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

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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: Broncoschopy, *Mycobacterium gordonae,* Pseudo-outbreak, Pulsed-field gel electrophoresis..

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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, parenteral 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

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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 antimycobacterial 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 1 - *Characteristics of patients with bal positive for m. gordonae.*

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

Pts (patients). CT (Computed Tomography). AFB (acid fast-bacilli). TST (Tuberculin skin test). INH (isoniazid). RIF (rifampin). PIR (pirazynamide). ETB (ethambutol). COPD (chronic obstructive pulmonary disease). LTBI (latent tuberculosis infection). HCV (Hepatitis C virus). Bronchiect. (bronchiectasis). Emp (emphysema). Tx (Transplant). NSCLC (non-small cell lung cancer). *Pt 1 died for pulmonary Aspergillosis (growth of *Aspergillus spp* from BAL, presence of galattomannan antigen from blood and BAL). §Pt 7 was cured with antibacterial therapy.

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 antimicobacterial 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 from water and surfaces of the endoscopy service (Table 2) including: 3 biopsy/suction channels and 2 connectors of the washer disinfector sampling by flushing of 50 ml of sterile saline (0.9%) and collecting in a sterile container (De Giusti *et al.*, 1993); 1 sample of tap water (100 ml) and 1 of the final rinse water of the washer disinfector (100 ml); 5 swabs from the internal surface of the autodisinfector, 1 swab from the water supply channel and 1 swab of the water supply channel of the washer disinfector. All samples were examined for mycobacterial growth by decontamination and inoculation into the MGIT tubes and Löwenstein-Jensen slants. All culture media were incubated at 36±1°C until mycobacterial growth was detected. Smears of colonies grown on the Löwenstein-Jensen medium from clinical and environmental samples were stained with the Ziehl-Neelsen (Beckton Dickinson).

TABLE 2 - *Enviromental samples positive for m. gordonae.*

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

Other species: *Achromobacter xylosoxidans* MDR (a). *Klebsiella pneumoniae* (b). *Sphyngomonas paucimobilis* (c). *Micrococcus luteus/lylae* (d). *Brevundimonas diminuta/vesicularis* (e). *Rhizobium radiobacter* (f).

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, HainLifescience 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% Tween80 (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 rmp), 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).

Bibliographic search

In order to collect data on the *M. gordonae* pseudo-outbreak related to broncoschopy, we performed a bibliographic search using both medical databases PubMed (Medline) and Scopus with the key words*: "Mycobacterium gordonae,* pseudo-outbreak, broncoschopy, contaminated hospital equipment", using the Boolean operator AND/OR. In addition to investigate non-indexed articles, we investigated the "grey literature", through Google.

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.

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 *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 *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 disinfector (Table 2). No *M. gordonae* was detected in the suction channel of the bronchoscope probably due to the presence of abundant multidrug-resistant microorganisms such as *Achromobacter xylosoxidans,* that may be able to mask and/or inhibit mycobacteria growth.

Molecular typing
The PFGE analysis revealed that all *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 *M. gordonae* (M.GOR 8) isolated from environmental samples (a swab from the water supply channel of the washer disinfector). This strain, compared with *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 *M. gordonae* associated with hospital water supply contamination (Prabaker *et al.,* 2015; Zlojtro *et al.,* 2015). Three

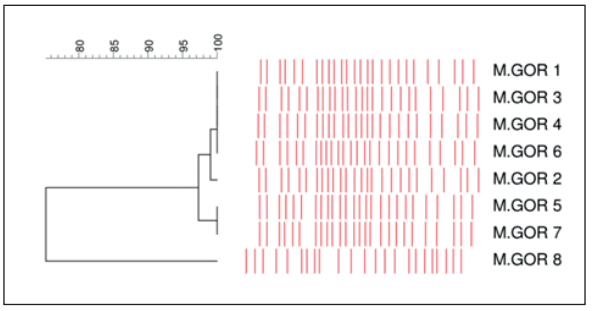

FIGURE 1 - *PFGE analysis of the strain of M. Gordonae. Dendrogram and PFGE patterns of XbaI-digested chromosomal DNA of M. gordonae (M.GOR) strains. M.GOR 1 to 7: M. gordonae strains isolated from patients' clinical samples (BAL). M.GOR 8: M. gordonae strain isolated from the environment. The dendrogram was constructed using Dice similarity coefficients (1.5% optimization and 1.5% position tolerance) and unweighted pair group method with averages.*

studies (Leclerc *et al.,* 1985; Stine *et al.,* 1987; Gubler *et al.,* 1992) dealt with broncoscopy procedures as a source of contamination for 41 out of 511 (8.00%) known specimens. Only one Italian study dealt with *M. gordonae* pseudo-outbreaks while the others were from various countries: in particular from USA (n. 7) and from France (n. 2).

DISCUSSION

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 *M. tuberculosis* and NTM (Gubler *et al.,* 1992; Wallace *et al.,* 1998). Literature data show that *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 *et al.,* 2001). Positive cultures of sputum and BAL for *M. gordonae* should be interpreted with caution since these cultures are more likely to represent contamination (Mazumder *et al.,* 2010; Johnson *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 with non sterile water (Kovaleva *et al.,* 2013). According to the Guideline Committee of the European Society of Gastrointestinal Endoscopy, after the disinfection phase the final rinse should be carried out with sterile water or bacterium-free 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 3 - *Pseudo-outbreak related to m. gordonae.*

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

Micro. (microorganism). Specim. (specimen). MG: *M. gordonae*. TBI: tuberculosis infection. Anti-mycob. (anti-mycobacterial).
MCH: *M. chelonae*. Ns: not specified. *3 specimens were positive for MG.

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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