Review

The role of epigenetics, bacterial and host factors in progression of *Mycobacterium tuberculosis* infection

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ABSTRACT

Tuberculosis (TB) infection caused by *Mycobacterium tuberculosis* (*Mtb*) is still a persistent global health problem, particularly in developing countries. The World Health Organization (WHO) reported a mortality rate of about 1.8 million worldwide due to TB complications in 2015. The Bacillus Calmette-Guérin (BCG) vaccine was introduced in 1921 and is still widely used to prevent TB development. This vaccine offers up to 80% protection against various forms of TB; however, its efficacy against lung infection varies among different geographical settings. Devastatingly, the development of various forms of drug-resistant TB strains has significantly impaired the discovery of effective and safe anti-bacterial agents. Consequently, this necessitated discovery of new drug targets and novel anti-TB therapeutics to counter infection caused by various *Mtb* strains. Importantly, various factors that contribute to TB development have been identified and include bacterial resuscitation factors, host factors, environmental factors and genetics. Furthermore, *Mtb*-induced epigenetic changes also play a crucial role in evading the host immune response and leads to bacterial persistence and dissemination. Recently, the application of GeneXpert MTB/RIF® to rapidly diagnose and identify drug-resistant strains and discovery of different molecular markers that distinguish between latent and active TB infection has motivated and energised TB research. Therefore, this review article will briefly discuss the current TB state, highlight various mechanisms employed by *Mtb* to evade the host immune response as well as to discuss some modern molecular techniques that may potentially target and inhibit *Mtb* replication.

1. Introduction

1.1. Epidemiology and transmission of tuberculosis

*Mycobacterium tuberculosis* (*Mtb*) is an etiological agent that causes tuberculosis (TB) infection. This pathogen is airborne and is transmitted from person to person through inhalation of contaminated droplet nuclei. Globally, an estimated 10.4 million people were infected with TB and 1.8 million casualties were reported in 2015 [236]. Countries with the highest TB burden include India, Indonesia, China, Nigeria, Pakistan and South Africa, respectively. These six nations account for approximately 60% of the global TB incidence [236]. Two distinct forms of TB are active and subclinical or latent TB infections. Patients with active infection exhibit typical TB clinical symptoms that include fever, chest pains, night sweats, anemia and weight loss [236] (Table 1). Nevertheless, people with LTBI may develop active TB, and this transition is called TB reactivation [236]. The risk of TB reactivation is 5–10% [1] and many individuals develop active TB within five years post-infection.

1.2. Pathogenesis of *Mtb*

*Mtb* gains access into the lungs following inhalation of droplets containing tubercle bacilli [2,3] (Fig. 1). The ability of *Mtb* to invade host cells [4] is determined by its virulence [5,6] and host immune competence. To gain entry into host cells, *Mtb* utilises its diverse surface ligands to interact with various host receptors and include complement [7], mannose [8], surfactant protein A [9], CD14 [10], macrophage scavenger receptors [11] and lectin DC-specific intracellular adhesion molecule-3 grabbing nonintegrin (DC-SIGN) [12]. The inhaled bacilli are then transported to the alveoli and engulfed by large phagocytic cells by *Mtb* antigens [236] (Table 1). Although these patients possess *Mtb*, they are unable to transmit the infection (Table 1). Nevertheless, people with LTBI may develop active TB, and this transition is called TB reactivation [236]. The risk of TB reactivation is 5–10% [1] and many individuals develop active TB within five years post-infection.
cells called macrophages. *Mtb* is an intracellular microorganism capable of evading host immunity and can persist within host macrophages for a long period of time. Proteins found in the bacterial cell wall, particularly glycoproteins are thought to be associated with evasion of the host immune response and *Mtb*-host interaction [13]. SodC is a bacterial glycoprotein found in the cell wall and has been shown to improve *Mtb* survival within host macrophages [14,15]. Bacterial clearance or containment depends on the host innate and adaptive immune responses (Fig. 1) and macrophages play a crucial role in engulfing infectious microbial cells. Following internalisation into host macrophages, most bacilli are killed, while the surviving bacterial cells are thought to inhibit phagosome maturation and replicate within phagosomes [16–18]. Extensive *Mtb* replication leads to disruption of macrophages and development of active TB disease.

### Table 1

<table>
<thead>
<tr>
<th>Active TB infection</th>
<th>Latent TB infection</th>
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<tr>
<td>Possess active <em>Mtb</em> in the body</td>
<td>Possess dormant <em>Mtb</em> in the body</td>
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<tr>
<td>Display abnormal chest X-ray, positive culture, smear or sputum test</td>
<td>Display normal chest X-ray and negative sputum test</td>
</tr>
<tr>
<td>Display clinical TB symptoms (fever, chest pains, night sweats, anemia and weight loss)</td>
<td>No clinical evidence of TB infection</td>
</tr>
<tr>
<td>May transmit infection</td>
<td>Cannot transmit TB</td>
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![Fig. 1. Infection cycle and transmission of *Mtb*. Mycobacterium tuberculosis is an airborne pathogen that is transmitted from person to person through inhalation of contaminated droplet nuclei. The bacterium elicits the host innate immune response by recruiting macrophages, which transports the pathogen across the lungs to other tissues. Subsequent recruitment of macrophages leads to inflammation (i.e. granuloma) comprised of macrophages and various immune cells. The newly formed inflammation advances infection by enabling *Mtb* dissemination to recruited macrophages. The adaptive immune response, mainly comprised of T-cells is mostly capable of decreasing bacterial replication and spread in immunocompetent hosts. However, failure to restrict bacterial growth leads to necrosis and allows *Mtb* replication and transmission to other hosts.](image-url)
However, in most infected hosts, alveolar macrophages recruit monocyte-derived dendritic cells and other macrophages to the lungs [19,20]. The recruited immune cells internalise, but rarely destroy bacterial cells and subsequently promote \textit{Mtb} replication within host tissues. During the same period, blood monocytes are released and aggregate at the infection site. The adaptive immune response mounted against \textit{Mtb} invasion is mainly T-cell-mediated [21–23] and is activated in about 2–3 weeks after infection (Fig. 1). Another glycoprotein Apa, is thought to control T-cell immunity through mannose receptors found on the surface of host macrophages [24,25]. Once the antigen-specific T-cells reach the site of infection, they multiply within primary lesions [26] and induce production of proinflammatory cytokines. These cytokines stimulate macrophages to destroy intracellular bacterial cells resulting in growth retardation. The inflammation site (i.e. granuloma) consists of extracellular growth of bacterial cells [27,28]. This particular stage of infection is called the latent phase, in which bacterial replication is inactivated. However, reactivation of \textit{Mtb} [29–31] may occur months or years when the host immune system becomes compromised. Importantly, there are a number of bacterial and host factors that play a key role in resuscitating TB infection and are discussed in sections 2 and 3 of this review. Furthermore, the various mechanisms by which \textit{Mtb} reprograms the host epigenetic processes to evade the host immunity following infection are also examined in section 4. Collectively, these mechanisms enable the pathogen to infect, survive and replicate within host cells while avoiding significant induction of the host immune response.

1.3. Diagnosis of \textit{TB} infection

Routine TB diagnosis entails detection of \textit{Mtb} in the sputum sample called the sputum smear microscopy method:

- The limitation with this method is that it only detects half the number of TB infections,
- It is unable to detect paucibacillary TB in HIV patients, and
- Is incapable of identifying drug-resistant strains [236].

As with most microbial infections, TB can be diagnosed using immunological tests involving the reaction between the host antibodies and \textit{Mtb} antigens. These include:

- \textit{In vitro} assay using the interferon-gamma release assay (IGRA) or
- \textit{In vivo} diagnostic assay using the tuberculin skin test (TST).

Although capable of diagnosing \textit{TB} infection, these two tests are unable to distinguish between active and LTBI [32], and also fail to predict the probability of \textit{TB} reactivation [33]. Due to distinct clinical properties, different methods are used to diagnose active and LTBI.

- WHO recommends TST or IGRA tests for diagnosing LTBI [1].
- Conversely, a number of methods are employed to test for active TB and include chest radiography, sputum smear technique and culture method.

Importantly, molecular techniques such as GeneXpert \textit{MTB/RIF}® [34–37] and line probe assays (LPAs) [38–41] are recommended for diagnosing active TB owing to their increased specificity and ability to identify drug-resistant \textit{TB} strains.

- GeneXpert \textit{MTB/RIF}® detects TB and the rifampicin resistant \textit{rpoB} gene with a turnaround time of about 2 h [34–37,42].
- Moreover, this technique can also be applied to test for paediatric TB and is as such highly recommended for preliminary diagnosis for patients exhibiting clinical symptoms of TB.

In contrast to the GeneXpert \textit{MTB/RIF}® which only identifies rifampicin resistant strains, LPAs have advanced utility as they are capable of detecting various drug-resistant \textit{TB} strains. Specifically, LPAs detect mutations within:

- the \textit{rpoB} gene associated with rifampicin resistance [40,43,44],
- \textit{katG} and \textit{inhA} genes conferring resistance to isoniazid [40,43,44],
- resistance to second-line anti-TB drugs [39], multi drug-resistant (MDR) [38,44] and extensively-drug resistant (XDR) \textit{TB} strains [41].

Furthermore, a rapid urine-based assay that detects lipoarabinomannan (LAM) has been developed for \textit{TB} diagnosis.

- This test detects LAM, a glycolipid secreted from the \textit{Mtb} cell wall [45,46].
- However, it is highly recommended that the LAM assay should not be used as a sole diagnostic tool owing to its low sensitivity of about 56% [47], and as such, must be employed in conjunction with other diagnostic methods.

In a more recent development, researchers have invented a rapid method of \textit{TB} detection.

- This method specifically detects living bacterial cells within minutes and emits fluorescence following incorporation of the trehalose-based dye into the cell membrane.
- However, bacteria treated with antibiotics exhibited decreased fluorescence while no fluorescence was emitted in heat-inactivated bacilli [48].
- Importantly, the degree of fluorescence was comparable between trehalose sugar and Auramine O-stained sputum samples obtained from TB patients.
- Conveniently, the trehalose dye rapidly detects live bacteria while minimising background fluorescence and does not require a washing step during sample preparation [48].
- Thus, this method may be applied as a rapid diagnostic tool to detect active \textit{TB} infection.
- However, further testing is required to validate the reproducibility, sensitivity and specificity of this novel diagnostic technique.

1.4. Prevention and treatment of \textit{TB}

The Bacillus Calmette-Guérin (BCG) vaccine is comprised of a live, attenuated strain of \textit{Mycobacterium bovis} Karlson & Lessel 1970 intended to trigger the host immunity and offer protection against TB and other mycobacterial infections. BCG is also used as a prophylactic agent to prevent tuberculous meningitis [49,50], Buruli ulcer [51,52], bladder cancer [53] and leprosy [54–56]. Traditionally, this vaccine is injected intracutaneously or intradermally, but research has been conducted to assess the advantage of respiratory administration [57]. This may be a useful approach as natural \textit{Mtb} infection occurs via the respiratory route. The BCG vaccine provides 60–80% protection against chronic forms of paediatric TB [49,58] and its activity towards pulmonary infection is depended on the geographical location of vaccinated individuals [59,60]. However, the major setbacks associated with the use of BCG vaccine include:

i. Though vaccination of neonates and infants confers improved protection, this does not extend to adults
ii. Moderate protective efficacy in some vaccinated individuals [59].
iii. Partial protection against chronic pediatric TB
iv. Limited efficacy against lung TB in adult patients
v. Revaccination of young adults, previously vaccinated at birth does not significantly enhance protective immunity [61].

Therefore, this necessitates the development of novel, potent and safe TB vaccine capable of protecting against various forms of TB.
including HIV-TB, pediatric TB, adult TB as well as drug-resistant TB. For this reason, an inactivated whole-cell vaccine comprised of *Mycobacterium vaccae*, has been tested for its ability to serve as an immune booster in HIV patients previously immunised with BCG vaccine [62]. For this trial, only HIV patients with a BCG scar and CD4+ counts of about 200 cells/microlitre were selected. The 1006 participants received 5 intradermal doses of *M. vaccae* while 1007 were in the placebo group. All participants were assessed at 3 months interval for up to 3.3 years. Impressively, the trial was shortened due to high immune protection and slow progression of disseminated TB following *M. vaccae* administration [62]. Importantly, this significant protection was achieved without serious side effects irrespective of viral load or CD4+ cell counts. This study illustrated that administration of *M. vaccae* to HIV patients with childhood BCG vaccination offers improved protection against TB without inducing serious adverse side effects. Therefore, data from this investigation indicates that the inactivated whole-cell vaccine may be employed as a strategy of preventing TB in HIV patients [62].

Since then, a number of TB vaccines have been tested with 12 entering clinical testing and include the MVA85A prophylactic agent that has been assessed in human phase 1 clinical trials [63]. This vaccine is comprised of IMX313, an adjuvant intended to enhance the host adaptive immune response. As such, the MVA85A-IMX313 vaccine was designed to improve immunity triggered by BCG immunisation. For this trial, 30 healthy adult patients previously vaccinated with BCG were selected and immunised with low MVA85A-IMX313 dosage (group A), standard MVA85A-IMX313 dosage (group B), or MVA85A (group C) [63]. Participants were followed for up to 6 months and evaluated for immunogenicity and safety. Data from this clinical trial indicated that both MVA85A-IMX313 and MVA85A triggered a marked elevation in interferon-gamma (IFN-γ).

There was no statistical significance between Ag85A ELISPot and cytokine induction in group B and C during the course of the study and only mild side effects were reported, irrespective of vaccine dosage. Thus, this investigation demonstrated that MVA85A-IMX313 was capable of inducing the host immune response without serious side effects and may be utilised to improve anti-TB immunity in patients previously vaccinated with BCG [63]. Unfortunately, a recent report indicated that the MVA85A vaccine displayed unsatisfactory efficacy and ineffective clearance of bacterial cells in *Mtb*-infected animals relative to BCG alone [64]. Additionally, the majority of data recovered from the MVA85A vaccine study was obtained from a single research group that included 40 primates and 192 animals. It has also been suggested that when the human clinical trial was at an advanced stage of preparation, data revealed that monkeys treated with a combination of MVA85A and BCG displayed high mortality than those treated with BCG alone [65].

Licensed first-line drugs employed to combat TB infection include rifampicin, isoniazid, ethambutol, pyrazinamide, rifapentine or rifabutin [66] (Table 2). Second-line anti-TB drugs include amikacin, kanamycin and capreomycin [67], while the third tier of TB therapeutics is comprised of linezolid, clofazimine, clarithromycin, amoxicillin and clavulanate as well as imipenem and cilastatin [66]. Bedaquiline is used in conjunction with other TB drugs to combat lung TB in adult patients with MDR TB strains [68] (Table 2). Importantly, TB clearance and symptom alleviation requires strict adherence to drug prescription and generally necessitates administration of anti-TB agents for 6 months to 2 years.

Improper use or early cessation of drug administration generally results in re-establishment of bacterial infection and also compromises efficacy by promoting emergence of drug-resistant strains [69]. Currently licensed therapeutic agents are capable of decreasing the risk of TB reactivation by about 60%. These agents have anti-TB efficacy of 60–90% and display sustained activity for up to 19 years [70]. Unfortunately, clinical complications, mainly hepatotoxicity have been reported following administration of some anti-TB regimens (WHO, 2015). The Centers for Disease Control and Prevention recommends the following first-line antitubercular drugs to be taken for 6–9 months to treat active TB infection:

1. Ethambutol
2. Isoniazid
3. Pyrazinamide, and

The approved drugs for LTBI treatment include:

1. 9 months daily administration of isoniazid,
2. 3 months weekly administration of rifapentine and isoniazid
3. 4 months daily administration of rifampicin

(https://www.cdc.gov/tb/topic/treatment/lptbi.htm)

2. Bacterial factors that resuscitate TB infection

There are bacterial factors that play a critical role in LTBI. These factors alter the virulent properties of *Mtb*, so that it invades tissues but remains dormant within infected host cells [71–73]. Due to decreased bacterial replication, the infected host will not develop clinical symptoms associated with TB infection [74]. The earliest resuscitation promoting factor (rfp), was identified in *Micrococcus luteus* and is also secreted by many members of Actinobacteria [75–77]. This resuscitation factor stimulates transition of bacteria from dormant to active phase and also promotes cell growth [75–77]. Rfp is structurally similar to

<table>
<thead>
<tr>
<th>TB drug</th>
<th>Mode of action</th>
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<tr>
<td>Amikacin</td>
<td>Interacts with the 30S ribosomal subunit and prevents protein synthesis</td>
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<tr>
<td>Bedaquiline</td>
<td>Prevents energy production by inhibiting the activity of adenine5′-triphosphate (ATP) synthase</td>
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<tr>
<td>Capreomycin</td>
<td>Binds to the 70S ribosomal subunit and inhibits protein synthesis</td>
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<tr>
<td>Clofazimine</td>
<td>Binds to Mtb DNA leading to growth retardation</td>
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<tr>
<td>Cycloserine</td>
<td>Alters the function of α-alanine in the Mtb cell wall resulting in impaired cell wall synthesis and decreased growth</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>Inhibits production of essential metabolites, leading to impaired metabolism and subsequent cell death</td>
</tr>
<tr>
<td>Ethionamide</td>
<td>Inhibits production of mycolic acid leading to disruption of Mtb cell wall</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>Inhibits synthesis of mycolic acid leading to bacterial cell wall disruption</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>Antagonizes the activity of DNA gyrase and topoisomerase IV, which are essential for DNA replication, transcription, recombination and repair</td>
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<tr>
<td>Moxifloxacin</td>
<td>Interacts with the A subunit of DNA gyrase, leading to blockage of DNA replication and transcription</td>
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<tr>
<td>Pap</td>
<td>Blocks the production of folic acid leading to impaired bacterial growth. This drug also inhibits production of mycobactin, causing iron depletion</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>Interacts with the ribosomal protein S1 (RpsA) to inhibit translation. This regimen also prevents fatty acid synthesis.</td>
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<tr>
<td>Rifabutin</td>
<td>Inhibits activity of RNA polymerase, which catalyses chain initiation</td>
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<tr>
<td>Rifampicin</td>
<td>Inhibits the activity of RNA polymerase required for chain initiation</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>Prevents chain initiation by inhibiting the activity of RNA polymerase</td>
</tr>
<tr>
<td></td>
<td>Binds to the 30S ribosomal subunit and inhibits protein synthesis</td>
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lytic transglycosylase enzymes [78] and C-type lysozyme and display maturalytic activity [75]. In a study conducted by Mukamolova et al., 2006 a mutation was introduced into the glutamate moiety [75]. This led to a significant decrease in resuscitation and peptideglycan hydrolytic properties of rpf and highlighted the importance of this resuscitation factor in cell wall degradation and growth stimulation [75].

*Mtb* has five homologues of *rpf* genes (*rpfA-E*) [79] which can be expressed in cell culture and animal models [80]. Interestingly, some of these *rpf* genes have been shown to participate during TB infection in humans [81]. Importantly, the *Mtb* *rpfA-E* genes function as a unit to promote bacterial growth in *vitro* and within infected host cells [80,82]. The *rpfA-E* complex stimulates *Mtb* transition from inactive to active phase and this process involves hydrolysis of the peptidegycan layer. In another study, individual *rpf* genes or mutated strains displayed delayed bacterial growth in cell culture and mice relative to the *ΔrpfA-E* strain and their growth was comparable to those of the wild type *Erdman* model were disrupted and the growth patterns of resulting deletion mutants were viable and their growth was comparable to those of the wild type *Erdman* strain in *vitro* and in *vivo*. Collectively, these findings suggest that although *rpf* gene is required for resuscitation in *M. luteus*, introduction of mutations within this gene does not seem to significantly alter *Mtb* growth kinetics in cell culture and mice [80].

In addition to the *rpf* proteins, the cAMP receptor proteins (Crps) play a critical role in modulating a wide variety of physiological processes in bacterial cells [84]. These include regulation of aerobic and anaerobic respiration, expression of virulent genes, nitro-gen fixation, denitrification, bacterial luminescence and some stages of nitrogen, carbon and sulphur metabolism [84]. Additionally, Crps are transcription factors that regulate transcription of cAMP by interacting with target promoters when there is a decline in cellular cAMP levels. A study was conducted to investigate the impact of deleting the *rv3676* gene which encodes a transcription factor for Crp protein in *Mtb*. The generated mutant resulted in impaired growth in cell culture and mice [84]. From the *in vitro* *Mtb* strain, microarray analysis revealed that 16 genes were downregulated in the mutant, but not in the wild type. Amongst these, 12 had sequences that were related to the consensus Crp binding domain, thus, suggesting the action sites of *rv3676*. Genes with improved expression profiling in the wild type included *ahpC*, *rpfA*, *whiB1* and *lprQ* [84]. In contrast to the wild type, activity of the *rpfA::lacZ* promoter complex was markedly decreased in the mutant. Moreover, specific interaction was observed between the product of the *rv3676* gene and *rpfA* promoter sequence, while no interaction was observed between the promoter and the mutant strain. Data gathered from this study indicates that *Rv3676* is essential for reactivation of dormant *MtB*, and that mutation of this gene leads to restricted growth and poor interaction with the promoter [84]. Ultimately, this results in impaired bacterial replication within host tissues.

Differential gene expression has also been observed in the *MtB* mutant strain devoid of the Crp. Thus, widespread transcriptional modulations were induced in the mutant strain during the log and death phases of bacterial growth [85]. Additionally, RNA sequencing also revealed subtle changes in the expression of genes containing the Crp binding site, while gene expression profiling in the mutant strain was associated with repression and binding, and resembled Crp modulation in *Escherichia coli* [85]. Since *Mtb* produces large amounts of cAMP which prolongs the bacterial survival in host macrophages, this prompted an investigation into the precise function of cAMP during *Mtb* pathogenesis [86]. In this investigation, the expression of 16 cyclases was studied and analysed under normal and stress-induced growth conditions. cAMP expression was significantly upregulated in response to heat stress, while stress conditions such as low pH, oxidative or nitrosative did not significantly alter cAMP levels [86]. Moreover, a marked increase in the expression of five cyclases following exposure to heat stress confirmed that cAMP plays a crucial role in regulating *MtB* response during heat stress conditions. Moreover, microarray data revealed that cAMP controls expression of genes such as *dnaJ*, *dnaK*, *grpE* and *Rv2025c*, all of which are induced by heat stress. The electro-phoretic mobility shift data highlighted that Crp recognises and binds to the 5′untranslated region of *dnaK* gene to modulate *MtB* survival under stress conditions [86].

In a different investigation, it was demonstrated that *Rv3676* Crp controls the expression of *whiB1* and *rpfA* genes that have been implicated to promote bacterial survival and TB reactivation [87]. Mechanistically, this *MtB* Crp has been shown to bind to two cAMP molecules at non-interacting regions, resulting in weak interactions that subsequently leads to moderate DNA binding. Two tandem Crp binding sites have been identified at the *whiB1* promoter region and include *Crp1* at −58.5 and *Crp2* at −37.5. Specific functions of these two Crp binding sites were elucidated following *in vitro* transcription studies that revealed that the *Crp1* site is involved in activation, while *Crp2* plays a crucial role in repression [87]. Therefore, these observations suggest that binding of Crp to *whiB1* promoter Crp1 induces transcription, however, high intracellular cAMP levels allows occupation of *Crp1* and *Crp2* sites and leads to repression. These features enable the *MtB* Crp to drive gene expression under normal and high cAMP concentrations [87].

Some resuscitation factors such as the Clp protease gene regulator, *Rv2745c* (clgR) are triggered by environmental stress [88]. Indeed, the *MtB-Rv2745c* mutant strain displays different physiological and biological characteristics once oxygen has been reintroduced into the growth medium. Genetic analysis confirmed differential gene expression between the *MtB-Rv2745c* mutant and wild-type strain. Furthermore, differential expression of genes within the DosR and *o^-t-o^-r* regulons was also reported in the mutant strain [88,89]. The upregulated genes include genes involved in transcription, protein synthesis and oxidative phosphorylation, all of which play a crucial role in promoting bacterial replication and survival within host tissues [90]. *Rv2745c* was also observed to be induced under low oxygen and reaeration conditions, thus, reinforcing the role of the clgR in establishing TB infection and reactivation. This illustrated the critical role that clgR plays in inducing Clp protease activity to effect protein degradation and *MtB* survival under stressful conditions. Therefore, induction of *Rv2745c* under different stress conditions leads to differential gene expression, which promotes *MtB* adaptation and survival within infected host cells [90].

An investigation was conducted to evaluate *MtB* proteomic reorganisation under exponential growth, dormancy and resuscitation [30]. Data from this undertaking revealed that under dormancy, the DosR regulon controls the expression of about 20% cellular proteins, while ribosomal protein levels remained consistent at 5–7%. These newly-acquired protein alterations may greatly affect the activity of cellular enzymes. Consequently, studying protein variations under different stress conditions may provide critical insight for drug [30] and vaccine development. A study focusing on *MtB* transcriptomic profiles during reactivation from hypoxia indicated that, reintroduction of oxygen induced a lag phase in which cellular metabolic and physiological functions preceded the onset of bacterial replication [89]. Specifically, the lag phase during reaeration was accompanied by repression of regulons required for bacterial persistence and included ClgR, DosR, MprA, sigma factors such as SigE and SigH, as well as metabolic
pathways involved in lipid uptake and catabolism. Conversely, an up-regulation of genes associated with metal transport, DNA repair and recombination and production of major cell wall components was also observed [89]. These findings indicated that reactivation has a significant impact on the Mtb lag phase by inducing differential gene expression which ultimately affects protein synthesis and functional characteristics [89].

In a similar study, the expression of 51 genes was studied in a dormant Mtb strain over a 40 day period using a Wayne culture model [91]. Findings emanating from this investigation revealed that the expression of many metabolic genes and certain sigma factors remained constant. However, genes associated with the dormancy regulon were upregulated at day 9, particularly the alpha-crystallin mRNA levels, which displayed more than 1000-fold increase relative to bacteria in the active phase of replication [91]. Interestingly, genes associated with persistent hypoxic response were upregulated at day 16 and included the sigma B and E transcription factors. During the same time frame, a significant downregulation of the fbpB gene involved in lag phase during dormancy was also observed. Expectedly, introduction of oxygen induced widespread gene expression in dormant Mtb cells within an hour and included resuscitation-promoting factors [91]. This study indicated differential gene regulation and functional characteristics within Mtb cells under hypoxic and oxygen conditions and may serve as a benchmark for therapeutic development.

Chief among bacterial resuscitation factors is the ability of Mtb to harbour various mutations that induce drug resistance. In recent years, different Mtb resistant strains have been described and include: MDR strains that are resistant to first-line anti-TB drugs such as rifampicin and isoniazid [92] and XDR strains that confer drug resistance to rifampicin, isoniazid, any fluoroquinolone and at least one of the 3 s-line drugs (e.g. capreomycin, kanamycin and amikacin) (WHO, 2018). Therefore, relative to people with susceptible TB, patients with MDR or XDR TB require a longer treatment schedule and must utilise more expensive second-line anti-TB drugs which have more side-effects compared to first-line TB therapeutics. Furthermore, qualities such as Mtb metabolism and cellular structure, ability to evade immune recognition and long-term monotherapy have also been implicated in driving bacterial adaptation, survival and subsequent drug resistance [93,94]. Other factors include non-compliance to the treatment schedule, drug misuse and incorrect prescription. Therefore, this necessitates the use of different anti-TB agents (i.e. combination therapy) to treat TB infection. This approach is advantageous as different drugs target various Mtb regions at the same time. This significantly decreases bacterial replication, which in turn, allows the host immunity to neutralise the microbial pathogen and improves patient recovery. Importantly, simultaneous targeting and inhibition of different Mtb cellular processes also reduces the emergence of drug resistant TB strains. Consequently, combination therapy generally leads to complete clearance of TB infection within 6–9 months, provided a strict treatment plan is adhered to.

Numerous mechanisms are employed by Mtb to induce genetic mutations that confer TB drug resistance. For example, rifampicin acts by inhibiting the activity of RNA polymerase which is required for chain initiation during transcription. This drug is active against both replicating and non-replicating TB strains [95]. The vast majority of rifampicin resistant strains have mutations in the rpoB gene that encodes the RNA polymerase β-subunit. This mutation induces conformational changes that negatively affects binding between the drug and bacterial cells and ultimately leads to drug resistance [96]. Specifically, mutations within the 507–533 codons [97], 516, 526 and 531 codons of rpoB gene have been implicated to be associated with rifampicin resistance [98,99]. Sequencing studies identified mutations within the rpoA and rpoC genes that code for the α- and β- RNA polymerase subunit in rifampicin resistant strains [100].

Another first-line anti-TB agent isoniazid acts by inhibiting the synthesis of mycolic acid and leads to disruption of bacterial cell wall. Unlike rifampicin which inhibits both replicating and non-replicating bacilli, isoniazid is only active against replicating bacterial cells. Resistance to this drug is as a result of mutations in genes such as aphC, inhA, katG, kasA and NDH. However, the key molecular mechanism responsible for isoniazid resistance is mutations within the katG and inhA genes or its promoter sequence [101,102]. The leading genetic mutation is the S315T within katG and results in the formation of an isoniazid product incapable of producing the isoniazid-NAD adduct required to accomplish antibacterial activity [103]. Another common mutation occurs within the inhA promoter region and results in a significant increase in the expression of inhA. Alternatively, this mutation may also occur within the promoter active site and results in decreased affinity for the isoniazid-NAD adduct [103]. Importantly, due to structural similarities between isoniazid and ethionamide, mutations in the inhA gene confer drug resistance to both these TB agents [104–106].

Selected second-line anti-TB therapeutics that have been rendered ineffective due to Mtb drug resistance include amikacin, kanamycin, capreomycin, fluoroquinolones and viomycin. Amikacin exerts its anti-TB activity by interacting with the 30S ribosomal subunit to prevent protein synthesis. The leading mutations associated with amikacin resistance are located at position 1400 and 1401 of the rrs gene. Mutations occurring at position 1483 [107,108] and within the eis promoter region have also been implicated in conferring drug resistance [109]. Generally, genetic mutations responsible for amikacin drug resistance also induce resistance to kanamycin owing to their similar mode of action and structural similarities [107,108]. On the other hand, capreomycin and viomycin bind to the 70S ribosomal subunit to prevent translation and are also structurally similar. The tlyA protein is an RNA methyltransferase enzyme that catalyses methylation of ribose sugar in ribosomal RNA. Therefore, mutations within the tlyA gene generates a tlyA protein devoid of methylation activity [110] and enhances Mtb drug resistance and survival in infected individuals.

Fluoroquinolones such as moxifloxacin bind to the A subunit of DNA gyrase to inhibit DNA replication and transcription. Interestingly, only type II topoisomerase enzyme (DNA gyrase) exists in Mtb and serve as the only target for fluoroquinolone drugs. This enzyme catalyses supercoiling of DNA molecule and is comprised of α and β subunits encoded by gyrA and gyrB genes, respectively. The common mechanism with which Mtb causes resistance to fluoroquinolones drugs is by inducing chromosomal mutations within the gyrA or gyrB gene regions. The majority of these mutations are located at position 90 and 94 of gyrA although mutations occurring at position 74, 88 and 91 have also been associated with conferring drug resistance towards fluoroquinolones [111,112]. Conversely, the presence of A90G and T80A mutations in gyrA has been linked with hypersusceptibility to various anti-TB fluoroquinolones [113].

Moreover, drug resistance has also been reported in new anti-TB regimens such as bedaquiline and delamanid. Bedaquiline was previously known as R207910 or TMC207 and acts by inhibiting the activity of adenosine 5′-triphosphate (ATP) synthase enzyme which is crucial for energy production. Bedaquiline is the first anti-TB therapeutic to target ATP synthase, thus making its mode of action unique. Nevertheless, the evasive Mtb bacterium has induced mutations that render the novel bedaquiline drug ineffective. Consequently, mutation in the apéE gene which codes for the F0 subunit of ATP synthase has been confirmed to be responsible for bedaquiline resistance [114]. The most common mutations found within the apéE gene among resistant strains are A63P, I66M, D28A/G, D32V, E61D and L59V [115]. Specifically, bacterial strains harbouring these mutations are capable of inducing drug resistance by interfering with binding of bedaquiline to ATP synthase. However, it has been reported that other mechanisms independent of apéE mutations may be responsible for drug resistance [116].

Another new anti-TB regimen is delamanid which has been demonstrated to be potent in inhibiting both susceptible and resistant TB strains [117,118]. Similarly to isoniazid, delamanid exerts anti-TB
activity by inhibiting production of mycolic acid. However, a mutation in the Rv3547 gene has been shown to render Mtb resistant to this anti-TB therapeutic [117]. Additionally, mutations within the fbiA, fbiB, fbiC, ddn and fgd1 genes have been implicated in conferring delamanid resistance in Mtb clinical isolates [119]. The fbiA, fbiB and fbiC gene cluster codes for coenzyme F420 which plays a crucial role in catalysing cellular redox reactions. Similarly, fgd1 codes for glucose-6-phosphate required for redox cycling reactions, while ddn encodes an F420-dependent nitroreductase that catalyse conversion of delamanid into desnitro form.

3. Host, comorbidities and environmental factors involved in TB resuscitation

In addition to bacterial factors, host immune competence, genetics, comorbidities and environmental factors play a major role in determining TB susceptibility or resistance. This section will discuss how these factors contribute in reactivating TB infection in host cells.

3.1. Host immune system

Upon establishing infection, the presence of Mtb in host cells is first detected by the innate immune system and at a later stage by the adaptive immune response involving T-cell immunity to combat infection [120]. Innate immune response directed against Mtb invasion mainly involves production of macrophages and intracellular signaling mechanisms [121]. Macrophages are mainly involved in phagocytosis (i.e. engulfing the causative agent); and this early phase is initiated by Toll-like receptors (TLRs) which recognise foreign antigens, leading to innate immune stimulation and subsequent secretion of macrophages. Specifically, TLR2 has been implicated in recognising bacterial antigen patterns and stimulating macrophage production [121], while TLR2 genotype T597C is involved in the recognition of Mtb antigens associated with TB meningitis [122]. A cell signaling protein such as Tu mour Necrosis Factor-α (TNF-α) has also been implicated to play a crucial role in TB modulation. In studies where patients [123] or non-human primates [124] were administered anti-TNF-α agents, a significant increase in Mtb dissemination was reported, thus, confirming the importance of TNF-α in bacterial regulation.

The adaptive immune response constitutes the second barrier of the immune response and can be divided into humoral and cell-mediated immunity. Humoral immunity involves production of antibodies, while cell-mediated immunity activates specialised cells to combat infection. Cell-mediated immunity, particularly T-cell mediated immunity is highly involved in regulating Mtb replication and dissemination within host tissues [21–23,120]. Specialised cells that participate in T-cell-mediated immunity include type 1 helper and type 2 helper cells and secrete proteinaceous molecules such as chemokines and cytokines [121,125–127]. CD4+ cells are the major cells involved in T-cell-mediated immunity [128] and mediate the production of cytokines [129]. An example of a cytokine involved in T-cell mediated immunity is IFN-γ, which stimulates alternate phagocytosis [130], antigen presenting cells (APCs) [129] and reactive nitrogen derivatives [131]. Furthermore, this cytokine promotes expression of the major histocompatibility complex (MHC) [129] and also regulates secretion of other cytokines [132]. Mtb can unintentionally elicit T-cell mediated immunity following:

i. Inhibition of macrophage-associated apoptosis [133],
ii. Escape of macrophage signaling pathways involving IFN-γ or
iii. Bypassing of other intracellular signaling pathways (mostly involving macrophages) [133].

3.2. Genetics and genetic mutations

Genetic mutations also contribute immensely in influencing the resuscitation of TB infection in host cells. The interferon-γR1 (IFN-γR1) mutation has been identified in children with extensive Mtb infection. This indicates that a mutation within the IFN-γR gene may promote the spread of TB infection [134]. Genetic mutations in the gene encoding interleukin-12 (IL-12) receptor β1 have been reported in individuals with persistent, chronic and disseminated Salmonella and Mtb infections [135]. Devastatingly, these individuals also displayed a marked decrease in the secretion of IFN-γ from both T- and Natural Killer (NK)-cells [135]. In an investigation conducted by Hoal-Van Helden et al., 1999, it was revealed that a genetic polymorphism in the gene encoding the Mannose Binding Protein (MBP) stimulated the dissemination of TB meningitis in the South African cohort [136]. Mtb dissemination was due to a significant reduction in the secretion of MBP, which is essential in countering TB meningitis [136]. Furthermore, it has been reported that genetic mutations in natural resistance-associated macropage protein1 (NRAMP1) [137], IFN-γ [138] and IL-1p/IL-1R [139] were implicated in pleural TB infection, while a defect in the P2X7 gene has been shown to exacerbates extra-pulmonary TB [140]. The role of genetics in TB resuscitation is further reinforced by a high incidence of concomitant infection between monozygotic (about 60%) relative to dizygotic twins (about 35%) [141] and increased extra-pulmonary infection in non-Caucasian groups [142]. Genetics may also be responsible for the increased disparity in IGRA and TST tests among children [143], infection rates and disease progression [84].

Genetic factors associated with TB susceptibility have been investigated using genome-wide linkage analysis (GWAS). GWAS has identified particular regions of the chromosomes (e.g. 2q21-2q24 and 5p13-5q22) to be responsible for producing consistent TST negative results [144]. In contrast to GWAS data, a high prevalence of IN10 promoter haplotypes is associated with a marked decline in the production of IN10 among TST positive patients [145]. Another GWAS-based study identified the “desert gene” located on chromosome 18 as a potential resuscitation factor for pulmonary TB and a locus on chromosome 11p13 as essential for protecting against development of pulmonary infection in the Ghana and Gambia cohorts [146]. Interestingly, these observations were not replicated when conducted in other population groups [147] and highlighted the critical role of genetics in the resuscitation of TB infection [84]. Genetic defects in the mononuclear phagocyte/T-helper cell type1 (Th1) have been observed in children with systemic TB infection [148]. Furthermore, primary immunodeficiencies coupled with multiple gene mutations that include IL12B, IL12BR1, ISG15, STAT1 and IFNGR1 were also reported in these children [149,150]. It has been proposed that mutations in TLR1, TLR2, Human Leukocyte Antigen (HLA) and vitamin D encoding genes are responsible for resuscitating pulmonary TB in adults [151].

A meta-analysis study revealed that mutation of the NRAMP1 gene was strongly associated with pulmonary TB in patients of Asian and African descent, but not the European population group [152]. This NRAMP1 gene polymorphism has also been documented in early TB disease in many infected children. This was further confirmed by the discovery of multiple NRAMP1 gene alleles in children relative to adults [153]. In addition to NRAMP1, various TOX gene alleles have also been implicated in the development of early pulmonary TB infection [154]. Recent data confirmed the important role played by genetics in determining various clinical properties including drug-resistance and susceptibility [155–157]. In addition to host immune system and genetics, other factors that determine Mtb susceptibility or resistance include exposure time, strain virulence, assorted comorbidities, patient age and environmental factors.

3.3. Comorbidities

Comorbidities play a major role in TB exposure, dissemination, resistance or susceptibility and host immune competence.
3.3.1. HIV-TB co-infection

HIV-positive patients are at a higher risk of acquiring active TB infection owing to their compromised immune systems [158]. Globally, Sub-Saharan Africa has the highest HIV incidence [159] and the majority of these individuals either have HIV-TB co-infection or are at a risk of developing active TB. Previous studies have documented that HIV-TB co-infection significantly increases the risk of TB reactivation [160], dissemination and development of clinical symptoms [161]. Additionally, investigations performed in regions with a high HIV burden demonstrated that temporal and spatial differences in TB prevalence were correlated with HIV prevalence [158,162]. This is because in patients with co-infection, TB stimulates HIV replication in infected organs while HIV increases TB chronicity [163-167]. The weak immune system (both innate and adaptive immune responses) promotes the interplay of these pathogens within infected host tissues, thus, resulting in disseminated TB and HIV infection [168].

Apart from patients with HIV-TB co-infection, the application of TNF inhibitory molecules as therapy for autoimmune diseases has also been identified as a risk factor for acquiring active TB infection [169]. These findings were corroborated by studies that demonstrated that TNF was an essential component of the immune response involved in the regulation of various microbial infections [170,171]. Consequently, it is important to screen for LTBI prior to utilizing anti-TNF agents as therapy for autoimmune diseases [123].

3.3.2. Diabetes

People with diabetes have a higher risk of contracting active TB infection [172]. The majority of these individuals are located in Sub-Saharan Africa and India and account for about 70% of the global diabetes prevalence [173]. In a study conducted by Jeon et al., 2008, it was reported that people with diabetes were three times likely to acquire TB infection relative to non-diabetic individuals [174]. Moreover, another study revealed that non-diabetic individuals had 6.9% smear-positive culture following completion of TB therapy relative to 22.2% positive results of patients with diabetes and TB [175]. Diabetes affects the functioning of the host immune response, thus, promoting development of active TB. Data obtained from animal and human studies indicated that diabetic mice exposed to Mtb had significantly higher bacterial burden relative to non-diabetic animals [176], while the secretion of IFN-γ and several cytokines weakened adaptive immunity in diabetic as compared to non-diabetic patients [177]. Additionally, TB is also responsible for inefficient glucose metabolism and glycemic regulation in diabetic individuals [178].

The mechanisms with which diabetes increases the likelihood of acquiring TB may be directly associated with hyperglycemia and inadequate secretion of insulin. Alternatively, diabetes may indirectly induce TB development by altering the function of lymphocytes and macrophages [178]. Diabetes specifically induces the development of pulmonary infection and not extrapulmonary TB [179]. TB-diabetes is prevalent in men and adult patients, and in patients with a higher body mass index. Importantly, treatment relapse and failure as well as mortality are markedly higher in patients with TB-diabetes than in non-diabetic TB patients [179]. Globally, it has been estimated that the number of individuals with TB-diabetes is comparable to the number of patients with HIV-TB co-infection [180]. In 2013, an estimated 1 million TB cases were correlated with diabetes and 15% of TB adult patients were also diagnosed with diabetes. Global prevalence of TB-diabetes was documented to be higher in India (302 000), China (156 000), South Africa (70 000), Indonesia (48 000), Pakistan (43 000), Bangladesh (36 000), Philippines (29 000), Russia (23 000), Burma (21 000) and Democratic Republic of Congo (19 000) [180, 181].

3.3.3. Malnutrition

Malnutrition can accelerate the onset or dissemination of active TB by depriving the body of useful nutrients that may otherwise participate in the host immune response [182]. Devastingly, TB can also induce malnutrition by altering the host metabolism, subsequently leading to loss of appetite [183]. In a BCG vaccine clinical trial conducted by Comstock et al., it was shown that underfed children were two times likely to develop TB as compared to their nourished counterparts [184]. In a separate study, it was reported that malnourished adults were more likely to acquire active TB relative to nourished individuals [185].

3.4. Patient age and environmental factors

3.4.1. Children

Children are more likely to contract TB infection than adults and previous investigations demonstrated that 60–80% of children become infected following exposure to a sputum smear-positive source [186,187]. It has also been reported that most children under the age of 2 years acquire TB from household sources, while older children mainly contract infection from the community. Sputum contaminated with Mtb is the primary source of infection for older children [188]. Most of the TB clinical symptoms manifest during the first year after primary infection, and children exposed to Mtb before the age of 2 have an increased risk of developing disease symptoms. However, the greatest risk of TB mortality in children is during the infancy years [189].

3.4.2. Smoking and alcohol

Previous studies have been conducted pertaining to the association between TB and smoking [190,191]. It has been shown that the probability of contracting TB was markedly higher in smokers than non-smokers [192]. Furthermore, individuals with active infection have a greater risk of TB-associated mortality [192]. It has also been noted that exposure to smoking (i.e. second hand smoking) may also contribute to contracting infection; however, smokers are at a greater risk of developing active TB infection [193]. Smoking exacerbates TB by weakening both the innate and T-cell mediated immunity by:

i. Impairing the activity of macrophages [194],
ii. Preventing efficient removal of mucosal secretions [195].
iii. Inhibiting the secretion of CD4+ cells owing to the nicotine component of cigarettes [194].

These attributes have also been implicated in the development of pulmonary TB among smokers [190]. Shang et al., 2011 indicated that mice infected with Mtb following exposure to cigarette smoke had a significantly higher bacterial load in the spleen and lungs, coupled with a marked decline in adaptive immune response relative to mice that were not exposed to cigarette smoke [196]. This observation inevitably confirmed that smoking increases the chronicity of TB infection by impairing both the innate and adaptive immune responses. Consumption of alcohol has also been strongly associated with active TB contraction and transmission in both low and high prevalent regions [197]. Additionally, it has been documented that the risk of acquiring active TB infection is markedly higher in people who consume more than 40 g (i.e. 40 ml) of alcohol on a daily basis and in individuals who have alcohol addiction [198]. As with smoking, alcohol has also been shown to exacerbate TB by weakening the host innate and adaptive immune signaling [199].

4. The role of epigenetics in TB infection

Epigenetics is the study of changes in gene expression that may be heritable, but does not change the DNA sequence. Epigenetic processes are involved in chromatin dynamics which subsequently controls various cellular mechanisms (Fig. 2). These include gene expression, DNA repair and recombination. Epigenetic changes may be inherited during cell division and may potentially be modified or deleted during development and differentiation. These genetic alterations occur as a result of different modifications on the DNA and histone proteins (Fig. 2). These modification processes are related to chromatin organisation and
dynamics and include ubiquitylation, phosphorylation, acetylation and methylation [200,201]. Importantly, these modifications must first participate in DNA replication to allow transmission to the offspring (i.e. heritability) (Fig. 2). A variety of microbial pathogens regulate these epigenetic processes to evade host immunity and cause diseases [202,203].

Previous studies have demonstrated the ability of microorganisms to regulate the host transcriptional process by introducing modifications in critical cellular mechanisms such as apoptosis as well as modification of genes involved in immunity and survival [204,205]. Evidently, microbial pathogens are capable of evading and reprogramming the host cellular mechanisms and transcription factors. It has also been documented that several bacteria are capable of inhibiting, degrading, inducing or modifying transcription factors and other important cellular proteins, thus, allowing evasion of the host immune response [204]. Similarly, Mtb is capable of reprogramming the host cellular machinery, leading to evasion of both the innate and adaptive immune responses followed by establishment of infection and dissemination. Specifically, Mtb reprograms epigenetic mechanisms by inducing changes in histone modification, DNA methylation and expression of non-coding RNA molecules.

4.1. Mtb infection alters histone modification

There is a wide variety of histone modifications which occur on different sites during transcription and include proline isomerisation, lysine, serine and poly-ADP ribosylation. These modifications alter the organisation, dynamics and function of the chromatin [201]. Active chromatin is associated with post-transcriptional modification in histone 3 lysine 4 residue trimethylation, while an inactive chromatin is correlated with histone 3 lysine 9 residue trimethylation [206]. These covalent chromatin modifications are mediated by various cellular enzymes and include histone methyltransferases (HMTs), histone acetyltransferases (HATs), histone deacetylases (HDACs), histone demethylases (HDMs), kinases, and phosphatases [207]. Histone acetylation catalysed by HATs is generally associated with activation of the chromatin by increasing the space between nucleosomes. Conversely, histone deacetylation performed by HDACs results in silencing of target gene expression [208]. Collectively, histone modifications are complicated and function in relation with other epigenetic processes to

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**Fig. 2. Epigenetic modifications.** (A) DNA methyltransferase catalyses the transfer of methyl to the DNA molecule. Subsequently, DNA undergoes epigenetic modification after addition of methyl to the cytosine bases. These modifications may participate in DNA replication and transcription, and may potentially be transmitted to the offspring. (B) Properties of histone proteins can be modified by addition of methyl (red), acetyl (blue) or phosphoryl moiety (orange). These epigenetic modifications participate in the reorganisation of the chromatin structure and transcriptional control. N represents the N-terminal tail modifications. The diagram (B) has been adapted from (www.whatisepigenetics.com/histone-modifications/) with some modifications. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
modulate gene expression.

To counter *Mtb* infection, activated T- and NK- cells produce significant amounts of IFN-γ. This in turn, activates the expression of MHC class II in various cell types. Binding of IFN-γ to the cell surface receptor stimulates the Janus tyrosine kinase-signal transducer and activator of transcription1 (JAK-STAT1) and regulates the transcription of genes such as CIITA transactivator. This transactivator participates in the transcription of MHC class II genes. Microbial evolution has enabled *Mtb* to prevent expression of genes such as CIITA, CD64 and HLA-DR, all which are elicited by IFN-γ to combat microbial invasion. It has been reported that *Mtb* regulates chromatin dynamics and histone modification at specific promoter sites to prevent efficient expression of these immune genes [209]. Inhibition of CIITA gene expression is as a result of histone deacetylation at the corresponding promoter site and attenuation of SWI/SNF interaction. This is followed by interaction between the transcriptional repressor C/EBP and CIITA promoter. It is thought that a 19 kDa protein may be responsible for triggering a sustained TLR2 signaling which leads to activation of various transcription factors [210].

Another study reported that prolonged immune stimulation directed against the *Mtb* susceptible strain (CDC1551) was capable of clearing the pathogen from host cells. Conversely, the *Mtb* resistant strain (H3N78) was able to evade the host immune signaling pathways and attenuate gene expression, which enabled the pathogen to survive and replicate within host cells [211]. This reinforced the ability of *Mtb* to reprogram the host epigenetic processes to evade immune detection and achieve optimal intracellular survival. Therefore, this strategy is beneficial with regard to bacterial survival and dissemination within host cells and has been employed by other microbial pathogens such as *Streptococcus bovis, Chlamydia pneumoniae, Campylobacter rectus, Helicobacter pylori* and hepatitis viruses.

### 4.2. *Mtb* infection alters DNA methylation

DNA methylation is essential for a wide variety of cellular mechanisms such as development, differentiation, gene silencing, reprogramming and stimulation of different diseases [212]. Methylation of CpG sites leads to silencing of gene expression by inhibiting the interaction between DNA binding or transcription factors and their respective binding sites on the CpG islands. Methylation of DNA promoter or enhancer regions generally results in transcriptional silencing or repression. In contrast to histone modification, DNA methylation introduces stable epigenetic alterations. These alterations are mainly irreversible and may lead to prolonged target gene silencing [212]. It has been shown that utilisation of BCG to initiate intracapillary epigenetic reprogramming in severe combined immunodeficient (SCID) mice led to prolonged survival relative to unvaccinated mice.

Impressively, this vaccine was also capable of reprogramming mononuclear phagocytes by using nucleotide-binding oligomerisation domain-containing protein2 (NOD2) to introduce epigenetic modifications at the level of histone H3 trimethyl lysine4 (H3K4me3) [213]. In a recent study, a marked variation in methylation profiles of inflammatory genes was reported in human macrophages following *Mtb* infection [214]. The promoter region of IL-17 receptor gene possessed the highest degree of methylation relative to macrophage receptors and other members of the IL-17 family. Subsequently, these signature methylation patterns may be utilised for *Mtb* diagnosis [214]. However, the degree of methylation induced within host cells depends on the virulence of *Mtb* strain, genotype and host immune competence.

The ability of *Mtb* to induce histone methylation and acetylation has also been evaluated [215]. In this study, *Mtb* triggered a significant decline in H3K4 methylation and CIITA pl acetylation in host macrophages following *Mtb* infection. Moreover, suppression of type I and IV CIITA was modulated by ESAT-6, using various regulatory mechanisms. Suppression of type IV CIITA expression was dependent on TLR2, while inhibition of type I CIITA expression was independent of TLR2 [215]. Data obtained from this study suggests that *Mtb* is capable of simultaneously inducing both methylation and acetylation in infected host cells, thus, indicating the versatility of this pathogen in effecting intracellular epigenetic modifications. The importance of DNA methyltransferase enzyme called MamA present in *Mtb* has been investigated. Suppression of this enzyme results in a global decline in gene expression. *Mtb* strains lacking the MamA protein are viable in cell culture, but are unable to survive under low oxygen conditions. This indicated that epigenetic modifications, in this case methylation enable *Mtb* to survive under drastic environmental conditions [216].

Various population groups exhibit different methylation profiles and CpG islands in the vitamin D receptor gene promoter after *Mtb* infection. Consequently, *Mtb* susceptibility is dependent on the degree of methylation and may potentially serve as a diagnostic tool to assess TB susceptibility or resistance [217]. Distinct acetylation has also been implicated in the overexpression of Matrix Metalloproteinases 1 (MMP 1) enzymes upon TB infection. Ghosh et al., 2016 demonstrated that the *Mtb* HU protein is modulated by post-translational histone modifications [218]. Acetylation of *Mtb* HU protein led to a significant decline in DNA binding, DNA compaction and genome decompaction. This data illustrated that acetylation-mediated epigenetics may allow *Mtb* to alter DNA binding and compaction properties within host cells, thus, facilitating reprogramming of cellular processes to promote its survival within infected host cells [218].

### 4.3. *Mtb* infection regulates expression of non-coding RNAs

The microRNAs (miRNAs) participate in various cellular processes such as oncogenesis, differentiation and apoptosis [219]. Additionally, these non-coding double-stranded RNA molecules also play a critical role in regulating gene expression during the RNA interference (RNAi) pathway. This RNAi mechanism is responsible for regulating gene expression in different eukaryotic organisms. *Mtb* infection may also affect miRNA expression profile within host tissues. A genome wide expression study revealed a marked variation in miRNA expression in human dendritic cells after *Mtb* infection [220]. Importantly, the miRNA molecules also participate in the regulation of chromatin structure dynamics, apoptosis and cell growth [221].

Previously, the role of miRNAs in TB infection has been assessed in human patients [222]. Relative to non-TB patients, a significant number of miRNAs were overexpressed in the serum recovered from active TB patients [222]. This data revealed that miRNAs are involved in reprogramming the host cellular machinery leading to development of active TB infection. Furthermore, it has been shown that BCG is capable of suppressing the expression of NK, CD4⁺ and CD8⁺ cells as well as miRNA-29 in macrophages infected with *Mtb* [223]. This reprograms various host cellular processes that stimulate the survival of *Mtb* within infected cells. Collectively, these studies confirmed that miRNAs effect epigenetic changes to reprogram the host cellular signaling pathway, thus, allowing survival and dissemination of *Mtb* in infected cells.

### 5. Current challenges and recommendations

There is evidence that proves that strides have been made in the field of TB research. The use of BCG vaccine is generally capable of triggering the host immune response [49,58] which subsequently leads to protective immunity against *Mtb* infection. The development of GeneXpert *MTB/RIF®* has revolutionised TB diagnosis by providing rapid analysis and detection of antibiotic resistant strains [236]. Importantly, researchers have identified various immunological markers that may be utilised to distinguish active and LTBI [224–226]. Despite these interventions, TB infection is still a major public health concern and BCG vaccination is unable to offer protective immunity to patients infected prior to immunisation. Devastatingly, the emergence of drug-resistant [227], MDR [228] and XDR [229] TB strains has been documented. Several factors have been implicated in the emergence of drug-
resistant TB strains, which include incorrect prescription, drug misuse and non-compliance to the treatment plan. Therefore, innovative strategies have to be applied for the development of new and effective anti-TB therapeutics. Such strategies will necessitate preliminary analysis in cell culture. Specifically, this will entail identification and characterisation of antibiotic resistant gene/s. This can be achieved by silencing the pathogenesis-causing gene using various gene silencing mechanisms. Additional in vitro studies will entail elucidation of pathogenesis and virulence of various drug-resistant TB strains.

Additionally, genome-wide mutational analysis and immune induction mediated by these drug-resistant strains also need attention. Transgenic mice comprising the Mtb genome may be utilised to study various bacterial properties. Importantly, mice containing both Mtb and HIV genomes may be exploited to investigate the effect of drug-resistant, MDR and XDR TB strains on pathology, disease progression and immune response. Furthermore, this animal model may also be employed for detailed analysis pertaining to immune stimulation, toxicity and drug efficacy. Research studies conducted in these transgenic animals may be extended to humanised mice. Since this model is comprised of implanted human cells, it provides better understanding of Mtb pathogenesis and dissemination. Therefore, this system is convenient for conducting a comprehensive analysis of antibacterial efficacy. Research studies conducted in cell culture and small animals also provides an opportunity for discovering new pathways, molecular markers, resistant genes, drug targets and diagnostic approaches that may aid in combating the TB disease burden.

Following rigorous cell culture analysis and mice studies, non-human primates may be employed to investigate various properties of drug-resistant TB strains. This animal model is well-suited for studying the TB infection cycle and screening of therapeutic agents as it possesses many similarities with humans [230]. As a consequence, data pertaining to efficacy, toxicity, immunological response and Mtb pathology recovered from this model will provide valuable insight for future human clinical trials.

Recently, various strategies of inhibiting target gene expression have been developed. They include the use of gene therapy such as RNAi [231] and gene editing technologies such as the clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein 9 (cas9) [232]. The RNAi mechanism is sequence-specific and may be conveniently applied to suppress any gene of interest (e.g. pathogenesis-causing gene) leading to translational suppression or mRNA degradation. Gene editing strategies may potentially be employed to cleave target Mtb DNA, resulting in the formation of double-stranded breaks. Subsequently, DNA repair enables additional binding and cleavage of target bacterial DNA. Mechanisms involved in DNA repair include homology directed repair (HDR) and non-homologous end joining (NHEJ) [233]. The HDR process induces accurate repair of disrupted target DNA by using homologous DNA sequences. Conversely, the NHEJ mechanism is error prone and induces deletions and insertions in the cleaved target DNA sequence. Consequently, gene editing techniques may be applied to attenuate Mtb replication following multiple cleavage cycles, resulting in mutation of target bacterial DNA. This decreased rate of bacterial replication within host cells may potentially lead to recovery and clearance of TB infection.

Design of molecules that inhibit binding of Mtb to various host cell surface receptors may prevent entry of the pathogen into host tissues [234]. Additionally, strategic design of agents that inhibit expression of genes encoding Mtb resuscitation promoting factors such as rpf and cAMP may inactivate bacterial replication and prevent subsequent bacterial dissemination. Alternatively, development of molecules that disrupt or alter expression of rpf and cAMP proteins may also be a feasible approach of preventing resuscitation of TB infection. Therefore, inactivation of the Mtb replication cycle, gene expression or protein function may aide the host immune system enough time to mount a potent immune response, leading to efficient clearance of the pathogen from the circulation. The decrease in bacterial replication in conjunction with appropriate use of licensed TB drugs may significantly bolster patient recovery and symptom withdrawal.

Ingestion of foods containing bioactive food constituents can alter microbial-induced epigenetics and modify gene expression at a transcriptional level [235]. Foods containing vitamin B12, betaine, choline and folate are capable of altering the DNA and histone methylation profiles by altering carbon metabolism. Some products of 1-carbon metabolism may change histone and DNA methylation and include S-adenosylhomocysteine which inhibits methyltransferases and S-adenosylmethionine which catalyses the transfer of methyl groups during the methylation process. Thus, diet containing bioactive components, nutritional supplements or a clinical condition that negatively affects the levels of S-adenosylhomocysteine and S-adenosylmethionine can modify the methylation of DNA and associated histone proteins. Other vitamins that affect methylation include biotin and niacin [235]. Biotin acts as a substrate during the biotinylation of histone proteins, while niacin participates in ADP-ribosylation of histones and histone acetylation. Specifically, bioactive food components inhibit the activity of enzymes that catalyse various epigenetic processes and include catechin and genistein which inhibit the activity of DNA methyltransferase.

Additionally, curcumin inhibits the function of histone acetyltransferases, while butyrate, diallyl sulhide and sulforaphane disrupt the activity of histone deacetylase. Inhibition of these essential enzymes attenuates the virulence of microbial pathogens [235]. Therefore, inclusion of appropriate nutrients and bioactive food components in the diet of TB patients may enhance their immune response and abolish Mtb-induced epigenetic alterations. Importantly, a diet rich in bioactive food components may also prevent reprogramming of the host cellular machinery as well as evasion of the innate and adaptive immune responses.

Production of a novel, potent, safe and broad-spectrum vaccine may provide long-term protection irrespective of various bacterial, host and environmental factors. This will go a long way in preventing TB infections, particularly in high endemic regions such as Asia and Africa, where the TB prevalence is unprecedented. Specifically, development of a novel vaccine will also greatly benefit individuals who are consistently exposed to Mtb such as healthcare workers and TB researchers. In sub-Saharan Africa, the TB epidemic is further complicated by a high number of patients presenting with HIV-TB co-infections. This poses a challenge with regard to decreasing disease burden, as there is a synergy between TB and HIV infection. This necessitates development of highly active antibacterial and antiviral therapies to counter these microbial pathogens. Conveniently, novel immunostimulants may be applied to boost the host immune system leading to eradication of deleterious HIV-TB co-infections. The use of immune stimulating agents may also be extended to combat various forms of TB bacteria including drug-resistant, MDR and XDR TB strains.

Importantly, development of novel and potent anti-TB agents targeting multiple sites within the bacterial genome may also be employed to counter emergence of drug-resistant strains. This strategy may increase drug efficacy and potentially decrease the number of TB escape mutants, thus, reducing drug resistance. Application of different anti-TB therapeutics may be advantageous in treating chronic and LTBI as well as TB-HIV co-infections, particularly in high endemic regions. Combinatorial therapy using conventional TB drugs, gene therapy and/or gene editing may improve TB treatment and also reduce drug resistance, as long-term monotherapy may ultimately lead to development of drug-resistant strains. Collectively, a multidisciplinary approach may be required to counter active and LTBI, and strict adherence to drug prescription and treatment plan will go a long way in eradicating Mtb infection and drug resistance.

Author contributions

Musa Marimani compiled the review article under the assistance and guidance of Aijaz Ahmad and Adriano Duse.
Conflicts of interest

We declare no competing interests.

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