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Review Article
Tuberculosis in Animals and Humans: Evolution of Diagnostics and Therapy

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Abstract
Tuberculosis caused by different species of genus *Mycobacterium* or different serotypes of various species are the leading cause of mortality among livestock, domesticated animals and humans alike. This leads to huge economic loss in terms of animal and human capital. Currently one third of global population is infected with tuberculosis (TB). There might be innumerable reasons for it being pandemic but proper diagnosis or lack of it is one of the major contributing factors for its global spread. In developing countries precise and reliable diagnosis has emerged out to be the major cause translating into high burden. The TB diagnosis has evolved over the time with changing needs from classical microscopic sputum smear analysis to rapid PCR based molecular diagnostics. Molecular techniques are becoming confirmatory diagnostic tools and advanced procedure for TB detection. Current review lays emphasis on the tuberculosis from lower animals to higher animals including human with respect to diagnostics, therapy and its improvisation over a decade.

Key words: TB bacilli, multi drug resistance TB, extensively drug resistance TB, sputum, PCR

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INTRODUCTION

Tuberculosis (TB) is a highly contagious disease that is affecting the animal as well as human population since the time immemorial. While infection in humans are chiefly from *Mycobacterium tuberculosis* leading to pulmonary TB (PTB), infection from other species may affect other parts of the body causing extra-pulmonary TB (ETB). Infection in domesticated livestock and wild animals from *M. bovis*, *M. avium* and rarely *M. tuberculosis* is responsible for the mass mortality as compared to the mortality from the combine of other infection. In the first half of 20th century, infection from animals to human through a process called zoonosis was quite common leading to the loss of both livestock as well as human capital. However, with the advent of pasteurization killing *M. bovis*, mortality in humans have reduced to a great extent. In the United Kingdom and other European countries farm animals or the domesticated animals are tested for the infection and are killed if tested positive for the infection[1-3]. Current review critically examines the array of TB diagnostic tools in terms of their accuracy, efficacy, affordability and evolution from classical TB diagnostics to modern molecular diagnostic protocols over a decade.

TUBERCULOSIS (TB) OF DOMESTICATED ANIMALS AND LIVESTOCK

Tuberculosis in horses: Equine TB is of rare occurrence nevertheless cases are reported where horse was found to be infected by *M. tuberculosis* and *M. bovis*. Infected horse displays the symptoms of granulomatous lymphadenitis in mediastinal spaces and tracheobronchial lymph nodes.

These infections are usually diagnosed by real time polymerase chain reaction (RT-PCR) and culture based techniques[4].

Sheep and goats: Sheep and goats are resistant to *M. tuberculosis* infection but are susceptible to *M. bovis* infection. It usually manifests in lungs and lymph nodes of infected animal. However, it may spread to other organs as well. The TB infection is contagious and infected animals can affect other animals as well. Diagnosis is usually performed by intradermal skin test utilizing purified proteins from *M. bovis* and *M. avium*[1-3,5].

Farmed and wild cervids: The visible symptoms of TB are produced by *M. avium* and *M. bovis* in the lymph nodes of the head and abscessation. Examples include farmed and wild cervids, including axis deer, fallow deer, roe deer, mule deer, sika deer, as well as red deer or elk or wapiti. Diagnosis is performed by tubercular skin test and *in vitro* cellular assays[1,3,5].

Hoofed animals: This category includes African buffalo, wood bison, North American bison, white-tailed and mule deer, lechwe, elk, brushtail possums and European badgers. These are usually susceptible to *M. bovis* infection. While, fennec fox, coyote, Arabian oryx, muntjac, impala, sitatunga, springbok, moles, voles, hares, eland, yak, bactrian camel, wildebeest, European wild goat, large spotted genet, tapir, moose, otters, feral water buffalo, hedgehogs, European wild boar, greater kudu, tiger, white and black rhinoceros and giraffe etc are susceptible to *M. tuberculosis* mediated infection. The *M. tuberculosis* is isolated from oryx, black rhinoceros, Asian elephant, addax and rocky mountain goats. Visible symptoms are in the form of lesions that vary in consistency from purulent (pus like) to caseous (necrotic) in lungs and regional lymph nodes with liver, spleen and serosal surfaces acting as major sites. Diagnostics involve tuberculin skin tests performed in the cervical region using *M. bovis* PPD[1-3,5].

Elephants: The TB infections in elephants are usually confined to captive domesticated elephants. As with other animals TB infection is usually confined to lung and the associated lymph nodes. Diagnosis via tuberculin skin test and *in vitro* immunologic test gives non-specific responses. Therefore, trunk washes should be used for diagnostic purposes. Combined drug therapy involving isoniazid and rifampin is recommended for treatment with continuous monitoring of blood to analyze the threshold concentration of drugs, enough to kill the TB bacilli[1,3].

Pigs: The TB in pigs is usually caused by *M. tuberculosis*, *M. bovis* and *M. avium* complex (*M. avium avium* and *M. avium hominissuis*). Infection is spread through shared contaminated grazing. Observable symptoms are in the form of granulomatous lesions present in cervical, submandibular and mesenteric lymph nodes. Lesions in their progressive stages are present in liver and spleen. Diagnosis includes intradermal test performed on the dorsal ear surface or vulva skin[1-3,5].

Cat and dogs: Dogs get TB infection chiefly from *M. tuberculosis*, *M. bovis* and rarely from *M. avium* complex or *M. fortuitum* having come from a human or bovine source. Tuberculous lesions are located in lungs, liver, kidney, pleura and peritoneum having grayish appearance with a non-calcified necrotic center. Tuberculin

skin test usually gives false negative results. Since dogs lives in close proximity with human so euthanasia is recommended instead of treatment[1,3]. Cats show high degree of susceptibility to *M. bovis*, *M. avium* complex or *M. microti*, *M. lepraemurium* but are usually resistant to *M. tuberculosis*. Clinical symptoms are in the form of granulomatous lesions in mesenteric lymph. These lesions were the cause for tuberculous cat in the Europe. Blood mediated transmission to lungs and localized lymph nodes may occur. Tuberculin test, which forms the gold standard for testing TB in animals is considered unreliable in case of cats and dogs and needs to be confirmed by radiography and ELISA[1-3,5].

Rabbit: The TB is extremely rare in rabbits, however, susceptibility to *M. bovis* and *M. avium* is reported. Rabbit gets the infection from exposure to infected animal or contaminated feed. The *M. avium* infection is caused by contact with domestic and exotic birds infected with *M. avium*. Tuberculin skin test forms the usual diagnostic procedure performed on abdominal skin[1,3].

Guinea pigs: The TB in Guinea pigs is caused by *M. tuberculosis*, *M. bovis*, serotypes of *M. bovis* and *M. avium*. Visible symptoms are present in the form of lesions in the parenchyma of gastrointestinal tract. As with other animals diagnosis involves tuberculin skin test that utilizes Purified Protein Derivative (PPD) of *M. bovis* and *M. avium*[3].

Non-primates: Non-primates get the infection from *M. tuberculosis*, *M. bovis* and *M. avium* in lungs (pulmonary TB and other organs (extra-pulmonary TB). Non-primates receive the infection from coming in close contact with infected human service providers. Modes of spread are aerosol with respiratory infection or the oral route. The TB bacilli may also be detected from urine. Diagnostics involve tubercular skin test where old tuberculin is preferred over Purified Protein Derivative (PPD) as it is more sensitive[1-3,5].

Aquatic mammals: Marine mammals gets the infection from *M. pinnipedii.* The causative organism *M. pinnipedii* is variant of *M. bovis* adapted and specific to seal. This has been isolated from tubercular lesions in seals and fur seals. Symptoms are produced in peripheral lymph nodes, spleen, peritoneum and lungs[1-3,5].

Bovine infection spread to humans: Infection by *M. bovis* or bovine infection can spread to human by contaminated unpasteurized dairy products, inhalation of infectious aerosols etc. However, it can be controlled by proper management and livestock surveillance programs. Bovine TB can be cured through antimicrobial drugs.

Tuberculosis in human: Tuberculosis (TB) is a long standing and one of the most primitive, epidemic disease of mankind[6-11]. Globally TB is one of the major cause for the mortality and morbidity in humans and other animals alike. Birds, rodents, reptiles and other animals can also contract *Mycobacterium* infection. Tuberculosis in cattles by *Mycobacterium bovis* is of grave concern for dairy and animal husbandry. Big animals like elephants can also get tuberculosis infection in captivity. It is believed that animals get this infection via 'Reverse zoonosis'[12-19]. The TB is highly infectious disease that spread through *M. tuberculosis* (Table 1). Approximately, 2 million people are killed by TB annually with addition of 8.6 million per year[20,21]. Amongst various causes, lack of economical and reliable diagnosis has huge impact on upsurge of TB. This becomes a huge challenge particularly with MDR/XDR/TB-HIV cases in developing countries and almost in all high burden countries. The World Health Organization (WHO) has approved many diagnostic methods and has evolved a special strategy as Supranational Reference Laboratory Network (SRLN) (Fig. 1) to provide diagnostic information and technical resource in addition to the strengthening of the diagnostic methods with special laboratory capacity in many countries[22-25].

Table 1: Morbidities due to different species of *Mycobacterium*

Species	Tuberculosis: Ghon focus/Ghon's complex
M. tuberculosis and *M. bovis*	Pott disease
	Brain (Meningitis, rich focus)
	Tuberculous lymphadenitis (tuberculous cervical lymphadenitis)
	Cutaneous (Scrofuloderma, erythema induratum, lupus vulgaris, prosector's wart, tuberculosis cutis orificialis, tuberculous cellulitis, tuberculous gumma)
	Lichen scrofulosorum, tuberculids (Papulonecrotic tuberculid)
	Primary inoculation tuberculosis, miliary, tuberculous pericarditis, urogenital tuberculosis, multi-drug resistant tuberculosis, extensively drug-resistant tuberculosis
M. leprae	Leprosy: Tuberculoid leprosy, borderline tuberculoid leprosy, borderline leprosy, borderline lepromatous leprosy, lepromatous leprosy, histoid leprosy

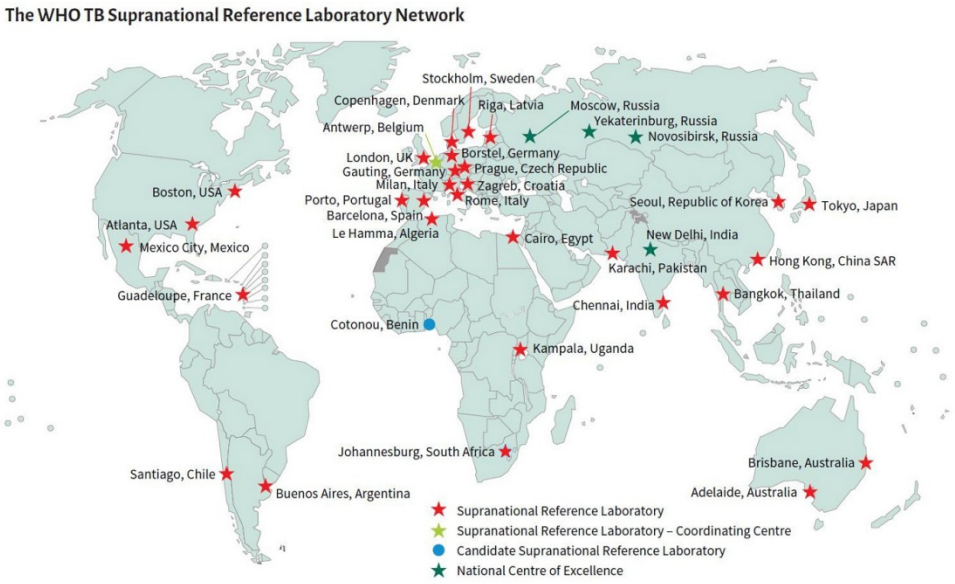

Fig. 1: WHO TB Supranational Reference Laboratory Network (SRLN)

About 36 counties are involved in SRLN network (Fig. 1) to improve and innovate the best diagnostics in terms of precision, reliability, portability and cost involved. The TB in most of the cases is often difficult to diagnose due to asymptomatic status of the patient or characters in phenotypic order for a long time. Slow progression of MTB usually take months or even year of latency. Methods are now available that facilitates direct detection of *Mycobacterium tuberculosis*. Basic diagnostics involve chest x-ray, sputum microscopy test, IGRA test and TB skin test. For cases where TB is associated with HIV or MDR/XDR cases, classical diagnostic methods are usually complemented with modern molecular diagnostic protocols[26-29]. However, these protocols are cost ineffective, not available easily and are still not optimized for commercial applications.

MYCOBACTERIUM PROFILE

Fatty acid and pathogenicity: Around 250 genes are involved in fatty acid metabolism of which 39 are involved in polyketide metabolism that produces coat of wax. The genes involved in fatty acid metabolism show evolutionary conservation that validates the importance of fatty acid in the pathogenicity. Cells stained with acid-fast staining show wrapped together due to the presence of fatty acid in the cell wall that stick together. High content of lipid, i.e., mycolic acid in the cell wall makes it highly resistant and pathogenic. Such type of cell wall prevents the fusion of bacterium containing phagosome with lysosome thus escape killing by antimycobacterial factors[30-35].

Host susceptibility: Tuberculosis has a definite genetic component. A certain type of genetic makeup predisposes an individual towards the mycobacterial infection. Group of rare genetic disorder called Mendelian susceptibility to mycobacterial diseases (MSMD) increases the likelihood of an individual to contract the disease. Modern research involving Genome Wide Association Studies (GWAS) also validates this[36-38].

Human-*Mycobacterium* co-evolution: Empirical evidences from phylogeny and phylogeography evidences have proved that *Mycobacterium* has migrated to distant parts of the globe along with its human host. Evolutionary history has traced back its origin to Africa from where it has migrated to other regions. Similarity found in the mitochondrial genome of *Mycobacterium* and human host suggests relationship between the two and co-evolutionary pattern. In any case, *Mycobacterium* must have evolved to increase its pathogenicity while human hosts have evolved to have better defense strategies[39-41].

Evolutionary spread: *Mycobacterium tuberculosis* complex (MTC) shows clonal spread pattern and human infecting species have been classified into seven spoligotypes (Table 2). Type 2 and 3 are closely related while type 3 is divided into two clades CAS-Kiii (Tanzania) and CAS-Delhi (Delhi and Saudi Arabia). Beijing strain is most pathogenic with population expansion of 500 fold[42-44].

Table 2: Clonal variation pattern of *Mycobacterium tuberculosis*

Spoligotypes	Human variant
Type 1	East African Indian and Manu Indians
Type 2	Beijing group
Type 3	Central Asian strain
Type 4	Ghana and Haarlem strain (H/T), Latin-America-Mediterranean (LAM) and X strains,
Type 5	*Mycobacterium africanum* in West Africa
Type 6	*Mycobacterium africanum* in West Africa
Type 7	Strain from Horn of Africa

TUBERCULOSIS DIAGNOSTICS: CONVENTIONAL APPROACHES

Traditional diagnosis methods: The TB is older than 6000 years in the realms of history of mankind, referred to as phthisis or white disease. In those times information about TB were scarce; diagnosis was based on productive cough of four or more week, hemoptysis, loss of weight, chest pain, chills, night sweat, fatigue and lot of sputum production constituting the preliminary information for TB diagnosis[45]. However, TB diagnosis has taken a giant leap since then from microscopic analysis of sputum to PCR and isotope based protocols[46-48]. Diagnosis of TB bacilli depends upon smear positivity in sputum sample, chest radiography and culture. Although several TB diagnosis methods are available but with known limitations. Robert Koch had discovered the tubercle bacillus in 1882 and thereafter methods of detecting these microorganisms were developed to assist the diagnosis of the disease. Thus, Acid Fast Bacilli (AFB) remain a cost effective method for staining TB bacilli[49].

Microscopy test of sputum smear or pulmonary TB test: Microscopic analysis of sputum smear is the most common method for TB diagnosis used worldwide particularly in developing or low/middle income countries making it a standard diagnostic method for detection of pulmonary TB[50]. This test involves microscopic analysis of sputum coughed by patient. Microscopic analysis leads to the visible detection of germs (bacilli), i.e., smear positive (Fig. 2). However, this test has its limitations when it comes to the detection of MTB that require culture test to confirm the presence of *Mycobacterium tuberculosis*. This test is rapid, affordable and accurate for normal pulmonary TB. Microscopic test is a common method of TB detection in Asian countries including India, Japan and China.

Culture method: Culture test requires a laboratory setup. Usually, sputum or phlegm is taken in a jar, if any MTB is present in the sample it could grow in culture medium

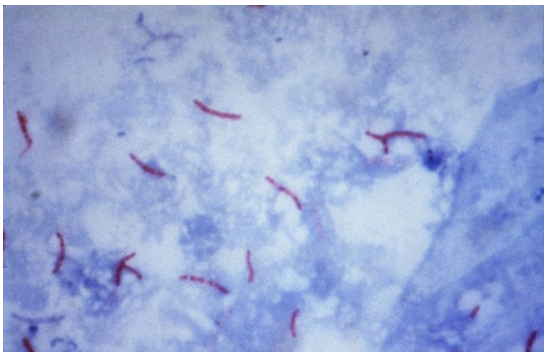

Fig. 2: Tuberculosis bacilli in Ziehl-Neelsen stain

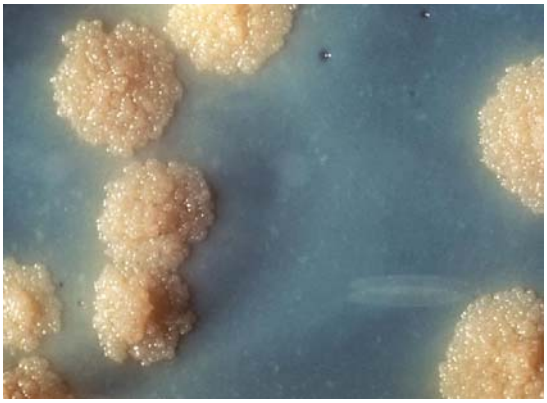

Fig. 3: Colonies of *Mycobacterium tuberculosis* grown on LJ media

forming colony of *M. tuberculosis* (Fig. 3). This test can detect TB like normal TB and drug resistant TB[51]. The culture based diagnosis takes 4-10 weeks.

Culture method requires fluorescence microscopy or auramix rhodamine staining following induction of sample with bronchodilator saline solution. There are many culture method available with different type of culture media such as Lowenstein-Jensen (LJ), Middlebrook media, JH9 and 7H10 or Kirchner. Microscopic Observatory Drug Susceptibility (MODS) culture assay is faster as compared to other culture methods. This type of diagnosis is commonly used worldwide. Besides conventional laboratory culture, modern automated system are also available such as VERSA TREK, BACTEC and MGIT (mycobacterial growth indicator tube).

Chest x-ray: Radiographs of chest x-ray indicates the pulmonary TB. Lung damage shows TB infection and its location. Damage, which appears as white patches, shows the presence of TB that could be further confirmed by other tests or diagnostic protocols (Fig. 4). However, x-ray appears

Table 3: Specification of currently available IGRAs

QuantiFERON-TB blood test	T-SPOT TB blood test
The QuantiFERON-TB blood test is relatively new and was started in 2005 by CDC followed by FDA approval in 2007	T-SPOT TB blood test is antigen detecting test, which is normally used in UK and some other European countries where it is called enzyme linked immune spot assay
QuantiFERON-TB blood test is highly sensitive	Process Peripheral Blood Mononuclear Cells (PBMCs) within 8 h or if T-cell Xtend is used takes 30 h
QuantiFERON is initial process which process whole blood within 16 h	This test counts the T-lymphocyte activation by MTB (Mabtech AB. ELISPOT, 2004, Oxford Immunotec Limited, T-SPOT TB 2004)
It involves antigen to detect TB bacteria in their blood (lymphocyte) following incubation with antigen (CDC, 2005). Works in ELISA format	Detects *M. tuberculosis*: Detection is based on the number of interferon-gamma f (IFN-g) producing cells (spots) in collected blood sample
The test measurement works through interferon-gamma (IFN-g) concentration when sample mixed with antigens (substances that can produce an immune response) derived from *M. tuberculosis*	

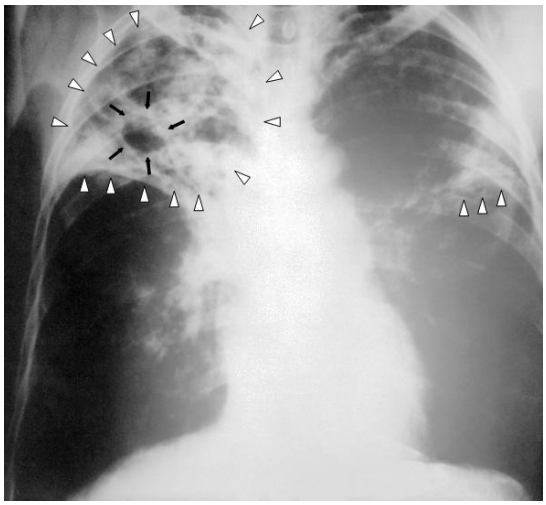

Fig. 4: Chest x-ray of a patient diagnosed with advanced stage of tuberculosis

normal in case of TB associated with HIV and other immune suppressed diseases thus giving false negative response. Chest x-ray identifying MTB appear as tree in bud sign on upper lobe. Chest x-ray report needs to be aligned with other diagnostic methods to confirm TB[52].

Chest x-ray is better only for acute pulmonary TB and redundant for extra pulmonary TB. Sometime other lung disease is mistakenly diagnosed as similar to pulmonary TB in X-ray called as mimicking pulmonary TB[53].

Diagnosis through skin test: Mantoux test and TST (Tuberculosis/Tuberculin skin test) depend on immune response to *Mycobacterium tuberculosis.* At the time of TST, a small amount of TB antigen is injected inside the top layer of skin, if the immune system of body comes in contact with the bacterium, skin colour changes to pale red. This test is non-confirmatory in nature and requires other tests to complement the finding. Mantoux tuberculin test involve intra-dermal injection of Purified Protein Derivative (PPD) followed by measuring the size of tuberculin indurations of 48-72 h, which measures immune response against 72-75 bacilli[10]. This test is commonly used in USA and other South American countries. The TST is also a method of diagnosis in other countries like UK where it is referred to as Heaf test with 4 on point scale detection[54].

Blood test for TB diagnosis: Blood test for TB diagnosis identifies parameters like hypocalcemia and hyponatremia with increased RBCs sedimentation rate. This test needs further confirmation to establish the infection. However, results are not sufficient to differentiate active or latent type. Interferon-Gamma Release Assays (IGRAs) are whole-blood tests that can aid in diagnosing *M. tuberculosis* infection[55-58].

This test is more common in developed countries like USA, UK or other European countries where blood test is available in three types-QuartiFERON, T-SPOT TB and ELISPOT. These tests are rarely available in India and other Asian countries. Two IGRAs that have been approved by the US Food and Drug Administration (FDA) are QuantiFERON-TB Gold In-Tube test (QFT-GIT) and SPOT TB test (T-SPOT) (Table 3).

MODERN MOLECULAR PROTOCOLS

Modified diagnostic test based on molecular and genetic based approaches: Increasing pathogenicity of tuberculosis bacterium and resistance to existing drug has made classical diagnostic protocols redundant paving the way for newer and modern PCR based molecular methods or radioisotope based fluorescence methods for detecting drug resistant TB. For instance, PCR based Ziehle-Neelsen stained sputum test, radioisotope based PCR, single PCR methods and multi-PCR SSP assay and DNA amplification of TB are available for molecular genetic analysis of TB. These improvised diagnostic protocols have facilitated the detection of more than 80% isoniazid (INH) and rifampicin (RIF) resistant TB[59,60]. Ziehle-Neelsen stained sputum test utilizes Ziehl-Neelsen acid fast stained slides, which uses silica based filter with PCR. The stained sputum sample on glass slide contains primer[61,62]. This

method use two set of primers-one based on the IS6110 sequence of *M. tuberculosis* and other based on protein antigen b (PAB). This protocol facilitates direct detection of pulmonary tuberculosis through PCR assay[63].

Multiplex allele-specific polymerase chain reaction (MAS-PCR): This protocol detects MDR/XDR TB. It is a relatively inexpensive and technically feasible technique for rapid detection of MDR-TB[64]. On other side, MTBDRSI assay is also available for rapid detection of drug resistance (Amikacin and almost all fluoroquinolones). This is a new type of molecular kit designed for specific detection of resistance against second line drugs. It works on a single strip and can be done directly on clinical sample[65]. In addition to this many Western countries use PCR-SSP (PCR-single strand conformational polymorphism) for confirmation of rifampicin resistance[66].

Xpert MTB/RIF: Xpert MTB/RIF test or assay is used for the diagnosis of pulmonary TB. This assay simultaneously detects *M. tuberculosis* complex (MTBC). This protocol utilizes capheild's gene Xpert Dx system that include PC, barcode scanner and software for running the test and viewing results[67]. Standard culture can take 2-6 weeks for MTBC to grow. This test can also detect resistance to rifampcin (RIF) and take around 3 weeks. The Xpert MTB/RIF assay is a Nucleic Acid Amplification Test (NAAT) that utilizes a disposable cartridge with the GeneXpert instrument system. A sputum sample is collected from the patient with suspected TB. The sputum is mixed with the reagent that is provided with the assay and a cartridge containing this mixture is placed in the GeneXpert machine. All processing from this point onward is fully automated. Additionally, assay can quickly identify possible multi-drug resistant TB (MDR TB). Resistance to rifampcin (RIF) is a predictor of MDR TB because resistance to RIF, in most instances co-exists with resistance to isoniazid (INH). Rapid diagnosis of RIF resistance potentially allows TB patients to start effective treatment much sooner than waiting for results from other types of drug susceptibility testing. However, this assay does not replace the need for smear with microscopy, culture of mycobacteria, acid-fast bacilli and growth-based drug susceptibility testing, in addition to genotyping for early discovery of outbreaks.

FAST-RIF or fluorometric assay: This protocol for susceptibility testing of rifampcin was developed around 2008 by the group at Stellenbosch University, South Africa. It is a fluorometric based assay to detect rifampin susceptibility of MTB. The FAST-RIF works on the principal of high resolution thermal melt analysis and determine the region of gene *rpo*B in MTB[68].

High Resolution Melting Analysis Assay (HRMA): The HRMA detects ofloxacin, rifampcin and isoniazid resistant MTB through mutation target[69,70]. It is a PCR based protocol that detects mutation in the genes that imparts resistance to isoniazid, rifampcin and ofloxacin. The HRMA is a routine test for detecting MDR-TB in developing countries. It is similar to Auto MODS assay, i.e., Microscopic Observation Drug Susceptibility (MODS) assay[71].

M-ARMS: The M-ARMS is utilized to detect only rifampin resistant MDR-TB. This protocol involves multiple amplification refractory mutation system PCR that works on single mutation system based on allele-specific priming. In this method, an oligonucleotide primer with a triple end complementarity to the sequence of a specific mutation coupled with a common primer is used in one PCR reaction. The M-ARMS involve chimeric primer and can detect mutation at many codon on *rpo*B gene of rifampin[72]. However, all the detection procedure including the conventional AFB-staining, skin tuberculin test and new generation modifying tests have some advantages as well as limitations (Table 4). There are some laboratory based commercially available diagnostic tests for TB that have been optimized by bio-laboratories or industries for improving diagnostic procedure (Table 5).

VACCINATION

Vaccines are permanent solution to the active and latent tuberculosis. The inherent limitations of BCG vaccination has forced scientist to look for other alternatives, the most promising being subunit vaccine[73]. MVA85A vaccine based on vaccinia virus is a subunit vaccine[74]. At global level, stop TB partnership, South African Tuberculosis Vaccine Initiative, Aeras Global TB Vaccine Foundation are spearheading the vaccine development research[75-78].

A research group under Professor Raghavan Varadarajan at Indian Institute of Science (IISc), Bangalore, India is already working on the HIV-AIDS vaccine. The vaccine in question will be a epitope based sub-unit vaccine[79]. On similar fashion vaccine for TB can be designed based on the capsule of TB bacilli. Subunit vaccine is the logical solution for TB as it would be based on the part of TB bacilli and free from the danger of using live weakened or dead bacterium for the

Table 4: Tuberculosis diagnostic procedures

Method	How does it works	Advantage	Disadvantage	Intended use	Limitations
AFB smear microscopy or pulmonary TB test	Sputum is collected from suspected TB person through coughing. A series of special stains are applied to the sample and the stained slide is examined under a microscope	Require training in microscopy, economical	Direct smear microscopy is relatively insensitive as at least 5,000 bacilli mL^{-1} of sputum are required for direct microscopy to be positive	Community	Low sensitivity, difficult to isolate viable forms from non-viable and drug-susceptible from drug-resistant strains
Culture method	Studying bacteria by growing them on media containing nutrients	Good sensitivity, gold standard	More complex and expensive than microscopy to perform as it requires specific equipment and enhanced laboratory facilities. Long period of assay to get the result	Referral lab	Long time to direct growth of bacteria, take weeks because of the slow growth of TB bacilli
Chest x-ray or radiography	White patches shows the presence of TB or an abnormal shadow may be visible on a chest x-ray	Results are available within hours	A normal chest x-ray cannot exclude extra pulmonary TB	Referral by clinician	Trained clinician needed, low specificity and sensitivity
Skin test	Involves injecting a small amount of fluid (called tuberculin) into the skin	Extensive clinical and published experience	TB skin test cannot tell if the person has latent TB or active TB disease	Community	Positive reaction in BCG vaccines
Interferon-Gamma Release Assays (IGRAs)	Fresh blood samples are mixed with antigens and controls	Results can be available within 24 h, highly specific for *M. tuberculosis*	Errors in collecting or transporting blood specimens or in running and interpreting the assay can decrease the accuracy of IGRAs	Referral to reference lab	Limited data on the use of IGRAs to predict who will progress to TB disease in the future
Xpert MTB/RIF assay	Based on Nucleic Acid Amplification Test (NAAT) that uses a disposable cartridge with the GeneXpert instrument system	It is fully automated and quickly identifies possible MDR TB	The Xpert MTB/RIF assay does not replace the need for smear with microscopy for acid-fast bacilli	Referral lab	Moderately trained personnel and equipment
Amplification Refractory Mutation System (ARMS)	In this method, an oligonucleotide primer work with PCR	High sensitivity, rapidity and detection of mutations in MDR-TB strains	More complex and expensive	Referral lab	Trained manpower, moderately trained personnel and equipment

Table 5: List of modern diagnostic kit for TB

Manufacture	Test
Advanced diagnostics	Tuberculosis rapid test
Ameritek USA	dBest one step tuberculosis test
Bio medical product	Rapid TB test
CTK Biotech	Onsite rapid test
Laboratorios Silanes	TB-instant test
Minerva Biotech	V Scan
Millennium Biotechnology	Immuno-sure TB plus
Pacific Biotech	BIOLINE tuberculosis test
Premier Medical	First response rapid TB card
Span Diagnostics	TB SPOT ver. 2.0
VEDA.LAB	TB-rapid test

vaccine. Subunit vaccine approach involves techniques from proteomics and biophysics, which relies largely on protein purification, folding and dynamics and finally biophysical characterization before submitting it for immunization[80-83]. Surface Plasmon Resonance (SPR) based biosensors are new highly evolved tool and technique to detect protein-ligand interaction in addition to rapid and sensitive diagnosis of biomarker proteins for TB detection[84].

CONCLUSION

Modern molecular diagnostic protocols require well equipped, state-of-art laboratory facilities that may not be easily available locally. Currently, most of the tools/techniques in demonstration or late-stage validation are sputum based and thus are likely to result in incremental gains in rate of TB detection. In addition to the lack of portability, cost involved is also a big deterrent before these modern protocols could realize their full potential particularly in the limited economic set up of developing countries.

SIGNIFICANCE STATEMENT

- Tuberculosis is the major cause of morbidity and mortality in animals and humans alike
- Precise and timely diagnosis is key to the successful treatment of TB
- Inaccurate diagnosis and incomplete treatment leads to drug-resistant TB (DR-TB)
- DR-TB and associated co-infections (AIDS) are making TB difficult to cure
- The TB diagnosis has evolved considerably from conventional SSM to DNA based molecular protocols

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