Journal of Hospital Infection (2007) 65, 143–148

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Prevalence of non-tuberculous mycobacteria in a hospital environment☆

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Received 6 February 2006; accepted 12 October 2006
Available online 14 December 2006

KEYWORDS
Cross-infection;
Non-tuberculous mycobacteria; Hospital Environment;
Waterborne infection

Summary In recent years, non-tuberculous mycobacteria (NTM) have emerged as an important cause of opportunistic nosocomial infections but there is little known about the isolation and identification of NTM in Korea. The aim of this study was to assess the prevalence of NTM in the hospital environment and identify the species. A total of 150 samples were collected from different parts of the hospital. NTM were isolated and identified by restriction fragment length polymorphism analysis of the gene encoding rpoB and partial sequencing analysis of *hsp65* and *rpoB*. In this study, 60 strains of NTM were isolated from 50 of the 150 samples. Half of the tap water samples (50 of 100) were positive for mycobacteria. An estimated 73.3% of the isolates were saprophytic, 21.7% were potentially pathogenic and 5% were unidentified. The presence of NTM in hospital tap water is not uncommon. Such water isolates might cause true nosocomial infection in immunocompromised patients, in addition to the risk of false-positive culture results.

Introduction

Non-tuberculous mycobacteria (NTM) are ubiquitous in natural environments.[1,2] These organisms are not particularly virulent in humans with a normal immune system but some species are pathogenic, causing pulmonary infection, skin lesions and

☆ This work was supported by the 2004 Inje University Research Grant.
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doi:10.1016/j.jhin.2006.10.004

disseminated disease.[2,3] In recent years, the increase in immunocompromised patients has led to a growing incidence of infections from NTM.

Without evidence of person-to-person transmission of NTM, it is proposed that humans are infected from environmental sources that may include aerosols, soil, food and equipment. Initially water was not considered to be an important source. There is increasing evidence, however, that water is the vehicle by which mycobacteria infect or colonize the human body.[4,5] Several kinds of NTM have been identified in ice and public drinking water and there have been reports showing that NTM can survive, persist, and grow in drinking water systems.[1,2,6,7] The presence of mycobacteria in tap water may lead to true infection or to a pseudo-infection. NTM are not killed by common disinfectants and can tolerate a wide range of pHs and temperatures that allows them to persist in hospital environments for long periods of time.[2,8] Any fluid reservoir that is not routinely and effectively decontaminated can act as a source of organisms. NTM can contaminate inadequately disinfected equipment used for clinical investigations or surgery, resulting in nosocomial infections.

In recent years, NTM have emerged as a major cause of opportunistic infections so the prevalence of NTM in hospital environments needs to be investigated. There are few publications that have reported on isolation and identification of NTM from hospital environments in Korea. The aim of this study was to assess the prevalence of NTM in the hospital environment and to identify the species.

Materials and methods

Collection and preparation of samples

A total of 150 samples were collected from various sources, including tap water ($N = 100$), drinking water from a water-purification system ($N = 5$), humidifiers and a ventilator ($N = 5$), hand or skin swabs from physician, nurse, and patients ($N = 13$), computer keyboard swabs ($N = 5$), surface swabs from other objects such as gowns ($N = 3$), dressing carts ($N = 4$) and sink surfaces in nurse work stations ($N = 8$) and others ($N = 7$). Water was collected from taps ($N = 91$) or showers ($N = 9$) at various sites in the hospital, including nurse workstations, sickrooms, restrooms, bathrooms of each ward and outpatient care rooms. The university hospital building is 26-years old and many of the tap water pipes were replaced seven years ago. All water systems of the hospital are composed of blended outlets with both hot and cold water. The water samples were collected from the hospital's incoming water supply pipe.

Samples were transported to the laboratory and processed the day of collection. After centrifugation at 3000 ***g*** for 30 min, most of the supernatant was removed. Half of each concentrate was inoculated after mixing with the same volume of 1% sodium hydroxide and the remainder was inoculated directly onto solid media for mycobacterial culture. The swab samples were added to 5 mL distilled water in 50 mL conical tubes, mixed by vortexing and processed in the same way as the tap water samples.

The plates were examined once a week for eight weeks. When colonies appeared, they were subjected to acid-fast staining and acid-fast isolates were examined by polymerase chain reaction—restriction fragment length polymorphism analysis (PCR-RFLP).

Species identification of NTM by PCR-RFLP

PCR-RFLP of the gene encoding the rpoB using the Myco-ID kit (M&D Inc, Wonju, Korea) was undertaken to identify the isolates. The primary principle and methods have been published.[9] The entire process was performed according to the manufacturer's instructions. DNA was extracted by boiling the sample for 5 min to lyse any mycobacteria. The PCR was carried out in a final volume of 50 μL consisting of 5 μL supernatant liquid and 45 μL 8-MOP premixed solution containing: 10 pmol of each primer, 2 mM $MgCl_2$, 40 mM KCl, 10 mM (pH 9.0) Tris—HCl, 200 μM concentrations of deoxynucleoside triphosphates, and 2.5 U *Taq* DNA polymerase. The PCR conditions were 94 °C for 5 min followed by 35 cycles of 94 °C for 20 s, 58 °C for 20 s, and 72 °C for 30 s and then extension at 72 °C for 10 min. The PCR mixture with DNA of the reference strain, *Mycobacterium tuberculosis* H37Rv, was used as the positive control and the PCR mixture without any DNA was used as the negative control. Amplification of the region of *rpoB* resulted in a 360-bp PCR product, which was digested by restriction enzymes for identification of NTM to the species level. The restriction enzymes MspI and HaeIII were used for analysis of the PCR products.

Additional identification of unidentified NTM

For the mycobacteria unidentified by Myco-ID, *hsp65* and *rpoB* partial sequencing analysis was used as previously described.[10,11] The PCR and sequencing primers were Tb11 F (5′-ACC AAC GAT