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Short Communication

An unusual outbreak of nontuberculous mycobacteria in hospital respiratory wards: Association with nontuberculous mycobacterial colonization of hospital water supply network

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ABSTRACT

The incidence and prevalence of pulmonary nontuberculous mycobacterial (NTM) infection is increasing worldwide arousing concerns that NTM infection may become a serious health challenge. We recently observed a significant increase of NTM-positive sputa samples from patients referred to respiratory disease wards of a large tertiary hospital in Rome. A survey to identify possible NTM contamination revealed a massive presence of NTM in the hospital water supply network. After decontamination procedures, NTM presence dropped both in water pipelines and sputa samples. We believe that this observation should encourage water network surveys for NTM contamination and prompt decontamination procedures should be considered to reduce this potential source of infection.

Introduction

Epidemiological studies suggest that the incidence and prevalence of pulmonary nontuberculous mycobacterial (NTM) disease is rising in different geographical areas around the world, including North and South America, Africa, and Asia. These findings have aroused concerns in the scientific community that NTM infection may become a serious health challenge [1,2].

NTM are a large family of microorganism defined as mycobacteria other than *Mycobacterium tuberculosis* and *Mycobacterium leprae*. Lung disease due to NTM ranges from immune-mediated reactions (such as "hot tube lung" in which exposure to antigens from microorganisms belonging to the *Mycobacterium avium* complex [MAC] that contaminates the hot water of indoor or outdoor pools causing a form of hypersensitivity pneumonia) to direct airway colonization that may eventually lead to lower airway infections [3]. Direct

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infection occurs more often in patients with structural lung diseases, such as chronic obstructive pulmonary disease, bronchiectasis, cystic fibrosis, and interstitial lung diseases, but it may also develop, and in some cases lead to death, in patients without a previously recognized lung disorder.

The disease onset is variable and characterized by symptoms that include cough, fatigability, asthenia, sweating, night fever, weight loss, and in some cases even hemoptysis, all of which are not specific and immediately suggestive of NTM. As a result, physicians may misdiagnose patients who therefore do not undergo any treatment or are inappropriately managed [4].

NTM are widely distributed in the environment and NTM organisms can be found with high isolation rates in soil and water [5–8]. There is no evidence of animal-to-human or human-to-human transmission of NTM and human disease is thought to be acquired from environmental exposure, although specific sources of infection in most cases cannot be determined.

Isolated epidemiological observations suggest that in Italy, as in other countries, a significant increase of NTM infections has taken place in the last few decades; however, its precise burden in terms of incidence and prevalence is not completely known [9].

We recently observed a significant increase of NTM-positive sputa samples from patients referred to respiratory disease wards of a large tertiary hospital in Rome. A survey to identify possible NTM contamination revealed a massive presence of NTM in the hospital water supply network. After decontamination procedures, NTM presence dropped both in water pipelines and sputa samples.

Materials and methods

Study population

Three early-morning sputum samples were collected from each individual referred to the respiratory wards of S. Camillo Forlanini Hospital between January 2011 and December 2013. Samples were tested using microscopy to detect acid-fast bacilli (AFB) and with culture for mycobacteria on Lowenstein–Jensen medium. Samples that were AFB positive were examined using INNO-Lipa Mycobacteria v2 (Immunogenetics NV Technologiepark, Ghent, Belgium) and ACCUPROBE (Gen-Probe Incorporated, San Diego, CA, USA) assays for mycobacteria identification as described by the manufacturer.

In order to exclude water supply contamination by NTM, in April 2013 samples from tap and shower water of the respiratory ward bathrooms and, as a control, from the main trunk which refills the entire hospital water pipeline network, and from faucets and shower water of other hospital buildings were collected in three different days (1000 mL each). The collected samples underwent identical procedures performed for NTM identification in sputa samples.

In May 2013, decontamination procedures (chlorination, thermal shock, and disposable filter installation on faucets) were performed. Thereafter and until December 2013, NTM presence was tested in sputa samples of all patients referred to the respiratory ward of the hospital as previously described.

Statistical analysis

Clinical and laboratory data were analyzed using Graph Pad Prism 4.0 (Graph Pad Software, San Diego, CA, USA). Data are expressed as percentage or mean ± standard deviation. Comparisons between the study groups were done with the nonparametric Mann–Whitney test and chi-square or chi-square trend test for frequency distribution analysis as more appropriate. Statistical significance was set at the two-tailed 0.05 levels.

Results and discussion

Sputa analysis showed a significant increase in NTM isolation rate in patients referred to the respiratory wards over time. In fact, in 2011 only 18% of 190 patients with sputa samples positive for AFB were positive for NTM. This percentage rose to 38.6% of the 251 AFB-positive patients in 2012, to 59.7% among the 67 AFB-positive patients in the 1st trimester of 2013, and to 53.33% of the 60 patients in the 2nd trimester (Fig. 1; $p < .0001$). The NTM subtype analyses revealed that MAC was the most frequent subtype with a frequency ranging from 61.5% to 87%, followed by *Mycobacterium gordonae* (2–19%), *Mycobacterium xenopi* (4–8%), and *Mycobacterium chelonae* (3–18%). *Mycobacterium fortitum*, *Mycobacterium simiae*, *Mycobacterium kansasi*, and *Mycobacterium abscessus* were also observed ranging from 0.4% to 2%.

Water supply network analysis demonstrated a massive NTM presence in the pipelines supplying the building of the respiratory wards compared with other building pipelines and to the main public trunk ($4 \times 10^4 \pm 0.5 \times 10^4$ CFU/L (colony forming unit/Liter) vs. $1 \times 10^3 \pm 0.2 \times 10^3$ and $1 \times 10^2 \pm 0.4 \times 10^2$, respectively, $p < .01$; Fig. 2). Most of NTM subtypes consistent with sputa analysis, were MAC (85%) followed by *M. gordonae* (15%) and *M. xenopi* (4%). Subsequently, strong decontamination procedures were put in place, including thermal shock and disposable filter installation on faucets. As a results, NTM contamination was no longer detected in

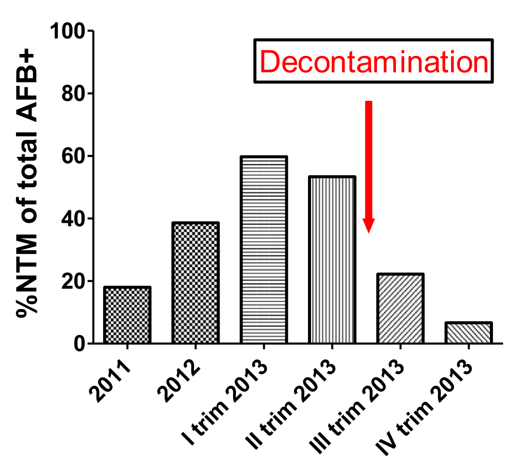

Fig. 1 – Incidence of nontuberculous mycobacterial (NTM) positive samples expressed as a percentage of total acid–alcohol fast bacilli (AFB) positive samples before and after decontamination procedures. *Note*: trim = trimester.

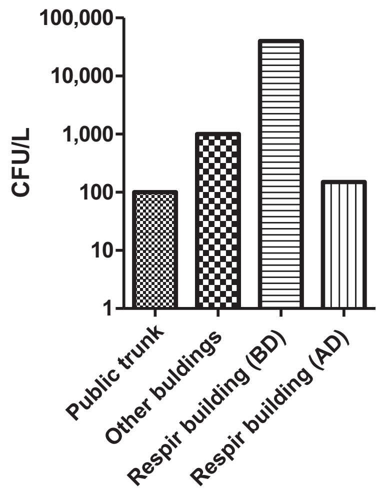

Fig. 2 – Nontuberculous mycobacterial presence in the main public trunk compared with pipelines supplying water to nonrespiratory buildings and to the building of the respiratory wards before and after decontamination procedures. *Note*: AD = after decontamination; BD = before decontamination; CFU/L = Colony forming unit/Liter; Respir = respiratory.

respiratory wards pipelines (Fig. 2) and more importantly a dramatic drop in NTM-positive sputa samples was observed in the following months, being 22% among the 36 AFB-positive patients in the 3rd trimester of 2013, and only 6.7% among the 30 AFB-positive patients in the 4th trimester ($p < .001$; Fig. 1).

The results that we have reported are consistent with previous observations of NTM outbreaks and pseudo-outbreaks in health care facilities where water supplies were recognized as potential sources of NTM contamination [4,10–12].

Even in normal conditions, water supply networks contain a microbial flora and NTM are a major component of it. NTM dissemination in the water supply network may be explained by the specific characteristics of these microorganisms. Several NTM species (e.g., *M. xenopi*, *Mycobacterium smegmatis*, *M. simiae*, and MAC) are thermophilic and are able to grow in warm water, even at temperatures as high as 55 °C, which are lethal for many other microorganisms [11].

Furthermore, NTM hydrophobicity, which is mainly due to their fatty acid and wax rich cell walls, enables their adhesion to solid substrates concurring to their characteristic resistance to antibiotics, disinfectants (e.g., chlorine), UV rays, and treatment with high temperatures. In addition, hydrophobicity may play an important role in determining the ability of these bacteria to form biofilms along water pipes. This film appears to be present in almost all collection and piping systems and likely provides the nutritional medium for the organisms [13,14]. Therefore, the colonization of hospital water networks by NTM is not surprising and may be facilitated by water disinfection against weaker microorganisms, which reduces competition for nutrients and lets NTM proliferate. Interestingly, in our observation the high NTM concentration in the pipeline network of the Respiratory Department might be related, at least in part, to water disinfection procedures through chemical treatments such as chlorination that took place in the previous years to minimize the risk of *Legionella* contamination [15].

Our observation is the first to clearly link a large NTM contamination of hospital water pipelines with an increase of NTM patient airway colonization. Importantly, the microbiology laboratory facilities of the hospital were located in a low NTM burden building, reducing the possibility of sample contamination after collection, during handling, and processing procedures. These findings are particularly interesting because NTM presence was observed in specimens from the respiratory tract of patients in whom these microorganisms could have caused a life-threatening disease. However, none of the patients met the criteria for NTM lung disease [4] and, accordingly, none of them were treated for NTM infection.

Importantly, decontamination procedures, especially the placement of disposable water filter taps, were able to significantly reduce both NTM isolation from water pipeline network and their isolation from sputa. We believe that our observation should encourage water network surveys for NTM contamination and prompt decontamination procedures should be considered to reduce this potential source of infection.

Conflicts of interest

The authors declare no conflicts of interest.

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