Pseudo-Outbreak of *Mycobacterium fortuitum* Due to Contaminated Ice Machines

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ABSTRACT

Objectives: We observed a 10-fold increase in isolation rates of *M. fortuitum* from respiratory specimens over a 3-month period. The majority of these isolates were from patients in contact with 1 of 3 wards at a tertiary care hospital. An epidemiologic investigation was undertaken to identify the source.
Methods: Environmental samples were collected from water/ice machines and tap water on affected and uninvolved wards as well as the Microbiology laboratory. All specimens were cultured with the use of a continuously monitored broth system for mycobacteria isolation. Positive samples for mycobacteria were sent to the regional Public Health Laboratory for identification. Case and control as well as environmental isolates were compared by genotyping using ERIC-PCR. A retrospective chart review was conducted to evaluate clinical impact.
Results: *M. fortuitum* was isolated from 20 respiratory samples from 17 patients. Surveillance cultures obtained from uninvolved wards were negative for mycobacteria. *M. fortuitum* was isolated from 3 ice machines on affected wards but not from tap water or water in the Microbiology laboratory. ERIC-PCR based typing revealed that patient isolates were identical or closely related, with the exception of one patient previously colonized. Isolates from environmental cultures were identical or closely related to patient isolates. All but one case was thought to represent transient colonization. One patient was changed from an anti-tuberculosis treatment regimen to a regimen for *M. fortuitum*; this resulted in clinical progression of tuberculosis that was later microbiologically confirmed.
Conclusion: The *M. fortuitum* pseudo-outbreak was due to contaminated ice machines. A negative clinical impact was observed in one patient.

INTRODUCTION

- Nosocomial outbreaks and pseudo-outbreaks caused by Non-tuberculous Mycobacteria have been recognized for more than 30 years and continue to be a problem[4]. Most of these pseudo-outbreaks have involved the rapidly growing Mycobacterial species. These Mycobacterial species are incredibly hardy and resist the activity of commonly used disinfectants. The reservoir is generally contaminated hospital water supplies[2,3,4].
- *Mycobacterium fortutium* is a rapidly growing Mycobacterium that is predominantly found in water and soil. Clinically, this organism causes primarily skin and soft tissue infection, subacute granulomatous disease and rarely, disseminated infections. Pulmonary disease is uncommon and therefore the presence of *M. fortuitum* in respiratory specimens usually reflects colonization rather than true infection.
- Typing of isolates, best achieved by genetic comparison, is particularly useful for outbreak studies and other epidemiological investigations[2,3,4]. Phenotypic typing methodologies have limited discriminative capacities. Genotypic methods are more discriminative and have been proven useful[2,3,4]. Newer approaches include pulse field gel electrophoresis (PFGE) and several polymerase chain reaction (PCR) based techniques. Enterobacter repetitive intergenic consensus (ERIC) PCR has been reported to be a useful tool for typing *M. fortuitum* isolates[1].
- The Microbiology laboratory at The Ottawa Hospital processes approximately 4000 specimens annually for Mycobacterial culture. In October 2013, we became aware of an abnormally high number of expectorated sputum and bronchialveolar lavage/wash (BAL/BRW) samples that grew *M. fortuitum*. An investigation of the processing and handling of specimens and cultures in the Mycobacteriology laboratory revealed no break in technique to explain the increase. This prompted an epidemiologic investigation to identify the source.

OBJECTIVES

1. To identify the source of a possible *M. fortuitum* pseudo-outbreak
2. To assess the relatedness of isolates through genotyping using ERIC-PCR
3. To determine the clinical impact of positive cultures

METHODS

- All sputum and BAL/BRW samples obtained for Mycobacterial culture were digested with 2% NaOH, Na citrate, and 0.5% N-acetyl, 1-cysteine for 15 minutes and stained with Auramine. Culture was performed by inoculating processed samples to Lowenstein-Jensen slants (LJ) and Mycobacteria growth indicator tubes (containing PANTA antibiotic mixture, MIGIT). *M. fortuitum* was identified based on negative GenProbe hybridization assays for *M. tuberculosis* complex, *M. avium-intracellulare* and *M. gordonae*, documentation of rapid growth and confirmation by the Ottawa Public Health Laboratory.
- Water and ice samples were obtained from ice machines throughout the 3 wards with and increase in *M. fortuitum* isolation: A, B, C; as well as other floors without an increase in *M. fortuitum*. Samples were also taken from the sink in the patient kitchen where the ice machine was located. Duplicate 50 mL samples were collected from each of the sites. All water samples were centrifuged (3000xg, 15 minutes) and the supernatant decanted to leave 10 mL of water in which the pellets were re-suspended. Duplicate samples were pooled and a MGIT and LJ slants were inoculated for Mycobacterial culture.
- Seventeen *M. fortuitum* isolates from 15 patients, 7 isolates from non-related patients from the previous 2 years and the Laboratory ATCC isolate were grown at 37ºC on Middlebrook 7H11 agar. DNA was extracted from one loopful of bacteria with the QIAamp DNA Mini Blood Kit after lysozyme (180uL) and sonication treatment from these isolates, as well as the environmental isolates.
- ERIC-PCR was performed using primers ERIC1 (5'-AtGTAAGCTCCTGGGGATTCAC) and ERIC2 (5'-AAGTAAGTGACTGGGGTGAGCG) as described by Sampaio et al[1]. PCRs were performed in a total volume of 25 µL containing 12.5 µL EcoTaq Plus Green Master Mix, 1 µM each primer and 4 µL extract. PCRs comprised 2 min at 94ºC, followed by 35 cycles of 94ºC for 45s, 52ºC for 1 min and 70ºC for 10 min, with a final extension at 70ºC for 20 min. Amplification was performed with GeneAMP PCR System 2720 Thermocycler.
- Amplified products were separated by electrophoresis with 1.4% agarose gel. Gels were analyzed by Ontario Public Health, using BioNumerics v. 6.1 software. Dendograms to illustrate the pattern relatedness among the isolates were constructed.
- A retrospective chart review was conducted to evaluate clinical impact.

Table 1. Case Characteristics

	# of Patients	Cluster
Unit A	3	1 (n=3)
Unit B	6	1 (n=5)
Unit C	3	1 (n=3)
Unit D	2	1 (n=1)
Outpatient	3	3, (n=3), 1 (n-1)
Specimens (n=20)		
Sputum	16	
BAL/BRW	4	

RESULTS

- There were 20 samples from 17 patients that were positive for *M. fortuitum*. All positive specimens were from patients admitted or seen at the General Campus.
- Three patients were seen as outpatients, 12 patients were admitted to 1 of 3 wards at one point prior to samples being taken and 2 patients were admitted to Ward D (Table 1). Of the outpatients one clinic was located in the General Cancer Center, one from the Infectious Disease Clinic, and one from the Cystic Fibrosis Clinic.
- Sixteen samples from 15 patients underwent ERIC PCR. There were 3 major clusters that were identified based on Dendogram analysis of ERIC PCR results (Figure 1). Isolates in the largest cluster, Cluster 1, were all obtained from patients on Wards A, B, C and D. Within this cluster the majority of strains from Wards A and B were identical, with the exception of 2 strains that had < 3-band difference indicating that they were closely related. Strains from Ward C and D were also closely related to other isolates. The majority of isolates obtained from outpatients and isolates from previous years were not included in Cluster 1 and are likely unrelated.
- Upon retrospective chart review it was determined that the culture results were not clinically relevant. There was 1 case where isolation of *M. fortuitum* had a negative clinical impact. The patient was HIV positive with a CD4 count of 74. She presented with pneumonia and a left breast cold abscess. She was also found to have splenomegaly with splenic lesions and a paravertebral abscess. Initially she was started on anti-tuberculous therapy but this was stopped when Mycobacterial sputum cultures grew *M. fortuitum*. She was changed to Doxycycline and Septra as treatment for *M. fortuitum*. Subsequently, Mycobacterial cultures of the aspirate from the left breast abscess grew *Mycobacterium tuberculosis*; this resulted in a delay in proper treatment of 1.5 months.
- Environmental samples from Ice Machines on Unit A, B and C were positive for *M. fortuitum*. ERIC profiles for the environmental isolates recovered from the ice machines were identical to the isolates in Cluster 1.

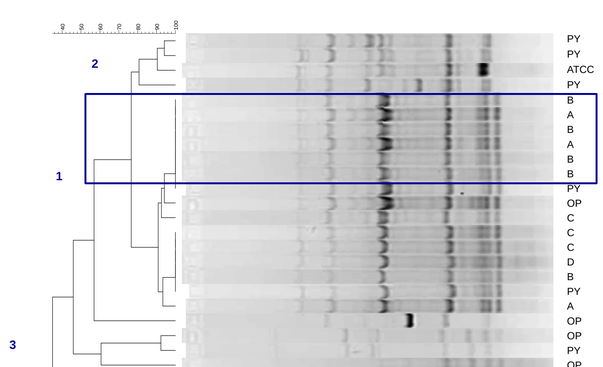

Figure 1. ERIC PCR profiles of *M. fortuitum* isolates, analyzed using BioNumerics v. 6.1; PY – Previous year isolate, OP – out patient, A – Ward A isolate, B – Ward B isolate, C – Ward C isolate, D – Ward D isolate

DISCUSSION

- Mycobacterial infections, especially among immunocompromised hosts, are potentially of extreme concern. We observed a significant increase in the proportion of patient respiratory samples that were positive for *M. fortutium*. Data from previous years showed that in 2012, 0.1 % of all specimens received for Mycobacterial culture grew *M. fortuitum*. Twenty *M. fortuitum* positive cultures were identified from 17 patients between the months of July through November 2013. This corresponded to an isolation rate of 1.5% of the total number of specimens received for mycobacterial culture; a greater than 10 fold increase.
- Upon retrospective review of patient charts, no case where *M. fortuitum* was isolated was thought to be clinically significant; they were thought to be due to transient colonization. Environmental sampling was then performed after laboratory contamination was ruled out.
- On the basis of the results of this molecular investigation by ERIC PCR and retrospective chart review, we suspect that this was a pseudo-outbreak linked to ice machines as the most probable source. According to nursing protocol patients are supplied water and ice from the ice machines located on their respective wards. This practice provided adequate exposure for colonization. All but 5 patients were found to have had contact with one of the 3 affected wards.
- In previous nosocomial pseudo-outbreaks that were attributed to shower water and contaminated ice, sputum cultures positive for *M. fortuitum* represented transient colonization or contamination but not infection[2,3]. The same conclusion was reached in this study. There was however, one case that resulted in a significant delay in adequate treatment.

CONCLUSION

- In summary, we report a nosocomial pseudo-outbreak of respiratory tract colonization with *M. fortuitum* that we believe had contaminated ice machines as its source.
- Molecular typing strongly supported the ice machine as the probable source of the pseudo-outbreak. Ice machines and other water sources should be considered as possible sources of nosocomial outbreaks of respiratory tract colonization with *M. fortuitum*.

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