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Short report

Surveillance and molecular characterization of non-tuberculous mycobacteria in a hospital water distribution system over a three-year period

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SUMMARY

A three-year surveillance of non-tuberculous mycobacteria (NTM) in a hospital water distribution system was conducted at a facility located in southern Alberta. NTM was not present in any intake water samples, but was found in 106/183 (58%) of endpoint samples across 15 sites over the study period. Two different species of NTM were identified, *Mycobacterium gordonae* (88/183) and *Mycobacterium avium* (34/183); with only one strain of each *M. gordonae* and *M. avium* found. Given the sensitive nature of a healthcare facility, attention should be paid to minimize potential impact of NTM from potable water sources on patient health.

Introduction

Non-tuberculous mycobacteria (NTM) are widely dispersed in the environment and are commonly isolated from water and soil. It is not uncommon to isolate NTM from potable water distribution systems since they can be resistant to many disinfectants and tend to persist in biofilms, making them difficult to eradicate. These opportunistic pathogens are typically non-virulent to healthy individuals but NTM are emerging as a major cause of infection, primarily in immunocompromised patients.[1] Reports have suggested that there is an increasing trend in the incidence of NTM disease, which is of concern.[2]

Hospital tap water may act as a vector for transmission by means of ingestion, inhalation, bathing/showering or contact with medical equipment. The colonization of NTM within a hospital water distribution system may lead to dissemination and infection in patients, causing increased morbidity and mortality. Several species of NTM have been identified in hospital water systems that have been implicated with nosocomial

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infections and outbreaks, illustrating the sensitive nature of a healthcare facility, and the role that water can play in NTM nosocomial infections.

The significant impact that waterborne nosocomial infections may have on patient health and the fact that NTM infections are escalating prompted this investigation of the water distribution system at a healthcare facility.

Methods

Water distribution system and study design

The study facility is a tertiary care hospital located in a large urban city in southern Alberta, with an innovative water distribution system designed to minimize microbial impact on patient health. The system incorporates instantaneous peripheral hot water heaters, constant recirculation loops in the hot water lines (two separate circuits, one maintained at 38 °C and one at 43 °C), UV light treatment (Trojan Technologies, London, Ontario, Canada) at the intake into the hospital, and without dead-end pipes. Samples were collected quarterly from 15 different surveillance sites throughout the hospital from April 2009 to July 2012. Intake water samples were collected immediately after entering the hospital both before and after UV treatment. Endpoint samples were collected from either automatic infra-red (38 °C) faucets, or manual dual tap faucets with cold and hot (43 °C) water. Additionally, during the October 2011 sampling period, 39 endpoint sites were sampled throughout the hospital to obtain a better understanding of mycobacterial distribution. The surveillance sinks were located in housekeeping closets ($N = 4$), unit kitchen areas ($N = 3$), patient care areas ($N = 4$), washrooms ($N = 2$), an emergency trauma room ($N = 1$), and in a clinical laboratory ($N = 1$).

Sample collection and NTM isolation

For automatic faucets, the first 1 L volume of water was collected and, for manual dual tap faucets, about 500 mL from the cold tap and 500 mL from the hot tap were combined to total 1 L. Each water sample was filtered through a 0.22 μm membrane filter (Millipore Corp., Bedford, MA, USA) and processed as per standard methods.[3] Four drops of the decontaminated concentrate were planted to a Lowenstein–Jensen agar slant containing pyruvate (Dalynn Biologicals, Inc., Calgary, Alberta, Canada). A duplicate set of slants was inoculated per sample, one incubated at 30 °C and one at 35 °C for up to 12 weeks, monitoring weekly for growth. Colonies presumptive for *Mycobacteria* spp. were identified either by polymerase chain reaction (PCR)–restriction enzyme analysis (PRA) or by using an Accuprobe Gen-Probe/pyrosequencing test at the Alberta Provincial Laboratory for Public Health (ProvLab, Calgary/Edmonton, Alberta, Canada).[4,5] Isolates from samples collected from April 2009 to July 2011 were identified using Gen-Probe/pyrosequencing, and isolates from October 2011 to July 2012 were identified by PRA.

PRA, DNA sequence analysis, and statistics

Genomic DNA was extracted from pure culture using the QIAmp DNA Mini Kit (Qiagen, Hilden, Germany). Using 5 μL of the extracted material, amplification of the partial *hsp65* gene was carried out using the primers Tb11 and Tb12 according to a previously published protocol.[6] Amplified products were separated by electrophoresis with a 1.2% agarose gel (Invitrogen, Carlsbad, CA, USA) at 90 V for 90 min. Restriction analysis was carried out using 20 μL of PCR product displaying positive bands for *hsp65* with *BstEII* (New England BioLabs, Ipswich, MA, USA) and *HaeIII* (Invitrogen) as per the manufacturer's instructions. The digested products were separated by electrophoresis with a 4% agarose gel at 90 V for 120 min.

DNA sequencing of the *hsp65* fragment was carried out on a portion of isolates (23 *Mycobacterium gordonae*, 12 *Mycobacterium avium*) to compare isolate clonality by the University of Calgary Core DNA Services. Sequences were aligned and manually verified for accuracy using BioEdit Sequence Alignment Editor (Version 7.1.3.0). The GenBank database was used to compare all sequences using the BLAST program from the National Center for Biotechnology Information. All statistical analysis was carried out using GraphPad Prism (GraphPad Software, La Jolla, CA, USA). A χ^2, two-tailed Fisher's exact test was performed and $P < 0.05$ was considered statistically significant.

Results

A total of 52 intake and 183 endpoint water samples were collected from 15 different sites distributed throughout the hospital. Mycobacteria were not present in any of the intake water samples, pre or post UV treatment throughout the course of the study. Of the 183 endpoint samples analysed, 106 (58%) were positive for NTM, and, of the 15 surveillance sites, all were positive for NTM at least once during the study period (Table I). Of the 39 endpoint sites tested from the October 2011 sampling period, 14 were positive (36%).

Two different species of NTM were identified: 72 samples contained *M. gordonae* alone, 18 contained *M. avium* alone, and 16 contained both *M. gordonae* and *M. avium*. Seventy-nine isolates were speciated by Accuprobe Gen-Probe or pyrosequencing, and 43 were speciated by PRA. Identification by PRA was validated against Gen-Probe/pyrosequencing and *hsp65* sequencing to ensure accuracy of speciation, and results indicated comparability (100%; 14 *M. gordonae*, 11 *M. avium*).

From the October 2011 sampling period, 11/39 (28%) sites were found to be positive for *M. gordonae* and 4/39 (10%) sites were positive for *M. avium*. All *M. gordonae* isolates had the same banding pattern of *BstEII* 235/120/85 and *HaeIII* 160/115/60 (Type I) and all *M. avium* isolates had the same banding pattern of *BstEII* 235/210 and *HaeIII* 130/105/60 (Type II). Moreover, sequence analysis on these isolates indicated that there was only one clone each of *M. gordonae* and *M. avium* in the system. Additionally, the endpoint sites that were chosen to investigate clonality in one site over time revealed that there was no clone variation over the study period (*M. gordonae*, $n = 12$; *M. avium*, $n = 8$).

NTM were isolated more frequently from manual dual tap (43 °C) sites [77/121 (63%)] than in automatic (38 °C) faucet sites [29/62 (47%)] ($P = 0.0394$), but the species of mycobacteria was not found to differ between the two types of faucets.