Pseudo-outbreak of *Mycobacterium gordonae* in a teaching hospital: importance of strictly following decontamination procedures and emerging issues concerning sterilization

Laura Scorzolini¹, Fabio Mengoni¹, Claudio M. Mastroianni¹, Rossella Baldan², Daniela M. Cirillo², Maria De Giusti³, Lucia Marinelli³, Alessia Cottarelli³, Lanfranco Fattorini⁴, Vincenzo Vullo¹

¹Infectious Diseases Unit, Department Public Health and Infectious Disease, “Sapienza” University of Rome, Italy; ²Emerging Bacterial Pathogens Unit, Department of Immunology, Transplantation and Infectious Diseases, IRCCS San Raffaele Scientific Institute, Milan, Italy; ³Hygiene Unit, Department Public Health and Infectious Disease, “Sapienza” University of Rome, Italy; ⁴Department of Infectious, Parasitic and Immune-Mediated Diseases, Italian National Institute of Health, Rome, Italy

**SUMMARY**

Aim of this study was to investigate a pseudo-outbreak of *Mycobacterium gordonae* analyzing isolates detected from clinical and environmental samples. *Mycobacterium gordonae* was detected in 7 out of 497 broncho-alveolar lavage (BAL) samples after bronchoscopy procedure in patients admitted to a teaching hospital between January and April 2013. During this pseudo-outbreak clinical, epidemiological, environmental and molecular investigations were performed. None of the patients met the criteria for non-tuberculous mycobacterial (NTM) lung disease and were treated for *M. gordonae* lung disease. Environmental investigation revealed *M. gordonae* in 3 samples: in tap water and in the water supply channel of the washer disinfector. All the isolates were subjected to genotyping by pulsed-field gel electrophoresis (PFGE). The PFGE revealed that only patients’ isolates presented the same band pattern but no correlation with the environmental strain was detected. Surveillance of the outbreak and the strict adherence to the reprocessing procedure and its supplies resulted afterwards in no detection of *M. gordonae* in clinical respiratory samples. Clinical surveillance of patients was crucial to establish the start of NTM treatment. Regular screening of tap water and endoscopic equipment should be adopted to compare the clinical strains with the environmental ones when an outbreak occurs.

**KEY WORDS:** Bronchoschopy, *Mycobacterium gordonae*, Pseudo-outbreak, Pulsed-field gel electrophoresis.

**INTRODUCTION**

*Mycobacterium gordonae* is a rare human pathogen despite its ubiquity in the environment (Weiss et al., 2012). The microorganism, previously known as the “tap water bacillus” or *Mycobacterium aquae*, was commonly isolated from water, soil and non-pasteurized fresh milk (Lessnau et al., 1993). *Mycobacterium gordonae* has a low virulence compared with others non-tuberculous mycobacteria (NTM), with a mortality rate of less than 0.1% (Weiss et al., 2012). Infection due to *M. gordonae* usually involves children, immunocompromised patients (AIDS, steroid therapy, carcinoma, transplant recipient), or patients with underlying diseases. However, *M. gordonae* can also cause disease in immunocompetent patients and in surgical patients through respiratory, cutaneous, parental and gastrointestinal exposure (Gonzales et al., 1971; Aguado et al., 1987; Weinberger et al., 1992; Bagarazzi et al., 1996; Bonnet et al., 1996; Harro et al., 1997; Rusconi et al., 1997; Phillips.
et al., 2001; Foti et al., 2009; Mazumder et al., 2010; Morimoto et al., 2015). Mycobacterium gordonae is the second most frequently identified species of NTM isolated in hospital laboratories and pulmonary samples and about 1% of clinical specimens are culture positive for M. gordonae (Good et al., 1982; Tokars et al., 1990; Metchock et al., 1999; Hoefsloot et al., 2013). Moreover, M. gordonae can often contaminate the hospital water supply causing nosocomial outbreaks or pseudo-outbreaks. Pseudo-outbreaks are defined as clusters of false infections or artefactual clusters of real infections (Weinstein et al., 1977). Several pseudo-outbreaks related to M. gordonae have been reported (Table 3). Since 1984, in view of several reports concerning the contamination of fiberoptic bronchoscopy by Mycobacterium tuberculosis and NTM, it was recommended to disinfect bronchoscopes and their accessories with both glutaraldehyde and iodophor solution to prevent contamination with mycobacteria (Davis et al., 1984). Bronchoscopes are used frequently, and their intricate design makes cleaning and disinfection difficult (Phillips et al., 2001). Early identification of a pseudo-outbreak is mandatory to avoid unnecessary antimonycobacterial treatment and delay the true diagnosis. The authors of this report tackled a pseudo-outbreak, most likely due to the contamination of bronchoscope equipment.

MATERIALS AND METHODS

Clinical and microbiological detection

During the period between January and April 2013, M. gordonae was isolated in 7 out of 497 BAL from patients who underwent bronchoscopy for various lung diseases including infections at the “Policlinico Umberto I” teaching hospital of Rome (Table 1). The criteria for classification and predisposing factors of NTM lung disease infection were in accordance with the standard criteria for the diagnosis of NTM infection (Griffith et al., 2007). Immunocom-

<table>
<thead>
<tr>
<th>Pts</th>
<th>Pulmonary disease</th>
<th>Symptoms</th>
<th>Comorbidity</th>
<th>CT Imaging</th>
<th>Presence AFB in BAL</th>
<th>TST reactive/AFB in sputum</th>
<th>Therapy</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pulmonary fibrosis</td>
<td>Cough and dyspnoea</td>
<td>Right cardiopathy</td>
<td>Ground glass and bilateral pulmonary infection</td>
<td>Yes</td>
<td>No/negative</td>
<td>INH+RIF+PIR+ETB stopped after 12 days</td>
<td>Dead*</td>
</tr>
<tr>
<td>2</td>
<td>COPD</td>
<td>Worsening of cough</td>
<td>HCV+ Fibrotic small nodules, bronchiect.</td>
<td>No</td>
<td>Yes/negative</td>
<td>INH+RIF</td>
<td>Cured</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>COPD</td>
<td>Worsening of cough</td>
<td>HCV+ bronchiect.</td>
<td>No</td>
<td>No/negative</td>
<td>No</td>
<td>Cured</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Emp.</td>
<td>Worsening of cough</td>
<td>None</td>
<td>Bullous cavity</td>
<td>No</td>
<td>No/negative</td>
<td>No</td>
<td>Lung Tx</td>
</tr>
<tr>
<td>5</td>
<td>No Recent dyspnea</td>
<td>Uterine cancer</td>
<td>Suspicious lesion and mediastinal lymphnode involvement</td>
<td>No</td>
<td>No/negative</td>
<td>No</td>
<td>NSCLC</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>No Recent dyspnea</td>
<td>None</td>
<td>Irregular nodule</td>
<td>No</td>
<td>No/negative</td>
<td>No</td>
<td>NSCLC</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>No Fever and dyspnea</td>
<td>None</td>
<td>Pleural effusion</td>
<td>No</td>
<td>No/negative</td>
<td>No§</td>
<td>Cured</td>
<td></td>
</tr>
</tbody>
</table>

promising condition was defined as having human immunodeficiency virus infection, diabetes, active malignancy, or receiving chronic immunosuppressive therapy. Demographic, clinical, radiological, microbiological and laboratory data were prospectively recorded and each patient was monitored until the definite lung disease was reached and clinical outcome was observed. Expert clinicians decided whether to start antimycobacterial treatment based on the potential risk and benefit of therapy for each individual patient, according to clinical, radiological and microbiological features. Microscopic examination for detection of acid fast bacilli (AFB) was performed in all BALs sent to the laboratory for the diagnosis of M. tuberculosis or other NTM infections. All clinical samples were digested-decontaminated using equal volumes of 4% NaOH with 0.5% N-acetyl-L-cysteine at room temperature. Mycobacteria growth indicator tube (MGIT) liquid medium vials (MGIT 960, Beckton Dickinson, Sparks, Maryland, USA) were inoculated after adding the required supplements according to the manufacturer's instructions. Löwenstein-Jensen slants (Beckton Dickinson, Sparks, Maryland, USA) were also inoculated at the same time as the MGIT tubes. Other respiratory samples were collected after the detection of M. gordonae from BAL. The health management of the hospital received the results of the clinical investigation.

Environmental investigation

In July 2013 an environmental investigation was carried out on the bronchoscopy service involved in all seven cases of detection of M. gordonae in BAL. The centre has three bronchoscopes to perform the service (about 20 endoscopies per week) and it uses an Olympus autodisinfector to reprocess bronchoscopes (peracetic acid 0.1% at 35°C for 5 minutes, followed by rinsing with tap water, without a bacterial filter and drying through air flow). After reprocessing, bronchoscopes are stored in plastic bags ready for reuse. Fourteen environmental samples were collected after the detection of M. gordonae from BAL. The health management of the hospital received the results of the clinical investigation.

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Sample No.</th>
<th>Positive No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flushing of biopsy/suction channel from Bronchoscopes</td>
<td>3</td>
<td>0 (a,b,c)</td>
</tr>
<tr>
<td>Flushing of connectors of the washer disinfecter</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Swabs of internal surfaces of the washer disinfecter</td>
<td>5</td>
<td>0 (d,e,f)</td>
</tr>
<tr>
<td>Tap water</td>
<td>1</td>
<td>1 (7.14)</td>
</tr>
<tr>
<td>Swab from inside tap</td>
<td>1</td>
<td>1 (7.14)</td>
</tr>
<tr>
<td>Final rinse water of the washer disinfecter</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Swab of water supply channel of the washer disinfecter</td>
<td>1</td>
<td>1 (7.14)</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>3 (21.43)</td>
</tr>
</tbody>
</table>


Molecular identification, typing and analysis

Mycobacteria were identified to the species level by conventional tests using the GenoType Mycobacterium CM assay according to the manufacturers' procedures (Arnika, HainLife-science GmbH, Nehren, Germany). The GenoType protocol consists of PCR amplification, hybridization of the PCR products to the strip, detection and interpretation of the results. Clonality of clinical and environmental isolates was determined by pulsed-field gel electrophoresis (PFGE), using the CHEF Genomic DNA
Plug kit (Bio-Rad). Bacteria were inoculated in 20 ml of 7H9 medium (Beckton Dickinson), modified by the addition of 10% ADC (Beckton Dickinson), 0.1% Tween 80 (wt/vol) (Sigma-Aldrich) and 0.5 M sucrose (Sigma-Aldrich), and were incubated at 37°C for 14 days. 1 mg/ml D-cycloserine was added to the culture, which was incubated at 37°C for a further 16 hours and then centrifuged at 12000 rpm for 5 minutes. The pellet was resuspended in 150 µl of cell suspension buffer (Bio-Rad), incubated at 75°C for 20 minutes with shaking (400 rpm), mixed with 150 µl of 2% low-melting-point agarose (Bio-Rad) at 55°C, and cast in plugs. Bacteria in plugs were lysed in 500 µl of lysis buffer containing 2 mg/ml lysozyme (Bio-Rad) for 4 hours at 37°C. The lysis buffer was then removed and plugs were incubated for 40 hours at 50°C in 500 µl of proteinase K buffer and 20 µl of proteinase K (Bio-Rad). Plugs were washed twice for 30 minutes at 55°C with TE buffer (Tris 10 mM, EDTA 1 mM, pH 8.0) containing 0.04 mg/ml of phenylmethylsulfonyl fluoride (PMSF) and 3 times for 30 minutes at room temperature with TE buffer. Genomic DNA was digested overnight at 37°C with 50U of restriction endonuclease XbaI in NE4 buffer as recommended by the manufacturer (Euroclone). Digested plugs were loaded into a 1% agarose gel prepared in TBE 0.5X buffer. PFGE was run in TBE 0.5X containing 100 µM thiourea (Sigma-Aldrich), using a CHEF DRIII system (Bio-Rad) at 14°C, 6V/cm voltage gradient, initial switch time of 3 s, final switch time of 12 s, run time of 20 hours, included angle of 120°. The band patterns obtained were interpreted as described by Tenover (Tenover et al., 1995). The analysis was performed using the InfoQuest FP (v5.1) software (Bio-Rad) and clustering was achieved using Dice coefficients and the unweighted pair group method with averages (UPGMA).

RESULTS

Clinical investigation

Clinical, radiological and microbiological characteristics of patients with *M. gordonae* isolated from BAL are shown in Table 1. Seven patients had undergone bronchoscopy for the following reasons: pulmonary infection in pulmonary fibrosis (pt 1), worsening of Chronic Obstructive Pulmonary Disease (COPD) (pts 2 and 3), screening for lung transplant in severe emphysema (pt 4), suspicion of pulmonary malignancy (pts 5 and 6), onset of pleural effusion (pt 7). According to the standard criteria of American thoracic Society (ATS) and Infectious Disease Society of America (IDSA) for diagnosis of NTM lung diseases infection (Griffith *et al*., 2007), no patients had any signs or symptoms indicative of mycobacterial lung disease. Nobody made mention of productive sputum, fatigue, malaise and chest pain to the clinician. No weight loss or hemoptysis were observed in the patients studied. Only one patient (pt 2) had radiological signs suspected most likely due to latent tuberculosis infection (LTI). The only patient who was smear-positive for AFB on direct examination of the BAL developed pulmonary infection due to the *Aspergillus spp* (pt 1). For all patients sputum examination for AFB and culture were negative for *M. tuberculosis* and NTM before and after bronchoscopy. No bacterial growth was detected from the pleural fluid of the patient with fever and pleural effusion. After bronchoscopy, no patient referred onset of chest pain, worsening of cough, sputum production, fever, shortness of breath. Concerning treatment adopted, only patient number 1 was treated with antituberculosis drugs (isoniazid, rifampin, pirazynamide and ethambutol) based on the observation of AFB in the BAL which were later identified as *M. gordonae*. The therapy was stopped after 12 days due to clinical and radiological worsening of the patient and microbiological isolation of *Aspergillus spp* and *Staphylococcus aureus* from BAL. The patient died three days later after the exact diagnosis.

Bibliographic search

In order to collect data on the *M. gordonae* pseudo-outbreak related to bronchoscopy, we performed a bibliographic search using both medical databases PubMed (Medline) and Scopus with the key words: “*Mycobacterium gordonae*, pseudo-outbreak, bronchoscopy, contaminated hospital equipment”, using the Boolean operator AND/OR. In addition to investigate non-indexed articles, we investigated the “grey literature”, through Google.
According to the Royal college of Physician guidelines for treatment of LTBI (2006), one patient (pt 2) received prophylaxis with isoniazid (INH) and rifampin (RIF) for three months. A pseudo-outbreak was suspected considering the discordance between the increasing number of \textit{M. gordonae} cultures' positive and the clinical finding of patients involved. The median time of positive culture detection was 9 days (range 5-13 days), immediately after the positive results environmental investigation was promptly started.

No NTM infection was observed during the six months of follow-up to the population study.

**Environmental investigation**

Environmental investigation was carried out and qualitative analysis of the environmental samples revealed the presence of \textit{M. gordonae} in 3 out of 14 samples (21.43%), in tap water, internal surface of the tap and into water supply channel of the washer disinfecter (Table 2). No \textit{M. gordonae} was detected in the suction channel of the bronchoscope probably due to the presence of abundant multidrug-resistant microorganisms such as \textit{Achromobacter xylosoxidans}, that may be able to mask and/or inhibit mycobacteria growth.

**Molecular typing**

The PFGE analysis revealed that all \textit{M. gordonae} strains isolated from patients’ BAL (M.GOR 1 to M.GOR 7) showed an identical pulsotype, suggesting that the pseudo-outbreak was sustained by a unique strain (Figure 1). It was possible to carry out the PFGE analysis of only one \textit{M. gordonae} (M.GOR 8) isolated from environmental samples (a swab from the water supply channel of the washer disinfecter). This strain, compared with \textit{M. gordonae} strains isolated from clinical samples, presented an unrelated pulsotype (Figure 1).

**Bibliographic research**

As shown in Table 3, we included 16 articles from 1976 to 2015 in the review. To our knowledge, during 2015 only two scientific articles were published regarding nosocomial pseudo-outbreaks due to \textit{M. gordonae} associated with hospital water supply contamination (Pra-baker \textit{et al.}, 2015; Zojo-tru \textit{et al.}, 2015). Three studies (Leclerc \textit{et al.}, 1985; Stine \textit{et al.}, 1987; Gubler \textit{et al.}, 1992) dealt with bronchoscopy procedures as a source of contamination for 41 out of 511 (8.00%) known specimens. Only one Italian study dealt with \textit{M. gordonae} pseudo-outbreaks while the others were from various countries: in particular from USA (n. 7) and from France (n. 2).

**DISCUSSION**

\textit{Mycobacterium gordonae} is the most commonly encountered NTM in water, with concentrations as high as 1,000 colony-forming units per liter (Lessnau, 2015). Contamination of bronchoscopes with or without infection of the patients has been reported for several pathogenic microorganisms, including \textit{M. tuberculosis} and NTM (Gubler \textit{et al.}, 1992; Wallace \textit{et al.}, 1998). Literature data show that \textit{M. gordonae} is less frequently involved in pseudo-outbreaks than other NTM (especially rapidly growing species) and, compared with other pathogenic species, it typically does not lead to infection (Phillips \textit{et al.}, 2001). Positive cultures of sputum and BAL for \textit{M. gordonae} should be interpreted with caution since these cultures are more likely to represent contamination (Mazumder \textit{et al.}, 2010; Johnson \textit{et al.}, 2014). However, if NTM are recovered from respiratory samples, it might be difficult
to discriminate if they are pathogenic or not, even considering the possibility of NTM affecting immunocompetent people, as described in the literature. On the other hand, the detection of NTM from sputum could delay the diagnosis of the other important lung diseases (Griffith et al., 2007). Therefore it is fundamental to carefully evaluate the patient’s clinical course and perform environmental investigation as soon as possible to determine the source. The diagnosis of NTM disease is complex and requires a multidisciplinary approach involving the clinician, microbiologist and radiologist. According to the criteria for NTM lung infection of American Thoracic Society clinical, radiological and microbiological evidence is required to achieve the definitive diagnosis. In addition, symptoms are not specific, most of the time the patient has a chronic cough due to underlying pulmonary disease and systemic symptoms are frequently not present. Radiological features are characteristic and finally microbiological evidence plays a fundamental role. When possible, histological or cytological analysis help to establish the diagnosis in difficult cases (van Ingen 2013). In the present study, all patients observed showed signs and symptoms unrelated to mycobacterial disease and only one patient had suspected radiological features most likely due to LTI, with TST reactive. Nevertheless, one patient received treatment for active tuberculosis. Concerning the clinical features of the other patients involved, the clinicians decided not to start antimycobacterial treatment according to the recommendation of ATS for NTM infection treatment. NTM lung disease is generally slowly progressive (relative to TB) so it allows enough time to collect multiple biological samples and microbiological data to be sure to reach the right diagnosis without embarking on long therapy with possible drug-related side-effects. In our experience, the detection of usually non pathogenic NTM as M. gordonae, the clinical presentation of patients and the same type of contaminated biological samples (BAL) made it possible to detect the pseudo-outbreak. The absence of M. gordonae in the following specimens of patients confirmed that no patient was colonized by the same microorganism. However the absence of AFB in the BAL and the long period needed for growth and identification of isolates, justified the delayed investigations and confirmed the high suspicion of contamination. According to recent reports by Diacon and Morimoto, a shorter time required to detect a positive culture and the subtype of M. gordonae could be used as the diagnostic criteria for M. gordonae pulmonary disease (Diacon et al., 2012; Morimoto et al., 2015). Prompt identification of the pseudo-outbreak and strict follow-up of involved patients is fundamental to avoid delayed real diagnosis and unnecessary therapy. In our population study the source of contamination was most likely derived from the bronchoscopy but the original source could not be identified probably due to different M. gordonae pulsotypes present in the environmental water. Moreover the delayed identification of the pseudo-outbreak caused delay in the environmental investigation. As widely described in the literature, NTM pseudo-outbreaks occurred in spite of several recommendations for disinfection of endoscopes (Kovaleva et al., 2013). Careful adherence to established decontamination guidelines is critical with endoscopy equipment. Many outbreaks and pseudo-outbreaks due to NTM and other waterborne pathogens have been related to rinsing flexible endoscopy due to NTM and other waterborne pathogens which have been related to rinsing flexible endoscopy with non sterile water (Beilenhoff et al., 2008). An environmental strategy to guarantee water quality for rinsing in automatic endoscope reprocessors should be the use of bacterial filters. Our experience shows that their absence on the water supply of the washer disinfector does not prevent contamination of waterborne pathogens (Table 2).

According to the guidelines of the World Health Organization, the European Commission directive (European Union Council Directive 98/83/EC) states that drinking water should not contain pathogenic microorganisms in a quantity or at a concentration able to adversely affect human health. However, the routine bacteriological examinations carried out on drinking water do not include a search for NTM, and frequently the endoscopy service companies do not include any device that achieves water ste-
rility. The ISO 15883-4:2008 (ISO 15883-4:2008) identified the responsibilities of the clinical service provider who must ensure the water quality for the washer-disinfector. Furthermore the lack of drying procedure has been also associated with many outbreaks of endoscopy-related

<table>
<thead>
<tr>
<th>Micro.</th>
<th>N° of Specim.</th>
<th>Contaminated Specim.</th>
<th>Source of contamination</th>
<th>Cause of contamination</th>
<th>Clinical Implications</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MG</td>
<td>7</td>
<td>Sputum</td>
<td>Aerosol-induction-machine</td>
<td>Contaminated tap water</td>
<td>Not specified</td>
<td>Gangadharam 1976</td>
</tr>
<tr>
<td>MG</td>
<td>52</td>
<td>Bronchial secretion</td>
<td>Local anesthetic</td>
<td>Dye additive for local anesthetic machines</td>
<td>None</td>
<td>Steere 1979</td>
</tr>
<tr>
<td>MG</td>
<td>32</td>
<td>Various</td>
<td>Water</td>
<td>Contaminated ice machines</td>
<td>Not specified</td>
<td>Panwalker 1986</td>
</tr>
<tr>
<td>MG</td>
<td>22</td>
<td>Bronchial secretion</td>
<td>Bronchoscope</td>
<td>Tap water used to rinse instruments</td>
<td>2 pts treated for TBI</td>
<td>Stine 1987</td>
</tr>
<tr>
<td>MG</td>
<td>46</td>
<td>Various</td>
<td>Culture medium</td>
<td>Antimicrobial solution in Bactec TB system</td>
<td>4 pts treated with anti-mycob. therapy</td>
<td>Tokars 1990</td>
</tr>
<tr>
<td>MG</td>
<td>3</td>
<td>Smears</td>
<td>Water</td>
<td>Contaminated deionized water unit</td>
<td>Not specified</td>
<td>Gubler 1992</td>
</tr>
<tr>
<td>MCH/MG</td>
<td>14*</td>
<td>Bronchial secretion</td>
<td>Bronchoscope</td>
<td>Contaminated tank of bronchoscopy washing machine</td>
<td>4 pts treated for TBI</td>
<td>Gubler 1992</td>
</tr>
<tr>
<td>MG</td>
<td>84</td>
<td>Clinical specimen</td>
<td>Water</td>
<td>Contaminated tap water used to mouth rinse before collection samples</td>
<td>None</td>
<td>Arnow 2000</td>
</tr>
<tr>
<td>MG</td>
<td>5</td>
<td>Sputum/gastric aspirate</td>
<td>Water</td>
<td>Contaminated water from refrigerator fountain</td>
<td>4 pts treated for TBI</td>
<td>Lalande 2001</td>
</tr>
<tr>
<td>MG</td>
<td>16</td>
<td>Bronchial secretion</td>
<td>Bronchoscope</td>
<td>Contaminated water of bronchoscopy washing machine</td>
<td>16 pts treated with anti-mycob. therapy</td>
<td>Rossetti 2002</td>
</tr>
<tr>
<td>MG</td>
<td>ns</td>
<td>Lung operative specimen</td>
<td>Water of surgical ward</td>
<td>Contamination of the apparatus for making super-oxidized water</td>
<td>None</td>
<td>Fujita 2002</td>
</tr>
<tr>
<td>MG</td>
<td>15</td>
<td>Smears</td>
<td>Water</td>
<td>Ground water contamination of water tank</td>
<td>Not specified</td>
<td>Moore 2005</td>
</tr>
<tr>
<td>MG</td>
<td>18</td>
<td>Various</td>
<td>Not found</td>
<td>Probable contaminated distilled water</td>
<td>None</td>
<td>Esteban 2006</td>
</tr>
<tr>
<td>MG</td>
<td>27</td>
<td>Respiratory cultures</td>
<td>Water</td>
<td>Contaminated ice machines</td>
<td>Not specified</td>
<td>Azalea 2008</td>
</tr>
<tr>
<td>MG</td>
<td>35</td>
<td>Clinical specimen</td>
<td>Water</td>
<td>Contaminated tap water</td>
<td>4 pts treated with anti-mycob. therapy</td>
<td>Prabaker 2015</td>
</tr>
<tr>
<td>MG</td>
<td>135</td>
<td>Pulmonary samples</td>
<td>Water</td>
<td>Contaminated cold tap water</td>
<td>64 pts treated with anti-mycob. therapy</td>
<td>Zloiotro 2015</td>
</tr>
</tbody>
</table>

infection due to NTM and other waterborne microorganisms (Beilenhoff et al., 2008), and the washer-disinfector performs only a short drying cycle by forced air. According to the American Guidelines (ASGE 2011), flushing with 70%-90% ethyl or isopropyl alcohol is recommended for drying of endoscope channel to reduce the risk of remaining pathogens. Thus any gap between the disinfection phase and rinse/drying cycle should be evaluated carefully. In our experience also the low temperature at 35°C during reprocessing cycle fails in the effectiveness of high disinfection. The contamination of medical instruments is related to the high resistance of mycobacteria and especially of M. gordonae, which is able to survive after treatment with 2% glutaraldehyde for more than 10 minutes and to the practice of rinsing medical instruments with tap water after immersion in the disinfectant (Collins, 1986; Stine et al., 1987). It has been reported that super-oxidized water is an effective disinfectant for Mycobacteria, however contamination of mycobacteria could have occurred inside the apparatus as well in the draining tubes (Ayliffe, 2000; Fujita et al., 2002).

To our knowledge, as shown in Table 3, pseudo-outbreaks due to M. gordonae have been associated with the environmental contamination of several supplements of hospital tap water and different medical equipment (endoscopes, local anesthetic, aerosol machine, super-oxidized water apparatus) by any step of use. As described in the literature, contamination with NTM can also occur after seemingly proper disinfection of the bronchoscopes has been performed (Davis et al., 1984). Even obtaining biological samples could result in contamination at any time from the collection of the specimen by the patient to the laboratory processing steps. It is generally accepted that the strategy to prevent infection due to endoscopy procedures should always include the molecular characterization of NTM and other pathogens.

Our study had some limitations: we did not find the correlation between clinical and environmental stains. This is probably due to the changing epidemiology of isolates from the water system during the relatively long time between the execution of clinical specimens and the microbiological results. Moreover only one out of three of environmental strains of M. gordonae was genotyped due to sample contamination.

Diagnosis of NTM lung disease remains challenging due to aspecificity of symptoms, so clinical observation, radiologic tools and microbiology data still represent the gold standard for diagnosis. Our results confirms that clinical survey, together with environmental and molecular investigations are valid tools to distinguish a pseudo-outbreak from true clinical infection. The adoption of this strategy could avoid unnecessary antimicrobial treatment and allow an optimal public health intervention. Regularly screening for NTM of the hospital water and endoscopic instrumentation should be recommended to compare the clinical strains with the environmental ones, when the outbreak occurs.

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