

***Mycobacterium avium* in a shower linked to pulmonary disease**

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ABSTRACT

Mycobacterium avium was isolated from hot and cold water samples and from sediment (biofilm) collected from the showerhead in the home of a woman with *M. avium* pulmonary disease lacking known *M. avium* risk factors. IS1245/IS1311 DNA fingerprinting demonstrated that *M. avium* isolates from the hot and cold water and showerhead sediment demonstrated a clonal relationship with the patient's *M. avium* isolate. The data provide evidence that showers may serve as sources of infection by waterborne *M. avium*.

Key words | aerosols, infections, mycobacteria, showers

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INTRODUCTION

Mycobacterium avium has been recovered from a variety of environmental compartments with which humans come in contact, including drinking water (duMoulin & Stottmeier 1986; Fischeder *et al.* 1991; Glover *et al.* 1994; von Reyn *et al.* 1994; Covert *et al.* 1999). In spite of the fact that *M. avium* isolates have been recovered from potable water samples, there have been few reports of DNA fingerprint matches between patient and epidemiologically-linked environmental isolates. DNA fingerprints of a number of *M. avium* isolates recovered from water samples were shown to match those of *M. avium* isolates recovered from AIDS patients who had been exposed to those waters (von Reyn *et al.* 1994). Further, *M. avium* infection has been associated with the presence of *M. avium* in hot tubs (Embil *et al.* 1997; Kahana *et al.* 1997; Mangione *et al.* 2001). In Florida, showers have been suggested as sources of mycobacterial pulmonary disease (Gorman 2002).

Herein we report an *M. avium* pulmonary infection in an individual without the known risk factors for *M. avium*

disease (Wolinsky 1979; Prince *et al.* 1989; Reich & Johnson 1991; Kennedy & Weber 1994; Falkinham 1996) whose IS1245/IS1311 DNA fingerprint showed a clonal relationship with those of *M. avium* isolates recovered from hot and cold water and sediment in a showerhead in her residence.

MATERIALS AND METHODS

Patient description

The patient was a 41 year old female referred to the National Jewish Hospital in September 2002 following discovery of nodules in the mid zone of the right lung involving the posterior segment and axillary region of the right upper lobe by CT scan. Three sputum cultures all yielded *Mycobacterium avium*. Prior to discovery of the nodules, the patient had been treated with a fluoroquinolone antibiotic for pneumonia in early 2001 and a recurrent

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episode in July 2002. The patient was started on a daily regimen of azithromycin and ethambutol and remained free of symptoms during a two year course of therapy.

Description of building

The patient resides on the second floor of a 13 story “prewar” building in the New York City metropolitan area. The building is served by New York City water and has a central, recirculating hot water heating system.

Isolation of mycobacteria from water

Following diagnosis of *M. avium* infection, water samples were collected in October 2002 using sterile containers. Three samples were collected from the residence’s bathroom. After flushing, 500 mL hot and cold water samples were collected from separate taps which were in regular use. The regularly used shower head was unscrewed and sediment was transferred to a sterile container and the biofilm collected with a cotton swab and placed in the same container and the container closed to prevent evaporation. Microbial cells in a 100 mL sample were pelleted by centrifugation ($5,000 \times g$ for 20 min). The supernatant liquid was discarded and the pelleted cells suspended in 1 ml sterile distilled water. Samples (0.1 ml) of the cell suspension were spread on five (5) Middlebrook 7H10 agar medium containing 0.5% (vol/vol) glycerol, 10% (vol/vol) oleic acid-albumin (Pethel & Falkinham 1989) enrichment, and additional malachite green to a final concentration of 0.001% (wt/vol) (M7H10 agar). That malachite green concentration does not inhibit mycobacterial growth, but prevents colony formation by most bacteria and fungi (Jones & Falkinham 2003). The plates were sealed with Parafilm[®] and incubated at 37°C. Colonies of putative acid-fast bacteria were picked after 10–14 days and streaked for isolation on M7H10 agar. Single isolated colonies were acid-fast stained. Isolates representing the different colony types identified were archived and the number of colonies of that type counted to provide the estimate of the number in the original sample. The PFGE fingerprint for each one of the representative isolates was identified and subjected to PFGE.

Isolation of mycobacteria from showerhead sediment

The showerhead sediment (biofilm) was scraped from the interior surfaces and suspended in 40 mL of sterile distilled water and vortexed at the highest setting for 60 sec. The suspension was centrifuged ($5,000 \times g$ for 20 min) and the supernatant discarded. The pellet was suspended in 2 ml sterile distilled water. Mycobacteria were recovered and enumerated by spreading 0.1 mL of the suspension on M7H10 agar as described above.

Identification of mycobacterial isolates

Acid-fast isolates from the primary isolation medium were streaked on Lowenstein-Jensen medium slants (BBL Microbiology Systems, Cockeysville, MD) and incubated at 37°C to assess growth rate. DNA was isolated from all strains and the nested PCR amplification of the 16S rDNA genes (Wilton & Cousins 1992) used to identify whether isolates were members of the genus *Mycobacterium* and belonged to either *M. avium* or *M. intracellulare*. Identification of *M. avium* and *M. intracellulare* isolates was confirmed by PCR amplification of the *hsp-65* heat shock protein gene followed by analysis of restriction endonuclease digestion products on agarose gels (Telenti *et al.* 1993).

IS1245/IS1311 restriction fragment length polymorphism (RFLP) analysis

IS1245/IS1311 DNA fingerprinting was performed as described by van Soolingen *et al.* (1998). Computer-assisted analysis of the IS1245/IS1311 DNA fingerprints was carried out by using the BioNumerics software version 4.0 (Applied Maths BVBA, Sint-Martens-Laten, Belgium). The DNA fingerprints were compared by using the Un-weighted Pair Group Method with arithmetic Average (UPGMA) clustering method and by using the Dice coefficient following the instructions of the manufacturer of BioNumerics. To measure the percentage of similarity between the DNA fingerprints 1% position tolerance and 1% optimization was used.

RESULTS

Recovery and identification of mycobacteria

Hot water (Hot-1 to 6), cold water (Cold-1 to 5), and showerhead sediment (Crud-1-6) all yielded either *M. avium* or *M. intracellulare* (Hot-6, Cold-2, and Crud-6) isolates. One cold water isolate (isolate Cold-5) was a member of the genus *Mycobacterium*, but the amplified *hsp-65* restriction fragment pattern did not match any published patterns. Because decontamination was not necessary, it was possible to estimate the colony-forming units (CFU) per unit volume or weight of the samples. Cold water yielded 2 colony-forming units (CFU) mL⁻¹ of both *M. avium* and *M. intracellulare* and hot water contained 2 CFU mL⁻¹ *M. avium*. The suspended sediment sample yielded both *M. avium* and *M. intracellulare* at 240 CFU mL⁻¹ of the sediment suspension for each. The sediment weighed approximately 0.2 gm leading to an estimated value of 1,200 CFU g m⁻¹ for each species.

Fingerprints of *M. avium* isolates

The results of IS1245/IS1311 fingerprinting (Figure 1) demonstrate the clonal relationship of the patient isolate with individual isolates from hot and cold water and showerhead sediment. All isolates, except two, showed the IS1245/IS1311 multi-copy pattern (Komijn *et al.* 1999; Johansen *et al.* 2005). The two exceptional isolates (i.e., Cold-1 and Crud-5) showed the characteristic cigarette pattern described by Mijs *et al.* (2002). This DNA fingerprint

pattern (data not shown) has only been found in cigarette isolates of *M. avium* (Eaton *et al.* 1995). The dendrogram of the IS1245/IS1311 DNA fingerprints of the patient and environmental *M. avium* isolates (Figure 1) demonstrates the clonal relationship between the patient's isolate and the individual isolates from hot and cold water and showerhead sediment. For example, the patient isolate shows a similarity of 72% with one isolate recovered from hot (Hot-5) and another recovered from cold water (Cold-4) and a similarity of at least 62% with the other water and sediment isolates, with the exception of the two with the cigarette pattern.

DISCUSSION

The data presented here provide evidence that the source of *M. avium* resulting in the patient's pulmonary disease may be the water in the shower. First, the isolates displayed 10–15 bands thereby providing sufficient discriminatory power (Hunter & Gaston 1988). Although no perfect fingerprint matches were obtained between the water or sediment isolates and that from the patient, two isolates, (i.e., Hot-5 and Cold-4) would be described as related (1–3 band differences, Figure 1). The data are also useful in providing an example of the genetic variation within *M. avium* isolates in water and showerhead sediment. It is likely that the clonal variation amongst *M. avium* isolates illustrated here (Figure 1), occurs in infected patients as well. If more isolates had been available from the patient, a perfect match might have been identified.

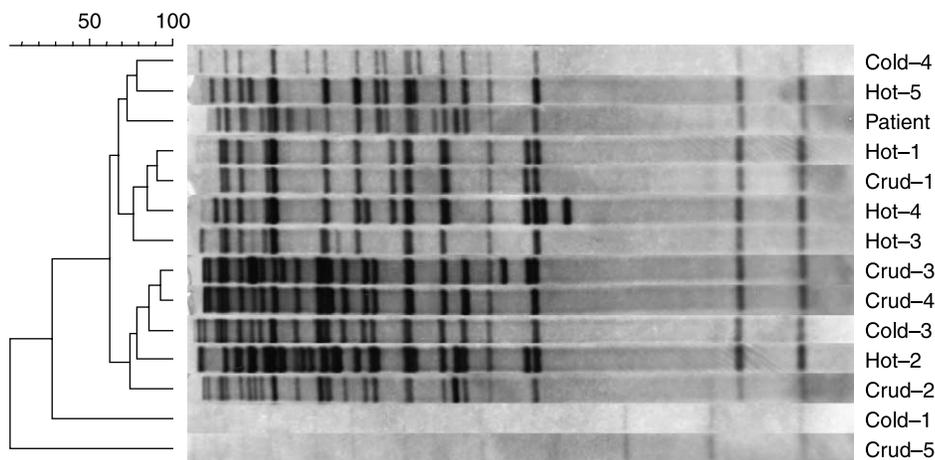


Figure 1 | IS1245/IS1311 RFLP patterns of patient and hot and cold water and showerhead sediment *M. avium* isolates.

To demonstrate the unique clonal relationship of the *M. avium* isolates described in this study, their IS1245/IS1311 fingerprint patterns were compared with those of 258 isolates recovered from pigs and humans (Komijn *et al.* 1999). None of the isolates had patterns indicating they belonged to the same clone as those from the patient and her water and showerhead sediment (data not shown). Only one human isolate had a similarity of 58.5%, whereas the other 257 isolates demonstrated similarities of less than 52.3%.

It is important to point out that the water and sediment samples were not decontaminated prior to plating concentrates on agar medium. This may be responsible, in part, for isolation of substantial numbers of the different *M. avium* isolates, because decontamination methods kill mycobacteria (Brooks *et al.* 1984). It has been our experience that *M. avium* can be directly isolated from drinking water samples, because they contain few other microorganisms (Falkinham *et al.* 2001). Further, many of the microorganisms (but not mycobacteria) found in drinking water are also susceptible to 0.001% malachite green present in the M7H10 agar used here.

It is not clear that the possible exposure to individuals, like the patient described here, can be reduced. Recirculating hot water systems carry high numbers of *M. avium* (duMoulin *et al.* 1988). In-line, point-of-use water filters can be placed on hot and cold water taps and showers to reduce microbial numbers. However, mycobacteria adhere to the filter material (e.g., granular activated charcoal) and it has been shown that microbial numbers, including *M. avium*, increase over time (Rodgers *et al.* 1999; Chaidez & Gerba 2004). Thus the filters must be replaced regularly.

CONCLUSIONS

M. avium and *M. intracellulare* could be isolated directly, without decontamination, from hot and cold water and showerhead sediment collected from a shower of a patient with *M. avium* pulmonary disease. IS1245/IS1311 restriction fragment length polymorphism patterns of several of the *M. avium* isolates showed that they were related to the patient's isolate. It is possible that aerosols generated in the shower and containing *M. avium* were the source of the patient's pulmonary infection.

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