ANTIBODY ENGINEERING THERAPEUTICS AGAINST TUBERCULOSIS: A REVIEW

Project Thesis submitted in partial fulfillment of the requirement for the degree of

Bachelor of Technology

in

Biotechnology

By

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Under the Guidance of

Dr. Rahul Shrivastava

to

May – 2022

DEPARTMENT OF BIOTECHNOLOGY AND BIOINFORMATICS
JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY,
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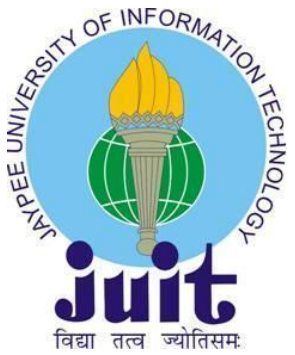

CERTIFICATE

This is to certify that the work titled **"ANTIBODY ENGINEERING THERAPEUTICS AGAINST TUBERCULOSIS: A REVIEW"**, submitted by **"SHARVI SOOD (181822)"** in partial fulfilment for the award of the degree of B. Tech in Biotechnology of Jaypee University of Information Technology, Solan has been carried out under my supervision. This work has not been submitted partially or wholly to any other University or Institute for the award of this or any other degree or diploma.

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CANDIDATE'S DECLARATION

I hereby declare that the work presented in this report entitled **"ANTIBODY ENGINEERING THERAPEUTICS AGAINST TUBERCULOSIS: A REVIEW"** in partial fulfilment of the requirements for the award of the degree of **Bachelor of Technology** in **Biotechnology** submitted in the Department of Biotechnology and Bioinformatics**,** Jaypee University of Information Technology Waknaghat is an authentic record of my work carried out over a period from August 2021 to May 2022 under the supervision of **Dr. Rahul Shrivastava** (Associate Professor) Department of Biotechnology and Bioinformatics.

The matter embodied in the report has not been submitted for the award of any other degree or diploma.

Sharvi Sood, 181822

This is to certify that the above statement made by the candidate is true to the best of my knowledge.

Dr. Rahul Shrivastava
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Date:

ACKNOWLEDGEMENT

It is indeed a great pleasure to thank all those individuals who have, directly or indirectly, contributed and extended their valuable assistance in the progress and completion of this project.

I thank **Dr. Sudhir Kumar**, Head of the Department of Biotechnology and Bioinformatics, for providing me the opportunity and facilities to carry out the project and for guiding and motivating me whenever required.

I owe my profound gratitude to my project supervisor **Dr. Rahul Shrivastava**, who took a keen interest and guided me all along in my project work titled**—ANTIBODY ENGINEERING THERAPEUTICS AGAINST TUBERCULOSIS: A REVIEW**. He provided all the necessary information for carrying out the project. His timely advice, conscientious scrutiny, and scientific approach taught me to work with precision and accuracy and this entity has been a great help to accomplish successful results. I am thankful to him.

I would also like to thank Ph.D. Scholar, **Ms. Ayushi Sharma,** who has guided me in all my experiments. She was there to explain every bit of my silly questions without any hesitation. Her guidance and timely suggestions made this project an easy task to accomplish.

It is my privilege to thank my parents for their constant motivation and encouragement. They were always there to provide an affectionate shoulder at odd times or when unsatisfactory results led to frustrations. I am and will always be indebted to their support and care.

Sharvi Sood, 181822

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ABSTRACT

Infectious diseases continue to be a major cause of illness and death known to mankind. Emerging and re-emerging pathogens create new infectious diseases that are no longer resistant to the available antimicrobials, or are incurable because treatment is not available. Therefore, there is a need for new treatments for the same, in the form of immunotherapeutic agents. These treatments will give maximum performance as adjuvants, aiming at growing the effectiveness of present-day antimicrobial chemotherapies. Immunotherapy or organic remedy is the remedy of sicknesses with the aid of suppressing the immune system. *Mycobacterium tuberculosis* turned into one of the first bacteria to be diagnosed because of the motive of a selected disease – Tuberculosis (TB). Various preventive measures, since its discovery, have been implicated, including vaccinations and antibiotics, with varying degrees of success. The main reason for this is that *M. tuberculosis* is an ancient human pathogen that has revolutionized the complex mechanisms of human resistance. Tuberculosis (TB) is a bacterial infection spread through the air that is caused by *M. tuberculosis* and primarily affects the lungs, though other organs and tissues may be affected, being pulmonary and extrapulmonary. There are 3 stages of TB: **exposure, latent, and active disease.** There are a few immunotherapies that are currently being tested and used to fight against tuberculosis, acting in these stages. **Isoniazid, Rifampicin, Ethambutol**, and **Pyrazinamide** are very effective drugs for treating tuberculosis, under **DOTS**. From this review, I have concluded that Immunotherapies will require randomized, placebo-controlled trials. The timing of those remedies is important, it ought to be aimed at decreasing the symptoms the patients experience along with the eradication of the disease, being active and/or latent.

Keywords: *tuberculosis; immunotherapy; antibodies; chemotherapy; vaccination*

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Chapter 1

Introduction

INTRODUCTION

Infectious diseases are a significant reason for morbidity and mortality in humans.

Emerging pathogens giving rise to new infectious illnesses have surfaced as proof against available antimicrobial drugs that can’t work against these infections due to the fact that remedy isn't available or in large part useless due to underlying host immune impairment [1].

The variety of people with a compromised immune system has risen rapidly, because of the HIV epidemic, and new advances in the treatment of most cancers, transplantations, and autoimmune diseases.

Hence, there may be a want for new processes to remedy infectious illnesses, furthermore immunotherapies. Likely, such healing procedures can be given in most cases as a side remedy, i.e., aimed to grow the efficacy of antimicrobial chemotherapy and repair or redirect the immune reaction to microbial pathogens (adjuvants).

ANTIBODY ENGINEERING stimulates the precise additives of the immune system or counteracts signals produced with the aid of using most cancer cells that suppress immune responses. Recent trials in immunology and biotechnology have caused an increase in clinically available cellular and immune treatment options that require the management of biologics and cells of various origins [2].

Chapter 2

Review of Literature

2.1 Introduction

Mycobacterium tuberculosis is a tuberculosis-causing pathogenic bacteria belonging to the Mycobacteriaceae family. *M. tuberculosis*, first discovered by Robert Koch in 1882, has a unique waxy coating on its cell surface due to the presence of mycolic acid. *M. tuberculosis* is Gram-positive due to this coating, which makes the cells impervious to Gram staining [3]. It attacks the lungs and is mostly a mammalian respiratory infection. The tuberculin skin test, acid-fast stain, culture, and polymerase chain reaction, is the most often used tuberculosis diagnostic test. To view *M. tuberculosis* under the microscope, an acid-fast test with auramine is done since this pathogen necessitates a huge amount of oxygen as it is an aerobic organism [4].

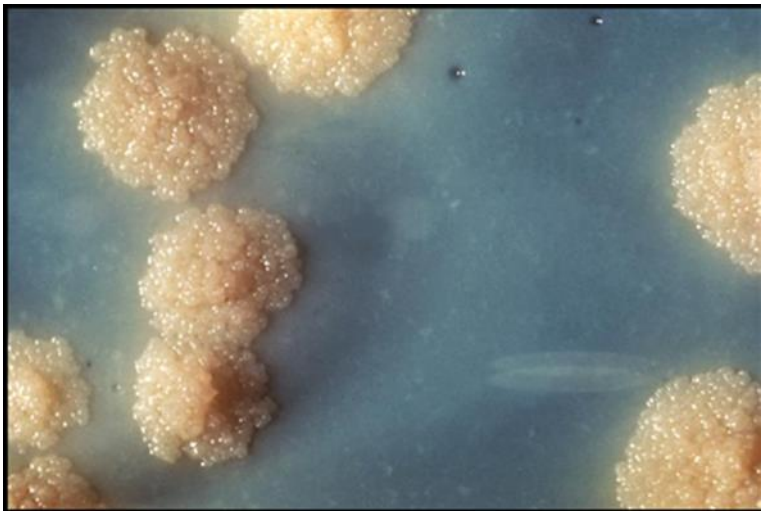

Figure 1:Microscopic view of M. tuberculosis colonies [3]

Tuberculosis (TB) remains a major global health problem. A third of the sector's population is infected with *M. tuberculosis*, and only about 10% of those who are infected develop TB; however, there are 9 million TB cases with 1.5 million deaths per year [5]. Standard prophylactic treatment for latent TB infection lasts 3 to 9 months and new TB cases require at least 6 months of treatment with more than one drug. This control of tuberculosis (that is latent) infection has apparently become quite tough because of the layout of resistance towards multidrug and extreme resistant tuberculosis. Attempts toward discovering new immunotherapies and TB drugs need to be intensified [6]. Immunotherapies should adapt with the immune system in people suffering from tuberculosis (latent) disease that is active and even

allows finer control of replication of *M. tuberculosis*. This evaluation narrates various kinds of proportion immunotherapies with the focus on those that had trials in humans [7].

The World Health Organization estimates that many countries have an increasing number of patients with multidrug-resistant TB (MDR), who have been tested for isoniazid and rifampin [5]. Adding to this, many more countries in the world with an unrestrained prevalence of MDR-TB will also be affected by the increasing number of significantly drug-resistant XDR-TB cases having resistance to drugs like isoniazid, fluoroquinolones, rifampicin, and aminoglycosides [6].

XDR-TB management is getting even more difficult day by day and the effects are also very unpredictable. Consequently, the most concerning public health problems worldwide like MDR and XDR-TB are because of the lack of good and effective treatments and the need for a much longer duration of treatment with second-line drugs. Chances for drugs to expand globally by immigration are urgently needed to scale up TB therapies that are under development [5]. The expansion in the development of the drug for TB was comparatively slow, and not even the slightest bit of the new drugs that are tested till now allowed the famous remedy for the same. Host-directed therapies and the use of immunomodulators is the hopeful method or technique to be explored for better management of TB [8].

2.2 History of *M. tuberculosis*

M. tuberculosis was called "tubercle bacillus," before it was identified on March 24, 1882, by Robert Koch, who was honoured with the Nobel Prize in Physiology or Medicine in 1905 for this same discovery; the bacterium is likewise called "Koch's bacillus".

M. tuberculosis has been around for an extended time; however, the term has been modified numerous times. The records of tuberculosis as disease started to take form in 1720, while the doctor Benjamin Marten defined it in his **" Theory of Consumption"**, *a pathogenic disease that travels through air and infects humans* [9].

2.3 Structure of *M. tuberculosis*

M. tuberculosis's molecular envelope is made up of 4 predominant layers:

(i) The plasma membrane or internal membrane

(ii) The peptidoglycan–arabinogalactan complex (AGP)

(iii) An asymmetrical outer membrane, that is covalently connected to AGP through mycolic acids

(iv) The external capsule [10].

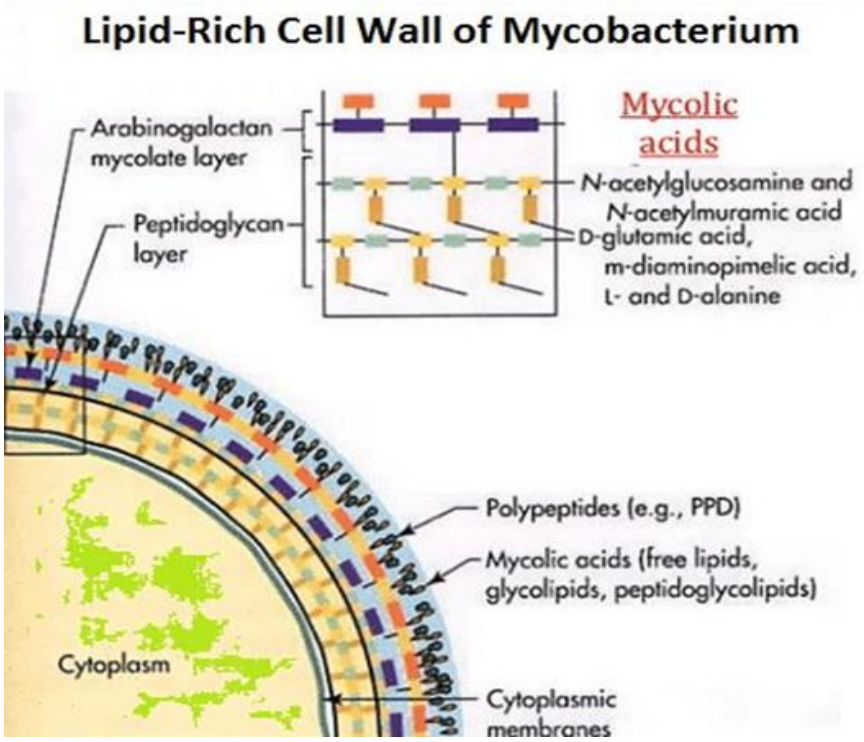

Figure 2: Cell wall Structure of M. tuberculosis [10]

2.4 Growth and Sporulation of *Mycobacterium*

In 2019, *M. tuberculosis*, *Mycobacterium africanum*, *Mycobacterium canetti*, *Mycobacterium bovis*, *Mycobacterium caprae*, *Mycobacterium microti*, *Mycobacterium pinnipedii*, *Mycobacterium mungi*, *and Mycobacterium orygis* have been determined as nine complex members of the genus Mycobacterium [11]. It needs oxygen to thrive, and whether or not it generates spores is debatable. It is likewise non-motile and in every 18–24 hours, *M. tuberculosis* divides [12]. When in comparison to microorganisms whose division durations are measured in minutes, this is especially slow (division in *Escherichia coli* takes place every 20 mins) [13].

2.5 Microscopic view of *Mycobacterium* cells

While other microorganisms are easily detected under microscope by Gram staining process. The mycolic acid present in the cell wall of the bacteria does not soak the dye. Acid-fast stains like Ziehl–Neelsen stain or luminous stains like auramine are utilized instead. Due to the embedded fatty acid in the cell wall, cells are bent regularly visible as rod-shaped and coiled together [14]. Large horseshoe-shaped cells are present in the nuclei of *M. tuberculosis* in tissues [15].

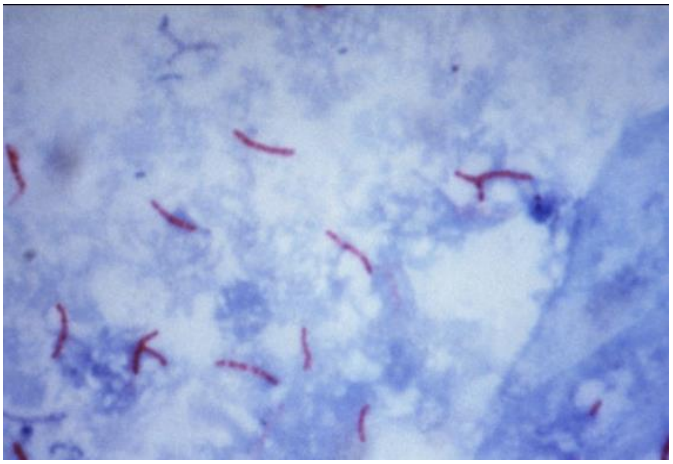

Figure 3: M. tuberculosis visualization using the Ziehl–Neelsen stain [14]

2.6 *M. tuberculosis* Culturing

In the laboratory, *M. tuberculosis* may be grown and has a modest growth rate as compared to other considerably researched bacteria, doubling once each day. Liquid media like Middlebrook 7H9 or 7H12, Lowenstein-Jensen media, and Middlebrook 7H11 or 7H10 media are all normally exercised [16]. On agar plates, colonies take many weeks to grow. It differs from other strains of mycobacteria as it produces catalase and niacin. Gene probes and MALDI-TOF are in addition assays, that may be used to validate its identity [17].

2.7 TB Latency and Host Immunity

More knowledge about the nature of interactions between the host-pathogen is needed to improve immunotherapy and the role of these in the treatment of tuberculosis infection and/or disease [7]. It's very interesting to know that only 10 percent of people infected with *M. tuberculosis* get infected from TB, and how most infected people manage or completely eradicate the infection, this is something that is not completely understood as to why this bacterium behaves in this way [18].

Response of the immune system can markedly adapt to the number of *M. tuberculosis* replicating actively in infected individuals, with associated adjustments in the risks of tuberculosis disease [20]. However, these responses are not sufficient for bacterial eradication because these subsets of T-cell number peak during decline in infection following a spontaneous immune control without removal of TB infection [21].

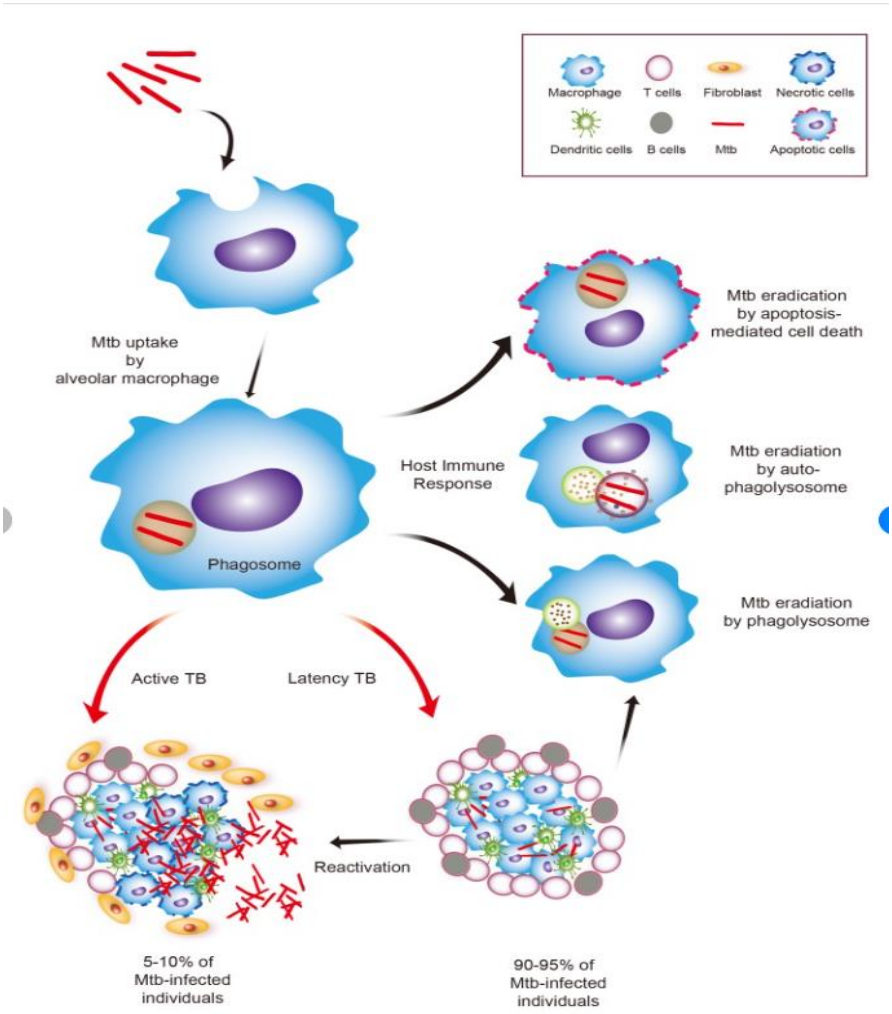

Figure 4: A schematic representation of the immune system of the host against the infection of M. tuberculosis [19]

2.8 *M. tuberculosis* Pathophysiology

The only known reservoirs of *M. tuberculosis* are humans, tuberculosis can be a contagious disease and can easily be transmitted through shaking hands, sharing eatables, or sharing personal belongings.

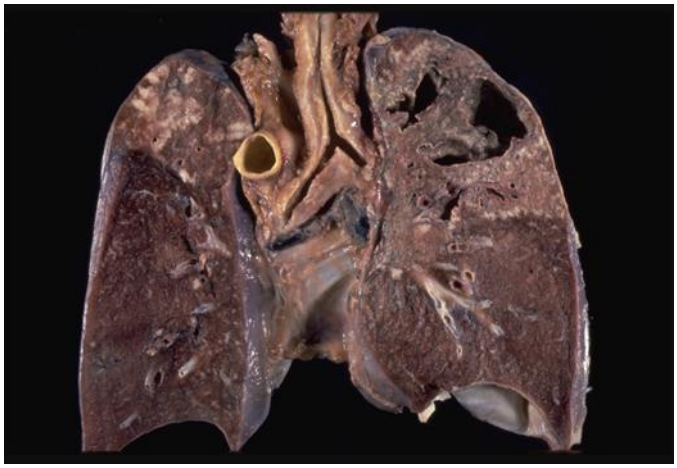

Figure 5: Tuberculosis infection in lungs [22]

Alveolar macrophages phagocytize *M. tuberculosis* in the lungs however, they are unable to kill or digest the bacterium. Its cell membrane prevents the phagosome from fusing with the lysosome, which contains antimicrobial properties [15]. *M. tuberculosis* inhibits the endosomal autoantigen 1 (EEA1), the bridging molecule. In addition, the diterpene isotuberculosinol stops the phagosome from maturing [23]. By neutralizing reactive nitrogen intermediates, the bacterium avoids being killed by macrophages. It has recently been discovered that *M. tuberculosis* secrets and coats itself with 1-tuberculosis nyc adenosine (1-TbAd), an antacid with the effective neutralizing pH functions that in turn cause fusion of lysosome [24].

The capacity to create *M. tuberculosis* mutants and the need to examine the gene products for their specific activity have made it possible to understand the disease and the related virulence factors [15]. The secretions of these products play an important role in disease progression. The cord factor (trehalose dimycolate), for example, is a virulence factor that helps the parasite survive in its host [25]. Mutations in the genes of *M. tuberculosis* strains have resulted in resistance to more than one TB treatment.

3.0 Strain variation observed in in *M. tuberculosis*

In the examination of tuberculosis outbreaks, strain typing is useful because it provides evidence for or against transmission from person to person. Take the case of individual A, who has tuberculosis and believes he got it from person B, so by using typing we can easily track

the infection [18]. Typing of *M. tuberculosis* strains was done using pulse-field electrophoresis in the 2000s. Variable numbers of tandem repeats (VNTR) have recently supplanted this method, which is technically easier to conduct and provides greater strain identification [26].

Antibiotic resistance in *M. tuberculosis* is usually caused by the accumulation of mutations in the target genes or a change in the drug's titration. If drug resistance is seen in *M tuberculosis* by both rifampicin and isoniazid, it is considered multidrug-resistant (MDR TB). XDR tuberculosis is also defined by resistance to both isoniazid and rifampin, as well as any fluoroquinolone and at least one of three injectable second-line medicines (i.e., amikacin, kanamycin, or capreomycin) [6].

3.1 Genomic Strain of *M. tuberculosis*

The *M. tuberculosis* genome was designed in 1998. It has a size of 4 Mil base pairs and 3,959 genes; 40 percent of these genes have been described, with another 44 percent having a putative function. There are six pseudogenes in the genome [27].

The waxy coat's evolutionary importance to pathogen survival is demonstrated by the huge number of conserved genes. Furthermore, experimental studies have recently proven the necessity of *M. tuberculosis* having a lipid layer composed of fats and cholesterol [28]. Isolated bacteria from infected mice’s lungs show a preference for fatty acid over carbohydrate substrates. Survival of *M. tuberculosis* is only possible due to the presence of these lipid layers that are classified as crucial during the infectious life cycle, especially during the chronic stage [15].

The deletion of a common N-terminal domain in these proteins’ hampers macrophage and granuloma development [29].

Antibiotic resistance pathways were studied using the genomes of numerous sensitive, ultra-resistant, and multi-resistant *M. tuberculosis* strains . The findings show previously unknown linkages of drug resistance genes, and they depict that some genes and intergenic areas may be implicated in drug resistance [30].

3.2 Evolution in *M. tuberculosis*

In Africa, the evolution of *M. tuberculosis* took place along with *Mycobacterium africanum, Mycobacterium bovis (Dassie's bacillus), Mycobacterium caprae, Mycobacterium microti, Mycobacterium mungi, Mycobacterium. orygis and Mycobacterium*, all of which infect a lot of animal species [31]. These *mycobacterium* species do not show species category because they are all embedded in the *M. tuberculosis* lineage [32]. An evaluation of over 3000 traces from 35 nations recommended an African beginning for this species

3.2.1 Co-evolution with Present-day Human Beings

There is a lot of debate about the age of *M. tuberculosis* and how it has correlations with humans. One takes a look at the comparison of the *M. tuberculosis* phylogeny versus the phylogeny of the human mitochondrial genome and observed that they have been very similar once [33].

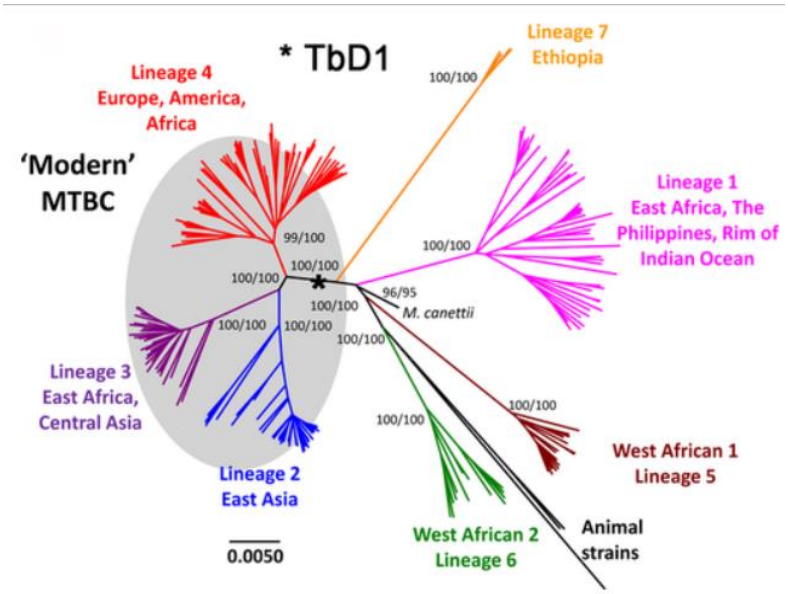

Figure 6: Genomic phylogeny of strains of M. tuberculosis [34]

Using this time scale, the observations found that the powerful populace length of *M. tuberculosis* accelerated around 10,000 years ago, implying that *M. tuberculosis* turned capable of being contagious to man through the dramatic increase in human host population density [35].

The latest observation was made that implied genome sequences from *M. tuberculosis* infected individuals extracted from 3,000 months old mummies, showing that the latest common ancestors lived not more than 5,000 years ago. Therefore, it has been predicted that the ancestors of *M. tuberculosis* thrived in humans in East Africa 3 million years ago [36].

3.3 Host Genetics

The nature of the *M. tuberculosis* interaction with the human host shows a pathogenic response [15]. A whole of the population showed genetic drift that increased susceptibility to mycobacterial infection, this, in turn, was found to be believed a group of rare disorders together known as Mendelian disorders [37].

In *M. tuberculosis*, genetic factors show host susceptibility, this was observed through certain early studies on the pathogen [38].

3.4 Vaccines

The BCG vaccine (bacille Calmette-Guerin) derived from *M. bovis* shows good results against in children but it is not so effective against adult pulmonary tuberculosis which is the most common form of tuberculosis today [39].

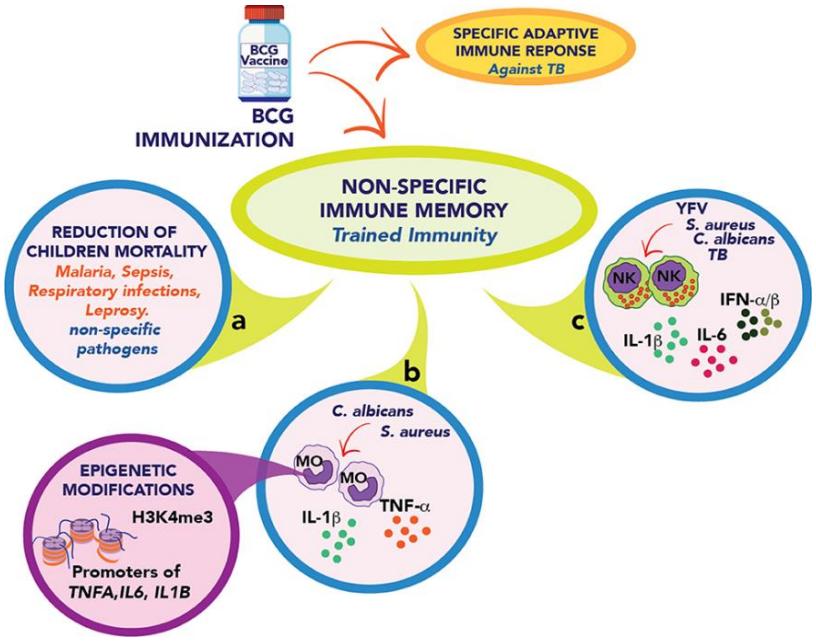

Figure 7: Schematic representation of trained immunity elicits by BCG immunization [40]

4.0 *M. tuberculosis*- Antibiotic resistance

M. tuberculosis is a clonal organism and is not able to do horizontal gene transfer to exchange DNA. The WHO in their report of 2019 said the effect of antibiotic resistance tuberculosis is 3-4 percent in new areas and 18 in already affected areas. There are geographical differences in the prevalence of drug-resistant tuberculosis, China, India, Russia, and South Africa have the highest rates of Antibiotic resistance-TB. Recent studies show drug resistance in areas such as Singapore and Australia, with significant increases in Papua New Guinea [5].

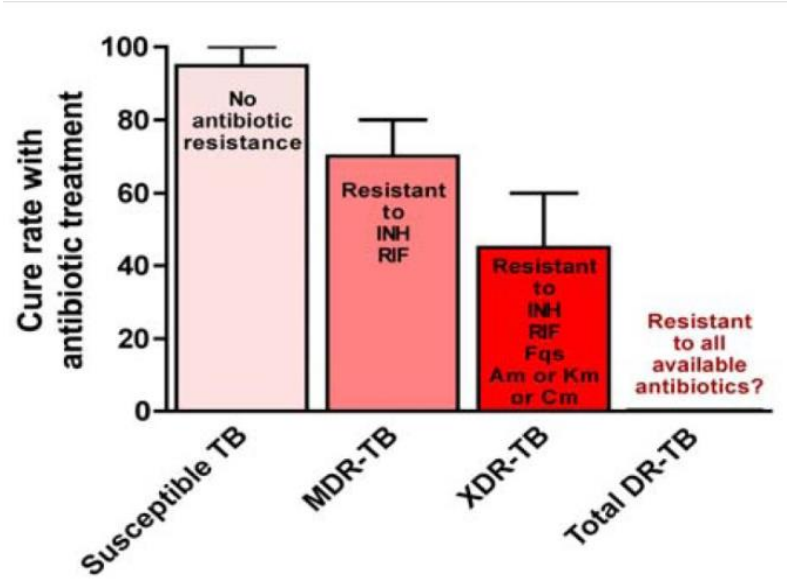

Figure 8: Antibiotic cure rates of TB based on drug resistance patterns[41]

The unique cell wall of *M. tuberculosis* is an intrinsic contributor to its antibiotic resistance. The mycobacterial cell, which is saturated with long-chain fatty acids or mycolic acids, forms a strong, relatively insoluble barrier. As a result, many antibiotics, including isoniazid, cannot target of pathogen and stop its working. However, the majority of them have encountered opposition [42].

4.1 Antibody Engineering Techniques

Several immunoglobulins evolved on a massive scale using traditional techniques because of the improvement of antibody-manufacturing strategies. Hybridoma technology has spread out

new techniques for the manufacturing of antibodies in response to antigens that are to be targeted by pathogens that are infectious, diseases that are malignant, autoimmune disorders, and many effective toxins [43].

The modified antibodies have been created for new healing medicines with progressed immune protective properties, together with enticing immune effector activities, fusion protein creation tumor, and tissue penetration, and the antibodies that have a very high affinity are directed in opposition to the targets that are to be conserved. In immunology, biotechnology, diagnostics, and healing medicine, superior antibody engineering strategies provide a huge variety of uses [44].

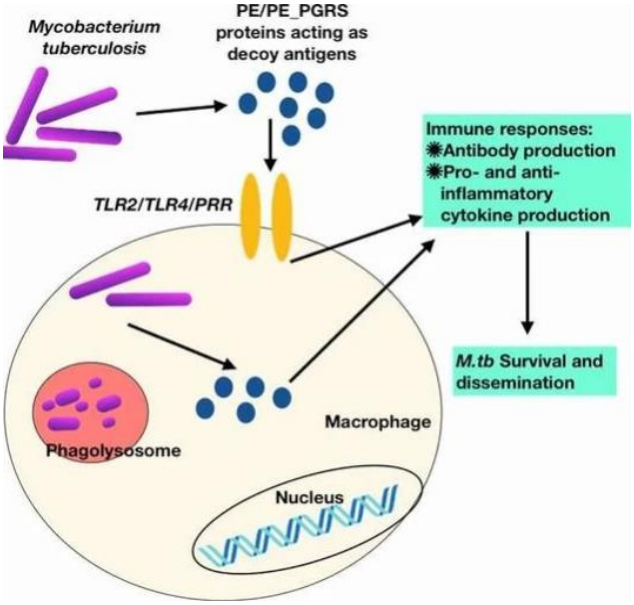

Figure 9: M. tuberculosis genome codes for proteins like PE/PPE/PE_PGRS, and they are intrinsically disarranged, non-essential, and antigenic [45]

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Immunoprotected molecules provide protection against diseases that are contagious or transmissible. Ultimately, immune sera from numerous animals had been acquired, pooled, and used as therapeutics. Since then, the management of infectious diseases consisting of diphtheria, tetanus, pneumococcal pneumonia, meningococcal meningitis, and toxin-mediated diseases has increased greatly in affected person survival [46].

4.1.1 Structure of the Antibody Molecule

Antibodies are made up of heavy chains, which are connected by bonds such as non-covalent and also disulphide (s-s) bridges. The binding of antibodies with antigens occurs with the help of a Single variable domain fragment which is specific for certain conformational epitopes because of the existence of complementary determining regions (long CDR3). The IgG antibody molecule is made from 4 chains of the polypeptide, consisting of the same-sized light chains and 2 same heavy chains, and can be thought of as forming a bendy structure in Y-form. Every chain has a variable (V) with an amino-terminal which is the antigen-binding site. The light chains are bound to the heavy chains via many non-covalent interactions and s-s bonds, and the V areas of the heavy and light chains pair in every arm of the Y to generate 2 same antigen-binding sites, which lie at the palm of the 'Y'. The Y stem, or Fc fragment, contains the carboxy-terminal domain names for heavy chains. The Fc fragment and hinge areas differ in antibodies of different isotypes but the general association of domain names is compared in all isotypes [47].

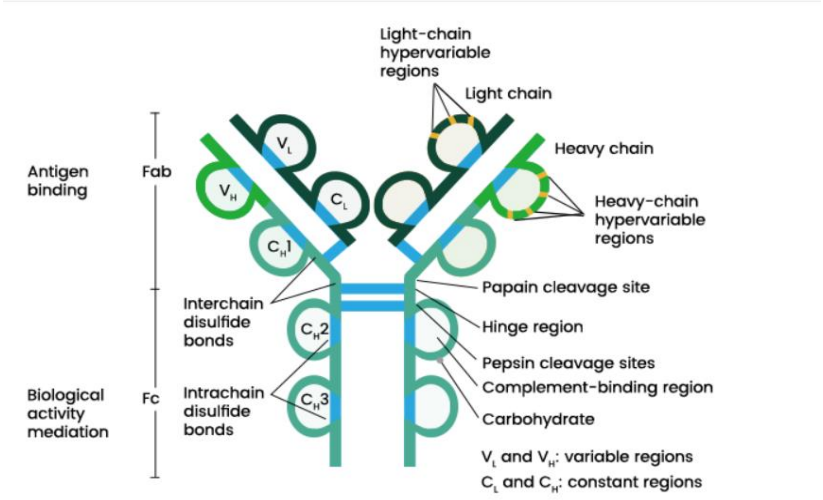


Figure 10: Structure of An antibody molecule [48]

All antibodies are constructed identically from the heavy and light polypeptide chains that are paired, and the well-known Ig is being used for all such types of proteins. Within this cutting-edge class, 5 precise groups of Ig—immunoglobulin M, immunoglobulin D, immunoglobulin G, immunoglobulin A, and immunoglobulin E—are present with their respective functions [47].

The periplasmic expression systems help in the pairing of VH and VL with the aid of providing optimum conditions to allow the production of practical antibody molecules [49].

4.2 Antibody Production

Experimentations on laboratory animals like mice, rabbits, etc, led to the use of antibody development using animal immunization in the late 1980s. The most important work in manufacturing of monoclonal antibody application is the incompetent response of two harmful antigens. Furthermore, most of the antibodies that are produced are kept humanized so that the immunogenicity is not altered [50]. In order to get the better of this hassle, antibodies for human beings were developed in vitro via antibody engineering technology which includes production fragments of antibodies that are immuno-modulatory, and also structures that are cell-free.

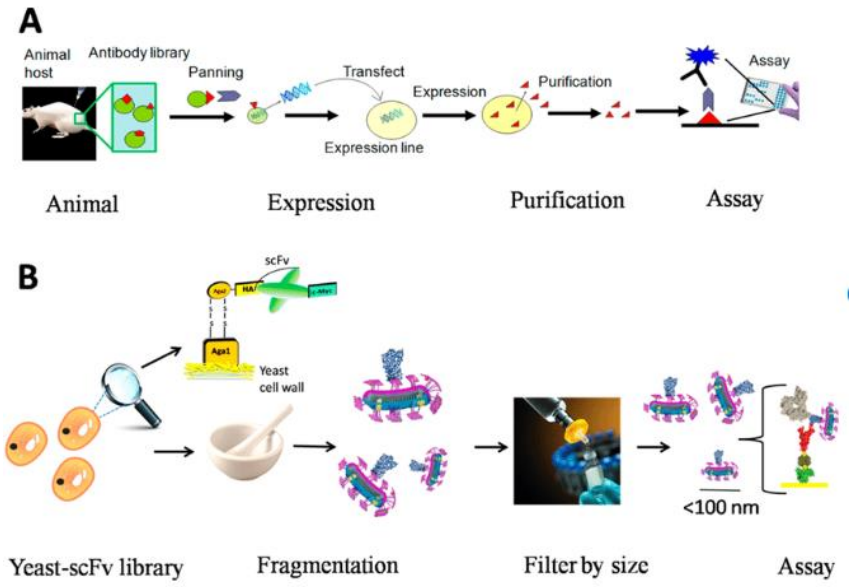


Figure 11: Traditional Antibody Production method. [51]

Various microorganisms like yeast which are systematically engineered using the phage technique gave a boost to the making of recombinant antibodies in invitro, which can be seen as a useful tool in today's molecular biology to study recombinant technology. George P. Smith first showed this approach of phage , who proved the occurrence of proteins that are exogenous

in nature on filamentous phage through fusion of the peptide that is of interest to gene number III. Recombinant antibody fragments that were first built were in microorganisms seventeen yrs. ago. The antibody production technology shows the use of titers and the immoderate anti-sera. Antigen production and animal immunizations are done using hybridoma technology and recombination generation [51].

4.2.1 Polyclonal Antibody

Polyclonal antibodies (pAbs) are a combination of more than one antibody with the useful beneficial aid of B-cells clones of animals. Polyclonal antibodies interpret more than one linear epitope with minimum conformational changes that consist of several antibodies of various affinities, from which a massive precipitating lattice is formed from antigens [52].

The quantity of antigen used impacts the immune reaction. Even small portions of impurities will bring about antibodies reacting greater to the impurity than to the given antigen [53].

Selection of Animal Species

The main components that affect the choice of animal species are the amount of polyclonal antibody required, phylogenetic dating among the animal and antigen, the age of the animal, the ease of getting blood samples, and the software where the polyclonal antibody is used [54].

Rabbits are favored because of their period and comparatively lengthy existence span. But, to produce huge quantities of pAbs, livestock at the side of sheep, goats and horses are used [50].

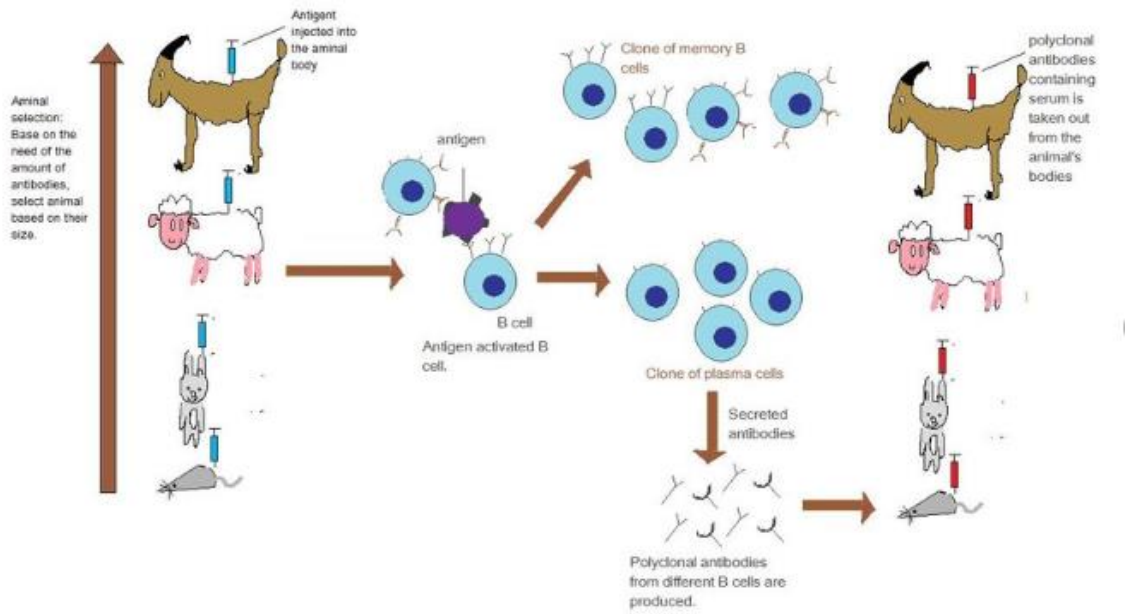


Figure 12: Production of polyclonal antibodies [55]

4.2.2.1 Antibody Preparation

The production method begins with the preparation of protein-based antigens, it is important to confirm the efficacy of the target antigen especially binding to the epitope. The specificity of polyclonal antibodies obtained, relies on the purity of the antigen used. Impurities (1% of the total) can also be immunodominant (e.g., with several microorganism antigens) and lead to antibodies that have a better affinity for the impurity than for the antigen of interest, purification could be a long and exhausting task [56].

4.2.1.2 Animal Immunization

The assembly of polyclonal antibodies depends upon the quantity of blood serum utilized, the evolutionary distance among the species from which the macromolecule of choice has been derived, the species of the animal to be immunized, and previous experience with the immunogens. Rabbits are the quality animal of choice as they're genetically divergent from the human and mouse assets of the proteins [57].

Adjuvants are accustomed to embellishing the production of Freund's Adjuvant, which is the most common type of adjuvant used in studies. In the presence of an associate adjuvant, the macromolecule substance is injected intramuscularly, intradermally, or subcutaneously into an animal of the chosen species. Booster protections are started after four to eight weeks once the priming immunization and are continued for few weeks. Before the first immunization, the animal is made to bleed and the liquid body substance extracted from whole blood. While the protein concentration has reached up to an acceptable degree, the manufacturing of polyclonal antibodies is done [58].

4.2.1.3 Antibody Purification

Macromolecule affinity purification will enhance IgG within the serum and eliminate the majority of undesirable proteins. However, there's still a huge quantity of non-unique IgGs in those preparations. In the past the protein is employed in assays comparable to ELISA, immunohistochemistry, Western blot, and so forth [59].

To isolate precise polyclonal antibodies from antiserum, antigen-particular affinity purification is often used. Affinity purification results from the removal of the major non-unique IgG fraction and enriches the fraction of immune globulin that significantly reacts with the goal-oriented antigen [60].

4.2.1.4 Quality Control

Purification, a series of management assessments is done to bind the high-quality polyclonal antibodies. Protein assessment is checked through absorption at 280 nm (A280). The purity of polyclonal antibodies is checked using the SDS page. To estimate the polyclonal antibody titer, an enzyme-linked-immunosorbent serologic assay is used [61].

4.2.1.5 Post-Immunization Remark

The blood is taken out at regular intervals to monitor animals every day for assessing the facet immunization effects. Animals' serum is analysed for displaying the antibody responses and for antibody extraction at the same time when sufficient amounts are produced [62].

4.2.1.6 Benefits of Polyclonal Antibodies

Costly method and is only able for utilizing in a single extraction. Polyclonal antibodies are a heterogeneous combination of antibodies that could bind to a big variety of antigenic epitopes. Subsequently, a small alternate in the epitopes of an antigen is much less to have an effect on polyclonal antibodies [52].

4.2.1.7 Risks of Polyclonal Antibodies

The affinity of pAbs to antigens might trade over the years thereby specifically because of numerous variations amongst batches. The purity and awareness tiers of a particular antibody are seen to be less in pAbs as compared with monoclonal antibodies [52]

4.2.2 Monoclonal antibodies

Monoclonal antibodies (mAb) are important reagents employed in medical specialty studies, for the identification of sicknesses, and remedy such diseases as infections and cancer. Those antibodies are created by cell lines or clones nonheritable from animals that are immunized with the substance. The mobile traces are produced by fusing B cells from the immunized animal with metastatic tumor cells. The antibodies that are monoclonal (mAbs), are considerably large homogenous and mono-specific medical biomolecules constituted of hybridoma cells. Monoclonal antibodies occur from a sole mobile clone in comparison to many cellular clones for polyclonal antibodies. [52]

4.2.2.1 The Approach to Antibody Production

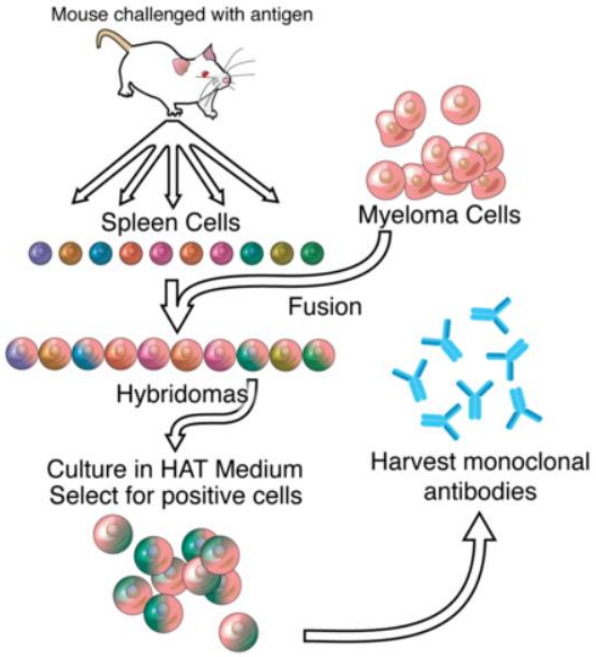


Figure 13: A general representation of the method used to produce monoclonal antibodies.[52]

Hybridoma generation is an excellent methodology for producing the high-end quality of monoclonal antibodies as it generates them in their original form.

The technology begins with animals' immunization with the help of an antigen of interest and titer of serum-antibody which is defined through ELISA. Towards the end, the elimination of the spleen occurs aseptically and the splenocytes produce hybridoma cells that get fused with myeloma cells [63].

These cells (Hybridoma cells) are cultured in ninety-six-well plates in the presence of a HAT (hypoxanthine-aminopterin-thymidine) selection medium to achieve high throughput screening.

After this, these cells (hybridoma cells) producing favored antibodies are then checked through traditional enzyme-related immunosorbent assay and novel nanoparticle-probed immunoassay [63].

Mass generation of these mAbs is done in vitro with the appropriate choice of media, shaker containers or flasks, and bioreactors (bench-scale). After checking these cells (hybridoma) move to wells that are comparatively bigger or in traditional containers which then further generated enough cells for cryopreservation and therefore supernatants for further experiments [46].

A disadvantage of this method is that it only produces a said number of thousands of clones after tedious experimentation. Invariably these clones are again used for clonal enlargement and mobile immortalization [63].

4.2.2.2 Various Applications of Monoclonal Antibodies

Monoclonal antibody-based remedies show specificity against a particular antigen. This attribute makes them suitable for different therapies. Diagnostic programs consist of biochemical analysis and imaging and for the detection of hormonal, tissue, and cellular products with the help of different immunoassays. After this, the imaging is performed using radiolabelled monoclonal antibodies for diagnostics of infections. Healing monoclonal antibodies have a wide variety of approaches which includes therapy for most cancers, autoimmune and cardiovascular diseases, and numerous other infectious diseases. [52].

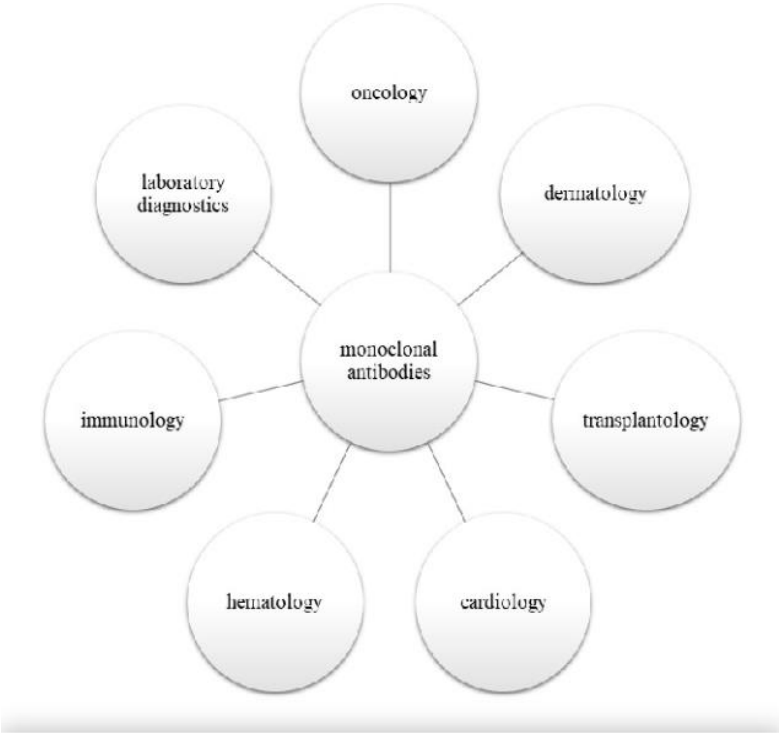


Figure 14: Applications of monoclonal antibodies [64]

4.2.2.3 Benefits of the Use of Monoclonal Antibodies

Hybridoma serves as an immortal source of monoclonal antibodies. The original antibody is maintained amongst the manufacturing batches. It is highly reproducible and scalable, with unlimited manufacturing source. One of the advantages is speed, sensitivity and specificity of assays. No need to preserve the animals. And also, antigens are immunogens that no longer are natural. Selection enables the picking of the right clones towards the specific antigen [52].

4.2.2.4 Risks of the Use of Monoclonal Antibodies

It is a time-consuming task which takes 6 months to 9 months. Production of monoclonal antibodies is expensive therefore we cannot repeat the process as we wish. Small peptide and fragment antigens may not be suitable monoclonal antibodies and won't recognize the original

antigen. Hybridoma culture can be prone to contamination. The technique is specifically for mice and rats and is difficult to perform on other animals. Greater than 99% of the cells cannot survive the production process – decreasing the range of useful antibodies that can be produced against an antigen [52].

4.2.3 Antibodies Specificity Against TB

For a long time, cellular-Mediated Immunity (CMI) modified due to the special protection mechanism against living pathogens. Occasionally *M. Tuberculosis* should be localized at cells at the onset of the infection within the upper extremity during the period of high incidence when rupture of tumor lesions occurs. Controlling the activity of intracellular pathogens can be done by combinations of both humoral and cellular immunogenicity [65].

4.2.3.1 Epidemiological Evidence of Antibody-Mediated Safety

With the experimentations done in the last few decades, a substantial amount of proof is there that these antibodies show positive effects on children and adults who have low IgG content in their bodies and cannot disrupt antigens against *M. tuberculosis*. Active IgA antibodies have been advised to be important in the protection of mucosal shielding and safety [66].

4.3 Experimental Studies with Antibodies

A substantial range of research using anti-mycobacterial antibodies was carried out way back in the nineteenth century. Those experiments may be classified into varied categories: Serum therapies, Mouse Polyclonal Antibodies, Human Polyclonal Antibodies, humour human immune globulin (hsIgA), and analysis with organism antibodies [67].

4.3.1 Serum Therapies

The immune system is generated from vaccinated animals that are further given to humans/animals. The results obtained would be beneficial, flexible, inconsistent, or contradictory [68].

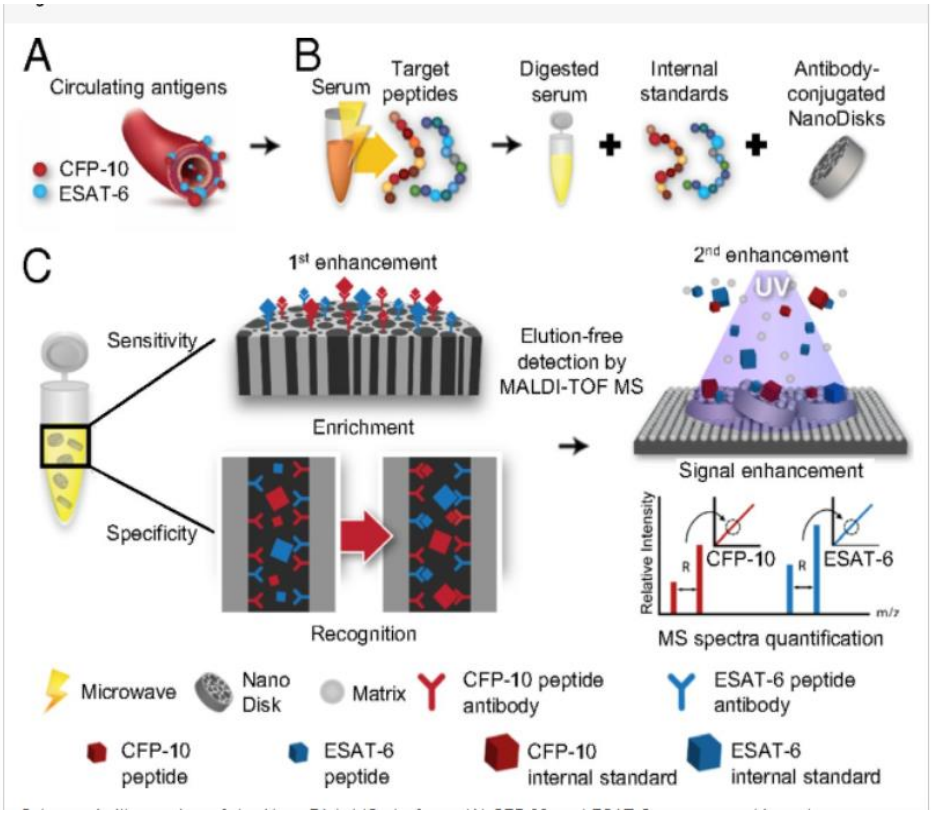


Figure 15: Schematic illustration of serum therapy in tuberculosis [69]

In the past 19th and early 20th centuries, a lot of experimentation was done and they tried to perceive serum therapeutics for TB. This research was inspired by the successful improvement of serum therapy in opposition to the spread of infectious diseases which included *Streptococcus pneumoniae*, *Neisseria meningitides*, and *Streptococci*, and several toxin-mediated diseases. Serum remedy is making of serum injections in animals and humans. For most sera taken from already immunized organisms with a specific pathogen, the active agent in the serum is therefore converted into an antibody [68].

To research whether the serum had direct bactericidal resident, anti-TR serum was blended with tubercle bacilli and incubated numerous times. An aliquot of the suspension was modified

and then injected into guinea pigs. Animals that were injected with serum-bacillus were incubated for a few hours died after 16 days , while those who received combinations incubated for 24 hours were seen to be alive for 6 weeks. The serum antitubercular properties were seen via again injecting anti-TR serum and tuberculin into the guinea pigs that survived. A 0.1ml quantity of serum is considered enough to inhibit tuberculin response seen from the invariable body temperature. A 0.5 ml of the said serum dose saved these guinea pigs from the harmful dose of tuberculin. Twenty human patients with early TB (analysis made microscopically) were made to give this dose every day of 1 ml of serum and were seen to show development within 6- 8 weeks; all were stated to expose development within 6 to 8 weeks. Although a number of side effects were also noted that, including temperature decline, cough, night-time sweats, decreased pulse rate and weight [68].

4.3.2 Polyclonal Antibodies In Mouse

A modern study re-examined the utility of immune blood serum within the context of a healing immunizing agent in competition to TB. In this observation, immune serum became generated with the help of mistreatment of the usage of immunized mice with RUTI. After chemotherapy, they were injected into the generated immune serum for up to 10 weeks. Mice that were injected with immune serum from RUTI-vaccinated animals confirmed significant decreases in lung CFUs in addition to decreased granulomatous response and abscess formation. These consequences suggest that defensive serum antibodies can be obtained through the useful means of vaccination and that the antibodies can usefully be combined with chemotherapy [66].

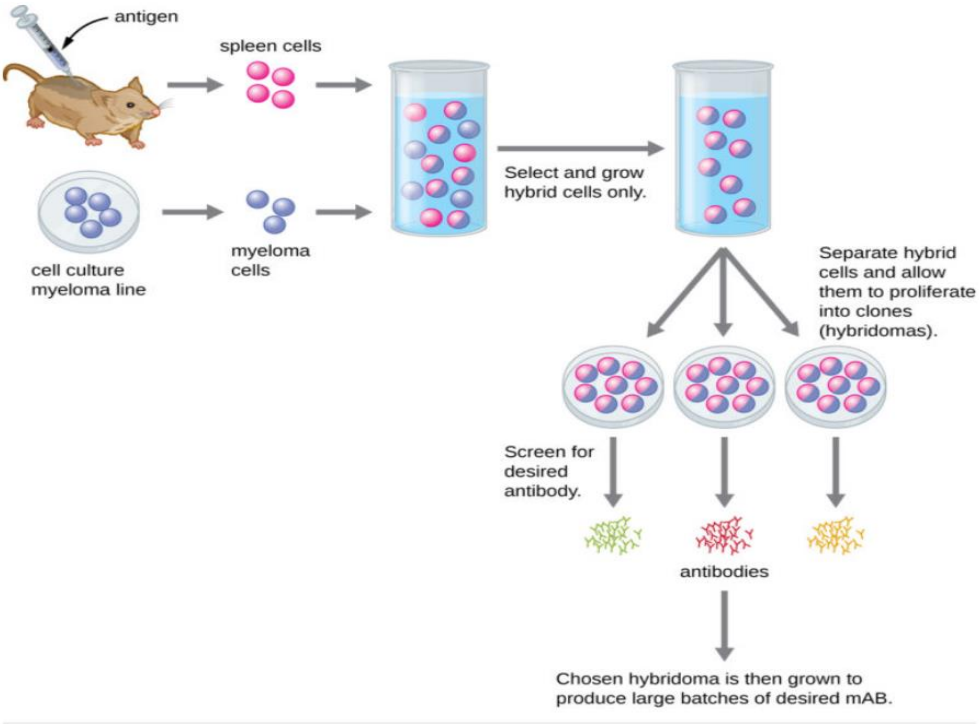


Figure 16: Polyclonal antibodies made from mouse's spleen in myeloma.[70]

4.4 Mechanism of Action of These Antibodies

Many of the antibody effects shown within the analysed study suggest that the best effect is shown by the monoclonal and polyclonal immune effect on *M. Tuberculosis*. Secretions located in the subcutaneous mucosal areas include large degrees of Igs, in particular, IgA . IgA has direct and indirect role to play in the fight against infectious agents, including microorganisms that disrupt the mucosal barrier [71].

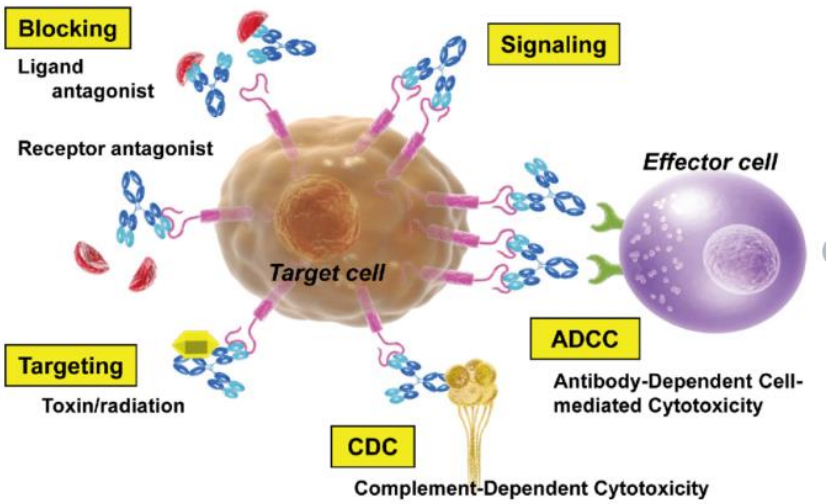


Figure 17: Mechanisms of action of therapeutic antibodies.[72]

At some stage in the extracellular tiers of intracellular facultative pathogens, Antibodies can be crucial. They'll act through a manner of interfering with adhesion, with the use of neutralizing pollutants, and with the resource of activating supplements. Furthermore, antibodies are also capable of penetrating recently infected cells, binding internalized pathogens, and enhancing substance processing [71].

4.5 Potential Use of Antibodies Against TB

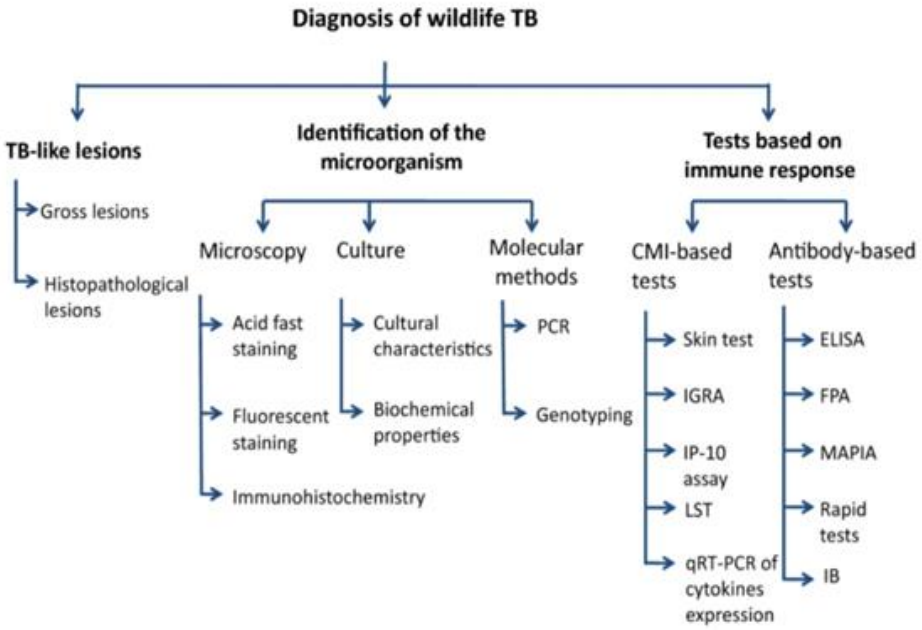


Figure 18: Diagnosis using tuberculosis antibodies [73]

It is predicted that the future of antibody treatment against TB may bring hope and numerous opportunities together with prevention and analysis of the disease [71].

4.5.1 Treatment

Antibody-based therapy is beneficial in many cases. Combined with certain chemotherapies, they can shorten the diagnosis for uncomplicated TB patients [29]. However, they would be in particular crucial within the treatment of people who are infected with Multidrug-Resistant (MDR) and strains that are extremely Drug-Resistant, in combination with usual treatment [74].

4.5.2 Prophylactic use

Immunosuppressive therapy may be performed in patients at high risk of TB. Keeping this in mind has been used in special antibody prophylactic strategies against many viruses including varicella, tetanus, respiratory syncytial virus (RSV), rabies, and Hepatitis B [75].

4.5.3 Vaccination

Vaccination is the use of a direct antibody response that may be autoimmune or cell mediated is considered a unique strategy for improving TB vaccines.

Polysaccharide conjugate vaccines

Vaccine agents of polysaccharide conjugate are shown to have a direct response of proteins against bacteria. However, the vaccine polysaccharide conjugate against the typhoid bacteria indicates the potential for this type of infectious diseases as a result of biological pathogens [76].

4.5.4 Diagnosis

Even though no serological assays are presently advocated for the prognosis of this tuberculosis, mainly because of the presence of negative outcomes and therefore wrong remedies, to different infectious agents. Various distinguishing serological checks are relatively very expensive, particularly in undeveloped countries. In a few instances, antibody responses can represent beneficial remedies. In a particular case of TB, many studies on antibody reactions have been suggested and there may be significant variability in the immune response to TB. This diversity has been caused by a number of factors associated with the pathogen (pressure fluctuations, small rotation, and a growing bacterial population) and some are in relation with the host, primarily, previous exposure to related antigens and the host gene [66].

Chapter 3

METHODS

1. Review of Literature

I have reviewed a few previously published works on antibody therapies against tuberculosis. From these research articles, I have analyzed the relevant protocols for different antibody therapies against tuberculosis. Firstly, I studied in detail the structure of the antibody molecule and its certain targets which can be used for creating specific therapies. Along with this, I studied the methodology of antibody production and how various animal species are utilized for the same.

From the vast data available on the internet, it was important to specify the main keywords for my search purposes, therefore, before beginning to read articles related to my project, I looked for these keywords. These are, therapeutics, antibodies, tuberculosis, antibodies preparations, tools for antibodies, etc., which made it simpler to carry out my project work.

This report focuses on the overview of methods carried out in specific areas such as antibiotic therapeutics for tuberculosis using qualitative and quantitative techniques. It aims to better understand the host's innate immune responses induced by a variety of *M. tuberculosis* protein antigens, as well as to identify potential TB vaccine candidates. It also includes recent advances in vaccine development strategies, such as the identification of new tuberculosis antigens using a variety of tools. A better understanding of the host-pathogen relationship, as well as the utility of mycobacterial antigens as novel vaccine candidates, will help to design the next generation of vaccines, as well as improve host protective immune responses while limiting immunopathology during *M. tuberculosis* infection.

The review and research articles for constructing this review article were taken from the publishers like Elsevier, Science Direct, Taylor and Francis, Nature, and Google Scholar and PubMed.

While completing this review report I followed the below-mentioned steps:

1. Defining the study objectives,
2. Reviewing the existing literature,
3. Filtering for inclusion, evaluating the quality of primary studies,
4. Extracting data
5. Analyzing data

Chapter 4

RESULTS

The following information was extracted from the review articles:

1. The WHO Global Tuberculosis 2021 Report – not so good news and turning the tide back to End TB

   It was pointed out that the WHO global TB report for 2021 paints a bleak picture of the worldwide TB epidemic's trajectory, including a halt in the annual fall in TB incidence, a drop in TB notifications, and an increase in anticipated TB mortality [5].

2. Antibody Engineering for Pursuing a Healthier Future

   Antibody engineering provides biomedical techniques for the identification of high-affinity peptides/proteins, receptor binding, protein-protein interactions, and epitope identification that are quick, cost-effective, and efficient. From a biotechnology and cell biology perspective, this powerful technology is applied in a number of systems to address various challenges. It's also utilised to make a variety of modified antibodies that can be directed at any drug molecule or very distinct conserved antigens. Antibody engineering has a wide range of biotechnological, biological, medical, and antibody applications in the development of new therapies for a variety of diseases. In vitro screening and selection strategies based on numerous high throughputs modified antibodies, immune effector functions, and high-affinity antibody fragments have resulted in the development of a number of innovative therapeutic medicines [67].

3. Development of therapeutic antibodies for the treatment of diseases

   Researchers have created many transgenic animals, including second-generation human chimeric mice and human mice increase the quality of antibody medicines. Transgenic animals are becoming more refined and advanced, providing more alternatives for drug development of antibodies in worldwide pharmaceutical companies. Three companies have produced all of the transgenic-derived mAbs approved for therapeutic use. High-affinity human antibodies can be acquired from the selected clones in the animals, depending on the immunization technique. Hybridoma technology is mostly used for this selection. These three transgenic animals have yielded 19 authorized human monoclonal antibodies (mAbs) [77].

4. The COVID-19/Tuberculosis Syndemic and Potential Antibody Therapy for TB Based on the Lessons Learnt From the Pandemic

   The therapeutic monoclonal antibody developed has the effector mechanisms as a conventional human antibody, including as neutralisation, complement activation, antibody-dependent cellular cytotoxicity (ADCC), and antibody-dependent phagocytosis (ADP). Due to the intracellular structure of the MTB virus, it's no surprise that researchers are concentrating their efforts on creating a TB therapy based on cell-mediated immunity (CMI). However, CMI cannot be relied on entirely to cure tuberculosis, especially in immunosuppressive situations such as HIV and even COVID-19, when T cell malfunction is found. The ramifications of the TB/COVID-19 syntomic are one of the most important lessons learned from the epidemic. There is a lot of evidence to back up these claims [78].

5. Antibodies and tuberculosis

   The only licenced TB vaccine is the Bacillus Calmette-Guérin vaccine, but it does not protect against humans. The development of this TB vaccine is thus a critical need for healthcare system. Although cell-mediated immunity is required for the control of latent infection, the assumption that this immunity is adequate for vaccine-induced prevention has recently been challenged. To guide future TB vaccine strategies, a deeper understanding of host defense against TB is required.

   Unlike cell-mediated immunity, the human antibody response to *M. tuberculosis* is believed to induce little immune authority over the course of infection. Humoral responses are common all through active TB disease and have even been linked to immunopathology. However, there is an indication that autoantibodies may limit the spread of *M. tuberculosis* and may also play a role in infection prevention via mucosal immunity. Furthermore, antibodies are now known to protect against a variety of intracellular pathogens by modifying immunity through Fc-receptor-mediated phagocytosis [71].

6. Quantification of circulating *M. tuberculosis* antigen peptides allows rapid diagnosis of active disease and treatment monitoring

Although serum therapy activity may theoretically be used to detect all current tuberculosis infestations, including TB cases, some NTM isolates express homologs, which may limit their value as biomarkers. We tested whether tryptic peptides could differentiate *M. tuberculosis*-derived homologs generated by other species because peptide sequence is the holy grail for protein discrimination. When MALDI-TOF MS was used to analyse recombinant protein tryptic digests, it discovered CFP-10 and ESAT-6 peptides with high signal-to-noise ratios, which were later reported by liquid chromatography-tandem mass spectrometry and showed strong *tuberculosis* specificity when aligned with protein sequences from 12 NTM species. Both peptides showed perfect homology with *Mycobacterium bovis*, a relatively uncommon form of tuberculosis, but they differed significantly from the two species that cause the large percentage of NTM diseases, *Mycobacterium avium* and *Mycobacterium intracellulare* [69].

Chapter 5

Discussion

During the completion of my review on the said topic, I interpreted that Immunotherapies will require randomized, placebo-controlled trials. The timing of those remedies is important, it ought to be aimed at decreasing the symptoms the patients experience along with the eradication of the disease, being active and/or latent.

Re-inspecting humoral immunity to tuberculosis infection in regard to naturally arising human antibodies from individual donors has sparked a surge of interest in antibody-mediated immunity to *M. tuberculosis* infection, but this optimism must be tempered with a realistic understanding of the many obstacles that must be faced before we can ascertain whether an antibody-based vaccine can be developed to prevent active tuberculosis infection. In the therapy of extensively drug-resistant TB, which is significantly more difficult and expensive to treat than ordinary TB patients, supplementary therapy using therapeutic monoclonal antibodies plays a role. Perhaps the first therapeutic application of the recent discoveries in recognizing *M. tuberculosis* antibody responses should be the use of the 21st century equivalent of serum treatment to combat the old adversary TB.

Chapter 6

EXPERIMENTAL WORK

6.1 Media Preparation and Sterilisation

Chemicals Required: Luria Broth Powder, LB Agar Powder, Nutrient Broth Powder, Nutrient Agar, Distilled water

Equipment Required: Conical flask, weighing balance, spatula, measuring cylinder, autoclave, cotton plugs, test tubes

Procedure followed:

1. Weighed the media according to the instructions provided and the volume needed, using the weighing balance.
2. Transferred the media powder to conical flasks.
3. Added distilled water using a measuring cylinder to make up the appropriate volumes.
4. Dissolved the media by stirring.
5. Added 10 ml liquid media (broth) to the test tubes
6. All test tubes and conical flasks were capped using tight cotton plugs.
7. Glassware containing the media is put in the autoclave and sterilized at 121º C for 15-20 minutes.

Results:

Sterilized solid and liquid media was prepared. The media was used 12-18 hours after preparation to check for any unwanted microbial contamination.

6.2 Culturing Microorganisms in solid and liquid media

Chemicals Required: Luria Broth, Nutrient Broth, LB Agar, Nutrient Agar (autoclaved), bacterial culture without contamination (*E. coli*), Test tubes and Flasks containing media

Equipment Required: Ethanol, inoculating loop, burner, cotton, sterilized Petri plates, Laminar Air Flow Chamber, parafilm, and incubator

Procedure followed:

Culturing in solid media:

1. The surface of the LAF was wiped clean with ethanol and cotton.
2. Placed all required material inside the LAF and the UV light was turned on for 10-15 minutes.
3. Heat the agar media to melt it.
4. Turned off the UV light, switched on the fan, and the light inside the LAF
5. With the burner lit, the Petri plates were opened and media was carefully poured into each of them (20-25 ml per plate).
6. The plates were allowed to sit for 25-35 minutes.
7. Using an inoculating loop, the culture was taken and streaked onto the plates. Some plates were also streaked in quadrants.
8. Plates were covered in parafilm and incubated overnight.

Culturing in liquid media:

1. The surface of the LAF was wiped clean with ethanol and cotton.
2. Placed all required material inside the LAF and the UV light was turned on for 10-15 minutes.
3. Heat the agar media to melt it.
4. Turned off the UV light, switched on the fan, and the light inside the LAF
5. With the burner lit, an inoculating loop was used to take the culture and inoculate the liquid media with *E. coli*.
6. Tubes and flasks were covered with cotton plugs and incubated overnight.

Results:

After 12-18 hours of incubation, isolated colonies could be seen on the streaked plates and the liquid media turned opaque indicating bacterial growth.

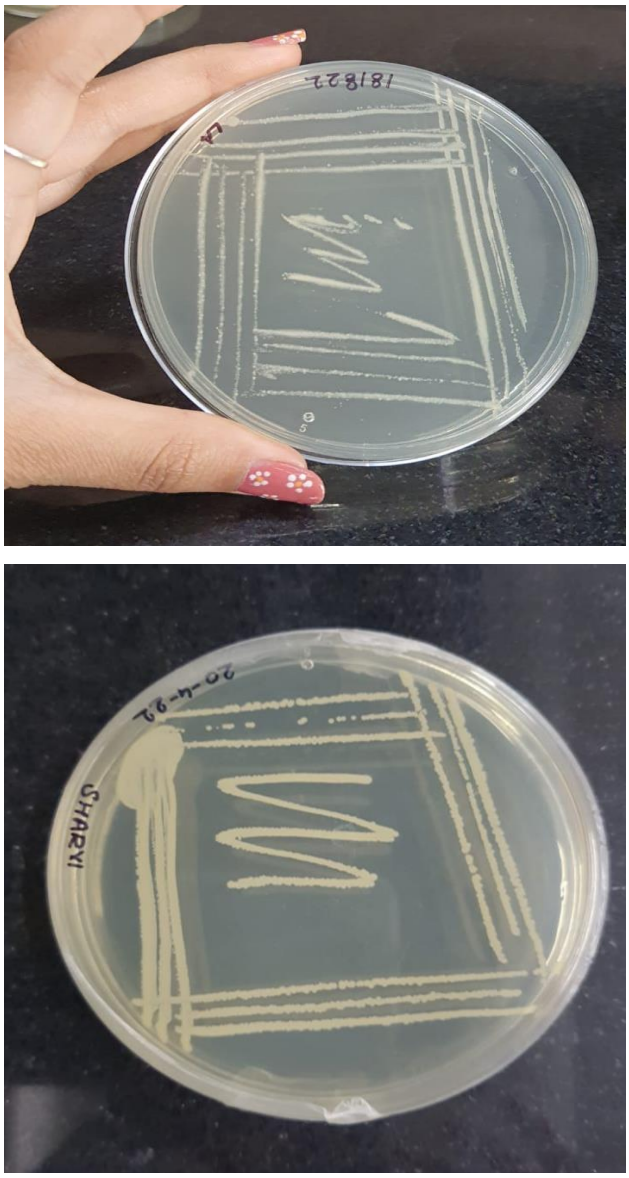


Figure 19: Quadrant streaking of E. coli

6.3 Gram Staining

Chemicals Required: Primary stain (crystal violet), Gram's Iodine, 95% alcohol or acetone, Safranin (counterstain)

Equipment Required: Glass slides, microscope, inoculating loop, distilled water, immersion oil, bacterial culture, dropper, burner

Procedure followed:

1. Using the inoculating loop a small amount of inoculum was put on a clean glass slide and using a dropper a drop of water was added to the inoculum and mixed using a needle.
2. The smear was heat-fixed.
3. Added crystal violet and kept it for 30 seconds to 1 minute.
4. Rinsed with water.
5. Added gram's iodine and kept it for 1 minute.
6. Rinsed with water.
7. Washed with 95% alcohol or acetone for 10-20 seconds.
8. Added Safranin for 1 minute.
9. Washed with water.
10. Air-dried and observed the slide under the microscope.

Results:

Pink *E. coli* cells were observed under the microscope at 100X.

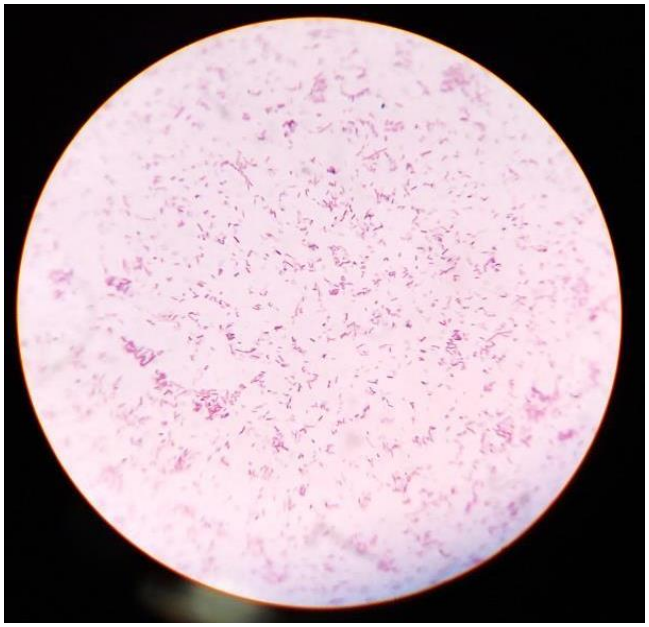


Figure 20: E.coli under microscope after Gram Staining

6.4 Acid-fast staining

Chemicals Required: Mycobacterial culture, Carbol Fuschin dye, Acid-alcohol (3% HCl in 95% alcohol), Malachite green

Equipment Required: Glass slides, inoculating needle, heating plate, dropper, water (distilled and tap water), immersion oil, and microscope

Procedure followed:

1. Prepared a bacterial smear on a clean glass slide by adding a needle of bacterial growth on a glass slide with a drop of water.
2. Mix the specimen with water and let it dry.
3. Air-dried smear and heat-fixed.
4. Covered the smear with carbol fuschin dye.
5. Heated the stain until vapours began to rise.
6. Allow the heated stain to remain on the slide for 5 minutes.
7. Washed off with running tap water
8. Wash the slide with acid-alcohol for 30 seconds.
9. Then it was rinsed with water.
10. Cover the smear with malachite green and kept it for 1-2 minutes.
11. Rinsed with water, wiped the back of the slide, and allowed the stained smear to air dry.
12. Examined the slide under the microscope under 100X objective.

Results:

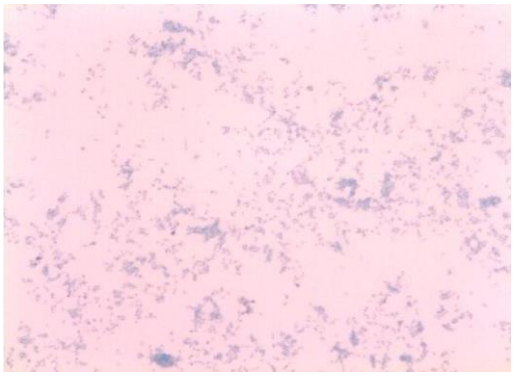


Figure 21: Non-acid-fast bacteria (blue colored)

6.5 Isolation of genomic DNA by phenol-chloroform method

Chemicals Required: Tris base, Tris-Cl, Phenol: Chloroform (1:1), absolute ethanol, SDS, Lysis Buffer, 1X TE Buffer, chilled isopropanol

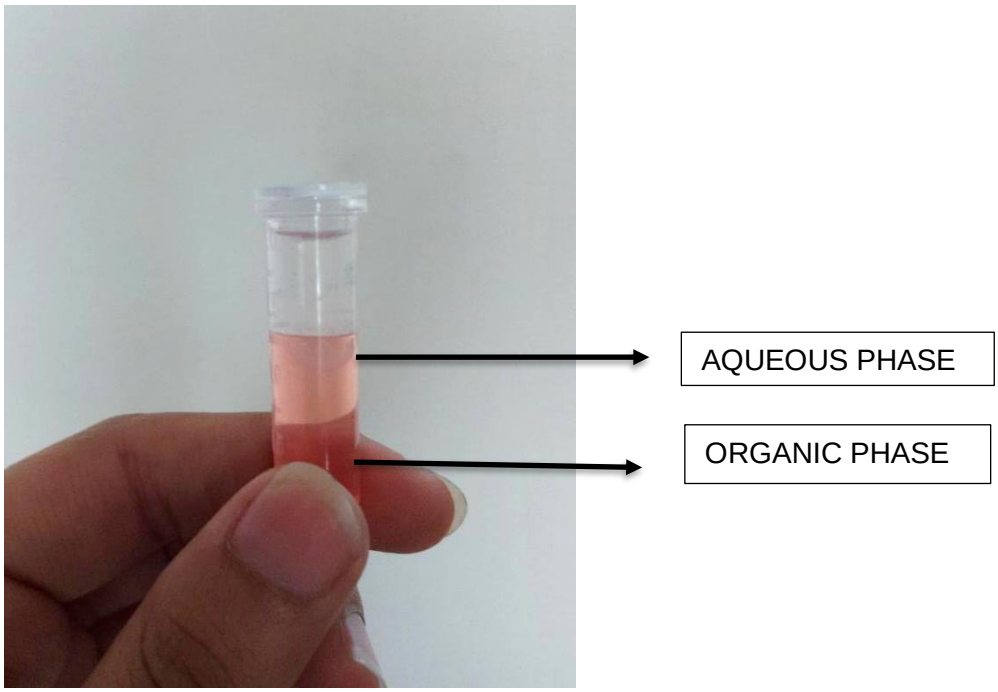
Equipment Required: *E. coli* culture, Luria broth containing flask, tarson tubes (50 ml), microfuge tubes (2 ml), Glass slides, centrifuge, Agarose gel electrophoresis buffers and apparatus, pipettes and tips, incubator (37 º C), ice bucket with ice, -20º C refrigerator, 4 º C refrigerator

Procedure followed:

1. A fresh flask containing LB Broth was inoculated and was incubated overnight at 37 º C.
2. From a fresh overnight culture, 10 ml culture was transferred to tarson tubes.
3. To get the cell pellet, the tubes were spun at 7,000 rpm for 10 minutes.
4. The supernatant was then discarded, and the pellet was resuspended in 4 ml Lysis buffer.
5. Completely vortex the tubes to allow proper mixing.
6. Incubated the tubes at 37 º C for 1 hour.
7. Added equal volumes of phenol: chloroform (2ml each) and mixed properly. The phenol used has saturated at pH 8.0 using Tris-Cl.
8. It was Centrifuged at 10,000 rpm for 10 minutes. After centrifugation, a white layer could be seen at the interface of organic and aqueous layers.
9. Carefully transferred the aqueous phase with a pipette to 2 ml microfuge tubes.
10. Repeated the phenol: chloroform step, but with 05. ml of each and spun for 10,000 rpm for 5 minutes.
11. Transferred the aqueous layer to a new tube and added 1 ml isopropanol for precipitation.
12. Incubated the tubes at -20 º C overnight.
13. Spun the tubes for 15 minutes at 4 º C.
14. Discarded the supernatant and rinsed the pellet with 1 ml of 70% ethanol.
15. Repeated the alcohol washing step
16. Resuspended the DNA in TE buffer
17. Agarose gel was loaded with DNA samples and electrophoresis was carried out to visualize the bands of genomic DNA.

Results:

Genomic DNA bands were visible.



The aqueous and organic phases separated

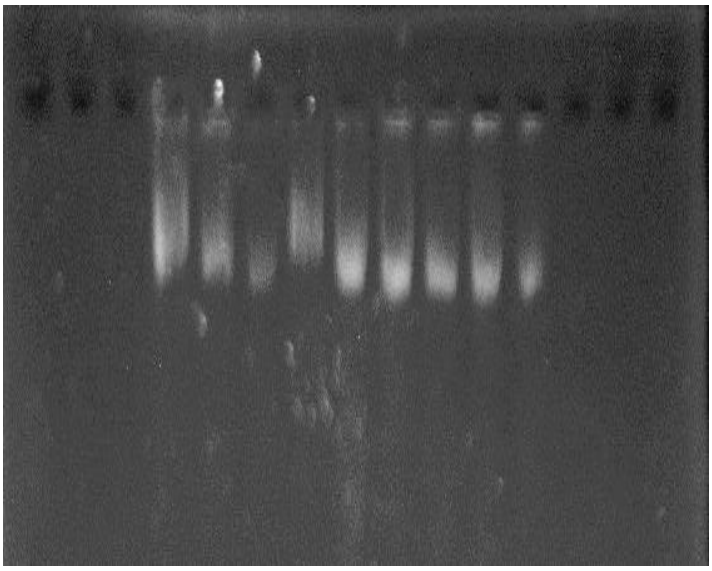


Figure 22: Genomic DNA (E. coli) visualized on 0.8% agarose gel.

6.6 Plasmid DNA Isolation using the phenol-chloroform method

Chemicals Required: Alkaline Lysis Buffers I, II, and III, 96% ethanol, 70% ethanol, chilled isopropanol, DNA loading dye (6X), EtBr

Equipment Required: pUC19 culture, Luria broth containing flask, tarson tubes (50 ml), microfuge tubes (2 ml), Glass slides, centrifuge, Agarose gel electrophoresis buffers and apparatus, pipettes, and tips, incubator (37 º C), ice bucket with ice, -20º C refrigerator, 4 º C refrigerator

Procedure followed:

1. A fresh flask containing LB Broth was inoculated and incubated overnight at 37 º C.
2. From a fresh overnight culture, 45 ml culture was transferred to tarson tubes.
3. To get the cell pellet, the tubes were spun at 7,000 rpm for 10 minutes at 4 º C.
4. Added 0.600 ml of ALS-I (GTE).
5. Completely vortexed the tubes to allow proper mixing.
6. Added 1.2 ml of SDS-NaOH solution (ALS-II) and inverted the tubes rapidly.
7. Incubated at 37 º C for 5 minutes, this gave rise to a slimy texture.
8. Carefully added 0.450 ml of acetate solution (ALS-III) to allow renaturation of circular DNA.
9. Mixed gently 5-6 times.
10. Incubated in ice for 30 minutes.
11. Centrifuged at 7,000 rpm for 20 minutes at 4 º C.
12. Transferred the supernatant to fresh tubes
13. Added equal volume (~1ml) of chilled isopropanol (1:1).
14. Incubated the tubes at -20 º C overnight.
15. Spun the tubes for 10,000 rpm for 15 minutes at 4 º C.
16. Discarded the supernatant and rinsed the pellet with 0.750 ml of 96% ethanol.
17. Centrifuged at 10,000 rpm for 15 minutes.
18. Discarded the supernatant and rinsed the pellet with 0.750 ml of 70% ethanol.
19. Centrifuged at 10,000 rpm for 10 minutes.
20. Discarded the supernatant
21. Air-dried the pellet by allowing the ethanol to evaporate.

22. Suspended the pellet in autoclaved distilled water (10 microliters) and mixed by tapping.

23. Briefly spun the tubes and then pooled plasmid DNA into one tube.

24. Cast 0.8% Agarose gel with 3 microliters of EtBr (50 ml) in an 8-well tank.

25. 5 microliters sample and 1 microliter loading dye were mixed and then loaded into the wells.

26. Agarose gel electrophoresis was carried out at 100V to visualize the bands of plasmid DNA.

Results:

Plasmid DNA was visualized.

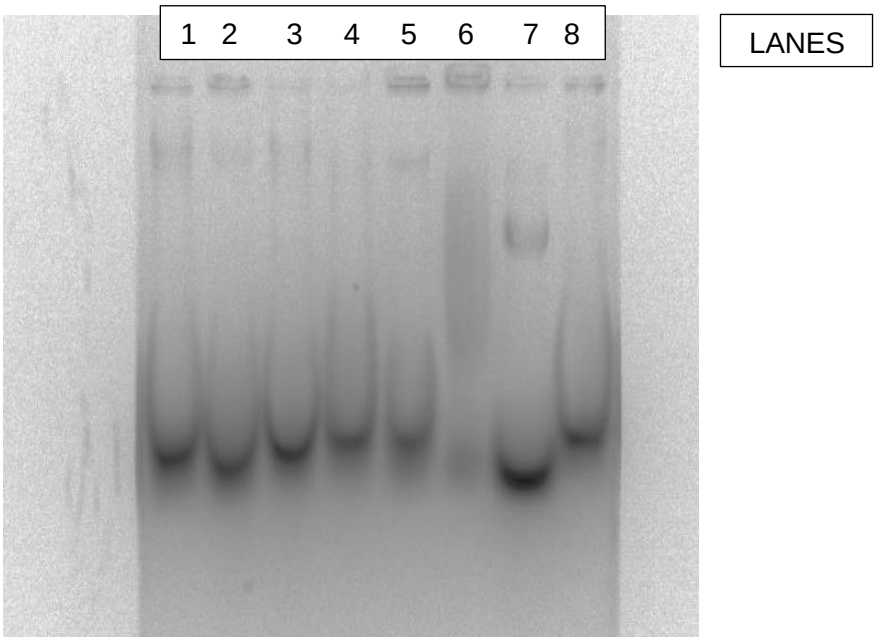


Figure 23: Plasmid DNA on 0.8% agarose gel, the first five lines are all pUC-19, the sixth is the pRT vector.

6.7 Antimicrobial Susceptibility test/ Disc Diffusion

Chemicals required: MHA Media, antibiotic discs, distilled water, 2% agar, liquid culture

Equipment required: distilled water, flasks, pouring plates, burner, ethanol, spreader, forceps, pipette, tips LAF

Procedure followed:

1. The MHA media was prepared (2% Agar+ MHA media) following the instructions given on the box.
2. Water was added to make up the required volume.
3. After the autoclave was performed and the media checked for contamination pouring was done.
4. After the media solidified, 1ml of liquid culture was pipette and poured inside the LAF
5. Using a spreader, the culture was carefully spread all over the media plate till it was completely dried.
6. Now the antibiotics disc was taken and evenly spread across the plate using a forceps
7. And plates were sealed using a parafilm and incubated overnight for the culture to grow.
8. The growth was noted and the disc parameter was measured using a scale specific for each antibiotic used.

Results: the diameter of the antibiotic was calculated.

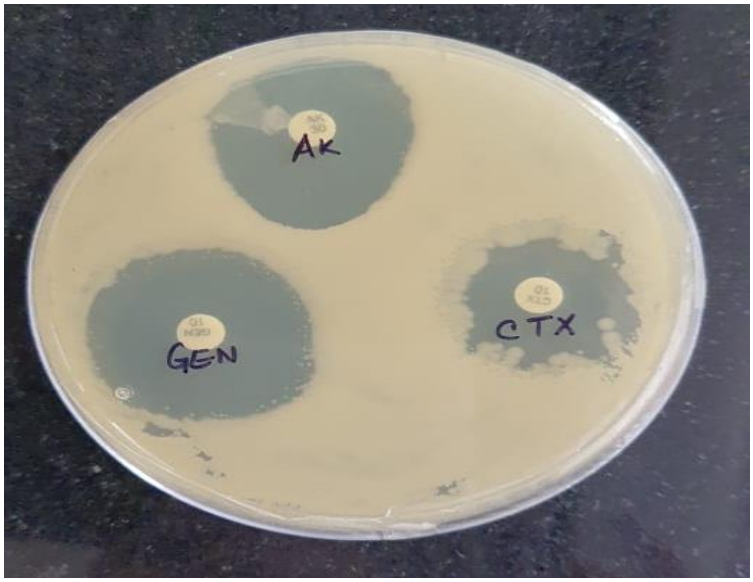


Figure 2: AST by Disk Diffusion Results

Antibiotic	Concentration (mcg/disk)	Observed Diameter (in mm)
Gentamycin (GEN)	10.00	31.0
Amikacin (AK)	10.00	27.0
Cephotaxime (CTX)	30.00	19.0

Table 1: Observed zones of inhibition against S. aureus.

Chapter 7

Appendix

7.1 Bacteriological media

All the media that was used in the experimental work was prepared in distilled water and autoclaved at 15 pounds per square inch for 15 min. unless indicated otherwise.

LB Broth (Luria Bertani Broth)

Tryptone	10 grams
Yeast extract	5 grams
sodium chloride	10 grams

The components of the broth were dissolved in 950 microliters of distilled water and the pH was maintained to 7.5 with 5N sodium hydroxide (NaOH) then, finally, the volume was made to 1000 mL with distilled water. Agar with a concentration of 1.5 % was added whenever a solid medium was prepared.

Nutrient Broth

Peptone	5 grams
Yeast extract	1.5 grams
Beef extract	1.5 grams
Sodium chloride	5 grams

The given components were dissolved in 950mL distilled water and the pH was adjusted to 7.5 with 5N NaOH finally, the volume was made to 1000 mL with distilled water.

7.2 Reagents for Acid Fast Staining

i. Carbol fuchsin (Primary stain)

Basic fuchsin	3 grams
Phenol	5%
Ethanol (96%)	10 ml

10 mL of Basic fuchsin prepared in 96% ethanol was mixed with 90 mL of phenol and then the solution was filtered through Whatman filter paper.

ii. Acid alcohol (Decolourizer)

Hydrochloric acid (conc.)	3 mL
Ethanol (96%)	97 mL

iii. Malachite green solution (Counterstain)

Malachite green	0.5 g
Distilled water	100 mL

7.3 Middle brook (MB)7H9 broth

MB7H9 broth base	4.7 g
Tween 80	1.5 mL (0.15%)
Glycerol	5 mL (0.5%)

The components were dissolved in 950mL distilled water and the pH was maintained at 7.2 with 5N NaOH finally, the volume makeup was done to 1000 mL with distilled water.

7.4 Reagents and Buffers

All the reagents and buffers for DNA and protein protocols were prepared in distilled water and sterilized by autoclaving for 15-psi pressure 15 minutes.

i. Tris-cl buffer

Tris-cl buffer of desired strength was prepared by dissolving an appropriate amount of Tris base in distilled water and the pH was adjusted with concentrated HCl. For bacteriological work 10 mM Tris-cl (pH 8.0) was prepared.

ii. Ethylene diamine tetra acetic acid (EDTA)

0.5 M solution of the disodium salt of EDTA was prepared in distilled water, pH was adjusted to 8.0 with NaOH and stored at 4 ^{0}C.

7.4Reagents for Genomic DNA isolation

i. TE Buffer

Tris-HCl (pH 8.0)	10 mM
EDTA	1 mM

ii. Lysozyme

Lysozyme	50 mg/mL in distilled H_2O

iii. Buffer Phenol

Phenol was melted at 60 ^{0}C in the water bath for 2 hours. 1M Tris-Cl (pH 8.0) was poured into the melted phenol and was incubated at room temperature for half an hour. Tris-Cl was removed using a glass pipette and pH of the phenol was noted. If the pH of phenol was maintained at 7.4 by repeatedly adding Tris-Cl. Then, it is stored submerged in 10 mM Tris-HCl (pH 8.0) in a dark bottle at 4 ^{0}C.

iv. Chloroform: phenol

The solution contains an equal amount of phenol and chloroform. The solution is stored in a dark bottle at 4 ^{0}C.

7.4.2 Buffers for Plasmid Isolation from *E. coli*

i. Alkaline Lysis Solution I

Tris-HCL (pH 8.0)	25 mM
EDTA (pH 8.0)	10 mM
Glucose	50mM

ii. Alkaline Lysis Solution II

NaOH	0.2N
SDS	1.0%

iii. Alkaline Lysis Solution III

5M Potassium acetate	60 mL
Glacial Acetic acid	11.5 mL
Distilled water	28.5 mL

7.4.3 Buffers for Electrophoresis

i. TAE Buffer (50 X)

Tris Base	242 g
Glacial Acetic Acid	57.1 mL
0.5 M EDTA (pH 8.0)	100 mL
Final Volume	1000 mL

REFERENCES

[1] National Institutes of Health (US); Biological Sciences Curriculum Study. NIH Curriculum Supplement Series [Internet]. Bethesda (MD): National Institutes of Health (US); *"Understanding Emerging and Re-emerging Infectious Diseases"*, 2007.

[2] L.M. Weiner, R Surana, S Wang., "Monoclonal antibodies: versatile platforms for cancer immunotherapy", *Nat Rev Immunol.*; 10(5):317-327, doi:10.1038/nri2744, 2010.

[3] M. Batt Sarah., E. Minnikin David, S. Besra Gurdyal; "The thick waxy coat of mycobacteria, a protective layer against antibiotics and the host's immune system". *Biochem J,*; 477 (10): 1983–2006. doi: https://doi.org/10.1042/BCJ20200194, 2020.

[4] American Thoracic Society and CDC. "Diagnostic standards and classification of tuberculosis in adults and children." *Am J Respir Crit Care Med*; 161(4): 1376– 1395 2000.

[5] J. Chakaya, E. Petersen, R. Nantanda, N. Mungai Brenda, Battista M. Giovanni, "The WHO Global Tuberculosis 2021 Report – not so good news and turning the tide back to End TB", *Pubmed.gov*, published online, 2021.

[6] K.J. Seung, S. Keshavjee, M.L. Rich, "Multidrug-Resistant Tuberculosis and Extensively Drug-Resistant Tuberculosis". *Cold Spring Harb Perspect Med*. 27;5(9):a017863. doi: 10.1101/cshperspect.a017863, 2015

[7] Abate, Getahun, and Daniel F. Hoft. "Immunotherapy for tuberculosis: future prospects." *ImmunoTargets and therapy* vol. 5 37-45. 2016, doi:10.2147/ITT.S81892

[8] Hawn, R Thomas "Host-directed therapeutics for tuberculosis: can we harness the host?." *Microbiology and molecular biology reviews : MMBR* vol. 77,4 : 608-27. doi:10.1128/MMBR.00032-13, 2013.

[9] I. Barberis., "The history of tuberculosis: from the first historical records to the isolation of Koch's bacillus." *Journal of preventive medicine and hygiene* vol. 58,1: E9-E12, (2017),

[10] A.V. Martynov, T.V. Bomko, T.N. Nosalskaya, Yu Lisnyak.V, E.A. Romanova, T.V. Kabluchko, T.A. Sidorenko, N.I. Igumnova, M.S. Pogorelaya, E.M. Shcherbak, V.I. Yukhimenko, B.S. Farber, & S.B. Farber., Tuberculosis as an infectious pathology of immune system. Annals of Mechnikov Institute, 3, 8–14, 2016

[11] G. Keith. Mansfield, G. Fox James, Chapter 16 - Bacterial Diseases, Editor(s): M. Robert, W. Lynn, T. Suzette, M. Keith, F. James, "The Common Marmoset in Captivity and Biomedical Research, Academic Press", *American College of Laboratory Animal Medicine*, Pages 265-287, ISBN 9780128118290, 2019

[12] B. Singh, J Ghosh, N. Islam, M. Dasgupta, S., A. Leif Kirsebom,.. "Growth, cell division and sporulation in mycobacteria". *Antonie van Leeuwenhoek, 98(2), 165–177,* (2010)

[13] Gengenbacher, Martin, and H. Stefan, E. Kaufmann. "*Mycobacterium tuberculosis*: success through dormancy." *FEMS microbiology reviews* vol. 36,3: 514-32, (2012).

[14] A. J Caulfield,., & N L Wengenack,.. "Diagnosis of active tuberculosis disease: From microscopy to molecular techniques". *Journal of Clinical Tuberculosis and Other Mycobacterial Diseases, 4, 33–43,* (2016).

[15] Smith, Issar. *"Mycobacterium tuberculosis pathogenesis and molecular determinants of virulence." Clinical microbiology reviews* vol. 16,3 (2003): 463-96.

[16] H. Saito., "Laboratory media for the cultivation of tubercle bacillus*"*. *Kekkaku.*; 73(5):329-37.,. PMID: 9637817, May 1998

[17] J. M. Balada-Llasat, K. Kamboj, P. Pancholi, "Identification of mycobacteria from solid and liquid media by matrix-assisted laser desorption ionization-time of flight mass spectrometry in the clinical laboratory." *Journal of clinical microbiology* vol. 51,9: 2875-9. (2013)

[18] B.R. Bloom, R. Atun, T Cohen, K.K. Holmes, S. Bertozzi, B.R. Bloom, "Major Infectious Diseases". Washington (DC): *The International Bank for Reconstruction and Development / The World Bank*;. Chapter 11, 3RD edition. 2017

[19] J.M. Yuk, & E. Jo. "Host immune responses to mycobacterial antigens and their implications for the development of a vaccine to control tuberculosis*". Clinical and Experimental Vaccine Research, 3(2), 155.* doi:10.7774/cevr.2014.3.2.155 (2014)

[20] D. M. Maurizio, L. Lorenzo, G. Luisa, "C. Elena; Immune Response to Mycobacterium tuberculosis: A Narrative Review*", Frontiers in Pediatrics*, Volume-7), ISSN=2296-2360 (2019)

[21] Cooper, M. Andrea. *"*Cell-mediated immune responses in tuberculosis." *Annual review of immunology* vol. 27 :393-422. doi:10.1146/annurev.immunol.021908.132703 (2009)

[22] Y. Rosen, "Cavitating pulmonary tuberculosis (gross pathology)". *Case study, Radiopaedia.org.* (accessed on 28 May 2022) https://doi.org/10.53347/rID-8633

[23] L. Ramachandra, J.L. Smialek, S.S. Shank, M. Convery, W.H. Boom, C.V. Harding. "Phagosomal processing of Mycobacterium tuberculosis antigen 85B is modulated independently of mycobacterial viability and phagosome maturation". *Infect Immun.* ;73(2):1097-105. doi: 10.1128/IAI.73.2.1097-1105.2005, 2005

[24] J. Buter, T Cheng, Y. Ghanem, M. Grootemaat, . E. Raman, S. Feng, X. Plantijn, A. R. Ennis, T., J Wang,., R Cotton,, N. Layre, E. Ramnarine, A. K. Mayfield, J. A. Young, D. C. Jezek Martinot, A. Siddiqi, N. Wakabayashi, S. Botella, H. Calderon, R. Murray, M. Moody, "*Mycobacterium* tuberculosis releases an antacid that remodels phagosomes". *Nature chemical biology*, *15*(9), 889–899. https://doi.org/10.1038/s41589-019-0336-0 (2019)

[25] K. Sakamoto, M. Jeong, R. Rhoades Allavena, S. Ehrt, H. Wainwright, G. David, K. Rohde, "Mycobacterial trehalose dimycolate reprograms macrophage global gene expression and activates matrix metalloproteinases." *Infection and immunity* vol. 81,3 : 764-76. doi:10.1128/IAI.00906-12, 2013

[26] PW Ei, WW Aung, J.S. Lee, G.E. Choi, C.L. Chang. "Molecular Strain Typing of *Mycobacterium* tuberculosis: a Review of Frequently Used Methods", *J Korean Med Sci*.;31(11):1673-1683. https://doi.org/10.3346/jkms.2016.31.11.1673 2016

[27] S.J Han,., T Song,., YJ Cho,., *Complete genome sequence of Mycobacterium tuberculosis K from a Korean high school outbreak, belonging to the Beijing family.* Stand in Genomic Sci 10, 78 (https://doi.org/10.1186/s40793-015-0071-42015).

[28] Ghazaei, Ciamak. "*Mycobacterium tuberculosis* and lipids: Insights into molecular mechanisms from persistence to virulence." *Journal of research in medical sciences : the official journal of Isfahan University of Medical Sciences* vol. 23 63, doi:10.4103/jrms.JRMS_904_17, 2018

[29] J. Qian C. Run, W. Honghai, Z, Xuelian, "Role of the PE/PPE Family in Host-Pathogen Interactions and Prospects for Anti-Tuberculosis Vaccine and Diagnostic Tool Design*"*, *Frontiers in Cellular and Infection Microbiology*, VOLUME-10, DOI=10.3389/fcimb.2020.594288 2020

[30] Palomino, J. Carlos, and A. Martin. *"*Drug Resistance Mechanisms in *Mycobacterium tuberculosis." Antibiotics (Basel, Switzerland)* vol. 3,3 317-40., doi:10.3390/antibiotics3030317, 2014.

[31] S., Mostowy, J., Inwald, S Gordon,., C., Martin, R Warren,., K Kremer, M. A. Behr, "Revisiting the Evolution of *Mycobacterium bovis*". *Journal of Bacteriology, 187(18), (6386–6395.* doi:10.1128/jb.187.18.6386-6395.2005 (2005).

[32] K. Seng Goh, E. Legrand, C. Sola, N. Rastogi, *"*Rapid differentiation of "Mycobacterium canettii" from other Mycobacterium tuberculosis complex organisms by PCR-restriction analysis of the hsp65 gene*." Journal of clinical microbiology* vol. 39,10 (2001): 3705-8. doi:10.1128/JCM.39.10.3705-3708.2001

[33] Brites, Daniela, and S. Gagneux. *"*Co-evolution of *Mycobacterium* tuberculosis and Homo sapiens*." Immunological reviews* vol. 264,1 6-24. doi:10.1111/imr.12264martin(2015)

[34] Adama, S. Bañuls, A. Laure & S. Godreuil & Nguyen, Van Anh.. "*Mycobacterium* tuberculosis: Ecology and evolution of a human bacterium". *Journal of Medical Microbiology*. 64. 10.1099/jmm.0.000171. (2015)

[35] Moule, G Madeleine, and D Jeffrey Cirillo. "*Mycobacterium tuberculosis* Dissemination Plays a Critical Role in Pathogenesis." *Frontiers in cellular and infection microbiology* vol. 10 65. 25 Feb., doi:10.3389/fcimb.2020.00065 2020

[36] R. Brosch, S. V. Gordon, M. Marmiesse, +11, P. Brodin, C. Buchrieser, K. Eiglmeier, T. Garnier, C. Gutierrez, G. Hewinson, K. Kremer, L. M. Parsons, A. S. Pym, S. Samper, D. van Soolingen, and S. T. Cole, *"*A new evolutionary scenario for the *Mycobacterium* tuberculosis complex*." Proceedings of the National Academy of Sciences of the United States of America* vol. 99,6 3684-9. doi:10.1073/pnas.052548299(2002):

[37] C.M., Stein, L Sausville,., C.. Wejse, "Genomics of Human Pulmonary Tuberculosis: from Genes to Pathways". *Curr Genet Med Rep* **5,** 149–166 https://doi.org/10.1007/s40142-017-0130-9 (2017).

[38] P Aravindan,. "Host genetics and tuberculosis: Theory of genetic polymorphism and tuberculosis." *Lung India : official organ of Indian Chest Society* vol. 36,3 244-252. doi:10.4103/lungindia.lungindia_146_15(2019):

[39] Dockrell, M Hazel, and G Steven Smith. *"*What Have We Learnt about BCG Vaccination in the Last 20 Years?*." Frontiers in immunology* vol. 8 1134. 13 Sep., doi:10.3389/fimmu.2017.01134,2017

[40] C. Camila, R. Díaz Angello, B. Susan M., Kalergis Alexis M.; "Could BCG Vaccination Induce Protective Trained Immunity for SARS-CoV-2?", Frontiers in Immunology Volume-11, 2020

[41] S. E Haydel,. ("Extensively Drug-Resistant Tuberculosis: A Sign of the Times and an Impetus for Antimicrobial Discovery". *Pharmaceuticals, 3(7), 2268–2290.* doi:10.3390/ph30722689(2010).

[42] Abrahams, A Katherine, and S Gurdyal Besra. *"*Mycobacterial cell wall biosynthesis: a multifaceted antibiotic target*." Parasitology* vol. 145,2 116-133. doi:10.1017/S0031182016002377(2018):

[43] Mitra, Sanchita, and P. Chaudhary Tomar. *"*Hybridoma technology; advancements, clinical significance, and future aspects*." Journal, genetic engineering & biotechnology* vol. 19,1 159. 18, doi:10.1186/s43141-021-00264-6 2021

[44] Goulet, R Dennis, and M William Atkins. *"*Considerations for the Design of Antibody-Based Therapeutics." *Journal of pharmaceutical sciences* vol. 109,1 74-103. doi:10.1016/j.xphs.2019.05.031(2020)

[45] T Sharma,.; A Alam,.; A Ehtram,.; A Rani,.; S.; Grover, N.Z Ehtesham,.; S.E Hasnain,. "The *Mycobacterium tuberculosis* PE_PGRS Protein Family Acts as an Immunological Decoy to Subvert Host Immune Response". *Int. J. Mol. Sci. , 23*, 525. https://doi.org/10.3390/ijms23010525 **2022**

[46] National Research Council (US) Division of Health Promotion and Disease Prevention. Vaccine Supply and Innovation. Washington (DC): National Academies Press (US); 1985.

[47] CA Janeway Jr, P Travers, M Walport, "Immunobiology: The Immune System in Health and Disease". *New York: Garland Science*; 5th edition, 2001.

[48] RZ Hamza, T Al-Talhi, AA Gobouri, WF Alsanie, SM El-Megharbel. "Are favipiravir and acyclovir with igG injections supplemented with vitamin d "suggested therapeutic option" can fight against covid-19?". Adv. Anim. Vet. Sci. 9(4): 549-554. 2021

[49] Sandomenico, Annamaria *"*Evolution of Escherichia coli Expression System in Producing Antibody Recombinant Fragments*." International journal of molecular sciences* vol. 21,17 6324. 31 Aug., doi:10.3390/ijms211763242020

[50] Percival-Alwyn, L Jennifer *"*Generation of potent mouse monoclonal antibodies to self-proteins using T-cell epitope" ,vol. 7,1 129-37. doi:10.4161/19420862.2014.985489(2015)

[51] Y. S Grewal,., M. J. A Shiddiky,., Mahler, S. M., Cangelosi, G. A., & Trau, M. "Nanoyeast and Other Cell Envelope Compositions for Protein Studies and Biosensor Applications". *ACS Applied Materials & Interfaces, 8(45), 30649–30664.* doi:10.1021/acsami.6b09263 (2016).

[52] NS Lipman, LR Jackson, LJ Trudel, F Weis-Garcia. "Monoclonal versus polyclonal antibodies: distinguishing characteristics, applications, and information resources". *ILAR Journal*.;46(3):258-268. doi: 10.1093/ilar.46.3.258 2005

[53] Reverberi, Roberto, and L. Reverberi. *"*Factors affecting the antigen-antibody reaction*." Blood transfusion Trasfusione del sangue* ,vol. 5,4 227-40. doi:10.2450/2007.0047-07(2007):

[54] M Leenaars,., & C. F. M., Hendriksen, "Critical Steps in the Production of Polyclonal and Monoclonal Antibodies: Evaluation and Recommendations*". ILAR Journal, 46(3269–279.* doi:10.1093/ilar.46.3.269, (2005)

[55] Panawala, Lakna. "*Difference Between Monoclonal and Polyclonal Antibodies*", (2017).

[56] S Sheng, F Kong. "Separation of antigens and antibodies by immunoaffinity chromatography*".Pharm Biol.* Aug;50(8):1038-44. doi: 10.3109/13880209.2011.653493. 2012

[57] Mapara, Manjeet, *"*Rabbit as an animal model for experimental research*." Dental research journal* vol. 9,1: 111-8. doi:10.4103/1735-3327.92960(2012)

[58] Leenaars, P.P.A.M., Hendriksen, C.F.M., Angulo, A.F. Koedam, M.A. and Claassen, E. "Evaluation of several adjuvants as alternatives to the use of Freund's adjuvant in rabbits. Veterinary Immunology and Immunopathology",40, , 225-241. (1994)

[59] B. Leitner, S Elke *"*Evaluation of immunoglobulin purification methods and their impact on quality and yield of antigen-specific antibodies*." Malaria journal* vol. 7 129., doi:10.1186/1475-2875-7-1292008

[60] Arur, Swathi, and Tim Schedl. *"*Generation and purification of highly specific antibodies for detecting post-translationally modified proteins in vivo*." Nature protocols* vol. 9,2 (2014): 375-95. doi:10.1038/nprot.2014.017

[61] N. Hadi & Valedkarimi, Zahra & A. Maleki, Leili & Abdolalizadeh, Jalal & Kazemi, Tohid & Esparvarinha, Mojghan & Majidi, Jafar. "Production and purification of polyclonal antibody against F(ab')2 fragment of human immunoglobulin G". *Veterinary Research Forum. 8.* (. 307-312. 2017)

[62] Darwish, A. Ibrahim. *"*Immunoassay Methods and their Applications in Pharmaceutical Analysis: Basic Methodology and Recent Advances*." International journal of biomedical science : IJBS* vol. 2,3): 217-35. (2006

[63] R Pasqualini, W Arap. "Hybridoma-free generation of monoclonal antibodies". *Proc Natl Acad Sci* U S A. 2004 Jan 6;101(1):257-9. doi: 10.1073/pnas.0305834101. Epub , PMID: 14688405; PMCID: PMC314172. 2003

[64] Maciej, S. Katarzyna; S. Katarzyna; W. Kamil; W. Jeremi; Z. Łukasz, *"*Clinical application of monoclonal antibodies in targeted therap*y." Journal of Clinical and Experimental Medicine* 17): 338-346. 2020

[65] B. Claudio, and G. Gutierrez. "*Mycobacterium* tuberculosis infection of host cells in space and time." *FEMS microbiology reviews,* vol. 43,4 341-361. doi:10.1093/femsre/fuz006(2019)

[66] A. Acosta, Y. Lopez, N. M. Nor, R. H. Pando, N. Alvarez, M. E. Sarmiento, and A. Glatman-Freedman, "The Role of Antibodies in the Defense Against Tuberculosis", in Tuberculosis - Current Issues in Diagnosis and Management. London, United Kingdom: IntechOpen, 2013.

[67] AF Saeed, R Wang, S Ling, S., Wang "Antibody Engineering for Pursuing a Healthier Future". *Front Microbiol*. Mar 28;8:495. doi: 10.3389/fmicb.2017.00495. PMID: 28400756; PMCID: PMC5368232. 2017

[68] G.Freedman, and A. Casadevall,*"*Serum therapy for tuberculosis revisi69ted: reappraisal of the role of antibody-mediated immunity against Mycobacterium tuberculosis*" Clinical microbiology reviews* vol. 11,3 514-32. doi:10.1128/CMR.11.3.514(1998)

[69] C. Liu, Z. Zhao, , J., FanLyon, C. J., Wu, H.-J., D., Nedelkov, Y Hu,., "Quantification of circulating*Mycobacterium* tuberculosis antigen peptides allows rapid diagnosis of active disease and treatment monitoring*". Proceedings of the National Academy of Sciences, 114(15),*) *3969–3974.* doi:10.1073/pnas.1621360114,2017.

[70] S. Carolyn, Ekaterina Dadachova and Arturo Casadevall, "Monoclonal Antibody-Based Therapies for Microbial Diseases," *Vaccine* 27 (2009): G38-G46

[71] Jacobs, J. Ashley., *"*Antibodies and tuberculosis*" Tuberculosis (Edinburgh, Scotland)* vol. 101 (: 102-113. doi:10.1016/j.tube.2016.08.0012016).

[72] Suzuki, Masami & Kato, C. & KATO, Atsuhiko. "Therapeutic antibodies: Their mechanisms of action and the pathological findings they induce in toxicity studies". *Journal of Toxicologic Pathology*. 28., 10.1293/tox.2015-0031. (2015).

[73] J.Thomas, A. Balseir, C.Gortázar, "Diagnosis of tuberculosis in wildlife: a systematic review*". Vet Res* ***52,*** 31. https://doi.org/10.1186/s13567-020-00881-y(2021)

[74] Joshi, M. Jyotsna "Tuberculosis chemotherapy in the 21 century: Back to the basics." *Lung India : official organ of Indian Chest Society* vol. 28,3 193-200. doi:10.4103/0970-2113.83977(2011)

[75] G. Guedes, "Tuberculosis and immunosuppression". *J Microbiol Exp.*;6(3):145-146. DOI: 10.15406/jmen.2018.06.00205, 2018.

[76] Ahsan, M. Jawed, *"*Recent advances in the development of vaccines for tuberculosis.*" Therapeutic advances in vaccines* vol. 3,3 66-75. doi:10.1177/2051013615593891(2015).

[77] RM Lu., YC., Hwang, IJ Liu, "Development of therapeutic antibodies for the treatment of diseases*'", Biomed Sci* **27,** 1). https://doi.org/10.1186/s12929-019-0592-z,2020.

[78] SA. Dass, V. Balakrishnan, N. Arifin, CSY Lim, F. Nordin, G.J. Tye. "The COVID-19/ Tuberculosis Syndemic and Potential Antibody Therapy for TB Based on the Lessons Learnt From the Pandemic", *Front Immunol.*;13:833715. doi: 10.3389/fimmu.2022.833715. PMID: 35242137; PMCID: PMC8886238. 2022 .

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1. Immunotherapies for Tuberculosis

   Sharvi Sood, Rahul Shrivastava

   E-short Presentation at: School of Life and Allied Science, ITM University, Raipur (Chhattisgarh) India. Held in Online mode on March 3, 2022.