Pathogenic Mycobacteria in Water

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Pathogenic Mycobacteria in Water

A Guide to Public Health Consequences, Monitoring and Management

Edited by
S. Pedley, J. Bartram, G. Rees, A. Dufour, J.A. Cotruvo

World Health Organization

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Contents

Preface

The World Health Organization's Water, Sanitation and Health Office, in collaboration with the US Environmental Protection Agency Office of Research and Development, is producing a series of expert workshops, reports and monographs on Emerging Pathogens in Water. This monograph was developed from the expert workshop entitled: Pathogenic Mycobacteria in Water that was held in Guildford, UK between the 18th and 20th September 2002. The sponsors of this workshop were the US EPA Office of Research and Development.

A total of 20 experts from 7 different countries, including those with expertise in medicine, clinical and water microbiology, epidemiology, regulatory policy, public health and water engineering, examined the range of issues associated with pathogenic mycobacteria in current and potential human-water interfaces.

Other workshops and publications in the series include:

- Heterotrophic Plate-Counts and Drinking-Water Supply
- H_2S Method for Detection of Faecal Contamination
- Severe Outcomes from Infectious Diseases Associated with Recreational Water Contact
- Respiratory Transmission of Faecally Excreted Viruses

- Toxic Cyanobacteria in Water
- Waterborne Zoonoses: Identification, Causes and Controls

These are all available from the World Health Organization website (www.WHO.int/water_sanitation_health/) or from the International Water Association (www.iwapublishing.com).

Non-tuberculous pathogenic mycobacteria, including those collectively referred to as *Mycobacterium avium* complex (MAC) have been associated with a variety of adverse health outcomes, including Crohn disease, cervical adenitis and pulmonary and skin infections. The bulk of interest in MAC infection in relation to water management revolves around the exposure of immunocompromised individuals (in particular HIV positive populations) to MAC in domestic water supply. The issues addressed included:

- the state of current knowledge on MAC
- health outcomes in high-exposure occupational groups
- examining the postulated causal link between *Mycobacterium avium* sub-species *paratuberculosis* (MAP*)* and Crohn disease
- risk assessment and risk management
- infections associated with non-tuberculous pathogenic mycobacteria
- analytical methods for the isolation and identification of non-tuberculous pathogenic mycobacteria
- environmental sources and routes of exposure
- controls, treatments and disinfection regimes

This monograph contains the results of the workshop deliberations largely based on the technical materials prepared by the participants prior to the workshops and the resultant scientific discussions. Each of the chapters in the monograph resulted from presentations by the experts in the various sessions of the workshop. The presentations were subsequently revised in the light of other presentations and the debate and discussion that reflected the collective experiences of the participants. The final monograph is the product of the Expert Workshop, all its chapters were redrafted and peer reviewed by members of the expert group and the editors.

We hope that you will find the document enlightening in describing the complex issue of non-tuberculous pathogenic mycobacteria and the role of water as a milieu for survival and transmission of infections associated with these organisms.

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Executive Summary

Mycobacteria are a large group of microorganisms that inhabit a diverse range of natural environments: some species are capable of infecting humans and animals. Apart from the highly significant mycobacterial pathogens, we may presume that the majority of people in the water industry, until recently, would not be familiar with environmental mycobacteria. Nevertheless, environmental mycobacteria are a frequent cause of infection, and there is a growing body of evidence to show that water is a significant vehicle for the transmission of these organisms.

The importance of the Pathogenic Environmental Mycobacteria (PEM), and especially the *Mycobacterium avium* Complex (MAC), was recognised with the discovery of disseminated infection in immunocompromised people, particularly people with HIV and AIDS. Yet there are many other forms of disease, both minor and serious, that are caused by PEM. This book provides a comprehensive review of PEM, the different types of disease that they are known to cause, and their distribution in water and other parts of the natural environment. It explores the role of water as a vehicle for the transmission of these pathogens and concludes with a discussion of the issues surrounding the control of PEM in drinking-water and the

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assessment and management of risks. The book is a valuable resource for sanitary and clinical microbiologists, public health professionals, water resource managers, water engineers, piped public water suppliers, regulators of water quality and facilities managers in medical and commercial buildings.

The history of human development and the growth of civilisation contains frequent reference to the role of infectious disease in forging social structures and deciding the fate of nations. Critical analysis of the historical record shows that at least some of the major infectious diseases circulating in the world today have existed relatively unchanged for several centuries, possibly even longer. In addition to these readily identifiable diseases, we may assume that there would have been in circulation a much greater number of other pathogens, causing a range of diseases that are unrecognisable from contemporary records or that were of insufficient virulence to be visible above the background of other social hazards. Notwithstanding the long, stable history of a few infectious diseases, the relationship between humans and pathogens is not static and from time-to-time new pathogens appear and present fresh challenges for society. In a brochure that accompanies this series of publications (Emerging Issues in Water and Infectious Disease, WHO, 2004) we are told that pathogenic microorganisms are subject to the same rules of evolution as all other organisms. The interaction between the pathogen and its environment leads to a modified, possibly more virulent strain of the pathogen, or to the emergence of a previously unrecognised form of the pathogen. As new types of pathogen emerge appropriate public health measures must be developed to control the spread of disease. Frequently, the public health response will impact the management of water resources, especially as drinking-water reserves and for recreational and agricultural use. The brochure highlights four drivers of emerging issues in water and infectious disease:

- New environments.
- New technologies.
- Scientific advances in water microbiology.
- Changes in human behaviour and vulnerability.

In order to inform the public about these emerging issues and to provide guidance to the water sector about the management options that are available to moderate their effects, the WHO and USEPA have initiated a series of meetings at which leading experts from different disciplines discuss the issues and work towards a consensus about the risks to public health and the measures required to control their impact. PEM have been identified as a group of microorganisms that are widespread in the environment and which appear to be an emerging cause of waterborne disease. This book is the final output from a meeting of experts held in Guildford, UK, in September 2002. The meeting brought together microbiologists, water quality

experts, public health experts and epidemiologists to present and analyse our knowledge of PEM and the particular issues surrounding their presence in water. The first drafts of the chapters were prepared from the presentations made by the experts. Subsequently, the chapters were redrafted and reviewed by members of the expert group, the editors, and other contributors.

The chapters in the book have been grouped into three sections, although this is not manifest in the contents. In chapters 2 to 5 the reader is introduced to the biology of PEM and is shown how this governs the ecology of the organisms. This section also describes the particular difficulties associated with the isolation, enumeration and identification of PEM and shows how a combination of classical and modern analytical methods are being used by laboratories to detect the organisms. In chapters 6 to 10 the book focuses on the range of infections caused by PEM and the role of water in the transmission of these infections. Chapters 10 and 11 form the final section of the book and deal with the particular issues in water treatment and water quality management that are presented by the characteristics of PEM. Every chapter is concluded with a list of the key research issues that, in the opinion of the authors, should be tackled in order to improve our understanding of the organisms, their ecology, their pathogenicity, and the role of water in the transmission of disease.

We have described earlier the concept of emerging issues in water and infectious disease and the key issues that form the theme of this series of books. Throughout this book you will find many examples of how PEM have gained prominence by exploiting the changing interface between humans and the environment, which is embodied in the key issues listed above. Chapter 1 provides a comprehensive summary of PEM and introduces topics that are dealt with in depth by the authors of later chapters. It is in this chapter that the reader will find the most conspicuous links with the key issues of water and infectious disease.

The relationship between PEM, in particular MAC, and the environment is developed in chapters 2 and 3. It is clear from the reviews presented in these two chapters that the ecology of PEM is complicated and poorly understood. As well as existing as a free-living resident of many environments, PEM may also form a symbiotic, or parasitic, relationship with certain species of amoebae and insect. These relationships may be fundamental to the transmission and pathogenicity of PEM. Furthermore, the unusual chemical structure and hydrophobicity of their cell wall leads to the organisms being concentrated at the interface between water and air; a particular issue for the dispersal of the organisms in aerosols. Another important aspect of the ecology of PEM is illustrated by the example of cervical lymphadenitis (chapter 7). Until recently, the principal cause of cervical lymphadenitis in children was *Mycobacterium scrofulaceum*; however, several groups of workers have reported a rapid shift in the main etiological agent of disease from *M. scrofulaceum* to *M. avium*. A significant feature of this shift is that it has

occurred concurrently in the UK, USA and Australia, and that it coincides with a sudden decline in the occurrence of *M. scrofulaceum* in water. The reason for this sudden change is not understood.

The authors of chapter 1 note that MAC was first recognised as a problem in people with HIV and AIDS. This theme is expanded in chapter 7, where the appearance and spread of disseminated MAC infection is discussed in the context of the emergence of HIV and AIDS and the vulnerability of individuals with impaired immunity: an example of changes in human behaviour and vulnerability. For these people, PEM in water and soil is a particular hazard, and exposure to these environments, for example at swimming pools or even contact with some potting composts, may give rise to a special risk of infection. The subject of changes in human vulnerability is also tackled by some of the other authors.

A common thread running through the book is the emergence of PEM as a problem organism in water systems. There can be little doubt that MAC and other PEM have existed in the aquatic and soil environments for a long time; in other words, they are not a recent contaminant. Yet it is only relatively recently that their significance for public health has been appreciated. This point can be illustrated by reference to Buruli Ulcer (BU), a disease caused by *Mycobacterium ulcerans*, which is described in chapter 8. The first description of disease symptoms consistent with BU was made in 1897, but it was not until 50 years later that the etiological agent was identified and described. Since 1980, evidence has emerged of the true extent of the disease and it is now the third most common mycobacterial infection after TB and leprosy. The authors of chapter 8 report the results of recent studies that suggest a link between *M. ulcerans* and a biting, aquatic insect. Based upon these observations we can speculate that BU is a vector-borne disease and that water may be an important environment for the proliferation of the vector. The authors of chapter 8 point out that this new development offers options for water resource management that may control the spread of the disease. In the context of the key emerging issues in water and infectious disease, BU embodies a number of themes, but principally it is an example of scientific advances in microbiology that have provided the tools that were necessary to detect *M. ulcerans* in aquatic insects.

The possible role of water in the transmission of Crohn Disease (CD) has stimulated a fascinating debate between microbiologists, public health professionals, and water quality scientists, which is reviewed in chapter 6. Currently, the link between *M. avium* subspecies *paratuberculosis* (MAP) and the waterborne transmission of CD has not been proven, but there is a body of epidemiological and microbiological evidence that would suggest that a link does exist, and that further research is needed. We await with interest the resolution of this debate. Once again, the possible role of water in the transmission of a pernicious disease is being unravelled. However, as new information is published

the possibilities for controlling the disease through effective water quality management strategies may become apparent.

Several of the chapters portray PEM as an opportunistic pathogen: a pathogen that can infect a compromised host but would not normally infect a healthy host. Yet these are not trivial infections as chapters 7 to 10 demonstrate. Once the organism has established an infection treatment can be very difficult, requiring the use of a combination of drugs for an extended period of time. Relapses are not uncommon. In some cases the treatment may involve surgical removal of the infected area. Water plays a significant role in the infection process. In chapter 8 the authors cite examples of people contracting PEM infections by contact with water used in a beauty treatment salon. One patient was left with a permanent scar on her leg. There is also evidence to suggest that body piercing may be a cause of PEM infection. These examples show how different uses of water, brought about by changing lifestyles, can present new challenges for public health protection, as well as emphasising the need for water users to be mindful of the possible risks associated with some of the more unconventional uses of water. Similarly, medical and dental establishments are not immune from opportunistic infections with PEM. Bronchoscopes that have been cleaned with water contaminated with PEM have been shown to be a cause of pulmonary infection (chapter 9).

New analytical methods are being developed all the time in all fields of microbiology. During the past 20 years, methods that use the detection, amplification, or sequencing of genetic material have emerged as major tools for analysts specialising in medical and environmental microbiology. These advanced methods have provided essential evidence to confirm PEM as a cause of some infections and for discovering new environmental reservoirs of the pathogen (for example, *M. ulcerans*). Yet the confirmation of PEM in many infections still relies upon the classical methods for culturing the organism in the laboratory. Chapter 5 describes the state of the art methods available for the isolation and identification of PEM, but points out that although many of the methods are accurate and precise, the first step in diagnosis often requires the growth of the organism on selective media. Similar limitations apply to the recovery of PEM from environmental samples. The chapter concludes that further work is required to improve the analytical methods that are available to microbiologists to expedite diagnosis and improve environmental monitoring.

Modern, rapid methods of analysis that detect specific nucleic acid sequences in the PEM are possible because of the major developments in our understanding of the biology and genetics of the organisms. In chapter 4, the authors expand upon the earlier descriptions of the ecology of MAC and other PEM and show how this can be affected by the biology of the organisms. The description of the genetics of the PEM provides an important insight into the many factors that control the ecology of the organisms.

Two factors control the presence and level of PEM in drinking-water distribution systems: ingress and regrowth. From our understanding of the biology and ecology of PEM (chapters 2, 3 and 4) we may surmise that drinking-water treatment will not eliminate PEM but, if operating satisfactorily, will significantly reduce the numbers that may be present in the source water to a level that represents a negligible risk to the general population. Ingress of PEM through leaks in the distribution system, and the regrowth of PEM in biofilms are potentially more significant events for drinking-water quality management. These issues are addressed in chapter 11.

The biology and ecology of PEM render them highly resistant to chlorine and the other chemical disinfectants used for the treatment of drinking-water. Chapters 1, 2 and 3 demonstrate that many sources of water, in particular surface water, will be contaminated with some species of PEM. Consequently, the early stages of water treatment – flocculation, sedimentation and filtration – are the most important barriers to the transfer of PEM into the water distribution system. Any breach of these treatment steps will not be remedied by chemical disinfection of the water. Even if PEM are removed from the source water, the potential for contamination to occur through breaches in the pipework, either from a burst water main or during maintenance work, is high. Once inside the network, PEM can readily integrate into the biofilm where they become an established member of the microbial community. Some studies carried out in drinking-water distribution systems have shown that slow growing PEM can be found at densities greater than 4000 per cm^2 in the surface biofilm, creating a potentially high frequency and level of exposure.

The book concludes by discussing the use of water management strategies for controlling exposure to PEM from water systems. In chapter 12, the authors argue that water safety plans (WSPs) can be an effective method for the control of PEM in water. To support their argument, the authors draw heavily upon the example of Legionella and the procedures that have been developed to control Legionella in water systems. WSPs are an important aspect of the 3rd edition of the WHO Guidelines for Drinking-Water Quality and the PEM are used to demonstrate how the risk assessment and management framework of a WSP can be an effective mechanism for the control of waterborne pathogens.

This book provides an in-depth review of PEM as an emerging waterborne pathogen, and examines their significance for public health and water resource management. PEM offer many challenges to the microbiologist for the development of improved analytical methods and monitoring procedures; to the public health sector to determine the full extent of the disease caused by PEM, their epidemiology and clinical management; and to the water sector, where PEM present significant problems in treatment and control. PEM highlight how holistic approaches to the management of water supplies may be the best, even the only option that is available to the water sector.

List of acronyms and abbreviations

AFB	acid fast bacillus
AOC	assimilable organic carbon
BCG	Bacillus Calmette-Guerin
BDOC	biodegradable dissolved organic carbon
bp	base pairs
BSA	bovine serum albumin
BU	Buruli Ulcer
CCL	contaminant candidate list
CD	Crohn disease
CDC	Centers for Disease Control and Prevention
CF	cystic fibrosis
cfu	colony forming units
CPC	cetyl pyridinium chloride
CR	congo red
DNA	deoxyribonucleic acid
ELISAs	enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency
G+C	guanine plus cytosine
GAC	granular active carbon

GPL	glycopetidolipids
H&E	haematoxylin and eosin
HAART	highly active antiretroviral therapy
HACCP	Hazard Analysis Critical Control Point
HPC	heterotrophic plate count
IFN-gamma	interferon gamma
IMS	immunomagnetic separation
IS	insertion sequences
ISH	*in situ* hybridization
ITS	internal transcribed spacer
JD	Johne disease
kb	kilobases
MAA	*Mycobacterium avium* subsp. *avium*
MAC	*Mycobacterium avium* complex
MAIS	*M. avium-intracelluslare-scrofulaceum*
MAP	*M. avium* subsp. *paratuberculosis*
mb	megabases
mce	mycobacterial cell entry
MLST	multi-locus sequence typing
MTB	*Mycobacterium turberculosis*
NTM	non-tuberculous mycobacteria
NTU	nephlometric turbidity units
OSHA	Occupational Safety & Health Administration
PCR	polymerase chain reaction
PEM	pathogenic environmental mycobacteria
PFGE	pulsed-field gel electrophoresis
PPD	purified protein derivatives
PRA	PCR restriction analysis
PVC	polyvinyl chloride
RFLP	restriction fragment length polymorphism
RGM	rapidly growing mycobacteria
RNA	ribonucleic acid
rRNA	ribosomal ribonucleic acid
SCOTS	selective capture of transcribed sequences
SDS	sodium dodecyl sulfate
ssGPL	serovar-specific gylcopeptidolipids
TB	tuberculosis
TU	turbidity units
USEPA	US Environmental Protection Agency
WHO	World Health Organization
ZN	Ziehl-Neelsen