Hospital water filters as a source of *Mycobacterium avium* complex

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Bronchoscopes and the filters used for washing them were found to yield high numbers of *Mycobacterium avium* isolates sharing the same repetitive sequence-based PCR (rep-PCR) fingerprint pattern as *M. avium* isolates recovered from patient samples collected by bronchoscopy. Water and biofilm samples collected from the bronchoscopy preparation laboratory yielded *M. avium*, *Mycobacterium intracellulare*, *Mycobacterium mageritense* and *Mycobacterium goodii*. Several *M. avium* and *M. intracellulare* isolates from water samples in the bronchoscopy laboratory had rep-PCR patterns matching those of patient bronchoscopy isolates. Five of the 22 (23%) *M. avium* patient bronchoscopy isolates and 42 of the 56 (75%) *M. intracellulare* patient bronchoscopy isolates could have been due to contamination from the water supply.

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INTRODUCTION

Reports of the isolation of non-tuberculous mycobacteria (NTM) from contaminated bronchoscopes appear regularly in the literature. Such investigations are usually prompted by the isolation of NTM from patient samples collected by bronchoscopy that are inconsistent with patient symptoms or conditions. In a number of instances, either inadequate bronchoscope disinfection (Rodrigues *et al.*, 2001; Wang *et al.*, 1995; Wheeler *et al.*, 1989) or contamination by a bronchoscope-washing machine (Brown *et al.*, 1993; Gillespie *et al.*, 2000; Gubler *et al.*, 1992; Kressel & Kidd, 2001; Takigawa *et al.*, 1995) was identified as the reason for the presence of NTM in the bronchoscopes. In addition, water used for washing bronchoscopes has been shown to be the source of NTM contaminating bronchoscopes (Bennett *et al.*, 1994; Nye *et al.*, 1990; Stine *et al.*, 1987; Vijayaraghavan *et al.*, 2006). In spite of these studies, there are only two reports where a DNA fingerprinting technique was employed to prove that the water used for washing the bronchoscopes was the source (Bennett *et al.*, 1994; Chroneou *et al.*, 2008).

The presence of NTM in the water supplies of bronchoscope-processing laboratories is consistent with their presence in hospital water (du Moulin *et al.*, 1988) and municipal drinking water (Falkinham *et al.*, 2001). However, source identification is not straightforward, as the wide genetic diversity within NTM species requires typing of patient and environmental isolates. In addition, in the absence of NTM colony counts, it is impossible to identify which source might be of primary importance and require remediation.

At a large general community hospital, *Mycobacterium avium* or *Mycobacterium intracellulare* isolates were recovered from 75 patient bronchoscopy samples collected over the period December 2007–February 2009, whose appearance was inconsistent with patient symptoms or conditions. The objective of our study was to collect samples from the hospital water system and bronchoscopy-processing laboratory, test them for the presence of mycobacteria, and, if recovered, compare the DNA fingerprints of the patient and environmental isolates.

METHODS

Patient (bronchoscopy) NTM isolates. Twenty-two *M. avium* and 56 *M. intracellulare* isolates cultured from patient bronchoscopy samples were obtained from the Maryland State Health Department Tuberculosis Laboratory.

Samples and NTM isolation, enumeration and identification. A total of 39 water and biofilm (swab) samples were collected from the bronchoscopy-processing laboratory (Table 1) by hospital staff and processed for the isolation, enumeration and identification of isolates as described by Falkinham *et al.* (2001). Briefly, 0.1 ml water samples were spread directly in triplicate on Middlebrook 7H10 agar medium (M7H10) and the number of c.f.u. (ml sample)^{−1} was reported (Table 1). Swabs used for the collection of surface biofilms were placed in 2 ml sterile tap water and vortexed, and 0.1 ml suspension was spread in triplicate on M7H10 agar and the results reported as c.f.u. per swab (Table 1). Six individual filters, all part of the same unit, were retrieved from the filter housings, placed in sterile bags and shipped to the Virginia Polytechnic Institute and State University laboratory for processing. For the filters, 1 cm² of the surface of both the inlet and outlet sides of the filters was swabbed and the swabs were placed in 2 ml sterile tap water and vortexed; 0.1 ml of the suspension was spread on M7H10 agar medium in triplicate and the results reported as c.f.u. cm^{−2}. All samples were spread directly on M7H10

Abbreviations: NTM, non-tuberculous mycobacteria; rep-PCR, repetitive sequence-based PCR.

Table 1. Hospital samples and mycobacterial recovery

Sample	Description	Water/swab*	Mycobacteria rep-PCR type	c.f.u.†
1	Final rinse water	61 ml	None	
2	Bronchoscope no. 1 pre-brushing	2 ml	None	
3	Bronchoscope no. 1 cleaning brush	11 ml	None	
4	Bronchoscope no. 1 post-brushing	35 ml	None	
5	Bronchoscope no. 2 suction port	Swab	None	
6	Bronchoscope no. 2 pre-brushing	33 ml	None	
7	Bronchoscope no. 2 biopsy port	Swab	<i>M. avium</i> type C	700
8	Bronchoscope no. 2 post-brushing	32 ml	None	
9	Bronchoscope no. 2 cleaning brush	16 ml	None	
10	Bronchoscope no. 3 suction port	Swab	None	
11	Bronchoscope no. 3 biopsy port	Swab	None	
12	Bronchoscope no. 3 pre-brushing	39 ml	None	
13	Bronchoscope no. 3 post-brushing	40 ml	None	
14	Bronchoscope no. 3 brush	10 ml	None	
15	Bronchoscope no. 4 biopsy port	Swab	None	
16	Bronchoscope no. 5 biopsy port	Swab	None	
17	Bronchoscope no. 5 suction port	Swab	None	
18	Bronchoscope no. 4 suction port	Swab	<i>M. avium</i> type B	1 400
19	Bronchoscope no. 5 pre-brushing water	29 ml	None	
20	Bronchoscope no. 5 brush	20 ml	None	
21	Bronchoscope no. 5 post-brushing	37 ml	None	
22	Bronchoscope no. 4 pre-brushing	33 ml	None	
23	Bronchoscope no. 4 brush	20 ml	None	
24	Bronchoscope no. 4 post-brushing	43 ml	None	
25	Water, pre-cleaning sink	Swab	<i>M. avium</i> no bands <i>M. intracellulare</i> type A <i>M. malmoense</i> <i>M. goodii</i>	70 10 100 870
26	Bronchoscope processor no. 1 ports	Swab	None	
27	Bronchoscope processor no. 2 ports	Swab	None	
28	Bronchoscope processor no. 3 ports	Swab	None	
29	Pre-wash cold water, spigot	300 ml	<i>M. intracellulare</i> type A <i>M. malmoense</i>	150 20
30	Pre-wash hot water, spigot	450 ml	<i>M. avium</i> no bands <i>M. intracellulare</i> type A <i>M. malmoense</i>	47 100 20
31	Valve box, hot water supply	Swab	<i>M. avium</i> type B	86 000
32	Valve box, cold water supply	Swab	None	
33	Pre-filter no. 1, cold water	250 ml	<i>M. avium</i> no bands <i>M. intracellulare</i> type A	3 3
34	Pre-filter no. 2, hot water	250 ml	<i>M. avium</i> no bands	2 810
35	Post-filter no. 2, hot water	400 ml	<i>M. avium</i> no bands	23
37	Post-filter no. 1, cold water	250 ml	None	
F-1	Charcoal pre-filter cold no. 1			
	Inside	Swab	None	
	Outside	Swab	None	
F-2	0.2 µm, post-filter cold no. 1			
	Inside	Swab	None	
	Outside	Swab	None	
F-3	Charcoal, pre-filter hot no. 2			
	Inside	Swab	<i>M. avium</i> no bands	150 000
	Outside	Swab	<i>M. avium</i> no bands	11 200
F-4	0.2 µm, post-filter hot no. 2			
	Inside	Swab	<i>M. avium</i> no bands	123 000
	Outside	Swab	<i>M. avium</i> type B	125 000

Table 1. cont.

Sample	Description	Water/swab*	Mycobacteria rep-PCR type	c.f.u.†
F-5	Endoscopy pre-filter			
	Inside	Swab	<i>M. avium</i> type B	60
	Outside	Swab	<i>M. avium</i> type B	3 040
F-6	Endoscopy filter			
	Inside	Swab	<i>M. avium</i> type B	20 000
	Outside	Swab	<i>M. avium</i> type B	90
Post-remediation measures				
38	Endoscope water, post filter and UV	900 ml	None	
39	Endoscope water, post filter	900 ml	None	

*Volume of water sample collected or volume of water in tube with bronchoscope brush. Swabs were placed in 2 ml sterile tap water and vortexed for 60 s at maximum speed.

†Results for water samples expressed as c.f.u. (ml sample)⁻¹, as c.f.u. per swab for swab samples and as c.f.u. cm⁻² for filter samples.

agar, as decontamination was not necessary. Representative colonies of each putative NTM type were counted and streaked for isolation, and acid-fast isolates were identified by nested PCR of the 16S rRNA gene (Wilton & Cousins, 1992) and the size of restriction fragments of the *hsp-65* gene amplified by PCR (Telenti *et al.*, 1993). Repetitive sequence-based PCR (rep-PCR) was employed for DNA fingerprint typing (Cangelosi *et al.*, 2004) using DNA isolated using an UltraClean Microbial DNA Isolation kit (MoBio Laboratories) following the manufacturer's instructions. For identification and typing, reactions were performed twice using two separate DNA samples for each isolate.

RESULTS

Recovery of NTM from samples

Mycobacteria, comprising *M. avium*, *M. intracellulare*, *Mycobacterium malmoense* and *Mycobacterium goodnae*, were recovered from 17 of the 51 hospital samples (33 %) (Table 1). Extremely high numbers of *M. avium* (~10 000–150 000 cm⁻²) were recovered from the inside and outside of the two filters in the hot water line used for washing bronchoscopes (Table 1). Considering the surface area of the filters (i.e. 50 × 6 cm diameter, and with a central 1 cm diameter hole), they harboured between 12 million and 188 million *M. avium* cells on the surface alone. *M. avium* was also recovered from two bronchoscope samples (Table 1).

Fingerprint comparisons

All *M. avium* and *M. intracellulare* isolates were fingerprinted by rep-PCR and unique band patterns were identified by letter (Fig. 1). Four unique band patterns (B, C, D and E) were displayed by the patient *M. avium* bronchoscopy isolates and six (A, F, G, H, I and J) by the *M. intracellulare* isolates (Fig. 1 and data not shown). Hospital sample *M. avium* isolates belonged to types B and C and *M. intracellulare* isolates belonged to type A (Table 1). Five of the 22 (23 %) *M. avium* patient bronchoscopy

isolates belonged to type B and 42 of the 56 (75 %) *M. intracellulare* patient bronchoscopy isolates exhibited a type A pattern.

Fig. 1. Rep-PCR patterns of patient (lanes 2–7, rep-PCR types B, C, A, F, G and H, respectively) and bronchoscope preparation laboratory (lanes 8–11, rep-PCR types B and C) isolates. Lanes: 1, 1 kb ladder; 2, *M. avium* type B; 3, *M. avium* type C; 4, *M. intracellulare* type A; 5, *M. intracellulare* type F; 6, *M. intracellulare* type G; 7, *M. intracellulare* type H; 8, *M. avium* isolate Sw-7-1 type B; 9, *M. avium* isolate W-18-1 type B; 10, *M. intracellulare* isolate W-29-1 type C; 11, *M. intracellulare* isolate W-30-2 type C.

DISCUSSION

The data demonstrated that isolation of mycobacteria from bronchoscopy samples was due to contamination of bronchoscopes during their preparation for use, based on the presence of the *M. intracellulare* rep-PCR type A and *M. avium* type B in filters and water in the bronchoscopy preparation laboratory. It is likely that the source of *M. intracellulare* rep-PCR type A contamination was the cold (sample 29) and hot (sample 30) water and that for *M. avium* rep-PCR type B was filter F-4 on the hot water line (Table 1). The endoscope filters also yielded *M. avium* rep-PCR type B (Table 1). The presence of *M. avium* and *M. intracellulare* in hot water is consistent with the heat resistance of the *M. avium* complex: more than 10% survive 3–4 h exposure to 60 °C (Schulze-Röbbecke & Buchholtz, 1992).

Remediation measures included a review of the procedures for meticulous cleaning of bronchoscopes and installation of new filters in the existing filtration equipment and a UV sterilization device. The existing non-microbiological, in-line cartridge-type water filters had been in place for more than 6 months. Point-of-use filters (0.1 µm pore size) that prevent the passage of micro-organisms were installed on all taps providing water or flushing bronchoscopes. It is planned that the filters will be changed every 3 weeks following the manufacturer's directions. Furthermore, bronchoscopes are now air dried vertically to avoid storage of bronchoscopes containing liquid. It is anticipated that these measures will prevent the filters becoming the NTM source (Hilborn *et al.*, 2006; Rodgers *et al.*, 1999). The numbers of *M. avium*, *M. intracellulare*, *M. malmoense* and *M. gordonae* were reduced to below detectable levels (i.e. <10 c.f.u. ml⁻¹) following the remediation measures (Table 1). The Centers for Disease Control and Prevention *Guidelines for Environmental Infection Control in Health-care Facilities* (<http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5210a1.htm>) includes guidelines for infection control of bronchoscopes.

A second noteworthy observation from this study was the very high numbers of *M. avium* in filters. This is believed to be the first report demonstrating that filters are a source of *M. avium* contaminating bronchoscopes. Although filters can be effective in reducing the numbers of *M. avium* in water lines, they can become sources of *M. avium*, presumably due to saturation and growth followed by release (Hilborn *et al.*, 2006; Rodgers *et al.*, 1999; Stine *et al.*, 1987). The water filters employed in the hospital described here were not microbiological filters, i.e. the pore size did not prevent the passage of NTM and other bacterial cells. The filters simply reduced the number of NTM passing through because of the longer pathway required for passage. However, as the filters trap nutrients, NTM can grow and eventually they pass through the filter. To be effective, the filters must be replaced at 3-week intervals to allow for continual removal of NTM from water (Rodgers *et al.*, 1999). Furthermore, high numbers of

NTM in the filters and consequently in the bronchoscopes could also have reduced disinfectant efficiency, as has been shown with *M. gordonae* (Jackson *et al.*, 1996).

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