Molecular characterization of nontuberculous mycobacteria in hospital waters: a two-year surveillance study in Tehran, Iran

Somayeh Moradi, Mohammad Javad Nasiri, Fazel Pourahmad and Davood Darban-Sarokhalil

ABSTRACT

Microbiological control of hospital waters as one of the main sources of nontuberculous mycobacteria (NTM) is important for the prevention of NTM-associated illness. This study aimed to investigate the prevalence of NTM in the hospital water systems of Tehran, Iran. A total of 218 samples from different hospital waters (i.e., tap water and medical devices such as humidifying cup of oxygen manometer, dialysis devices, nebulizers, and dental units) were included in this study. Phenotypic and molecular tests were used to identify the isolated organisms to species level. Of 218, 85 (39.0%) samples at 37 °C and 87 (40.0%) samples at 25 °C were identified as NTM. Using *hsp*65-sequencing method, *Mycobacterium lentiflavum* was the most frequently encountered, followed by *M. gordonae* and *M. paragordonae*. No significant difference was seen in frequency and species in mycobacteria isolated at 37 °C and 25 °C temperatures. Humidifying cup of oxygen manometer had the most contaminated water among the investigated water distribution systems in hospitals. Isolation of NTM from hospital water sources is a serious public health problem in Iran and merits further attention by health authorities. Establishment of microbiological monitoring systems for hospital waters and expanding the number of facilitated laboratories are strongly recommended.

Key words | hospital, Iran, nontuberculous mycobacteria, oxygen manometer, water

Somayeh Moradi
Davood Darban-Sarokhalil (corresponding author)
Department of Microbiology, School of Medicine,
Iran University of Medical Sciences,
Tehran,
Iran
E-mail: *davood_darban@yahoo.com*

Mohammad Javad Nasiri
Department of Microbiology, School of Medicine,
Shahid Beheshti University of Medical Sciences,
Tehran,
Iran

Fazel Pourahmad
School of Veterinary Medicine,
Ilam University,
Ilam,
Iran

INTRODUCTION

Infections caused by nontuberculosis mycobacteria (NTM) have been recently reported as an important public health problem in many parts of the world (Prevots & Marras 2015) NTM infections are rarely spread from person to person and are mainly acquired from environmental sources including water, drinking water supplies, soil, etc. (Prevots & Marras 2015). They can cause serious infections in immunocompromised patients, including pulmonary disease which may resemble tuberculosis (TB) as well as cervical lymphadenopathy and skin and soft tissue infections (Griffith *et al.* 2007). NTM are often resistant to disinfectants and antibiotics, including anti-TB drugs, and treatment for one species may not be effective for others (Soni *et al.* 2016; Aguilar-Ayala *et al.* 2017). As NTM are usually resistant to disinfectants, it is not uncommon to isolate NTM from water distribution systems (Crago *et al.* 2014). NTM colonization and persistence within a hospital water distribution system may lead to dissemination and infection in patients (Vaerewijck *et al.* 2005; Castillo-Rodal *et al.* 2012; Mullis *et al.* 2013; Crago *et al.* 2014; Falkinham 2015, 2016). In countries such as Iran, where TB is endemic, we have suggested that there is an increasing prevalence of NTM disease, which is of concern (Nasiri *et al.* 2015, 2018a, 2018b). While monitoring hospital water distribution systems for

doi: 10.2166/wh.2019.294

NTM is not a routine practice in Iran and because of the significant impact that these infections may have on patients, the investigation of NTM seems to be important. Thus, the present study aimed to evaluate the frequency of NTM in hospital waters in Tehran, Iran.

MATERIALS AND METHODS

Sample collection

A total of 218 samples from January 2016 to December 2017 was collected from tap water and medical devices (such as oxygen manometers, dialysis devices, nebulizers, and dental units) of different wards of the six hospitals in Tehran, the capital of Iran. Approximately 50 mL of each sample was collected in a sterile glass bottle, transferred to the laboratory in an icebox and analyzed within 24 hr. Stranded procedure recommended by the Centers for Disease Control and Prevention (CDC) was used to produce water for hemodialysis machines (Sehulster *et al.* 2003).

Sample preparation and culture

Water samples were centrifuged for 30 min at 3,000 rpm. The sediment (3 mL) was transferred to two sterile containers, and was decontaminated by our method (1.5 mL of NaOH 1% and SDS 3%) for 30 min at room temperature. Phenolphthalein and phosphoric acid 40% was used for the neutralization step. The samples were then centrifuged for 30 min at 3,000 rpm. The supernatant was discarded and the sediments of each treated sample were used to prepare a Ziehl–Neelsen smear and inoculated into two separate batches of Lowenstein Jensen (LJ) medium containing cyclohexamide 0.5 g and incubated at temperatures 37 °C and 25 °C for two months (Khosravi *et al.* 2016).

Phenotypic identification of mycobacteria

All mycobacterial isolates were grown on LJ medium and examined for growth rate, macroscopic and microscopic morphological features, growth at different temperatures, and also a set of biochemical tests including Tween 80 hydrolysis, nitrate reduction, niacin production, arylsulfatase, urease production, tellurite reduction, salt tolerance, and catalase production according to standard procedures (Nasiri *et al.* 2018a, 2018b).

Molecular assignment of isolates to NTM

IS6110-based polymerase chain reaction (PCR) assay was used for differentiation of NTM from *Mycobacterium tuberculosis* complex. Genomic DNA, for *IS6110*-based PCR assay, was extracted using QIAamp DNA Mini Kit (QIAGEN, USA) according to kit instructions. A 123 bp fragment of insertion element *IS6110* of the *M. tuberculosis* complex was used as a target and amplified using previously described PCR primers (Eisenach *et al.* 1990). Genomic DNA of *M. tuberculosis* H37Rv (ATCC27294) and *M. fortuitum* (ATCC 49404) were used as positive and negative controls, respectively. The assay is negative for NTM species due to the absence of *IS6110* (Eisenach *et al.* 1990).

*hsp*65-PCR restriction analysis (PRA)

An approximately 441 bp fragment of *hsp*65 gene was amplified by PCR using two specific primers Tb11 (50-ACCAA CGATGGTGTGTCCAT-30) and Tb12 (50-CTTGTCGAA CCGCATACCCT-30). PCR products were digested with 5 U of restriction enzyme *Hae*III and *Bst*II for 24 hours at 37 °C (Telenti *et al.* 1993). The pattern of digested products was analyzed using 8% polyacrylamide gel. *M. fortuitum* (ATCC 49404) and double distilled water were used as positive and negative control in all PCR experiments, respectively. Species identification was performed using algorithms proposed by Roth *et al.* (2000) and Telenti *et al.* (1993).

PCR and sequencing of *hsp*65 and *rpoB*

A 441 bp fragment of *hsp*65 gene was amplified by PCR with two specific primers Tb11 (5′-ACCAACGATGGTGTGTC-CAT-3′) and Tb12 (5′-CTTGTC GAACCGCA-TACCCT-3′) (Telenti *et al.* 1993). The cycling condition was 95 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 62 °C for 30 s, and 72 °C for 1 min and finalized with 72 °C for 5 min. Distilled water was used as a negative control. PCR products of *hsp*65 genes were analyzed on 1% agarose gel electrophoresis. For confirmation of results, a number of

samples were randomly examined for *rpoB* gene by using two specific primers MycoF (50-GGCAAGGTCACCCCG AAGGG-30) and MycoR (50-AGCGGCTGCTGGGTGAT CATC-30) (Adékambi *et al.* 2003).

Analysis of sequence data

The obtained sequences for each isolate were aligned separately and compared with all existing relevant sequences of mycobacteria retrieved from GenBank database at the NCBI website via the nucleotide BLAST search.

RESULTS

Out of 218 samples from different water sources of hospitals in Tehran, 85 (39.0%) samples at 37 °C and 87 (40.0%) samples at 25 °C were identified as NTM using conventional and molecular methods (Tables 1 and 2). Using *hsp*65-PRA and sequencing methods, *M. lentiflavum* (n: 72, 84.7%) was the most frequently encountered, followed by *M. gordonae* (n: 11, 13.0%) and *M. paragordonae* (n: 2, 2.3%) at 37 °C (Table 3). No significant difference was seen in frequency and species in mycobacteria isolated at temperatures of 37 °C and 25 °C. Randomly selected NTM isolates were also confidently identified by *rpoB* gene.

The frequency of NTM species in different hospital water sources are presented in Tables 4 and 5. Humidifying cup of oxygen manometer had the most contaminated water among the investigated water distribution systems in the hospitals.

DISCUSSION

There are several reports of nosocomial infections caused by waterborne NTM, indicating that this route of transmission

Table 1 | Frequency of mycobacterial isolates in water sources of hospitals incubated at 37 °C

	Water samples ($n = 218$)			
Hospital (No. of samples)	**Positive (%)**	**Negative (%)**	**Contaminated[a] (%)**	**Total (%)**
No. 1 (43)	8 (3.7)	8 (3.7)	27 (12.3)	43 (19.7)
No. 2 (27)	19 (8.7)	1 (0.5)	7 (3.2)	27 (12.4)
No. 3 (25)	10 (4.6)	9 (4.1)	6 (2.8)	25 (11.5)
No. 4 (51)	3 (1.4)	45 (20.6)	3 (1.4)	51 (23.4)
No. 5 (65)	39 (17.9)	17 (7.8)	9 (4.1)	65 (29.8)
No. 6 (7)	6 (2.7)	0	1 (0.5)	7 (3.2)
Total	85 (39)	80 (36.7)	53 (24.3)	218 (100)

[a]Contaminated by bacteria other than NTM.

Table 2 | Frequency of mycobacterial isolates in water sources of the hospitals incubated at 25 °C

	Water samples ($n = 218$)			
Hospital (No. of samples)	**Positive (%)**	**Negative (%)**	**Contaminated[a] (%)**	**Total**
No. 1 (43)	6 (2.7)	8 (3.7)	29 (13.3)	43 (19.7)
No. 2 (27)	22 (10)	1 (0.5)	4 (1.85)	27 (12.4)
No. 3 (25)	7 (3.25)	12 (5.5)	6 (2.7)	25 (11.5)
No. 4 (51)	5 (2. 3)	41 (18.8)	5 (2.3)	51 (23.4)
No. 5 (65)	40 (18.4)	19 (8.7)	6 (2.7)	65 (29.8)
No. 6 (7)	7 (3.25)	0	0	7 (3.2)
Total	87 (39.9)	81 (37.2)	50 (22.9)	218 (100)

[a]Contaminated by bacteria other than NTM.

Table 3 | Frequency of different species of Mycobacterium isolated at 37 °C and 25 °C identified by *hsp*65 gene

	Number of isolates (%)	
Mycobacterium species	**37 °C**	**25 °C**
Mycobacterium lentiflavum	72 (84.7)	70 (80.4)
Mycobacterium gordonae	11 (13)	14 (16)
Mycobacterium paragordonae	2 (2.3)	2 (2.3)
Mycobacterium fortuitum	0 (0)	1 (1.1)
Total	85 (100)	87 (100)

may cause significant impact on patients (Crago *et al.* 2014). This study found that an unexpected number of the examined isolates throughout the hospital water distribution systems over the two-year surveillance were NTM, in particular *M. lentiflavum* and *M. gordonae*. Although *M. lentiflavum* and *M. gordonae* are generally considered non-pathogenic, there are cases describing infections caused by these mycobacteria. Similar findings were reported by Khosravi *et al.* (2016) in Khuzestan province of Iran, in which of 77 culture-positive mycobacteria isolated from 258 hospital water samples, *M. fortuitum* and *M. gordonae* were among the most prevalent isolates. Likewise, in other studies conducted in Iran, *M. gordonae* and *M. lentiflavum* were among the most prevalent NTM isolated from water samples (Bahram *et al.* 2012; Moghim *et al.* 2012; Azadi *et al.* 2016). Our results were also relatively similar to other investigations on hospital waters in the world (Angenent *et al.* 2005; Hussein *et al.* 2009;

Table 4 | Frequency of mycobacterium species in different water sources of hospitals incubated at 37 °C

Water sources	Number of samples	Number of positive samples	NTM species (based on analysis)
Oxygen manometer (humidifying cup)	66	38	*M. lentiflavum (34), M. gordonae (4)*
Tap water	51	26	*M. lentiflavum (20), M. gordonae (4), M. paragordonae (2)*
Dialysis devices	69	6	*M. lentiflavum (4), M. gordonae (2)*
Water distillation unit	5	1	*M. lentiflavum (1)*
Nebulizers	13	5	*M. lentiflavum (5)*
Humidifier	4	4	*M. lentiflavum (3), M. gordonae (1)*
Shower heads	6	2	*M. lentiflavum (2)*
Drinking fountain	2	2	*M. lentiflavum (2)*
Dental units	1	1	*M. lentiflavum (1)*
Neonatal incubator	1	0	

Table 5 | Frequency of mycobacterium species in different water sources of hospitals incubated at 25 °C

Water sources	Number of samples	Number of positive samples	NTM species (based on analysis)
Oxygen manometer (humidifying cup)	66	34	*M. lentiflavum (29), M. gordonae (5)*
Tap water	51	32	*M. lentiflavum (24), M. gordonae (6), M. paragordonae (2)*
Dialysis devices	69	5	*M. lentiflavum (3), M. gordonae (2)*
Water distillation unit	5	1	*M. lentiflavum (1)*
Shower heads	6	5	*M. lentiflavum (5)*
Humidifier	4	2	*M. lentiflavum (1), M. fortuitum (1)*
Nebulizers	13	5	*M. lentiflavum (5)*
Drinking fountain	2	2	*M. lentiflavum (2)*
Dental units	1	1	*M. lentiflavum (1)*
Neonatal incubator	1	0	

Genc et al. 2013). In a study in Turkey, a total of 160 water samples from hot and cold water in two hospitals was examined (Genc et al. 2013). Out of 33 isolated strains, 20 (60/6%) were *M. lentiflavum* and the remainder were *M. gordonae* (30/3%) and *M. peregrinum* (9/1%) (Genc et al. 2013). Furthermore, in our recent publication we showed that *M. gordonae* was among the dominant pathogens recovered from clinical samples, highlighting the role of hospital tap water as a vector for transmission of the bacteria (Nasiri et al. 2018a, 2018b).

Due to the risk of NTM-contaminated hospital water supplies for nosocomial infections, preventive measures should be considered. For water supplies, it is suggested to prevent water stagnating in water supplies and to regularly disinfect regions at risk (i.e., reservoirs and drinking fountains) (Dailloux et al. 1999). Furthermore, in the tap water used to rinse haemodialyzers, the use of water inlet filters is recommended (Dailloux et al. 1999). For healthy people, a number of common measures should be indicated, namely, disinfection of wounds in the case of an accidental injury and protecting them from water.

M. gordonae was the second most common NTM in our study. This mycobacterium is usually found in water, soil, and raw milk and is known as saprophytic and a rarely pathogenic bacterium in humans (Wolinsky 1979; Jarikre 2011). *M. gordonae* (the tap water bacillus) has been reported responsible for infections including skin and soft tissues infections (Gengoux et al. 1987; Modilevsky et al. 1989), lung disease (de Gracia et al. 1989), and liver and peritoneal infections (Kurnik et al. 1983). Based on recent studies, *M. gordonae* is one of the most common mycobacterium isolated in hospital waters (Angenent et al. 2005; Genc et al. 2013).

M. lentiflavum, another prevalent NTM in the current study, was first described in 1996 (Springer et al. 1996). This mycobacterium has been shown to grow in a wide range of temperatures (22 to 37 °C), and can cause human disease in both immunocompetent and immunocompromised individuals (Springer et al. 1996; Cabria et al. 2002; Tortoli et al. 2006; Marshall et al. 2011). In a study conducted in Australia in 2001 and 2008, the relationship between genotype and geographical similarities between *M. lentiflavum* isolates from patients and drinking water was evaluated (Marshall et al. 2011). Based on their results, genotypes of the environmental clone of *M. lentiflavum* were close to the strains that were isolated from patients. This finding suggests that drinking water can be the source of infection for *M. lentiflavum*. Similarly, in the study of Torvinen et al. (2004) over 90% of the mycobacteria isolated from water belonged to *M. lentiflavum* and *M. gordonae*. Likewise, in South Korea, Lee et al. (2008) showed that 65% of the samples obtained from drinking water were *M. lentiflavum*. Although for setting up the decontamination method we evaluated several protocols, we should mention that due to the method used for decontamination, some species might not have been isolated.

The rising number of NTM isolated from hospital waters in Iran may have several negative effects on public health status. Importantly, most TB laboratories in Iran are not equipped to perform NTM species identification; thus, culture reported positive for TB may likely be the result of laboratory contamination with environmental NTM. Consequently, NTM may be misdiagnosed as TB which results in unnecessary anti-TB treatment (Nasiri et al. 2015).

Another important finding in our study is the contamination of medical devices such as oxygen manometer (humidifying cup), nebulizers and hemodialysis fluid with mycobacteria which is a significant health risk for hospitalized patients. Recently, Heidarieh et al. (2016) in Iran indicated that a diverse bacterial community, containing predominantly mycobacteria, was detected in a hospital's hemodialysis distribution system. Due to poor immune system in these patients, microbiological monitoring of water used for hemodialysis and other medical devices is very important for the prevention of NTM-associated illness (Montanari et al. 2009; Heidarieh et al. 2016). Recently, transmission of *M. chimaera* from heater–cooler units during cardiac surgery has been reported (Sax et al. 2015; Sommerstein et al. 2016). Thus, it is recommended that standard protocols for the evaluation of microbial contamination should be used for regular monitoring and identification of NTM in medical devices.

CONCLUSION

Isolation of NTM from hospital water sources is a serious public health problem in Iran and merits further attention

by health authorities. The establishment of microbiological monitoring systems for hospital waters, and expanding the number of facilitated laboratories are strongly recommended.

ACKNOWLEDGEMENTS

This study has been supported by Deputy of Research and Technology, Iran University of Medical Sciences, Grant No. 25606–30-01-94. All contributing authors declare no conflicts of interest.

REFERENCES

Adékambi, T., Colson, P. & Drancourt, M. 2003 rpoB-based identification of nonpigmented and late-pigmenting rapidly growing mycobacteria. *Journal of Clinical Microbiology* **41** (12), 5699–5708.

Aguilar-Ayala, D. A., Cnockaert, M., André, E., Andries, K., Gonzalez, Y., Merchand, J. A., Vandamme, P., Palomino, J. C. & Martin, A. 2017 In vitro activity of bedaquiline against rapidly growing nontuberculous mycobacteria. *Journal of Medical Microbiology* **66** (8), 1140–1143.

Angenent, L. T., Kelley, S. T., Amand, A. S., Pace, N. R. & Hernandez, M. T. 2005 Molecular identification of potential pathogens in water and air of a hospital therapy pool. *Proceedings of the National Academy of Sciences* **102** (13), 4860–4865.

Azadi, D., Shojaei, H., Pourchangiz, M., Dibaj, R., Davarpanah, M. & Naser, A. D. 2016 Species diversity and molecular characterization of nontuberculous mycobacteria in hospital water system of a developing country, Iran. *Microbial Pathogenesis* **100**, 62–69.

Bahram, N. E., Ensieh, S., Shrareh, M., Jamshid, F., Hossein, F., Ghasemian, S. H., Sadat, H. N., Tahmineh, N., Farzaneh, S. & Nima, B. 2012 Isolation and phenotypic identification of non-tuberculous mycobacteria existing in Isfahan different water samples. *Advanced Biomedical Research* **1**, 18.

Cabria, F., Torres, M.-V., García-Cía, J.-I., Dominguez-Garrido, M.-N., Esteban, J. & Jimenez, M. S. 2002 Cervical lymphadenitis caused by Mycobacterium lentiflavum. *The Pediatric Infectious Disease Journal* **21** (6), 574–575.

Castillo-Rodal, A., Mazari-Hiriart, M., Lloret-Sánchez, L., Sachman-Ruiz, B., Vinuesa, P. & López-Vidal, Y. 2012 Potentially pathogenic nontuberculous mycobacteria found in aquatic systems. Analysis from a reclaimed water and water distribution system in Mexico City. *European Journal of Clinical Microbiology & Infectious Diseases* **31** (5), 683–694.

Crago, B., Ferrato, C., Drews, S., Louie, T., Ceri, H., Turner, R., Roles, A. & Louie, M. 2014 Surveillance and molecular characterization of non-tuberculous mycobacteria in a hospital water distribution system over a three-year period. *Journal of Hospital Infection* **87** (1), 59–62.

Dailloux, M., Laurain, C., Weber, M. & Hartemann, P. 1999 Water and nontuberculous mycobacteria. *Water Research* **33** (10), 2219–2228.

de Gracia, J., Vidal, R., Martin, N., Bravo, C., Gonzalez, T. & Riba, A. 1989 Pulmonary disease caused by Mycobacterium gordonae. *Tubercle* **70** (2), 135–137.

Eisenach, K. D., Cave, M. D., Bates, J. H. & Crawford, J. T. 1990 Polymerase chain reaction amplification of a repetitive DNA sequence specific for Mycobacterium tuberculosis. *Journal of Infectious Diseases* **161** (5), 977–981.

Falkinham, J. O. 2015 Environmental sources of nontuberculous mycobacteria. *Clinics in Chest Medicine* **36** (1), 35–41.

Falkinham, J. O. 2016 Current epidemiologic trends of the nontuberculous mycobacteria (NTM). *Current Environmental Health Reports* **3** (2), 161–167.

Genc, G. E., Richter, E. & Erturan, Z. 2013 Isolation of nontuberculous mycobacteria from hospital waters in Turkey. *APMIS* **121** (12), 1192–1197.

Gengoux, P., Portaels, F., Lachapeile, J., Minnikin, D., Tennstedt, D. & Tamigneau, P. 1987 Skin granulomas due to Mycobacterium gordonae. *International Journal of Dermatology* **26** (3), 181–184.

Griffith, D. E., Aksamit, T., Brown-Elliott, B. A., Catanzaro, A., Daley, C., Gordin, F., Holland, S. M., Horsburgh, R., Huitt, G. & Iademarco, M. F. 2007 An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *American Journal of Respiratory and Critical Care Medicine* **175** (4), 367–416.

Heidarieh, P., Hashemi Shahraki, A., Yaghoubfar, R., Hajehasani, A. & Mirsaeidi, M. 2016 Microbiological analysis of hemodialysis water in a developing country. *Asaio Journal* **62** (3), 332–339.

Hussein, Z., Landt, O., Wirths, B. & Wellinghausen, N. 2009 Detection of non-tuberculous mycobacteria in hospital water by culture and molecular methods. *International Journal of Medical Microbiology* **299** (4), 281–290.

Jarikre, L. 2011 Disseminated mycobacterium-gordonae infection in a nonimmunocompromised host. *QNRS Repository* **2011** (1), 1952.

Khosravi, A. D., Hashemi Shahraki, A., Hashemzadeh, M., Sheini Mehrabzadeh, R. & Teimoori, A. 2016 Prevalence of non-tuberculous mycobacteria in hospital waters of major cities of Khuzestan Province, Iran. *Frontiers in Cellular and Infection Microbiology* **6**, 42.

Kurnik, P. B., Padmanabh, U., Bonatsos, C. & Cynamon, M. H. 1983 Case report: Mycobacterium gordonae as a human hepato-peritoneal pathogen, with a review of the literature. *The American Journal of the Medical Sciences* **285** (1), 45–48.

Lee, E.-S., Lee, M.-Y., Han, S.-H. & Ka, J.-O. 2008 Occurrence and molecular differentiation of environmental mycobacteria in surface waters. *Journal of Microbiology and Biotechnology* **18** (7), 1207–1215.

Marshall, H. M., Carter, R., Torbey, M. J., Minion, S., Tolson, C., Sidjabat, H. E., Huygens, F., Hargreaves, M. & Thomson, R. M. 2011 *Mycobacterium lentiflavum* in drinking water supplies, Australia. *Emerging Infectious Diseases* **17** (3), 395–402.

Modilevsky, T., Sattler, F. R. & Barnes, P. F. 1989 Mycobacterial disease in patients with human immunodeficiency virus infection. *Archives of Internal Medicine* **149** (10), 2201–2205.

Moghim, S., Sarikhani, E., Esfahani, B. N. & Faghri, J. 2012 Identification of nontuberculous mycobacteria species isolated from water samples using phenotypic and molecular methods and determination of their antibiotic resistance patterns by E-test method, in Isfahan, Iran. *Iranian Journal of Basic Medical Sciences* **15** (5), 1076–1082.

Montanari, L. B., Sartori, F. G., Cardoso, M. J. d. O., Varo, S. D., Pires, R. H., Leite, C. Q. F., Prince, K. & Martins, C. H. G. 2009 Microbiological contamination of a hemodialysis center water distribution system. *Revista do Instituto de Medicina Tropical de São Paulo* **51** (1), 37–43.

Mullis, S. N. & Falkinham III, J. 2013 Adherence and biofilm formation of Mycobacterium avium, Mycobacterium intracellulare and Mycobacterium abscessus to household plumbing materials. *Journal of Applied Microbiology* **115** (3), 908–914.

Nasiri, M. J., Dabiri, H., Darban-Sarokhalil, D. & Shahraki, A. H. 2015 Prevalence of non-tuberculosis mycobacterial infections among tuberculosis suspects in Iran: systematic review and meta-analysis. *PloS One* **10** (6), e0129073.

Nasiri, M. J., Dabiri, H., Fooladi, A. A. I., Amini, S., Hamzehloo, G. & Feizabadi, M. M. 2018a High rates of nontuberculous mycobacteria isolation from patients with presumptive tuberculosis in Iran. *New Microbes and New Infections* **21**, 12–17.

Nasiri, M. J., Heidary, M., Azimi, T., Goudarzi, H., Tabarsi, P., Sarokhalil, D. D. & Feizabadi, M. M. 2018b Mycobacterium simiae pulmonary disease in Iran: a systematic review and meta-analysis. *New Microbes and New Infections* **26**, 118–123.

Prevots, D. R. & Marras, T. K. 2015 Epidemiology of human pulmonary infection with non-tuberculous mycobacteria: a review. *Clinics in Chest Medicine* **36** (1), 553–567.

Roth, A., Reischl, U., Streubel, A., Naumann, L., Kroppenstedt, R. M., Habicht, M., Fischer, M. & Mauch, H. 2000 Novel diagnostic algorithm for identification of mycobacteria using genus-specific amplification of the 16S-23S rRNA gene spacer and restriction endonucleases. *Journal of Clinical Microbiology* **38** (3), 1094–1104.

Sax, H., Bloemberg, G., Hasse, B., Sommerstein, R., Kohler, P., Achermann, Y., Rössle, M., Falk, V., Kuster, S. P. & Böttger, E. C. 2015 Prolonged outbreak of Mycobacterium chimaera infection after open-chest heart surgery. *Clinical Infectious Diseases* **61** (1), 67–75.

Sehulster, L., Chinn, R. Y., Arduino, M. J., Carpenter, J., Donlan, R., Ashford, D., Besser, R., Fields, B., McNeil, M. & Whitney, C. 2003 Guidelines for environmental infection control in health-care facilities. *Morbidity and Mortality Weekly Report. Recommendations and Reports* **52** (10), 1–42.

Sommerstein, R., Rüegg, C., Kohler, P., Bloemberg, G., Kuster, S. P. & Sax, H. 2016 Transmission of Mycobacterium chimaera from heater–cooler units during cardiac surgery despite an ultraclean air ventilation system. *Emerging Infectious Diseases* **22** (6), 1008.

Soni, I., De Groote, M. A., Dasgupta, A. & Chopra, S. 2016 Challenges facing the drug discovery pipeline for non-tuberculous mycobacteria. *Journal of Medical Microbiology* **65** (1), 1–8.

Springer, B., Wu, W.-K., Bodmer, T., Haase, G., Pfyffer, G. E., Kroppenstedt, R. M., Schröder, K., Emler, S., Kilburn, J. O. & Kirschner, P. 1996 Isolation and characterization of a unique group of slowly growing mycobacteria: description of Mycobacterium lentiflavum sp. nov. *Journal of Clinical Microbiology* **34** (5), 1100–1107.

Telenti, A., Marchesi, F., Balz, M., Bally, F., Böttger, E. & Bodmer, T. 1993 Rapid identification of mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis. *Journal of Clinical Microbiology* **31** (2), 175–178.

Tortoli, E., Mattei, R., Russo, C. & Scarparo, C. 2006 Mycobacterium lentiflavum, an emerging pathogen? *Journal of Infection* **52** (6), e185–e187.

Torvinen, E., Suomalainen, S., Lehtola, M. J., Miettinen, I. T., Zacheus, O., Paulin, L., Katila, M.-L. & Martikainen, P. J. 2004 Mycobacteria in water and loose deposits of drinking water distribution systems in Finland. *Applied and Environmental Microbiology* **70** (4), 1973–1981.

Vaerewijck, M. J., Huys, G., Palomino, J. C., Swings, J. & Portaels, F. 2005 Mycobacteria in drinking water distribution systems: ecology and significance for human health. *FEMS Microbiology Reviews* **29** (5), 911–934.

Wolinsky, E. 1979 Nontuberculous mycobacteria and associated diseases. *American Review of Respiratory Disease* **119** (1), 107–159.

First received 10 December 2017; accepted in revised form 2 January 2019. Available online 1 February 2019