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REVIEW ARTICLE

Environmental Mycobacteria: A Threat to Human Health?

Mónica Rosenblueth, Julio C. Martinez-Romero, Mariana Reyes-Prieto,
Marco A. Rogel, and Esperanza Martinez-Romero

In many cases, bacterial pathogens are close relatives to nonpathogens. Pathogens seem to be limited lineages within nonpathogenic bacteria. Nonpathogenic isolates are generally more diverse and widespread in the environment and it is generally considered that environmental bacteria do not pose a risk to human health as clinical isolates do; this may not be the case with mycobacteria, but environmental mycobacteria have not been well studied. It is documented that several environmental mycobacteria constitute a source for human infections. Diverse mycobacterial environmental isolates are rarely involved in human disease. Environmental mycobacteria may have a role in degradation of different compounds. Environmental mycobacteria have had a long interaction with humans, maybe as long as the human species, and may have contributed to human evolution.

Introduction

Most bacteria are innocuous and even beneficial to different organisms; they play a central role in biogeochemical cycles. Pathogens seem to be limited lineages within a larger group of environmental or commensal isolates and many bacterial pathogens are closely related to nonpathogenic bacteria. Further, some of the human pathogenic bacteria seem to be recently evolved bacterial lineages such as *Mycobacterium tuberculosis*, *Yersinia pestis*, *Burkholderia mallei*, and *Bacillus anthracis* (Fabre *et al*., 2004; Gutierrez *et al*., 2005; Losada *et al*., 2010). *Escherichia coli* and *Bacillus* are the best-studied bacteria; they contain pathogenic and nonpathogenic strains or species. In some bacteria, the genetic bases of pathogenicity have been elucidated (Groisman and Casadesus, 2005; Luna *et al*., 2006; Schuch *et al*., 2009; Wang *et al*., 2010) in relation to pathogenicity islands (Hacker and Kaper, 2000), plasmids, or phages (encoding toxins) that exist in pathogenic bacteria. There is the general notion that environmental bacteria do not represent a risk to human health. Environmental isolates lacking virulence genes would be nonpathogenic and harmless. Does this hold for mycobacteria? Are there also harmless mycobacteria? Do some mycobacterial environmental isolates lack virulence genes? Environmental mycobacteria are widespread. Mycobacteria have had a long and well-known interaction with humans; tuberculosis and leprosy have been dreaded diseases for millennia.

Excellent reviews have been published on environmental and nontuberculous mycobacteria (Falkinham, 2003; Primm *et al*., 2004; van Ingen *et al*., 2009; Alvarez-Uria, 2010; Kazda *et al*., 2009) and on the pathogenic mycobacteria (Cosma *et al*., 2003). The information contained therein will not be repeated here and we will only cover the pertinent literature addressing the new questions posed here.

A Context of Different Environmental and Clinic Bacteria

In a comparative genomic analysis of klebsiella (Reyes-Prieto *et al*., unpublished), we found that there was an uneven distribution of virulence genes in environmental and clinical klebsiellas. There were virulence genes in klebsiellas obtained from the environment, so it is not assured that environmental bacteria would be completely safe. However, *Klebsiella variicola* plant isolates (from maize plants) had fewer virulence genes than clinical *Klebsiella pneumoniae* isolates. A different epidemiological dynamics has been suggested for *K. pneumoniae* and *K. variicola* (Martínez *et al*., 2004). *K. pneumoniae* isolates are transmitted from human to human and *K. variicola* isolates most probably from the environment to human. *K. variicola* isolates fix nitrogen, but this is not a characteristic of *K. pneumoniae* (Rosenblueth *et al*., 2004). Nitrogen fixation is advantageous in the environment, especially in association with plants but probably not so in human patients. In *Klebsiella*, genes determining nitrogen fixation as well as genes involved in cellulose or pectin (natural products existing only in plants) degradation allowed us to recognize that the human-infecting *K. variicola* should be environmental borne (Martínez *et al*., 2004). Nitrogen fixing genes may be easily lost when not needed. A *K. variicola* strain isolated as a maize endophyte promoted increased crop yields when used in agriculture as inoculant for wheat and maize in the United States (Iniguez *et al*., 2004). This strain was no longer used in agriculture after virulence genes were detected in its genome.

Interestingly, genes that are found in human bacterial pathogens (considered virulence determinants) such as type

Centro de Ciencias Genomicas, Universidad Nacional Autonoma de Mexico, Cuernavaca, Morelos, Mexico.

III secretion systems are also required in plant colonization in *Rhizobium* (Marie *et al*., 2001), a well-known plant mutualist that has been safely used in agriculture as plant inoculants for over 100 years. In *Azoarcus*, a rice endophyte with promising biotechnological applications, cable pili are needed for plant colonization (Böhm *et al*., 2007), and they are required for epithelium colonization by pathogenic bacteria (Urban *et al*., 2005). Some plant-associated bacteria with genes for eukaryotic infection could be potential human pathogens and sick people, especially immune-compromised patients, could be at risk. Bacterial species from *Stenotrophomonas*, *Serratia*, *Klebsiella*, and *Salmonella enterica* Typhimurium that have been reported as plant endophytes (Araujo *et al*., 2001; Gyaneshwar *et al*., 2001; Dong *et al*., 2003; Barac *et al*., 2004; Martínez *et al*., 2004; Rosenblueth *et al*., 2004) are also human pathogens or opportunistic pathogens. The use of potential human pathogens as crop inoculants is not recommended as an agricultural practice (Parke and Gurian-Sherman, 2001; Rosenblueth and Martínez-Romero, 2006).

Bacteria that are able to use many nutrients and are highly resistant to different stresses may be capable to colonize many plants and it is not surprising that some endophytes (associated bacteria inside the plant) may be human pathogens or opportunistic human pathogens (Rosenblueth and Martínez-Romero, 2006); a similar argument has been considered to explain that very successful biocontrol agents are opportunistic human pathogens (Parke and Gurian-Sherman, 2001). Among rhizospheric (on roots) bacteria, opportunistic human pathogens are commonly found as well (Berg *et al*., 2005).

The Amazon River, the water of which is considered suitable for human consumption, contains *Chromobacterium violaceum*, which confers the river its color by producing violacein. It has been reported that *C. violaceum* or *Agrobacterium* (plant pathogen relative to *Rhizobium*) may cause serious human disease in rare cases (Alnor *et al*., 1994; Teoh *et al*., 2006). Nevertheless, clinical agrobacteria are phylogenetically distant from other agrobacterial species (Aujoulat *et al*., 2011) and a similar situation seems to occur in *Burkholderia*. In burkholderias, most of the highly pathogenic bacteria are a distinct phylogenetic group from the mostly nonpathogenic plant and other environmental isolates (Caballero-Mellado *et al*., 2004). We have isolated *Acinetobacter* as plant endophytic bacteria from bean seeds (López-López *et al*., 2010) that are not closely related to the clinical *Acinetobacter* isolates (Dijkshoorn *et al*., 2007). Only some strains of *Vibrio cholerae* are highly virulent and there is a larger diversity of nonpathogenic environmental isolates (Faruque *et al*., 2004). Most nonpathogenic mycobacteria are phylogenetically separated from the slow-growing mycobacteria that contain mostly pathogens (*M. tuberculosis*, *M. leprae*, *M. bovis*). This contrasts to rhizobia, wherein no human pathogens occur in the slow-growing group (*Bradyrhizobium*), but have been detected in the fast-growing bacteria related to *Agrobacterium*, which produce tumors in plants (Aujoulat *et al*., 2011). *Afipia*, close relatives to bradyrhizobia, have been regularly recovered from various clinical samples and may be associated with nosocomial infections (La Scola *et al*., 2002).

Previously described examples illustrate that some clinical isolates are distinct from environmental populations. On the other hand some environmental isolates and human pathogens may be undistinguishable. In *Burkholderia*, clinical strains have been reported to group with rhizospheric and bioremediation strains (Baldwin *et al*., 2007). *Burkholderia vietnamiensis* is a nitrogen-fixing rice isolate that has been used for rice fertilization (Govindarajan *et al*., 2008). Recently, its environmental use has been banned in many countries because of its belonging to the cepacia complex that poses a high risk for people with cystic fibrosis (Holmes *et al*., 1998). Similarly, environmental and clinical *Pseudomonas aeruginosa* were equally virulent in model hosts (Vives-Flórez and Garnica, 2006).

Environmental Mycobacteria and Human Disease

Mycobacteria are very diverse, with over 140 species currently reported. In recent years, a large number of new mycobacterial species have been described from the environment. There are indications that environmental mycobacterial diversity is much broader than we suspected (Primm *et al*., 2004; our own unpublished results). There is compelling evidence that human mycobacterial infections may be acquired from the environment (Wolinsky, 1979; Primm *et al*., 2004; De Groote *et al.* 2006; van Ingen *et al*., 2009). However, it is clear that as mycobacteria seem to be everywhere human contact with environmental mycobacteria is inevitable (the same holds for *P. aeruginosa*) and the mild transient infections that mycobacteria may produce may be asymptomatic and self-cured. Nevertheless, nontuberculous mycobacteria are increasingly encountered as human pathogens (van Ingen *et al*., 2009). Around 20% of the species have been isolated both from clinics and the environment. Around 30% of mycobacterial species (the numbers may vary in the different surveys, and many environmental isolates have not been designated as formal species) have not been isolated from clinics but only from the environment. Many have not been characterized with regard to their effects on human health. Mycobacterial species considered to be innocuous (Tortoli, 2003) are indicated in a phylogenetic tree (Fig. 1). Many environmental isolates belong to the group of fast-growing mycobacteria (Pitulle *et al*., 1992), but they have also been detected in the slow-growing group.

Environmental mycobacteria are found in soil, rivers, oceans, pipes distributing drinking water (Falkinham *et al*., 2001; Le Dantec, 2002; Dailloux *et al*., 2003; September *et al*., 2004; Young *et al*., 2005), or sewage and have also been found in bath showers and other manmade sites. It has been recommended that people with lung diseases avoid mycobacterial aerosols (Falkinham, 2003) from contaminated sources. Hospital shower mycobacteria are the same strains as those found in patients and have been recognized as a source of nosocomial infections (Falkinham *et al*., 2008).

Mycobacterium avium is found in soil and dust and may survive for a long time outside hosts (Whittington *et al*., 2004). Recently, an increase (not due to better sampling or detection procedures) has been found in infections with *M. avium* in the aging population from The Netherlands with chronic obstructive pulmonary disease (van Ingen *et al*., 2010a). *M. avium* strains have been isolated from patients with AIDS (Bellamy *et al*., 2004) and seem to be a major cause of death. The high incidence of *M. avium* infections is correlated with high numbers of these bacteria in environmental or drinking water (Glover *et al*., 1994; Pierce, 2009), but dust from potting soil (especially peat) may also be a source of human infections (De Groote *et al*., 2006; De Groote and

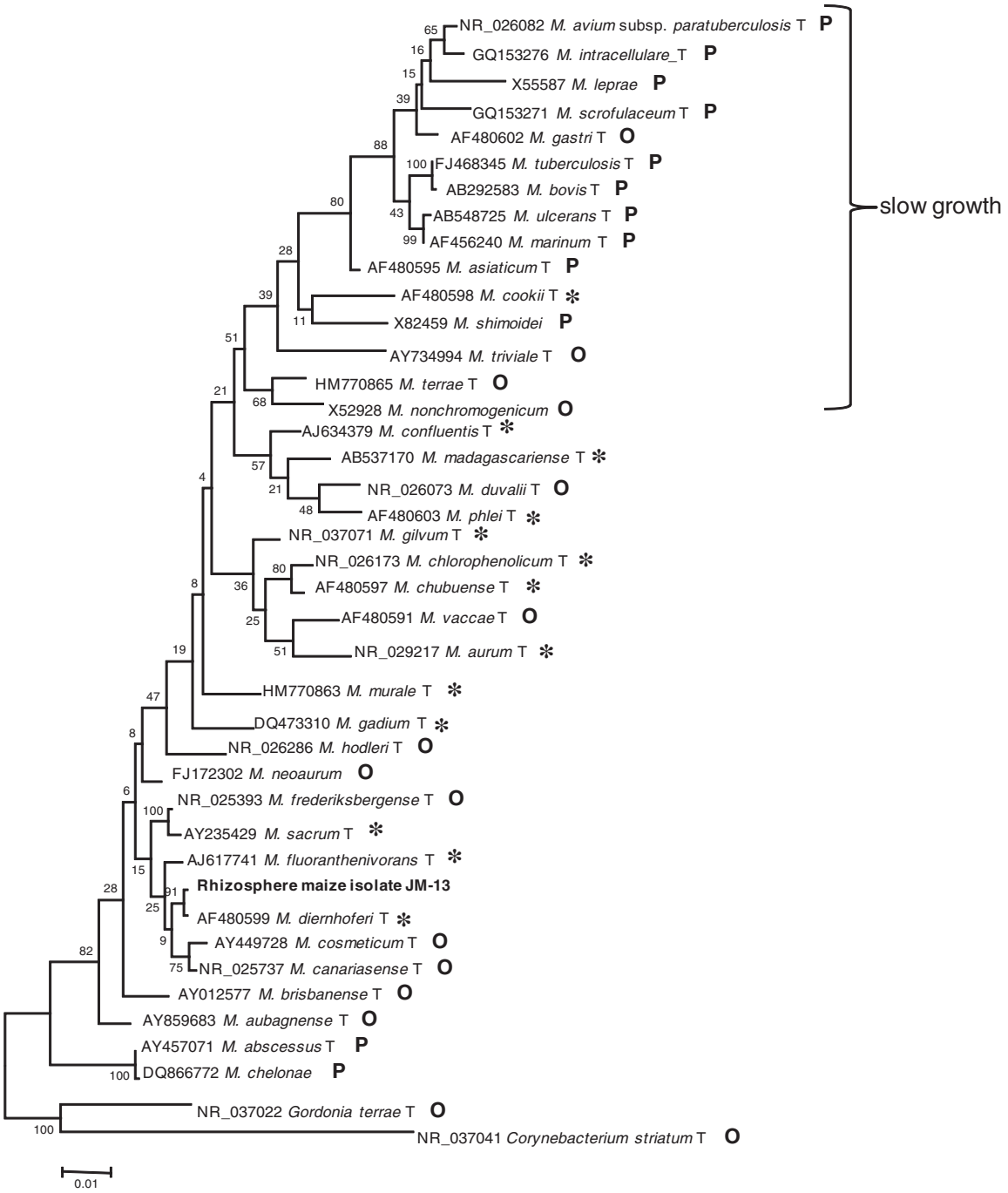

FIG. 1. 16S rRNA phylogeny of some *Mycobacterium*, species constructed with maximum likelihood under model GTR and rooted with *Gordonia terrae* and *Corynebacterium striatum.* T, type strain; *, nonpathogenic, purely environmental; O, oportunistic or rare pathogenic; P, highly pathogenic. Bolded terms indicate maize rhizosphere.

Huitt, 2006). High incidence of mycobacterial infections have been found in gardeners. The use of protective antidust masks is recommended. Genomic islands have been identified in *M. avium* subspecies *paratuberculosis*, which is implicated in cases of Crohn's disease, but are not present in *M. avium* subspecies *avium*, which infects immunocompromised patients (Wu *et al*., 2006). Environmental or human *M. avium* are not the same populations as those obtained from birds (Johansen *et al*., 2009).

Mycobacteria have also been found in swimming pools (Leoni *et al*., 1999). Otomastoiditis has been detected in children and is caused by *Mycobacterium abscessus*, and it is attributed to their swimming in pools (van Ingen *et al*., 2010b). Finding mycobacteria in such environments is related to their resistance to chloride (Taylor *et al*., 2000) and the capacity of mycobacteria to make biofims (Schulze-Robbecke *et al*., 1992; Ojha *et al*., 2005). Bacteria in biofims are highly resistant to different antimicrobials (Stewart and Costerton, 2001).

Pathogens may have their reservoirs in the environment and this may be a source of human infections, as is the case with *Mycobacterium ulcerans*, which thrives in water plants and aquatic insects (Marsollier *et al*., 2002, 2004; Mosi *et al*., 2008).

In the most acid river in the world, the Tinto River in Spain, a novel species was identified resembling *Mycobacterium shimoidei* (Garcia-Moyano *et al*., 2007). *M. shimoidei* was found as a rare clinical isolate (Tsukamura, 1988) and has been also found in water distribution systems. From the Thames River in London, *Mycobacterium fluoranthenivorans* was identified (Boden *et al*., 2008); this species had been isolated from contaminated soil with polycyclic aromatic hydrocarbons from an abandoned gas-producing mine in Germany (Hormisch *et al*., 2004).

The Han River in Seoul Korea was studied with a culture-independent approach using a restriction enzyme analysis of the *hsp65* gene obtained by PCR and its sequence (Lee *et al*., 2008). *M. gordonae*, *M. terrae*, *M. kumamotonense*, *M. peregrinum*, *M. intracellulare*, *M. holsaticum*, *M. aichiense*, *M. phocaicum*, *M. arupense*, *M. brisbanense*, *M. chubuense*, *M. gilvum*, *M. lentiflavum*, *M. gadium*, and other nonclassified *Mycobacterium* sp. were identified. River-borne mycobacteria include bacteria that are not innocuous.

Using culture-dependent and -independent approaches (with *rpoB*- and *hsp65*-specific primers and sequencing), we analyzed mycobacteria (Sachman-Ruiz *et al*., 2009) from two rivers in Mexico, one highly polluted (Apatlaco River) and the other not (Tembembe River). A large diversity of fast-growing mycobacteria and also *M. avium* was detected in the non-polluted Tembembe River and in the Apatlaco River, which crosses an important urban area in Cuernavaca, Morelos in Mexico. It is unknown whether mycobacteria, especially *M. avium*, from the polluted Apatlaco River, which is in close contact with a large population, may be a source of human infections. Our data highlighting a potential risk prompted the authorities to start building collectors and sewage plants for these waters.

Mycobacteria have been reported in a river at the Mexican-United States border, Rio Grande (Bland *et al*., 2005). Mycobacterial presence was correlated with enterobacterial numbers meaning that this river carried bacteria excreted by humans. In the United States, new rules allow 0 enterobacteria per 100 mL potable water.

In developed countries, lymphadenitis in babies and small children are due to mycobacterial infections in mandibular and lymph nodes (Wolinsky, 1995); surprisingly, there has been a shift in the species involved, from *Mycobacterium scrofulaceum* to *M. avium* (Wolinsky, 1995). This shift has been related to the increases of *M. avium* in water (Primm *et al*., 2004).

Mycobacteria in Human Evolution

As there is now evidence that different rivers (Boden *et al*., 2008; Lee *et al*., 2008; Sachman-Ruiz *et al*., 2009) and lakes (van Ingen *et al*., 2009) around the world naturally contain mycobacteria, it seems probable that humans who have drunk water from rivers and lakes for hundreds of thousand years have ingested mycobacteria for most of our living history. As mycobacterial composition may not be the same everywhere and may depend on environmental conditions, local human populations may have been selected for their resistance to the local mycobacteria. In a sense, this must have shaped human evolution, and this can also be applied to many other bacteria of which we know little about. A closer contact with soil and soil mycobacteria may have arisen with agriculture, dating from only around 10,000 years ago, followed by a rise in contact with farm animals and their bacteria.

Mycobacteria in Plants

Mycobacteria have been found as wheat endophytes (Conn and Franco, 2004). When studying the bacterial community associated with plant roots we found mycobacteria related to *Mycobacterium diernhoferi* in the maize rhizosphere (Fig. 1). This species is considered nonpathogenic and has been previously found together with *Mycobacterium vanbaalenii*, *Mycobacterium mageritense*, *Mycobacterium austroafricanum*, *Mycobacterium chubuense*, or *Mycobacterium chlorophenolicum* in contaminated soils (Cheung and Kinkle, 2001).

Upon leaving Cuernavaca, the Apatlaco River is used for irrigation of a large agricultural area where maize and other crops are grown. We wondered whether mycobacteria could proliferate in plants irrigated with mycobacteria-contaminated water. A pending question in mycobacterial research is which are the actual sites of mycobacterial replication. Bacteria form biofilms on plant roots and mycobacteria seem to be seed species for biofilm formation in different niches. We found no mycobacteria on maize roots irrigated with the contaminated river water (from the Apatlaco River crossing Cuernavaca) in laboratory assays using culture-dependent and -independent approaches. A large diversity of other bacteria was encountered in the irrigated roots but not in control plants (not shown).

Beneficial Mycobacteria

Kazda *et al.* (2009) published a review on the biological role of mycobacteria in the environment. Some mycobacteria have been recognized as having biotechnological advantages because of their capacity to degrade petroleum-contaminated soils and may be used in bioremediation (Cheung and Kinkle, 2001; Coleman and Spain, 2003; Hormisch *et al*., 2004; Leys *et al*., 2005; Hennessee *et al*., 2009).

Among the fast-growing mycobacteria, *Mycobacterium vaccae* is used in vaccination (Yang *et al*., 2010). Environmental mycobacteria could stimulate immunity against pathogenic mycobacteria and help build resistance to them (Black *et al*., 2001; Fine, 2001); they possibly prevent asthma or allergies (Wang and Rook, 1998; Black, 2001). The health impacts of environmental bacteria were extensively reviewed by Primm *et al.* (2004). It was also reported that environmental mycobacteria interfere with the response to vaccination (Brandt *et al*., 2002; Young *et al*., 2007; Mendoza-Coronel *et al*., 2011).

Environmental Mycobacteria Predisposition for Human Infections

It seems that several environmental isolates have genes that predispose them to infect humans. General characteristics of mycobacteria are their resistance to stress, extreme temperatures, acidity, chlorides, and starvation. These may be predisposing characteristics to resist defense responses during infections. Are fast-growing mycobacteria less tolerant to such stresses?

Mycobacteria are old prokaryotic lineages, and for a long time, they have probably constituted prey for protozoa. Many are able to survive inside amoebas, and consequently, they have a long experience colonizing and persisting inside eukaryotic cells (Harriff *et al*., 2008).

Mycobacteria, like other bacteria, have genetic plasticity, their genes may be lost or acquired, and genetic variation may determine degrees of pathogenicity (Brosch *et al*., 2001; Bhatt *et al*., 2002; van der Sar *et al*., 2004; Wang and Derbyshire, 2004; Gutierrez *et al*., 2005). Environmental mycobacteria could be recipients of pathogenicity determinants from pathogenic mycobacteria (Picardeau and Vincent, 1997).

Conclusion

Are environmental mycobacteria a threat to human health? This is still an open question and it depends on the environmental mycobacteria and as a corollary on the environment. Many environmental mycobacteria remain unknown. The human impact on mycobacterial ecology and diversity has been recognized, with human activities causing a selection in the environment of more stress-resistant mycobacterial species such as *M. avium* in place of *M. scrofulaceum* (Primm *et al*., 2004), and the culprit here may be the choride (added by humans) in water. For some environmental mycobacteria, there is no doubt that they are pathogens, and others may be less virulent and could even prevent pathogen infections (Fine, 2001). As discussed above, mycobacteria seem to have a great capacity to colonize humans and animals and have a long and extensive history of interactions with humans. In most mycobacterial species, the genetic basis of pathogenicity is unknown. It is difficult to definitively ascertain whether a particular strain or a newly found species from the environment may be a potential or an opportunistic pathogen. The fact that an environmental bacterium is not found in clinics does not warrant the conclusion that it is not pathogenic. Unless adequate pathogenicity tests are performed with strains to be used in bioremediation or other biotechnological applications, there should be restrictions to handle them or to deliver them to the environment. We still know very little about mycobacteria. "More information about the pathogenesis and treatment of NTM (nontuberculous mycobacteria) is needed . . . physicians and scientists should join efforts to . . . studies of specific species . . . in the different groups of human hosts" (Alvarez-Uria, 2010).

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Address correspondence to:
Dr. Esperanza Martinez-Romero
Centro de Ciencias Genomicas
Universidad Nacional Autonoma de Mexico
Cuernavaca 62210
Morelos
Mexico

E-mail: emartine@ccg.unam.mx

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