HHS Public Access

Author manuscript

Am J Infect Control. Author manuscript; available in PMC 2023 April 02.

Published in final edited form as:

Am J Infect Control. 2023 April ; 51(4): 454–460. doi:10.1016/j.ajic.2022.06.013.

Investigation of a cluster of rapidly growing mycobacteria infections associated with joint replacement surgery in a Kentucky hospital, 2013–2014 with 8-year follow-up

Matthew R. Groenewold, PhD, MSPH[a,b,*], **Andrea Flinchum, MPH, BSN, RN, CIC, FAPIC**[b], **Aravind Pillai, PhD, MBBS, MPH**[b], **Stacey Konkle, PhD, MPH**[b], **Heather Moulton-Meissner, PhD**[c], **Pritish K. Tosh, MD**[d], **Douglas A. Thoroughman, PhD, MS**[a,b]

[a]Career Epidemiology Field Officer Program, Centers for Disease Control and Prevention, Frankfort, KY, USA

[b]Division of Epidemiology and Health Planning, Kentucky Department for Public Health, Frankfort, KY, USA

[c]Division of Healthcare Quality Promotion, Centers for Disease Control and Prevention, Atlanta, GA, USA

[d]Division of Infectious Diseases, Mayo Clinic, Rochester, MN, USA

Abstract

Background: We describe the investigation of a nosocomial outbreak of rapidly growing mycobacteria (RGM) infections and the results of mitigation efforts after 8 years.

Methods: A cluster of RGM cases in a Kentucky hospital in 2013 prompted an investigation into RGM surgical site infections following joint replacement surgery. A case-control study was conducted to identify risk factors.

Results: Eight cases were identified, 5 caused by *M. wolinskyi* and 3 by *M. goodii*. The case-control study showed the presence of a particular nurse in the operating room was significantly associated with infection. Environmental sampling at the nurse's home identified an outdoor hot tub as the likely source of *M. wolinskyi*, confirmed by pulsed-field gel electrophoresis and whole genome sequencing. The hot tub reservoir was eliminated, and hospital policies were revised to correct infection control lapses. No new cases of RGM infections have been identified as of 2021.

[*]Address correspondence to Matthew R. Groenewold, PhD, MSPH, Centers for Disease Control and Prevention, NIOSH, 1090 Tusculum Ave. MS R-17, Cincinnati, OH 45226. gyr5@cdc.gov.

Conflicts of Interest: Andrea Flinchum wishes to disclose that she serves as a board member on the Certification Board of Infection control and Epidemiology and that, in 2015, she received support from CDC to attend the APIC national meeting to give an oral presentation describing the outbreak and investigation that is the subject of this manuscript. All other authors report nothing to disclose.

Ethics approval and consent to participate: As an outbreak investigation, this activity was determined to be non-research public health practice and was exempted from IRB review. This activity was reviewed by CDC and was conducted consistent with applicable federal law and CDC policy (45 C.F.R. part 46.102(l)(2), 21 C.F.R. part 56; 42 U.S.C. §241(d); 5 U.S.C. §552a; 44 U.S.C. §3501 et seq).

DISCLAIMER

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Discussion: Breaches in infection control practices at multiple levels may have led to a chain of infection from a nurse's hot tub to surgical sites via indirect person-to-person transmission from a colonized health care worker (HCW).

Conclusions: The multifactorial nature of the outbreak's cause highlights the importance of overlapping or redundant layers of protection preventing patient harm. Future investigations of RGM outbreaks should consider the potential role of colonized HCWs as a transmission vector.

Keywords

Outbreak investigation; *M. wolinskyi* ; Surgical site infection; Healthcare-associated infections; Non-tuberculosis mycobacteria

BACKGROUND

Rapidly growing mycobacteria (RGM) comprise a subset of the non-tuberculosis mycobacteria that usually grow within 7 days of subculture into solid media. They are widely distributed in the environment and able to survive extreme temperature and nutrient deficient environments.[1] RGMs can be found in soil, water, hospital environment, pharmaceuticals, and contaminated reagents. Some clinically important species of RGM include members of the *M. fortuitum* group and the *M. abscessus* group, including *M. chelonae*. As pathogens, these organisms are usually associated with lung, skin, and soft tissue infections. They are increasingly recognized as causes of post-traumatic and post-surgical wound infections, and are often associated with surgical implantation of devices, such as joint replacement.[2-4] Infections caused by members of the *M. smegmatis* group, which includes *M. smegmatis* and 2 additional species first described in 1999, *M. wolinskyi* and *M. goodii*,[5] are much rarer. Until recently only 21 cases of human infection with *M. wolinskyi* had ever been published and were predominantly associated with surgical wound infections, followed by cardiovascular infections.[6,7] Exposure to nonsterile water or breach in sterile procedures are the usual causes of outbreaks in surgical settings.[8] At least 1 outbreak of post-surgical RGM infections associated with a colonized health care worker (HCW) has been reported.[9] In that case, the causative agent, a strain of *M. wollinskyi*, was recovered from an outdoor hot tub regularly used by the HCW, a surgeon. Based on the identification of the colonized HCW's hot tub as the outbreak reservoir, the name *M. jacuzii* was proposed for the strain of *M. wolinskyi* involved.[9]

Although the number of reported cases of RGM infections remains relatively low, these infections appear to be increasing in health care settings globally.[10-12] An upsurge in complex medical and surgical procedures, the increasing number of vulnerable and immunocompromised individuals, as well as enhanced detection could be contributing to the observed rise in cases.[13] In light of the increasing incidence of RGM infections, we report on an outbreak investigation of RGM infections at a Kentucky Hospital during 2013-2014[14] to characterize its likely cause as well as results of mitigation efforts after 8 years.

On August 13, 2013, the Kentucky Department for Public Health was notified by a local health department of four cases of *M. wolinskyi* infection at Hospital A. In each of the 4 cases, *M. wolinskyi* was isolated from diagnostic specimens collected from patients

subsequent to clinical evidence of infection. *M. wolinskyi* had never before been isolated from a patient at Hospital A, and the large national reference laboratory used by the hospital for diagnostic testing had only seen 3 other cases in the previous 2 years. All cases at Hospital A had under-gone joint replacement surgery at that hospital during October-December 2012, 3-8 months prior to isolation of *M. wolinskyi*, and were patients of 1 orthopedic practice. The Health care Associated Infections (HAI) Program of Kentucky Department for Public Health launched an epidemiological investigation into the outbreak in conjunction with the local health department and the infection control department of Hospital A. The objectives of the investigation were to determine the extent of the outbreak, identify the source of the infections and the mode of transmission, and to implement effective control measures.

METHODS

Case definition

For this investigation, a case was defined as a surgical site infection or other infection involving skin, soft tissue, bone, or a joint which was culture positive for any RGM, occurring on or after October 1, 2012, in a patient who had joint replacement surgery at hospital A during the 12 months before the infection.

Case identification

Case identification efforts included retrospective review of microbiology laboratory records and prospective surveillance for new infections. Inpatient and outpatient medical records were reviewed for each case-patient to identify any common exposures, such as clinic locations, visit dates, and procedures.

Infection control, environmental and laboratory investigation

In September 2013, initial observations of multiple joint replacement procedures at Hospital A were conducted by trained HAI evaluators of the investigation team. The purpose of these observations was to identify possible sources of exposure to contaminated water or fluids in the operating room (OR), as well as to assess compliance with hospital infection control policies, and proper aseptic surgical technique by OR personnel.

During the same time frame, environmental samples were collected from surfaces using swabs, and bulk samples were collected from water sources, including ice from ice-machines. Health care worker colonization status was assessed for all staff involved in the surgical procedures of the case patients using samples collected from hands with handwipes, hair follicles from eyebrows and scalp, and swabs of the nares, pinnae, and scalp.

Environmental samples were submitted to the U.S. Centers for Disease Control and Prevention (CDC) for isolation and identification of RGM species. Any acid-fast bacilli (AFB) found in environmental samples were presumptively identified using PCR-restriction fragment length polymorphism analysis of the heat-shock protein 65 gene and confirmed by 16s rRNA and *rpoB* gene sequencing. Preserved clinical isolates were provided to the CDC, via the Kentucky State Public Health Laboratory, by the reference laboratory that made the

initial identifications. Clinical and environmental isolates were compared using molecular typing by pulsed-field gel electrophoresis (PFGE) and by whole genome sequencing (WGS) performed by the CDC. Details of the laboratory methods used to isolate and identify RGM species are provided in the supplementary appendix.

Case-control study

A case-control study was conducted after the identification of the fifth case, the first of the *M. goodii* cases, in September of 2013, to identify risk factors associated with case status. The study included the 5 cases identified by the time of the study and 20 unmatched controls randomly selected from among all patient who had joint replacement surgery at Hospital A between October 2012 and March 2013. Data for the study were abstracted from subjects' electronic medical records. Unadjusted odds ratios (ORs) and their exact 95% confidence intervals were calculated using exact logistic regression for sex, operating room used, day and time (morning or afternoon) of surgery, and separately for each of the 68 HCWs present in the operating room at any time during any of the case-patient surgeries. Exact logistic regression was also used to regress age on case status, with the OR indicating the estimated change in the odds of being a case associated with a 1-year increase in age. Data were analyzed using STATA 16.0 (College Station).

This activity was reviewed by CDC and was conducted consistent with applicable federal law and CDC policy (45 C.F.R. part 46.102(l) (2), 21 C.F.R. part 56; 42 U.S.C. §241(d); 5 U.S.C. §552a; 44 U.S.C. §3501 et seq).

RESULTS

In total, 8 cases were identified during this investigation, 5 caused by *M. wolinskyi* and 3 caused by *M. goodii*. During the period October 1, 2012-March 31, 2014, there were 2,737 joint replacement surgeries (1,634 hips and 1,103 knees) performed at Hospital A, indicating an overall attack rate of 0.3%. Table 1 describes the clinical characteristics of patients identified with RGM infections. Five of the 8 case patients were female and the median age was 65 years (age range: 36-75). Seven cases had total knee replacement surgery and one had a hip replacement surgery. The incubation period, defined as the time from the date of the initial joint replacement surgery to the date of first RGM-positive specimen collection, ranged from 79 to 288 days with a median of 173 days. Seven cases had deep incisional or organ/space infections involving the surgical site or the replaced joint, while 1 case had remote organ/space infection, a lumbar discitis. Five patients required surgical revision of their joints as a result of the RGM infections.

Figure 1 shows the timeline of cases identified during the outbreak. All case onset dates occurred during January 2013 through March 2014. After the initial notification of the outbreak in August 2013, the Kentucky Department for Public Health and local health department investigators reviewed case patients' inpatient and outpatient medical records. No common outpatient clinic locations, appointment dates, procedures or other risk factors could be identified from the cases' outpatient medical records. What the cases did have in common, in addition to the orthopedic practice, was joint replacement surgery at Hospital A.

This led the investigation to focus on the hospital's orthopedic surgery suite operating rooms (ORs) and post-anesthesia care unit (PACU). Surgery observations were made, and environmental samples were collected from the ORs and PACU in September 2013. Observation of several joint replacement procedures failed to identify any sources of exposure to non-sterile water or fluid medical products. No lapses in hospital infection control policies or of surgical asepsis and no improper surgical techniques were observed. All surgical attire was hospital laundered. When asked about the operation of the facility's HVAC system, Hospital A reported that the temperature in the surgery suite was kept higher than usual. This was reportedly done to reduce the relative humidity in the ORs and maintain it within the desired range. RGMs were cultured from water samples collected from operating room scrub sinks, recovery ward ice-machines, and a portable cold-therapy unit reservoir, but none contained *M. wolinskyi* or *M. goodii*. Species recovered included *M. sphagni*, *M. mucogenicum*, and *M. abscessus*.

The case-control study included the first 5 cases identified, the initially identified cluster of four *M. wolinskyi* cases and the first of the *M. goodii* cases. Cases and controls in the case-control study did not differ significantly in terms of age or sex, and no significant association was found between case status and operating room, day of surgery or time of surgery (Table 2). However, the presence of one non-scrubbed circulating nurse, HCW 1, in the operating room was significantly associated with case status. The circulating nurse was present in 5 of 5 case procedures and 6 of 20 control procedures (OR: 21.8, $P = .009$)[1]. Additional observations focusing specifically on HCW 1 were undertaken. HCW 1's surgical dress, scrub and surgical techniques were observed and compared to those of other surgical personnel. No differences or deficiencies were noted.

The identification of the sixth case (*M. wolinskyi*) in January 2014, with a surgery date of April 2013, and the observation that HCW 1 was also present during that surgery led to the implementation of interim control measures, including exclusion of HCW 1 from the operating room. The nurse was temporarily reassigned to administrative duties.

Samples were collected from HCW 1, including from their home and environment. HCW 1's hands, nares, ears, scalp and hair samples were cultured for RGM. Environmental samples included water from the home shower, washing machine and an outdoor hot tub. Swab samples were collected from the shower head, washing machine, hot tub jets and filter membrane.

While the HCW 1 samples were being analyzed, the seventh and eighth cases (both *M. goodii* cases) were identified in March of 2014, with surgery dates in October and November of 2013 respectively. HCW 1 was not documented to have been in the OR for the seventh case patient surgery but was working in the surgical suite on a different case in a different OR at the time. HCW 1 was present in the OR during the eighth case patient's surgery.

All bodily samples collected from HCW 1 were negative for RGM. However, *M. wolinskyi* grew from the hot tub water sample and *M. goodii* grew from each of 2 swab samples from

[1]Median unbiased estimate (MUE) of the odds ratio and its exact *p*-value reported. CMLE estimate of the odds ratio and the upper bound of its 95% CI are undefined because there were no unexposed cases, resulting in a zero cell count.

the hot tub filter membrane. The four *M. wolinskyi* clinical isolates available for analysis at the time were found to be closely related to the four *M. wolinskyi* isolates from the hot tub water sample by PFGE (Fig 2) and by WGS (Fig 3). While the 2 *M. goodii* isolates from the hot tub filter were found to be closely related to each other (93% similarity), neither was found to be related to either of the 2 clinical *M. goodii* isolates available at the time. The clinical *M. goodii* isolates were found to be possibly related to each other (88% similarity). RGM species such as *M. wolinskyi* and *M. goodii* have sufficient genetic diversity to make PFGE reliable for strain typing of these organisms.[15]

After the identification of the seventh and eighth cases, additional focused joint replacement surgery observations found important lapses in surgical infection control practices that had not been recognized previously. Long-sleeved jackets were not consistently worn by non-scrubbed OR personnel and head coverings worn by non-scrubbed personnel did not consistently cover the nape of the neck. Consequently, excessive amounts of exposed skin were observed. There was also significant undocumented movement of surgical personnel between ORs during cases. These observed practices were contrary to guidelines promulgated by the Association of periOperative Registered Nurses (AORN)[16,17] and the Association of Surgical Technologists[18] at the time.

Based on the investigation findings, Hospital A implemented the following mitigation measures. First, the hospital revised its policies to ensure that all AORN guidelines are followed. Particularly during joint replacement procedures, the skin of all surgical personnel is to be as completely covered as can be achieved, including the wearing of surgical hoods and long-sleeved jackets snapped closed with the cuffs down to the wrists for non-scrubbed personnel. Administrative steps were also taken to minimize traffic in and out of ORs during cases. Second, short-wave ultraviolet (UVC) light bulbs were installed in all air handling units servicing surgical or procedural spaces for air-stream disinfection. Finally, pursuant to an agreement between Hospital A and HCW1, the implicated hot tub was removed from use and eliminated as a possible reservoir for further infection.

After mitigation efforts were implemented, no additional cases were detected. The hospital infection control staff continued to perform ongoing surveillance for additional cases and no new cases of *M. wolinskyi* or *M. goodii* infection have been identified as of September 2021.

DISCUSSION

We investigated a nosocomial outbreak of RGM infections caused by *M. wolinskyi* and *M. goodii* among patients who had joint replacement surgery at a Kentucky hospital. We believe that this was the largest reported outbreak of *M. wolinskyi/M. goodii* infections in the United States at the time of the investigation. No evidence of environmental contamination with the specific outbreak organisms was found in the hospital. The case-control study indicated that the presence of a particular surgical HCW in the OR was significantly associated with infections. Based on this finding, we conducted environmental sampling of the HCW's home and identified an outdoor hot tub at the HCW's home as the possible source of *M. wolinskyi*. These results were confirmed by PFGE and WGS

analyses comparing clinical and environmental mycobacterial isolates. We hypothesize that the HCW's use of the hot tub led to transient skin colonization. The HCW reported using the hot tub predominantly in cooler months which coincides with case dates ranging from January to March. The hot tub use and laboratory findings suggest a likely explanation for where the pathogens originated, and the challenge became how to identify the mode of transmission from the HCW to patients in operating room suites. Focused infection control rounds were conducted in the operating room suites that led to the identification of infection prevention and control breaches. Infection prevention and control breaches included: frequent movement throughout the space by health care workers, doors to operating rooms left open, and bare skin exposed among non-scrubbed personnel. We hypothesize that the higher than usual temperature maintained in the ORs to control relative humidity discouraged non-scrubbed personnel from wearing their long-sleeved jackets snapped closed with the cuffs down to the wrists.

It has been reported that people can shed more than 10^7 skin squames as they move about and the squames are light enough to travel on air currents.[19] Current AORN guidelines state that "doors to the operative or invasive procedure room should be kept closed as much as possible except during the entry and exit of patients, required personnel, and necessary equipment," since frequent door opening of an operating room suite by personnel can contribute to air contamination and increase the risk for a surgical site infection.[19] The combination of these breaches and a HCW transiently colonized with *M. wolinskyi* shedding skin squames suggests a possible chain-of-infection pathway. The laboratory data, demonstrating a close relationship between the cases and the HCW's hot tub samples further supports this conclusion.

Based on the investigation findings, Hospital A revised its policies, mandating that all AORN guidelines relating to surgical attire that were in place at the time be followed. However, in the years following the outbreak described here, the thinking around surgical attire for non-scrubbed personnel has evolved. The 2020 revisions to the AORN Guidelines for Perioperative Practices no longer include the recommendation that long sleeves be worn in the restricted and semi-restricted areas, except during patient skin antisepsis.[20] This change was based on recent research suggesting that surgical attire—such as the use of long sleeves by non-scrubbed personnel and specific types of head coverings—is not associated with the risk of surgical site infection.[21-26] Indeed, the idea that covering exposed skin with long-sleeved surgical attire can contain contaminated skin squames and thereby prevent surgical site infections has recently been called "archaic."[22,26] However, our finding that a cluster of surgical site infections was likely due to contamination from an intermittently colonized circulating nurse in the OR highlights the potential role of skin squames as a source for surgical site infection. Preventing such infections deserves continued attention and research, particularly for procedures involving implants—such as joint prostheses—which "have the greatest potential for wound infection, and the most significant consequences, from intraoperative airborne particle settling."[27,28]

The multifactorial nature of the cause of this outbreak highlights the importance of a systems approach to infection prevention and control and patient safety. A systems approach emphasizes the use of multiple overlapping or redundant layers of protection in conjunction

to prevent harm to patients.[29] For an outbreak to occur, multiple gaps in the layers of patient protection must align, resulting in infections. The gaps in this outbreak included improper attire being allowed in the ORs, possibly due to inappropriate temperature and humidity control, frequent movement throughout the space by health care workers, and doors to ORs being left open. The alignment of these gaps could have allowed contaminated skin squames shed from a colonized HCW to travel on air currents and infect patients, potentially including 1 patient in a different OR from the one where the colonized HCW was documented to be working.[2] The systems approach to patient safety seeks to minimize the number and size of gaps in the layer of patient protection, making it less likely that they will align and cause patient harm.

In response to the findings of this investigation, hospital policies were revised to correct the infection control lapses which led to the infections. Implementation of these measures was aimed at reducing the risk of surgical site infections not only from those microorganisms that caused the current outbreak, but also from other microorganisms, that might be present on any HCW's skin. The source of the *M. goodii* infections is not as clear as that of the *M. wolinskyi* infections, given that the organisms from the clinical samples were not found to be related to those from the environmental sample taken from HCW1's hot tub. However, the growth of *M. goodii* from the same hot tub that was the reservoir for the *M. wolinskyi* infections is suggestive. In any case, rectification of the identified deficiencies in basic infection prevention and control practices was expected to mitigate that risk as well.

In addition to the need for the kind of primary prevention exemplified by the implementation of and strict adherence to established infection control measures, this outbreak also highlights the importance of secondary prevention measures in the form of early recognition, reporting and investigation of suspected nosocomial outbreaks. A combination of effective infection surveillance, prompt cluster reporting to public health authorities, and a thorough investigation comprising coordinated epidemiological, environmental, and laboratory elements resulted in the identification of a specific point source and likely transmission route for this outbreak. These findings resulted in the outbreak being interrupted by the application of interim control measures and then permanently resolved by the implementation of ongoing mitigation measures. This response depended for its success on close and effective collaboration among Hospital A, the local and state health departments, and the CDC.

This outbreak also represents at least the second documented instance of person-to-person nosocomial transmission of *M. wolinskyi* from a colonized HCW to a patient, the first being the 2003 outbreak reported by Rahav et al[9] The remarkable similarities between the outbreaks—infection after surgeries involving device implantation, transmission by a colonized surgical HCW vector, and an outdoor hot tub reservoir—highlight the importance of considering this mechanism in the investigation of future outbreaks. An important difference—one involving an operating surgeon, the other involving a non-scrubbed

[2]This could have occurred due to HCW1's undocumented presence in that OR or due to contamination from the corridor, given the observation that OR doors were often left open, which could reduce the effectiveness of positive pressure, [30] and that undocumented movement of surgical personnel between ORs frequently occurred.

circulating nurse—suggests that direct patient contact or presence in the sterile field is not necessary for such transmission to occur.

CONCLUSION

This report describes the investigation of one of the largest outbreaks of surgical site infections due to RGMs in the United States. Future outbreak investigations of RGMs should always consider the potentially important role of colonized HCWs as a vector of transmission. Nonetheless, an outbreak of this nature often indicates breaches in infection control practices at multiple levels, which in this case may have led to an uninterrupted chain of infection from a HCW's hot tub to surgical sites. As RGM infections are increasing globally, this report highlights the importance of strict adherence to the recommended practices for prevention of transmissible infections.

APPENDIX

Laboratory Methods for Isolation and Identification of RGM in Environmental Samples and Clinical Isolates

Isolation from environmental samples

Swabs: Swab heads were broken off and placed into Middlebrook 7H9 broth, mixed well with a vortex, and incubated at 30°C overnight. Broth demonstrating growth/ showing turbidity were plated (100 μL) on to TSA II with 5% Sheep's Blood or Middlebrook and Cohn 7H10 with OADC, incubated overnight at 30°C, and examined for growth.

Water: Three approaches were used to process the water samples due to on-going developments within the outbreak to improve isolation of *M. wolinskyi*: (A) The initial batch of 7 water samples were processed by membrane filtration in 10 mL, 100 mL, and 250 mL aliquots and filtered through a 0.45 μm gridded filter, in addition to 100 μL aliquots being plated. All samples were placed on to Middlebrook and Cohn 7H10 with OADC and incubated at 30°C overnight. (B) The next 2 samples were treated for 30 minutes with 0.005% cetylpyridium chloride to reduce background organisms prior to filtration (10 mL aliquots) and being plated (100 μL). (C) The final 3 water samples were assayed for detection of *M. wolinskyi*: 10 mL, 100 mL, and remaining volumes of approximately 250 mL aliquots were filtered through 0.45 μm gridded filters, placed on Middlebrook and Cohn 7H10 with OADC, and incubated at 30°C overnight.

Hand samples: Hand samples collected with commercial hand cleaning wipes were placed in Phosphate Buffered Saline with 0.02% Tween 80 (90 mL), homogenized at 260 rpm for 1 minute in a stomacher, concentrated by centrifugation (2700 *x*g, 20 min), plated (100 μL), and incubated at 30°C overnight. The remaining sample was added to Middlebrook 7H9 broth and incubated at 30°C overnight. Broth demonstrating growth/ showing turbidity were plated (100 μL) on Middlebrook and Cohn 7H10 with OADC and incubated at 30°C overnight.

Hair samples: Hair samples were placed in Middlebrook 7H9 broth (5 mL) and incubated at 30°C overnight. Broth demonstrating growth/ showing turbidity were plated (100 μL) on Middlebrook and Cohn 7H10 with OADC and incubated at 30°C overnight.

Species identification

Suspect isolates were stained using the Kinyoun acid-fast staining method. Positive Acid-Fast bacteria were identified using PCR-restriction fragment length polymorphism analysis (PRA) to presumptively identify as *M. wolinskyi*[1]. The hsp65 gene was amplified by PCR of cell-free lysates, and the presence of its 440bp amplification product was verified by gel electrophoresis (1% agarose gel, 80V for 70 minutes). Two aliquots of the product were digested with either BstEIII or HaeII restriction enzymes. The digests were then run on a 3% agarose gel at 70V for 3 hours. The base pair size of each band was estimated by comparison with a Low Molecular Weight Ladder (NE Biolabs). PRA was performed by entering fragment size data into “PRA Site,” a web-based analysis tool. 16s rRNA and rpoB gene sequencing was used to confirm ID of *M. wolinskyi*.

Pulsed-Field Gel Electrophoresis: Molecular typing was performed by pulsed-field electrophoresis (PFGE). Molecular chromosomal DNA was prepared as described previously[2,3] with the following modifications: the DNA plugs from the *M. wolinskyi* isolates were digested with the restriction endonucleases AseI and XbaI; restriction fragments were separated with CHEF Mapper XA Pulsed Field Electrophoresis System (Bio-Rad Laboratories). 50 mM thiourea was added to the running buffer. PFGE running conditions were initial switch time of 3 seconds and final switch of 20 seconds with total run time of 20 hours. *Salmonella* serotype Braenderup (H9812 strain) was used as the universal standard. The genetic relatedness of the isolates was analyzed by BioNumerics software (Applied Maths). Similarity of PFGE patterns was based upon Dice coefficients and a dendrogram was built using the unweighted-pairing group method. The Tenover criteria[4] were used to interpret the comparison of the patient isolate PFGE patterns; patterns were classified as indistinguishable (100% similarity), closely related (1-3 bands difference), possibly related (4-6 band difference) or unrelated (>7 band difference).

Whole Genome Sequencing and Analysis: Colonies from the confirmed *M. wolinskyi* cultures were suspended in sterile DI water and boiled for 10 minutes, followed by DNA extraction with a Maxwell automated extractor (Promega). DNA was sequenced using the Illumina MiSeq using 2 × 250 reads, producting ~2.2 million reads per isolate. DNA library preparation was performed by the CDC Biotechnology Core Facility. Contigs were generated from those reads and subsequently joined into scaffolds. SNP discovery was based on k-mer analysis, using kSNP v2[5], and required no selection of a single reference genome. SNP matrices were created from all the SNPs and used for building maximum likelihood trees.

References

1. Martin A, Uwizeye C, Fissette K, et al. Application of the hsp65 PRA method for the rapid identification of mycobacteria isolated from clinical samples in Belgium. Journal of Microbiological Methods. 2007;71(1):39–43. [PubMed: 17719666]

2. Vanitha JD, Venkatasubramani R, Dharmalingam K, Paramasivan CN. Large-restriction-fragment polymorphism analysis of *Mycobacterium chelonae* and *Mycobacterium terrae* isolates. Applied Environmental Microbiology. 2003;69:4337–41. [PubMed: 12839827]

3. Wallace RJ Jr, Zhang Y, Brown BA, Fraser V, Mazurek GH, Maloney S. DNA large restriction fragment patterns of sporadic and epidemic nosocomial strains of *Mycobacterium chelonae* and *Mycobacterium abscessus*. Journal of Clinical Microbiology. 1993;31:2697–701. [PubMed: 8253968]

4. Tenover FC, Arbeit RD, Goering RV, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. Journal of Clinical Microbiology. 1995;33:2233–9. [PubMed: 7494007]

5. Gardner SN, Hall BG (2013) When Whole-Genome Alignments Just Won't Work: kSNP v2 Software for Alignment-Free SNP Discovery and Phylogenetics of Hundreds of Microbial Genomes. PLoS ONE. 2013;8(12):e81760. [PubMed: 24349125]

References

1. Binder AM, Adjemian J, Olivier KN, Prevots DR. Epidemiology of nontuberculous mycobacterial infections and associated chronic macrolide use among persons with cystic fibrosis. Am J Respir Crit Care Med. 2013;188:807–812. [PubMed: 23927602]

2. Herold RC, Lotke PA, MacGregor RR. Prosthetic joint infections secondary to rapidly growing Mycobacterium fortuitum. Clin Orthop Relat Res. 1987;216:183–186.

3. Eid AJ, Berbari EF, Sia IG, Wengenack NL, Osmon DR, Razonable RR. Prosthetic joint infection due to rapidly growing mycobacteria: report of 8 cases and review of the literature. Clin Infect Dis. 2007;45:687–694. [PubMed: 17712751]

4. Buser GL, Laidler MR, Cassidy PM, Moulton-Meissner H, Beldavs ZG, Cieslak PR. Outbreak of nontuberculous mycobacteria joint prosthesis infections, Oregon, USA, 2010–2016. Emerg Infect Dis. 2019;25:849. [PubMed: 31002056]

5. Brown BA, Springer B, Steingrube VA, et al. Mycobacterium wolinskyi sp. nov. and Mycobacterium goodii sp. nov., two new rapidly growing species related to Mycobacterium smegmatis and associated with human wound infections: a cooperative study from the International Working Group on Mycobacterial Taxonomy. Int J Syst Evol Microbiol. 1999;49:1493–1511.

6. Hernández-Meneses M, González-Martin J, Agüero D, et al. Mycobacterium Wolinskyi: A new non-tuberculous mycobacterium associated with cardiovascular infections? Infect Dis Ther. 2021;10:1–8.

7. Nagpal A, Wentink JE, Berbari EF, et al. A cluster of Mycobacterium wolinskyi surgical site infections at an academic medical center. Infect Contr Hosp Epidemiol. 2014;35:1169–1175.

8. Schnabel D, Esposito DH, Gaines J, et al. Multistate US outbreak of rapidly growing mycobacterial infections associated with medical tourism to the Dominican Republic, 2013–2014. Emerg Infect Dis. 2016;22:1340. [PubMed: 27434822]

9. Rahav G, Pitlik S, Amitai Z, et al. An outbreak of Mycobacterium jacuzzii infection following insertion of breast implants. Clin Infect Dis. 2006;43:823–830. [PubMed: 16941361]

10. Braga JR, do Prado M, Aparecida e, et al. Rapidly growing mycobacterial infections associated with plastic surgery: an epidemiological description. Afr J Microbiol Res. 2021;15:120–124.

11. Alcaide F, Peña M, Pérez-Risco D, et al. Increasing isolation of rapidly growing mycobacteria in a low-incidence setting of environmental mycobacteria, 1994–2015. Eur J Clin Microbiol Infect Dis. 2017;36:1425–1432. [PubMed: 28321580]

12. Turner NA, Baker AW. Rapidly growing mycobacteria in transplant: Evolving epidemiology and treatment options. In: Morris MI, Kotton CN, Wolfe C (eds) Emerging Transplant Infections. Springer, Cham. 2021: 1–35.

13. Ahmed I, Tiberi S, Farooqi J, et al. Non-tuberculous mycobacterial infections—A neglected and emerging problem. Int J Infect Dis. 2020;92:S46–S50.

14. Groenewold MR, Russell ES, Konkle SL, et al. Investigation of a cluster of rapidly-growing mycobacteria infections associated with joint replacement surgery in a Kentucky hospital. Paper

presented at: Council of State and Territorial Epidemiologists 2014 Annual Conference. Nashville, TN; 2014.

15. Jagielski T, van Ingen J, Rastogi N, Dziadek J, Mazur PK, Bielecki J. Current methods in the molecular typing of Mycobacterium tuberculosis and other mycobacteria. Biomed Res Int. 2014;2014: 645802. [PubMed: 24527454]
16. AORN. Recommended Practices for Surgical Attire. Perioperative Standards and Recommended Practices. Denver, CO: AORN; 2011:57–72.
17. Braswell ML, Spruce L. Implementing AORN recommended practices for surgical attire. AORN J. 2012;95:122–137. quiz 38-40. [PubMed: 22201576]
18. Association of Surgical Technologists. Standards of Practice for Surgical Attire, Surgical Scrub, Hand Hygiene and Hand Washing. LIttleton, CO: AST; 2008.
19. AORN. Guidelines for Perioperative Practice. Denver: AORN. Inc; 2019. 2019.
20. AORN. AORN Guidelines for Perioperative Practice 2020: Association of PeriOperative Registered Nurses. AORN; 2020.
21. Shallwani H, Shakir HJ, Aldridge AM, Donovan MT, Levy EI, Gibbons KJ. Mandatory change from surgical skull caps to bouffant caps among operating room personnel does not reduce surgical site infections in class I surgical cases: a single-center experience with more than 15 000 patients. Neurosurgery. 2018;82:548–554. [PubMed: 29447369]
22. Farach SM, Kelly KN, Farkas RL, et al. Have recent modifications of operating room attire policies decreased surgical site infections? An American College of Surgeons NSQIP review of 6,517 patients. J Am Coll Surg. 2018;226:804–813. [PubMed: 29408507]
23. Markel TA, Gormley T, Greeley D, et al. Hats off: a study of different operating room headgear assessed by environmental quality indicators. J Am Coll Surg. 2017;225:573–581. [PubMed: 29106842]
24. Elmously A, Gray KD, Michelassi F, et al. Operating room attire policy and health care cost: favoring evidence over action for prevention of surgical site infections. J Am Coll Surg. 2019;228:98–106. [PubMed: 30359824]
25. Haskins I, Prabhu A, Krpata D, et al. Is there an association between surgeon hat type and 30-day wound events following ventral hernia repair? Hernia. 2017;21:495–503. [PubMed: 28631104]
26. Stapleton EJ, Frane N, Lentz JM, et al. Association of disposable perioperative jackets with surgical site infections in a large multicenter health care organization. JAMA surg. 2020;155:15–20. [PubMed: 31642891]
27. Eickhoff TC. Airborne nosocomial infection: a contemporary perspective. Infect Contr Hosp Epidemiol. 1994;15:663–672.
28. American Society of Anesthesiologists. ASA Guidelines for Surgical Attire. Washington, D.C: ASA; 2019.
29. CMPA. Quality improvement: Patient safety. The Canadian Medical Protective Association (CPMA); 2021… Retrieved from; https://www.cmpa-acpm.ca/en/education-events/goodpractices/the-healthcare-system/quality-improvement-patient-safety.
30. Pokrywka M, Byers K. Traffic in the operating room: a review of factors influencing air flow and surgical wound contamination. Infect Disord-Drug Targets. 2013;13:156–161. [PubMed: 24001332]

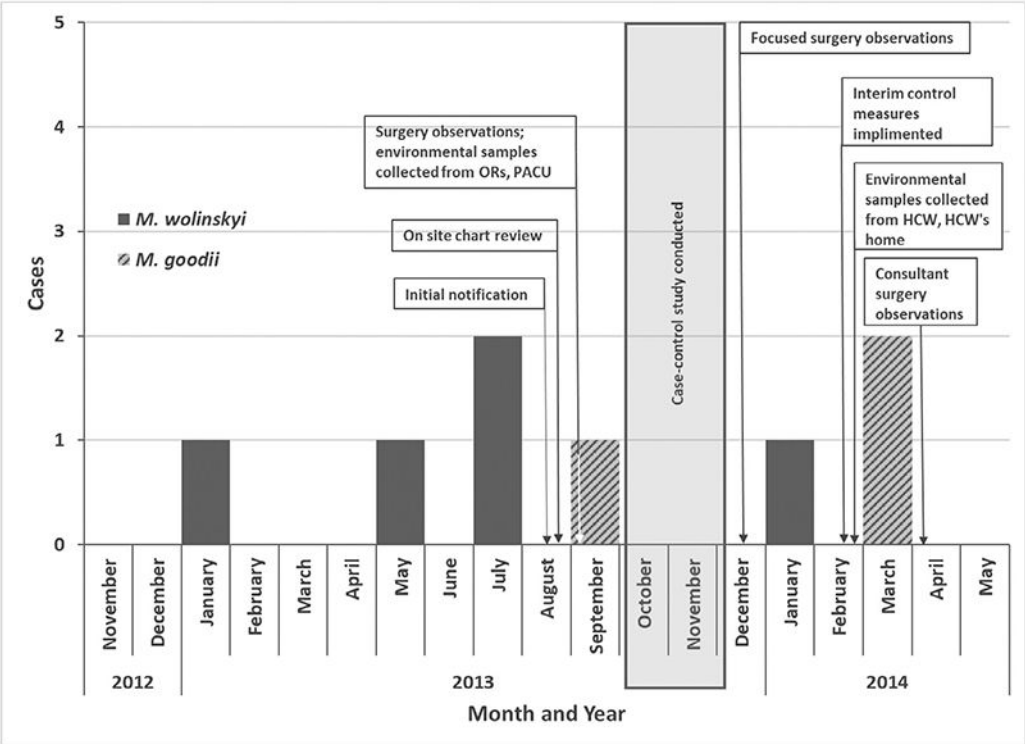


Fig 1.

Cases of rapidly-growing mycobacteria infections at hospital A by date of diagnosis and species—Kentucky, November 2012-May 2014.

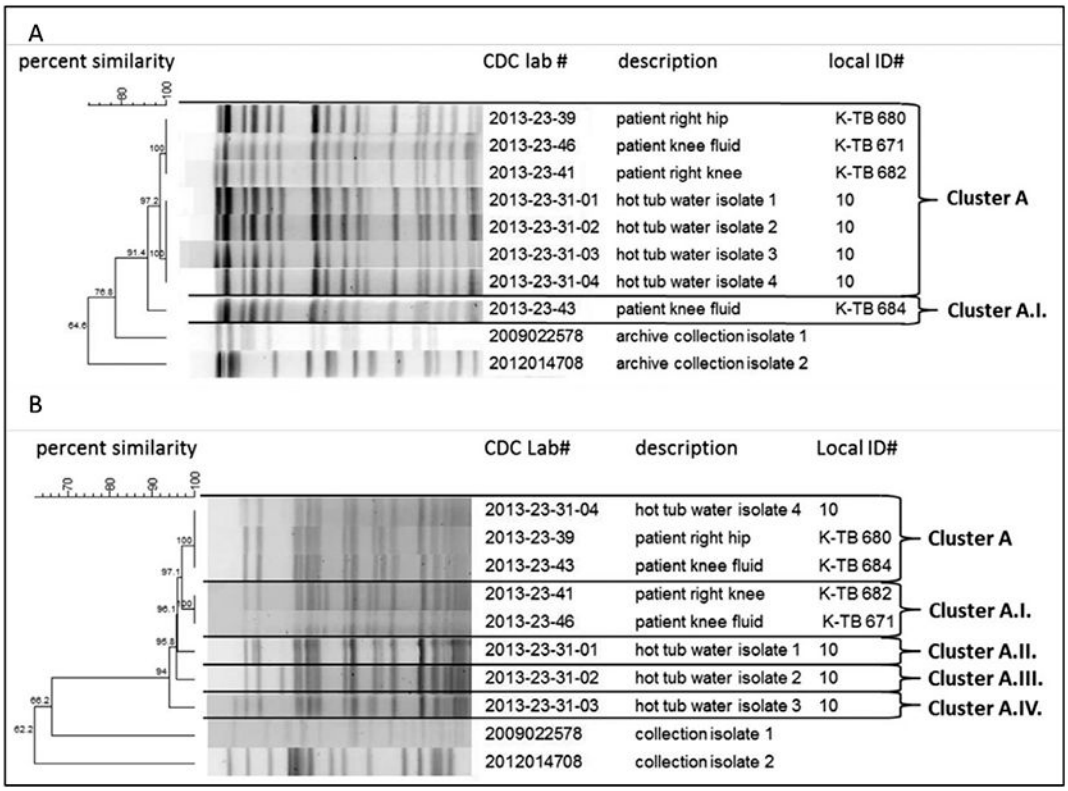
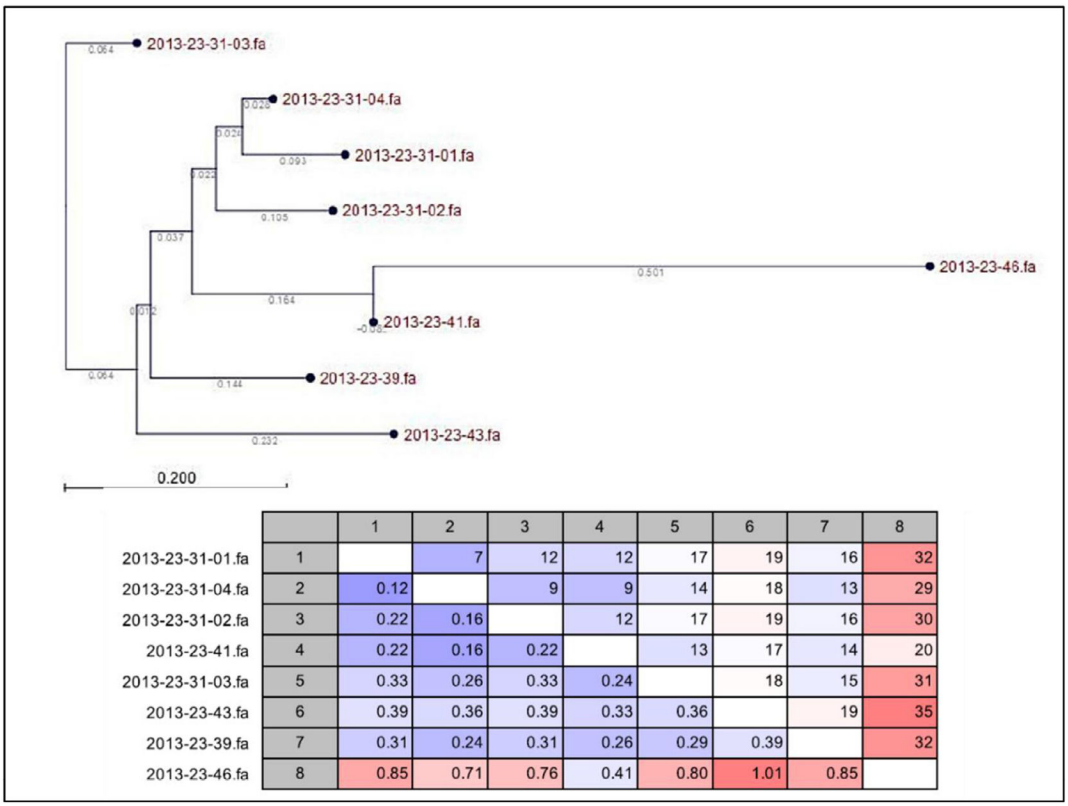


Fig 2.

PFGE Dendrogram of AseI (A) and XbaI (B) restriction digests of M. wolinskyi isolates* from an outbreak of rapidly-growing mycobacteria infections at a Kentucky hospital.

*Includes four clinical isolates from 4 outbreak case-patients and 4 environmental isolates grown from a bulk water sample collected from a hot tub, plus 2 controls.



	1	2	3	4	5	6	7	8
2013-23-31-01.fa	1		7	12	12	17	19	16	32
2013-23-31-04.fa	2	0.12		9	9	14	18	13	29
2013-23-31-02.fa	3	0.22	0.16		12	17	19	16	30
2013-23-41.fa	4	0.22	0.16	0.22		13	17	14	20
2013-23-31-03.fa	5	0.33	0.26	0.33	0.24		18	15	31
2013-23-43.fa	6	0.39	0.36	0.39	0.33	0.36		19	35
2013-23-39.fa	7	0.31	0.24	0.31	0.26	0.29	0.39		32
2013-23-46.fa	8	0.85	0.71	0.76	0.41	0.80	1.01	0.85	

Fig 3.

Unrooted single-nucleotide polymorphism (SNP) matrix alignment tree and differences (upper right corner) and average SNPs per sequence length* (lower left corner) for *M. wolinskyi* isolates† from an outbreak of rapidly-growing mycobacteria infections at a Kentucky hospital. *Scale bar represents average SNPs per sequence length. †Includes four clinical isolates from four outbreak case-patients and four environmental isolates grown from a bulk water sample collected from a hot tub, plus 2 controls.

Table 1

Selected clinical characteristics of case-patients

Characteristic	No. (%)
Female	5 (63)
Median age, y (range)	65 (36-71)
Procedure	
Total knee replacement	7 (88)
Hip replacement	1 (12)
Median incubation period, days (range)	173 (79-288)
Infection site	
Surgical site infection	7 (88)
Remote (discitis)	1 (12)
Organism	
M. wolinskyi	5 (63)
M. goodii	3 (37)
Required revision of joint	5 (63)

Table 2

Case-control study results

Characteristic	Cases no. (%)	Controls no. (%)	OR* (95% CI)
Age (mean, yrs)	59	64	0.95 (0.84-1.05)†
Sex			
Female	3 (60)	14 (70)	Ref
Male	2 (40)	6 (30)	1.5 (0.1-17.4)
Procedure			
Total Knee Replacement	4 (80)	17 (85)	Ref
Hip Replacement	1 (20)	3 (15)	1.4 (0.2-2.4)
Operating Room			
A	1 (20)	9 (45)	Ref
B	2 (40)	3 (15)	4.6 (0.2-353.0)
C	2 (40)	4 (20)	3.6 (0.1-264.8)
D	0 (0)	4 (20)	1.8 (0.0-70.2)
Day of Surgery			
Monday	2 (40)	5 (25)	1.6 (0.1-28.3)
Tuesday	2 (40)	8 (40)	Ref
Wednesday	0 (0)	3 (15)	1.3 (0.0-19.7)
Thursday	1 (20)	4 (20)	1.0 (0.0-25.3)
Time of Surgery			
AM	3 (60)	11 (55)	Ref
PM	2 (40)	9 (45)	0.8 (0.1-8.9)

Abbreviations: OR = Odds Ratio; CI = Confidence Interval.

*Conditional Maximum Likelihood Estimate of the Odds Ratio.

†OR indicates the estimated change in the odds of being a case associated with a 1-year increase in age.