

# 1

## Introduction

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### 1.1 ENVIRONMENTAL MYCOBACTERIA

Environmental mycobacteria can be found in diverse environments and most appear to exhibit a saprophytic lifestyle. However, some have the ability to infect animals, birds and humans, and have evolved mechanisms by which they can invade and grow within host cells. Because the number of organisms shed back into the environment from infected animals can be relatively small, and heavy and widespread colonization of some environments occurs, it remains rather unclear what role animal/human infection plays in the life-cycle of many of these organisms.

Because these organisms are widespread in the environment, and there is little evidence that person-to-person transmission is common, there is an implicit assumption that environmental mycobacterial infections derive from water, food, the environment or contact with animals. There is evidence to support this assumption in many cases, although the source of infection in most

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remains unclear. A variety of mycobacterial species causing human disease have been linked to contaminated water (Table 1.1). However, some of these links can result from diagnostic uncertainty associated with differentiating contamination of patients or their specimens from human disease caused by environmental mycobacteria; a topic that is dealt with in later chapters.

Members of the MAC are responsible for the majority of non-*M. tuberculosis* mycobacteria infections in developed countries (Horsburgh 1996). The main presentations of MAC are lymphadenitis in children, respiratory infection in the elderly, respiratory, intestinal and disseminated disease in HIV-positive people and infections in people with other immunocompromising conditions. In areas of developing countries where *M. ulcerans* is endemic, ulcerative disease can cause severe disability (refer to Chapter 8).

## 1.2 NOMENCLATURE AND TYPING

As methods for differentiating strains improve, the need to accurately type and name organisms becomes important for our understanding of the disease epidemiology. With environmental mycobacteria this is no less important. Much of the literature in the last twenty years contains terms like MAC and MAIS for a group of organisms with different properties and virulence characteristics. MAC includes *M. intracellulare*, *M. avium* and its subspecies MAA, MAP and *M. avium* subspecies *sylvaticum*. Because MAP is very slow growing, fastidious and requires special media for its isolation, the term MAC is usually used to indicate members of the complex other than MAP, although strictly speaking MAP is a member. *M. lepraemurium* is related to *M. avium* and causes a leprosy like disease in mice, rats and cats (Rojas-Espinosa & Lovik 2001). Like MAP it is difficult to cultivate and very slow growing.

The MAIS complex includes *M. scrofulaceum*, *M. avium* and *M. intracellulare*, although MAIS is a less commonly used term. The term *M. avium-intracellulare* is for most purposes identical to MAC. One of the difficulties of using this nomenclature is that differences in the individual species have not been highlighted and therefore the epidemiology is less clear. There is support for strains from humans and pigs being reclassified as *M. avium* subspecies *hominissuis* because of their differences from bird strains, and this reclassification may be useful in revising our understanding of the epidemiology of this group of diseases (Mijs *et al.* 2002). Another example where typing can be useful is with MAP. There are typing differences between the organisms found in sheep and cattle and a lack of clarity about the zoonotic origin of organisms derived from humans.



**Table 1.1** Evidence of pathogenic *Mycobacterium* species in water identified by selected references

Species	Natural waters	Drinking water	Drinking water biofilm	Sewage	Hospital water	Hospital equipment	Hot water systems	Recreational water	Injections	Industrial water	Damp buildings	Mine water
	<i>al.</i> (1995)											
<i>M. marinum</i>												Slosarek <i>et al.</i> (1994)
<i>M. terrae</i>	Tuffley & Holbeche (1980)	Jin <i>et al.</i> (1984)	Schulze-Robbecke <i>et al.</i> (1992)		Lockwood <i>et al.</i> (1989)			Dailloux <i>et al.</i> (1980)	Zenone <i>et al.</i> (1999)		Huttunen <i>et al.</i> (2001)	
<i>M. tusciae</i>		Tortoli <i>et al.</i> (1999)										
<i>M. xenopi</i>	Torkko <i>et al.</i> (2000)	Sniadack <i>et al.</i> (1993)			Wright <i>et al.</i> (1985)	Bennett <i>et al.</i> (1994)	Wright <i>et al.</i> (1985)	Slosarek <i>et al.</i> (1994)				

### 1.3 UNDERSTANDING THE DISEASE

Primary *M. avium* infections, including non-tuberculous lymphadenitis (inflammation/swelling of the lymph nodes), can occur in children, although infection is more common in patients with pre-existing pulmonary disease. Most diagnosed infections occur in people who are severely immunocompromised. Infections can affect the respiratory and gastrointestinal tracts and may produce a generalized infection. MAA is the most frequent mycobacterial subspecies isolated from patients with AIDS and often causes serious disseminated disease: *M. intracellulare* is more common in immunocompetent people (Guthertz *et al.* 1989).

#### 1.3.1 The epidemiology of environmental mycobacteria

As a result of the dramatic increase in HIV-associated MAC infection in developed countries, MAC has received more attention than many other environmental mycobacteria. Disease can result from infection by one or more strains of different species (Conville *et al.* 1989), serotypes (Dawson 1990) or genotypes (Arbeit *et al.* 1993; Mazurek *et al.* 1997) of MAC, sometimes in combination with a mycobacterium other than MAC (Levy-Frebault *et al.* 1987; Falkinham 1996), including MTB (Epstein *et al.* 1997; Raju & Schluger 2000). HIV-positive patients frequently have a variety of concurrent infections, such as respiratory co-infection with *Pneumocystis carinii* (Raju & Schluger 2000). The observation that higher rates of disseminated MAC in AIDS patients occur in developed rather than developing countries is probably due to differences in both exposure and immunity (von Reyn *et al.* 1996) and high rates of infection with MTB.

Mycobacterial infections linked to contaminated hospital water have been recognized for many years (Wallace *et al.* 1998), and MAC has been isolated from hospital waters (Graham *et al.* 1988), particularly hot water systems (du Moulin *et al.* 1988). However, diagnostic difficulties can result from contamination of patients or their specimens by the use of non-sterile water during sample processing (Stine *et al.* 1987; Graham *et al.* 1988). It is likely that hospital waters represent a source of infection for immunocompromised patients (du Moulin *et al.* 1988; Peters *et al.* 1995). du Moulin *et al.* (1988) and Gurtler (1994) have suggested that contaminated showers may be a source of infection for HIV patients, although, in contrast, a case-control study of the risk factors for MAC in HIV-positive patients found that showering was protective (Horsburgh *et al.* 1994). A study of mycobacteria in swimming pool water in Finland found no MAC among the many mycobacteria isolated (Iivanainen *et*

*al.* 1999c), but MAC were recovered from pools and spas in the Netherlands (Havelaar *et al.* 1985). MAC infections have also been linked to hot tub use (Embil *et al.* 1997; Mangione *et al.* 2001), and there is some evidence that use of water transported over long distances represents an increased risk for infection (du Moulin *et al.* 1985).

A range of environmental mycobacteria have been recovered from bronchoscopes, bronchoscopy specimens (Dawson *et al.* 1982; Stine *et al.* 1987) and other clinical specimens from patients whose infections are related to the use of bronchoscopes. Mycobacteria have also been isolated during pseudo-epidemics where contaminated bronchoscope washers have been implicated as the source of infection (Gubler *et al.* 1992; Sniadack *et al.* 1993; Maloney *et al.* 1994; Wallace *et al.* 1998; Kressel & Kidd 2001; see also Chapter 10).

A prospective cohort study of AIDS patients in developed and developing countries found patient rates of disseminated MAC were 10.5-21.6% in New Hampshire, Boston and Finland compared to 2.4-2.6% in Trinidad and Kenya ( $p < 0.001$ ) (von Reyn *et al.* 1996a). PPD skin test reactions greater than or equal to 5 mm were present in 20% of patients from Kenya compared to 1% at other sites ( $p < 0.001$ ). Among patients from the United States and Finland, multiple logistic regression indicated that occupational exposure to soil and water was associated with a decreased risk of disseminated MAC; whereas low CD4 count, swimming in an indoor pool, history of bronchoscopy, regular consumption of raw or partially cooked fish/shellfish and treatment with granulocyte colony-stimulating factor were associated with an increased risk of disseminated MAC (von Reyn *et al.* 1996).

MAC have been isolated from a variety of supermarket foods (Argueta *et al.* 2000), and the apparent similarity between some food and clinical isolates suggests that food may be an important source of infection (Yoder *et al.* 1999). MAC isolates can be relatively resistant to heating and will survive pasteurization if present in sufficient numbers in raw milk (Grant *et al.* 1996). Not surprisingly, MAC have been recovered from raw and pasteurized milk (Hosty & McDurmont 1975). As part of an epidemiological study of patients with MAC and AIDS in San Francisco, food samples from the houses of patients were cultured for MAC. The organism was recovered from only 1 of 397 food samples, suggesting that this was not the principal route of transmission (Yajko *et al.* 1995). A significant association has been found between MAC infection in HIV-positive people and the consumption of hard cheese (Horsburgh *et al.* 1994). MAC have also been recovered from cigarettes (Eaton *et al.* 1995), and it has been suggested that this may contribute to MAC disease in smokers (Falkinham 1996). There is also some evidence that MAC infections are more common in people such as miners and farmers who are exposed to dust (Falkinham 1996).

### 1.3.2 Risk factors

People throughout the world are exposed to environmental mycobacteria. However, substantial differences in exposure occur at different ages, as measured by skin tests, and in different geographical regions. The overall picture suggests greater exposures in developing countries. While exposure to environmental mycobacteria may confer some resistance to infection with *M. tuberculosis*, it also appears to reduce the effectiveness of the BCG vaccine in protecting against *M. tuberculosis*. The links between HIV and TB within the developing world in particular make it important to determine whether environmental mycobacteria are contributing indirectly to the burden of disease associated with the HIV pandemic. In the developed world, MAC disease has been a significant contributor to the morbidity and mortality associated with HIV. However, since the introduction of HAART to control HIV replication, MAC disease has become less of a clinical problem in these patients.

## 1.4 PATHOGENIC MYCOBACTERIA IN WATER

In 1997, Hunter reported that about eight species of mycobacteria had been associated with waterborne transmission of human disease (Hunter 1997). These species included *M. avium* complex, *M. fortuitum*, *M. goodii*, *M. marinum*, *M. scrofulaceum*, *M. terrae*, *M. ulcerans*, and *M. xenopi*. Today the list continues to grow, with the possible addition of *M. chelonae*, *M. immunogenum*, *M. abscessus*, *M. kansasii*, *M. ulcerans*, *M. szulgai*, *M. simiae*, *M. palstre* and MAP. There has been an increase in the number of potentially pathogenic mycobacterial species whose transmission route is associated with water (Table 1.1). This, in part, is due to industrial and institutional exposures that have resulted in respiratory infections that have previously been misdiagnosed. In some cases, the causative agents had yet to be described, e.g. *M. immunogenum* in metal-working fluids (Shelton *et al.* 1999). No doubt further species will emerge as water-related infections as both our epidemiological and monitoring tools continue to improve.

### 1.4.1 Water supply

Water remains an important potential source of human exposure to environmental mycobacteria. Organisms such as MAP, although difficult to recover from environmental samples, are excreted in large numbers in the faeces of infected animals and are likely to be present in source waters that are abstracted for drinking-water.

Some species, such as *M. kansasii*, can colonize cold water distribution systems whilst *M. xenopi* and *M. avium* are more commonly associated with hot water systems. In contrast, *M. marinum* in swimming pools or aquaria can cause infection of skin abrasions. Contamination of liquids that are injected can cause abscesses (*M. chelonae*, *M. fortuitum*), and other iatrogenic infections are linked to contaminated endoscope washers and renal dialysis fluid.

The difficulty with investigating the waterborne transmission of mycobacteria is firstly that the infections are generally sporadic (there are some outbreaks), secondly there are a variety of sources of exposure other than water, and thirdly that the typing schemes that are routinely available are not sufficiently discriminating to confidently identify whether isolates from the environment are the same as those from associated patients.

#### **1.4.2 Recently reported cases of waterborne mycobacterial disease**

Table 1.2 lists a sample of recently reported cases of environmental mycobacterial diseases. It is important to remember that the vast burden of Buruli ulcer (caused by *M. ulcerans*) in tropical countries is not reflected in this table. An association between water exposure and Buruli ulcer in endemic areas is suspected, but the evidence is inconclusive (refer to Chapter 8). In fact, a recent study suggests that the disease may be at least partially vectorborne, transmitted by an aquatic insect (Marsollier *et al.* 2002).

For MAP, the connection with CD is controversial (refer to Chapter 6, the article by Sechi *et al.* 2001 and response by Roholl *et al.* 2002). At present, there would appear to be no epidemiological studies linking MAP in water with human disease. From a public health stand point this is a much-needed area of research/clarification.

### **1.5 GLOBAL BURDEN OF DISEASE**

According to the World Health Report for 2002 (WHO 2002), mortality due to infectious diseases accounts for 19.3% (10 932 000 of 56 554 000) of total deaths. In terms of the burden of disease, as measured in DALYs, the contribution from infectious diseases is 24.5% (359 377 000 of 1 467 257 000) with TB, HIV/AIDS and malaria responsible for nearly half of this burden (WHO 2002).

**Table 1.2** Waterborne outbreaks of disease caused by environmental mycobacteria, or where water is strongly implicated in transmission

<b>Organism</b>	<b>Disease</b>	<b>Source</b>	<b>Nos. infected</b>	<b>Reference</b>
MAC	Pulmonary disease	Hot tub	Family of 5	Mangione <i>et al.</i> 2001
MAC	Pulmonary disease	Hot tub	Family of 5	Embil <i>et al.</i> 1997
MAC	Cutaneous infection	Circulating bath water	3	Sugita <i>et al.</i> 2000
MAC	Hypersensitivity pneumonitis	Hot tub	2 case studies	Rickman <i>et al.</i> 2002
<i>M. fortuitum</i>	Furunculosis	Whirlpool footbaths at a nail salon	110	Winthrop <i>et al.</i> 2002
<i>M. fortuitum</i>	Respiratory tract colonization	Hospital ice machine	19	Labombardi <i>et al.</i> 2002
<i>M. fortuitum</i>	Respiratory tract colonization	Hospital ice machine	47	Gebo <i>et al.</i> 2002
<i>M. chelonae</i>	Pseudo-outbreak	Contaminated endoscopy washer	-	Kressel & Kidd 2001
<i>M. chelonae</i>	Cutaneous abscesses	Tap water contaminated instruments in liposuction	34	Meyers <i>et al.</i> 2002
<i>M. immunogenum</i>	Hypersensitivity pneumonitis	Metal removal fluids	Several case studies	Shelton <i>et al.</i> 1999
<i>M. abscessus</i>	Sporotrichoid dermatosis	Public bath	2 case studies	Lee <i>et al.</i> 2000a
<i>M. marinum</i>	Cutaneous infection	Aquarium management	3 case studies	Dorrnsoro <i>et al.</i> 1997
<i>M. marinum</i>	Ulcerated nodule	Aquarium	14 month old girl	Speight & Williams 1997
<i>M. kansasii</i>	Cellulitis	Swimming at a beach	1 immuno-compromised patient	Hsu <i>et al.</i> 2002
<i>M. ulcerans</i>	Ulcerative disease	Irrigation waters	29	Ross <i>et al.</i> 1997
<i>M. szulgai</i>	Keratitis	Intraoperative contamination from ice water	5	Holmes <i>et al.</i> 2002
<i>M. palstre</i>	Cervical lymphadenitis	Potential for water exposure	1 child + veterinary and natural water isolates	Torkko <i>et al.</i> 2002

In the USEPA's Mycobacteria: Drinking-water fact sheet (EPA, 2002), the CDC estimates that 1.8 out of 100 000 individuals are infected by non-AIDS related mycobacterial diseases each year in the USA. The burden within the American AIDS population has been as high as 50% (primarily MAC) (Horsburgh 1991), although antiretroviral therapy has reduced this rate in recent years (Horsburgh *et al.* 2001). Two and a half million people are estimated to be living with HIV in industrial countries (United States, Canada, Australia, New Zealand, Japan, and Western and Eastern Europe), most of who have or will have access to antiretroviral therapy. In developing countries, where HIV/AIDS prevalence can exceed 30% in the adult population (e.g. Botswana, Lesotho, Swaziland and Zimbabwe) (AIDS 2002), access to antiretroviral therapy can be extremely limited. In fact, the AIDS 2002 Barcelona report suggests that a total of only 158 168 people have received or are receiving antiretroviral therapy in "low and middle income" countries (AIDS 2002). However, the burden of mycobacterial diseases may not be as high as in developed countries, at least for MAC-related infections. This is thought to be due, in part, to the relatively short survival times of people after conversion to AIDS (Maartens 2002).

Based on CDC estimates for the USA, the global burden of non-AIDS related mycobacterial diseases would exceed 110 000 per year. However, applying American infection rates to other countries may be very unrealistic, particularly where nutritional and other indicators of health status suggest high susceptibility to disease. Of particular note are the numerous endemic areas for Buruli ulcer (at least 32 countries) caused by *M. ulcerans*. Although considered the third most common mycobacterial infection of humans after TB and leprosy, the actual burden of this disease is unknown (<http://www.who.int/gtb-buruli/>). Potential transmission by an insect vector raises specific issues related to public health and the potential to manage the disease (refer to Chapter 8).

At the end of 2001 over 40 million adults and children were reported to be living with HIV/AIDS: over 71% in sub-Saharan Africa. Although at present, rates of infection with MAC within the HIV/AIDS population in developing countries may be lower than in developed countries, any improvement in longevity of AIDS sufferers may place them at greater risk from these opportunistic infections.

People in different parts of the world appear to be exposed to MAC and other environmental mycobacteria in different ways (von Reyn *et al.* 1993, 1996). Evidence of exposure in Africa is based on skin tests (von Reyn *et al.* 1993, 1996; Fine *et al.* 2001) and INF $\gamma$  responsiveness to MAC PPD using whole blood (Black *et al.* 2001). The evidence suggests that people in Malawi are commonly exposed to environmental mycobacteria. The growing evidence that environmental mycobacterial exposure reduces the ability of people to develop an effective immune response to TB following BCG vaccination means that

protection programmes may be influenced by exposure. This has clear public health implications in determining the effectiveness of BCG vaccination programmes (Black *et al.* 2001; Fine *et al.* 2001). It remains unclear whether differences in such immunological tests are a good measure of exposure to MAC or represent the extent of sub-clinical disease.

Estimating the amount of infection and disease associated with environmental mycobacteria is both difficult and inherently inaccurate. This is true even in HIV where disease caused by MAC can affect a significant proportion of the population. The extent of MAC contamination of the environment suggests that people are commonly exposed to this and other environmental mycobacteria but do not necessarily develop an immune response following such exposures. The substantial regional variation in reported infections with different environmental mycobacteria makes global generalization problematic.

### **1.5.1 Crohn disease and Johne disease**

The causes of CD have been the subject of much microbiological and epidemiological interest over the last 30 years. There is some microbiological and clinical data that suggests that MAP is involved with the pathology of this disease, and possibly even its causation. One reason MAP has attracted interest is its role in JD in herbivores, and the relative similarity of the pathology of the two conditions. However, CD appears to have a genetic component and causation may have multiple factors. The isolation of MAP from pasteurized cows' milk has led to concerns that people may be exposed to this organism through the food chain. Also, as many agricultural animals excrete large numbers of MAP in their faeces, it is reasonable to assume that source waters for drinking-water supplies may be contaminated with this organism. So far, MAP has not been isolated from drinking-water in England, although molecular studies are beginning to indicate the potential presence of MAP in surface waters (Chapter 6). There are technical difficulties in isolation from both clinical and environmental samples that make confirmation of the causative role of MAP in CD and the assessment of environmental exposure difficult. It is likely that water will prove to be one of a number of sources from which people are exposed to MAP. While there is little current evidence that improving drinking-water treatment will have any impact on the incidence of CD, water companies and utilities should consider whether any action is required to adopt a more precautionary approach. A detailed discussion of the links between MAP and CD is included in Chapter 6.

## 1.6 CONTROL MEASURES

The preceding sections show that there is growing evidence that people are acquiring mycobacterial infections from organisms present in water, food and the environment. However, exposure to these organisms without the development of overt symptoms, but with the development of an effective immune response, is relatively common. Therefore, should we be trying to reduce or increase people's exposure in order to reduce the risks of infection?

There are clear winners and losers in this immunological conflict. People who are at a vulnerable stage of development (in particular children and the elderly) or are immunocompromised (HIV), and are immunologically naïve to the particular environmental mycobacteria, may succumb to infection if exposed. In the developed world, infection with environmental mycobacteria is sufficiently uncommon to make prevention through mass immunization an unattractive solution. We are in a position in which the majority of people must be relatively resistant to most environmental mycobacteria, given their ubiquity in the environment, and further reduction in environmental exposure is unlikely to significantly affect risk. There is, however, some rationale for reducing individual exposures to some potential sources, such as whirlpool baths and showers that might represent a significant risk of infection. For particular "at risk" populations (e.g. HIV-positive individuals) there is practical risk advice for reducing the chances of exposure to MAC.

## 1.7 GENOMIC AND EVOLUTIONARY PERSPECTIVES

Basic information on the characteristics of mycobacterial genomes has been important in understanding their biology, classification and taxonomy for many years (Imaeda *et al.* 1982; Bercovier *et al.* 1986; McFadden *et al.* 1987b; Antoine *et al.* 1988). The recent projects to sequence a number of *Mycobacterium* species (Table 1.3) have resulted in an expanding dataset that can be used for comparative genomics (Behr *et al.* 1999; Brosch *et al.* 2000, 2001; Cole, 2002a; Gordon *et al.* 2002; Schoolnik 2002), vaccine and drug candidate selection (Cockle *et al.* 2002; Cole 2002b) and pathogenic and evolutionary genetics (Brosch *et al.* 2001; Fitzgerald & Musser 2001). There is extensive gene loss in the strictly parasitic mycobacterial species (e.g. *M. tuberculosis*, *M. leprae*) (Brosch *et al.* 2001) and IS may be responsible for these losses (Ho *et al.* 2000).

**Table 1.3** *Mycobacterium* genome sequencing projects

<b>Organism</b>	<b>Genome size/characteristics</b>	<b>Institution sequencing genome</b>
<i>M. avium</i> 104	The genome is about 5.48 mb long and has a G+C content of 68.5%	The genome sequence is nearing completion at the Institute for Genomic Research
<i>M. bovis</i> AF2122/97	The genome is 4 345 492 bp long, with a G+C content of 65.63%.	The Sanger Institute with the Unité de Génétique Moléculaire Bactérienne, Institut Pasteur, and the Veterinary Laboratories Agency. Funded by Beowulf Genomics and the Ministry of Agriculture Fisheries and Food.
<i>M. leprae</i>	The complete sequence is 3 268 203 bp in length with a G+C content of 57.8%. There are 1604 protein-coding genes and 1116 pseudogenes.	The Sanger Institute with the Unité de Génétique Moléculaire Bactérienne, Institut Pasteur. Funded by the New York Community Trust.
<i>M. marinum</i>	The genome is approximately 6.589 Mb with a G+C content of around 65%. At present there are 138 601 reads totalling 59.714 Mb and giving a theoretical coverage of 99.99% of the genome. At present, there are 88 contigs > 1kb (65 contigs > 2kb).	The Sanger Institute with the University of Washington, Seattle; the Unité de Génétique Moléculaire Bactérienne, Institut Pasteur; Monash University and Monash Medical Centre, Australia; the Department of Microbiology, University of Tennessee, Knoxville. Funded by Beowulf Genomics.
<i>M. microti</i> OV254	A minimal set of 50 bacterial artificial chromosome clones that covers almost all of the genome of <i>M. microti</i> OV254	
<i>M. paratuberculosis</i> K10	The genome sequencing is now complete, and the annotation is in progress. The genome is a single circular chromosome 4 830 869 bp in length, with 69.30% G+C content, and contains over 4000 open reading frames.	University of Minnesota funded by the US Department of Agriculture – Agricultural Research Service.
<i>M. smegmatis</i> MC2 155		The Institute for Genomic Research funded by National Institute of Allergy and Infectious Disease.
<i>M. tuberculosis</i> H37Rv	The sequence is 4 411 529 bp long with an overall G+C content of 65.6%, and with 3924 predicted protein coding genes. (Cole <i>et al.</i> 1998)	The Sanger Institute with clones supplied by the Unité de Génétique Moléculaire Bactérienne, Institut Pasteur. Funded by the Wellcome Trust
<i>M. tuberculosis</i> CDC 1551		National Center for Biotechnology Information, National Institute of Health, Bethesda, Maryland, USA

## 1.8 KEY RESEARCH ISSUES

What, then, are the important areas where research needs to be targeted in order to protect public health in the longer term? Given the apparent associations between exposure to environmental mycobacteria and lack of responsiveness to BCG vaccination there needs to be an approach to the development of an improved TB vaccine, preferably one that offers protection against the important environmental mycobacteria as well as greater protection against TB.

There is a limited understanding of the social, environmental and personal risk factors responsible for initiating disease caused by most of the environmental mycobacteria and this is aggravated by limited use of typing and environmental testing in investigating individual cases of infection. Greater effort needs to be put into sensitive typing methods and their use in elucidating the epidemiology of these diseases. Sequencing the genomes of further environmental mycobacteria will undoubtedly benefit our long-term understanding of these organisms.

The challenge of the next decade will be to convert our improved knowledge of the organism, immunology, disease and risk factors into a strategy to reduce the morbidity and mortality caused by these organisms that is applicable worldwide. This will require targeted responses to key individual mycobacterial pathogens such as *M. ulcerans* and coordination of these responses with WHO Programmes concerned with TB.