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Mycobacterium genus

The genus *Mycobacterium* is a diverse member of the family *Mycobacteriaceae*, consisting of over 100 species and ten subspecies. These include pathogens *M. tuberculosis* and *M. leprae;* opportunist pathogens (particularly in the immunocompromised) and saprophytic, generally not pathogenic species. All *Mycobacterium* spp. are aerobic, non-spore forming, Gram positive coccobacilli (with or without branching). In addition, they are characterised by a differentiating cell wall structure containing mycolic long chain fatty acids, which makes them resistant to decolourisation with acid/alcohol and allows identification from most other species using “acid-fast” staining. Most species will grow slowly, requiring at least five days of incubation and may require particular growth conditions such as a liquid media; therefore, routine culture methods are not appropriate for detection of these organisms and may be slower than is ideal, particularly when there are public health or treatment implications.

***Mycobacterium tuberculosis* complex (MTBC)**

Tuberculosis (TB) is a disease caused by a group of closely related slow growing organisms collectively named the *Mycobacterium tuberculosis* complex (MTBC). These organisms can infect a range of mammals including humans and affect different organs of the body such as lymph nodes, lungs, kidney, brain, larynx and bone. On inhalation, MTBC tubercles may be destroyed by the immune system, may cause an active infection or may become latent in the body, able to reactivate later in life (in ~10%) - therefore creating a reservoir in the human population.

The MTB complex comprises ***M. tuberculosis, M. bovis*, *M.bovis* bacille Calmette-Guérin (BCG), *M. caprae, M. africanum, M. pinnipedii, M. microti, M. orygis, M. mungi*, *M. suricattae*** *(*formerly *M. bovis),* **dassie bacillus, chimpanzee bacillus,** and the rare, smooth-colony-morphology tubercle bacillus named ***M. canettii****.*

The MTBC shares identical MALDI-TOF profiles and 16S rRNA gene sequences, with 99% identity at the nucleotide level for some species. However, despite these similarities, complex members differ significantly in morphology, biochemistry, host spectra, disease patterns in animals, antimicrobial susceptibility testing (AST) data, geographic ranges, and epidemiological patterns. MTBC organisms are slow growing with a generation time of >24 hours in laboratory media, therefore many weeks are needed to identify the organism using culture.

Mycobacterium tuberculosis

M. tuberculosis has killed more than 100 million people over the last century and the current global burden is vast, estimated to infect one-third of the world population. In 2022 an estimated 10.6 million people developed tuberculosis (TB) and 1.3 million died, making it the second leading infectious cause of death after SARS-Cov-2 and Human immuno-deficiency virus (HIV), (World Health Organization, 2023). Most cases are in low- and middle-income countries and cases in the UK are often travel or immigration associated. Pulmonary TB is the commonest presentation; however, TB may also be disseminated (miliary) or found in non-respiratory sites. Disease is often associated with HIV, however there are also social risk factors associated with TB such as imprisonment, alcohol and drug misuse, and homelessness.

Mycobacterium avium complex (MAC)

The term *M. avium* complex previously referred to only three species, *M. avium, M. intracellulare and M. chimaera*, with three named subspecies of *M. avium: M. avium* subsp*. avium; M. avium* subsp*. paratuberculosis; and M. avium* subsp*. Silvaticum* identified. However, since 2018, recent literature review and phylogenetic analyses have further defined the MAC, with twelve published species now comprising the group, including: ***M. avium, M. intracellulare, M. chimaera, M. colombiense, M. arosiense, M. vulneris, M. bouchedurhonense, M. timonense, M. marseillense, M. yongonense, M. paraintracellulare*** and ***M. lepraemurium*** (van Ingen *et al.,* 2018)***.***

In patients who are immunocompetent, MAC organisms, may invade the bronchial tree, pre-existing areas of bronchiectasis, or old cavities; in susceptible hosts MAC organisms can cause disseminated infection. Infections with *M. avium* may also cause cervical lymphadenitis in young children. These organisms are often present in water supplies and may contaminate specimens. *M. chimaera,* a slow growing non-tuberculosis *Mycobacterium* (NTM) found in the environment has also been implicated in several cases of endocarditis or deep infection following cardiac surgery involving the use of cardiac bypass equipment.

Mycobacterium leprae

M. leprae is a very slow growing species which causes the disease leprosy or chronic granulomatous disease which can affect the skin, peripheral nervous system and mucous membranes. Around 60% of new cases are diagnosed in India with fewer in Brazil and Indonesia; infection is also a major cause of disability in developing countries worldwide. In the UK infections are typically related to travel and residence abroad, particularly South Asia.

Mycobacterium kansasii

Pulmonary infection is the most common form of disease caused by *M. kansasii*, usually in patients with pre-existing chronic lung disease or pneumoconiosis, although infections can occur in other parts of the body. It is a photochromogen, that is, light is required for colonies to become pigmented.

Mycobacterium gordonae

This is a common aquatic species which has rarely, and disputably, caused disease in patients who are immunosuppressed. It is a common contaminant of clinical samples.

Mycobacterium malmoense

M. malmoense usually causes pulmonary and lymph node diseases, but disseminated and other extra pulmonary disease have also been reported. Diagnosis of *M. malmoense* infection is as for other *Mycobacteria*, although incubation times may need to be as long as 12 weeks before colonies become visible on solid media.

*Mycobacterium marinum**

M. marinum is the causative organism of 'fish tank' or 'swimming pool' granuloma, a localised skin lesion following contamination of an open wound or abrasion with water from fish tanks, swimming pools and natural areas of fresh or salt water. This species has an intermediate growth rate with an optimum growth temperature of 28-30°C.

*Mycobacterium ulcerans**

M. ulcerans is a strictly human pathogen that can cause of skin lesions or osteomyelitis in various global areas, including Australia ('Bairnsdale ulcer') and South-East Asia; Uganda and other parts of Africa ('Buruli ulcer'); and in Central/South America. Infection may lead to a chronic progressive painless ulcer, which can occasionally present in travellers from endemic areas. The organism can be difficult to isolate in the laboratory – it is more sensitive to standard decontamination methods than other mycobacteria, it is slow growing (6-12 weeks) and requires incubation at 30-33°C.

**Due to their very close phylogenetic relationship, M. marinum and M. ulcerans are unable to be differentiated using sequencing; however, relevant clinical detail e.g. 'contact with water/fish tanks' and 'recent travel to/from endemic countries', is typically sufficient to aid clinical diagnosis.*

Mycobacterium xenopi

M. xenopi is another comparatively common cause of NTM pulmonary disease in certain geographic areas. It is thermophilic, with an optimum growth temperature of 45°C; and, similar to *M. malmoense*, grows comparatively slowly at 37°C. It can be isolated from various environmental sources, including hot-water taps, and hence may also be a cause of specimen contamination.

M. haemophilum

Requires haemin or other iron containing compounds to be cultured, has an optimum growth temperature of 28-30°C, can also cause lymphadenitis in otherwise healthy paediatric patients.

Rapid growing species

Mycobacteroides abscessus, Mycobacteroides chelonae, Mycolicibacterium fortuitum (formerly Mycobacterium)

These, and related species, are well recognised as the cause of skin and soft tissue infections. These mycobacteria may infect long-term vascular catheters and other medical devices. Such organisms have been found in lavage fluids obtained by bronchoscopy and may be associated with false positive diagnoses. Although variation is found in some subspecies, the optimum growth temperature of these organisms lies between 30-33°C.

M. abscessus more so than the other NTM are an increasing problem for the cystic fibrosis patient group. Testing should be considered in cystic fibrosis patients who show deteriorating lung function but where no clear pathogen has been identified. The Cystic Fibrosis Trust microbiology standards recommend routine screening of NTM at least once a year for all patients able to produce sputum and all *M. abscessus* positive isolates should be referred to the appropriate reference laboratory for strain typing. Other slow growing and rapid growing *Mycobacterium* species isolated from clinical specimens have recently been identified using the molecular approach.

Diagnosis:

Clients may wish to send specimens where a *Mycobacterium* species are suspected clinically, where growth has been identified in liquid or *Mycobacterium* specific solid culture, or where acid-fast bacilli have been visualised on a stain. Specimens typically received include lower respiratory samples such as bronchoalveolar lavage (BAL), sputum, tissues (e.g. lymph nodes, fixed in wax etc.) and cerebrospinal fluid (CSF); or culture media where a client may suspect they have grown an organism from this range of specimens.

Our assay:

At Micropathology Ltd, we use a qualitative, semi-nested PCR assay which targets the internal transcribed spacer (ITS) region between the 16S rDNA and 23S rDNA genes of Mycobacteria, which contains genus-specific and species-specific sequences which may be distinguished using DNA sequencing.

UKAS accredited sample types for our assay include: BAL, sputum, CSF and tissues. Other sample types may be tested and are reported alongside an appropriate caveat stating that the assay is not UKAS accredited for testing such sample types.

Turnaround times are stated in our laboratory user handbook with results usually available in practice much sooner than the given time frame. Where there is a delay, we are usually confirming a result and addressing clinical data given with the specimen.

References

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