

# Molecular Approaches to Subspecies-Level Identification of *Mycobacterium avium*

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## DESCRIPTION

*Mycobacterium avium* is a species of Non-Tuberculous Mycobacteria (NTM) that includes several subspecies, some of which are of major clinical significance due to their ability to cause disease in humans and animals. This pathogen primarily affects individuals with compromised immune systems or those with underlying lung diseases, but it can also infect otherwise healthy individuals. The *Mycobacterium Avium* Complex (MAC) comprises *M. avium* and other closely related species, including *M. intracellulare*, and is one of the most common causes of NTM infections worldwide. Accurate subspecies-level identification of *M. avium* is important for diagnosis, treatment, and epidemiological tracking, as different subspecies exhibit varying pathogenicity, antimicrobial resistance, and clinical outcomes. This article focuses on the importance, methods, and challenges of accurately identifying clinically significant subspecies of *M. avium*.

### Clinical significance of *M. avium* subspecies

The *M. Avium* Complex (MAC) is composed of several subspecies that differ in their pathogenic potential and host preference. The three main clinically significant subspecies of *M. avium* are:

***M. Avium* subsp. *Hominissuis* (MAH):** This is the most common subspecies responsible for human infections. It primarily causes pulmonary disease in individuals with chronic lung conditions, such as Chronic Obstructive Pulmonary Disease (COPD) or bronchiectasis. MAH is also known for causing disseminated infections in immunocompromised individuals, particularly those with HIV/AIDS. The subspecies is ubiquitous in the environment and can be found in soil, water, and dust.

***M. avium* subsp. *Paratuberculosis* (MAP):** This subspecies is primarily associated with Johne's disease in ruminants, a chronic intestinal condition. However, it has also been implicated in human Crohn's disease, although this link remains controversial. MAP is challenging to culture and identify due to its slow growth

and specific nutritional requirements, which complicates its detection in clinical and environmental samples.

***M. avium* subsp. *avium* (MAA):** While primarily a pathogen of birds, MAA can occasionally cause infections in humans, especially in immunocompromised individuals. In avian species, it leads to tuberculosis-like symptoms, and its zoonotic potential raises concerns about its transmission to humans through contaminated water or poultry products.

Given the differences in clinical presentations, treatment responses, and transmission routes, accurate subspecies-level identification of *M. avium* is essential for tailored therapeutic strategies and effective public health interventions.

### Methods for subspecies identification

Traditionally, the identification of *M. avium* has relied on phenotypic methods such as culture characteristics, biochemical testing, and microscopy. However, these methods lack the resolution needed for subspecies-level differentiation. Over the past two decades, molecular techniques have revolutionized the identification of *M. avium* subspecies, offering higher accuracy and faster turnaround times. Polymerase Chain Reaction (PCR) assays targeting specific genomic regions have been developed to distinguish *M. avium* subspecies. For instance, IS900 PCR is commonly used to identify *M. avium* subsp. *paratuberculosis*, while other assays target subspecies-specific genes or genomic islands. Multiplex PCR techniques allow for simultaneous detection of multiple subspecies in a single assay, improving efficiency. Multilocus Sequence Typing (MLST) involves sequencing several housekeeping genes to provide a detailed genetic profile of an isolate. This method has been particularly useful in differentiating closely related *M. avium* subspecies. By comparing the sequence data to a reference database, researchers can accurately identify the subspecies of an isolate and infer its evolutionary relationships. Whole Genome Sequencing (WGS) offers the highest resolution for identifying *M. avium* subspecies. WGS allows for comprehensive analysis of an isolate's entire

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**Received:** 05-Aug-2024, Manuscript No. MDTL-24-34284; **Editor assigned:** 07-Aug-2024, Pre QC No. MDTL-24-34284 (PQ); **Reviewed:** 21-Aug-2024, QC No. MDTL-24-34284; **Revised:** 28-Aug-2024, Manuscript No. MDTL-24-34284 (R); **Published:** 04-Sep-2024, DOI: 10.35248/2161-1068.24.14.503.

**Citation:** Berbers N (2024). Molecular Approaches to Subspecies-Level Identification of *Mycobacterium avium*. *Mycobact Dis*. 14:503.

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genome, including its virulence factors, antibiotic resistance genes, and evolutionary history. This method is particularly valuable in research settings and for tracking outbreaks of *M. avium* infections. Although expensive and resource-intensive, WGS is becoming more accessible as sequencing technologies continue to advance. Matrix Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) mass spectrometry has emerged as a rapid and accurate tool for identifying mycobacterial species, including *M. avium*. However, its utility for subspecies-level identification is still being refined. By analyzing the protein spectra of isolates, MALDI-TOF can differentiate between species but may not always offer the resolution needed for distinguishing between closely related subspecies. Improvements in reference databases and analytical algorithms are likely to enhance its subspecies-level identification capabilities in the future.

## CONCLUSION

Accurate subspecies-level identification of *Mycobacterium avium* is critical for improving patient outcomes, guiding treatment decisions, and informing public health strategies. Molecular methods, particularly PCR, MLST, and WGS, have significantly

advanced the ability to differentiate clinically significant subspecies. Continued research into the genetic and phenotypic diversity of *M. avium* will further improve diagnostic accuracy and lead to better management of infections caused by this complex group of mycobacteria.

## CHALLENGES AND FUTURE DIRECTIONS

Despite advances in molecular methods, accurate subspecies-level identification of *M. avium* remains challenging due to the genetic similarity among subspecies and the presence of genetic diversity within subspecies. Moreover, the environmental ubiquity of *M. avium* adds complexity to distinguishing between pathogenic and non-pathogenic strains. Rapid diagnostic techniques must balance accuracy with cost-effectiveness, especially in resource-limited settings where *M. avium* infections are common. Standardization of diagnostic methods and the creation of comprehensive genomic databases will play a pivotal role in improving subspecies identification. Additionally, integrating molecular diagnostics with clinical data will enhance the understanding of the clinical relevance of specific *M. avium* subspecies.