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Epidemiology of Infection by Nontuberculous Mycobacteria

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INTRODUCTION

The Nontuberculous Mycobacteria

The nontuberculous mycobacteria include those *Mycobacterium* species that are not members of the *Mycobacterium tuberculosis* complex; hence the use of the terms "nontuberculous mycobacteria" and "mycobacteria other than tuberculosis." As the incidence of tuberculosis fell, infection by those mycobacteria became more readily recognized. Earlier, the organisms

carried the epithet "atypical," originating from the mistaken belief that they were unusual *M. tuberculosis* strains. In a truly benchmark review, the case for treating these mycobacteria as disease entities was presented in convincing fashion (547). That review summarized the evidence that nontuberculous mycobacteria were able to cause disease and established the validity and importance of studies of nontuberculous mycobacteria. That review has been followed by a growing body of reports describing the role of nontuberculous mycobacteria in

human and animal disease. Further, the criteria for demonstrating that a particular nontuberculous mycobacterium was responsible for disease (e.g., repeated isolation, absence of other pathogenic mycobacteria) were also provided (547). A 1979 international conference in Denver, Colo., sponsored by the National Jewish Hospital further stimulated studies of nontuberculous mycobacteria and provided the first evidence of studies of the epidemiology, pathogenicity, taxonomy, and molecular genetics of nontuberculous mycobacteria (114). Both documents are starting points in any proposed investigations of nontuberculous mycobacteria.

Nontuberculous Mycobacterial Disease before the AIDS Epidemic

Before the advent of the AIDS epidemic, disease caused by nontuberculous mycobacteria was pulmonary, confined to cervical lymph nodes, limited to skin, or, in rare cases, disseminated (547). Pulmonary disease was found predominantly in males in the sixth decade of life. Most patients had predisposing lung conditions (e.g., pneumoconiosis or black lung) or worked under conditions where they were exposed to dusts (e.g., farming). In the absence of evidence of person-to-person transmission, it was proposed that humans were infected from environmental sources via aerosols (547). Regional differences in the frequency of persons showing evidence of prior nontuberculous mycobacterial infection (135) were consistent with that hypothesis. It was also proposed that infection with environmental nontuberculous mycobacteria could provide some degree of immunity to *Mycobacterium tuberculosis* infection (136, 465).

The major pulmonary mycobacterial pathogens were *Mycobacterium kansasii*, *Mycobacterium avium*, and *Mycobacterium intracellulare*. Whether one or the other predominated depended upon the locale; *M. kansasii* disease was mostly urban, with many cases in the central United States (5). *M. intracellulare* and *M. avium* disease was principally rural and centered in the southeastern United States (5). In some regions, the incidence of disease caused by nontuberculous mycobacteria was increasing (5).

Nontuberculous mycobacterial disease was not exclusively pulmonary. *Mycobacterium scrofulaceum* was found to be the causative agent of cervical lymphadenitis in children (547). *Mycobacterium marinum* infections were found principally in the skin and were associated with cuts or abrasions and exposure to aquaria or swimming pools or occupation in the fishing industry (547, 569). In these cases, transmission from water was the postulated route of infection (547, 569).

Nontuberculous Mycobacterial Disease after the AIDS Epidemic

The picture of nontuberculous mycobacterial disease has been radically changed by emergence of the AIDS epidemic throughout the world. Some 25 to 50% of patients with AIDS in the United States and Europe are infected with nontuberculous mycobacteria (232, 233, 235, 390, 452). The rise in the incidence of nontuberculous mycobacterial disease has accelerated rapidly since the first reports of nontuberculous mycobacterial disease in AIDS patients in 1982 (567).

In some parts of the world, infection by acid-fast bacilli in AIDS patients could be due to either *M. tuberculosis* or *M. avium* complex. Because of the need to institute appropriate public health and chemotherapeutic measures immediately, the causative agent must be identified. In addition to use of DNA probes for rapid identification, clinical and biological features can be used to distinguish between *M. tuberculosis* and

M. avium infection. *M. avium* infection is more often disseminated, with acid-fast organisms being isolated more commonly from either blood or stool, and patients are more likely to have digestive problems, anemia, and leukopenia and to be infected with cytomegalovirus than are patients infected with *M. tuberculosis* (54).

AIDS has also had a direct impact upon the picture of nontuberculous mycobacterial disease (37, 79, 81, 452, 564, 565). Before the AIDS epidemic, and still today in immunocompetent individuals, nontuberculous mycobacterial disease was primarily pulmonary. In contrast, in AIDS patients and immunodeficient individuals, nontuberculous mycobacterial disease is usually disseminated. Further differences between immunodeficient patients (e.g., those with AIDS) and immunocompetent patients are seen in mycobacterial skin infections. Infections of the skin or joints in immunocompetent individuals are usually associated with trauma (e.g., injury and surgery) or corticosteroid injections. In contrast, skin and joint infections in AIDS patients are not associated with trauma or corticosteroid use (439).

The impact of nontuberculous mycobacteria on morbidity and mortality of AIDS patients (81, 235, 374, 452) has stimulated the initiation of studies of the epidemiology, ecology, genetics, molecular biology, and physiology of nontuberculous mycobacteria. Furthermore, understanding the taxonomy and relationship between members of this diverse group of organisms has been important. In addition, the rise in nontuberculous mycobacterial disease has stimulated the development of rapid methods for recovery and identification of nontuberculous mycobacteria.

Current Trends in the Epidemiology of Nontuberculous Mycobacterial Disease

Nontuberculous mycobacterial disease is found in patients in the developed world and rarely in patients in the developing world. In the United States and Europe, where the incidence of tuberculosis is low, the incidence of nontuberculous infections is high in AIDS patients (232, 233, 235, 374, 390, 452). In addition to the fact that mycobacterial disease in AIDS patients is usually disseminated, there has been a change in the distribution of mycobacteria causing disease. Disease in AIDS patients is caused principally by *M. avium*. Therefore, the frequencies of disease caused by other mycobacterial species (e.g., *M. kansasii* and *M. intracellulare*), which were high in the era before the AIDS epidemic, are now low. That is not to say that the incidence of disease caused by other nontuberculous mycobacteria has fallen; it has not. Those infections have simply been outnumbered by the large number of AIDS-associated *M. avium* infections.

Because mycobacterial disease in immunocompromised or AIDS patients is principally disseminated, questions have been raised concerning the portal of entry of the mycobacteria. Before the advent of AIDS and in the absence of person-to-person spread of nontuberculous mycobacteria, pulmonary infection was thought to be due to an aerosol route of infection (547). The only exceptions were cervical lymphadenitis caused by *M. scrofulaceum* in children and cutaneous granulomas caused by *M. marinum*, in which exposure to water was the route of infection (547, 569). However in AIDS patients, infection could possibly occur via the lungs, gastrointestinal tract, or both. Thus, a wider range of sources and modes of transmission has had to be investigated in the AIDS era.

Nontuberculous mycobacterial disease (caused primarily by *M. avium* in AIDS patients) is found throughout the United States. In fact, the distribution of *M. avium* disease in AIDS

patients in the United States is different from the distribution of persons reacting to PPD-B, an antigen prepared from an *M. intracellulare* strain (135). *M. intracellulare* is a relative of *M. avium* and is included as a member of the *M. avium* complex. Those data showed that the incidence of PPD-B reactors was highest in the southeastern United States (135). Two studies have examined the geographic distribution of *M. avium* complex disease in the United States among AIDS patients. In a 1990 study, the frequency of *M. avium* disease among AIDS patients is lowest in the southeast (555). In a 1995 study of homosexual men with AIDS (229), the percentage of men with *M. avium* infection, as an initial AIDS illness, was higher in Baltimore (6.9%) and Los Angeles (5.6%) than in Chicago (2.6%) and Pittsburgh (0%). There are alternative explanations to account for those observations. First, exposure to environmental mycobacteria may protect against later acquisition of mycobacterial disease (136, 465). Second, the distribution of PPD-B reactors measured in 1958 to 1965 (135) may not represent the distribution of members of the *M. avium* complex today.

The possibility that the natural population of the *M. avium* complex and *M. scrofulaceum* is changing is supported by evidence that there has been a shift in the frequency of isolation of nontuberculous mycobacteria recovered from children suffering from cervical lymphadenitis (87, 549) and the serovars of *M. avium* complex organisms causing infections in animals (487). Up to 1979, *M. scrofulaceum* was the most frequent cause of cervical lymphadenitis in children (547). In contrast, recent reports have established that *M. avium* is now the most common causative agent of lymphadenitis in children in England (87) and in the United States (549). The shift in the United States occurred during the decade 1970 to 1979 (549). There has been a change in the serotypes of *M. avium* recovered from tuberculous lymph nodes in swine (487). Serovars 1 and 2 accounted for 74.5% of isolates recovered from tuberculous lesions in swine over the period 1973 to 1982; for the period 1983 to 1992, they accounted for only 52.7% (487). The percentage of serovars 4 and 8 among isolates from swine was 10.3% for the period 1973 to 1982 and 27.8% for the period 1983 to 1992 (487).

M. avium complex organisms have been repeatedly isolated from drinking water distribution systems in the United States and the rest of the world (98, 130, 131, 516, 517). These mycobacteria, rather than those in the environment (150, 291, 517), may be the major source of *M. avium* complex strains infecting AIDS patients.

In Africa and other parts of the developing world, where the incidence of tuberculosis is high, the incidence of nontuberculous mycobacterial disease in AIDS patients is low (366, 377). The absence of nontuberculous mycobacterial disease among AIDS patients in Africa is not due to an absence of the organisms in the African environment; they are present in large numbers (133, 517). Possibly, African AIDS patients die of other causes before they reach a stage at which nontuberculous mycobacterial disease develops.

Chemotherapy of Nontuberculous Mycobacterial Infections

Chemotherapy of nontuberculous mycobacterial infections has been problematic because of the relative resistance of nontuberculous mycobacteria to a wide range of antibiotics. Consequently, there has been a search for new and more effective antimycobacterial antibiotics and new targets for antimycobacterial therapy. Also, methods for rapid assessment of mycobacterial susceptibility to antibiotics have been developed (12, 252). Further, because most mycobacteria are intracellular

pathogens, the mammalian host cells (e.g., macrophages) serve as a barrier to the delivery of drug to the intracellular environment. Accordingly, novel methods for drug delivery, including incorporation of drugs in vesicles or liposomes, have been attempted.

There are a number of published guidelines for prophylaxis and treatment of nontuberculous mycobacterial infections, especially directed toward *M. avium*. These include American Thoracic Society Guidelines on the diagnosis and treatment of disease caused by nontuberculous mycobacteria (526) and the reports of the U.S. Public Health Service Task Force on Prophylaxis and Therapy for *M. avium* complex (339, 507). In addition, a number of publications have reviewed results from prophylaxis and treatment studies (38, 138, 178, 242). Because of that literature, the following discussion will focus on the physiological characteristics of nontuberculous mycobacteria that serve as barriers to effective chemotherapy. Two characteristics, high cell surface hydrophobicity and a permeability barrier, are thought to contribute to resistance to a wide range of antibiotics.

The relative hydrophobicity of nontuberculous mycobacteria is thought to restrict the activity of most hydrophilic drugs. That observation has spurred the synthesis and testing of hydrophobic derivatives of drugs with borderline antimycobacterial activity. Generally, hydrophobic derivatives have shown greater antimycobacterial activities (i.e., lower MICs) (412). An example is rifabutin, a hydrophobic derivative of rifampin which has been shown to be effective in prophylaxis and treatment of nontuberculous mycobacterial infections (178, 375).

It is believed that, with the possible exception of the rapidly growing mycobacteria, the major determinant of antibiotic resistance in nontuberculous mycobacteria is the presence of a cell wall-associated permeability barrier (364, 403, 405). Evidence for low permeability of cells of *Mycobacterium chelonae* to hydrophilic molecules (e.g., penicillins, glucose, glycerol, glycine, and leucine) has been presented (255). To overcome that barrier, antibiotic agents whose target is the cell wall (e.g., ethambutol) have been used in combination with others whose target is different (e.g., streptomycin, ciprofloxacin, or rifampin) (223, 224, 227, 406, 407). The need to identify other effective, synergistic combinations of antimycobacterial drugs has been underscored (247).

One of the major problems facing clinicians treating AIDS patients suffering from *M. avium* complex infections has been the lack of correlation between in vitro susceptibility and therapeutic effectiveness (243). This has led to studies comparing different methods of susceptibility testing (244) and demonstration that the growth stage of mycobacteria influences antibiotic susceptibility (558). However, methods of susceptibility testing may not be entirely at fault. Because mycobacteria are intracellular parasites, antibiotics must penetrate phagocytic cells to reach their target (408, 409, 554). Antibiotics such as azithromycin, which are concentrated in tissue and phagocytic cells, are expected to be effective against intracellular pathogens (221).

The lack of correlation between in vitro susceptibility and therapeutic efficacy may be because AIDS patients can be infected with more than one strain of a particular species or two or more representatives of different species. Thirty-eight percent of Australian AIDS patients were infected with more than a single *M. avium* serovar (119). Fifteen percent of a group of AIDS patients in Boston were infected with more than a single *M. avium* strain based on patterns of large restriction fragments analyzed by pulsed-field gel electrophoresis (PFGE) (13). In a more recent study of AIDS patients in New England, 33% were infected with more than one *M. avium*

complex isolate as judged by PFGE (463). Mixed infections involving two or more different nontuberculous mycobacterial species, while not as frequent, have been reported as well (52, 319, 497). In clinical practice, it is unusual for susceptibility testing to be performed on more than a single isolate from a patient or specimen. Because nontuberculous mycobacterial isolates of the same (163, 515) or different (547) species vary widely in antibiotic susceptibility, test results from a single strain cannot serve to guide the choice of therapy when more than a single strain or species is present.

Resistance to commonly used antimycobacterial drugs has been reported in the *M. avium* complex as well as other nontuberculous mycobacteria. Rather than broad-spectrum resistance, resistance in these cases has been to a single antibiotic, often the one used for therapy (i.e., acquired resistance). Resistance has emerged in *M. avium* complex strains during single-drug prophylaxis and treatment trials involving clarithromycin (109, 111–113, 209). That event has reinforced the view that single-drug therapy, especially for nontuberculous mycobacterial infections, should be avoided. Mechanisms of resistance to specific antibiotics have been identified; most involve changes in the target and probably are due to chromosomal mutations. Examples include quinolone (67), isoniazid (544, 570), streptomycin (524), and clarithromycin resistance (357). Recently, resistance to rifampin was shown to be due to ribosylation of the drug (107). There has been only one report of transposon-encoded antibiotic resistance involving sulfonamide resistance in *Mycobacterium fortuitum* (336). Plasmids were not shown to be associated with aminoglycoside resistance in *M. fortuitum* (238). Genetic determinants encoding tetracycline resistance have been detected in *M. fortuitum* (third biovariant) and *Mycobacterium peregrinum* (381). Isolation of an isoniazid-tolerant, isonicotinate-degrading *Mycobacterium* strain resembling *Mycobacterium aurum* (298, 299) suggests that isoniazid resistance could be a consequence of degradation of the antibiotic.

One final possible mechanism for antibiotic resistance in nontuberculous mycobacteria is adaptation. Adaptive resistance requires continual exposure to the drug; in its absence, resistance is lost. Because of the slow growth of nontuberculous mycobacteria, cells are capable of producing agents (e.g., proteins, pigments, and polysaccharides) which antagonize the activity of antibiotics. For example, exposure of cells of *M. intracellulare* to clofazimine results in induction of carotenoid pigmentation which might protect against the lethal action of that drug (530). It is expected that drugs able to prevent adaptation (e.g., mRNA or protein synthesis inhibitors) would act synergistically.

Detection, Recovery, and Identification of Nontuberculous Mycobacteria

The increased incidence and prevalence of nontuberculous mycobacterial infections have led to studies and development of methods for improved recovery, rapid detection, and rapid identification (438). Reports of mixed infections involving at least two different nontuberculous mycobacterial species (52, 319, 497) or two different isolates of the same species (463) emphasize the importance of careful examination of specimens.

A variety of techniques have been tested in hopes of improving recovery of mycobacteria from patient samples. Biphasic culture media, lysis concentration (e.g., isolator tubes), and CO₂ formation from [¹⁴C]palmitic acid (e.g., the BACTEC system) have been shown to be superior to conventional methods of direct culture on egg-containing media. Care must be

taken to avoid interculture contamination by mycobacteria when using the BACTEC system (92, 369, 511). Although some selective media have been developed (170), they are not useful for all mycobacteria. Published research has established the use of enzymes to improve the recovery of nontuberculous mycobacteria from soil and other natural samples (492), presumably by releasing cells from particulate matter; it is possible that enzyme treatment (e.g., polysaccharidases) of patient specimens (e.g., feces and sputum) will also improve recovery of nontuberculous mycobacteria from these sources.

In spite of those improvements, cultures of slowly growing mycobacteria are still subject to overgrowth and contamination. Thus, decontamination of environmental samples (e.g., water or soil) and some types of patient samples (e.g., sputum and stool) is required. The fact that decontamination reduces the number of viable, colony-forming mycobacteria (56, 450) has prevented an unambiguous identification of the portal of entry of nontuberculous mycobacteria in patients with AIDS. Determination of whether mycobacterial infection occurs first in the lungs or in the gastrointestinal tract is problematic because of the lack of sensitivity of detection as a result of the reduction of mycobacterial numbers by decontamination (74, 253). In addition, the numbers of nontuberculous mycobacteria in environmental samples may be underestimated because of stress placed on the organisms in the environmental (e.g., chlorine) or mycobacterial (e.g., autoclaving results in the formation of hydrogen peroxide) (28) media.

PCR methods have been developed for detection of mycobacteria (e.g., *M. tuberculosis*). These methods are in their infancy and offer a great deal of promise. In addition, they may provide evidence of mycobacteria in numbers greater than those measured by culture techniques. In a study of groundwaters, numbers of acid-fast CFU were significantly smaller than numbers calculated on the basis of acid-fast staining cells counted in a Petroff-Hauser counting chamber (337). Such a finding suggests that there are uncultivable mycobacterial cells and raises questions of their role in disease.

Because it is possible to collect large sample volumes from possible environmental sources of nontuberculous mycobacteria (e.g., tap water or potting soil), the sensitivity of detection is very high (e.g., one mycobacterium per liter of water). Unfortunately, the relevance of such high sensitivities to patient risk is unknown. For example, the presence of one mycobacterium in a liter of drinking water may be of no significance for infection in AIDS patients.

Ecology and Physiology of Nontuberculous Mycobacteria

At the outset, it is important to point out that the ecology and physiology of the nontuberculous mycobacteria are significantly different from those of *M. tuberculosis*. Many nontuberculous mycobacteria are free-living saprophytes that have been detected in and isolated from a wide variety of environments, including water, soil, dust, and aerosols. The exceptions to date include *Mycobacterium ulcerans*, *Mycobacterium haemophilum*, and *Mycobacterium genavense*. In addition to their presence in natural environments, nontuberculous mycobacteria have been recovered from drinking-water distribution systems throughout the world (86). Thus, the history of exposure involves interactions with natural and constructed sources, rather than infected patients (as has been the case for *M. tuberculosis* infection). Nontuberculous mycobacteria found in the environment or drinking-water distribution systems are not contaminants from another source; they are residents able to grow, persist, and survive. Members of the *M. avium* complex and *M. scrofulaceum* (172) and *M. fortuitum* and *M. chelonae* (70) grow

in water. The resistance of nontuberculous mycobacteria to disinfection (69, 70, 195) undoubtedly contributes to the ability of a number of nontuberculous mycobacteria (e.g., *Mycobacterium xenopi*, *M. avium*, *M. fortuitum*, and *M. chelonae*) to persist in drinking-water systems (60, 130, 131, 153, 230, 354, 462, 516, 517). Furthermore, the presence of nontuberculous mycobacteria in hospital water supply systems and their resistance to disinfectants has led to nosocomial infections involving bronchoscopes contaminated with *M. intracellulare* (120) or *M. chelonae* (189, 344, 471, 483).

Because of the widespread presence of nontuberculous mycobacteria in natural and constructed environments, studies of their physiology are relevant. Studies of the physiology of nontuberculous mycobacteria have provided an understanding of the geographic distribution of mycobacterial species and identify those factors influencing their numbers. My colleague, Bruce Parker, coined the term "physiologic ecology" to describe that aspect of mycobacterial studies. Nontuberculous mycobacteria grow over wide ranges of temperature, pH, salinity, and oxygen tension. Thus, nontuberculous mycobacteria can be isolated from a wide variety of environmental samples. Numbers of total mycobacteria in Finnish brook waters correlated positively with the presence of peatlands, precipitation, chemical oxygen demand, water color, and concentrations of Fe, Al, Cu, Co, and Cr (240). A specific example of the relationship between mycobacterial physiology and ecology is provided by the *M. avium* complex. They grow best between pH 5 and 5.5 (170, 400) and microaerobically, approximately 2 to 5 mm below the surface of semisolid mycobacterial media (144a). Furthermore, *M. avium* complex strains grow equally well in waters with and without salt (i.e., up to 2%) (172). Consequently, *M. avium* complex organisms can be found in large numbers in brackish swamps and estuaries of the southeastern coastal United States (150, 291).

One additional characteristic may also contribute to the widespread presence of nontuberculous mycobacteria in the environment. Nontuberculous mycobacteria are relatively resistant to heavy metals and oxyanions (73, 149, 475). In fact, some isolates of *M. avium*, *M. intracellulare*, or *M. scrofulaceum* were abnormally resistant to cadmium, mercury, silver, and tellurite (149). The fact that five of seven mercury-resistant isolates were isolated from water samples collected in regions of reported heavy metal pollution (149) demonstrates that mycobacterial physiology is a determinant of their ecology and geographic distribution. Furthermore, metal metabolism and metal requirements directly influence *M. avium* complex populations (e.g., *M. avium* numbers in natural waters directly correlate with zinc levels [291]). Possibly, the persistence of *M. avium* complex organisms in hospital water systems (98, 131, 516) and drinking-water distribution systems (130, 517) is because many systems use galvanized (i.e., zinc-coated) pipes.

Nontuberculous mycobacteria, primarily rapidly growing mycobacteria, have been shown to be responsible for degradation of a wide variety of novel compounds, including acetone (61, 62), alkanes (368), benzene (61, 62), chlorinated phenolics (197), dioxane (61, 62), ethene (200), fluoranthene (48), humic and fulvic acids (291), morpholine (59), nitrotyrene (212), paraffin (378), pentachlorophenol (506), phenanthrene (48, 190), polycyclic aromatic hydrocarbons (529), polyhalogenated phenolics (196, 376), propane (84), pyrene (212), pyridines (including isonicotinate) (298, 299), styrene (61, 62), trichloroethylene (520), trinitrotoluene (518), uric acid (148), and vinyl chloride (199). In addition, nontuberculous mycobacteria are capable of metabolizing and transforming sterols and are used in industrial processes for sterol production (231, 249, 451, 519). The fact that nontuberculous mycobacteria can de-

grade compounds not readily used as carbon and nitrogen sources by other microorganisms may contribute to their ability to inhabit certain environments in spite of their relative slow growth. Mycobacteria in large numbers have been found associated with sphagnum vegetation (277). Also, the high levels of humic and fulvic acids found in coastal swamps correlated with large *M. avium* complex numbers (291).

Physiological traits (e.g., hydrophobicity) directly influence transmission (e.g., aerosolization) and hence exposure of susceptible individuals. Hydrophobic mycobacteria are easily collected from aerosols (10, 542) and more readily aerosolized by natural processes (145, 171, 383, 542). Hydrophobicity also influences the distribution of nontuberculous mycobacteria in waters. Hydrophobic mycobacteria collect at air-water interfaces and thus are found in large numbers at water surfaces (542). Hydrophobicity is probably also responsible for the formation of biofilms (449), which, in turn, would contribute to the persistence of nontuberculous mycobacteria in water delivery systems.

Virulence of Nontuberculous Mycobacteria

Nontuberculous mycobacteria are opportunistic pathogens. They are capable of causing lymphadenitis and infections of the lungs, skin, soft tissue, bursa, joints, tendon sheath, and bones (37, 38, 526, 547). Infection with *M. kansasii*, *M. haemophilum*, or *M. chelonae* is characterized by subcutaneous nodules or abscesses (282, 526, 546). In most cases of pulmonary infection in immunocompetent individuals, there is some predisposing condition (e.g., chronic lung disease) (547). In immunocompetent individuals, nontuberculous mycobacterial infections of the skin, soft tissue, bursa, joints, tendon sheath, and bones are often associated with trauma or surgical wounds (156, 439, 526). Disseminated cutaneous infections caused by *M. chelonae* are associated with prior corticosteroid therapy (523). In patients with AIDS or those with profound immune deficiency, infection is often disseminated. Symptoms in patients with AIDS often include fever, weight loss, diarrhea, and anemia (37, 38). Cutaneous or soft tissue infections in patients with AIDS are not associated with trauma, unlike the situation in immunocompetent patients (156, 439, 523). The number of nontuberculous mycobacterial cells that are required to provoke disease is unknown for most species, even when coupled to some predisposing condition.

There has been progress in identifying virulence factors of nontuberculous mycobacteria. Many studies focusing on virulence factors have been performed with *M. avium* complex organisms. Potential virulence factors include colonial type (446), prevention of acidification of phagocytic vesicles (478), prevention of phagosome-lysosome fusion (160), production of an electron-transparent zone around cells (404), resistance to inhibitory serum constituents (105), delay in tumor necrosis factor secretion by infected host cells (164), uptake by intestinal epithelial cells (330), production of receptors for macrophage binding (402), and the ability of mycobacteria to replicate in macrophages (106, 164). *M. ulcerans* has been shown to produce a cytotoxic activity (410). The presence of phenolic glycolipids and their ability to scavenge oxygen radicals (72) or inhibit human lymphoproliferative responses (157) have also been suggested as virulence factors of mycobacteria. Transformants of *Mycobacterium smegmatis* overexpressing a gene for cyclopropane mycolic acid synthase (*cmal*) are hydrogen peroxide resistant, suggesting a role for this modification in environmental survival and virulence (566). *M. tuberculosis* H37R_a DNA fragments endowing *Escherichia coli* transformants with the ability to enter and survive inside HeLa cells have also been

cloned (15). Selection for rapid growth of *M. tuberculosis* H37R_a transformants harboring strain H37R_v DNA fragments led to cloning of a gene (*ivg*) responsible for rapid growth of *M. tuberculosis* in vivo (384).

There has been long-term interest in the role of catalase activity and virulence of mycobacteria since the observation that isoniazid-resistant isolates of *M. tuberculosis* from southern India lacked catalase activity and were of low virulence compared with isoniazid-susceptible isolates (250). Also, strains of *M. kansasii* with high catalase activity were more virulent than strains with low catalase activity (474). Recently, loss of catalase activity by a strain of *Mycobacterium bovis* through mutation in the *katG* gene was shown to reduce virulence (544). In contrast, mutation in the *inhA* gene, which, like *katG* mutations, confers isoniazid resistance, did not reduce virulence (544). Introduction of a wild-type *katG* gene restored isoniazid susceptibility to *M. tuberculosis* isolates resistant to the drug (570). However, it appears that the mechanism by which pathogenic mycobacteria resist oxygen stress is unique, on the basis of the observation that *M. tuberculosis* and *M. avium* lack an intact regulator gene for the oxygen stress response (*oxyR*) whereas it is intact in the saprophyte *M. smegmatis* (455).

A number of newly developed methods will greatly assist the identification of virulence genes of nontuberculous mycobacteria. They include methods for mRNA isolation (30, 286, 385) and labeling with 5-fluorouracil (2). Techniques for the isolation and characterization of mRNA (394) and proteins (8) in mycobacterial cells within phagocytic cells will lead to identification of genes induced upon phagocytosis. Another technique that offers promise for identification of virulence genes is use of subtractive hybridization between virulent (e.g., *M. tuberculosis* H37R_v) and avirulent (e.g., *M. tuberculosis* H37R_a) strains (285).

Reports that plasmids were more common in *M. avium* complex isolates recovered from AIDS patients than in isolates from immunocompetent individuals or the environment (101, 266) suggested that plasmids encoded virulence genes. Unfortunately, studies to test that hypothesis were flawed by comparisons between nonisogenic, unrelated strains (i.e., *M. avium* with *M. intracellulare*) (165, 411). Furthermore, attempts to cure plasmids from strains of the *M. avium* complex have failed (103, 146).

In addition, growth conditions have been shown to influence virulence of nontuberculous mycobacteria. Growth of *M. marinum* in cell cultures was observed at 33°C but not at 37°C (401) as would be expected of an organism that predominantly causes cutaneous infections. Growth of *M. intracellulare* at 45°C (443) and growth of *M. fortuitum* in the presence of propane (380) increase virulence.

Taxonomy of Nontuberculous Mycobacteria

A variety of approaches have been used to characterize the systematics and taxonomy of the mycobacteria (320, 533). Results from cultural and biochemical tests have been analyzed by numerical taxonomic techniques to identify clusters of related mycobacteria (533, 535, 536). Those studies have been extended and confirmed by studies of semantides, namely, cellular macromolecules such as protein, RNA, and DNA. Serological analysis of catalases of nontuberculous mycobacteria has provided data for the development of phylogenetic trees illustrating the relationships between mycobacterial species (534). Particularly valuable information has been gained from the study of mycobacterial rRNA. 16S rRNA gene sequence analysis shows that there appear to be two groups of mycobacteria:

the slowly growing and rapidly growing mycobacteria (473). Knowledge that there are species-specific rRNA gene sequences has led to the development of nucleic acid probes for the identification and detection of nontuberculous mycobacteria (44, 294) and the phylogeny and definition of mycobacterial species (420). In spite of the progress in both the understanding of the relatedness of different mycobacterial species and the development and application of molecular tools to studies of the taxonomy of the genus *Mycobacterium*, there is still a great deal to be learned. For example, 16S rRNA gene sequence studies of representatives of the *M. intracellulare* species identified three different sequences (288). Phenotypic testing and numerical taxonomic techniques have identified a third cluster of mycobacterial isolates, related to but distinct from *M. avium* and *M. intracellulare* (537). By using these examples, it was pointed out that phenotypic characteristics and 16S rRNA gene sequence data must be obtained for the same strains used in those two studies (288). Possibly, one of the *M. avium* complex-related clusters identified by phenotypic and numerical analysis includes one of the three 16S rRNA sequence groups of *M. intracellulare*.

Another approach used for taxonomic studies that has resulted in development of diagnostic tools is the isolation and characterization of unique DNA sequences. For example, identification of a transposable genetic element unique to *Mycobacterium paratuberculosis* (185) has led to the development of a DNA probe-based detection test to distinguish this organism from other members of the *M. avium* complex with which *M. paratuberculosis* shares 100% sequence similarity (241).

Genetics of Nontuberculous Mycobacteria

Some progress has been made in describing the genetic basis of variation in the nontuberculous mycobacteria. Most of the information to date has been focused on members of the *M. avium* complex and rapidly growing mycobacteria. Information concerning the genome, the presence of extrachromosomal DNA, the presence of transposable genetic elements (e.g., transposons), and the interaction between mycobacteriophage and host cells is available. An excellent summary of the available information is presented in a review (350).

Early studies on the mycobacterial genome resulted in estimates of the size of the genome and relationships between mycobacterial species based on DNA-DNA hybridization data (18–21, 241). That work suggested that mycobacteria have a rather large genome (21). Extensions of that work established that *M. avium*, *M. intracellulare*, and *M. scrofulaceum* are distinct species (18, 19) and that *M. avium*, *M. paratuberculosis*, and “wood pigeon mycobacteria” are members of the same species (442). Current efforts to sequence the genomes of *M. tuberculosis* and *Mycobacterium leprae* (228) should provide data of direct relevance to understanding the genome of nontuberculous mycobacteria.

Unlike many other species of bacteria, which have multiple rRNA genes (e.g., *E. coli* with seven and *Streptomyces lividans* with six [480]), the mycobacteria have a small number of rRNA genes. Slowly growing species, i.e., *M. tuberculosis*, *M. leprae*, *M. avium*, *M. paratuberculosis*, *M. intracellulare*, *M. simiae*, and *M. marinum*, were shown to have only a single copy of the 16S, 23S, and 5S rRNA genes (39, 260, 480). In contrast, most rapidly growing species had two copies (39, 127), except *M. chelonae* and *M. abscessus*, which have one (127). Because of the relationship between the number of ribosomes in cells and the rate of protein synthesis, it is possible that the small number of rRNA genes limits the rate of protein synthesis and hence the rate of growth in the slowly growing mycobacteria.

A number of mycobacterial genes for catabolism and biosynthesis have been cloned. Oligonucleotide probes were used to clone and sequence the inducible amidase of *M. smegmatis* (324). The genes involved in carotene pigment synthesis in *M. aurum* have been cloned in *M. smegmatis* (236), as have the genes encoding the serotype 2 glycopeptidolipid antigen of *M. avium* (34). Genes involved in the synthesis of lysine (e.g., the diaminopimelic acid decarboxylase gene, *lysA* [11]) and aromatic amino acids (e.g., the 5-enolpyruvylshikimate-e-phosphate synthase gene, *aroA* [167]) have been cloned by complementation in *E. coli* auxotrophs. Those successes suggest that mycobacterial promoters are recognized in *E. coli*. This has been confirmed by demonstration that DNA sequences from *M. bovis* BCG (460) and *M. paratuberculosis* (489) can serve as promoters in *E. coli*. However, that finding does not prove that the promoters are used as such in mycobacteria. In fact, it was shown that only one of the two transcriptional initiation sites in the rRNA operon of *M. bovis* BCG was recognized by *E. coli* (480).

The organization of genes in the chromosome of mycobacteria has some similarities to and differences from what is known in other bacteria. First, although the rRNA genes are linked in an operon (39, 160, 161, 260, 480), not all genes that are found in operons in other bacteria are linked in operons in mycobacteria. Identification of an *M. smegmatis* DNA fragment able to complement a histidine auxotroph of the same strain (i.e., the *hisD* gene) showed that the *hisD* and *hisC* genes were linked (219). However, unlike *E. coli*, the other genes involved in histidine biosynthesis (e.g., *hisG*) were not linked, suggesting that the genes were not part of a single operon and may be regulated in a manner different from that found in *E. coli* (219).

The key protein of recombination and DNA repair, the *recA* gene product, has been cloned by complementation of RecA^- mutants of *E. coli* (372) or by hybridization with a *recA* gene probe to genomic fragments (117). The *M. tuberculosis recA* gene was able to confer ethyl methanesulfonate resistance upon RecA^- strains of *E. coli*, did not increase UV light (254 nm) resistance, and increased recombination proficiency (117, 372). An unexpected finding was that the coding capacity of the clone was substantially larger than predicted from the size of the mycobacterial *recA* gene product (117). Although the mycobacterial *recA* gene product was approximately the same size as and reacted with antibody against the *E. coli recA* gene product (117, 372), the mycobacterial *recA* gene shared nucleotide sequence homology with the *E. coli recA* gene only at its 5' and 3' ends (117). Further work demonstrated that the product of the *M. tuberculosis recA* gene was an 85-kDa precursor protein that was spliced to yield the mature 38-kDa *RecA* protein (116). The presence of that *recA* "protein intron" was limited to members of the *M. tuberculosis* complex; it was absent in the *recA* genes of nontuberculous mycobacteria (118).

Extrachromosomal genetic elements, namely, plasmids, have been discovered in members of the *M. avium* (including *M. scrofulaceum*) (146, 360) and *M. fortuitum* (304) complexes. Unfortunately, only a few species have been examined in a systematic manner. Plasmids are widespread in members of the *M. avium*, *M. intracellulare*, and *M. scrofulaceum* group (360). Plasmids in members of the *M. avium* complex and *M. scrofulaceum* are large, and strains often have as many as three to six plasmids (360). In some strains, almost 30% of the total DNA of the strains is plasmid DNA (360). Plasmids have been shown to encode mercury (359) and copper (144) resistance and restriction and modification (102) in members of the *M. avium* complex and *M. scrofulaceum*. Plasmids were more common in

M. avium complex isolates recovered from AIDS patients than in those from non-AIDS patients and the environment (101, 266). Although it has been suggested that plasmids encode virulence functions in *M. avium* complex strains (101, 165, 411), there have been no studies in which isogenic plasmid-carrying and plasmid-free strains were compared. In addition, there has been no demonstration of a relationship between plasmid presence and antibiotic resistance (159), but those studies were flawed by the absence of isogenic plasmid-carrying and plasmid-free strains. Mycobacterial plasmids in the slowly growing nontuberculous mycobacteria are quite stable (103, 146). Because identification of plasmid-encoded functions requires either isolation of plasmid-free segregants, plasmid transfer, or complete sequencing of plasmids, identification of plasmid-encoded genes has been slowed. The ability of isolates of *M. chelonae* to degrade morpholine appears to be plasmid encoded (532). Cryptic plasmids have been found in members of the *M. fortuitum* complex (304). One of those *M. fortuitum* plasmids was used to construct a useful mycobacterial cloning vector (305), and methods for transformation of plasmid DNA into mycobacterial cells have been developed (251).

A number of transposable genetic elements have been found in nontuberculous mycobacteria (345). Sulfonamide resistance in a strain of *M. fortuitum* is due to the presence of a transposable genetic element (336). Tetracycline resistance in isolates of *M. peregrinum* and the third biovariant of *M. fortuitum* appears to be due to the presence of *tetK* and *tetL* genes, which are normally associated with transposable genetic elements in other bacteria (381). *M. paratuberculosis* strains carry a unique transposable element, IS900, and a probe based on that element has been developed for identification of that pathogen (185). Discovery that the presence of IS901 in strains of the *M. avium* complex was associated with increased virulence in mice (302) has led to its use in epidemiological studies (301). Mycobacterial transposons have been successfully used as tools in genetic analysis. Transposon mutagenesis has been successfully employed by using IS611 in *M. smegmatis* (192), and IS900 was used to promote the integration of a foreign gene into the genome of *M. smegmatis* (143).

Although there are a number of mycobacteriophages whose hosts include nontuberculous mycobacteria (100, 495), only a few have been studied in detail. In addition, few associations have been found between the presence of temperate phage in lysogenic mycobacterial strains and phenotypic characteristics (264, 329, 495). In particular, changes in colonial morphology, nitrate reductase activity, and Tween 80 hydrolysis were associated with lysogeny in *M. smegmatis* (264). However, mycobacteriophages have been suggested to be valuable tools for studies of mycobacteria (201, 202). The origin of replication of mycobacteriophage D29 has been cloned and could be used for construction of mycobacterial cloning vectors because of its wide host range (312). The temperate phage L5, whose entire genome has been sequenced (203), may serve as a stable cloning vector for the expression of antigens in vaccine strains of *M. bovis* BCG (313). Furthermore, promoters of mycobacteriophage L5 may be used for expression of cloned genes in mycobacteria (201, 202). Finally, L5 clones containing luciferase genes have been developed for rapid assessment of antibiotic susceptibility in strains of *M. tuberculosis* (252).

Tools for Epidemiologic Investigations of Nontuberculous Mycobacteria

In addition to the impetus for rapid detection and identification, the dramatic rise in nontuberculous mycobacterial disease has stimulated the development and study of markers for

epidemiologic typing. The ideal marker would (i) be stable, (ii) be measurable in every isolate (typeability), and (iii) have sufficient alternative forms to permit high levels of discrimination between strains. Also, the measurement techniques should be simple, inexpensive, and rapid.

Mycobacterial typing methods of long standing include serotyping (498, 555) and phage typing (100). Unfortunately, those techniques fail to type a sufficient frequency of strains (i.e., not all isolates react with antisera or autoagglutinate or react with phage). Serotyping lacks utility for epidemiologic studies of nontuberculous mycobacterial disease in AIDS patients, because most patients are infected with a rather narrow range of serotypes (555). Biotyping is limited by the number of characteristics (e.g., pigment, catalase, and urease activity in *M. avium*) (205, 396). All three typing methods are limited in utility, because measurement relies upon gene expression, which can be influenced by cultural conditions. Multilocus enzyme electrophoresis typing has proven useful in typing *M. avium* isolates (531, 556) and isolates of *M. fortuitum*, *M. chelonae*, and *M. abscessus* associated with infections following cardiac surgery (525). Although the technique offers a high level of discrimination because of the number of different types, it is technically difficult and the phenotype can be influenced by environmental (e.g., medium composition) conditions.

Newer typing methods based on analysis of DNA (and thus not subject to variation in phenotypic expression) have been developed for epidemiologic studies of nontuberculous mycobacteria. They include plasmid typing (527, 562); restriction fragment length polymorphism (RFLP) analysis of chromosomal DNA (198), of rRNA genes (76), or of plasmid DNA (266); rRNA spacer sequencing (161, 162); and large-restriction-fragment (LRF) analysis involving PFGE (13, 321, 343). These methods examine a stable molecule that is present in all isolates (i.e., the typeability is 100%). Although the methods are all conceptually simple, they require knowledge of sophisticated molecular biological techniques and instruments. Unfortunately, to date, there has been no study comparing the results of different DNA typing methods when the same collection of nontuberculous mycobacterial strains was used. However, as demonstrated below, these methods have been successfully used for typing mycobacterial isolates.

EPIDEMIOLOGY OF *MYCOBACTERIUM KANSASII*

Epidemiology of *M. kansasii* Infection

The AIDS epidemic has had a striking effect on the frequency of disease caused by individual nontuberculous mycobacterial species, especially the *M. avium* complex, but not necessarily *M. kansasii*. In certain parts of the world before the appearance of the AIDS epidemic, *M. kansasii* infections were more common than *M. avium* complex infections. In southeastern England over the period from 1977 to 1984, the number of cases of mycobacterial disease caused by *M. kansasii* was larger than the number caused by *M. avium* complex but still smaller than the number of *M. xenopi* cases (560). In Wales, there were 143 pulmonary *M. kansasii* infections compared with 41 *M. avium* complex pulmonary infections over the period from 1952 to 1978 (256). In fact, over the period from 1953 through 1970, there was an increase in the incidence of *M. kansasii* infections in Wales (387). In Texas, over the period from 1967 to 1976, there was an increase in the number of cases of *M. kansasii* infection and there were approximately twice the number of *M. kansasii* cases as *M. avium* complex cases (5). The number of *M. kansasii* isolates submitted to the Centers for Disease Control and Prevention is quite variable (174, 176),

and certain areas, including Louisiana, have large numbers (546). In New Orleans, the number of cases of *M. kansasii* infection among human immunodeficiency virus (HIV)-infected individuals doubled from July 1984–January 1988 to July 1988–June 1991 (546).

Predominance of *M. kansasii* infections among nontuberculous mycobacterial infections in immunocompetent individuals is not the case worldwide. In Japan, the majority of nontuberculous mycobacterial lung infections were caused by the *M. avium* complex over the period from 1971 to 1979 (503) and 1971 to 1984 (501). The number of pulmonary infections caused by *M. kansasii* also rose during those periods (501). *M. kansasii* infections are rare in Australia (123). In British Columbia, the number of *M. avium* complex infections far exceeds the number due to *M. kansasii* (246). Among isolates submitted to the Centers for Disease Control and Prevention for identification in 1979, 18.4% were *M. avium* complex and 3.3% were *M. kansasii* (174). In a southern California hospital between 1971 and 1981, 12 patients with *M. kansasii* pulmonary disease and 15 with either *M. avium* or *M. intracellulare* were identified (179). *M. avium* complex cases predominated among cases in Virginia over the period from 1970 to 1979 (284). In Virginia, the prevalence of *M. kansasii* infections was almost four times higher in the first half of the period 1970 to 1979 than in the second half (284), suggesting a change in the epidemiology of these two mycobacterial species.

By contrast, over the period from 1984 to 1992, there has been a 10-fold increase in the number of *M. avium* complex infections compared with *M. kansasii* infections in England (561). The recent shift to a predominance of *M. avium* over *M. kansasii* infections is not always the case. In Zurich, Switzerland, there was no change in the prevalence of *M. kansasii* infections over the period from 1983 to 1988 (124). In New Orleans, the increase in *M. kansasii*-HIV coinfection has paralleled the increase in AIDS cases (546).

As has been the case with disease caused by other nontuberculous mycobacteria, *M. kansasii* disease in immunocompetent patients is primarily pulmonary (79, 124, 179). In only 1 of 42 immunocompetent New Orleans patients with *M. kansasii* disease was the infection disseminated (546). In addition, there are often predisposing lung conditions. Among 154 patients with pulmonary *M. kansasii* disease in Great Britain, 33 had pneumoconiosis and another 31 were coal miners or steelworkers or worked in dusty conditions (256). In a study of 12 patients with *M. kansasii* pulmonary infections in southern California, 7 had preexisting pulmonary disease and 3 reported exposures to dusts (179). *M. kansasii* has been isolated from sputum samples collected from a patient with cystic fibrosis (217). In addition to pulmonary disease, cervical lymphadenitis caused by *M. kansasii* has been found in children (87, 179).

M. kansasii pulmonary disease in immunodeficient patients (e.g., those with AIDS) can be disseminated or exclusively pulmonary (546). In a study of 49 HIV-infected individuals with *M. kansasii* coinfection, 32 (65%) had pulmonary infections only and 17 (35%) had disseminated infections (546). Thirteen of the *M. kansasii*-infected HIV-positive patients were also infected with *M. avium* complex and six patients had disseminated infections with both *M. kansasii* and *M. avium* complex (546). In another study of 19 HIV-infected patients with a median CD4⁺ count of 49 cells per mm³, 14 (74%) had pulmonary disease only and the remainder had disseminated infections (316). Disseminated *M. kansasii* disease has been found in other groups of AIDS patients (398, 454). Cutaneous *M. kansasii* infections have been found in both immunosuppressed and immunocompetent patients (53).

Characteristics of *M. kansasii*

M. kansasii is a slowly growing photochromogen that grows over a range from 32 to 42°C; there is no growth at 45°C (258). Isolates produce both catalase and nitrate reductase and hydrolyze Tween 80 (258). These characteristics form the basis for the identification of *M. kansasii*. *M. kansasii* isolates with strong catalase activity are more virulent (474). Like other nontuberculous mycobacterial species, individual isolates demonstrate colonial variation (258). The genetic basis for that variation is unknown.

There appears to be some genetic diversity among *M. kansasii* isolates recovered throughout the world (426). Almost all isolates of *M. kansasii* from Australia, Japan, and South Africa reacted with either of two *M. kansasii*-specific DNA probes (237); isolates from Belgium and Switzerland less frequently reacted with either probe (426). Sequences of 16S rRNA were different for the probe-negative isolates, but all were similar to the 16S rRNA sequence of *M. kansasii* (426). A probe prepared from a highly repeated DNA element from *M. tuberculosis* (427) demonstrated that there were five different RFLP patterns among isolates reacting with an *M. kansasii*-specific probe (426). In addition to an RFLP type seen in European and Australian isolates, there was a unique South African RFLP type (426, 427). Distinct RFLP types were also seen among the isolates that did not react with the *M. kansasii*-specific probe (426, 427).

Sources of *M. kansasii*

There have been a number of reports documenting the presence of *M. kansasii* in water samples. It has seldom been recovered from soil. *M. kansasii* isolates of the same phage type as those isolated from patients in the coal-mining provinces of Limburg and Rotterdam have been recovered from drinking-water distribution systems in the Netherlands (141, 142). Other investigators have reported the isolation of *M. kansasii* from tap water and shower heads (22, 273, 328, 354). *M. kansasii*-like mycobacteria were recovered from water and soil samples collected in Cardiff, Wales (387). *M. kansasii* was shown to be capable of surviving in water for up to 12 months (265) but incapable of long-term survival in soil (265, 479). Those reports prompted the hypothesis that infection by *M. kansasii* occurs via an aerosol route (85).

The reports of successful isolation of *M. kansasii* stand in contrast to other studies in which *M. kansasii* was not found in the environment. For example, no *M. kansasii* isolates were recovered from a variety of water samples collected in and around Cleveland, Ohio (180), and from soils collected in the environs of Houston, Texas (263), or Cleveland, Ohio (550). In addition, *M. kansasii* was not recovered from water samples collected in New Hampshire, Boston, Massachusetts, Finland, Kenya, and Zaire (517) and soil samples collected in Uganda (133). Because *M. kansasii* disease is found in urban as opposed to rural areas (5, 547), failure to recover *M. kansasii* from rural New England, Finland, and Africa is expected. A long-term study of *M. kansasii* in tap water demonstrated that isolation was intermittent, which suggests why some investigators have failed to recover *M. kansasii* from that source (86).

There have been a few reports of isolation of *M. kansasii* from other types of samples. *M. kansasii* has been isolated from the tissue of 1 of 193 feral pigs in Australia (96).

Risk Factors for *M. kansasii* Infection

Risk factors for *M. kansasii* infection include preexisting pulmonary disease, cancer, and alcoholism (261, 546). A report

of recovery of *M. kansasii* from a patient with cystic fibrosis (217) suggests that this inherited disease may also be a risk factor for *M. kansasii* infection. Preexisting pulmonary diseases include pneumoconiosis (256), chronic obstructive pulmonary disease (179), and impaired ventilatory function (6). In a 1988 review of nontuberculous pulmonary infections in patients admitted to a Toronto hospital, 8 (9%) of 89 patients had infections that were caused by *M. kansasii*, and of those 8 patients, 5 had underlying lung disease, 3 had chronic liver disease, 2 smoked, and 2 reported alcohol abuse (91). No common risk factors could be identified among the remaining patients. Persons in occupations in which dust is generated, such as coal mining, steelmaking, and glass manufacturing, are also at risk for *M. kansasii* infection (23, 179, 379). In British Columbia, *M. kansasii* isolates came from a portion of the province where smelting was a major industry (246). Based on the finding that the rate of increase of *M. kansasii* disease in New Orleans has paralleled the rise in AIDS in Louisiana (546), geography appears to be a risk factor for *M. kansasii* disease.

In a demographic study of pulmonary disease caused by *M. kansasii* in Texas, it was reported that cases were significantly more likely to come from urban than rural areas and that patients were unlikely to have Spanish surnames (5). In Japan, most *M. kansasii* lung infections were found in Tokyo (501, 503). Prior nocardiosis was associated with *M. kansasii* infection in cardiac allograft recipients (458).

It is logical that immunodeficiency is a risk factor for *M. kansasii* infection. Immunodeficiency and the attendant low CD4 cell count appear to predispose patients to dissemination of infection (53, 316, 546). In a retrospective study of 49 *M. kansasii*-infected human immunodeficiency virus (HIV)-positive patients in New Orleans, 35 (71%) were cigarette smokers and only 1 had preexisting pulmonary disease (546). Risk factors for cutaneous *M. kansasii* infection include systemic illness, immunosuppression, skin pathology, or exposure to *M. kansasii*-contaminated water (53). However, there has not been a significant rise in the number of *M. kansasii* infections since the start of the AIDS epidemic in southeastern England (561), underscoring the possible role of geography in *M. kansasii* epidemiology.

Chemotherapy of *M. kansasii* Infections

In most studies of treatment of *M. kansasii* infections, rifampin has been included along with other antimycobacterial drugs (4, 179, 217, 391, 526). Combinations that have been used include rifampin, streptomycin, isoniazid, and ethambutol (4); rifampin, ethambutol, and amikacin (217); and rifampin, isoniazid, and ethambutol (391). The American Thoracic Society-recommended combination is rifampin, isoniazid, and ethambutol with or without streptomycin, given for 18 months (526). Some isolates of *M. kansasii* are resistant to low concentrations of streptomycin or isoniazid, and most are resistant to high concentrations of pyrazinamide (526) and *p*-aminosalicylic acid (4). Among *M. kansasii* isolates recovered from HIV-infected patients in New Orleans, 40 of 43 were susceptible to 1 µg of rifampin per ml, 29 of 39 were susceptible to 2 µg of streptomycin per ml, 12 of 37 were susceptible to 0.2 µg of isoniazid per ml, and 26 of 38 were susceptible to 0.5 µg of ethambutol per ml (546). The New Orleans patients were treated with a triple-drug regimen of isoniazid, rifampin, and either ethambutol or pyrazinamide (546). Drug combinations including rifampin were more effective in clearing infection within 4 months than were combinations lacking rifampin (4, 391).

EPIDEMIOLOGY OF *MYCOBACTERIUM MARINUM*

Epidemiology of *M. marinum* Infection

Historically, the frequency of *M. marinum* infections has been quite sporadic, because of the irregular exposure of persons to *M. marinum*-contaminated waters (86, 489, 569). Large numbers of *M. marinum* infections in any calendar year have usually been associated with an outbreak in a swimming pool (86). Infections or outbreaks are also associated with occupational hazards, such as exposure of skin abrasions to *M. marinum* (e.g., during fishing) (569). Over the last 10 years, there have been fewer reports of sporadic outbreaks, and consequently there has been a small but steady number of reports describing *M. marinum* infection. Furthermore, there has been no change in the prevalence and frequency of *M. marinum* infections in the developed world since the advent of the AIDS epidemic. That may reflect a more narrow range of *M. marinum* habitats and predisposing conditions for *M. marinum* infection of humans.

M. marinum infections in immunocompetent humans appear to be restricted principally to the extremities, with few systemic infections. In Nottingham, England, 4 of 21 cases of cervical lymphadenitis were caused by *M. marinum* (87). Restriction of *M. marinum* infection to the extremities or cervical lymph nodes is consistent with the fact that *M. marinum* fails to grow above 30°C in some laboratory culture media (73, 80). Furthermore, *M. marinum* fails to grow in cell cultures at 37°C (401). However, that is not necessarily a sufficient restriction, because *M. haemophilum* also fails to grow above 30°C in laboratory media yet causes disseminated infections in AIDS patients (282). The restriction of *M. marinum* infections to the extremities may be different in immunodeficient patients (e.g., AIDS patients) or immunosuppressed patients. There have been several recent reports of disseminated *M. marinum* infections in such patients (306, 484). Cutaneous *M. marinum* infection has been described in an HIV-infected individual who kept a large freshwater aquarium that he cleaned regularly (418).

M. marinum Infection in Fish

In contrast to an unchanged annual incidence of *M. marinum* infection in humans, there appears to be an increase in the frequency of *M. marinum* infection in cultured or hatchery-confined fish. Over a 6-year period, *M. marinum* infection was detected at significant levels in hatchery-confined chinook salmon in Australia (16). Cultured striped bass were reported to be infected with *M. marinum* over several seasons (208). As expected, *M. marinum* infection has been reported in freshwater ornamental fish, salmon, sturgeon, and bass (307). It is likely that the high population densities and the presence of high levels of excreted carbon and nitrogen compounds in the recirculated water in hatcheries contribute to the growth of *M. marinum* (16, 208). Transmission of *M. marinum* between fish and between fish and amphibians through cocultivation or exposure to *M. marinum*-contaminated water has been reported (80).

Characteristics of *M. marinum*

M. marinum is a photochromogenic mycobacterium whose growth is somewhat intermediate between those of the truly slowly and rapidly growing species (258). *M. marinum* growth does not occur above 32°C upon initial isolation, which distinguishes that species from *M. kansasii* (258). However, *M. marinum* strains maintained on laboratory media will grow at

37°C, although less luxuriantly than at 28°C (80, 499). *M. marinum* lacks catalase and nitrate reductase activities, and some but not all isolates hydrolyze Tween 80 (258). *M. marinum* colonial variants have been observed.

Sources of *M. marinum*

The source of *M. marinum* infection in humans is water containing the mycobacteria. Evidence linking infection with waterborne *M. marinum* has come from studies of *M. marinum* infection in persons exposed via aquaria (3, 27, 418) or of persons employed in the fisheries industry (569).

Even though water has been implicated as the source of *M. marinum* infection, it may simply be the vector of fish-borne *M. marinum*. It has been proposed that *M. marinum* infection in hatchery-confined chinook salmon was transmitted through eggs and not through feed, because trout that were given the same food were free of *M. marinum* infection (16). In the same study, it was proposed that infection was not maintained by fish feeding on dead fish (16). In another study, however, when the practice of feeding fish carcasses to hatchery-raised fish was decreased and pasteurized feed was used, *M. marinum* infection was reduced (382). Unfortunately, because two possible sources of *M. marinum* were changed in that study, assignment of either one as the risk factor (or both) is impossible.

Risk Factors for *M. marinum* Infection

The two major risk factors for *M. marinum* infection in nonimmunocompromised patients are exposure to *M. marinum*-infested waters and the presence of superficial cuts or abrasions. Consequently, fishermen are at risk for infection, especially if they perform tasks during which the hands can be cut (569). By extension, a clear risk factor for HIV-infected persons or patients with AIDS would be similar exposure. It would be best if persons in those categories avoided both freshwater and saltwater aquaria.

The advent of widespread high-density, intensive fish farming is a new risk factor for *M. marinum* infection. Contact with the recirculated water or with *M. marinum*-infested fish could lead to infection through superficial cuts.

Chemotherapy of *M. marinum* Infection

Suggested chemotherapeutic regimens for treating *M. marinum* infections include minocycline or doxycycline, trimethoprim-sulfamethoxazole, or rifampin plus ethambutol (526). Another suggested regimen includes rifampin, ethambutol, and amikacin (217). *M. marinum* isolates are resistant to isoniazid and pyrazinamide and have an intermediate susceptibility to streptomycin (526).

EPIDEMIOLOGY OF THE *MYCOBACTERIUM AVIUM* COMPLEX

Case History of an *M. avium* Complex Infection in an AIDS Patient

A 30-year-old homosexual man presented with a 2-month history of dysphagia and dyspnea in June 1981 (567). A chest roentgenogram demonstrated the presence of apical infiltrates. Bronchial washings contained *Pneumocystis carinii*, and esophageal biopsy specimens yielded *Candida albicans*. In addition, several colonies of *M. avium* complex were isolated from sputum cultures. The patient was treated with intravenous sulfamethoxazole, trimethoprim, and amphotericin B. Upon release from the hospital, he was treated with continuing sulfamethox-

azole, trimethoprim, and ketoconazole. In September 1981, he was again seen, complaining of fever. A chest roentgenogram was normal, but endoscopy revealed the presence of erosive esophagitis. The patient's condition deteriorated, with progressive encephalopathy. The day before the patient died in November 1981, cultures of cerebrospinal fluid yielded *M. avium* complex. At autopsy, *M. avium* complex was isolated from cultures of lung and blood samples.

Epidemiology of *M. avium* Complex Infection

Infections caused by members of the *M. avium* complex (i.e., *M. avium*, *M. intracellulare*, and *Mycobacterium* species X) in immunocompetent patients are principally pulmonary. Pulmonary *M. avium* complex infection has been reported throughout the world, including the United States (5, 284, 547), Japan (501, 503), and England and Wales (445). In Texas, the number of *M. avium* complex pulmonary infections rose over the period from 1967 to 1979 (5). Cases in the United States and Great Britain have historically appeared in white males 45 to 65 years old with predisposing lung conditions (425, 445, 547). Those conditions include pneumoconiosis and silicosis, due to chronic and long-term exposure to dusts as a result of occupations (e.g., coal mining and farming) (445, 547). In one study of 45 patients with *M. avium* complex pulmonary disease, 33 (73%) patients had preexisting pulmonary disease, 17 smoked (38%), and 15 (33%) reported alcohol abuse (91). The prognosis in *M. avium* complex pulmonary infections was strongly influenced by the associated disease (425).

Members of the *M. avium* complex have also been reported to be responsible for soft tissue infections in immunocompetent patients. Six cases of granulomatous tenosynovitis of the hand or wrist caused by *M. avium* complex have been reported (213). In five of the six cases, infections followed local surgery, trauma, or corticosteroid injection (213).

In addition to the historical patient profile of *M. avium* complex infection, two new categories of immunocompetent patients have been identified. Women with structural changes in the chest leading to reduced pulmonary function have been shown to be at risk for *M. avium* complex pulmonary infection and disease (248). Members of the *M. avium* complex have been isolated from patients with cystic fibrosis (7, 283, 287). *M. avium* complex isolates were recovered from 13 of 87 (283) and 7 of 64 (7) cystic fibrosis patients. It is not known whether the presence of *M. avium* complex contributes in any manner to the morbidity or mortality of cystic fibrosis patients.

A recent review of cases of mycobacterial lymphadenitis among children in Nottingham, England, for the period from 1979 to 1990 has demonstrated that most of the infections were caused by members of the *M. avium* complex (87). Twenty-one cases were identified; nine of these were caused by *M. avium* complex, and only one was caused by *M. scrofulaceum* (87). In southeastern England, the number of cases of lymphadenitis caused by members of the *M. avium* complex has risen over the period from 1973 to 1993 (184). *M. avium* complex is also the predominant cause of cervical lymphadenitis in children in the United States (549). Although previous studies had shown that *M. scrofulaceum* was responsible for most of the infections (547), a recent review of cervical lymphadenitis in children established that there was a rather abrupt change from *M. scrofulaceum* to *M. avium* complex in the 1970s (549). These data suggest that the numbers of *M. scrofulaceum* may be falling and the numbers of *M. avium* complex may be rising in the environment.

The number of cases of pulmonary *M. avium* complex disease in immunocompetent patients has been overwhelmed by

the high frequency (e.g., 25 to 50%) of *M. avium* complex infections in AIDS patients in the United States (204, 235, 373, 564, 565), Australia (119), Brazil (26), Germany (390), and southeastern England (184, 560, 561). In addition, *M. avium* complex infection is prevalent among HIV-infected children in the United States (432). These infections are disseminated, rather than restricted to the lungs as in immunocompetent patients (37, 38, 202, 235, 374, 564, 565). A major portion of the increase in *M. avium* complex disease in the United States must be due to infection in AIDS patients and not to an increase in the prevalence of *M. avium*. In a study in San Francisco, no increase in *M. avium* complex infection in immunocompetent patients had been detected (373). However, the prevalence of *M. avium* complex may be rising in other regions of the United States. AIDS patients with *M. avium* complex infection survive for a shorter time (median, 7.4 months) than do AIDS patients without this infection (median, 13.3 months) (235).

The most common manifestation of *M. avium* complex in patients with AIDS is mycobacteremia (429). In a study of 55 AIDS patients, 47 (85%) had mycobacteremia, with *M. avium* complex recovered from blood (365). In another study, pulmonary *M. avium* disease was rare (i.e., 2.5%) among AIDS patients with disseminated *M. avium* disease (267). None of the *M. avium* complex-infected AIDS patients had preexisting pulmonary disease (267). Endobronchial (355) and intra-abdominal (424) *M. avium* complex infections and a case of erosive arthritis (126) have been reported in AIDS patients. Anemia (85% of patients [38]), anemia with impaired erythropoiesis (168), fever (38), night sweats (38), and diarrhea (29, 38) have been associated with disseminated *M. avium* complex infections in AIDS patients.

Studies of African AIDS patients have failed to demonstrate disseminated *M. avium* complex infection (e.g., mycobacteremia) (366, 377). The absence of *M. avium* complex infections in African AIDS patients is not due to inherent resistance, because *M. avium* complex isolates were recovered from 11 of 60 African AIDS patients who were being treated in Belgium (398). Furthermore, *M. avium* complex organisms were recovered from stools of 7 of 60 HIV-positive patients (12%) and 9 of 41 HIV-negative patients (22%) in Kinshasa, Zaire (83). Mycobacteria were isolated significantly less often from patients with diarrhea (6 of 40 [15%]) than from those without diarrhea (27 of 61 [44%]) (83). Finally, the absence of disseminated *M. avium* infections in African AIDS patients is not due to the absence of *M. avium* in the African environment. *M. avium* complex isolates have been recovered from water (395, 517) and soil (395) samples collected in Zaire, water samples collected in Kenya (517), and water and soil samples collected in Uganda (133). *M. avium* complex isolates recovered from Ugandan soils and waters were of the same serotypes and had the same groups of plasmids as *M. avium* complex isolates recovered from AIDS patients in United States and Europe (133).

Infection by *M. avium* complex in AIDS patients tends to occur late in HIV disease and is most closely related to the CD4⁺ cell count and not to age, gender, or race (232, 374). Infection is rare or absent in AIDS patients whose CD4⁺ cell counts are greater than 200 but becomes increasingly common in AIDS patients as their CD4⁺ cell count falls below 100 or less (232, 374). The highest frequency of disseminated *M. avium* complex disease is in patients with CD4⁺ cell counts below 10 (232, 374).

The frequency of *M. avium* and *M. intracellulare* isolates is different in AIDS and non-AIDS patients. Approximately 90% of *M. avium* complex isolates from AIDS patients were iden-

tified by DNA probe as *M. avium*, whereas approximately 40% of *M. avium* complex isolates from non-AIDS patients (i.e., pulmonary) were *M. intracellulare* by DNA probe (129, 193). Pigmented *M. avium* complex isolates are frequently isolated from AIDS patients (281) and these strains segregate unpigmented variants (476). The pigmentation of *M. avium* complex isolates recovered from lymph nodes of children was not reported (87). In the United States, *M. avium* complex serotypes 4 (40%), 8 (17%), and 1 (9%) predominate among AIDS patients (555) whereas serotypes 8 (13%), 16 (10%), 4 (7%), 9 (7%), and 19 (7%) predominate among immunocompetent patients (498). In Sweden (225, 226) and Germany (428), other serotypes predominate in AIDS patients. Because of the predominance of a limited number of serotypes, serotyping has limited utility for epidemiologic studies of *M. avium* complex infection in AIDS patients.

Polyclonal *M. avium* Complex Infection in AIDS Patients

Infection of AIDS patients with more than a single nontuberculous mycobacterial species or clone of *M. avium* complex appears common. Mixed *M. avium*-*M. intracellulare* (93) and mixed *M. avium*-*M. simiae* (319) infections in AIDS patients have been reported. In Australia, 17 of 45 AIDS patients (38%) were infected with *M. avium* complex strains of different serovars (119). This is probably an underestimate of polyclonal infection, because different DNA fingerprint patterns have been seen in isolates of the same serotype (537). In a group of patients examined in the United States, 2 of 13 AIDS patients were infected with two strains on the basis of LRF profiles resolved by PFGE (13). In another study involving LRF comparisons by PFGE, 4 of 12 patients (33%) were infected with two different *M. avium* complex strains (463). The reported studies were performed with a small number of patients and isolates; the actual percentage of polyclonal infection is probably higher. In a study of 18 AIDS patients from whom 3 individual blood isolates were recovered, 9 (50%) had isolates with different plasmid profiles (132a). Proof of polyclonal infection is important, because it provides one possible explanation for the inability to correlate the outcome of antibiotic treatment with susceptibility patterns. In fact, differences in antimicrobial susceptibility patterns have been documented among different strains isolated from the same patient (515).

Characteristics of the *M. avium* Complex

Members of the *M. avium* complex are slowly growing, non-pigmented, acid-fast bacilli that are unable to hydrolyze Tween 80 (258). Although cultural and biochemical tests can be used to distinguish *M. avium* from *M. intracellulare* (242, 320, 441, 491, 496), there are isolates whose characteristics are intermediate between those of *M. avium* and *M. intracellulare* (205, 396, 536). Mycobacteria, including members of the *M. avium* complex, can be identified by high-pressure liquid chromatography (HPLC) analysis of mycolic acids (66, 173) or by analysis of cell wall fatty acids by gas-liquid chromatography (464). A third member (species?) of this group, called *Mycobacterium* species X, has been distinguished on the basis of hybridization to rRNA gene probes, but these isolates have not been well characterized (104). *M. avium* complex strains can be distinguished from *M. simiae* on the basis of the photochromogenicity of *M. simiae*, from *M. xenopi* on the basis of the formation by *M. xenopi* of stick-like projections in colonies and "birds-nest" microscopic appearance, from *M. bovis* because it fails to grow at 25°C, and from *M. malmoense* because of its ability to hydrolyze Tween 80 (183).

Today, DNA probes based on rRNA gene sequences (Gen-Probe, San Diego, Calif.) can be used to identify members of the *M. avium* complex and the serotypes reacting with either the *M. avium* or *M. intracellulare* probes can be identified (435, 494, 537). The *M. avium* probe also reacts with *M. paratuberculosis* (493). A DNA probe based on the insertion sequence IS900 can be used to distinguish between those two species, since this sequence is present in *M. paratuberculosis* and absent in *M. avium* (185).

There are a number of methods for typing *M. avium* complex isolates that are useful for epidemiological studies. Serotyping was the first method developed (444). A large number of serotypes based on peptidoglycolipids or Schaefer antigens have been identified (498) and have proven useful in demonstrating polyclonal infection in patients with AIDS (119) and identifying common serotypes infecting AIDS and non-AIDS patients in the United States (234, 556), Sweden (225, 226), and Germany (428).

Three insertion sequences offer promise for use in epidemiologic studies of the *M. avium* complex. IS1110 (216), IS1245 (191), and IS1311, which shares 85% DNA similarity with IS1245 (422), have all been shown to yield enough different types that they can be used in epidemiologic investigations.

Recently, other discriminatory epidemiologic markers, including multilocus enzyme electrophoresis (525, 531, 556) and analysis of LRF patterns of genomic DNA separated by PFGE (13, 343), have been developed. LRF analysis of *M. avium* complex isolates has established the wide genetic variability of members of the complex, including members of the same serotype (13, 343), polyclonal infection in AIDS patients (13, 463), persistence of a single LRF type in a water delivery system (516), and the identity of *M. avium* isolates from patients and water samples to which they were exposed (516). Using a DNA probe consisting of IS900 and linked chromosomal DNA, it was shown that European and U.S. *M. avium* isolates share an RFLP pattern whereas isolates from Africa share a different pattern (398). LRF patterns have also been used as a quality control tool in a clinical mycobacteriology laboratory and as a tool for distinguishing true outbreaks of *M. avium* complex infection from pseudo-outbreaks due to laboratory-associated contamination (63).

Physiologic Ecology of the *M. avium* Complex

The frequency of isolation and the number of organisms of the *M. avium* complex correlate with certain physiochemical characteristics of their natural habitat (i.e., physiologic ecology). *M. avium* complex organisms are recovered in larger numbers from waters and soils with low pH, low dissolved-oxygen content, and high organic matter (i.e., humic and fulvic acid) content (57, 291). *M. avium* complex organisms are found in large numbers in acidic, brown water coastal swamps of the southeastern United States, where all those conditions are met (291). In addition, *M. avium* complex numbers correlate directly with zinc concentration (291). *M. avium* complex organisms grow in natural fresh and brackish (i.e., up to 2% salinity) waters (172). In laboratory media, *M. avium* complex organisms have a pH optimum for growth of 5 to 5.5 (170, 400). *M. avium* strains grow at 45°C, unlike *M. intracellulare* strains, which can grow to only 42°C (258, 430, 499). From these characteristics, coupled with the relative chlorine resistance of mycobacteria (70, 195), it is not surprising that *M. avium* complex organisms are found in municipal drinking-water systems and that *M. avium* is concentrated by hospital hot-water distribution systems (131, 516, 517). The persistence of *M. avium* complex in water systems (516) may be due, in part, to the

correlation between *M. avium* numbers and zinc; many hot and cold water distribution system pipes are galvanized (namely, zinc coated).

Sources of the *M. avium* Complex

In the absence of evidence of person-to-person spread of *M. avium* complex, an environmental source of *M. avium* complex was proposed (547), and *M. avium* complex isolates have been recovered from almost every corner of the world and from a great variety of samples. *M. avium* complex isolates have been recovered from water samples collected from ponds in New Zealand (274) and Germany (33), sphagnum bogs in Germany (277), swamps in the southeastern United States (291), rivers in Japan (239), public baths in Japan (437), the Mediterranean (512), rivers and wastewater systems in France (513), and rivers and lakes in Finland and Kenya (517), Zaire (395, 517), and Uganda (133). A variety of natural waters, including fresh, brackish, and ocean, in the United States have yielded *M. avium* complex (150, 180, 517). Regional differences in the prevalence of *M. avium* complex in the environment (150) may be balanced by the fact that *M. avium* complex organisms are present in public drinking-water systems (130, 309, 517).

M. avium complex isolates have been recovered from soils collected in Wales (387), Australia (414, 415), Brazil (97), Uganda (133), Texas (263), Cleveland (550), and the southeastern United States (57). In a study of the local environment of HIV-infected individuals in San Francisco, *M. avium* complex organisms were rarely recovered from tap water or food samples but were recovered from 55% of samples of potted soils (552). Tap water samples in Berlin also failed to yield *M. avium* complex (1.7% of samples), although other nontuberculous mycobacteria were recovered frequently (42% of samples) (389). In addition, *M. avium* complex organisms have been recovered from aerosol samples collected in Virginia (542) and house dusts collected in Australia (416) and Japan (502).

In addition to waters and soils, *M. avium* complex organisms have been isolated from bronchoscopes (120), raw milk (132), and cigarettes (134).

At least one source, water, has been identified as a source of *M. avium* complex organisms infecting some AIDS patients. Comparison of LRFs separated by PFGE of *M. avium* complex isolates from AIDS patients and from waters to which they were exposed showed identical patterns (516). Three AIDS patients who lived in separate locations, each with its own water supply, were infected with *M. avium* complex strains with the same LRF patterns. An *M. avium* complex strain with the same pattern was recovered from the hot water system of a single hospital where each patient showered and drank water (516). Two patients whose only common site of exposure was a hospital were infected with *M. avium* complex strains with an identical LRF pattern, which was the same as that of an *M. avium* complex strain recovered from the hospital water system (516). Finally, one patient with AIDS was infected with an *M. avium* complex strain whose LRF pattern was identical to that of an isolate recovered from the Charles River (516). This is a powerful technique, because patterns of unrelated *M. avium* complex isolates are highly polymorphic and stable (13, 343).

Route of *M. avium* Complex Infection

Pulmonary *M. avium* complex infection in non-AIDS patients most probably occurs via aerosols (150, 542, 547). Members of the *M. avium* complex are readily aerosolized from water and can be recovered from droplets that, if sufficiently small (i.e., $\leq 5 \mu\text{m}$), can enter the alveoli of the human lung

(10, 147, 383). Cervical lymphadenitis in children most probably results from the ingestion of *M. avium* complex organisms in natural waters, swimming pools, or drinking-water systems (87, 547, 549). To date however, there have been no reports (no attempts?) of fingerprint comparisons of environmental *M. avium* complex strains with isolates from patients with pulmonary infections or cervical lymphadenitis.

It is not known whether the entry point for *M. avium* complex in AIDS patients is the lungs or the gastrointestinal tract. Evidence of pulmonary or gastrointestinal *M. avium* complex infection is predictive of dissemination to blood (74, 253). Sixty percent of patients developed *M. avium* complex bacteremia approximately 7 months following the appearance of the organism in stool specimens and 9 months following its appearance in respiratory specimens (74). Isolates were not fingerprinted in those studies, preventing the tracing of blood isolates to stool or sputum. Decontamination procedures for stool and sputum specimens result in the loss of viable cells (56). Consequently, the inability to detect small numbers of *M. avium* complex organisms in cultures of those specimens makes it difficult to determine whether the portal of entry in AIDS patients is the lungs or gastrointestinal tract. It is also possible that aerosol transmission of *M. avium* complex between AIDS patients occurs because *M. avium* complex cells are readily aerosolized (171, 383). Furthermore, a fecal-oral route of infection may also occur. The availability of typing methods could be used to determine whether that is possible.

Risk Factors for *M. avium* Complex Infection

Risk factors for pulmonary *M. avium* complex infection in immunocompetent individuals include cigarette smoking with the associated chronic obstructive pulmonary disease, pneumoconiosis, bronchiectasis, prior tuberculosis or other mycobacterial disease, alcoholism, and pulmonary alveolar proteinosis. Employment in dust-generating occupations, such as coal mining and farming, is also a risk factor (547). In a study of 45 patients with *M. avium* complex disease, 33 (73%) had underlying lung disease (27 had chronic obstructive pulmonary disease), 17 (38%) smoked heavily, and 15 (33%) reported alcohol abuse (91). In a study of 19 patients with pulmonary alveolar proteinosis, *M. avium* was recovered from bronchoalveolar lavage samples in 8 (57%) of 14 smokers and was not isolated from the 5 nonsmokers (545). That evidence, coupled with the fact that *M. avium* complex strains can be recovered from cigarettes (133), suggests that cigarette smoking may be a source for the *M. avium* complex.

Risk factors for soft tissue infections in immunocompetent individuals are local traumas caused by surgery, injury, or injection (213).

Profound immunodeficiency, such as seen in late-stage AIDS patients, is clearly a risk factor for disseminated *M. avium* complex infection. Geography is evidently a risk factor for disseminated *M. avium* complex disease in AIDS patients. Homosexual men with AIDS were more likely to have disseminated *M. avium* disease as an initial AIDS-related illness if they resided in Baltimore (6.9%) and Los Angeles (5.6%) than if they lived in Chicago (2.6%) or Pittsburgh (0%) (229). Prior infection with cytomegalovirus was also highly correlated with disseminated *M. avium* complex infection in homosexual men with AIDS (229). Immunodeficiency as a consequence of immunosuppression as employed for heart and kidney transplantation would also be considered a risk factor.

Chemotherapy and Prophylaxis of *M. avium* Complex Infection

Because of the lack of success of antibiotic therapy of *M. avium* complex infections in the pre-AIDS era (547) and knowledge that members of the *M. avium* complex were resistant to a wide variety of antibiotics, including antimycobacterial antibiotics, a wide variety of treatment regimens have been tested for their efficacy in clearing *M. avium* complex infections (38, 138, 242, 279, 339, 507, 526, 564, 565). In addition, a number of newer drugs, alone or in combination, have been used in prophylaxis trials (178, 339, 375, 507), some of which are under way.

It is likely that one reason for the lack of uniform success in chemotherapy of *M. avium* complex infections is the wide diversity in antibiotic susceptibility of *M. avium* complex isolates (163, 234, 515). That, coupled with the possibility that a substantial number of AIDS patients are infected with more than a single *M. avium* complex isolate (13, 119, 463), has undoubtedly contributed to the lack of success in treating *M. avium* complex infections (515).

A variety of antibiotics, alone or in combination, are effective in reversing symptoms in infected patients and eradicating or reducing *M. avium* complex numbers (38, 138, 217, 242, 275). A large number of different antibiotics and combinations have been used in trials. In one commentary, the results from eight different regimens were reviewed (138); in a second, three regimens were evaluated (292); and in a third, six different drug combination regimens and four single-drug regimens were reviewed (38). In addition, trials of liposome-encapsulated drugs have been performed (38). The reports were uniform in demonstrating that combinations including macrolides (e.g., clarithromycin or azithromycin), rifabutin, or both were effective in clearing *M. avium* complex infection and abolishing disease symptoms (38, 111–113, 138, 292).

Certain antibiotic combinations have synergistic activities against *M. avium* complex isolates in vitro (223, 224, 227, 280, 406, 407, 408, 553) and in macrophages (407, 409, 554). A postantibiotic effect of antimycobacterial drugs against *M. avium* complex isolates has also been reported (40). Those studies should guide the choice of drug combinations for use in patients.

Prophylaxis of *M. avium* complex infection in AIDS patients has also been judged. The antibiotic rifabutin has been shown to be effective in preventing or delaying the onset of *M. avium* complex infection in AIDS patients and is the only drug with Food and Drug Administration approval for this use (178, 375). In one of the studies, the risk of *M. avium* disease in AIDS patients was halved (375).

Unfortunately, in both single-drug (209) and combination (223) regimens, drug-resistant isolates have been recovered. When examined, relapse of symptoms coincided with the emergence of an *M. avium* complex isolate resistant to one of the drugs used for treatment (111–113, 217). A recent study involving a technique for DNA fingerprinting has demonstrated that in three of four patients in whom clarithromycin or azithromycin resistance emerged during therapy, the resistant isolate was a mutant of the original, macrolide-susceptible *M. avium* isolate (259a). In the fourth patient, the clarithromycin-resistant isolate was unrelated to the original, clarithromycin-susceptible isolate (259a). Either it was an undetected drug-resistant member of the original *M. avium* population (515), or it was a drug-resistant isolate that infected the patient during therapy. Emergence of drug-resistant mutants during therapy is probable since *M. avium*-infected AIDS patients harbor large numbers of organisms (209).

EPIDEMIOLOGY OF *MYCOBACTERIUM PARATUBERCULOSIS*

Epidemiology of *M. paratuberculosis* Infection

M. paratuberculosis is the causative agent of Johne's disease in cattle. Johne's disease is a slow, progressive infection of the intestine in cattle. *M. paratuberculosis* infection can result in diarrhea and wasting of the infected cattle. Approximately, 10% of dairy cattle and 33% of dairy herds are infected in the state of Wisconsin (468). The principal route of infection has been from mother to calf. Recently, it was reported that 12% of healthy but infected cattle had *M. paratuberculosis* in milk and almost 9% of fetuses were infected before birth (468). That data suggested that *M. paratuberculosis* infection in cattle may not be limited to the intestinal tract but may be disseminated.

In addition to well-documented evidence of *M. paratuberculosis* as the causative agent of Johne's disease in cattle, there has been evidence linking mycobacteria, including *M. paratuberculosis*, with Crohn's disease in humans. Crohn's disease is a chronic, inflammatory disease of the gastrointestinal tract. Histopathological studies demonstrating the presence of a granulomatous reaction in infected tissue suggested that the disease could possibly be of mycobacterial origin. There are two relevant pieces of evidence.

Epidemiologic studies of the incidence and geographic distribution of Crohn's disease have suggested that it is caused by an infectious organism. A study of two French families demonstrated clustering of Crohn's disease. The disease appeared among siblings in 7- to 23-month periods, and recurrences appeared every 4 to 8 years (510). Crohn's disease cases in Cardiff, Wales, were shown to be clustered in areas near the River Faff (341), and clustering of Crohn's disease cases has been observed in a Cotswold village (9). The presence of those clusters has led Hermon-Taylor (214, 215) to propose that clustering is due to the transmission of *M. paratuberculosis* via water from fields where infected cattle have defecated.

Chiodini et al. (75, 77, 78) isolated an acid-fast organism that resembled *M. paratuberculosis* from tissue of a patient with Crohn's disease. The initial isolate was shown to be identical to *M. paratuberculosis* on the basis of DNA-DNA hybridization (563), although it should be remembered that *M. avium* (which was not included in that study) and *M. paratuberculosis* share 100% DNA-DNA hybridization values (491). Since the initial reports, other investigators have independently reported isolation of *M. paratuberculosis*-like organisms from tissue of patients with Crohn's disease (182, 215, 353). Isolation of *M. kansasii* from tissue of patients with Crohn's disease after long-term culture has also been reported (64). It should be pointed out that culture of such tissue rarely yields *M. paratuberculosis* (182, 331, 563), mycobacteria, or any other microorganisms (510).

DNA hybridization studies have implicated both *M. paratuberculosis* and the wood pigeon mycobacterium in Crohn's disease. Two acid-fast bacilli were isolated from Crohn's disease tissue. One of the isolates was stably mycobactin dependent and, when an IS900-derived probe was used, gave a restriction fragment polymorphism banding pattern identical to that of *M. paratuberculosis*, confirming the data of others (351, 353). The other isolate did not retain the mycobactin dependence upon subculture and yielded an RFLP banding pattern identical to that of the wood pigeon mycobacteria (353). In addition, by using the same RFLP technique, a wood pigeon isolate was identified from a patient with ulcerative colitis and *M. avium* was identified from a patient with carcinoma of the colon (353).

Results of a survey to detect the presence of *M. paratuberculosis* DNA in tissue samples from patients with Crohn's disease or ulcerative colitis and those without inflammatory bowel disease have been reported (440). Detection of *M. paratuberculosis* was performed by PCR amplification of the *M. paratuberculosis*-specific insertion sequence IS900, with DNA isolated from the tissue samples (440). DNA sequences able to be amplified by the IS900-specific primers and yielding a fragment of the expected size were detected in tissue from 26 of 40 Crohn's disease patients (65%), from 1 of 23 patients with ulcerative colitis (4%), and from 5 of the 40 control patients (13%) (440). It was reported that the PCR primers used did not amplify the related insertion sequence, IS901, found in the wood pigeon mycobacterium strains (440). Again, note that not all tissue from Crohn's disease patients contained IS900.

Characteristics of *M. paratuberculosis*

M. paratuberculosis is a member of the *M. avium* species as judged by DNA-DNA hybridization (491). Features that distinguish *M. paratuberculosis* from other members of the *M. avium* complex are its stable mycobactin dependence (491) and the presence of multiple copies of a unique insertion element, IS900 (185, 351, 352). The mycobactin dependence is stable upon prolonged serial transfers of cultures and is thus distinguishable from the mycobactin dependence of other members of the *M. avium* complex (i.e., the wood pigeon strains of *M. avium*), which is seen only in primary culture and is lost on serial transfer (491). These strains contain an IS900-related insertion sequence, IS901. With IS900 as a probe, three RFLP types have been identified in animal isolates of *M. paratuberculosis*. Bovine and ovine strains shared the same RFLP types (types A and B), whereas a single caprine isolate had a unique RFLP pattern (type C) (388). Uniformity of RFLP types was observed within one herd over a 2-year period, and different types appeared to predominate in different regions of the Czech Republic and Slovakia (388).

Sources of *M. paratuberculosis*

Transmission of *M. paratuberculosis* in cattle has been thought to be principally from infected to uninfected cattle and from infected mothers to their young. Thus, culling infected animals from herds has been successful in reducing the incidence of disease, as has isolation of newborns from their infected mothers (468). Although the major risk factor for Johne's disease in cattle is the presence of an infected cow in a herd, it is likely that transmission occurs through exposure to *M. paratuberculosis*-contaminated materials (e.g., feces, milk, grass, or feed).

One route for infection of humans, leading to Crohn's disease, has been proposed. Hermon-Taylor et al. (214, 215) proposed that evidence of clustering of cases along a river course suggested that *M. paratuberculosis* organisms shed by infected cattle were entering the watershed and the river. Thus, human infection would occur by drinking or being exposed to the contaminated water. Evidence for the presence of *M. paratuberculosis* in milk (214) suggests that inadequate pasteurization could lead to infection.

Risk Factors for *M. paratuberculosis* Infection

Evidence of geographic clustering of Crohn's disease cases suggests that an additional risk factor for Crohn's disease would be location (9, 341, 510). If it ultimately proves the case that *M. paratuberculosis* and wood pigeon strains of *M. avium* are the causative agents of Crohn's disease, contact with milk,

water, or soil contaminated with either organism may lead to infection.

EPIDEMIOLOGY OF MYCOBACTERIUM SCROFULACEUM

Case History of an *M. scrofulaceum* Infection

A 35-year-old male arc welder had repeated attacks of pleuritic pain, and a chest roentgenogram demonstrated the presence of a thin-walled cavity in the right upper lobe of the lung (548). *M. scrofulaceum* was recovered from one of a number of sputum cultures. It was serotype 42. Over the following months, 12 sputum cultures were free of mycobacteria, but 1 year later, five cultures were positive for *M. scrofulaceum* serotype 42. At that time, the patient was feeling well. Two years after the cavity had been first seen, a repeat roentgenogram demonstrated that the cavity had enlarged and the wall had thickened. The right upper lobe was resected, and culture of the contents of the cavity yielded *M. scrofulaceum* serotype 42.

Epidemiology of *M. scrofulaceum* Infection

Historically, *M. scrofulaceum* infection has been associated with cervical lymphadenitis in children (179, 547). Disease is limited to the cervical or mandibular lymph nodes. Surgical resection of the infected node, rather than antibiotic treatment, is the preferred treatment (179, 547). In recent years, the frequency of cervical lymphadenitis caused by *M. scrofulaceum* has declined among the total cases of cervical lymphadenitis, and there are now more cases of disease caused by *M. avium* complex than *M. scrofulaceum* (87, 549). A subcutaneous *M. scrofulaceum* infection of the thigh has also been reported (179).

There have been a variety of reports of pulmonary *M. scrofulaceum* infection in adults (248, 548). *M. scrofulaceum* disease in adults has been associated with arc welding (548). The predisposing conditions can include pneumoconiosis, preexisting *M. tuberculosis* lesions, chronic bronchitis, emphysema, bronchiectasis, and lung malignancies (548). Disseminated *M. scrofulaceum* infection has also been reported in patients with AIDS (235) and in patients with and without immune deficiency (439). Chronic ulcerative and nodular skin lesions and lung disease in an AIDS patient have also been reported (439).

In addition to disease in humans, *M. scrofulaceum* infection has been detected in a laboratory mouse colony in which transmission was suspected to be due to oral ingestion of fecal material (504). *M. scrofulaceum* has also been detected in plant tissue culture (482). The mycobacteria may have infected the plant cell tissue culture via water; alternatively, the plants were already infected with *M. scrofulaceum*. The latter suggests that *M. scrofulaceum* may be a member of the normal plant flora.

Characteristics of *M. scrofulaceum*

M. scrofulaceum is a slowly growing, Tween 80-nonhydrolyzing relative of the *M. avium* complex. *M. scrofulaceum* has many genetic, ecologic, biochemical, and cultural characteristics in common with *M. avium* and *M. intracellulare*. In fact, mycobacterial isolates with cultural and biochemical characteristics intermediate between those of *M. avium* and *M. scrofulaceum* have been described (205, 396). Furthermore, *M. scrofulaceum* strains that carry plasmids present in *M. avium* and *M. intracellulare* strains have been found (266, 360).

In the past, there has been reference to the *M. avium*, *M. intracellulare*, and *M. scrofulaceum* complex. Although *M. scrofulaceum* has plasmids in common with members of the *M.*

avium complex (266, 360), *M. scrofulaceum* is a distinct species (19, 447). Furthermore, because *M. scrofulaceum* is rarely found in patients with AIDS, unlike members of the *M. avium* complex, it is best to treat *M. scrofulaceum* as a distinct entity (447). Some *M. scrofulaceum* isolates belong to established *M. avium* complex serotypes, while others belong to serotypes that are distinct from the *M. avium* complex serotypes (447, 537) and can be distinguished from members of the *M. avium* complex on the basis of the former's carotenoid pigmentation and presence of urease and catalase activities (258). *M. scrofulaceum* whole-cell catalase activity is quite energetic, and the enzyme is heat stable, unlike that of members of the *M. avium* complex (258, 342, 447). *M. scrofulaceum* isolates can grow at 42°C (258).

Because *M. scrofulaceum* has been isolated from natural waters (150) and is closely related to members of the *M. avium* complex, it is surprising that so few cases of *M. scrofulaceum* disease occur in AIDS patients. Perhaps this species has become less prevalent in the environment or is absent in waters that serve as sources of infection for AIDS patients (e.g., drinking water) (516). Possibly, it lacks some characteristic (as does *M. intracellulare*) that is necessary for virulence in AIDS patients. All explanations are likely. Recent results from surveys over the period from 1990 to 1993 demonstrate that *M. scrofulaceum* is no longer as prevalent in natural waters as before (i.e., from 1970 to 1980) (147, 150, 291) and that *M. scrofulaceum* is absent in drinking-water distribution systems (517). Evidence that *M. avium* complex serotypes 1, 4, and 8 are most commonly found in infections of AIDS patients (555) and that *M. avium* predominates over *M. intracellulare* among isolates from patients with AIDS (129, 193) supports the hypothesis that virulence for patients with AIDS is limited to a narrow spectrum of mycobacteria.

Sources of *M. scrofulaceum*

M. scrofulaceum has been readily isolated from natural lakes and rivers (150) and aerosols (542). Isolation from natural waters is expected for an organism causing cervical lymphadenitis in children (547). The geographic distribution of *M. scrofulaceum* appears to be limited by the temperature of the natural waters, because few *M. scrofulaceum* isolates were recovered from waters collected in the northeastern United States compared with the number recovered from southeastern waters (188). Although *M. scrofulaceum* was readily isolated in large numbers from southeastern U.S. water samples collected over the period from 1975 to 1980 (150), its numbers and frequency of isolation appear to be lower in samples collected in the same geographic area 10 years later (147, 291). That observation, coupled with evidence that the frequency of *M. scrofulaceum* infections is falling among patients with cervical lymphadenitis (87, 549), suggests that the prevalence of *M. scrofulaceum* in the natural environment is falling. Further, on the basis of recent survey data, *M. scrofulaceum* appears to be rare, if not absent, in drinking waters and distribution systems (517).

There are other possible sources for *M. scrofulaceum*, other than natural waters. *M. scrofulaceum* has been isolated from raw milk (137) and has been found as a contaminant in plant tissue culture (482). It is possible that *M. scrofulaceum* is a natural epiphyte of plants.

Risk Factors for *M. scrofulaceum* Infection

It is likely that the major risk factor for *M. scrofulaceum* cervical lymphadenitis in children is exposure to waters containing *M. scrofulaceum* (547). As is the case for members of

the *M. avium* complex, risk factors for *M. scrofulaceum* disease in adults are predisposing lung conditions (548). Those include pneumoconiosis, preexisting tuberculosis, chronic bronchitis, emphysema, bronchiectasis, and lung malignancies (548). Arc welding is a risk factor for *M. scrofulaceum* pulmonary infection (548). Trauma to skin or a joint is a risk factor for *M. scrofulaceum* cutaneous or joint infection in immunocompetent individuals (439). Immunodeficiency is a risk factor for disseminated cutaneous or pulmonary (or both) *M. scrofulaceum* infection (439).

Chemotherapy of *M. scrofulaceum* Infection

M. scrofulaceum and *M. avium* complex isolates have similar antibiotic susceptibility patterns (163). Although there have been no studies comparing the efficacy of different drugs for treatment of *M. scrofulaceum* infection, it is logical that regimens effective against *M. avium* complex (38, 138, 339, 526) might prove effective against *M. scrofulaceum*. Furthermore, since susceptibility to clarithromycin can be measured and results can be correlated with clinical efficacy (439), it is possible to determine whether clarithromycin would be useful in treatment of *M. scrofulaceum* infection. On the basis of similarity of susceptibilities to antimycobacterial drugs, it is expected that rifabutin prophylaxis, effective for prevention of *M. avium* complex infection (178, 375) would prove useful for *M. scrofulaceum* prophylaxis.

EPIDEMIOLOGY OF MYCOBACTERIUM SIMIAE

Case History of a Mixed *M. simiae* and *M. avium* Infection

A 43-year-old male native of the Congo employed as a hospital attendant denied homosexuality or intravenous drug use (319). In March 1981, the patient presented with herpes zoster infection; this was followed in January 1982 by diarrhea related to amoebiasis. The diarrhea persisted, and upon evaluation in France, he was diagnosed with AIDS in March 1983 and was found to have *Candida* esophagitis and acute colitis. Blood and stool cultures yielded *Shigella flexneri*, and the patient had 100 T cells per mm³ with a T4/T8 ratio of 0.125. In June 1983, *Cryptosporidium* oocysts were seen in stool examination and *Nocardia asteroides* pneumonia was diagnosed. The pneumonia was cleared by antimicrobial therapy. The patient was released and showed a weight gain by December 1983. In May 1984, the patient was readmitted because of massive weight loss. Biopsy specimens of duodenal tissue showed evidence of acid-fast microorganisms, and cultures of duodenal and rectal biopsy specimens and blood yielded *M. simiae* and *M. avium*. The mycobacteria were identified on the basis of cultural and biochemical characteristics and, importantly, mycolic acid profiles by HPLC. The patient requested a return to his homeland and was lost to follow-up.

Epidemiology of *M. simiae* Infection

M. simiae was described as a novel *Mycobacterium* sp. infecting monkeys in 1965. A total of 33 mycobacterial isolates were recovered from 69 imported feral monkeys; 18 were identified as the new species, *M. simiae*, and 4 as a new related species, *Mycobacterium asiaticum* (540).

Pulmonary disease caused by *M. simiae* has been reported in the United States, Israel, Thailand, and France (35, 46, 297, 310, 472). In addition, there have been reports of disseminated disease caused by *M. simiae* (319, 423). In Berlin, *M. simiae* was recovered from 1 of 16 AIDS patients who were suffering from nontuberculous mycobacterial infections (390). Another mixed

M. simiae-*M. avium* complex infection has also been reported in an AIDS patient (497), and *M. simiae*-like organisms have been recovered from AIDS patients (521).

Characteristics of *M. simiae*

M. simiae is a photochromogenic mycobacterium that grows on standard mycobacterial culture media without additional growth factors (541). It grows optimally at 37°C and slowly at 25°C, and it fails to grow at 40 and 20°C (540). *M. simiae* isolates grow over the pH range 5.5 to 7.5 (400). *M. simiae* produces niacin, unlike other nontuberculous mycobacteria, and has urease but lacks phosphatase and nitrate reductase activities (541). The pattern of cell-free antigens of *M. simiae* is unique, although approximately half are shared with members of the *M. avium* complex (540). There appear to be two serotypes represented among the *M. simiae* strains collected and serotyped to date (541). *M. simiae* and *Mycobacterium habana* are synonymous (358, 490, 541).

M. simiae is unique among mycobacteria in that part of its 16S rRNA gene sequence is similar to those sequences shared by slowly growing mycobacteria, while another portion resembles sequences shared among rapidly growing mycobacteria (420). *M. simiae* shares many features with members of the *M. avium* complex and *M. scrofulaceum* (47) and can be incorrectly identified as a member of the *M. avium*, *M. intracellulare*, and *M. scrofulaceum* intermediate group (205, 396). For example, *M. simiae* is incapable of hydrolyzing Tween 80, is pigmented, and has urease activity (541), like some members of the *M. avium*, *M. intracellulare*, and *M. scrofulaceum* intermediate group (205, 396). Fortunately, there are distinct, reproducible differences in the pattern of antigens (541) and mycolic acids (317, 363) between *M. simiae* and members of the *M. avium* complex and *M. scrofulaceum*. Although *M. simiae* and *M. malmoense* have the same pattern of mycolic acids, *M. malmoense* can hydrolyze Tween 80.

Sources of *M. simiae*

M. simiae can be transmitted between animals. Twenty-six percent of healthy monkeys caged in groups with infected monkeys became infected with *M. simiae* over a 13- to 90-day period of contact (540). Although the exact mechanism of transmission was not identified in that study, the data suggest that transmission from animal to animal may occur, possibly via aerosolized organisms.

M. simiae has been isolated from the feces of HIV-positive and -negative humans in Kinshasa, Zaire (83), and from the stools of healthy Europeans (399). Possibly, the infection in monkeys described above (540) occurred through a fecal-oral route.

Like members of the *M. avium* complex and *M. scrofulaceum*, *M. simiae* has been isolated from water (310). Unfortunately, there have been no systematic studies of its distribution in the environment, so it is only conjecture that *M. simiae* is an environmental mycobacterium, although its isolation from water makes this a likely factor.

Risk Factors for *M. simiae* Infection

As is the case for other nontuberculous mycobacteria, AIDS and presumably immunodeficiency are risk factors for *M. simiae* infection (319). In addition, exposure to possible environmental sources of *M. simiae* or to an animal or another person infected with *M. simiae* may be a risk factor for infection (73, 540). Geography may be a risk factor for *M. simiae* infection, because isolation appears restricted to a few regions

of the world, such as Arizona (174), Havana (541), and Israel (310).

Chemotherapy of *M. simiae* Infection

Guidelines for chemotherapy of *M. simiae* infections suggest that drug regimens proposed for or with efficacy for treatment of *M. avium* complex infections be used (526). However, susceptibilities should be measured by recommended methods (243). Unfortunately, responses to treatment have been mixed (35).

EPIDEMIOLOGY OF *MYCOBACTERIUM MALMOENSE*

Case History of an *M. malmoense* Infection in an AIDS Patient

In February 1989, a 41-year-old homosexual man presented with a history of weight loss, intermittent diarrhea, and persistent fever with night sweats (568). He had been diagnosed as HIV positive in 1986. In October 1990, the patient presented with a 3-week history of severe night sweats, fever, productive cough, and severe diarrhea. The CD4 lymphocyte count was 10/mm³, and the patient was anemic (presumably as a result of zidovudine therapy). A roentgenogram of the chest showed infiltration of the right upper lobe, which had been absent 7 months earlier. Sputum, feces, and bronchoalveolar lavage fluid contained acid-fast bacilli. Isoniazid, pyrazinamide, and rifampin therapy was started, but after the laboratory reported the presence of nontuberculous mycobacteria (4 weeks), rifabutin, clofazimine, ethambutol, and isoniazid replaced the initial regimen. After 7 weeks, the patient's symptoms had not changed; *M. malmoense* was identified from cultural and biochemical tests and fatty acid profiles. Gradually, over a 6-month period, the diarrhea and coughing were reduced, and after 10 months of drug therapy, the diarrhea disappeared, coughing was almost absent, and stool and sputum cultures were negative for acid-fast bacilli.

Epidemiology of *M. malmoense* Infection

M. malmoense was first described in 1977 as a new species of the nontuberculous mycobacteria, causing pulmonary disease, on the basis of a unique set of biochemical and cultural characteristics shared by seven independent isolates (448). Since that initial report, almost 200 cases of *M. malmoense* infection have been reported throughout the world, including England (88, 257, 259), Scotland (158), and Scandinavia (269). Although the number of cases is rather small, the incidence of *M. malmoense* infections is rising in England (88, 257, 259, 561), Scotland (158), and Scandinavia (269). In Sweden, it is the most common cause of mycobacterial infections after *M. tuberculosis* and the *M. avium* complex (222). *M. malmoense* is the second most frequent cause of lymphadenitis in southeastern England, after the *M. avium* complex (184).

Among immunocompetent individuals, infection is restricted principally to the lungs (124). Over the period from 1983 to 1988, three *M. malmoense* infections occurred in Zurich, Switzerland, among 513 HIV-negative individuals with nontuberculous mycobacterial infections (124). Two patients had pulmonary infections, and the third had tenosynovitis (124). In a survey conducted in Finland from 1977 and 1986, among 12 children with cervical adenitis, two of the infections were caused by *M. malmoense* (268).

Preexisting chronic lung disease in immunocompetent individuals or immunodeficiency alone appears to be associated with *M. malmoense* infection. In a group of eight Welsh men

with pulmonary disease caused by *M. malmoense*, all either had pneumoconiosis or had been exposed to coal dust (256). In immunodeficient individuals (e.g., AIDS patients), *M. malmoense* infection is often disseminated (568). A cutaneous *M. malmoense* infection in an immunocompromised patient (166) and an *M. malmoense* infection in a patient with hairy cell leukemia (55) have also been reported.

Characteristics of *M. malmoense*

M. malmoense can be distinguished from other slowly growing mycobacteria on the basis of its cultural and biochemical characteristics (509, 535). It is quite slow growing, requiring at least 6 weeks for primary isolation (448, 568). Growth is not increased by subculturing, suggesting that there is no conditional auxotrophy (448). Growth can be enhanced by acidic pH (270, 271, 400) or inclusion of pyruvate in a low-pH medium (271). *M. malmoense* has a number of characteristics in common with the *M. avium* complex, with the exception that it hydrolyzes Tween 80 (448). *M. malmoense* grows microaerophilically, fails to grow at 45°C, and has a heat-labile (i.e., 68°C) catalase (448). Upon inoculation of *M. malmoense* into guinea pigs, there was no generalized infection. Four of seven hens given injections of *M. malmoense* had lesions in the liver and spleen from which *M. malmoense* could be isolated in large numbers, and two of the seven hens died of the infection (448).

M. malmoense can be easily missed in the laboratory (222). First, *M. malmoense* requires a longer incubation period than other nontuberculous mycobacteria for colony formation (448). Second, media used routinely for cultivation of mycobacteria do not support luxuriant growth of *M. malmoense*; instead, low-pH, pyruvate-containing media are recommended (271). Further, incubation of cultures at 30 to 33°C has also been recommended for *M. malmoense* isolation (222). Isolation of *M. malmoense* from cervical lymph node samples was higher with the BACTEC system than with standard Lowenstein-Jensen culture medium (222). Because of these unique characteristics, it is possible that the frequency of *M. malmoense* isolation is underrepresented.

Like the *M. avium* complex, *M. malmoense* strains are quite diverse. Five types of surface glycolipid patterns could be distinguished in 29 clinical isolates of *M. malmoense* (272). In addition, two major and three minor rRNA ribotypes were identified from the same group of strains (272). There was no relationship between glycolipid and ribotype groups (272).

Sources of *M. malmoense*

M. malmoense has only infrequently been recovered from the environment. The organism has been isolated from natural waters in Finland (240) and soil in Japan (436). The paucity of reports may not reflect its absence from waters and soils but, rather, the difficulty in culturing *M. malmoense* (222). It is possible that the increases in the prevalence of *M. malmoense* infections in England (88) and Scotland (158) before the full impact of the AIDS epidemic did not simply reflect growing awareness of its existence but, rather, some change in the numbers of *M. malmoense* or in human exposure.

M. malmoense has been isolated from feces from HIV-positive individuals in Kinshasa, Zaire (83), and from stools of healthy Europeans (399). It is possible that *M. malmoense* is a human commensal.

Risk Factors for *M. malmoense* Infection

Among immunocompetent individuals, pulmonary conditions such as pneumoconiosis caused by chronic exposure to

dusty working conditions (e.g., coal mining) are risk factors for *M. malmoense* infection (256). Profound immunodeficiency, such as found among AIDS patients, is also a risk factor for *M. malmoense* infection (568).

Chemotherapy of *M. malmoense* Infection

To date, there have been no large-scale studies of efficacy of different antibiotic regimens on the treatment of *M. malmoense* infections. Recommendations for *M. malmoense* treatment are similar to those for treatment of the *M. avium* complex and include rifampin, ethambutol, and streptomycin (526). Rifampin, ethambutol, and amikacin were used successfully in treatment of two *M. malmoense* infections (217). Currently, a multicenter study is being conducted in Europe comparing two regimens for the treatment of pulmonary *M. malmoense*, *M. avium* complex, and *M. xenopi* infections: rifampin, ethambutol, and isoniazid, and rifampin and ethambutol (217).

EPIDEMIOLOGY OF MYCOBACTERIUM SZULGAI

Epidemiology of *M. szulgai* Infection

M. szulgai, first described in 1972 (333), has been isolated from patients throughout the world, including Japan (503), Wales (333), British Columbia (246), and the United States (73, 174, 327). Infection is principally pulmonary (73, 246, 327, 333, 503). In one study of 14 cases of *M. szulgai* infection, 10 (71%) were pulmonary (547), and in another study of 27 cases, 18 (67%) were pulmonary (327). Other *M. szulgai* infections involved the bursa, tendon sheaths, bones, lymph nodes, and skin (327, 547). There appears to be no seasonal or geographic clustering of cases or isolations (327). Disseminated *M. szulgai* infection has been reported in AIDS patients (175) and immunocompromised patients (327).

Characteristics of *M. szulgai*

M. szulgai is scotochromogenic at 37°C but photochromogenic at 25°C (73, 258). Pigmentation at 37°C is weak upon primary isolation (73). *M. szulgai* isolates grow from 25 to 37°C but fail to grow at 42°C. Unlike other scotochromogens, *M. szulgai* hydrolyzes Tween 80 (73, 258, 327). *M. szulgai* isolates have 68°C-resistant catalase activity and nitrate reductase and urease activities but lack acid phosphatase activity (177, 499). *M. szulgai* fails to grow in 5% NaCl. Although distinguishing *M. szulgai* from other nontuberculous mycobacteria solely on the basis of phenotypic features is difficult (535, 536), the species can be identified on the basis of lipid patterns seen by thin-layer chromatography (258). The sequence of the 16S rRNA gene shows that *M. szulgai* is on a branch with *M. malmoense*, to which it is closely related (420).

Sources of *M. szulgai*

There have been no systematic studies designed to identify the possible sources of *M. szulgai* infection. On the basis of the worldwide distribution of cases and the similarity of risk factors for *M. szulgai* and infection by other environmental nontuberculous mycobacteria (e.g., *M. avium* complex), it is likely that the sources of *M. szulgai* infection are environmental (327). The association between injury and *M. szulgai* infection in joints (327) suggests an environmental source for the organism.

Risk Factors for *M. szulgai* Infection

Chronic lung disease, cigarette smoking, and high alcohol consumption appear to be risk factors for *M. szulgai* lung infection (327). In the case of infections of the joints or bursa, trauma due to injury was associated with *M. szulgai* infection in approximately two-thirds of patients (327). Two-thirds of patients with cutaneous *M. szulgai* infection had undergone corticosteroid injection and treatment (327).

Chemotherapy of *M. szulgai* Infection

Isoniazid, rifampin, ethambutol, and streptomycin have been used for treatment of *M. szulgai* infection (327). In vitro susceptibility test results can be used to guide the choice of therapy (327). Surgery has been used in some cases of infection involving the joints or lymph nodes (327).

EPIDEMIOLOGY OF *MYCOBACTERIUM XENOPI*

Case History of an *M. xenopi* Outbreak

In 1990, an increase in the number of sputum samples yielding *M. xenopi* was noted in Prague (462). *M. xenopi* was recovered repeatedly from the sputum of 13 of 21 individuals reported to be infected by the organism. Those 13 had clinically significant disease. Water was collected from the faucets and showers in apartments where 11 of the 13 patients resided. *M. xenopi* was recovered from hot and cold water samples from 5 (45%) of those apartments and from 5 of 17 (29%) adjoining apartments. The organism was not recovered from water samples collected from 3 waterworks, 6 reservoirs, and 10 hydrants serving the flats.

Epidemiology of *M. xenopi* Infection

Recovery of *M. xenopi* from patient samples has not been uniform throughout the world. *M. xenopi* recovery was rare in Australia (349, 417), Japan (95), and Zimbabwe (500). In contrast, *M. xenopi* was often recovered from clinical material in Wales (332, 334), southern England (32), the northwest coast of Europe (45, 140, 386), and Toronto, Canada (91). In the report of Canadian patients with pulmonary disease caused by nontuberculous mycobacteria, 34 of the 89 patients identified were infected with *M. xenopi* (91).

In the United States, before the advent of HIV infection and AIDS, the frequency of *M. xenopi* infection was low (128) and occurred in clusters (36, 98), even up to 1984 (485). A recent study of nontuberculous mycobacteria, other than *M. avium* complex and *M. gordonae*, recovered from cultures of specimens from 86 patients at a hospital in the New York City metropolitan area revealed that over the period from 1981 to 1990, 33 isolates of *M. xenopi* were recovered (453). Patients whose samples yielded *M. xenopi* were significantly more likely to be infected with HIV than were other patients (453). Those data suggest that the frequency of recovery of *M. xenopi* will rise in the United States.

Recent reports from Europe have continued to document the recovery of *M. xenopi*, now from patients with AIDS. Nontuberculous mycobacteria were recovered from 16 of 72 AIDS patients (22%) in a Berlin hospital (390). *M. xenopi* was recovered from 1 of the patients (6%), *M. avium* complex was recovered from 14 (88%), and *M. simiae* was recovered from 1 (6%) (390). It is interesting that nontuberculous mycobacteria were not recovered from 134 patients with non-HIV-related immunosuppression (390).

As is the case for infection by members of the *M. avium* complex, *M. xenopi* infection in non-AIDS patients has been

principally pulmonary (124, 295, 332, 349, 417, 457). Among a group of 513 non-HIV-positive patients with nontuberculous mycobacterial infections recorded in Zurich, Switzerland, over the period from 1983 to 1988, four had *M. xenopi* infections (124). In all four, the infection was pulmonary (124). Preexisting pulmonary disease is a risk factor for *M. xenopi* disease in immunocompetent individuals. In Wales, of 9 men with pulmonary *M. xenopi* infection, 3 had pneumoconiosis and 1 was a coal miner (256). Liver transplantation is also a risk factor for *M. xenopi* infection. The frequency of *M. xenopi* infection in both adults and children following liver transplantation (i.e., approximately 1%) is higher than the frequency of nontuberculous mycobacterial infection in the general population (348). *M. xenopi* infection has been reported in kidney transplant recipients as well (322, 539).

Infection in AIDS patients is usually disseminated (139, 390); however, it can be exclusively pulmonary. *M. xenopi* pulmonary infections were detected in two HIV-infected men who lacked a history of pulmonary disease or AIDS-defining conditions (254). Initially, because of the lack of conditions associated with nontuberculous mycobacterial pulmonary infections (e.g., one patient even had a CD4⁺ cell count of 340), the patients were thought to be infected with *M. tuberculosis* (254).

Clustered outbreaks of *M. xenopi* disease have occurred in Europe and the United States. In an apartment complex in Prague, Czech Republic, and in a hospital in New Haven, Conn., infection was correlated with the presence of *M. xenopi* in the city's drinking-water system (230, 462) or in the hospital's hot water system (98), respectively. In the New Haven outbreak, 16 of the 20 *M. xenopi* isolates had the same antibiotic susceptibility pattern (98). *M. xenopi* isolates from the hospital's hot water supply system were not tested for antibiotic susceptibility. A cluster of *M. xenopi* pseudoinfections in a Michigan hospital were traced to tap water and a hot water tank that was used for disinfection of bronchoscopes (36). Two of the three different LRF patterns of the *M. xenopi* patient isolates observed by PFGE were identical with patterns in hospital water isolates (36). Surveys of drinking-water systems in other parts of the world have also revealed its presence (60, 354).

Characteristics of *M. xenopi*

The major distinguishing characteristic of *M. xenopi* is its ability to grow at 45°C but not at 28°C (258, 338, 499). In addition, *M. xenopi* colonies are unique, forming irregular, stick-like projections best observed by phase-contrast microscopy (338), and isolates are arylsulfatase positive in 3 days (258). These characteristics are important because *M. xenopi* isolates can be confused with *M. avium* owing to the similarity of results in cultural and biochemical tests (258, 499). For example, *M. xenopi* strains are pigmented and grow at 45°C and *M. avium* isolates from AIDS patients are often pigmented (281) and can grow at 45°C (499).

In spite of the characteristics that *M. xenopi* shares with members of the *M. avium* complex, it is a distinct mycobacterial species, as evidenced by its 16S rRNA gene sequence (420). Thus, *M. avium* complex-specific nucleic acid probes fail to react with *M. xenopi* isolates (338). Methods involving either a non-rRNA-directed nucleic probe (392) or PCR based on the *M. xenopi* 16S rRNA gene sequence (151) have been developed for *M. xenopi* identification. In addition, *M. xenopi* isolates display a unique pattern of mycolic acids by HPLC (173). Growth that is slower than that of members of the *M. avium* complex further distinguishes *M. xenopi* from that group (499).

Sources of *M. xenopi*

There is a great deal of epidemiologic evidence supporting the hypothesis that the source of *M. xenopi* infection is water supplies (86, 230, 462). *M. xenopi* has been isolated from tap water samples and shower heads (85, 187, 273, 354). However, *M. xenopi* has not been found in all hot water systems and, as noted above, most cases are clustered (338). The fact that the organism can grow at 45°C is consistent with its isolation from hot water samples (462). The absence of *M. xenopi* from water treatment plants, reservoirs, and distribution systems is probably due to the inability of the organism to grow at temperatures below 28°C (462). Lack of detectable levels of *M. xenopi* in water distribution systems distinguishes it from the *M. avium* complex, which is found repeatedly (130, 517). Fortunately, proof that *M. xenopi* isolates from water are responsible for disease is possible by comparing LRF patterns of genomic DNA by PFGE (36).

The presence of *M. xenopi* in water samples collected in homes, coupled with its absence in the water distribution system, suggests that the organism proliferates in water-heating systems. There has been no report of studies of that possibility. In addition, the mechanism of transmission of *M. xenopi* from water to infected patients requires definition. In non-AIDS patients, infections have been primarily pulmonary (230, 462), and it has been suggested that *M. xenopi* infection has occurred principally via generation of aerosols, perhaps in showers (85). In AIDS patients with disseminated infection (110, 139), infection could occur via aerosolization or the gastrointestinal route.

M. xenopi has rarely been recovered from sources other than domestic, heated water distribution systems. Although there have been reports of the isolation of *M. xenopi* from animals (e.g., pigs), the frequency has been low compared with recovery of *M. avium* complex and *M. bovis* (96, 488). In a survey of mycobacteria in 2,036 porcine tissue samples collected throughout the United States, only 1 sample yielded *M. xenopi* (488). Only two isolates of *M. xenopi* were recovered from tissue samples from 193 feral pigs in Australia (96). Bird dung does not appear to be a source of *M. xenopi* (514).

Risk Factors for *M. xenopi* Infection

In immunocompetent individuals, a variety of factors appear to be associated with pulmonary disease caused by *M. xenopi*. In a study of 34 patients with *M. xenopi* pulmonary disease, all 34 had underlying lung disease (32 had chronic obstructive pulmonary disease), 22 (65%) smoked heavily, 18 (53%) reported alcohol abuse, 15 (44%) had chronic liver disease, 13 (38%) had cardiovascular disease, and 10 (29%) had a previous gastrectomy (91).

Recent reports from throughout the world suggest that, as with other mycobacteria, AIDS is a risk factor for *M. xenopi* infection (390, 453). In addition, exposure to water systems that contain *M. xenopi* is a risk factor for *M. xenopi* infection in non-AIDS patients (462). It is not known whether mechanical features of the distribution system or heater or physiochemical parameters of the water itself are predictive of the presence of *M. xenopi*. For example, *M. avium* complex numbers in natural waters are influenced by levels of oxygen, zinc, and organic matter (291).

Chemotherapy of *M. xenopi* Infection

Drug therapy of *M. xenopi* infections has resulted in a mixture of success and failure (24, 526). Combinations of rifampin and streptomycin and of isoniazid, rifampin, and ethambutol

have shown success (526). The results of the current ongoing multicenter European study comparing two regimens for the treatment of pulmonary *M. malmoense*, *M. avium*, and *M. xenopi* infections (i.e., rifampin, ethambutol, plus isoniazid versus rifampin plus ethambutol) (217) will provide valuable guidance.

EPIDEMIOLOGY OF *MYCOBACTERIUM ULCERANS*

Clinical Features of *M. ulcerans* Infection: the Buruli Ulcer

The earliest presentation of *M. ulcerans* infection is appearance of skin papules, or as a subcutaneous nodule, which later invades the dermis (207). The papules or nodules ulcerate, and there is necrosis of the subcutaneous fat. Extracellular acid-fast organisms are found in large numbers in the necrotic areas surrounding the ulcerated regions. Occasionally, the necrosis extends into muscle and even bone. Sometimes *M. ulcerans* infection appears as diffuse edema of a limb. Lesions are commonly found on the limbs but in children can occur on the face and trunk. The lesions heal slowly, even with treatment, and leave scars reminiscent of burns.

Epidemiology of *M. ulcerans* Infection

Although the first description of skin ulceration due to *M. ulcerans* was in Australia in 1948, the majority of cases have been found in Africa along the Nile River, particularly in Buruli County, Uganda (505). Accordingly, the disease was called the Buruli ulcer. However, the organism is not restricted to Australia and particular areas of Africa, for cases have been described in other countries in Africa, Central and South America, New Guinea, Malaysia, and Sumatra (206). The incidence of Buruli ulcer appears to be increasing in West Africa (335). Unusually for a nontuberculous mycobacterial species, there have been no reports of *M. ulcerans* infection in AIDS patients (233). However, even though the *M. avium* complex is present in the African environment, there have been almost no cases of *M. avium* complex disease in African AIDS patients.

A common feature of *M. ulcerans* disease is that infected individuals often reside in swampy areas or river valleys with many river courses in tropical regions of the world (25, 206, 505). Proximity to water is not the only factor; in Madi, Uganda, disease was confined to the river's edge on the hilly side of the Nile but was found up to 10 miles from the river on the side where the land was flat (25).

Skin testing with an antigen prepared from *M. ulcerans* (i.e., burulin) revealed that the incidence of *M. ulcerans* infection was low in the areas where the infection was not endemic (25), suggesting that the organism was absent in those areas. In contrast, the incidence of skin test reactors was high in areas of Uganda where the infection was endemic (25), suggesting the presence of subclinical infections. Because of the equally high incidence of infection in both males and females of all age groups in certain areas, it would appear that *M. ulcerans* is widespread in those environments.

Characteristics of *M. ulcerans*

M. ulcerans has rarely been included in comprehensive studies of mycobacterial taxonomy and physiology. *M. ulcerans* grows slowly at both 25 and 37°C, although growth at 25 and 28°C is more luxuriant than at the higher temperature (499). Growth is preferentially microaerobic (258), and *M. ulcerans* lacks catalase activity (499), although it should be pointed out that semiquantitative catalase measurements do not reflect true catalase activity (342). A single colony type (rough) is

found on agar media; no colony variants are apparent (499). Some pigmented strains of *M. ulcerans* have been isolated (259). *M. ulcerans* appears to be a fastidious organism on the basis of its inability to grow on a variety of carbohydrates in the presence of either glutamate or ammonia nitrogen (499). One confirmatory test for identification of *M. ulcerans* is mouse footpad inoculation. The mouse footpad will swell 3 to 6 weeks following inoculation with *M. ulcerans* (258).

M. ulcerans fails to grow on Sauton medium unless supplied with Tween 80 (499), suggesting that it has a fatty acid requirement. This finding would agree with its extracellular location in the lipid-rich areas surrounding the sites of infection (89, 90).

In the one study of DNA relatedness of mycobacteria which included *M. ulcerans*, it was shown that the organism was most closely related to *M. marinum* (241). The mycolic acid pattern of *M. ulcerans* was also similar to that of *M. marinum* (317).

Sources of *M. ulcerans*

Although *M. ulcerans* has never been isolated from the environment, water is one possible source (25). In a study relating the frequency of disease to the water sources used for drinking and crop irrigation, it was shown that cases were found in 6% of families using boreholes, 25% of families using seasonal swamps, and 53% of families using permanent swamps (25). However, these data cannot rule out other sources, because only the permanent swamps were located close to the Nile River. There was no association between frequency of disease and proximity to the river for families using the two other water sources (25). Other evidence suggesting that river water was not a likely source came from studies of a refugee community in an area where *M. ulcerans* is endemic (505). The refugees who almost immediately began to get the Buruli ulcer upon settling within this area rarely used the river water (505).

As an alternative to the hypothesis that water is the source of *M. ulcerans*, Barker (25) proposed that vegetation could serve as a source of *M. ulcerans*. Hayman (206) has proposed that *M. ulcerans* infection is associated with disturbance of the rain forest. It is likely that intimate contact with vegetation would be prevented by steep hillsides, thus explaining the absence of *M. ulcerans* disease in persons who reside on the Nile River bank where the hillside is steep next to the river (25). Although mycobacteria have been isolated from plant material, none were identified as *M. ulcerans* (25). Recovery of an *M. ulcerans*-like, slowly growing *Mycobacterium* isolate from plant material collected in Zaire was reported by Portaels (395).

It has also been suggested that *M. ulcerans* transmission can occur via aerosolization (206), as has been shown for members of the *M. avium* complex (145, 171, 383, 542). Aerosolized *M. ulcerans* possibly present in recycled sewage water used to irrigate a golf course was proposed as the route of infection in Australia (262). In fact, it was shown that cells of *M. ulcerans* could be aerosolized from suspensions of tap water (206).

The possibility that *M. ulcerans* requires fatty acids for growth and is microaerobic is not in conflict with the hypothesis that the organism is widespread in the environment. First, plants are sources of fatty acids. Second, microaerobic habitats predominant in swamps (291). Thus, the hypothesis that *M. ulcerans* exists in association with tropical, swamp-dwelling plants is consistent with its physiology.

Risk Factors for *M. ulcerans* Infection

Clearly, a major risk factor for *M. ulcerans* infection is residence in close proximity to a river in a tropical area of the world (25, 335, 505). Studies attempting to link human behav-

ior to the frequency of disease identified farming activities near a river as a risk factor (335). Protection against *M. ulcerans* infection is provided by wearing long pants (335). Previous mycobacterial infection, evidenced by a tuberculin reaction of less than 4 mm, offers some protection against *M. ulcerans* disease (467). Protection is equivalent to that afforded by BCG vaccination (467).

Chemotherapy of *M. ulcerans* Infection

M. ulcerans has not been included in any of the studies of antimicrobial susceptibility of mycobacteria. However, in the few studies that have included *M. ulcerans*, it has been shown that the organism is susceptible to *p*-aminosalicylic acid and ethambutol and resistant to isoniazid and rifampin (258, 499).

EPIDEMIOLOGY OF *MYCOBACTERIUM HAEMOPHILUM*

Case History of an *M. haemophilum* Infection in a Patient with Hodgkin's Disease

Generalized skin granulomata and subcutaneous abscesses developed in a patient with Hodgkin's disease (469). Aspirated pus samples from the abscesses yielded acid-fast rods. Attempts to culture the acid-fast rods were all unsuccessful, until media containing hemolyzed sheep erythrocytes were employed. Slowly growing mycobacteria whose optimal growth temperature was 30°C (no growth at 37°C) and which had an absolute requirement for hemoglobin or hemin for growth were isolated. Subsequently, the organism was identified as a representative of a heretofore unrecognized species, *M. haemophilum*. Antimycobacterial drug therapy including streptomycin, ethambutol, isoniazid, and topical *p*-aminosalicylic acid ointment was instituted, and the lesions healed about 6 months later.

Case History of an *M. haemophilum* Infection Associated with an Outbreak in the New York Metropolitan Area

M. haemophilum infection in four patients occurred in the same New York City hospital over a 7-month period (282). One patient with AIDS had three prior episodes of *Pneumocystis carinii* pneumonia and was found to have tender, pruritic, nodular skin lesions. Acid-fast bacteria were found in biopsy specimens of arm and thigh lesions. Initial treatment with isoniazid, ethambutol, and rifampin was modified following recovery and identification of *M. haemophilum*. Regression of the lesions occurred over a 10-month period while the patient was treated with doxycycline, rifampin, and amikacin, but new lesions appeared when therapy was halted. Institution of combined rifampin, amikacin, and ciprofloxacin therapy led to regression of lesions but was followed by the appearance of bilateral pneumonia and isolation of *M. haemophilum* from sputum. Intravenous multidrug therapy reduced the extent of pulmonary infiltrates, and *M. haemophilum* was no longer recovered from sputum. At autopsy, *M. haemophilum* was recovered from a lymph node, the adrenal gland, and the spleen. Death was attributed to *M. haemophilum* pneumonia.

Epidemiology of *M. haemophilum* Infection

Both cutaneous (115, 326, 347) and disseminated (31, 282, 362, 421, 559) *M. haemophilum* infections have been reported. The majority of instances of *M. haemophilum* infection have occurred in immunocompromised patients (300, 486), namely, renal transplant recipients (52, 218, 361) and AIDS patients (125, 326). Simultaneous infection by *M. haemophilum* and *M.*

xenopi in a renal transplant patient was reported (52). On the basis of the observation that *M. haemophilum* skin lesions in renal transplant patients developed 5 to 6 months after surgery, the incubation period was proposed to be 2 to 4 months (122). Generally, disseminated infections have been found in severely immunodeficient individuals (282, 326, 421). However, *M. haemophilum* infection has not been restricted to the immunocompromised. There have been two reports of *M. haemophilum* lymphadenitis in healthy children (14, 121).

Since its initial discovery in 1976, the literature presents a picture of a regular, albeit low frequency of *M. haemophilum* isolation from patients throughout the world until approximately 1990 (477, 538). There was one report in 1988 of two cases of *M. haemophilum* infection in a renal dialysis facility (181). However, in the period from 1990 to 1991, a cluster of *M. haemophilum* infections appeared in hospitals in the New York City metropolitan area (71, 282, 477).

Restriction fragments of *M. haemophilum* DNA from isolates recovered from patients from different geographic areas were compared by PFGE to determine whether this technique could be used in epidemiologic studies of this species (557). Isolates from Albany, N.Y.; New York City; Florida; and Texas had unique LRF patterns (557). Among 16 isolates recovered from patients in the metropolitan New York City area, 6 different LRF patterns were identified (557). However, 12 of the isolates from New York City had the same pattern and 6 came from the same hospital (557). Because it is unlikely that the patients shared a history of exposure to possible *M. haemophilum* sources, the data suggest that the source of the *M. haemophilum* infecting the patients was within the hospital. Not only did this study demonstrate the utility of LRF typing by PFGE for *M. haemophilum*, but also it demonstrated that there existed unique LRF types of *M. haemophilum* in different geographic areas and even unique types in different hospitals (or at least a narrow range of types).

Characteristics of *M. haemophilum*

There are two noteworthy characteristics of *M. haemophilum*. First, it has a requirement for hemin or ferric ammonium citrate (122, 367, 397, 433, 469, 470). Neither mycobactin nor ferric chloride could replace hemin or ferric ammonium citrate (122). Catalase also failed to stimulate growth in the absence of hemin (469). Second, the optimal temperature for growth appears to be between 30 and 32°C (122, 397, 469, 470), although a number of isolates of *M. haemophilum* were capable of growth at 37°C on Middlebrook 7H10 agar medium containing hemin (326).

Growth of *M. haemophilum* was stimulated and colonial morphology was altered by incubation in 7% CO₂ (326). Possibly the response of *M. haemophilum* to CO₂ was due to a stimulatory effect of carbon dioxide directly or was mediated through a pH effect. *M. haemophilum* is of low virulence. Animals inoculated with *M. haemophilum* develop few if any signs of infection (1, 367, 469), with the exception of amphibians (469) and unless the animals have been treated with steroids (1). The low virulence of *M. haemophilum* for laboratory animals, except amphibians, may be due to a restriction of growth provided by the animal's body temperature.

Because of the unique growth requirements, it is likely that the frequency of isolation of *M. haemophilum* and its impact on the morbidity and mortality of immunocompromised patients are underestimated (477). The mycobacterial skin infections caused by an unidentified species in immunocompromised patients could have been due to *M. haemophilum* (152). A num-

ber of reports have described novel or rapid methods for isolation and identification of *M. haemophilum* (109, 346, 508).

Neither the hemin/ferric ammonium citrate requirement nor the temperature optimum for growth appears to be due to simple, single point mutations, as opposed to deletions, insertions, or multiple mutations. Variants able to grow at 37°C or without hemin or ferric ammonium citrate have not been isolated (470). The hemin requirement distinguishes *M. haemophilum* from a class of isoniazid-resistant isolates of *M. tuberculosis* (154, 155). Furthermore, the inability of *M. haemophilum* to grow at 37°C is not due to some unknown auxotrophy. *M. haemophilum* grew intracellularly in human fibroblast monolayers at 30°C but failed to grow at 37°C (469). *M. haemophilum* cells released from lysed fibroblasts failed to grow, even at 30°C (469). Thus, the optimal temperature for growth and the nutritional requirements were the same in laboratory media and in human cells (469).

Cultural and biochemical characterizations of *M. haemophilum* strains have established that the organism is distinct from *M. ulcerans* and *M. marinum*, two other mycobacteria that cause granulomatous skin disease. Rather, *M. haemophilum* shares a great deal of similarity with members of the *M. avium* complex (122, 397, 469, 470). *M. haemophilum* is incapable of hydrolyzing Tween 80 and lacks nitrate reductase activity. It was reported that isolates of *M. haemophilum* lack catalase activity (469). However, the test used for measurement (430) can yield false-negative results (342). Lack of measurable catalase activity prompted studies of the aerotolerance of strains of *M. haemophilum*. Although *M. haemophilum* failed to grow under conditions of low oxygen tension generated by the GasPak system (BBL Microbiology Systems, Cockeysville, Md.), it was capable of growing approximately 3 to 4 mm below the surface of semisolid media (470).

Colonial pigmentation may be variable in *M. haemophilum* (470), perhaps influenced by the age of colonies (122). In addition, colonial pigmentation could be influenced by colony type, as it is in members of the *M. avium* complex (476). Colonial variation (i.e., rough and smooth colonies) has been reported in *M. haemophilum* strains (122, 469, 470).

In spite of the similarity between members of the *M. avium* complex and *M. haemophilum*, the latter does not share any of the glycopeptidolipid antigens (i.e., Schaefer antigens) used for serotyping the *M. avium* complex (122). Sompolinsky et al. (470) did detect some cross-reactivity between serum from rabbits given injections of live *M. haemophilum* cells and cells of *M. marinum* or *M. ulcerans*. In fact, an *M. haemophilum*-specific antigen and a reactive antibody have been prepared (122). Other unique and distinguishing characteristics include a unique pattern of methylated fatty acids demonstrated by gas-liquid chromatography (470), the presence of an *M. leprae*-related phenolic glycolipid antigen (42), and the presence of a distinct pattern of mycolic acids shown by thin-layer chromatography (397). Thus, there appears firm evidence that *M. haemophilum* is a distinct mycobacterial species.

Sources of *M. haemophilum*

Two factors influence the probability of infection by any microorganism: exposure and dose. Evidence of cervical lymphadenitis caused by *M. haemophilum* in healthy children (14, 121) suggests that the source of *M. haemophilum* is environmental. *M. haemophilum* grows over a wide range of pH (397), which is of adaptive value for an environmental organism. In this model, healthy children would be exposed to *M. haemophilum* residing in various environmental compartments (e.g., water and soil). Originally, evidence that *M. haemophi-*

lum requires hemin argued against a free-living environmental source (469). However, the discovery that ferric ammonium citrate (i.e., chelated iron) can substitute for the hemin requirement (122) removed that constraint. This model then requires that there exist environmental sources for chelated iron (e.g., humic and fulvic acids).

Frogs (*Bufo* and *Rana* spp.) infected with *M. haemophilum* and maintained at 30°C, but not at higher or lower temperatures, developed systemic infections (469). Acid-fast organisms were found in the liver and kidneys at death 8 to 20 days following injection of *M. haemophilum* (469). Perhaps amphibians are a reservoir for *M. haemophilum*, carrying the organism during periods of low temperature (i.e., below 30°C). Release of *M. haemophilum* from such a reservoir could occur during periods of high temperatures through death of the infected amphibians.

Water is a possible source of infection in immunocompromised patients. Evidence that *M. haemophilum* isolates from different infected patients in the same hospital had identical LRF patterns supports the hypothesis that the patients were infected from a single, common source. Although *M. haemophilum* strains have not been tested, most mycobacteria are resistant to chlorination (70, 86, 195). On the basis of their similarity to other water-borne mycobacteria (e.g., *M. avium* complex), *M. haemophilum* may be able to persist in water in spite of chlorination. Because *M. haemophilum* fails to grow at temperatures above 35°C (469), the organism may be restricted to cold water delivery systems, unlike *M. xenopi* and *M. avium*, which can tolerate high temperatures.

Reports of *M. haemophilum* infection in individuals undergoing dialysis (52, 69, 181) suggest the frightening possibility that kidney dialysis instruments may be the source of infection. Mycobacteria as a group are relatively resistant to disinfectants (43, 70, 344, 431) and there are reports of instrument-mediated infection by mycobacteria (120, 189, 314, 471) and mycobacterial infections associated with dialysis centers (68).

Risk Factors for *M. haemophilum* Infection

A major risk factor for *M. haemophilum* infection is immunodeficiency brought about by either immunosuppressive drugs or HIV infection. In the latter case, infection has been associated with low (e.g., <100) CD4⁺ cell counts (477). Renal dialysis alone or transplantation and the attendant immunosuppression may also be risk factors for *M. haemophilum* infection (52, 218, 361, 459).

Evidence of disseminated infection (e.g., to lungs) (282) raises the intriguing question of how an organism that is incapable of growing at 37°C can proliferate in the human body. Cutaneous infection is consistent with an optimum temperature for growth of 30 to 32°C for *M. haemophilum*. *M. haemophilum* grows within fibroblasts at 30°C but not 37°C (470), demonstrating that at least that cell type fails to provide a nutritional suppressor for the growth temperature limitation. Either the whole human organism can suppress the growth temperature limitation, or the explanation lies elsewhere.

In the absence of any recovery of *M. haemophilum* from the environment (e.g., water), it is only speculative that exposure to *M. haemophilum* in the environment (e.g., ponds) or infected amphibians (e.g., frogs) would lead to infection in healthy individuals.

Chemotherapy of *M. haemophilum* Infection

A variety of regimens for antibiotic treatment of *M. haemophilum* infections have been attempted. A number of these regimens have been reviewed (477). In many instances, the

initial and final treatment regimens have been different (477). Treatment regimens leading to disappearance of symptoms of *M. haemophilum* infection most often included ciprofloxacin and rifampin (477). Other reported regimens for *M. haemophilum* infection have included combinations of streptomycin, ethambutol, isoniazid, and *p*-aminosalicylic acid (469); doxycycline, rifampin, and amikacin (282); or rifampin, amikacin, and ciprofloxacin (282). In the regimen including *p*-aminosalicylic acid, that antibiotic was applied as an ointment (469). The emergence of resistance to rifamycins during a course of rifamycin therapy in a single patient was described (41).

The antibiotic susceptibility pattern of a number of *M. haemophilum* strains has been measured (41, 122, 282, 326, 397, 469, 477). Although no standard method exists for measuring the susceptibility of *M. haemophilum* to antibiotics, the results show some useful uniformity. Generally, members of the species were resistant to isoniazid, streptomycin, ethionamide, and ethambutol and susceptible to *p*-aminosalicylic acid, amikacin, clofazimine, clarithromycin, quinolones (ciprofloxacin, ofloxacin, and sparfloxacin), and rifamycins. No systematic study of the influence of *M. haemophilum* colony type upon antibiotic susceptibility has been performed.

In one report, isoniazid was described as weakly active against *M. haemophilum* (41); in another, the isolates were described as resistant. Interpretation of isoniazid susceptibility of *M. haemophilum* is complicated by the fact that hemin is incorporated into the medium used for growth and susceptibility measurements. Hemin inhibits the activity of isoniazid against *M. tuberculosis* (293). It has been suggested that the isoniazid resistance of *M. haemophilum* could be due to the lack of catalase activity (469). There is a long history of the concomitant appearance of loss of catalase activity and appearance of isoniazid resistance in *M. tuberculosis* (570).

EPIDEMIOLOGY OF *MYCOBACTERIUM GENAVENSE*

Case History of an *M. genavense* Infection in a Patient with Hemophilia A and AIDS

A 13-year-old boy with hemophilia A presented with a 2-week history of loss of appetite and weight, vomiting, abdominal distension and backache (371). Symptoms did not regress upon treatment with factor VIII. Examination of a fine-needle biopsy specimen of a retroperitoneal lymph node showed granulomas with large numbers of intracellular acid-fast bacilli. Serological tests for HIV were positive. The patient was treated with rifampin, pyrizinamide, ethambutol, amikacin, and ciprofloxacin for 3 weeks. He recovered slowly and was released after 3 weeks. Two months later, the patient was cachectic and, while being treated with amikacin, rifampin, and ethambutol therapy, experienced abdominal cramps, meteorism, and ileus. Under parenteral nutrition and continuation of drug therapy, his condition slowly improved. Anemia required recurrent transfusion of erythrocytes. Ten months after diagnosis, the patient was alive, free of acid-fast bacilli in stool, and under oral clarithromycin, rifampin, and ethambutol therapy.

Epidemiology of *M. genavense* Infection

In 1992, a disseminated infection caused by a hitherto undescribed acid-fast *Mycobacterium* species was described in an AIDS patient (49). The organism was unusual in that repeated attempts to culture it in laboratory media were unsuccessful (49). In addition, there was novelty in that the organism was not described on the basis of cultural or biochemical features, other than that it was difficult to culture (49). Identification was

based on PCR amplification and sequencing of the resulting amplified 16S rRNA gene fragment. *M. genavense* has been recovered from both adults (49) and children (371) with AIDS. The poorly growing, unidentified mycobacteria isolated from patients with AIDS (220) may also be *M. genavense*.

M. genavense, its name notwithstanding, appears to have a wide geographic distribution. *M. genavense* isolates have been recovered from Europe (49) and North America (99). The high frequency of recovery of *M. genavense* from AIDS patients in Seattle was rather striking in light of the few other reports of its isolation in the United States (99). The authors speculated that this could have been because they routinely incubated BACTEC vials for up to 8 weeks (i.e., 2 weeks longer than recommended). Also, the authors reported that there had been distinct periods when *M. genavense* had been isolated and times when it had not been isolated (99). If that periodicity in recovery did not represent the true situation, they hypothesized that failure to recover *M. genavense* could have been due to changes in the Seattle water supply, the BACTEC 13A broth, or the therapy of the AIDS patients (99).

M. genavense has also been recovered from tissue from caged, indoor birds at autopsy (228a). Because other birds in the cages were uninfected, it was proposed that there was no transmission between birds (228a). There were no infected humans in the homes where the birds were kept; therefore, the possibility of bird-to-human transmission could not be demonstrated (228a).

Characteristics of *M. genavense*

In a comprehensive study of the characteristics of seven *M. genavense* isolates recovered from patients in Seattle, growth in the BACTEC 13A vials appeared considerably later than had been found for *M. avium* complex (i.e., 38 to 42 days compared with 14 to 20 days) (99). Colony formation was observed on Middlebrook 7H11 broth medium containing agar and mycobactin J when inocula from BACTEC 13A vials were used (99). Another group found pinpoint acid-fast colonies after a 4-week incubation of Middlebrook 7H10 agar medium containing human blood and inoculated with culture from BACTEC 13A broth medium (325).

The whole-cell fatty acid pattern of the seven *M. genavense* isolates was similar to that of *M. fortuitum* and distinct from that of *M. simiae* (99). Mycolic acids of the seven *M. genavense* isolates were also identical and resembled those of *M. simiae* and *M. malmoense* (99). Hence, *M. genavense* appears to have a unique set of characteristics that distinguish it from other mycobacteria, especially *M. simiae*. *M. genavense* isolates had urease and catalase activities and were incapable of Tween 80 hydrolysis (99). However, with respect to any negative test, the authors pointed out that the absence of activity might have been due to the poor growth of the *M. genavense* cells (99). Furthermore, as has been pointed out by other authors (194), lot-to-lot variations in media and supplements to media may influence the growth of *M. genavense* (99).

Because growth is slow and sparse, it is not yet clear whether growth of *M. genavense* can be stimulated by addition of hemin, mycobactin, or pyruvate (99, 442). Although colony formation on Middlebrook 7H11 medium required mycobactin J (99) and colony formation on Middlebrook 7H10 medium required human blood (325), the organism grew in BACTEC 13A medium which lacks mycobactin. Growth occurred at 37°C but not at 30°C, although it would be expected that growth would be extremely slow at 30°C (99, 442). There was no growth under anaerobic conditions, but measurement of growth under microaerophilic conditions was not attempted (425).

Sources of *M. genavense*

The source of *M. genavense* infection in humans is unknown (371), although an intestinal source has been proposed (99) since a variety of uncharacterized, fastidious mycobacteria have been isolated from intestinal tissue (331) and feces (399). Because the growth requirements of *M. genavense* are unknown, it is not possible to exclude any environmental source. Although *M. genavense* has been recovered from indoor pet birds (228a), there has been no association between appearance of *M. genavense* infection and the presence of birds in the infected person's environment.

Risk Factors for *M. genavense* Infection

Profound immunodeficiency or AIDS would appear to be a predisposing risk factor for *M. genavense* infection, although there have been only a few reports describing *M. genavense* infection (371). The incidence of *M. genavense* infection is likely to be higher in AIDS patients than is reflected by the number of cases described to date. First, *M. genavense* infection may be undetected because of the slow growth and fastidious requirements of the organisms. Furthermore, *M. genavense* infection may occur in combination with other mycobacterial infections of AIDS patients (e.g., *M. avium* complex). In that case, it is likely that the patient will be diagnosed with an *M. avium* infection and treated accordingly. *M. genavense* infection should be considered in individuals with profound immunodeficiency, spiking fevers, severe gastrointestinal symptoms, acid-fast bacilli in stool samples, and negative mycobacterial blood cultures (371). In addition, *M. genavense* infection may occur in immunocompetent individuals without AIDS. Detection of such infections would also be problematic.

Chemotherapy of *M. genavense* Infection

Few reports of chemotherapy of *M. genavense* infections are available. Antibiotic treatment regimens of a hemophilia patient infected with *M. genavense* included rifampin, pyrazinamide, ethambutol, amikacin, and ciprofloxacin, followed by amikacin, rifampin, and ethambutol and finally by clarithromycin, rifampin, and ethambutol (371). Combined amikacin, clofazimine, clarithromycin, rifampin, ethambutol, and ciprofloxacin were successful in treating another child with AIDS and disseminated *M. genavense* infection (371). A patient with AIDS infected with *M. genavense* was successfully treated with clarithromycin (340).

EPIDEMIOLOGY OF *MYCOBACTERIUM FORTUITUM*, *MYCOBACTERIUM CHELONAE*, AND *MYCOBACTERIUM ABSCESSUS*

Case History of an *M. chelonae* Infection

A 39-year-old woman with a history of immunodeficiency and lymphadenopathy presented with a skin lesion from which *Mycobacterium terrae* was isolated (245). Antimycobacterial drug therapy consisting of ethionamide, ethambutol, rifampin, and streptomycin was initiated. When drug therapy was halted, the symptoms appeared again, and she was treated with ethambutol and rifampin. Four years later, she presented with a nodular skin rash, fever, and sweats. Culture of skin biopsy specimens yielded *M. chelonae*. Drug therapy this time included erythromycin, amikacin, ciprofloxacin, and ansamycin. *M. chelonae* continued to be recovered from skin biopsy, lymph node, blood, and sputum specimens. In spite of antimicrobial drug therapy, the patient died of progressive infection 2 years later.

Epidemiology of *M. fortuitum*, *M. chelonae*, and *M. abscessus* Infection

Almost all diseases caused by rapidly growing mycobacteria in humans are due to *M. fortuitum*, *M. chelonae*, and *M. abscessus* (522). Cases have been described throughout the world, including Japan (501, 503), Sweden (461), and the United States (108, 522, 528, 547).

Diseases include traumatic (528) and surgical wound (522, 525, 527) infections, skin and soft tissue infections (156, 245, 523), pulmonary infections (186, 528), and catheter-associated infections (522). Surgical wound infections associated with cardiac bypass surgery (525) or augmentation mammoplasty (82, 527) are often clustered and occur more frequently in Texas and the southern coastal region of the United States.

M. fortuitum, *M. chelonae*, and *M. abscessus* do not have the same likelihood of causing infection in different locations in the body. For example, *M. fortuitum* is responsible for the majority of infections following cardiac bypass surgery (525) or augmentation mammoplasty (527). In contrast, skin and soft tissue infections are due to *M. chelonae* and *M. abscessus* (245) and pulmonary infections are most often caused by *M. abscessus* (186). These patterns may reflect the environmental source and route of infection, especially in cases of nosocomial infection. For example, in two outbreaks of sternal wound infection following cardiac bypass surgery, isolates from possible environmental sources were similar or identical to isolates from patients (525). The genetic and phenotypic heterogeneity of rapidly growing mycobacterial isolates recovered from infections following cardiac bypass surgery or mammoplasty suggests that the isolates were unrelated and probably came from local sources rather than from contaminated surgical materials or devices (525, 527).

Other cases of infection caused by these species include prosthetic valve endocarditis caused by *M. chelonae* (413) and post-thoracotomy sternal osteomyelitis due to *M. fortuitum* (419), sternotomy wound infections involving *M. fortuitum* (562), and augmentation mammoplasty (82). There have also been a number of reports describing corneal infections caused by rapidly growing mycobacteria (108, 311, 315, 543, 551, 571). Those reports identified the causative organism as *M. fortuitum*. However, the changes in the taxonomy of the group and the fact that most rapidly growing mycobacteria recovered from corneal infections today are *M. chelonae* (522) suggest that the earlier isolates were possibly *M. chelonae*, not *M. fortuitum*.

There have been reports of the isolation of rapidly growing mycobacteria (50, 466), *M. fortuitum* (7, 283), and *M. chelonae* (283) from sputum of patients with cystic fibrosis. The clinical significance of the rapidly growing mycobacteria recovered from these patients was not investigated. However, death of a patient with cystic fibrosis was attributed to *M. fortuitum* infection in one case (137). A large multicenter investigation of the prevalence and significance of nontuberculous mycobacterial infections in cystic fibrosis patients has been initiated.

Cases of disseminated *M. fortuitum* and *M. chelonae* infections in the United States over the period from 1978 to 1991 have been reported and reviewed (245). Of the 54 patients reviewed, 40 had skin involvement and 75% of the total had a history of fever (245). Eighty percent of all the patients had some underlying disease or condition and fell into three groups (245). Patients in the first group did not have cell-mediated immune deficiency but did have an underlying condition or disease. These included kidney transplantation, chronic renal failure, and collagen vascular disease (245). Infections were limited, for the most part, to the skin, and the probability of

clearing the infections and symptoms was high (245). Patients in the second group had cell-mediated immune deficiency including lymphoma and leukemia. For these individuals, infection involved many organs and the prognosis for survival was very low, with only a 10% survival rate (245). The third group of patients had underlying diseases of great variety (e.g., carcinoma and anemia) but no impaired cell-mediated immunity. Of the 12 patients in this group, 7 died, principally those with some type of carcinoma (245). Survival of patients was associated with a lower degree of involvement of organs and better general health than for those who died. Thus, patients whose infection was limited to the skin and were without carcinoma or cell-mediated immune deficiency survived (245).

Disseminated infection in 10 of 11 kidney transplant patients and all 9 patients with chronic renal failure was caused by *M. chelonae* (94, 245). There was no other evidence of a bias in infections in any other groups of patients with disseminated disease (245).

Characteristics of *M. fortuitum*, *M. chelonae*, and *M. abscessus*

Since 1986, there have been significant changes in the names and taxonomy of the rapidly growing mycobacteria as a result of studies of DNA-DNA hybridization (127, 303, 318, 393) and 16S rRNA RFLP comparisons (127, 393). In some cases, biovariant and subspecies designations have been dropped because a group met the criteria for species designation (318, 420, 522). Species identification of rapidly growing mycobacteria is important, because the members differ in their susceptibilities to different antibiotics (522).

Measurement of the extent of DNA-DNA hybridization between rapidly growing mycobacteria demonstrated that *M. fortuitum*, *M. chelonae*, and *M. abscessus* are distinct species (303, 318). Phylogenetic analysis of 16S rRNA sequences demonstrated that *M. fortuitum*, *M. abscessus*, and *M. smegmatis* belonged to a unique tight, distinct cluster (420). Biovariants of *M. fortuitum* have been distinguished on the basis of sequence differences in 16S rRNA genes (290).

M. fortuitum, *M. chelonae*, and *M. abscessus* can be distinguished from one another and from other rapidly growing mycobacteria on the basis of a number of cultural and biochemical tests (303, 318). For several of the tests (i.e., growth on MacConkey agar medium and growth in 5% NaCl), the response is influenced by the temperature of incubation (303). *M. fortuitum* isolates grow at 43°C, grow on MacConkey agar medium at 37°C, grow in 5% NaCl at 37°C, have nitrate reductase activity, and are unable to utilize citrate as the sole carbon source (303, 318). *M. chelonae* isolates do not grow at 43°C, do not grow on MacConkey agar medium at 37°C, do not grow in 5% NaCl at 37°C, lack nitrate reductase activity, and utilize citrate as the sole carbon source (303, 318). *M. abscessus* isolates do not grow at 43°C, grow on MacConkey agar medium at 37°C, grow in 5% NaCl at 37°C, lack nitrate reductase activity, and are unable to utilize citrate as the sole carbon source (303, 318). These and other tests can be used for identification of other rapidly growing mycobacteria isolated from clinical and environmental sources (303, 318).

Rapidly growing mycobacteria have been shown to be responsible for degradation of a number of novel compounds. *Mycobacterium vaccae* has been reported to degrade alkanes (84), acetone, benzene, dioxane, styrene (61, 62), and trichloroethylene (61, 62, 520) and to degrade and transform sterols (519). *M. aurum* is capable of degrading ethene (199) and vinyl chloride (200) and of degrading or transforming sterols (231, 249, 519). *M. fortuitum* isolates can degrade polyhalogenated

phenols (376) and pentachlorophenol (506) and can degrade or transform sterols (231). *Mycobacterium phlei* can degrade and transform sterols (249). In addition, on the basis of the characteristics of growth, it is likely that those unidentified nontuberculous mycobacterial isolates reported to degrade a variety of other novel compounds are rapidly growing mycobacteria as well.

Source of *M. fortuitum*, *M. chelonae*, and *M. abscessus*

M. fortuitum, *M. chelonae*, and *M. abscessus* are widely distributed in the environment in relatively large numbers. Caution should be exercised in interpreting many reports describing the isolation of individual rapidly growing species from environmental samples, because of the changes in names and taxonomy. They have been isolated from freshwater rivers and lakes, seawater, and wastewater from hospitals (512), from animal drinking troughs (33), and from soils (263, 387). *M. fortuitum* was found in water samples collected from ponds, canals, and swamps in Germany (33) and in water samples collected in Zaire (395). In addition, *M. peregrinum* was isolated from ice in an ice machine (308).

Although an early systematic search for mycobacteria in drinking water showed that rapidly growing mycobacteria were seldom recovered (180), a 1984 review documented reports of recovery of *M. fortuitum* and *M. chelonae* (and probably *M. abscessus* as well) from drinking-water samples (86). A recent study has documented their presence and measured the numbers of *M. fortuitum* and *M. chelonae* organisms in drinking water as well (153). *M. fortuitum*, *M. chelonae*, and *M. abscessus* can grow in distilled water (70). Although neither *M. chelonae* nor *M. abscessus* can grow at 42°C, they were recovered in large numbers from a hot water drinking-water distribution system (153). Because nontuberculous mycobacteria are relatively resistant to chlorine (70, 195), it is likely that *M. fortuitum*, *M. chelonae*, and *M. abscessus* in water distribution systems can survive and even grow on the nutrients in the water or in biofilms (449). In fact, *M. chelonae* was found as a contaminant in a gentian violet solution (434). Their growth may be stimulated by the presence of novel compounds such as polyhalogenated phenols (376) and pentachlorophenol (506) in polluted natural waters or water distribution systems.

There is evidence that the environment is the source of rapidly growing mycobacteria that infect patients. Isolates of *M. fortuitum* and *M. abscessus* recovered from a water bath in an operating room had the same patterns of susceptibility to antimicrobial agents, multilocus enzyme electrophoresis patterns, and plasmids as did *M. fortuitum* and *M. abscessus* isolates from patients following cardiac bypass surgery (525). The availability of those techniques, as well as methods for comparison of rRNA RFLP patterns (562), will permit the identification of sources and routes of infection by rapidly growing mycobacteria.

Soil is another habitat for *M. fortuitum*. *M. fortuitum* has been recovered from a variety of soils in the northern (550) and southern (263) United States, Wales (387), and Zaire (395). Although those reports did not assign any of the rapidly growing isolates recovered from soils to the species *M. chelonae* or *M. abscessus*, it is clear from the characteristics of the strains that many could have been *M. chelonae* or *M. abscessus*. *M. fortuitum* and *M. chelonae* (and possibly *M. abscessus*) are able to colonize and survive in soils (479). Therefore, it is unlikely that *M. chelonae* and *M. abscessus* are absent in soils. As was suggested for waters, it is likely that the growth of pathogenic rapidly growing mycobacteria would be stimulated

by the presence of novel compounds such as polyhalogenated phenols (376) and pentachlorophenol (506) in polluted soils.

In addition to soil and water, *M. fortuitum* has been isolated from raw milk (132) and from tissue samples of feral buffaloes (210). *M. chelonae* (and possibly *M. abscessus* too) has been found frequently in sphagnum vegetation in Sweden, Norway, and Germany (277) and was responsible for infections in numbats in the Perth Zoo (169). Evidence of prosthetic valve endocarditis caused by *M. chelonae* (413) suggests that the replacement valves can be contaminated with *M. chelonae*.

Risk Factors for *M. fortuitum*, *M. chelonae*, and *M. abscessus* Infection

Because of the widespread presence of *M. fortuitum*, *M. chelonae*, and *M. abscessus* in the environment and drinking-water systems, everyone is exposed to these rapidly growing mycobacteria. In addition, exposures to contaminated solutions (e.g., gentian violet) (434), medical equipment (e.g., bronchoscopes) (522), and materials used in surgery (e.g., prosthetic heart valves) (413) are risk factors for infections by rapidly growing mycobacteria.

Risk factors for pulmonary infections caused by *M. fortuitum* or *M. abscessus* included previous or current mycobacterial disease, cystic fibrosis, bronchiectasis, and chronic vomiting (186). Risk factors for localized skin or soft tissue infections were trauma or surgery (523). Risk factors for one of the three groups of patients with disseminated *M. chelonae* or *M. abscessus* infections included kidney transplantation, collagen vascular disease, corticosteroid therapy, or chronic renal failure (245). Corticosteroid therapy was also associated with *M. chelonae* infection (245). Cell-mediated immunity defects, lymphoma, and leukemia were risk factors for a second group (245). Corticosteroid therapy and chronic renal failure were also risk factors for catheter-associated infections caused by rapidly growing mycobacteria (522).

Chemotherapy of *M. fortuitum*, *M. chelonae*, and *M. abscessus* Infections

Like the slowly growing nontuberculous mycobacteria, *M. fortuitum*, *M. chelonae*, and *M. abscessus* are resistant to many of the antimycobacterial drugs (451, 522). The presence of tetracycline resistance genetic determinants in members of the *M. fortuitum* group (381) clearly precludes the use of tetracyclines. Susceptibility testing is useful in guiding the choice of antibiotic therapy (481, 522). *M. fortuitum* strains are susceptible to amikacin, ciprofloxacin, sulfonamides, cefoxitin, and imipenem (522, 526). *M. chelonae* strains are susceptible to amikacin, tobramycin, and imipenem and resistant to cefoxitin (522, 526). Strains of *M. abscessus* are susceptible to amikacin and cefoxitin (522, 526). All three species are susceptible to clarithromycin (58) and azithromycin (522), and a multidrug regimen including clarithromycin has been used to successfully treat *M. abscessus* infections (370).

NEWLY DESCRIBED NONTUBERCULOUS MYCOBACTERIA

New *Mycobacterium* Species

New *Mycobacterium* species have been discovered and distinguished from other species by 16S rRNA gene sequences. Unfortunately, because of their recent discovery, there is insufficient information concerning their epidemiology. Two new, slowly growing, nonpathogenic species have been isolated from sphagnum vegetation in New Zealand (*Mycobacterium*

cooki) (278) and in Ireland (*Mycobacterium hiberniae*) (275). *Mycobacterium celatum* sp. nov. (65) and *Mycobacterium intermedium* sp. nov. (356) are two newly described slowly growing mycobacterial species that have been isolated from patient samples. *M. intermedium* should not be confused with *Mycobacterium interjectum*, which is another slowly growing mycobacterium related to *M. simiae* (296). Slowly growing mycobacteria, isolated repeatedly from sputum samples collected from patients with pulmonary infections in Finland and Somalia, which resembled yet were distinct from *M. xenopi* and the *M. avium* complex have been described (51). Four of the Finnish isolates belonged to type I of *M. celatum*, and the one Somali isolate belonged to type II of *M. celatum* (65). The remaining Finnish isolates form a new species, *Mycobacterium branderi*, that is related to *M. celatum*, *M. cookii*, and *M. xenopi* on the basis of 16S rRNA gene sequence (296). The *M. branderi* isolates were resistant to isoniazid and rifampin and susceptible to ethambutol and streptomycin (51). Mycobacteria that grew slowly in liquid media but failed to grow on solid media have been isolated from AIDS patients (220, 456). Possibly, they include representatives of *M. genavense*.

A new and possibly pathogenic rapidly growing mycobacterial species, *Mycobacterium brumae*, defined on the basis of DNA-DNA hybridization, has been isolated from water, soil, and sputum samples in Barcelona, Spain (323). Three other new, nonpathogenic rapidly growing mycobacterial species have also been described: *Mycobacterium confluentis*, isolated from human sputum (289); *Mycobacterium madagascariense*, isolated from sphagnum vegetation (276); and *Mycobacterium alvei*, isolated from human sputum, soil, and water samples (17).

Characteristics of *M. celatum*, *M. intermedium*, and *M. branderi*

The maximum temperature for growth is 37°C for *M. celatum*, 41°C for *M. intermedium*, and 45°C for *M. branderi*. *M. intermedium* is photochromogenic, while *M. celatum* and *M. branderi* are nonpigmented. Strains of *M. intermedium* hydrolyze Tween 80 and have catalase and urease activities (356). Strains of *M. celatum* fail to hydrolyze Tween 80 and have catalase and lack urease activity (65). *M. branderi* strains fail to hydrolyze Tween 80 and lack both catalase and urease activities (296). Strains of all three species have arylsulfatase activity.

Antibiotic susceptibilities for *M. celatum* and *M. intermedium* have been reported. Both are resistant to 1 µg of isoniazid per ml and susceptible to 5 µg of ethambutol per ml (65, 356). *M. celatum* is resistant to 1 µg of rifampin per ml and susceptible to 2 µg of streptomycin per ml and 2 µg of ciprofloxacin per ml (65). *M. intermedium* is susceptible to 1 µg of rifampin per ml and resistant to 2 µg of streptomycin per ml (356).

CONCLUDING REMARKS

Challenges

The number, morbidity, and mortality of AIDS patients infected with nontuberculous mycobacteria, principally *M. avium*, are major challenges facing physicians and mycobacteriologists today. Specifically, the challenges include (i) developing or identifying antimicrobial agents with efficacy in prophylaxis and therapy of nontuberculous mycobacterial infections; (ii) assessing the impact of polyclonal and mixed mycobacterial infection; (iii) developing methods, such as PCR and HPLC, for rapidly detecting the presence of nontuberculous mycobac-

teria, even in patient specimens containing substantial numbers of nonmycobacterial microorganisms; (iv) adapting methods for typing individual strains (i.e., clones) within a species of nontuberculous mycobacteria to identify sources of infection; and (v) determining whether prevention of nontuberculous mycobacterial infection is possible, either through antimycobacterial drug therapy or through reduction of exposure of patients to sources of the mycobacteria. In addition, responses to these challenges must proceed while vigilance for new, undescribed mycobacterial species is increased. Evidence of increased frequencies of recovery of seldom-encountered nontuberculous mycobacteria (in the United States), such as *M. malmoense*, *M. xenopi*, and *M. genavense* (453), is an additional challenge.

A number of observations suggest that nontuberculous mycobacterial infection may not always occur with the same clinical symptoms, especially in patients with AIDS. First, nontuberculous mycobacterial disease may be mistaken for tuberculosis. The clinical picture of *M. xenopi* pulmonary disease resembled *M. tuberculosis* disease in two patients with AIDS (254). Second, mycobacterial species with low-temperature optima for growth should not be excluded from consideration as agents of disseminated mycobacterial infection. Disseminated infection caused by either *M. marinum* (306, 484) or *M. haemophilum* (421, 559) has been reported. Finally, a number of nontuberculous mycobacterial species not normally encountered as agents causing cutaneous infection (e.g., *M. kansasii*) have been isolated from AIDS patients with cutaneous infections (53).

Knowledge of the epidemiology of nontuberculous mycobacterial infections in immunocompetent individuals should not be forgotten in responding to the needs of AIDS patients. For example, because the geographic distribution of *M. xenopi* in water distribution systems is not uniform (86) and *M. xenopi* disease occurs in clustered outbreaks (462), it is expected that clusters will occur among AIDS patients. This may also be the case for *M. haemophilum* and presents a challenge for studies designed to identify its source(s).

Remaining Questions

A large number of questions remain, in spite of the marvelous and intensive progress that has been made in the last 15 years. The following list is by no means exhaustive, but it is provided with the hope that this review can recruit new persons to the study of the mycobacteria.

(i) Why does *M. avium* infection predominate in AIDS patients? Is it (a) because *M. avium* has special virulence factors, (b) because the source of infection is drinking water distribution systems where *M. avium* may predominate, or (c) because of a disappearance of *M. intracellulare* from natural and drinking waters?

(ii) Why is *M. avium* infection rare among African patients with AIDS? Is it (a) because these persons succumb before *M. avium* infection can appear or (b) because they are not normally exposed?

(iii) Why does *M. avium* persist in drinking-water distribution systems? (a) Does it form biofilms and grow in such systems? (b) Do physiologic and biologic factors lead to the persistence of nontuberculous mycobacteria in drinking-water distribution systems?

(iv) What are the sources of the newly discovered nontuberculous mycobacteria, such as *M. haemophilum* and *M. genavense*?

(v) What are the mechanisms of virulence of the nontuberculous mycobacteria? (a) Is there a role for environmental

selection in the development of virulence? (b) Does selection for resistance to light- and organic matter-catalyzed production of toxic oxygen metabolites lead to enhanced survival in infected humans?

(vi) What are the mechanisms of antibiotic resistance in nontuberculous mycobacteria? (a) Does cell surface hydrophobicity provide a barrier to antibiotic entry, and (b) are mycobacteria capable of adapting and hence surviving antibiotic challenge?

(vii) Does the ability of nontuberculous mycobacteria to metabolize a variety of novel substances influence their ecology and epidemiology?

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