The genus *Mycobacterium* is composed of ~100 named species that are characterized by complex, lipid-rich cell walls and, as a consequence, the characteristic acid-fast staining property. Until recently mycobacterial species were classified according to cultural and biochemical properties, but genetic differences are now used for this purpose, notably sequence differences in the 16S ribosomal RNA that correlate closely with the phenetic differences previously used for speciation. Application of such technology has led to the description of many new species over the last few years, although the majority is encountered rarely. The published mycobacterial species, which in November 2001 numbered almost 100, are included in the List of Bacterial Names with Standing in Nomenclature (website: http://www.bacterio.cict.fr/).

The genus contains two obligate pathogens—the *Mycobacterium tuberculosis* complex and *Mycobacterium leprae*. In this context it is important to note that the *M. tuberculosis* complex contains several mycobacteria that have been given separate specific names (ie, *Mycobacterium bovis*, *Mycobacterium africanum*, *Mycobacterium microti*, and *Mycobacterium canetti*) even though on DNA analysis they are all merely variants of *M. tuberculosis*. The other species live freely in the environment and are thus often termed environmental mycobacteria (EM) or nontuberculous mycobacteria (NTM). EM are found particularly in water and wet soil, and some species colonize piped water supplies. Mycobacteria are divisible into two main groups, the rapid and slow growers, and they vary greatly in their nutritional requirements. Thus many grow on simple media but some, as outlined below, require supplements for growth in vitro.

The EM commonly encountered as causes of human pulmonary disease are listed in Table 1.

**Epidemiology**

Unlike tuberculosis (TB), disease due to EM is rarely, if ever, transmitted from patient to patient. Infection is transmitted from the environment; pulmonary disease is probably due to inhalation of aerosols of water containing the bacilli. Thus the incidence of disease due to EM is independent of that of TB but is determined by the number, distribution, and species of EM in the environment and the susceptibility of the human population. In regions where TB is common only a small minority of cases of pulmonary mycobacterial disease will be due to EM. By contrast, in regions where TB is rare, such as rural areas of Europe and the United States, a much higher proportion of pulmonary mycobacterial disease is due to EM. There are geographical variations in distribution of the species of EM; while the *Mycobacterium avium* complex (MAC) occurs worldwide, others such as *Mycobacterium xenopi* and *Mycobacterium malmoense* are restricted to certain regions. In addition, the distribution of species varies with time, possibly as a result of environmental changes [1].

Because many microbiological health services in the tropics are overwhelmed by the diagnostic burden of TB there is little or no incentive to devote limited resources to the isolation and identification of EM...
Table 1
The environmental mycobacteria causing human pulmonary disease

<table>
<thead>
<tr>
<th>Species</th>
<th>Characteristic</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. abscessus</em></td>
<td>A rapid grower, usually regarded as a subspecies of <em>M. chelonae</em></td>
</tr>
<tr>
<td><em>M. chelone</em></td>
<td>A rapid grower</td>
</tr>
<tr>
<td><em>M. avium</em></td>
<td>Usually included with <em>M.intracellular</em> in the <em>M. avium</em> complex</td>
</tr>
<tr>
<td><em>M. intracellular</em></td>
<td>Usually included with <em>M. avium</em> in the <em>M. avium</em> complex</td>
</tr>
<tr>
<td><em>M. fortuitum</em></td>
<td>A rapid grower</td>
</tr>
<tr>
<td><em>M. gordonae</em></td>
<td>A frequent contaminant of sputum but also an occasional pulmonary pathogen</td>
</tr>
<tr>
<td><em>M. kansasii</em></td>
<td>Particular associated with industrial dust disease</td>
</tr>
<tr>
<td><em>M. malmoense</em></td>
<td>A species isolated with increasing frequency in Europe</td>
</tr>
<tr>
<td><em>M. scrofulaceum</em></td>
<td>A cause of cervical lymphadenopathy (scrofula) and also, occasionally, pulmonary disease</td>
</tr>
<tr>
<td><em>M. simiae</em></td>
<td>Causes pulmonary disease in monkey handlers</td>
</tr>
<tr>
<td><em>M. szulgai</em></td>
<td>A rapid grower causing disease similar to tuberculosis in patients with underlying lung disease and alcoholics</td>
</tr>
<tr>
<td><em>M. xenopi</em></td>
<td>A species of limited geographical distribution</td>
</tr>
<tr>
<td><em>M. celatum</em></td>
<td>Similar to <em>M. avium</em>. Causes disseminated disease in immunosuppressed patients</td>
</tr>
</tbody>
</table>

Species causing single reported cases or small numbers only are not included

from sputum. Accordingly, very little is known of the incidence and nature of pulmonary disease due to EM in the tropics. The clinical descriptions of such disease (outlined below) are thus, of necessity, derived principally from experience in temperate countries. In the limited number of detailed microbiological surveys in the tropics EM has been isolated from the sputum of some patients who have persistent symptoms of lower respiratory tract infection.

In Lagos, Nigeria, for example, 102 mycobacterial isolates were obtained from 668 such patients, of which 87 were *M. tuberculosis*, four were *M. bovis*, and 11 were EM (six *M. avium*, four *Mycobacterium kansasii*, and one *Mycobacterium fortuitum*). Without the bacterial studies the patients infected with *M. bovis* and EM would probably have been treated for classical TB [2]. In Guinea-Bissau, mycobacteria were cultured from the sputum of 206 patients with clinically suspected TB; 189 (36 HIV-positive) yielded *M. tuberculosis* and 17 (2 HIV-positive) yielded MAC [3]. It was therefore suggested that, where it commonly occurs, infection by MAC could be responsible for misdiagnoses of TB and apparent treatment failures.

By contrast, although strains of MAC corresponding to the types causing human disease are common in water and soil in Uganda, HIV-related human disease due to this complex are rarely encountered in that country [4,5]. The reason for this is unknown, although various hypotheses have been suggested [6].

Being common in the environment, notably in water sources, exposure of the human population to EM by drinking and bathing and through cuts and abrasions is a regular occurrence. The risk of such regular contact progressing to overt disease is small, except in the profoundly immunosuppressed. On the other hand, such exposure may result in what has been termed “immunologically effective contact” [7]. This contact may be sufficient to induce low levels of tuberculin reactivity. This must be taken into consideration when using tuberculin testing in epidemiological studies or for diagnosis in individual patients. In addition, there is growing evidence that infection by EM may profoundly affect immune responses to subsequent infection by members of the *M. tuberculosis* complex, *M. leprae*, or vaccination by Bacille Calmette-Guérin (BCG).

Extensive studies in Malawi indicate that the nature of the impact of EM on immune responses is related to the species of EM to which the population is exposed. Thus persons sensitized to rapidly growing mycobacteria such as *M. fortuitum* had a reduced risk of developing both TB and leprosy—a factor relevant to the development of novel vaccines [8]. A further study in Malawi showed that peripheral blood leucocytes of those sensitized by natural exposure to MAC and *Mycobacterium scrofulaceum* showed a trend toward reduced in vitro interferon responses on stimulation with *M. tuberculosis* antigens [9]. This trend, though not statistically significant, further indicates that sensitization by EM modifies immune responses to the major pathogenic mycobacteria.

Immune reactions elicited by exposure to EM have been postulated as the cause of the wide geographical variation in the protective efficacy of BCG, and various explanations of this effect have been advanced. According to one explanation, repeated immunologically effective contact with EM builds up protective immunity that reaches, in some regions, levels of protection equivalent to that conferred by BCG. Under such circumstances BCG vaccination would appear to have no protective effect. Another explanation is that immune responses in TB are qualitatively different, with some eliciting protective immune responses and others leading to tissue-necrotizing hypersensitivity reactions and progression of
the disease. There is increasing evidence that the "choice" between the two responses is determined by the activity of type 1 and type 2 cytokines, which, in turn, are the result of the involvement of Th1 and Th2 helper T cells in the immune response [10]. Thus a predominantly Th1 response facilitates protective immunity while a superimposed Th2 response is associated with tissue necrosis. When given neonatally BCG affords protection against TB and induces a Th1 response, but later in life it either boosts or fails to down-regulate an environmentally determined harmful Th2 component and therefore fails to protect from—and may even predispose to—active TB [11]. A third hypothesis supported by a mouse model is that environmental sensitization by some species such as M avium, but not others including M fortuitum and Mycobacterium chelonae, prevents the multiplication of BCG in tissues, which is essential for the development of protective immunity [12]. These hypotheses are not mutually exclusive.

Virulence and pathogenicity of environmental mycobacteria

Many questions surround the determinants of virulence of M tuberculosis and M leprae, and even less is known about the virulence of EM. A mouse model has revealed three patterns of pathogenicity among a group of recently described EM. After intravenous inoculation most tested species replicate progressively in the livers and spleens of immunocompetent mice, but some replicated in these organs in interferon-gamma- (IFN-γ-) deficient mice but not in immunocompetent mice (eg, Mycobacterium heidelbergense and Mycobacterium intermedium). Others were eliminated in both types of mice (eg, Mycobacterium confluens and Mycobacterium lentiflavum) [13]. The relevance of these findings to human disease remains to be established, but they suggest that IFN-γ is not a crucial determinant of protection in all forms of mycobacterial disease.

There are little data on the relative virulence of EM in humans. While some species such as MAC and M kansasii are recognized as pathogens, others such as Mycobacterium gordonae are often isolated as non-pathogenic contaminants of the respiratory tract but only occasionally as causes of overt disease [14]. In the presence of immunosuppression all EM must be regarded as potential pathogens, but some species are particularly associated with disease. For unknown reasons HIV-positive patients are particularly prone to developing diseases from certain genetic variants of MAC.

Human disease due to environmental mycobacteria

Some species of EM cause opportunistic diseases in humans and animals. Infection is acquired by consuming water, inhaling aerosols, or through penetrating injuries of the skin. Three principal classes of disease due to EM (in addition to pulmonary disease) have been described.

Post-inoculation lesions

Post-inoculation lesions usually affect skin or subcutaneous tissues following a traumatic inoculation. This category contains two named mycobacterial diseases—Buruli ulcer and swimming pool (or fish tank) granuloma caused, respectively, by Mycobacterium ulcerans and Mycobacterium marinum. Post-injection abscesses are usually caused by the rapidly growing species Mycobacterium abscessus, M chelonae, and M fortuitum, which have on occasion also caused deeper lesions and disseminated disease following cardiac surgery.

Lymphadenitis

Lymphadenitis usually affects the cervical lymph nodes in otherwise healthy children aged <5 years. Lymphadenitis in older persons usually indicates HIV infection or some other form of immunosuppression. Many mycobacterial species are involved.

Disseminated disease

Localized, non-pulmonary lesions in the kidney, bones, joints, and central nervous system have been described, but they are exceedingly rare. Most diseases in this category are multi-focal or widely disseminated. Such diseases are almost always associated with some form of congenital or acquired immune defect, including post-transplant immunosuppressive therapy. Most cases at present occur in patients with AIDS, and most are caused by MAC.

Pulmonary disease due to environmental mycobacteria

Pulmonary disease due to EM may occur as a component of disseminated infection, but often disease affects only the lungs. Four main categories of pulmonary disease are encountered. First, disease occurs in middle-aged or elderly patients, usually men with a history of lung disease. Second, disease occurs in otherwise apparently healthy persons although, as out-
lined below, some may have minor and covert immune defects. Third, disease occurs in children with more severe immune defects or predisposing pulmonary disease, notably cystic fibrosis. Forth, disease occurs in profoundly immunosuppressed patients, of which HIV infection is the prevalent cause worldwide.

**Pulmonary disease in those with predisposing lung disease**

Most patients are men with a history of smoking, bronchiectasis, chronic obstructive lung disease, rheumatoid lung, healed TB, or exposure to industrial dusts as a result of mining, sandblasting, or welding. Risk factors have been evaluated in South African gold miners with pulmonary mycobacterial disease. In one study [15] 51 patients with disease due to EM and 425 with TB were similar with regard to age, education, home region, and smoking habits. Those with disease due to EM were more likely to have been previously treated for TB, have worked longer underground, or have evidence of silicosis. Patients with disease due to EM were less likely to be HIV positive (35.3%) than those with TB (48.8%), although the difference was not statistically significant. Pulmonary disease due to *M. kansasii* is particularly associated with underlying lung damage such as pneumoconiosis or silicosis, which lead to slowly progressive and insidious disease in miners and other workers [16, 17]. This species has been recognized since 1977 as the most common cause of EM pulmonary disease in South African gold miners [18]. The disease occurs in both HIV-positive and HIV-negative patients, and most had radiological evidence of silicosis. Disease due to *M. kansasii* in HIV-positive gold miners differs from that occurring in HIV-positive patients without the risks associated with mining. Thus, in miners the disease occurs much earlier in the course of HIV infection, with the CD4+ T cell counts being significantly higher, and clinically it more closely resembles the disease in HIV-negative patients [19]. It has been noted that assessment of the clinical significance of sputum isolates of *M. kansasii* in this group of patients by means of the American Thoracic Society guidelines [20] is not straightforward [21].

Old TB lesions may be colonized by EM. In one study in Japan, three quarters of mycobacteria isolated from sputum ≥ 1 year after completion of therapy for TB were EM [22]. The presence of such mycobacteria could lead to a false diagnosis of recurrence of TB. In some cases disease due to *M. xenopi* has been superimposed on aspergillosis in old cavities; this disease has a poor prognosis and response to therapy [23].

**Pulmonary disease in apparently healthy persons**

A number of cases, mostly caused by MAC, have been reported in elderly people, principally non-smoking women who have no other evidence of lung disease [24]. It has been postulated that such disease in women is associated with the ladylike practice of coughing very quietly and gently, thereby suppressing the clearance of sputum. The disease has accordingly been termed the Lady Windermere syndrome after the fastidious aristocrat in Oscar Wilde’s play “Lady Windermere’s Fan” [25]. If the disease continues undetected for years, cavities develop in the lung and respiratory failure may ensue. The causative organisms include MAC and *M. kansasii* and, less frequently, *M. xenopi*, *M. scrofulaceum*, *Mycobacterium szulgai*, *Mycobacterium malmoense*, *Mycobacterium simiae*, *Mycobacterium celatum*, and *M. chelonae*. A similar type of pulmonary disease due to EM has also been reported in apparently immunocompetent men. In a study in the United States 42 immunocompetent male patients with no evident predisposing factors had EM in their sputum and five of these patients met the American Thoracic Society’s criteria for diagnosis of mycobacterial pulmonary infection. All responded well to therapy [26].

A bizarre characteristic of ten otherwise healthy patients with diffuse pulmonary disease due to EM (*M. avium* in nine cases) was that they all bathed in hot tubs. Although termed “hot tub lung,” further studies are required to confirm whether the use of such tubs is an important predisposing factor [27].

Although the patients described above appeared clinically and immunologically normal, it is possible that they had minor immune defects. Some patients with pulmonary disease due to EM have, on detailed investigation, been found to have such defects, although it is not clear whether these were a cause or a consequence of the disease [28].

**Pulmonary disease in children**

Though rare in childhood, a few cases caused by MAC, *M. chelonae*, and *M. fortuitum* have been reported in children with cystic fibrosis [29, 30]. Children with deteriorating lung function should be screened for EM because therapy can, in some cases, halt the deterioration.

Familial susceptibility to disease, often potentially fatal, due to BCG and EM but not, surprisingly, *M. tuberculosis* is due to various types of mutations in four genes. These mutations result in eight different clinical disorders, all characterized by impaired cell-
mediated immune responses mediated by IFN-γ [31]. Because these disorders vary in severity and require different therapeutic strategies, identification of the underlying genetic defect is important. In addition, a determination of the reason why this congenital immune defect predisposes patients to disease due to EM could shed useful light on the nature of protective immunity in TB.

Pulmonary disease in immunosuppressed persons

Mycobacteria, both *M. tuberculosis* and EM, are common causes of lung disease in HIV-positive patients. In general, the isolation of EM, notably MAC, from the respiratory tract of an HIV-positive person is more likely to be clinically significant than from an HIV-negative person. Cough is a common complaint irrespective of HIV status, but HIV-positive patients are more likely to have fever. Abnormal chest radiographs are common, with HIV-positive patients being more likely to have diffuse abnormalities [32]. In one study [33] a specific diagnosis was made in 20 of 25 HIV-positive patients with cavitating lung lesions. Bacteria, often more than one species, were the cause in 17 patients. Mycobacteria were isolated in eight patients. Mediastinal or hilar lymphadenopathy and additional ill-defined, non-cav-itating, nodular opacities were seen more frequently in patients with mycobacterial pathogens.

**Diagnosis**

**Bacteriological studies**

Since there are no typical clinical features of NTM disease diagnosis depends on having a high index of suspicion. Guidelines for the diagnosis of disease due to NTM are given in the following box. Definitive diagnosis is made by bacteriological examination, but it is important to distinguish true disease from mere contamination of sputum by these ubiquitous organisms. Traditionally, EM are identified and distinguished from other species of mycobacteria after isolation by culture on solid media or, where facilities are available, by one of the automated liquid culture systems. Most strains grow on the egg-based Löwenstein–Jensen medium, but some species require nutritional supplements such as iron for *Mycobacterium haemophilum* and mycobactin (a mycobacterial lipid with iron-chelating functions) for some members of the *M. avium* complex. In certain centers nucleic acid-based methods are increasingly used to identify clinical and other isolates and to directly detect them in specimens.

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**Guidelines for diagnosing NTM pulmonary disease**

- Clinical and radiological features indicative of mycobacterial disease
- Acid-fast bacilli smear-positive and/or moderate/heavy growth of NTM on culture in two clinical specimens (sputum or bronchial lavage)
- Absence of other pathogens. Other common causes of disease have been excluded (eg tuberculosis, aspergillosis etc)
- Underlying host conditions (eg alcoholism, AIDS, immunosupression, chronic lung disease, cancer, cystic fibrosis etc)
- Underlying host conditions (eg alcoholism, AIDS, immunosupression, chronic lung disease, cancer, cystic fibrosis etc)
- Failure of clearance of the NTM from clinical specimens within two weeks of initiation of specific antimycobacterial therapy
- Where sputum evaluation in cavitary and non-cavitary disease is negative:
  - Transbronchial or open lung biopsy has histopathological features of mycobacterial disease and grows NTM on culture
  - Transbronchial or open lung biopsy does not grow the organism but has histopathological features of mycobacterial disease and other reasonable causes for granulomatous disease have been excluded
One major problem in the diagnosis of pulmonary disease due to EM is the establishment of the clinical significance of these organisms isolated from sputum and other pulmonary specimens by culture or detected by nucleic acid-based techniques.

There are no absolute criteria for distinguishing true pulmonary disease due to EM from contamination or colonization, but the American Thoracic Society [20] and the British Thoracic Society [34] have issued very similar guidelines for reaching a diagnosis, which may be summarized as:

- Compatible chest radiograph or computerized tomography appearances including infiltrates, multiple nodules, multi-focal bronchial disease, and cavities
- Compatible clinical symptoms
- Exclusion of other causes of the symptoms and radiological signs, including TB
- Three sputum specimens positive on culture within a 12-month period or two culture-positive specimens if they are also positive for acid-fast bacilli on microscopic examination
- Heavy growth on culture and acid-fast bacilli seen on microscopic examination of one specimen of bronchial washings
- Isolation of mycobacteria from a “sterile” site including lung tissue obtained by transbronchial biopsy or open-lung biopsy

It should, however, be noted that these diagnostic criteria were developed principally with respect to disease caused by the common pathogens MAC, M kansasii, and M abscessus. Further clinical experience is required to evaluate the relevance of these criteria to the less frequent—and possibly less pathogenic—EM. Additionally, as outlined below, these criteria are of limited value in determining the significance of isolates of EM from certain high-risk groups such as miners with silicosis.

Washing of bronchi draining areas of the lung showing nodular opacities on a CT scan is useful in the differentiation of causative from casual isolates of MAC and for making a diagnosis when sputum is negative on culture. In addition, bronchial washing is more likely to aid diagnosis than transbronchial biopsy even though the latter reveals characteristic granuloma formation [35].

The presence of an obvious cause of immunosuppression, such as HIV infection, does not per se indicate that an isolated EM is causing the disease. In a study based on the clinical, bacteriological, and radiographic diagnostic criteria advocated by the American Thoracic Society [20] MAC isolates were only considered clinically significant in 7 of 46 HIV-positive patients and 1 of 34 HIV-negative patients [32]. The diagnostic problems are further illustrated by a study in a Dutch TB center where EM were isolated from 27 patients (25 HIV-negative and two HIV-positive patients) but were only considered to be pathogenic in 14 patients. The detection of EM led to unnecessary or inappropriate treatment (including treatment for TB) in 17 of the patients, and a diagnosis of malignant disease was delayed in two patients [36].

### Radiological examination

Although some differences in radiological features between TB and diseases due to EM—and even between different species of EM—have been described, there is so much overlap in these features that a radiological determination of the cause is not possible in the individual patient [37]. In one study a radiological feature noted in some cases was described as a cluster of homogeneous shadows 1 cm across surrounding a translucent zone, with line shadows radiating from each lesion [38]. Some patients, notably non-smokers with no other evidence of lung disease, tend to have nodular lesions localized to the middle lobe or the lingula [25]. When MAC causes chest disease in immunocompetent individuals, there are three categories of chest radiograph patterns seen in clinical practice.

The most common appearance is similar to that of apical post-primary TB, with or without cavities. It is not possible to differentiate this disease from TB, although the cavities have been described as being thinner and smaller.

Somewhat common are patchy, nodular opacities in any zone of the lung, which on CT scanning are shown to be associated with local bronchiectasis.

Least common is the isolated pulmonary nodule. Mediastinal lymphadenopathy and pleural effusion are also rare.

Bizarre and rapidly changing radiological appearances are seen in patients with AIDS and other immunosuppressive disorders. Chest radiographs may appear normal in up to one third of AIDS patients with EM pulmonary disease. As with TB, diffuse appearances and lymph node enlargement are more common than in immunocompetent persons and cavitation is less common.

### Therapy of disease due to environmental mycobacteria

There have been few major controlled clinical trials of the therapy of pulmonary disease due to EM.
Those that have been conducted have been based on triple regimens of rifampicin, isoniazid, and ethambutol [34]. More work has been conducted on disseminated MAC disease in AIDS patients, but there is anecdotal evidence that the regimens developed for this condition are suitable for therapy of pulmonary disease due to the commonly encountered, slow-growing EM, especially MAC and M kansasii. Effective regimens are principally based on ethambutol with one of the newer macrolides such as azithromycin 600 mg daily and clarithromycin 500 mg twice daily [39]. These regimens are currently the subject of clinical trials being conducted by the British Thoracic Society. Some regimens also include rifampicin or rifabutin, although because of mutual inhibition of activity, care must be observed in the use of rifamycins in patients who are receiving antiretroviral therapy. Disease due to M kansasii often responds to shorter and simpler regimens based on ethambutol and rifampicin [40]. In all cases the length of therapy is determined by the time taken to clear the EM from the sputum, with therapy usually being continued for 1 year after sputum clearance.

There are limited data on regimens for disease due to rapidly growing mycobacteria, notably M abscessus and M chelonae. Success has been achieved with various combinations of clarithromycin, quinolones, cephalosporins, amikacin, and imipenem, but because clinical responses are notoriously unpredictable surgical excision of localized lesions should be considered [41,42].

In vitro drug susceptibility testing has been used to determine therapeutic regimens, but its use is limited owing to a poorly understood lack of correlation between the results such testing and clinical responses to treatment [43].

Summary

Pulmonary disease due to EM occurs worldwide, and its prevalence has increased as a consequence of the HIV pandemic. It is not often detected in the tropics owing to a lack of laboratory facilities, but when sought it has been found. In addition to HIV infection certain occupations such as mining render the work force more susceptible to disease and calls for a revision of working conditions. Resolution by therapy can be achieved in many cases. As the prevalence of TB diminishes worldwide—and hopefully it will in the wake of the resurgence of interest and the widespread application of the World Health Organization’s Directly Observed Therapy Short Course (DOTS) strategy—disease due to EM will become relatively more important and will necessitate revised strategies in clinical, microbiological, and public health approaches to mycobacterial disease.

References

[13] Ehlers S, Richter E. Differential requirement for interferon-gamma to restrict the growth of or eliminate some


