Identification of mycobacteria

- The conventional method of identifying mycobacteria is by Ziehl Nelson (ZN)staining and culture growth on media. Specific media containing malachite green like the widely used egg-based Lowenstein Jensen (LJ) and agarbased media such as Middlebrook7H10/11 are used for isolation.
- Mycobacterial isolation by culture can be supplemented with histopathology and other clinical presentations such as signs, symptoms, radiological scans, cytolopathology, history and antibiotic susceptibility.
- Nucleic acid based amplifications by targeting genes like hsp65, rpoB, 16SrRNA, dnaJ, insertional sequences etc. is commonly applied for accurate and relatively rapid identification of mycobacteria.
- Multiplexing of primers targeting regions for genus confirmation as well as identification of species or complexes in a single tube reaction are also some good approaches for NTM and MTBC differentiation.



Fig 5. Mycobacterial identification at Animal Health Division, ICAR, Meghalaya, through partial amplification of hsp65 gene (441bp). L-Ladder; Lane 1- M. tuberculosis; Lane 2- M. smegmatis; Lane 3 to 24- Field isolates; Lane 25-Negative control; Lane 26-NTC.

Conclusion

Important distinguishing features of NTM and MTBC includes lower virulence of NTM; human-to-human transmission of M. tuberculosis is caused by inhaling bacilli-containing expectorated aerosol and through ingestion ofM. bovisthrough contaminated milk, while NTM infections are mainly acquired directly from the environment. Although M. tuberculosis, M. lepraeand M.bovis as a zoonotic pathogen are the best known human pathogensfrom the group, an increasing number of NTM are being constantly designated as human and animal pathogens making it

crucial for identification and discrimination of the pathogenic entities from the usual contaminants or the saprophytes. As pulmonary NTM can manifests TB, cautiousdiagnosis and treatment is also called for while managing TB cases in thisTB-laden country. Eradication programs for bovine tuberculosis has been implemented in several countries which involves farm visits, inspection in abattoirs, controlled movement of herds, testing and culling of infected herds. However in countries like India, slaughtering of cows are prohibited due to religious sentiments, due to which the sick herds are isolated in separate sheds. Although vaccination is practiced in humans, treatment of these infected cattle are rarely attempted as it is expensive, requires long duration and care.

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Mycobacteria







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Mycobacteria

Mycobacteria are a group of non-motile organisms presenting structures which may be straight or mildly curved rods and are at times branched depending on the growth stage. Mycobacteria as a group consist of five genusMycobacterium, Mycocilibacterium, Mycolicibacter, Mycolicibacillus, Mycobacteroidesandoverall comprises over 184 different speciescomprising opportunistic pathogens and intracellular parasites with size ranging from 0.2 to 0.6 x 1.0 to 10 µm.Mycolic acids (MAs), 2-alkyl, 3-hydroxy long-chain fatty acids (FAs), are the hallmark of the cell envelope of Mycobacterium tuberculosis and related species in these genera playing a crucial role in cell architecture and impermeability of the cell wall. Also called the acid-fast organisms, Ziehl-Neelsen(ZN) staining method is used to identify these bacteria which are capable of retaining the primary stain when treated with an acid.



Fig1.Mycobacteroides chelonaein Lowenstein Jensen (LJ) medium.



Fig 2. Acid-fast bacilli following Ziehl Neelson staining of mycobacteria.

Mycobacterium tuberculosis bacillus complex (MTBC)

Given the genetic relatedness of the tuberculosis causing species, nine species currently are classified as members of the MTBC. The human-adapted ecotypes include Mycobacterium tuberculosis, M. canetti and M. africanum while animal-adapted ecotypes comprises M. bovis, M. microti, M. caprae, M. orygis, M. pinnipedi, and M. mungi. M. microti is known to be a rodent pathogen, M. caprae of goats and M. bovis an established pathogen in cattle with zoonotic risk to humans.M. tuberculosisis the entity responsible for causing tuberculosis (TB). M. tuberculosisinfections primarily involves the lungs and is capable of affecting many other sites which is then called as extrapulmonary tuberculosis. In 2017, the WHO reports that globally, India accounts for more TB cases (27%) annually than any other country. Out of the estimated global annual incidence of 104 lakh TB cases, 28 lakhs were estimated to be in India with a mortality of 4.8 lakh in 2015.

Zoonotic tuberculosis

M. bovis is a member of MTBC and basically causes tuberculosis in cattle which is known as bovine tuberculosis. The World Organization for Animal Health (OIE) considers it to be an important zoonotic disease with a socioeconomic and public health impact that affects the international trade of livestock and animal products.Itcan cause tuberculosis in humans which cannot be distinguished clinically or radiologically from tuberculosis caused by M. tuberculosis.M. bovishas a very wide host range and can infect almost all mammals with the highest incidence seen in the bovine population which acts as a principle reservoir. Other species susceptible to M. bovis include cats, dogs, buffalo, bison, goats, pigs, deer, wild boars, badgers, possums etc. and infection caused in these organisms, including man are spill over hosts. Other than cattle, pigs and other household livestock which are fed with wastes of human house dwellings can lead to tuberculosis infection in the food tract and closely located lymph nodes.Zoo animals are prone to infection by TB as they constantly interact with large numbers of visitors and are exposed to stressful surrounding through continuous activities under enclosed structures. They also can be infected with the foods fed which is contaminated with the bacilli. Co-transmission is very common and likely when animals and humans live in closely situated or overlapping habitats.



Nontuberculous mycobacteria (NTM)

Besides the members of MTBC that cause diseases in human, mycobacterial infections other than those caused by MTBC are attributed to the larger group of the genus comprising the Nontuberculous Mycobacteria (NTM). These organisms are ubiquitous in the environment (water, soil, cigarettes, vegetables, animals, amoeba, bronchoscopes) and are also occasionally responsible for infection in man (disseminated infections, cutaneous granulomas, lymphadenitis, post-operative wound infections), animal (granulomatous enterocolitis, tuberculous thelitis, Johne's disease), birds (avian tuberculosis), fishes (fish tank granuloma) and other animals as opportunistic pathogen. M. leparewhich causes leprosy is a non-cultivable bacteria of this large group.



Fig 4. Immunologically compromised pet animals could harbor potential pathogenic mycobacteria



Fig 3. An emaciated cow suspected with tuberculosis