# NON-TUBERCULOSIS MYCOBACTERIA IN HOSPITAL WATER SUPPLIES

A. VANTARAKIS[1], A. TSINTZOU[1], A. DIAMANDOPOULOS[2] and M. PAPAPETROPOULOU[1] *

[1] *Environmental Microbiology Lab, Medical School, University of Patras, Greece and* [2] *Renal Ward, Patras General Hospital 'St. Andrew', Patras, Greece*

*E-mail: papapet@hol.gr*

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**Abstract.** Non-tuberculosis mycobacteria (NTM) were found in 10 (15.6%) out of 64 examined samples of tap water collected from 64 different points from the water supplies of the five main hospitals of the city of Patras (Peloponnese, Greece), during a six month period. The range of mycobacteria isolated from all the hospital water supplies was from 5-205 CFU $L^{-1}$. The identification of non-tuberculosis mycobacteria was performed with PCR-Restriction Enzyme Analysis (PCR-REA). The dominant isolates were *Mycobacterium chelonae* (3), *Mycobacterium gordonae* (2), *Mycobacterium flavescens* (1) and *Mycobacterium terrae* (1). One strain was unidentifiable and another two were lost during subculturing. All examined samples were negative for faecal indicators. The periodical examination of potable water of certain hospital units (such as the ones taking care of immunocompromised hosts, haemodialysis units, endoscopy units, Intensive Care Units etc.) for non-tuberculosis mycobacteria is considered worthwile.

**Key words:** hospital, non-tuberculosis mycobacteria, PCR-REA, water supply

## 1. Introduction

Tap water harbors non-tuberculous mycobacteria (NTM) which are generally more resistant to chemical disinfection than other bacteria (Pelletier *et al*., 1988). These opportunistic bacilli are widely distributed in soil and water (Wallace, 1987).

The presence of NTM in the tap water of a hospital may lead to nosocomial infections e.g. intravascular device infection, prosthetic valve endocarditis, peritonitis, meningitis associated with ventricular shunt, pulmonary infections due to the aerosol spread from shower heads and dialysis-related infections (Gonzales *et al*., 1978; Lohr *et al*., 1978, Kumar and Varkley, 1980; Kurnik *et al*., 1983; Horak *et al*., 1986; Wallace, 1987). In addition, pseudoepidemics due to non-tuberculosis mycobacteria in the hospital water supplies have been described (Lowry *et al*., 1974; Brown *et al*., 1993). This study was carried out using samples of the tap water from different wards of the five main hospitals in the town of Patras (SW Greece, 200,000 inhabitants) in order to examine both the microbiological quality and the possible presence of non-tuberculosis mycobacteria in their water supplies.

* Corresponding author: Maria Papapetropoulou, Associate Professor, Environmental Microbiology Lab, Medical School, University of Patras, P.O. Box 1045, Patras 261 10, Greece.

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## 2. Material and Methods

### 2.1. Study area and sample collection

Sixty four water samples were collected from different points (one sample/ point) within the distribution system of the five main hospitals (St. Andrew Hospital, University Hospital, 409 Satellite Hospital of Obstetrics and Gynecology, Hospital of Thoracic Diseases, and Karamandanio Children's Hospital) of the city of Patras (Peloponnese, SW Greece, 200,000 inhabitants) during a six month period (September 1994–February 1995). A sample from two different points of all hospital wards (two samples per ward), were taken for analysis.

The distribution systems use ground water which is empirically chlorinated (not using an automatic dosimetric appliance). Free chlorine levels measured at site using the APHA method (1989), ranged between 0 and 1 mg $L^{-1}$.

One litre of sample was collected in dark sterile bottles containing 1 mL of 10% (w/v) $Na_2S_2O_3$solution for the analysis of both mycobacteria and enteric bacteria. The samples were placed in a portable cooler (4–8 °C) and transported to the laboratory. They were refrigerated and analyzed within 6–24 hr from sampling. Samples were collected at the tap after running at full force for 5 min.

### 2.2. Microbiological techniques and media

All water samples were tested for: *total coliforms* (TC), *faecal coliforms* (FC), *faecal streptococci* (FS), according to Standard Methods (APHA, 1989).

Two hundred mL of each sample were decontaminated with 0.005% of CetylPyridinum Chloride (CPC) (Serva, Germany) as Schultze-Robbecke *et al*. (1991) and Neumann *et al*. (1997) proposed. After an exposure time of 30 min the samples were filtered through membrane (pore size 0.45 $\mu$m, Gelman, Germany). The filter membrane was rinsed with 300 mL of sterile water to remove residual CPC, plated on Middlebrook 7H10 OADC agar (Difco,Laboratories, USA) and incubated at 35 °C in a 5% $CO_2$ atmosphere. The plates were examined every two days for the first week and once a week thereafter for two months.

Colonies with characteristic morphology, pigmentation and growth rate were examined for acid fast bacilli using the Ziehl-Neelsen procedure. Colonies of acid fast rods were counted to determine the concentration of mycobacteria in terms of CFU $L^{-1}$ (Colony forming units per liter). In each sample two to five colonies with the same characteristics were subcultured on Lowenstein-Jensen agar for preservation until further treatment.

These representative colonies grown on Lowenstein-Jensen agar were identified by polymerase chain reaction combined with restriction enzyme analysis (PCR-REA) (Telenti *et al*., 1993).

2.2.1. *Sample Preparation*
A loop of mycobacteria grown on solid medium (Lowenstein-Jensen) was suspended in 200 $\mu$l double-distilled $H_2O$ and boiled for 5 min at 95 °C. The DNA was extracted with the help of Micromix Kit 660 (Talent, *Trieste*, Italy).

2.2.2. *Amplification*
Ten microliters of lysate were added to each reaction tube. The composition of the PCR mixture (50 $\mu$l) was 100 mM KCl, 20 mM Tris-HCl (pH 8.0), 1.5 mM $mgCl_2$, 0.2% Triton X100, 200 $\mu$M (each) deoxynucleotide triphosphate, 0.5 $\mu$M (each) primer, 1.25 U of Taq Polymerase (Finnzymes, Finland). The reaction was subjected to 45 cycles of amplification (1 min at 94 °C, 1 min 60 °C, 1 min at 72 °C); this was followed by 10 min of extension at 72 °C. Primers Tb11 and Tb12 as described by Telenti *et al*. (1993), common to all mycobacteria were used.

2.2.3. *Contamination Precautions*
Amplification tubes for PCR-restriction enzyme pattern analysis were prepared in a separate PCR-clean room.

The extraction of DNA was performed in a different room (PCR-dirty room). The samples were added to the PCR mixture in the 'dirty' room, where restriction analysis was performed as well.

2.2.4. *Restriction Analysis*
Two restriction enzymes were used, BstEII and HaeIII (New England Biolabs, USA). Aliquots of the PCR product were digested, following the instructions of the manufacturers. The quantity of the PCR product used, was dependent on the intensity of the zone shown in the gel. For BstEII digestion, 10 $\mu$l of PCR product was added directly to a mixture containing 0.5 $\mu$l (5U) of the enzyme, 2.5 $\mu$l of restriction buffer (No. 3) and 11.5 $\mu$l of water and the mixture was incubated for at least 60 min at 60 °C. Similarly, 10 $\mu$l of PCR product was digested at 37 °C in a solution containing HaeIII enzyme, the corresponding buffer (No. 2) and water.

2.2.5. *Evaluation of the Restriction Patterns*
After digestion, 12 $\mu$l of the mixture was loaded into a 3% Nuisive Agarose (FMC products, Denmark). Fragments were visualised by ethidium bromide staining and UV light, and were photographed by a Ultra Violet Product (UVP, U.K) programme. Their molecular weights were estimated by the use of GelBase software. The identification was performed with the help of the algorithm prepared by Telenti *et al*. (1993). Non-tuberculosis mycobacterial strains (personal collection) provided by the reference laboratory for mycobacteria in Greece (Director Dr E. Marinis) were used for quality control of the molecular method in our laboratory.

Table I
Species of non-tuberculosis mycobacteria isolated from hospital water supplies

| Hospital/ ward or unit | Number of positive samples | Mycobacteria CFU/L | Species identification |
|---|---|---|---|
| St. Andrew (20 samples examined) | | | |
| Urological | 1 | 10 | Unidentified |
| Urological | 1 | 5 | *M. terrae* |
| Dialysis | 1 | 205 | *M. chelonae* |
| U.H.P.[a] (26 samples examined) | | | |
| Urological | 1 | 115 | *M. chelonae* |
| Internal medicine | 1 | 33 | *M. chelonae* |
| Internal medicine | 1 | 35 | Not analyzed |
| Dermatological | 1 | 90 | Not analyzed |
| Ear nose throat | 1 | 20 | *M. flavescens* |
| 409[b] (4 samples examined) | | | |
| Baby unit | 1 | 20 | *M. gordonae* |
| Birth unit | 1 | 10 | *M. gordonae* |
| Hospital of thoracic diseases (10 samples examined) | – | 0 | – |
| Karamandanio children's (4 samples examined) | – | 0 | – |

[a] University Hospital of Patras, Greece.
[b] 409 Satellite Hospital of Obstetrics and Gynecology, Patras, Greece.

## 3. Results

Non-tuberculosis mycobacteria were found in 10 (15.6%) out of 64 examined samples. All ten positive samples came from the three of the five hospitals (St. Andrew hospital, University Hospital (U.H.P) and 409 Satellite Hospital of Obstetrics and Gynecology). In the St. Andrew hospital, 3 out of 20 (15%) of the analyzed samples were found positive, whereas in the U.H.P., 5 out of 26 samples (19.2%) were positive. In the 409 Satellite Hospital of Obstetrics and Gynecology, 2 out of 4 samples examined were found positive. No mycobacteria were detected in any of the analyzed samples (14 samples) taken from the other two hospitals.

Numbers varying from one to 100 CFU $L^{-1}$ were found in 8 (12.5%) of the samples and from 101–1000 CFU $L^{-1}$ in 2 samples (3.1%). The range of the mycobacteria detected was 5–205 CFU $L^{-1}$ in the St. Andrew Hospital, 20–115 CFU $L^{-1}$ in the U.H.P and 10–20 CFU $L^{-1}$ in the 409 Satellite Hospital.

The non-tuberculosis mycobacteria isolated from the examined samples and the specific wards, are shown in Table I. The strains were identified as *Mycobacterium chelonae* (3 samples), *Mycobacterium gordonae* (2 samples), *Mycobacterium flavescens* (one sample) and *Mycobacterium terrae* (one sample).

The patterns obtained with the above strains corresponded to those described by Telenti *et al*. (1993). One strain could not be identified with the Telenti *et al*. (1993) method, giving irrelevant patterns. Two rapidly growing strains were not tested (due to contaminating by fungi and lost during the subculturing process).

No enteric bacteria were isolated in any of the samples.

## 4. Discussion

The bacteriological quality of drinking water is determined routinely by estimation of the presence of faecal indicators (*Total Coliform* bacteria (TC), *Faecal Coliform* bacteria (FC), *Faecal Streptococci* (FS)).

These bacteriological markers do not seem to predict the presence of some potentially pathogenic microorganisms like non-tuberculosis mycobacteria in water. In our study, all tested samples of tap water were found negative for faecal indicators while in ten out of the 64 (15.6%) non-tuberculosis mycobacteria were found. The number of positive samples was rather low compared to other investigator's findings (Wright *et al*., 1985). The ranges of NTM detected between hospitals varied probably because of the fact that each hospital was supplied by a different source of potable water. The species isolated from the tap water at the different wards and units were: *Mycobacterium flavescens*, *M. gordonae*, *M. chelonae* and *M. terrae*. *M. terrae*, being much more common in soil (Bionde *et al*., 1982), was isolated only once from the urological ward of the St. Andrew hospital where soil became mixed accidentally with tap water after repeated interruptions of the water supply.

Samples from the tap water from the haemodialysis ward of the St. Andrew hospital showed contamination with *M. chelonae*. The same species was isolated again after water had been treated through reverse osmosis (R.O). But these R.O. results have not been included in our statistics. During this period, a considerable number of patients in this center had experienced chills and fever while the routine cultures for common bacteria were found negative. The symptoms ended after proper disinfection procedures and repair of the reverse osmosis equipment. A similar case has been described by Bolan *et al*. (1985). These cases emphasize the pathogenicity of these water-associated microorganisms and the need for special care. (High level disinfection of water treatment systems and frequent monitoring for non-tuberculosis mycobacteria seems necessary at the various haemodialysis centers).

Hospital infections control officers and microbiologists must be aware of the presence of the non-tuberculosis mycobacteria in the water supply systems of hospital, not only because of the risk of causing hospital of acquired infections

but also to avoid diagnostic confusion. The colonization of water supplies by mycobacteria is a likely source for the contamination of clinical specimens (Wright *et al*., 1985). Frequently, non-tuberculosis mycobacteria have been isolated from broncho-alveolar lavage fluid, the presence of which has been associated with their presence in the hospital water supply (Lowry *et al*., 1974; Bolan *et al*., 1985; Stine *et al*., 1987; Nye *et al*., 1990; Brown *et al*., 1993).

Some patients and especially AIDS patients may be infected by ingestion (Iseman, 1989). In endoscopy units where the endoscopes are rinsed after immersion in disinfectant, it seems worthwhile to evaluate for the presence of non-tuberculosis mycobacteria in the tap water used (British Society of Gastroenterology, 1988).

Faecal indicators should not be used as an indicator of a water supply's safety as negative results for faecal indicators do not exclude the presence of other potentially pathogenic bacteria such as mycobacteria. On the contrary, there may be a negative association between them (Kazda *et al*., 1973a, b). This is not the case for other microorganisms present in tap water like *Legionella*, where the presence of diverse bacterial flora supports its growth in potable waters (Wadowsky *et al*., 1985).

We do not believe that routine examination of all hospital tap waters for non-tuberculosis mycobacteria is necessary since their culture and identification is expensive and time consuming. However, for units taking care of immunocompromised hosts, as well as Haemodialysis or Endoscopy units, Intensive Care Units etc., it seems rational to periodically examine the water supply systems of these units for non-tuberculosis mycobacteria.

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