

VOLUME 9

ISSUE 2

2023

ISSN 2454 – 3055



**INTERNATIONAL
JOURNAL OF
ZOOLOGICAL
INVESTIGATIONS**

***Forum for Biological and
Environmental Sciences***

Published by Saran Publications, India



Neoteric Drifts in Tuberculosis-A Global Pandemic: A Review

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Received: 2nd December, 2023; Accepted: 22nd December, 2023; Published online: 25th December, 2023

<https://doi.org/10.33745/ijzi.2023.v09i02.150>

Abstract: *Mycobacterium tuberculosis* has coherently existed with humans for the past centuries resulting in reduced quality of life and high mortality rate. Over years many treatment strategies and plans to eradicate TB globally have been formulated. MTB has wrecked human life and is a cause of great concern and despair in less privileged countries. Rigorous research on antibiotics and vaccines are being conducted to impede the transmission of *Mycobacterium tuberculosis*. Even after major breakthrough in scientific field mycobacteria has developed mechanism to supersede the harmful effects rendered by antibiotics. Thus, creating a generation of highly adaptable strains referred as multi-drug resistance tuberculosis which has caused great havoc throughout the world. This review will give an overview on tuberculosis and recent trends in combatting this highly infectious pathogen.

Keywords: *Mycobacterium tuberculosis*, Multi-drug resistance, Macrophage, Ziehl-Neelsen staining, Antibiotics

Citation: Sakharkar Nilima G., Nikam Priya V., Mane Namrata S. and Thakare Vinod M.: Neoteric drifts in tuberculosis-A global pandemic: A review. Intern. J. Zool. Invest. 9(2): 1338-1343, 2023.

<https://doi.org/10.33745/ijzi.2023.v09i02.150>



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Introduction

Tuberculosis caused by pathogenic bacteria *Mycobacterium tuberculosis* which originated over many years ago, continuously striving to be one of the deadliest pathogens procuring a second position to be most infectious disease after HIV (Barberis *et al.*, 2017). Tuberculosis has existed in our societies over a long time with different names, in the 18th century it was known as phthisis and consumption, in the 19th century it evoked despair among people giving the name The Great White Plague. The spread of this bacteria

has continued over centuries but a significant discovery made on March 24 1882 by Robert Koch paved the way for eradication efforts. Robert Koch discovered that *Mycobacterium tuberculosis* caused the deadly tuberculosis infection. Since then, plentiful studies have helped humans understand better about tuberculosis infection to take the necessary precautions (Frith, 2014).

Mycobacterium tuberculosis is a rod shaped, non-motile acid-fast bacteria with a size of about 3-3.5 μm which occurs in pairs or clumps. The

primary detection method for mycobacterium is staining using Ziehl-Neelson technique. It is the most widely used acid stain method, other staining methods are using auramine rhodamine dyes or Kinyoun's modification (Ananthanarayan and Panicker, 2013). Tuberculosis infection can persist throughout life in asymptomatic individuals which is referred as latent infection. This highly contagious infection can be classified under respiratory infection. This highly potent bacteria ravage the host through air transmission in the form of cough, or sneeze. The active form of TB occur during reactivation of latent TB. The detection and management of latent form of TB is highly critical in prevention and control (Kiazyk and Ball, 2017). The characteristic feature of *Mycobacterium tuberculosis* to evade immunity makes it a challenging task to cure TB infection. This virulent bacterium has developed mechanisms to escape innate immunity and delaying adaptive immunity, earlier it was noted that it inhibits apoptosis and promotes necrosis of the host cell (Behar *et al.*, 2011), but later studies have proved that mycobacteria is responsible for necroptosis which is alternate method of cell death showing phenotypes of both necrosis and apoptosis (Dhuriya and Sharma, 2018). Unlike other strains of mycobacteria, MTB does not form spores during stress, it undergoes a state of dormancy where the bacteria remain in a dormant stage for a prolonged period of time in the host and develops latent infection after few years. Re-emergence of infection is most in cases noted during deterioration of health (Gengenbacker and Kaufmann, 2012). The projected milestone for End TB strategy is conceived as 80% reduction in the incidence rate and 90% reduction in mortality rate by 2030. In 2017, Moscow declaration hosted by Russian Federation held a conference to discuss sustainable developmental goals to end TB. In 2018, UN general assembly high level meeting was conducted to discuss urgent global response. An important factor which controls the rate of TB infection are antibiotic such as rifampicin, isoniazid, ethambutol, pyrazinamide. These antibiotics have significantly reduced mortality

however, the over usage of these antibiotics have led to the onset of MDR tuberculosis (multidrug resistance TB). Such multi-drug resistance TB prove to be unresponsive to first line anti-TB drugs such as rifampicin and isoniazid. Though, it renders response to second line anti-TB drugs, it requires extensive chemotherapy. Such MDR strains of *Mycobacterium tuberculosis* are a cause of huge public health concern. MDR spreads through an MDR infected person or patient who did not undergo proper treatment regime. The rate of spread of tuberculosis can only be prevented by early detection methods such as tuberculin test or IFN- γ release assay (IGRA). People with adverse health conditions or HIV infections are to be strictly monitored. HIV patients have high risk factor for tuberculosis infection. Other molecular diagnostic techniques available for TB detection includes Nucleic Amplification Tests (NAAT), real time PCR, loop mediated isothermal amplification (Eddabba and Benhassiou, 2018).

Pathogenesis:

The transmission of TB takes place through aerosols where bacilli are dispersed through air from an infectious person. Once the bacilli enter the human host it develops infection in lungs or other extra-pulmonary sites such as respiratory tract, digestive tract, and damaged skin and mucous membrane. The entry of bacteria in the host activates innate and adaptive immunity. Then a Global pandemic primary response against the pathogen is initiated by innate immunity whereby it recognizes pathogen associated molecular patterns (PAMPs) through PRR resulting in the activation of pro-inflammatory cytokines such as IFN α , TNF, IL-1 β , IL-12. Adaptive immunity is initiated when live mycobacteria are presented to naïve T cells to impede bacterial proliferation. A central response to mycobacterial infection is activation of macrophages which may result as cell necrosis, apoptosis and survival of infected macrophages (Heemskerk *et al.*, 2015). The mycobacterium- macrophage interaction leads to binding of MTB to cell surface receptors of

macrophages, phagosome-lysosome fusion, inhibition of MTB growth through activation of cytokines, NO, and free radicals, antigen presentation. The mycobacteria are able to survive intra-cellularly by preventing the phagosome maturation or lysis of phagolysosome. The inhibition can be stimulated due to structural composition of mycobacteria or the production of cytokines like IFN α followed by stimulation of IL-10, recruitment of TACO protein also known as coronin1 responsible for processing the active bacilli, downregulation of LAMP1, secretion of PKng, regulation by transcription factor NF-kB (Zhai *et al.*, 2019). Another important characteristic feature of *Mycobacterium tuberculosis* is granuloma formation. Granuloma are compact cell mass made up of monocytes, macrophages, foamy macrophages, dendritic cells, epithelioid cells, neutrophils, multi-nucleated giant cells. This layer is further surrounded by lymphocyte cells which forms a solid structure. Granuloma formation begins when MTB phagocytosed by alveolar macrophages exudes into epithelial cells, monocytes from nearby blood vessels begins the formation of granuloma. The fibrous capsule of granuloma contains typical caseous debris which acts as a reservoir for thousands of *Mycobacterium tuberculosis* bacilli. These bacilli are released into air whenever the person coughs. Virulence proteins such as ESAT6 triggers MMP-9 which recruits new macrophages into granuloma then these infected macrophages establish secondary granulomas which disseminate infection further (Ndlovu and Maraklala, 2016). MTB is known to contain complex components in the cell wall such as mycolic acid, long chain fatty acids, Wax D, phosphatidic acid which are responsible in conferring the characteristic caseating necrosis. Another glycolipid occurring in the cell wall known as cord factor demonstrates protective and non-toxic actions to prevent phagocytosis by macrophage during primary infection and triggering caseating necrosis during secondary infection. Phthiocerol dimycocerosate is another glycolipid which targets lipid organization and

perturbs its properties to facilitate receptor mediated phagocytosis to invade macrophages (Sanyaolu *et al.*, 2019).

Immune escape mechanism:

Macrophages activation is a classic phenomenon observed during entry of any pathogen. A pathogen encounter by the immune cells trigger a cascade of factors such as IFN γ , Tc1, Th1, NK cells and ROS. The activation of macrophages to eliminate pathogens depends on the competence of the immune system. In case of weak immunity *Mycobacterium tuberculosis* pertains in a latent form followed by resuscitation when immune system weakens further. Further complex mechanism takes place to sabotage the immune system. MTB is intracellular bacteria which is taken up inside the cell due to action of various phagocytic receptors like binding to FC- γ evades lysosomal degradation and binding to mannose capped lipoarabinomannan impairs phagosomal maturation (Bussi and Gutierrez, 2019; Bhat and Yaseen, 2018). MTB employs full proof mechanism to subvert immunity such as prevention of phagosomal maturation. This process results in a dynamic change in the composition of luminal components resulting in acidification to impede growth of bacteria. Acidification of phagosome can be considered as an important contributing factor for which a proper mechanism is not known. MTB employs tyrosine phosphatase A PtpA causing a defective recruitment of vacuolar ATPase and reduction in the levels of lysosomal hydrolases. Another mechanism was controlled blockage of endocytic Rab Gtpase. Rab5 which is an early endosomal protein is converted into Rab7, a late endosomal protein (Upadhyay *et al.*, 2018). to a great extent. Calmette and Guerin (1900) started their experiment to produce anti-tuberculosis vaccine. It was a result of fortuitous observation which led to the discovery of BCG vaccine. Immense scepticism against the vaccine grew after tragic disaster in Lübeck. Over the recent years many trials were conducted to increase the efficiency of the vaccine which includes subunit vaccine, Modified BCG, attenuated *Mycobacterium*

tuberculosis. The current BCG vaccine is a live vaccine obtained from *Mycobacterium bovis* isolate which is sub cultured several times in potato-glycerine-ox bile medium. *M. bovis* is a zoonotic tuberculosis bacterium which inflicts tuberculosis in cattle. The BCG vaccine due to it being sub cultured in various location across the world has diverged to a great extent from its original ancestral strain, which in turn leads to difference in efficiency and protection (Martin, 2005; Luca and Mihaescu, 2016). The immunogenicity of the vaccine was reportedly not affected by different BCG strains being used in clinical trials but illustrates more significant difference due to various culture media. A recent clinical trial of MVA85A designed to enhance BCG efficiency responded with moderate immunogenicity but failed to confer protection. High variability in BCG vaccines poses a question for better vaccine candidates. MTBVAC attenuated vaccine conferring deletions in *phoP* and *fadD26* and live recombinant vaccine VPM1002 with urease gene deletion and listeriolysin expression that perforates phago-lysosomal membrane. Both these vaccines have entered phase I clinical trial. Logical priming vaccines are also under trials which maintains childhood protective effects. H1 vaccine which is a fusion protein of 85b with ESAT6 and many more which are presently under phase I clinical trials. Viral vector vaccines such as an Adenovirus Hu35 expressing Ag85A, Ag85B and TB10.4MVA85A, a Modified Vaccinia virus Ankara expressing antigen 85A; AdHu5Ag85A a recombinant human type 5 adenovirus expressing antigen 85A; and AERAS-402, are supplemented to boost BCG vaccine response. Hence an effective vaccination strategy must be employed to achieve the goal of End TB (Manjaly Mcshane, 2015; Davenne and Mcshane, 2016).

Trends for better detection:

The primary and foremost way to achieve complete eradication of TB is through proper and precise monitoring. TB is highly prevalent in developing and developed countries where there are resource limitations. The low availability of

resources and infrastructure in such places develop clusters of *Mycobacterium tuberculosis* infected people. Kinyoun cold staining method, Gabetts cold staining method, Tan thiam hok staining method are all old and less robust method for microscopic detection of AFB. Sputum smear is the most commonly used specimen due to less expense incurred and easy availability. Ziehl-Neelsen staining method is the most prevalent method in resource limited settings, carbol fuchsin tends to be primary stain and methylene blue is the counter stain. This staining technique has been made obsolete by WHO due its high variability. Fluorescent staining have gained much popularity ever since 1990s after its synthesis by Adolf Von Bayer. Fluorescent staining is also preferred over Ziehl-Neelsen staining due to its high sensitivity. Auramine- rhodamine staining is used to visualize acid fast bacilli, where the fluorescent dyes will bind to the nucleic acid (Dezemon *et al.*, 2014; Caulifield and Wegenack, 2016). The highly expensive nature of the technique poses a great challenge in poor countries. Fluorescein diacetate technique is a vital technique which confirms active TB bacteria. It utilizes the principle of intracellular FDA hydrolysis where non-fluorescent FDA is converted into green fluorescent compound only in the presence of live bacteria. This technique also reduces over diagnosis of Tb by avoiding confusion due to dead bacilli (Kanade *et al.*, 2016).

Trends for better diagnosis:

There has been a change in paradigm from conventional diagnostic methods to modern molecular approaches. The most common conventional technique was the Mantoux test where a small amount of tuberculin is injected intradermally and after 45-72 h skin induration is monitored. This test is highly variable and can confer false positive and negative results. Other diagnostic option involved chest X-ray which is also not an accurate diagnostic method. Recent interventions in molecular approaches have made infectious disease diagnosis easier and faster. Nucleic acid amplification test is utilized in multi-

bacillary disease when there is a high mycobacterial load and positive AFB smear would indicate active tuberculosis. In case of positive AFB smear and negative NAAT indicates non-tuberculosis mycobacteria. There are 3 varieties of NAAT test which includes Amplified Mycobacterium Tuberculosis Direct (MTD) Test (Gen-Probe, Inc), Amplicor Mycobacterium Tuberculosis Test (Roche Molecular Systems, Inc), and WHO endorsed Xpert/RIF MTB assay (Martinez *et al.*, 2018). Whole genome sequencing is a fast and rapid method which can detect mutations better than Xpert MTB assay. It can detect mutation in MDR tuberculosis where polymorphism of rifampicin resistance determining region *rpoB* is detected. It acts as a predictive tool in determining the genomic regions responsible for drug resistance phenotype and also determines genetic relatedness. Earlier for WGS culturing of MTB, which is a slow growing bacterium, was required resulting in major time consumption. Later a reliable and low-cost mycobacterial growth indicator tube from which DNA can be rapidly extracted is used. WGS has changed the overall scenario for MDR TB. Earlier drug resistance was detected using drug susceptibility test which required more time compared to the rapid WGS. Recently WGS of sample without prior requirements of specimen culture, are under consideration (Nurwidya *et al.*, 2018).

Potential for next pandemic:

The world has not heaved a sigh of relief from the current pandemic caused by coronavirus. It has taken a toll worldwide. *Mycobacterium tuberculosis* also known to a highly infectious pathogen should not be considered lightly. Negligence in human part in controlling tuberculosis outbreaks may prove to be fatal and may lead to an onset of another pandemic. MTB is an airborne pathogen and may pose a grave threat due to globalization. Nowadays, People can travel from one part of the world to another within a few hours. *Mycobacterium tuberculosis* has a unique characteristic to remain in latent form in the host. During latent infection the host

will not feel any physical abnormality. An increasing trend of multi-drug resistance tuberculosis is detected which demonstrates the increasing ability of bacteria to adapt to present situation. Such adaptations in the long run may also result in latent form to release infectious bacilli. WHO has recommended protective measure to prevent TB infections. Patients with infectious TB should be rigorously monitored and should not be allowed in flights with more than 8 h journey. In case of necessary travel infectious persons should cover mouth and nose to prevent spreading of infection. Proper detection, diagnosis and monitoring are the key steps to achieve the goal to end TB (Saleem and Azher, 2013).

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