Microbial Pathogenesis 117 (2018) 237–242

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

Review on emergence of drug-resistant tuberculosis (MDR & XDR-TB) and its molecular diagnosis in Ethiopia

Muthupandian Saravanan[a,**], Selam Niguse[a], Mahmud Abdulkader[a], Ephrem Tsegay[a], Haftamu Hailekiros[a], Atsbeha Gebrekidan[a], Tadele Araya[a], Arivalagan Pugazhendhi[b,*]

[a] Department of Medical Microbiology and Immunology, School of Medicine, College of Health Science, Mekelle University, Mekelle 1871, Ethiopia
[b] Innovative Green Product Synthesis and Renewable Environment Development Research Group, Faculty of Environment and Labour Safety, Ton Duc Thang University, Ho Chi Minh City, Viet Nam

ARTICLE INFO

Keywords:
M. tuberculosis
Emergence
Drug resistance
Molecular diagnostics
miRNA
MDR & XDR-TB
Anti-tuberculosis drugs

ABSTRACT

Tuberculosis (TB) remains a major global health problem and ranks as the second leading cause of death among deaths caused by infectious diseases worldwide. Although the availability of short-course regimens as first-line anti-tuberculosis drugs, the emergence of drug-resistant *Mycobacterium tuberculosis* strains pose a major challenge to the prevention and control efforts of national tuberculosis programs (NTPs). *M. tuberculosis* changes its cellular environment with the mechanisms that have been evolved since prehistoric times. The interactions between the bacteria and the host environment have been studied well. However, the studies at molecular level began to emerge recently including expression profiling of micro RNA (miRNA) and literature survey revealed that researchers find more information about their regulatory role in biological processes including immune response to infectious agents like mycobacteria. In developing countries, including Ethiopia, the burden of tuberculosis and or drug resistance profile of *M. tuberculosis* remains largely unexplored, mainly due to lack of quality controlled second-line laboratory tests and also lack of knowledge on molecular diagnostics. This review describes the disease etiology, pathogenesis, epidemiology, molecular mechanism and advanced molecular diagnostics for precision MDR-TB diagnosis.

1. Introduction

Tuberculosis (TB) is an infectious disease caused by the bacillus *M. tuberculosis* complex it typically affects the lungs (pulmonary TB) but can affect other sites as well (extrapulmonary TB). The disease is transmitted through aerosol infection when people who are sick with pulmonary TB expel bacteria. In healthy people, infection with *Mycobacterium tuberculosis* often causes no symptoms, since the person's immune system acts to “wall off” the bacteria. The symptoms of active TB of the lung are coughing, sometimes with sputum or blood, chest pains, weakness, weight loss, fever and night sweats. Without treatment, mortality rates are high. Treatment using combinations of *anti*-TB drugs, developed in the 1940s and 1950s, can dramatically reduce mortality rates [1].

Despite the availability of highly efficacious treatment for decades, TB remains a major global health problem. It causes ill health among millions of people each year and ranks alongside the human immunodeficiency virus (HIV) as a leading cause of death worldwide. According to the 2014, World Health Organization (WHO) reports showing that there were nine million new tuberculosis (TB) cases and 1.5 million tuberculosis (TB) deaths [2].

In developing countries, the mortality rate is predominant due to TB than any other infectious diseases. This is directly indicated by less health care access, as well as higher exposure to unhealthy and crowded living and working conditions, undernutrition, HIV infection, diabetes mellitus, smoking, alcohol and drug abuse, and several other TB risk factors [3].

Ethiopia is one of the developing countries, having experienced a major increase in the burden of TB, presents with the most serious public health challenges. It was highly afflicted by the TB pandemic and is ranked second after Nigeria in Africa and seventh among the 22 high TB burden countries worldwide [4].

Drug resistance in TB threatens the National Tuberculosis Control Programme in numerous low and middle-income countries (LMIC) and the major problem is multidrug resistance TB (MDR-TB). It is more challenging in developing countries like Ethiopia with more than 5000

* Corresponding author.
** Corresponding author.
E-mail addresses: saravanan.muthupandian@mu.edu.et (M. Saravanan), arivalagan.pugazhendhi@tdt.edu.vn (A. Pugazhendhi).

https://doi.org/10.1016/j.micpath.2018.02.047
Received 9 January 2018; Received in revised form 21 February 2018; Accepted 23 February 2018
Available online 24 February 2018

estimated MDR-TB patients each year [5,6].

Multidrug-resistant (MDR) tuberculosis (TB), defined as disease caused by *Mycobacterium tuberculosis* strains with resistance to, at least, isoniazid (INH) and rifampicin (RIF), is a growing public health and clinical problem worldwide [7]. The emergence of extensive drug resistance (XDR) tuberculosis (TB) defined as TB that is resistant to isoniazid, rifampicin, quinolones, and at least 1 of 3 injectable SLDs (i.e., kanamycin, capreomycin, or amikacin) in every region of the world has raised additional alarms about the future of TB control. TB drug resistance is caused by inadequate therapy enabling selection and growth of resistant populations (i.e., acquired resistance) or by infection with a drug-resistant strain (i.e., primary resistance) [8].

The effective control of TB is based on the immediate detection of *M. tuberculosis*, followed by the prompt implementation of adequate anti -TB therapy [9]. The emergence of strains resistant to the major *anti*-TB drugs speeds up the need for rapid methods for the identification of resistant *M. tuberculosis* strains in order to treat the disease effectively and, at the same time, to prevent the spread of resistant strains. One of the tools was molecular methods of diagnosis is effective, rapid, and detects a resistant strain of TB [10].

Diagnosis is established by laboratory methods requiring advanced laboratory capacity; however, these methods are not available in most resource-limited settings. Compared with the treatment of drug-susceptible TB, treatment of MDR-TB is longer, more complicated, more expensive, and less successful. Although efforts to expand drug-susceptibility testing (DST) and the availability of second-line drug (SLD) therapy have been emphasized over the past decade, the majority of MDR-TB cases that occur globally are still undiagnosed and untreated [11]. Therefore the objective of the present review is to overview the prevalence, MDR, XDR and its molecular diagnosis of TB in Ethiopia.

2. Etiology

TB is an infectious disease caused by *M. tuberculosis* complex (MTC) bacteria, which has an endemic character and worldwide distribution. The MTC comprises closely related species responsible for strictly human and zoonotic TB. The complex consists of seven species and subspecies including *M. bovis*, *M. africanum*, *M. microti*, *M. canetti*, *M. caprae* and *M. pinnipedi* [12].

Despite the different species tropisms, the MTC is characterized by 99.9% or 3 greater similarities at the nucleotide level and possess identical 16S rRNA sequence [13]. *M. tuberculosis* is a large non-motile and non-spore forming rod shaped bacillus grouped under the order actinomycetales. The bacteria in the group are known for the high lipid content in their cell wall. The cell wall is the most distinctive anatomical feature of the bacteria. It is constituted by an inner peptidoglycan layer, which seems to be responsible for the shape forming property and the structural integrity of the bacterium. The lipid coat confers the distinctive characteristics of the group: acid fastness, extreme hydrophobicity, and resistance to weak disinfectants. It probably also contributes to the slow growth rate of some species by restricting the uptake of nutrients [14].

M. tuberculosis strains can be classified into a number of major clades according to defined evolutionary markers. It is hypothesized that strains comprising these clades have evolved different properties, which may influence a local strain population structure. This evolved different properties growth was exclusively attributable to drug-susceptible strains. Recent evidence suggests that these differences likely reflect enhanced pathogenicity rather than transmissibility. The rapid emergence of different strains demonstrates adaptation to conditions within the study community and poses a grave challenge to future TB control [15]. Although TB may manifest itself in any tissue, the lung represents the main port of entry and is an important site for disease manifestation [14].

3. Epidemiology

Ethiopia is one of the 22 high burden countries (HBCs) and TB remains one of the leading causes of mortality due to communicable diseases in the country. According to 2014, WHO report showed the prevalence and incidence of all forms of TB are 211 and 224/100,000 population, respectively. Excluding HIV related deaths; TB mortality was estimated to be 32 per 100,000 populations in 2013. Among estimated all new TB cases, 13% are HIV co-infected. Moreover, Ethiopia is also one of the high TB/HIV and multi-drug resistant TB (MDR TB) burden countries. According to the recent national TB drug resistance surveillance report, 2.3% of new TB cases and 17.8% of previously treated TB cases were estimated to have MDR [2].

Furthermore, it was the first cause of hospital death. Some of the main reasons suggested for the widespread of pulmonary tuberculosis in Ethiopia are HIV infection, neglect of tuberculosis program, rapidly growing slums with crowded living conditions, lack of access to modern health care and deficient medical services, Poor nutritional status/poverty, increased virulence and/or increased dose of bacilli. Increased susceptibility of infants and the elderly. For infants, contact with non-smear positive cases is even significant. Miscellaneous: Hormonal therapy, diabetes mellitus (three to four times' increase of risk), alcoholism, silicosis, etc. [16].

4. Pathogenesis

A healthy adult exposed to relatively low numbers of bacteria generally clears them before considerable damage to the lung. But if the phagocytic cells do not clear the infection, new T cells, polymorph nuclear cells (PMNs) and more macrophages continue to be attracted to the area where bacteria are growing [17]. Mycobacteria may also be transported to other organs via the lymph vessels or bloodstream and produce dissemination foci there. The host eventually develops granulomas, foci fibrosis, scar, and calcify but the infection remains clinically silent. In about 10% of infected persons, the primary TB reactivates to become organ TB either within months or after a number of years. Reactivation begins with a caseation necrosis in the center of the granulomas (also called tubercles) that may progress to cavitation and frequently stems from old foci in the lung apices. Tissue destruction is caused by cytokines among which tumor necrosis factor alpha (TNFα) appears to play an important role. These cytokines are also responsible for the cachexia associated with tuberculosis. The body's immune defenses have a hard time in containing necrotic tissue lesions in which large numbers of Mycobacterium cells occur [18].

In some cases where the phagocytes fail to kill the bacteria, the T cells and macrophages wall off the growing lesion with a thick fibrin coat. The walled-off lesion is called tubercle. Tubercles eventually calcify, giving rise to the hard-edged lesions visible in chest x-rays. Phagocytes unsuccessfully trying to kill the bacteria cause considerable damage to lung tissue by releasing lysosomal enzymes and by producing TNF. Initially, the areas where bacteria are growing have a thick, cheese-like appearance (caseous necrosis). As bacteria continue to grow and phagocytes continue to enter the area, the necrotic region becomes much more liquid. A person with liquefied lesions is more contagious than lesions in caseous necrosis [19].

5. Factors driving drug-resistant tuberculosis

MDR and XDR tuberculosis arise because of inadequate or interrupted administration of first-line treatment. If patients are given too few drugs, for too short a period, or given drugs to which infecting strains are partly drug resistant, the resistant strains are favored and will eventually predominate in the body. However, once drug-resistant strains of *M. tuberculosis* have been selected and occur in a community, they are directly transmitted to others. Many factors then propagate the spread of drug-resistant tuberculosis (Fig. 1) and lead to the widespread