-based estimates for countries with data before 2004 are not shown. MDR-TB is a subset of RR-TB (WHO 2019).

M.tuberculosis resistant to isoniazid but not rifampicin is called isoniazid-monoresistant, whereas M tuberculosis resistant to both rifampicin and isoniazid is called multidrug-resistant. *M.tuberculosis* resistant to rifampicin but susceptible to isoniazid, or with unknown susceptibility to isoniazid, is called rifampicin monoresistant. However, because most rifampicin monoresistant tuberculosis with unknown susceptibility to isoniazid is resistant to that drug, rifampicin-monoresistant tuberculosis is routinely treated as multidrug-resistant tuberculosis (Lange *et al.* 2019).

It is known that resistance to INH and RMP is a key factor in determining the effectiveness of the currently recommended standard treatment regimens. The elucidation of the mechanism of action of these drugs, which was accomplished only recently, has led to the development of new rapid diagnostic methods (Sajduda *et al.* 2004), The development of resistance to these two drugs, means that the efficacy of standard anti-TB treatment is reduced by up to 77% (Viedma *et al.* 2002).

Rifampicin is a major compound of anti-tuberculosis chemotherapy. As resistance to rifampicin is rarely found without associated resistance to other tuberculostatics, rifampicin resistance is a good marker for MDR-TB. Moreover, rifampicin resistance is a good predictor of poor treatment outcome (Morcillo *et al.* 2002), More than 90% of RIF-resistant TB-causing isolates are also resistant to INH (Heep *et al.* 2001).

Resistance to anti-TB drugs occurs during selective multiplication of drug-resistant mycobacteria which spontaneously emerge. These resistant mutants then are able to flourish and replace the wild-type strains when therapy is inadequate due to either a suboptimal number of medications or low serum drug levels (Seaworth and Griffith 2017). MDR strains carry multiple mutations in different resistance-related genes, and each mutation results from an independent mutational event. An MDR strain is thus the product of a multistep process, in which a progressive accumulation of genetic alterations occurs and results in the selection of a viable and fit bacterium (Meacci *et al.* 2005).

2.2.1 Pathogenesis

Tuberculosis resistance arises spontaneously but at a low and predictable de-novo rate, and not by horizontal gene transfer In view of the large bacterial burdens of up to 10^9 colony-forming units per patient and bacterial replication, pre-existing M tuberculosis subpopulations resistant to one drug might be expected occur in some patients, although the probability of pre-existing drug resistance to two or three drugs (as calculated by multiplication of mutation rates) is infinitesimally small. In the classic understanding of emergence of drug resistance with monotherapy, initial therapy kills off most of the

susceptible bacterial subpopulation, but allows the pre-existing drug resistant subpopulation to continue to replicate, eventually replacing the drug-susceptible population (Figure 2.3) (P. K. Dheda *et al.* 2014).

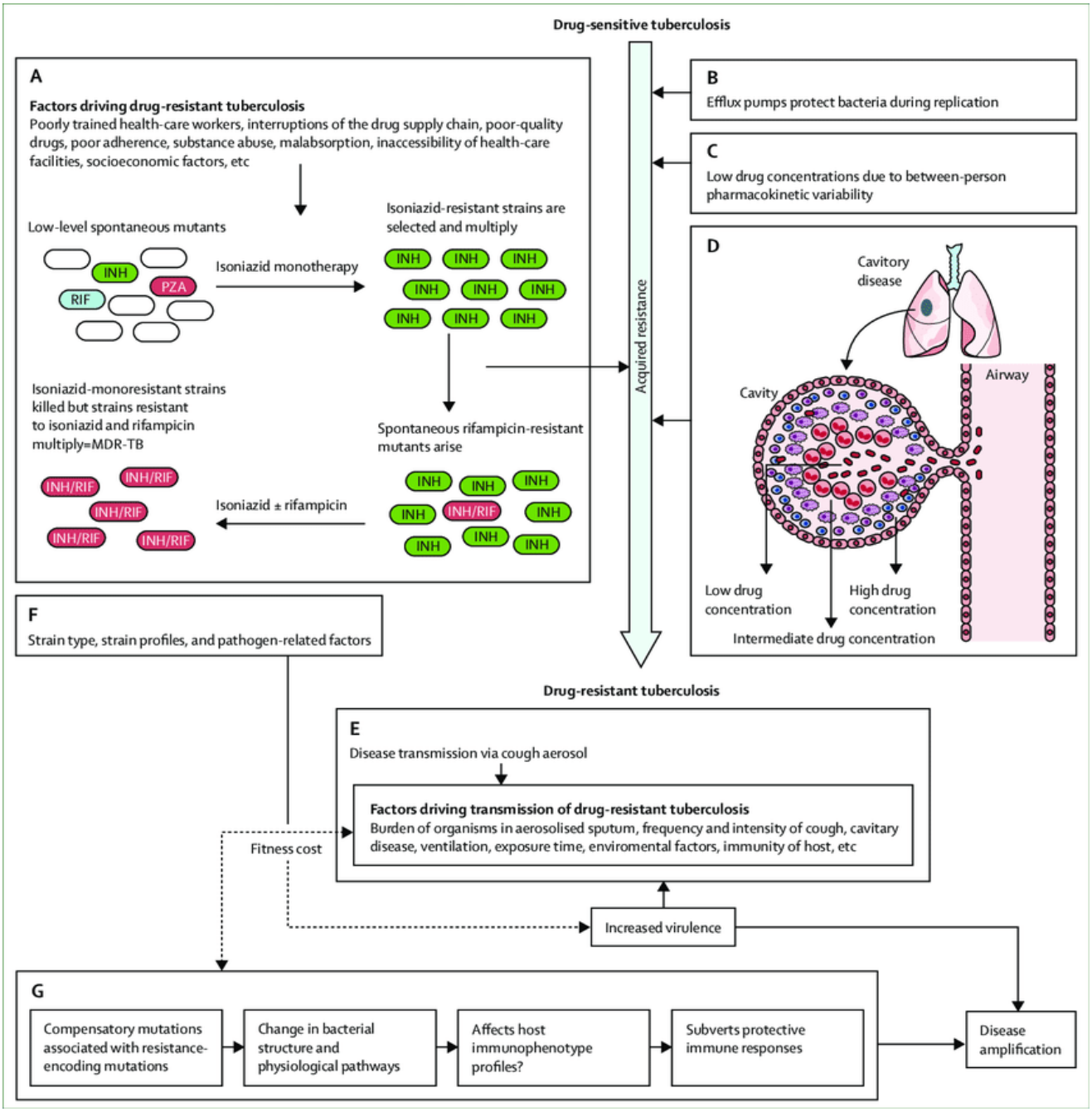

Figure 2.3 The pathogenesis of drug-resistant tuberculosis (Dheda *et al.* 2014)

The traditional interpretation of resistance development is that sequential drug resistance develops through fragmented treatment (A), which can be fuelled by several programmatic and socioeconomic factors. However, resistance can develop despite excellent adherence.

Several factors, including efflux pumps (B), between-person pharmacokinetic variability (C), and extensive immunopathology in the lung resulting in differential drug penetration into granulomas and cavities (D) might all drive site-specific drug concentrations below minimum inhibitory concentrations, thus probably enabling drug resistance. After acquired drug resistance develops, person-to-person transmission might constitute the major route of spread (E). Strain-specific genotype, newly acquired drug-encoding mutations, and compensatory mutations that can affect fitness cost (and hence transmission) might also interact (F). Compensatory mutations could be associated with changes in structure and physiological pathways, which could affect host immune response and thereby potentially subvert protective responses and drive progressive disease (G) (Dheda *et al.* 2014).

2.3 Diagnosis of mumtidrug reistant tuberculosis

The diagnosis of multidrug resistant tuberculosis is based on the detection of resistance to rifampicin and isoniazid in the causative organism in a clinical sample, usually sputum. The quality and quantity of the sputum sample can affect test performance. An increasing array of new tools for the diagnosis of multidrug resistance has become available in recent years, for the detection of either phenotypic (growth based) or genotypic (molecular markers) resistance. The use of specific tests in different regions is heterogeneous and most regions will use several different tests in combination (Millard, Ugarte-gil, and Moore 2015).

All patients with tuberculosis should undergo drug susceptibility testing (DST) because only testing those perceived to be at risk will miss up to half of multidrug resistant cases (Millard, Ugarte-gil, and Moore 2015). For MDR-TB, rapid molecular diagnostic tests such as the Xpert MTB/RIF, a cartridge-based automated molecular assay which was endorsed by WHO in 2016 as an initial test for TB and detection of RIF resistance, has significantly increased case detection and improved RIF resistance identification from sputum. It is a polymerase chain reaction (PCR) test which requires limited technical expertise and is able to produce a result within 2 hours (Park *et al.* 2019). Recently, a new version of the test, Xpert MTB/RIF Ultra, has been introduced to the market. The Xpert MTB/RIF Ultra has higher sensitivity than Xpert MTB/RIF in patients with paucibacillary disease and in patients with HIV, though at the expense of a decrease in specificity. In a preliminary study using cerebrospinal fluid samples, Xpert MTB/RIF Ultra appeared to be more sensitive in those with suspected TB meningitis (Lange *et al.* 2018). The conventional smear microscopy results can take up to 2 days and have a variable sensitivity from 32–94%. Conventional culture methods can take up to 8 weeks for the final report. First-line drug susceptibility testing takes a further 2–3 weeks, but if there is evidence of resistance then a

second-line DST is undertaken which takes a further 2–3 weeks, introducing more delay before a full DST profile is available for resistance cases and meaning a possible delay in appropriate treatment of MDR-TB (Park *et al.* 2019).

Phenotypic direct drug susceptibility testing delivers a result direct from inoculation of the sputum sample thus does not need to be sub-cultured. Samples are inoculated into media containing drugs at specific critical concentrations such that the presence of growth (whether detected by a color change or visual identification of microscopic growth) is indicative of phenotypic resistance. These methods are rapid, non-commercial, and low cost making them well suited to resource constrained settings with a high burden of tuberculosis (Millard, Ugarte-gil, and Moore 2015).

2.4 Treatment of multidrug resistant tuberculosis

Treatment of MDR-TB is challenging because it requires the administration of at least five anti-TB drugs, many associated with significant adverse reactions, for at least 9 to 20 months. Selection of drugs to make up the regimen should be based on the past treatment history, known patterns of resistance, and DST data, when available. At least two drugs should have good bactericidal activity, and one to two drugs should have good sterilizing activity, to assure cure and avoid relapse. The WHO updated its guidelines for the programmatic management of drug-resistant TB in 2016, with additional updates related to new drugs including Bedaquiline and Delamanid, as well as shorter course regimens (Daley and Caminero 2018), (Figure 2.4) illustrates historical timelines of discovery of tuberculosis drugs.

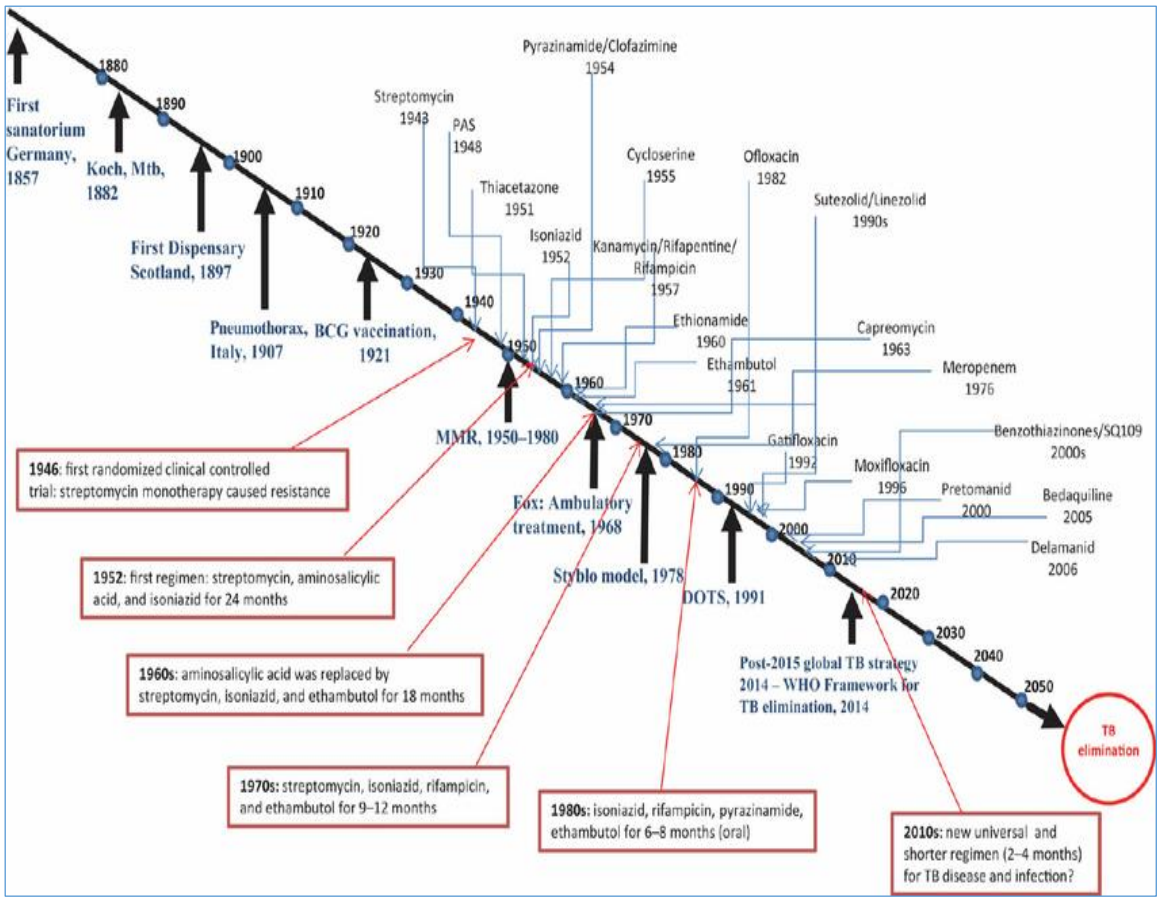

Figure 2.4 Historical timelines of discovery of tuberculosis drugs and introduction of tuberculosis treatment regimens used at programmatic level.(MMR; mass miniature radiograph, PAS; para-aminosalicylic acid) (Schito *et al.* 2015)

Bedaquiline is a diarylquinoline which inhibits bacterial ATP synthase, and acts on drug-sensitive and resistant M tuberculosis , killing both replicating and non-replicating bacilli. Delamanid is the first treatment in the novel nitro-dihydroimidazooxazole class that inhibits mycolic acid synthesis (Park *et al.* 2019).

Treatment regimens for MDR-TB are complex. They rely on drugs with reduced efficacy and increased toxicity (Kurz, Furin, and Bark 2016), none of the five anti-TB drugs administrated are as potent as rifampicin or isoniazid and all of which are more toxic and less well tolerated. Countries with access to quality assured drug susceptibility testing for second line drugs (Figure 2.5) are likely to offer an "individualized" regimen tailored to a patient's resistance pattern. Countries without this facility are likely to offer a "standardized" regimen, which is broadly similar for all patients and based on historical resistance patterns and which drugs have

been used in that region. Even in the context of individualized regimens, initial treatment is often empirical (and based on similar principles to standardized treatment), pending second line drug susceptibility testing (Millard, Ugarte-gil, and Moore 2015).

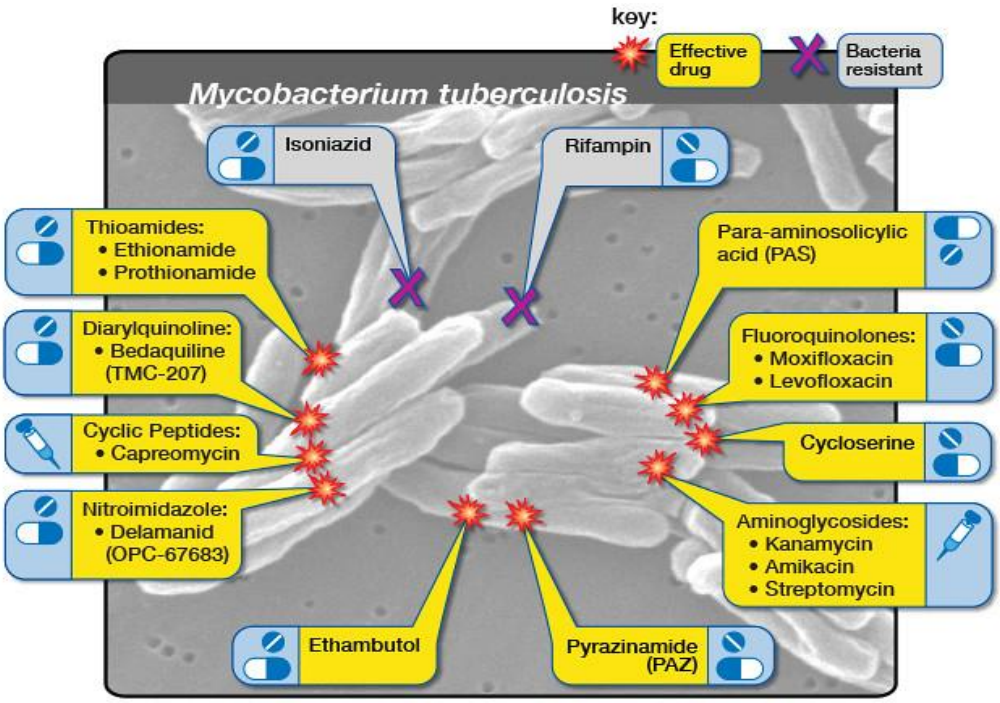

Figure 2.5: Multidrug-Resistant Tuberculosis (MDR TB) and Second-Line Treatments (NIAD 2016)

The WHO regrouped the second-line drugs (SLDs) based on current evidence on their effectiveness and safety. In the new scheme, drugs are divided into groups A to D, with subgroups D-1, D-2, and D-3. Drugs in groups A to C are listed in order of general preference (Park *et al.* 2019; Seaworth and Griffith 2017). Group A includes the later generation fluoroquinolones, group B includes injectables, group C includes other core second-line drugs, and group D comprises add-on drugs that are used in drug resistance or intolerance. Delamanid or bedaquiline can be added if there is quinolone or injectable resistance. Para-aminosalicylic acid and carbapenems with clavulanate are also reserve drugs (Table 2.1) (Park *et al.* 2019).

Table 2.1 Anti-mycobacterial drugs recommended for the treatment of MDR/RR-TB (Daley and Caminero 2018).

Category		Type	Drugs
A		Fluoroquinolones.	• Levofloxacin. • Moxifloxacin. • Gatifloxacin.
B		Second-line injectable.	• Amikacin. • Capreomycin. • Kanamycin. • Streptomycina.
C		Second-line agents (in order of decreasing preference).	• Ethionamide/prothionamide. • Cycloserine/terizidone. • Linezolidb. • Clofazimine.
D	D-1	Add-on agents.	• Pyrazinamide (PZA). • Ethambutol. • High-dose INH.
	D-2	Add-on agents.	• Bedaquiline. • Delamanid.
	D-3	Add-on agents.	• p-Aminosalicylic Acid (PAS). • Imipenem/Meropenemc. • Amoxicillin/clavulanate (thioacetazone)

Two types of standardized multidrug-resistant tuberculosis treatment regimens (i.e., a long and a short treatment regimen) are recommended by WHO. They differ by drug combinations and treatment duration (Lange *et al.* 2019).

The longer conventional MDR-TB regimen should consist of at least five effective TB drugs during intensive phase (up to 8 months) including pyrazinamide, and four core second-line TB medications, one from group A, one from group B and at least two from group C. If the minimum of effective TB medicines cannot be composed, an agent from group D-2 and other agents from D-3 may be added to bring the total to five. It is also recommended that this regimen can be further strengthened by high-dose isoniazid and/or ethambutol (Park *et al.* 2019). This intensive phase has historically been characterized by the use of an aminoglycoside (amikacin or kanamycin) or a polypeptide (capreomycin) delivered parenterally . This approach has been intended to provide greater bactericidal activity during the time when the bacillary burden is highest and reducing the number of drugs in the continuation phase to reduce risk of toxicity and intolerability caused by the multidrug regimen at a phase when the microbial burden has diminished (Nahid *et al.* 2019).

At least three, preferably four, effective medications are recommended in the continuation

phase of therapy. This phase is extended 12 months past the initial phase for a total duration of therapy of 20 months in patients who have not had prior treatment with second-line drugs. Both, intensive and continuous guidelines support extension of treatment up to 24 to 26 months for those with poor response and/or previous failed MDR-TB treatment (Seaworth and Griffith 2017).

In the 2016 WHO guidelines, a 9- to 12-month shorter course regimen was recommended for the first time. The shorter course regimen can be used in HIV-infected persons with pulmonary disease, children and adults who have not previously been treated with second line drugs and in whom resistance to fluoroquinolones and second-line injectable agents has been excluded or is considered highly unlikely. It is not recommended during pregnancy and while breastfeeding or for extrapulmonary disease (Daley and Caminero 2018; Seaworth and Griffith 2017). If the patient does not meet this recommended criteria for shorter course regimen, then an individualized regimen should be used ideally with the addition of new or repurposed drugs such as bedaquiline, delamanid, and/or linezolid (Daley and Caminero 2018).

The WHO short-course regimen is standardized and divided into two phases. The initial intensive phase is 4 months (which can be extended to 6 months if sputum conversion is slow) and includes high-dose gatifloxacin (or moxifloxacin), kanamycin, ethionamide (or prothionamide), clofazimine, high-dose INH, PZA, and ethambutol. This is followed by the 5-month-long continuation phase of treatment, which includes highdose gatifloxacin (or moxifloxacin), clofazimine, PZA, and ethambutol (Seaworth and Griffith 2017).

2.5 Risk factors for multidrug resistant tuberculosis

The mechanism of drug resistance can be caused by genetic factors, factors related to previous treatment and other factors such as comorbidity with diabetes mellitus. Although there is some evidence which postulate host genetic predisposition is the basis for the development of MDR-TB, changes in the genomic content is the major underlying event in the emergence of variants strains in the M. tuberculosis complex (Rumende 2018).

Studies have been conducted to explore risk factors of developing MDR-TB worldwide, including cross-sectional studies, case–control studies, and others. Many studies have discovered that previous TB treatment, gender, age, alcohol abuse, poor past compliance to treatment, abandonment of treatment, and being registered as migrants were associated with developing MDR-TB (Zhang *et al.* 2016), the most powerful predictor for the presence of MDR-TB is a previous history of treatment of TB (Mulu *et al.* 2015).

Patients with previous TB treatment are difficult to manage and might be infectious for a longer period of time. 'Previous treatment' may mean a relapse after a successful treatment, a return after treatment discontinuation, a treatment failure, or any other types (other types include patients with an unknown previous history; with unknown outcome of that previous treatment; and/or who have returned to treatment with smear-negative pulmonary TB or bacteriologically negative extrapulmonary TB) (Rifat *et al.* 2015).

Two common comorbidities, HIV and diabetes mellitus, have been inconsistently associated with drug-resistant tuberculosis (K. Dheda *et al.*, 2019).

recently, along with the convergence of the diabetes mellitus (DM) and TB epidemics, the high prevalence of DM among MDR-TB patients is a serious cause for concern, with a range of 10–23% of MDR-TB patients having DM (Alvarez *et al.* 2015), The additional risk of DM for the development of MDR-TB, however, remains controversial. Many previous studies have found a 2.1 to 8.8 times increased risk of MDR-TB among TB patients co-morbid with diabetes. In addition, observational studies from Georgia and Mexico showed that TB patients with DM had a higher risk of developing MDR-TB. In contrast, several others reported that there is no increased risk of MDR-TB among TB patients who have DM. Similarly, none but one of the previously conducted systematic reviews and meta-analysis reported DM as an independent risk factor for MDR-TB (Tegegne *et al.* 2018).

People living with HIV are at a higher risk of developing MDR and XDR tuberculosis associated with increased mortality, and greatly reduced survival time. HIV and MDR-TB are equally balanced deadlier combinations. Even if the impact of HIV infection on MDR-TB is of great public health importance, the relationship between the two infections is not yet clearly understood. Findings from different studies on associations of HIV co-infection and drug resistance among patients with TB have been contradictory (discordant) (Mesfin *et al.* 2014).

Chapter 3

Methods

3.1 Search strategy

3.2 Criteria for considering studies for this review

3.3 Screen, identification and selection of publications based on the established criteria

3.4 Data collection

Chapter 3

Methods

3.1 Search strategy

This systematic review was conducted on associated factors for multidrug resistant tuberculosis. studies have been identified from PubMed database, using a combination of the following key terms “tuberculosis”, “risk factors”, “multidrug resistant tuberculosis” by applying medical subject headings (Mesh): ("Risk Factors"[Mesh]) AND "Tuberculosis, Multidrug-Resistant"[Mesh], this search was performed without using any filter or language restrictions.

3.2 Criteria for considering studies for this review

Experimental, cross-sectional, case control and cohort studies reporting associated risk factors for multidrug resistant tuberculosis in adult patients eighteen years and older with confirmed diagnosis of active pulmonary or extrapulmonary tuberculosis were included in this systematic review. Multidrug resistant tuberculosis is defined by resistance of *mycobacterium tuberculosis* against at least rifampicin and isoniazid (Lange *et al.* 2018). Descriptive studies, case-report, case-series and studies including patients with surgical or non-medical therapy, as well as studies reported in languages other than English and French were excluded. Studies reporting HIV co-infection alone as a high risk factor for multidrug resistant tuberculosis were as well excluded in order to ensure homogeneity in the studies included. Risk of bias, sample size or length of follow-up period were not considered as exclusion criteria.

3.3 Screen, identification and selection of publications based on the established criteria

Title/abstracts were reviewed in relation to the inclusion/exclusion criteria. Unless the abstract clearly described one or more exclusion criteria, the full text was examined to determine whether or not it met the inclusion criteria. Relevant titles/abstracts that met the inclusion criteria were selected then full texts were examined, articles have been removed if full text wasn’t available. For the relevant titles with missing abstracts, full texts were directly screened if available.

3.4 Data collection

Data from all included studies have been extracted and collected, data extracted included: region or country of study, study period, risk factors identified and study design.

Chapter 4

Results and discussion

4.1Results

4.2 Discussion

Chapter 4

Results and discussion

4.1 Results

PubMed search on July 17th, 2020 yielded 788 result, one duplicate have been removed after checking duplicates twice, first using MENDELEY REFERENCE MANAGER, then manually. From the 787 records, 647 were excluded after title/abstract reading, that's for not being relevant to the research question and not meeting the defined inclusion criteria, and seven articles were excluded for unavailability of full text, results are documented in a Flowchart (Figure 3.1). For the remaining 133 studies selected based on title/abstract relevance, full texts were assessed if they meet the eligibility criteria and 111 of them were excluded : for including patients under the age of eighteen (56), irrelevant content to the research question (12), reporting factors affecting MDR-TB treatment outcome (5), did not report risk factors for MDR-TB (8), reporting factors associated to MDR-TB mortality (2), the age of the included patients wasn't mentioned (4), reporting rates of anti-tuberculosis drug resistance (3), Reporting factors contributing to the prevalence of multidrug resistant tuberculosis (20), including patients with surgical therapy (1), excluded studies and reasons of exclusion are documented in (Table 3.1).

There were twenty two studies that met the inclusion criteria among which several factors were reported as significant risk factors associated to MDR-TB development. The majority of the studies demonstrated interruption and previous TB treatment as significant risk factor (18), two of them pointed out alcohol abuse, male sex (2), immigration (2), un-employment/occupation (3), contact with persons infected with drug-resistant strains (7), precarious living conditions (3), past smoking status (2), type 2 diabetes mellitus (2), HIV infection (2), Presence of a cavity on chest radiography (3). This factors, others and characteristics of the included studies are documented in (Table 3.2). Geographically, studies were mostly undertaken in the Asian region (9), Africa (7), Americas region (3) and Europe (2). In the Asian region, studies originated from Iran, China, India, Bangladesh, Indonesia, Korea, Malaysia and Thailand. In the African region, studies originated from Botswana, Sudan, Mali, Uganda, and three studies from Ethiopia. In the Americas region, studies originated from Mexico and two studies from Peru. In the European region, studies originated from France and Europe.

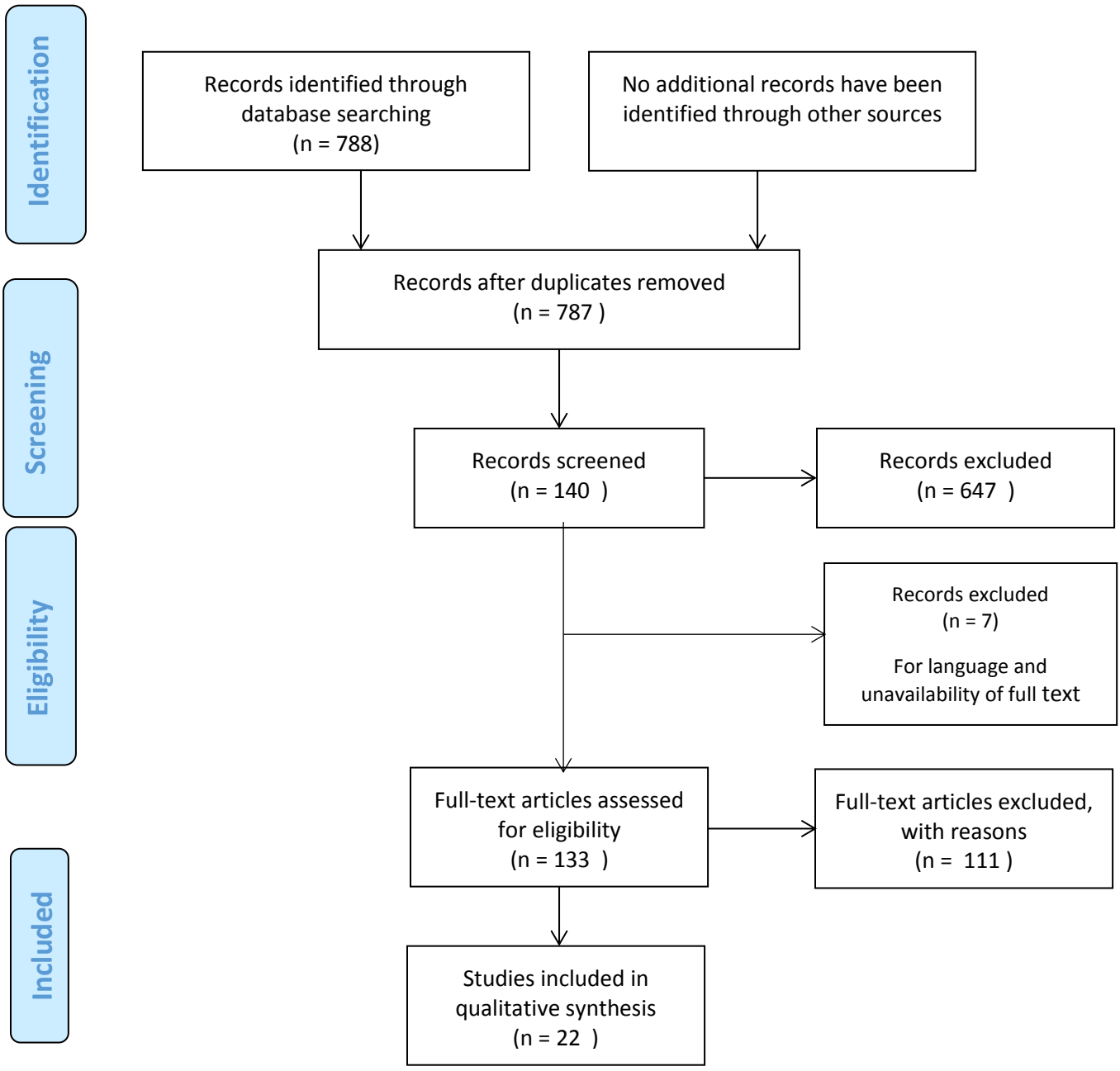

Figure 3.1: Flow Chart for Selection of Studies on associated risk factors for multidrug-resistant tuberculosis.

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

Table 3.1 Studies excluded from the systematic review and reasons of exclusion

Citation	Reasons of exclusion
(Casal *et al.* 2005) (Telles *et al.* 2005) (Marahatta *et al.* 2015) (Ricks *et al.* 2012) (Sharma *et al.* 2003) (Zhang *et al.* 2016) (Lu *et al.* 2019) (Kritski *et al.* 1997) (Bäcker *et al.* 2013) (Abate *et al.* 1998) (Girardi *et al.* 1996) (Mesfin *et al.* 2018) (Kritski *et al.* 1997) (Choi *et al.* 2007) (Shen *et al.* 2009) (Green *et al.* 2010) (Z. Liu, Shilkret, and Finelli 1998) (Panda *et al.* 2016) (Jacobs, Pelissari, and Pinto 2018) (Tessema *et al.* 2012) (Id *et al.* 2019) (H E Jenkins *et al.* 2014) (Island *et al.* 2019) (Z. Yang *et al.* 2018) (Gerais, n.d.) (Elmi *et al.* 2009) (Peng *et al.* 2017) (Skrahina *et al.* 2013) (Okethwangu *et al.* 2019) (Ullah *et al.* 2016) (Jitmuang, Munjit, and Foongladda 2015) (Thi *et al.* 2013) (Study, Zhang, and Wang 2016) (Q. Liu *et al.* 2013) (Ignatyeva *et al.* 2015) (Santo 2017) (Alrajhi and Abdulwahab 2001) (Clark *et al.* 2005) (A Cetin Tanrikulu *et al.* 2008) (Chen, Huai, *et al.* 2013) (Stosic *et al.* 2018) (Suárez-García *et al.* 2009) (Abdullah Cetin Tanrikulu, Abakay, and Abakay 2010) (Faso *et al.* 2009) (Law *et al.* 2008) (Alene *et al.* 2019) (Wahab *et al.* 2009) (Desissa, Workineh, and Beyene 2018) (Ms *et al.* 2013)	Study including patients aged under eighteen years old.

(Sb *et al.* 2010) (Tudó *et al.* 2004) (Zignol *et al.* 2012) (Massi *et al.* 2011) (Gupta *et al.* 2011) (Fourati *et al.* 2015) (Weltman and Rose 2015)	
(Becerril-Montes *et al.* 2013) (Moniruzzaman *et al.* 2006) (Pavlenko *et al.* 2018) (Tsao *et al.* 2002) (Dispensary *et al.* 2005) (Churchyard *et al.* 2000) (Falzon, Infuso, and Aït-Belghiti 2006) (S. Yang *et al.* 2020) (SB 2010) (Perez-Navarro *et al.* 2017) (Liao *et al.* 2017) (Oliveira *et al.* 2019)	Content irrelevant to the research question.
(Miller *et al.* 2012) (Ferrara *et al.* 2005) (Ormerod 2005) (Jaber and Ibrahim 2019)	Reporting factors affecting multidrug resistant tuberculosis treatment outcome and not factors associated to MDR TB development.
(Ramazanzadeh *et al.* 2006) (El *et al.* 2006) (Lalor, Perkins, and Thomas 2020) (Conners *et al.* 2016) (Ferro *et al.* 2011) (S. Wang *et al.* 2012) (CHRISTOPHER *et al.*, n.d.) (Otero *et al.* 2011)	Did not report risk factors for multidrug resistant tuberculosis.
(Sun *et al.* 2015) (Republic and Thomayer 2010)	Reporting risk factors related to multidrug resistant tuberculosis mortality.
(Muchena *et al.* 2017) (Helen E Jenkins *et al.* 2014) (PRITCHARD *et al.* 2003) (Rumende 2018)	Patient's age not mentioned.
(Schaberg *et al.* 1995) (Brito *et al.* 2010) (Hirano *et al.* 1996)	Reporting rates of anti-tuberculosis drugs resistance.

(Liang *et al.* 2012) (Chen, Wang, *et al.* 2013) (Mdivani *et al.* 2008) (Long and Langlois-Klassen 2013) (Bojorquez *et al.* 2013) (Robert *et al.* 2003) (Ulmasova *et al.* 2011) (Mekonnen *et al.* 2015) (Otu *et al.* 2014) (Tembo and Malangu 2019) (Daniel and Osman 2011) (Vashakidze *et al.* 2009) (Lomtadze *et al.* 2009) (Boonsarngsuk, Mangkang, and Pitak Santinirand, n.d.) (S. F. Wang *et al.* 2016) (Lv *et al.* 2017) (Timire *et al.* 2019) (Ejaz *et al.* 2010) (Prakash *et al.* 2015)	Reporting factors contributing to the prevalence of multidrug resistant tuberculosis.
(Bagheri *et al.* 2013)	Including patients with surgical therapy.

Table 3.2 Studies included in the systematic review

Citation	Study period	Region/ country	Study design	Risk factors identified
(Zetola *et al.* 2012)	Between 1 May 2010 and 30 April 2011	Botswana	Case-control study	Alcohol abuse
(Merza *et al.* 2008)	From December 2000 to June 2005	Iran	a retrospective analysis	Age < 45, Male sex, Previous treatment, Immigration, Poor living conditions, Un-employment
(Elduma *et al.* 2019)	From May 2017 to February 2019.	Sudan	case-control study	Interruption of TB treatment, previous history of TB treatment, contact with MDR-TB patients, and being between 25 years and 60 years of age
(Y.-X. Liu *et al.* 2015)	2001, 2004, 2005, 2003 and 2008	China	Ecological study design	TB prevention, health resources, health services, TB treatment, TB detection, geography and climate.
(Gaborit *et al.* 2017)	2002-2016	France	Case-control	Recent immigration, precarious living conditions, history of incarceration, history of

				substance abuse, previous history of tuberculosis, at-risk exposure.
(Pardini *et al.* 2009)	2003-2005	Abkhazia (Georgia)	Cross-sectional	Beijing infection, infection with strains of ABK-1genotype, history of previous anti-tuberculosis treatment.
(Baya *et al.* 2019)	Between January 2007 and December 2016	Mali	Cross-sectional	Younger age, failure of prior TB treatment, lower number of prior TB therapy courses, history of close contact with a TB patient and better physical condition.
(Vadwai *et al.* 2012)	June to November 2009	Mumbai, India	Case-control	Previous treatment history with a Fluoroquinolone and an injectable other than streptomycin.
(Rifat *et al.* 2014)	between September 2012 and April 2013	Bangladesh	Case-control	Previous tuberculosis treatment, age 18 to 45 years, some education up to secondary level, service and business as occupation, past smoking status, and type 2 diabetes.
(Alvarez *et al.* 2015)	1998-2013	Mexico	Case-control	Diabetes mellitus, previous anti-TB treatment, duration of the first anti-TB treatment.
(Rifat *et al.* 2015)	from September 2012 to mid-April 2013	Bangladesh	Case-control	Incomplete treatment which includes treatment discontinuation due to treatment failure, adverse reactions to anti-TB medicine, and hospitalization for TB complications during previous TB treatment
(Tan *et al.* 2017)	From January to June in 2013	Lima, Peru	Case-control	Exposure to TB patients, family with financial difficulties, history of other chronic respiratory diseases, and history of smoking.
(Saktiawati and Subronto 2018)	between 2010 and 2014	Yogyakarta, Indonesia	A retrospective cohort study	Type 2 Diabetes mellitus.
(Mulisa *et al.* 2015)	From October 1, 2013 to March 30, 2014	Oromia region of Ethiopia.	Case-control	Occupation (being a farmer), known TB contact history, alcohol use, HIV infection, previous known

				TB history, previous TB treatment outcome.
(Lee *et al.* 2009)	Between January 2001 and December 2006	Korea	Retrospective review	Presence of a cavity, previous treatment of tuberculosis.
(Günther *et al.* 2015)	January 2010– December 2011	Europe	Prospective cohort study	Previous TB treatment and patient age <45 years, male sex and current homelessness, previous treatment for TB and contact with persons infected with drug-resistant strains.
(Dessalegn *et al.* 2016)	/	Addis Ababa, Ethiopia	Case-control	History of previous TB, more than one TB episode, pulmonary type of TB, and treatment with a Category II regimen.
(Mohd, Azhar, and Kamaludin 2015)	From April 2013 until April 2014.	Malaysia.	Case-control	Previously treated patients, have positive sputum smear at the 2nd month of treatment, living in suburban areas
(Temple *et al.* 2008)	From July 2003 through November 2006	Kampala, Uganda.	Cohort study	history of treatment failure, presence of cavities on chest radiograph, and multiple previous episodes of TB
(Demile *et al.* 2018)	September 2014 to August 2015	Ethiopia	Cross-sectional study	HIV infection, TB contact history.
(Chuchottaworn *et al.* 2015)	Between January 2007 and December 2013.	Thailand	Case-control	Two or more episodes of prior pulmonary TB, duration of illness > 60 days, having a sputum AFB smear grading 2+ or 3+, presence of cavities, presence of pleural effusion, and presence of atelectasis.
(Brewer *et al.* 2011)	between August 1, 2008 and December 12, 2008	Lima, Peru	population based case-control study	recent MDR-TB household contact.

4.2 Discussion

MDR-TB are defined as strains that are resistant to at least isoniazid (INH) and rifampicin (RIF)(Daley and Caminero 2018). These two anti-tuberculosis drugs form the backbone of conventional first line treatment for tuberculosis (Millard, Ugarte-gil, and Moore 2015). Infected patients with MDR-TB strains are practically incurable by standard first-line treatment (Seung, Keshavjee, and Rich 2015). This antibiotic resitantce classification is to consider since during treatment. (Millard, Ugarte-gil, and Moore 2015).

In all settings, patients who have previously been treated for tuberculosis are at higher risk for developing MDR-TB (Millard, Ugarte-Gil, and Moore 2015). Globally, in 2018, an estimated rate of 3.4% (95% confidence interval [CI]: 2.5–4.4%) of new cases and 18% (95% CI: 7.6–31%) of previously treated cases had developed MDR/RR-TB (WHO 2019). In the current review, the majority of included studies reported previous history of tuberculosis treatment as a risk factor strongly associated with the development of MDR-TB, most of it was from Asia (Iran, India, Bangladesh, Korea, and Malaysia) (Merza *et al.* 2008) (Vadwai *et al.* 2012) (Rifat *et al.* 2014)(Lee *et al.* 2009) (Mohd, Azhar, and Kamaludin 2015), while a Thailand study specified that having a treatment failure or default as treatment outcome is associated with MDR-TB (Chuchottaworn *et al.* 2015). In the African region, a Sudanese study revealed that 67.9% of patients had a history of previous TB treatment and showed improvement during first-line TB treatment. This was the most common reason for interrupting TB treatment by the concerned patients. This study agrees with a Bangladesh investigation results, in which the authors reported that TB patients who interrupted treatment had a 4 times higher risk of becoming infected with MDR-TB (Elduma *et al.* 2019). Several studies from Mali, Ethiopia as well as ones from Europe and Americas regions (Mexico) were in line with the finding of the previous ones (Baya *et al.* 2019) (Demile *et al.* 2018) (Mulisa *et al.* 2015), (Günther *et al.* 2015) (Alvarez *et al.* 2015).

Several risk factors have been pointed out in numerous studies associated with MDR-TB such as: age (Merza *et al.* 2008)(Elduma *et al.* 2019) (Rifat *et al.* 2014) (Günther *et al.* 2015); immigration (Merza *et al.* 2008) (Gaborit *et al.* 2017); gender (Merza *et al.* 2008) (Günther *et al.* 2015); precarious conditions (poor living conditions, unemployment, homelessness) (Merza *et al.* 2008) (Gaborit *et al.* 2017) (Mulisa *et al.* 2015) (Günther *et al.* 2015); contact with MDR-TB patients (Elduma *et al.* 2019) (Elduma *et al.* 2019) (Baya *et al.* 2019) (Tan *et al.* 2017) (Mulisa *et al.* 2015) (Günther *et al.* 2015); history of incarceration (Gaborit *et al.* 2017); alcohol intake (Zetola *et al.* 2012)(Mulisa *et al.* 2015); smoking (Rifat *et al.* 2014) (Tan *et al.* 2017); diabetes mellitus (Rifat *et al.* 2014) (Alvarez *et al.* 2015) (Saktiawati and

Subronto 2018); HIV infection (Mulisa *et al.* 2015) (Demile *et al.* 2018) and presence of cavities on chest radiograph (Temple *et al.* 2008) (Chuchottaworn *et al.* 2015).

Recently, the high prevalence of diabetes mellitus among MDR-TB patients, has become a serious cause for concern with a range of 10–23% (Liu *et al.* 2017). In a Mexican study, it was found that there was a relationship between pulmonary MDR-TB and diabetes mellitus in 47.2% of cases (Alvarez *et al.* 2015). An Indonisian study (Yogyakarta) recommends for clinicians to undertake an early screening test of MDR-TB for such group of patients (Saktiawati and Subronto 2018). These findings agreed with those of Liu *et al.* 2017 that indicated, through pooling analyses, that DM was an independent risk factor for MDR-TB, especially for primary MDR-TB comorbidity. Effective measures need to be implemented to promoting early diagnosis of MDR-TB, and followed by intensive treatment and follow-up.

The association between MDR-TB and HIV has been highlighted since the earliest reports of the spread of MDR-TB among immune-compromised patients (WHO, 2014). This relationship has not yet been clearly understood. Findings from different studies on associations of HIV co-infection and drug resistance among patients with TB have been discordant. Some institution based studies found strong increased risks for MDR-TB among patients co-infected with HIV. However, further studies found no increased risk and remains less clear in community based studies (Mesfin *et al.* 2014). In the current review, there was only one included study from a tertiary armed force referral and teaching hospital Oromia, Ethiopia (Mulisa *et al.* 2015) that reported human immunodeficiency virus as a significant predicting factor for MDR-TB. The other studies showed no significant association.

Epidemiological studies have been equivocal about the statistical significance of the association between HIV-positivity and MDR-TB. It is clear that HIV-positive patients are vulnerable to drug-resistant TB. This was best illustrated by the rapid and deadly spread of extensively drug-resistant TB (XDR-TB) among HIV-positive patients in South Africa and elsewhere (who 2014).

A previous systematic review conducted by Suchindran, Brouwer, and Van Rie in 2009 aiming to summarize evidence on the association between HIV infection and MDR-TB, could not demonstrate an overall association between both affections but suggested through their results that HIV infection is associated with primary MDR-TB.

From a microbiological point of view, the most remarkable association between a mycobacterial genetic background and drug resistance documented thus far has been described for strains of the so-called Beijing lineage. These strains have been found to be

involved in outbreaks and the transmission of MDR TB in several areas worldwide (Niemann *et al.* 2010). A particular feature of the Beijing genotype is the higher mutation rates, that facilitate the emergence of drug resistance. The strong association between the Beijing genotype and drug-resistant TB is evidenced by the higher proportion of this lineage among MDR-TB and/or XDR-TB in comparison to drug-susceptible TB (Niemann *et al.* 2010). In our review, an included study from Abkhazia (Georgia) mentioned that being infected with Beijing genotype is strongly associated with both MDR-TB and transmission (Pardini *et al.* 2009). This was confirmed by another study from the same region which reported that the Beijing epidemic has reached Georgia and is associated with a high rate of recent transmission, especially in prison populations, and high MDR rates (Niemann *et al.* 2010)

The World Health Organization highlights numerous social determinants that affect the risk of developing resistance. Special attention was placed to poverty, poor living conditions, various causes of social vulnerability and reduced access to and availability of health services (Stosic *et al.* 2018). Two studies reported that gender, specifically males, who are more at risk for developing MDR-TB (Merza *et al.* 2008) (Günther *et al.* 2015). A certain age section was reported as a risk factor, (Merza *et al.* 2008), (Rifat *et al.* 2014) and (Günther *et al.* 2015) revealed that people aged under 45 years old are more at risk for developing MDR-TB, while (Elduma *et al.* 2019) reported that being between 25 and 60 years of age is associated with developing MDR-TB.

Chapter 5

Conclusion

In the current narrative review we highlighted the major risk factors that could be associated with the development of multidrug resistant tuberculosis. Identification of patients with these predictors will facilitate careful monitoring and control of drug resistance. The discordance between studies from different regions wordlwide, regarding the association of different risk factors with MDR-TB indicates that this epidemic is associated with region-specific risk factors, especially with regard to the association of social determinants with this resistance to anti-tuberculosis antibiotics, which denote that studies of this association should be undertaken regionally. Furthermore, it should be noticed that the lack of data in the African continent constitutes a major issue that should be urgently addressed since this region of the world accounts for over eighty per cent of TB cases. Additionnally, studies of different designs are needed aiming to establish the causality and strength of the association of chronic diseases, particularly HIV and diabetes mellitus, with MDR-TB. Drug susceptibility tests for patients with high risk of developing MDR-TB could be the optimal solution for an optimal treatment of TB positive cases.

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