There are more than 90 identified species of the genus Mycobacterium, of which over 50 are implicated in human disease\(^1,2\). Two species, *Mycobacterium tuberculosis* (tuberculosis) and *Mycobacterium leprae* (leprosy), are usually pathogens only of humans.

Mycobacteria are extremely diverse and inhabit various similarly diverse ecological niches. The vast majority are environmental organisms, found naturally in waterways, soil and other environments\(^1\). *Mycobacterium* spp. are encountered with increasing frequency as opportunistic pathogens of humans, due to increased levels of immunosuppression in the population, selection of these organisms by disinfection, and improved diagnostic techniques. Contention exists regarding an appropriate collective term for this group of organisms, but they will be referred to here as 'environmental mycobacteria'.

Examples of clinical syndromes attributable to mycobacteria and some examples of causative species are given in Table 1. Environmental mycobacterial infections are of particular concern due to their high levels of resistance to commonly used antibiotics, including antituberculous drugs\(^3\), highlighting the importance of identification to the species level.

Prior to the appearance of the human immunodeficiency virus (HIV), environmental mycobacterial infection was seen most often in patients with some degree of immunosuppression, such as the elderly (usually with chronic pulmonary disease resulting in changes in macrophage activity), and cancer or organ transplant patients undergoing long-term immunosuppressive treatment\(^4\).

The advent of the acquired immunodeficiency syndrome (AIDS) epidemic drastically changed the pattern of environmental mycobacterial infection throughout the world. In 1996, it was reported that 25-50% of patients with AIDS in the United States and Europe were infected with *M. avium* complex\(^5\). This problem has been addressed by the introduction of highly active antiretroviral therapy (HAART), which has greatly reduced the occurrence of opportunistic infections in AIDS patients\(^1\). Resistance to HAART drugs is not uncommon, however, and this situation must be monitored carefully to detect a resurgence of *M. avium* infections.

Mycobacteria are characterised by a thick, waxy cell wall. The high lipid content of the cell wall confers innate resistance to common disinfectants and antimicrobials, and the hydrophobic cell surface allows for easy aerosolisation, a common route of transfer to human and animal respiratory systems\(^1\). Resistance to chlorine and biocides commonly used for microbial decontamination, for example of drinking water supplies, directly explains the prevalence of mycobacteria, particularly members of the *M. avium* complex in these environments\(^6\).

Children are a unique indicator of this phenomenon, as they are particularly prone to mycobacterial cervical lymphadenitis whilst gaining their first set of teeth. Prior to the 1970s, this was usually caused by *M. scrofulaceum*, but, when water chlorination was adopted, it was replaced by *M. avium*, probably because of the higher level of chlorine resistance of the latter\(^7\).

Mycobacterial populations are also capable of survival in flowing systems, due to their ability to form biofilms\(^8\). Tolerance of nutrient deprivation and extreme temperatures of various species also contributes to persistent colonisation of environments such as hot water systems and ice machines, in hospitals and other institutions, and in private

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**Table 1. Clinical syndromes.**

<table>
<thead>
<tr>
<th>Clinical syndrome</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmonary disease</td>
<td><em>M. tuberculosis</em>, <em>M. avium</em> complex, <em>M. chelonae/abscessus</em>, <em>M. kansasii</em></td>
</tr>
<tr>
<td>Lymphadenitis</td>
<td><em>M. scrofulaceum</em>, <em>M. avium</em> complex</td>
</tr>
<tr>
<td>Skin, soft tissue and skeletal infections</td>
<td><em>M. ulcerans</em>, <em>M. marinum</em>, <em>M. fortuitum</em> complex, <em>M. abscessus/chelonae</em></td>
</tr>
<tr>
<td>Catheter-related blood-stream infections in immuno-compromised hosts</td>
<td><em>M. avium</em> complex, <em>M. fortuitum</em> complex</td>
</tr>
<tr>
<td>Disseminated disease in AIDS patients</td>
<td><em>M. avium</em> complex, <em>M. tuberculosis</em></td>
</tr>
</tbody>
</table>
homes. M. avium complex organisms have also been isolated from food sources and cigarettes, and various other sources involving human interactions with the environment.

The search for antimycobacterial therapies is continuing, but is hampered by high levels of resistance to many classes of antibacterial agents, the length of time taken to kill mycobacteria (resulting in the need for long-term multiple-drug regimens), and poor correlation between in vitro susceptibility and in vivo response to therapy. Environmental mycobacterial infections are particularly difficult to treat, often requiring the use of a macrolide antibiotic (clarithromycin or azithromycin), combined with one or more other antimycobacterial agents, such as ciprofloxacin, (or a newer fluoroquinolone), ethambutol and rifabutin.

Treatment of AIDS patients is particularly complicated, as rifabutin and clarithromycin can interact with protease inhibitors. Analysis of Peruvian Amazon plant compounds has identified promising new compounds displaying antimycobacterial activity, and honey has also been shown to possess antimycobacterial properties in vitro.

Environmental mycobacteria make up the greater proportion of isolates received for identification in Australian Mycobacterial Reference Laboratories. Historically, species identification was performed by investigating the phenotypic properties of the organism, a laborious and time-consuming process.

Many isolates with unique or diverse phenotypic characteristics were encountered, and were subsequently assigned to the closest matching known species, due to lack of more sensitive means of discrimination. Subsequently, analysis of the fatty acids or mycolic acids extracted from the unique mycobacterial cell wall, by high performance liquid chromatography (HPLC), was found to be a highly discriminatory technique, providing characteristic profiles for most mycobacterial species.

More recently, molecular methods have drastically improved differentiation and taxonomy of mycobacterial species. Of these, DNA sequencing of the mycobacterial 16S rRNA gene, and analysis of two hypervariable regions is the most common, and has proven to be a powerful taxonomic tool. Analysis of generated sequence data is performed using public databases, which have historically been subject to poor quality control. This has resulted in inherent problems such as incomplete and erroneous sequences. Quality controlled databases are now in use, but they do not assist in the identification of ‘novel’ or as yet unidentified species. Further complicating matters, is the high degree of sequence homology in mycobacteria (>91%), and lack of agreement in the requirements, (genotypic and phenotypic), for defining a novel or ‘new’ species.

In the last 14 years, 42 new species of mycobacteria have been officially recognised, with over half of these isolated from clinical specimens. As humans continue to interact with and influence the natural environment, our exposure to potentially pathogenic environmental mycobacteria will continue to increase. Disinfection selects for the presence of these highly resistant organisms, and this will particularly affect the ever-increasing numbers of immunosuppressed persons in populations.

Antimicrobial resistance is perhaps the most significant issue regarding patient care. Information must be gathered on particular Mycobacterium species to allow for proper treatment regimens for significant infections, as species differ in virulence and sometimes have characteristic susceptibility patterns.

Further taxonomic studies of the mycobacteria are crucial to improving our understanding of the role these organisms play in infections in the immunocompromised, and also to enable the formulation of successful treatment regimens.

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References