

Potentially Pathogenic, Slow-Growing Mycobacteria Released into Workplace Air During the Remediation of Buildings

Sirpa Rautiala,¹ Eila Torvinen,² Pirjo Torkko,^{2,3} Sini Suomalainen,⁴
Aino Nevalainen,² Pentti Kalliokoski,⁵ and Marja-Leena Katila³

¹Kuopio Regional Institute of Occupational Health, Kuopio, Finland

²National Public Health Institute, Kuopio, Finland

³Kuopio University Hospital, Kuopio, Finland

⁴University of Helsinki, Helsinki, Finland

⁵University of Kuopio, Kuopio, Finland

*Construction workers' exposure to airborne viable mycobacteria was studied during the remediation of three moldy and two nonmoldy buildings. Furthermore, the concentrations of airborne fungal and actinobacterial spores were determined. The samples for the microbial analyses were collected using a six-stage impactor and an all-glass impinger sampler, and by filter sampling. Specific mycobacteria media and nonselective media were used for the cultures. The samples were cultured for the total numbers of rapidly growing and slow-growing mycobacteria, and the isolates obtained were identified to the genus or species level. Mycobacteria were recovered from the air during the remediation of two of the moldy buildings and one nondamaged building. Concentrations of mycobacteria up to 160 cfu/m³ were detected. A total of 43 mycobacterial isolates was recovered. Most of the isolates were slow-growers, only two rapid-growing strains being detected. The 38 identified isolates belonged to potentially pathogenic species, including *Mycobacterium avium* complex, *M. scrofulaceum*, and *M. fortuitum*, and to saprophytic species, including *M. nonchromogenicum* and *M. terrae*. Mycobacteria were the most often detected in samples taken with a six-stage impactor. They were found in buildings with both high and low concentrations of fungi. In conclusion, mycobacteria, both potentially pathogenic and saprophytic species, may be released into the indoor air during the remediation of buildings.*

Keywords airborne mycobacteria, construction work, indoor air

Address correspondence to: Sirpa Rautiala, Kuopio Regional Institute of Occupational Health, P.O. Box 93, 7071 Kuopio, Finland; e-mail: sirpa.rautiala@ttl.fi.

Construction workers have been found to be exposed to high concentrations of fungal and actinobacterial spores during the remediation of moldy structures.^(1,2) Such exposure has also been found

to be associated with an increased prevalence of respiratory symptoms, including dry cough, runny nose, eye irritation, and hoarseness.⁽³⁾ It has been suggested that actinobacterial and fungal spores released from damaged building materials would cause these kinds of health effects. However, there are several other biologically active microorganisms (e.g., bacteria)^(4,5) present simultaneously although they have not been well characterized. Currently, the role of various microorganisms as possible causal agents of the health effects remains obscure.

Mycobacteria are acid-fast, slow-growing Gram-positive bacteria. The most harmful mycobacteria for man are the human tuberculosis and leprosy bacilli. Environmental mycobacteria, which are also called atypical mycobacteria, or mycobacteria other than *Mycobacterium (M.) tuberculosis*, are heterotrophic species that take part in the decomposition of organic matter. Environmental mycobacteria occur in soil and natural waters,^(6,7) but also in man-made water environments.^(8,9)

Environmental mycobacteria can be divided into two main groups on the basis of their growth rate. The rapid-growing mycobacteria are seen as visible colonies grown from a dilute inoculum in less than 7 days, while the slow-growing mycobacteria usually need several weeks before visible colonies appear.⁽¹⁰⁾ In general, slow-growers are usually potentially pathogenic to humans and animals, while many of the rapid-growing mycobacteria are regarded as nonpathogenic.

Infections caused by environmental mycobacteria are becoming increasingly common in the United States and other parts of the world.^(11,12) There is no evidence of person-to-person transmission, and the environment is regarded as the primary source of infections. Slow-growing mycobacteria (e.g., *M. avium*, *M. intracellulare*, and *M. scrofulaceum*) can cause

chronic pulmonary diseases resembling tuberculosis, cervical lymphadenitis, and skin and other soft-tissue infections.^(11,12) Mycobacteria are also effective stimulators of the immune system (i.e., they are capable of stimulating macrophages to produce inflammatory mediators such as cytokines).^(13,14) Furthermore, aerosolized mycobacteria have been associated with hypersensitivity pneumonitis among metal workers.⁽¹⁵⁾ Evidently, the sources of environmental mycobacteria are still insufficiently known, as is their importance as a causal agent of occupational diseases.

The slow growth rate of mycobacteria makes it difficult to isolate them from samples rich in other microorganisms. Incubation times of 6 weeks to 5 months are mostly needed for the detection of slow-growing mycobacteria. Decontamination is an essential part of their culturing, necessary for the destruction of more rapid-growing flora. If too harsh, decontamination becomes detrimental, not only to contaminating flora, but also to mycobacteria, and, if too mild, it may allow the growth of fast-growing contaminating microorganisms, which prevent the growth of mycobacteria. In addition, selective media are needed for the isolation of slow-growing mycobacteria.

Environmental mycobacteria have only recently been taken into account in the study of moldy indoor environments and assessments of their adverse health effects. Rapid-growing mycobacteria have been isolated from the indoor wall of a children's day care center, where workers had reported work-related upper respiratory symptoms.^(5,16) No previous results have been reported on the occurrence of slow-growing mycobacteria in indoor air. To identify their possible role as exposing agents via inhalation, we examined whether mycobacteria can be isolated from air during the remediation of moldy buildings and buildings without detectable mold damage. In parallel with mycobacterial sampling, the occurrence of fungi and actinobacteria were also studied to determine whether there is any association between concentrations of mycobacteria and other microorganisms.

MATERIALS AND METHODS

Sampling Strategy

Viable airborne mycobacteria, fungi, and actinobacteria were sampled in five buildings during the dismantling of the structures (Table I). Three of the studied buildings had suffered from water damage and had visible microbial growth on the surfaces of the structures. In Buildings 3 and 4, the water damage had occurred recently, but in Building 5 the water damage was older, having occurred years earlier. In these damaged buildings, air samples were taken during the dismantling of moldy materials. The two reference buildings had no known history of water damage and had no signs of mold growth on their surfaces, but they were undergoing another type of remediation. Air samples were taken during the dismantling of nondamaged wall materials in these buildings. All the air samples were taken as close to the remediation process as possible, generally within 5 meters of the worker.

TABLE I. The Buildings Studied

Building	Reason for Mold Growth	Remediation Work Examined
1. Nonmoldy	—	removal of the top layer of a concrete wall by grinding
2. Nonmoldy	—	dismantling of a wooden panel wall
3. Moldy	water leaks	dismantling of a wall made of gypsum board
4. Moldy	leaks from pipes	dismantling of both a wall made of gypsum board and wooden floor
5. Moldy, old	leaks from pipes	dismantling of a wooden floor

One set of three parallel samples for mycobacteria was collected using a six-stage impactor, an all-glass impinger, and by filter sampling at each site. Air samples for fungi and actinobacteria were only taken with impactors, one to four samples being taken during the remediation work in each building. In all, 15 air samples for mycobacteria and 42 samples for fungi and actinobacteria were taken. Furthermore, one outdoor air sample was taken as a control at each sampling site using impactor, impinger, and filter sampling.

Isolation of Microorganisms

Impactor Samples

Impactor samples were taken with six-stage cascade impactors⁽¹⁷⁾ (Model 10-800, Andersen Samplers, Inc., Atlanta, Ga.) calibrated at a flow rate of 28.3 L/min. The mycobacterial samples were collected onto petri dishes containing Mycobacteria 7H11 agar (Difco Laboratories, Detroit, Mich.) supplemented with OADC enrichment (100 mL/L), malachite green (25 mg/L), and cycloheximide (500 mg/L).⁽¹⁸⁾ Dichloran glycerol agar (DG18)⁽¹⁹⁾ was used for xerophilic fungi, and 2% malt extract agar (M2)⁽²⁰⁾ was applied for hydrophilic fungi. For actinobacteria, tryptone-yeast-glucose agar (TYG)⁽²¹⁾ was used for the collection. The collection time varied between 5 and 15 min for all the samples.

After the collection, the mycobacterial plates were sealed with parafilm, packed in a plastic bag and incubated at 30°C for 6 months. If a plate became visibly contaminated, which was considered a sign that other microorganisms were growing, it was eliminated from the incubation. The plates for fungi and actinobacteria were incubated at 25°C for 7 days. In the TYG media, only actinobacteria were counted. The concentrations of microorganisms were calculated using a positive hole correction method⁽¹⁷⁾ and expressed as colony forming units/m³ (cfu/m³).

All-Glass Impinger Samples (AGI-30)

An AGI-30 impinger was filled with deionized water (40.5 mL) and sterilized. The flow rate used varied from 10.2 to 12.3 L/min and the sampling times from 30 to 125 min. The volume of the AGI-30 impinger solution was aseptically measured after the sampling. Ten milliliters of the solution was fixed in 2% (w/v) formalin. The rest of the solution was centrifuged (8600 g, 4°C, 15 min, Sorvall RC-5B, E.I. du Pont Nemours and Co., Wilmington, Del.) and the sediment was suspended in 5 mL of sterile deionized water. This suspension was decontaminated with NaOH (final concentration 0.5 M) for 15 min.

After centrifugation in the same manner as used for the aforementioned solution, the sediment was neutralized by adding 30 mL of sterile deionized water, and the sample was centrifuged, as for the aforementioned solution. The sediment was resuspended in 400 μ L of sterile deionized water, and 50 μ L was inoculated onto two parallel slopes of each of the following growth media: (a) egg medium supplemented with glycerol, pH 6.5; (b) egg medium supplemented with Na-pyruvate, pH 6.5; (c) egg medium supplemented with glycerol, pH 5.5; and, (d) egg medium supplemented with Na-pyruvate, pH 5.5.^(22,23) All the media contained cycloheximide (500 mg/L). The samples were incubated as described earlier.

Filter Samples

Filter samples were collected on polycarbonate membrane filters (diameter 37 mm, pore size 0.4 μ m; Nuclepore Corp. Cambridge, Mass.) with a flow rate of 2 L/min. The sampling time varied between 30 and 120 min depending on the length of the remediation work. The microorganisms were eluted from the filter to 5 mL of sterile peptone water and shaken on a laboratory shaker for 15 min. After the elution, the suspension was decontaminated and cultivated in the same manner as the described AGI-30 impinger samples.

Identification of Mycobacteria

Each colony type appearing on the growth medium was examined for acid fastness by Ziehl-Neelsen staining. Acid-fast isolates were subcultured and identified using an identification scheme for mycobacteria based on the gas liquid chromatographic (GLC) analysis of cellular fatty acids and alcohols and mycolic acid cleavage products as described earlier in detail.⁽²⁴⁾

In addition to the analysis for the growth and biochemical characteristics, the isolates were analyzed using commercial DNA probes for *M. avium* complex (AccuProbe; GenProbe, Inc., San Diego, Calif.).⁽²⁴⁾ The isolates positive with the *M. avium* complex probe were tested further using species-specific probes for *M. avium* and *M. intracellulare*. The isolates were assigned as MAC X if they were positive with the *M. avium* complex probe but negative with the *M. avium* or *M. intracellulare* specific probe.⁽²⁵⁾ The AccuProbe complex negative isolates that had a GLC profile typical of MAC and gave a negative result in the Tween 80 hydrolysis test

were sequenced for partial 16S rDNA as described earlier in detail.⁽²⁴⁾

RESULTS

Mycobacteria were recovered from two of the three moldy buildings and from one of the two nonmoldy buildings (Table II). The total isolation frequency of the sites studied was 60% and that of the samples was 33%. The concentrations of mycobacteria determined for the positive sites varied from 5 to 160 cfu/m³ during the dismantling of the structures. None of the outdoor air samples yielded mycobacteria in culture (detection limits 2–68 cfu/m³). Mycobacteria were the most often detected when sampled with a six-stage impactor. The highest numbers of viable mycobacteria were detected in the moldy building where the water damage had occurred years earlier.

A total of 43 mycobacterial isolates were recovered for the identification tests. Most of the isolates (95%) were slow-growers. Only two rapid-growing isolates were detected. Twenty-four isolates were identified by GLC fatty acid analysis combined with biochemical testing as *M. avium* complex. Among them, 11 hybridized with commercial probes. Three were identified as *M. avium*, and eight were MAC X complex (Table II). The 13 isolates with a typical GLC profile of *M. avium* complex, but negative with the *M. avium* complex probe, were also analyzed by 16S rRNA gene sequencing. One of them was identified as *M. scrofulaceum*, and 12 were grouped very closely with *M. intracellulare*. Compared with the sequence of the type strain of *M. intracellulare*, the isolates in this group were found to have three to five deviations within the 1000 bases analyzed, covering both hypervariable regions of the 16S rRNA gene.

The other slow-growing isolates were identified as *M. terrae* or *M. nonchromogenicum*. Four isolates remained without a precise identification. One of the isolated rapid-growing mycobacteria represented a potentially pathogenic species, *M. fortuitum*; the other remained unidentified.

The concentrations of fungal spores in air varied between 10² and 10⁴ cfu/m³ during the remediation of the moldy buildings and between 10² and 10³ cfu/m³ in the nonmoldy buildings (Table II). In the outdoor air samples, the concentrations of fungal spores varied between 10¹ and 10² cfu/m³, depending on the time of year of the sampling. No actinobacteria were found in the indoor or outdoor samples.

DISCUSSION

To our knowledge, this is the first report of slow-growing environmental mycobacteria isolated from indoor air samples. Slow-growing mycobacteria have earlier been isolated from air only when sampled above river waters.^(18,26) In the river water studies, mycobacteria of the *M. avium-intracellulare-scrofulaceum* (MAIS) complex were recovered in 0% to 75% of the air samples collected, depending on the

TABLE II. Concentrations of Microorganisms (cfu/m³) and Species of Mycobacteria Isolated During the Dismantling of Structures

Building	Mycobacteria ^A						Fungi ^B
	Six-Stage Impactor		AGI-30 Impinger		Filter Sampling		Six-Stage Impactor
	cfu/m ³	Species	cfu/m ³	Species	cfu/m ³	Species	cfu/m ³ (range)
1. Nonmoldy	b.d. ^C		b.d. ^D		b.d. ^E		130–1200
2. Nonmoldy	5	unidentified, rapid-growing chromogenic species (n = 1)	b.d. ^D		b.d. ^E		190–600
3. Moldy	b.d. ^C		b.d. ^D		b.d. ^E		120–85,000
4. Moldy	7	unidentified, slow-growing nonchromogenic species (n = 1)	b.d. ^D		b.d. ^E		5020–16,000
5. Moldy, old water damage	150	<i>M. avium</i> (n = 3) MAC X (n = 6) <i>M. intracellulare</i> -like (n = 11) <i>M. scrofulaceum</i> (n = 1) <i>M. terrae</i> (n = 7) <i>M. fortuitum</i> (n = 1) isolates belonging to unidentified, slow-growing, nonchromogenic species (n = 3)	29	MAC X (n = 1) <i>M. intracellulare</i> -like (n = 1) <i>M. terrae</i> (n = 1)	160	MAC X (n = 1) <i>M. terrae</i> (n = 4) <i>M. nonchromogenicum</i> (n = 1)	8200–25,000
Outdoor	b.d. ^C		b.d. ^D		b.d. ^E		2–980

Notes: *M.* = *Mycobacterium*; b.d. = below detection limit.

^AEach result represents one sample.

^BN = number of samples (1–4 per building site) total N = 28.

^CDetection limit = 2 cfu/m³ for 15 min sampling and 7 cfu/m³ for 5 min sampling.

^DDetection limit = 5–19 cfu/m³.

^EDetection limit = 18–68 cfu/m³.

environment and season studied. The maximum concentrations of MAIS complex in the aerosol samples, collected using a six-stage impactor, were 70 cfu/m³.⁽²⁶⁾ In the indoor air of other occupational environments, such as a metalworking industrial facility, rapid-growing environmental mycobacteria have recently been detected.^(15,27)

M. avium, capable of causing tuberculosis-like infections in humans and animals, was recovered from indoor air during the dismantling of the structures. Nonpathogenic mycobacterial species were also isolated. Although the nonpathogenic species are unlikely to cause respiratory infections in healthy individuals, they are known as strong immunostimulators. Some of the isolates recovered in our study have been shown to be capable of activating inflammatory mechanisms in both human and murine macrophage cell lines.^(13,14) This result applied to both the potentially pathogenic and nonpathogenic species tested.

Low numbers of mycobacteria were detected in the air of both the nondamaged and moisture-damaged buildings during the dismantling work. The highest concentration of mycobacteria was found in the moisture-damaged building, where a wooden floor was dismantled during the sampling. In the nondamaged building where mycobacteria were isolated, the sampling was performed during the dismantling of a wooden panel wall. The mycobacteria may have been released from the wooden structures because they have earlier been isolated from wood-based materials such as wood shavings and sawdust.^(28,29) Mycobacteria may also be found in other types of wetted building materials. A new, rapid-growing mycobacterial species has recently been isolated from the gypsum board liner of a moisture-damaged day care center.^(5,16)

Mycobacteria were found in buildings with both high and low concentrations of fungi. In fact, mycobacteria were not found in a moldy building, where the highest concentration of fungal spores was measured. However, the small number of samples limits drawing the conclusions of the relationship between the concentrations of mycobacteria and the fungal and actinobacterial spore concentrations.

Six-stage impactor sampling proved to be the most successful method for sampling mycobacteria resulting in mycobacterial detection in both low and high concentrations. This is probably because the bacteria are impacted directly onto the growth medium without the decontamination step needed with AGI-30 impinger and filter samples. Decontamination is an essential part of mycobacterial culture, necessary for the destruction of more rapid-growing flora.⁽¹⁰⁾ We used 0.5 M NaOH for the decontamination of the AGI-30 impinger and filter solutions. However, even this treatment did not completely inhibit the growth of contaminating microorganisms found in the air samples of the moisture-damaged buildings. The lowest yield of mycobacteria was detected with the AGI-30 impinger. In addition to the losses in the decontamination phase, some mycobacteria may have escaped with the bubbling water droplets during the sampling.^(30,31) Furthermore, the detection limit of this method was higher than in the six-stage impactor sampling. The filter sample in Site 5 gave the highest concentration of mycobacteria, but the

low total number of samples does not allow further conclusions about the characteristics of the different samplers.

With the six-stage cascade impactor, mycobacteria were isolated at Stages 1 to 5. The size classes retained by the Stages from 6 to 1 of the sampler are 0.65–1.1, 1.1–2.1, 2.1–3.3, 3.3–4.7, 4.7–7.0, and >7.0 μm , as given by the manufacturer (Andersen Samplers, Inc.). This information indicates that mycobacteria were present in particles small enough to penetrate the alveolar region of the lungs.

CONCLUSION

Slow-growing mycobacteria are among the microorganisms that may grow in water-damaged or aged building materials and be released into the air of worksites during the dismantling of damaged structures. Because of their potentially pathogenic and strongly immunostimulating properties, their health importance as a component of bioaerosol in construction work deserves further study.

ACKNOWLEDGMENT

This study was supported by a grant from the Finnish Work Environment Fund.

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