

Original Article

Clinical and operational impact of a four-year-long bronchoscopy-associated pseudo-outbreak of *Mycobacterium mucogenicum*

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Abstract

Objective: To report on the investigation of a pseudo-outbreak of *Mycobacterium mucogenicum* and examine its clinical and operational impact.

Design: Outbreak investigation, retrospective cohort study.

Setting: Academic medical center in Los Angeles, California.

Patients: Patients whose bronchoalveolar lavage (BAL) cultures grew *M. mucogenicum* from 2020–2024.

Methods: We performed an institutional outbreak investigation of *M. mucogenicum*, reviewed electronic medical records of a subset of affected patients (2023–2024), and assessed the operational impact.

Results: The incidence of *M. mucogenicum* in BAL cultures at Hospital A increased from 6.1% (29/473) in 2020 to 18.6% (29/156) in the first quarter of 2024. Epidemiologic investigation revealed non-sterile ice baths used to cool uncapped sterile syringes during bronchoscopy procedures as the contamination source. Next generation sequencing linked clinical isolates to *M. mucogenicum* recovered from a perioperative ice machine. Nearly all (157/160) clinical isolates grew from nocardia media rather than acid-fast bacilli media. Among 154 patients, including 51 (33.1%) who were highly immunocompromised, no true infections were identified. Thirty-nine (25.3%) patients were referred to infectious diseases for consultation, seven (4.5%) underwent additional workup, and only one received targeted treatment. The pseudo-outbreak incurred 458 hours of microbiology technologist and infection preventionist time and cost the laboratory \$88,426.

Conclusions: A four-year pseudo-outbreak of *M. mucogenicum* traced to contaminated ice baths used during bronchoscopy resulted in unnecessary infectious disease referrals and substantial operational and financial burden to the institution. Avoidance of non-sterile ice use in procedures prevents costly and burdensome pseudo-outbreaks of environmental mycobacteria in healthcare settings.

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Introduction

Mycobacterium mucogenicum, like other nontuberculous mycobacteria (NTM), is ubiquitous in the environment as well as household and hospital potable water, largely due to its relative resistance to chlorine.^{1,2} Contamination of central lines by tap water has been

linked to healthcare-associated *M. mucogenicum* bacteremia.^{3–5} More commonly reported, however, are pseudo-outbreaks of *M. mucogenicum* and other rapid-growing mycobacteria due to ice and tap water contamination of clinical respiratory cultures. Many of these have been associated with bronchoscopy and linked to inadequate disinfection of bronchoscopes.^{2,6,7} More recently, bronchoscopy-related pseudo-outbreaks of *Mycobacterium mucogenicum* and *Mycobacterium chelonae* have been linked to the use of non-sterile ice during bronchoalveolar lavage (BAL).^{8,9} The clinical and financial impact, however, has not previously been reported.

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Our health system, which includes two academic medical centers in Los Angeles, CA (Hospital A and Hospital B), experienced a large pseudo-outbreak of *M. mucogenicum* from patients undergoing bronchoscopy beginning in 2020. Despite an initial infection prevention (IP) investigation in 2020, the pseudo-outbreak lasted until 2024 when a second IP investigation identified the source of specimen contamination. We describe our IP investigation and mitigation plan and an analysis of the clinical and operational impact of the pseudo-outbreak on our health system.

Methods

Retrospective review of cases

We reviewed the electronic medical records (EMR) of all patients growing *M. mucogenicum* from BAL cultures obtained in the operating room (OR) of Hospital A during the 15 months prior to our investigation (1/1/2023 to 3/31/2024). For patients with more than one positive BAL culture during this period, only the first case was included in our review. Data regarding patient sex, age, immunocompromised status, Infectious Diseases (ID) referral, diagnostic workup, receipt of antimicrobials, and clinical outcome were abstracted from the EMR. Patients were considered immunocompromised if they had any of the following conditions at the time of bronchoscopy: primary immunodeficiency, human immunodeficiency virus (HIV, with or without antiretroviral therapy), solid organ or bone marrow transplant, hematologic malignancy, active cancer with receipt of chemotherapy within the past 3 months, receipt of corticosteroids (any dose) for >14 days, or receipt of any other immunosuppressive medication at the time of bronchoscopy.

Environmental testing

Environmental swabs were collected from both dispenser chutes and drip tray, along with 50 mL water samples from the machine's input line and melted ice. E-swabs of surfaces and water samples in screw-cap containers were spun in a vortex mixer for 30 s, and 100 µL of each was plated on buffered charcoal yeast extract (BCYE) and BCYE select agar—a method adapted from *Nocardia* culture protocols, as most clinical isolates in this study grew in *Nocardia* cultures. Standard mycobacterial culture and identification techniques were employed.

Genetic relatedness analysis of clinical and environmental isolates

Three representative environmental isolates from the ice and one from water culture were analyzed for genetic relatedness to three representative patient isolates from BAL collected on Aug 12, 2020 (at the start of the pseudo-outbreak) and to six clinical isolates collected in April 2024 (at the time of IP investigation) using Whole Genome Sequencing (WGS). Frozen 2020 isolates were revived on BCYE agar and all morphotypes present were sequenced (3, 2, 2 morphotypes respectively) for the completeness of the study. Organism identification was confirmed by MALDI-TOF as well as WGS. The mycobacteria WGS identification workflow on Illumina MiSeq was described previously.¹⁰ The whole genome sequences were mapped to the reference genome *Mycobacterium mucogenicum* ssp. *phocaicum* NZ_AP022616. Single nucleotide polymorphism (SNP) analysis was performed using CLC Genomic workbench's SNP analysis workflow (Qiagen, USA).

Analysis of laboratory and institutional costs

The laboratory cost per isolate was calculated by combining the average hourly wage of a Clinical Laboratory Scientist (CLS) technologist (\$80) in Los Angeles and the microbiological reagent cost. Infection prevention effort was calculated by reviewing emails and schedules from the two pseudo-outbreak investigation periods (2020, 2024). The following activities were considered related to the investigation: chart review of reported patients, observations of bronchoscopy procedures, education to perioperative staff, and meetings with the IP, hospital leadership, environmental services, and facilities teams.

Results

Infection prevention investigation and mitigation plan

In 2020, the Clinical Microbiology Laboratory notified the IP team about a cluster of *M. mucogenicum* cases from patients undergoing bronchoscopy in the Hospital A OR. The number of BAL cultures growing *M. mucogenicum* had increased from five in 2016 to 29 in 2020 (Figure 1). An IP investigation at that time revealed that there were no deficiencies in endoscope reprocessing and that the isolates were not genetically related. Pulmonologists who were interviewed denied the use of non-sterile ice or water during the procedure. Given that the proportion of positive BAL cultures was unchanged between 2016 and 2020 (5.4% vs 6.1%), we concluded that the increase in cases was likely due to an increase in procedure volume and recommended avoiding non-sterile ice or water during BAL.

In April 2024, a veteran pulmonologist at Hospital B who recently began performing bronchoscopies at Hospital A notified IP about a high proportion of *M. mucogenicum* growth from BAL cultures obtained at Hospital A compared to Hospital B. Further investigation revealed a steady increase in *M. mucogenicum*-positive clinical cultures at our institution from 2016 to 2024, with nearly all cultures being sent from the perioperative area of Hospital A (Figure 1), and a significant difference in the rate of positive perioperative cultures between Hospital A and Hospital B in 2024 (18.6% vs 1.3%). IP observation of bronchoscopies performed in the OR of Hospital A revealed that sterile saline syringes were uncapped and placed in a tub of non-sterile ice for bleeding control during BAL (Figure 2). During BAL, a significant amount of non-sterile ice had melted and mixed with the sterile saline which was then flushed into the patient's lungs and suctioned back for collection into a sterile cup for culture. The ice used during the procedure came from a single perioperative area ice machine (Scotsman HID540A). A 0.5 µm point-of-use filter was in place between the water inlet pipe and the ice machine. The hospital's water disinfectant residual levels, pH and temperature were closely monitored during this time and were within goal. Review of cleaning logs revealed no significant gaps in quarterly ice machine disinfection or filter exchange. Visual examination of the ice machine revealed significant corrosion on the ice dispenser chute.

In May 2024, IP met with perioperative leadership to immediately discontinue the practice of placing uncapped sterile saline syringes in a non-sterile ice bath. Hospital A adopted Hospital B's practice of placing a bottle of sterile saline in a non-sterile ice bath and pouring the chilled sterile saline into a sterile basin to be drawn up with a single-use syringe as needed during BAL. The perioperative ice machine was removed from use and

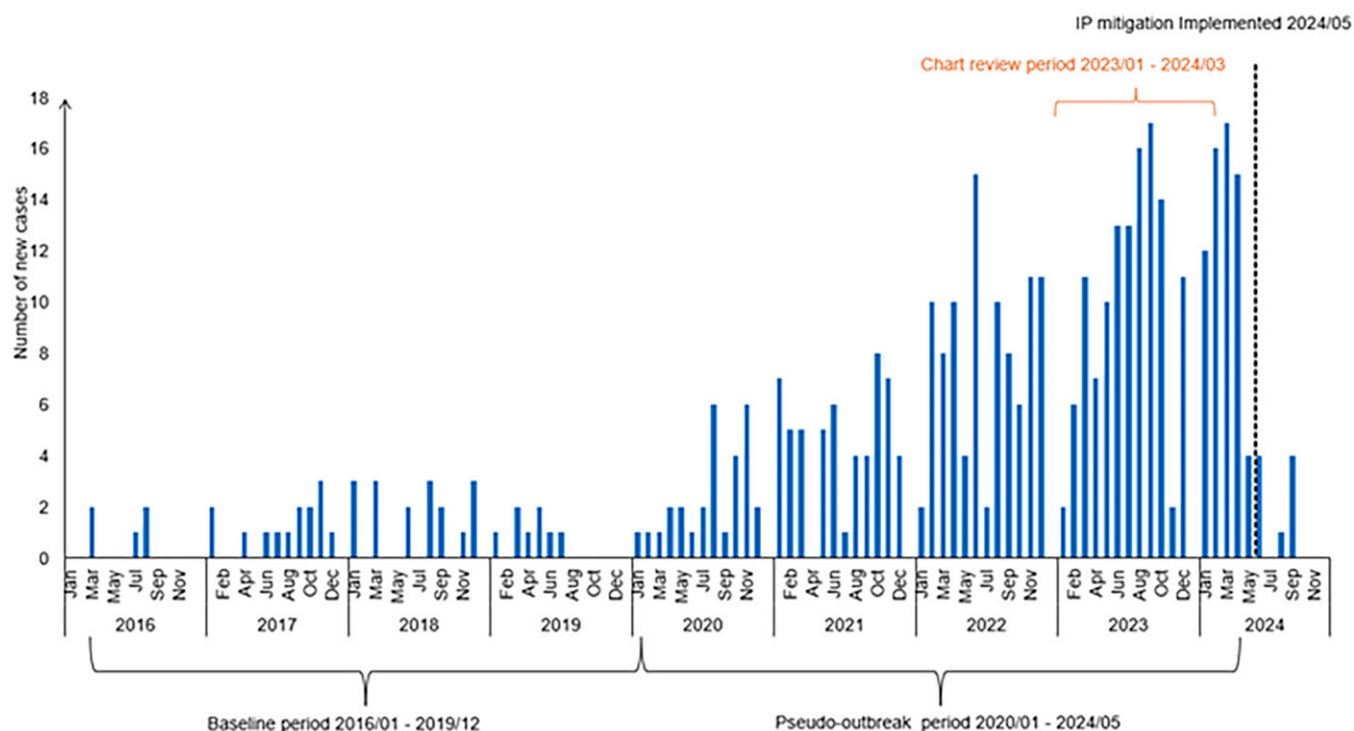


Figure 1. Epidemic curve of *Mycobacterium mucogenicum* cases identified in hospital a between 2016 and 2024.



Figure 2. Uncapped sterile saline syringes left in a tub of non-sterile ice for use during bronchoalveolar lavage.

replaced with a new ice machine, new point-of-use filter, and new tubing between the filter and the machine. Routine *M. mucogenicum* surveillance from BAL cultures was initiated. In the postintervention period from June to December 2024, the proportion of BAL cultures growing *M. mucogenicum* had decreased to 2% (Figure 1).

Retrospective review of cases

From Jan 2020 to March 2024, 358 BAL cultures obtained from 313 patients in Hospital A's OR grew *M. mucogenicum*. Twenty-one patients grew *M. mucogenicum* twice, five patients grew *M. mucogenicum* three times each, and two patients grew *M. mucogenicum* in several cultures (nine and seven, respectively). Most of these patients with two or more positive cultures (20/28) grew *M. mucogenicum* in different bronchoscopy procedures.

During the 15-month period prior to the outbreak investigation, 154 patients grew *M. mucogenicum* 160 times from their BAL cultures (Table 1). Notably, nearly all (157/160) of these clinical specimens initially grew *M. mucogenicum* in nocardia cultures which were then reflexed to acid-fast bacilli cultures. The median age was 69 years (range 21 to 91 years). Immunocompromised patients (33.1%) were more likely to receive an ID referral [OR 2.13, 95% CI (1.008 to 4.5)] ($P = .045$) but were not significantly more likely to receive additional workup [OR 1.54, 95% CI (0.33 to 7.19)] ($P = .58$) for their positive culture. The seven (4.5%) patients who received additional workup had repeat CT imaging (5/7), additional AFB cultures sent (2/7) or cell free metagenomic sequencing sent (1/7). One patient was started on antibiotics for *M. mucogenicum* infection, which were discontinued after a rash developed due to trimethoprim-sulfamethoxazole. This patient was later seen by ID and felt to have culture contamination. No patients subsequently developed true infection due to *M. mucogenicum*.

Environmental surveillance and genetic relatedness analysis

From the environmental surveillance, *M. mucogenicum* was isolated from ice and water supplied to the ice machine but not from the surfaces of the ice dispensing chutes or the drip tray (Figure 3). The environmental and patient isolates were confirmed as *M. mucogenicum* ssp. *phocaicum* using WGS. SNP analysis

Table 1. Demographic and clinical data for retrospective case review

Total	N = 154 (%)
Age Range	
20 to 39	4 (2.6)
40 to 59	30 (19.5)
60 to 79	105 (68.2)
≥ 80	15 (9.7)
Sex	
Male	76 (49.4)
Female	78 (50.6)
Immunocompromised	
Yes	51 (33.1)
No	103 (66.9)
Immunocompromise reason	
Cancer ^a	34 (66.7)
Transplant status	10 (19.6)
Other ^b	7 (13.7)
Referred to ID	
Yes	39 (25.3)
No	115 (74.7)
Additional workup ^c	
Yes	7 (4.5)
No	147 (95.5)
Considered a contaminant	
Yes	68 (44.2)
No	4 (2.6)
Result not acknowledged in chart	82 (53.2)
Outcome	
No development of true infection	135 (87.7)
Death unrelated	9 (5.8)
No follow up	10 (6.5)

^aCancer defined as hematologic malignancy or receipt of chemotherapy within past 3 months.

^bOther includes HIV, immunosuppressive medication or on steroids >14 days.

^cAdditional workup included further imaging, repeat sputum or blood cultures, and Karius testing.

revealed that the environmental isolates were closely related to patient isolates collected in April 2024 (4 to 92 SNPs apart) but not genetically related to the patient isolates from 2020 (>63,000 SNPs apart) (Figure 4). Even among the 2024 samples, two distinctive clusters were noticeable, with the isolates from PT5 closely related to isolates from Ice 1 and 2 and the water sample (6–92 SNPs) while the other cluster comprised isolates from PT1–4 and 6 and Ice 3 (4–26 SNPs). These two clusters were over 44,000 SNPs apart, suggesting a multi-strain reservoir of *M. mucogenicum* colonizing the ice machine.

Analysis of laboratory and institutional costs

We estimated that the cost to the microbiology laboratory for workup of each isolate was \$247 and that the total cost during the

pseudo-outbreak was \$88,426 (Table 2). The cost of replacing the ice machine was \$10,086. In addition, approximately 100 hours of infection preventionist time was devoted to the pseudo-outbreak investigation and mitigation.

Discussion

Bronchoscopy-related pseudo-outbreaks of NTM, including *M. mucogenicum*, linked to contaminated ice machine ice and water have been previously described;^{8,9} however, the clinical and financial impacts have not previously been studied. Similar to prior reports, we found that discontinuing the practice of using non-sterile ice and water during these procedures was highly effective at curtailing the pseudo-outbreak.^{8,9} We also showed that clinical isolates were genetically related to environmental isolates from the ice machine. Our study was much larger than previously reported pseudo-outbreaks, lasting over four years, and involving 313 patients.

Although our institution replaced the entire ice machine, tubing, and point-of-use filter, we suspect that stopping the use of all non-sterile ice or water was the primary intervention responsible for ending the pseudo-outbreak, as NTM is ubiquitous in hospital potable water and difficult to eradicate from ice machines.^{1,11,12} Studies have shown that 61–91% of hospital water and ice are contaminated by NTM,^{1,13} and that concentrations of NTM are significantly higher in ice and water collected from ice machines compared to water in faucets.¹⁴ The internal water reservoir of an ice machine can promote growth of non-thermophilic NTM given its low temperature and water stagnation. In our study, the WGS of clinical *M. mucogenicum* isolates from 2020 were unrelated to clinical or environmental isolates from 2024, suggesting that the ice machine was a reservoir for multiple strains of *M. mucogenicum*. Adherence to standard cleaning and disinfection and point-of-use filter may be insufficient to prevent growth of NTM in ice machine water and ice. In previously reported pseudo-outbreaks, enhanced cleaning of the ice machine alone was unsuccessful in eliminating growth of NTM.^{12,15} Point-of-use filters with ≤0.2 µm pore size have been recommended to prevent entry of NTM,¹⁶ but the size of the prefilter between the water inlet pipe and the ice machine at our institution was 0.5 µm. Cazals et al showed that the largest amplification of NTM occurred within the tubing connecting the outlet of the prefilters to the ice machine.¹⁴ It is interesting that we did not isolate other NTM species in our environmental or BAL cultures, and we hypothesize that this may be due to *M. mucogenicum*'s ability to grow in cold and in distilled water, as well as its relative resistance to chlorine.² Unlike other studies, we did not find a link to contaminated bronchoscopes through inadequate disinfection,^{2,6,7} and our pseudo-outbreak ended without requiring sterilization or removal of bronchoscopes from circulation. We also did not find the ice machine drip tray to be a source of environmental contamination, unlike other studies.¹⁷

Our retrospective review of *M. mucogenicum* revealed surprisingly few clinical adverse consequences of our pseudo-outbreak, even among a highly immunocompromised population. Only 25.3% of cases were referred to ID for consultation (more likely for immunocompromised hosts), 4.5% had further workup performed, and only one patient was treated unnecessarily with antimicrobials. We believe that this was because the four pulmonologists at Hospital A who commonly perform outpatient bronchoscopy procedures became accustomed to the growth of *M. mucogenicum* and disregarded these results, either documenting

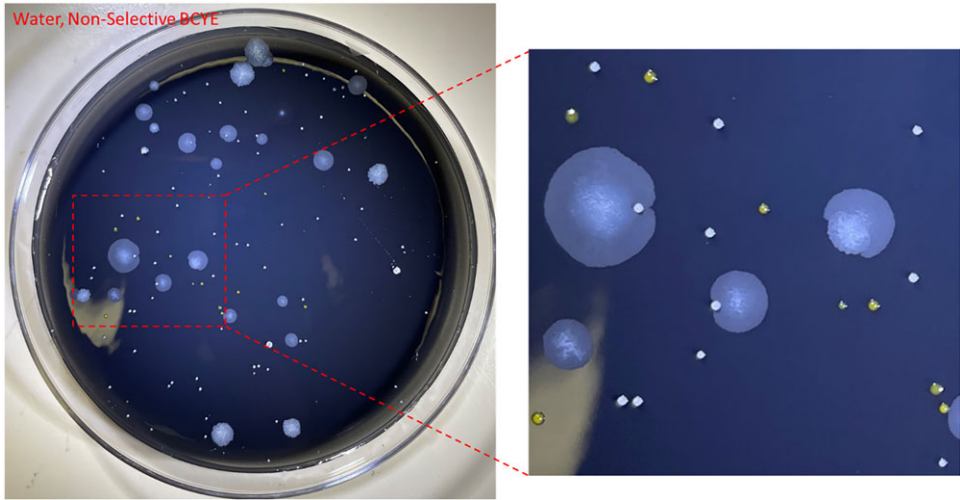


Figure 3. *Mycobacterium mucogenicum* isolated from a perioperative ice machine water source at hospital A. Environmental culture on buffered charcoal yeast extract (BCYE) non-selective agar shows *M. mucogenicum* colonies (large, slightly wrinkled, off-white) alongside other environmental flora (small, yellow or white, convex mucoid colonies).

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
2024 PT1	1	0	6	9	18	44484	24	44482	44411	4	44414	64280	64908	65266	65023	65024	64396	65022
2024 PT2	2	6	0	3	22	44488	18	44486	44405	8	44408	64276	64904	65262	65019	65020	64392	65018
2024 PT3	3	9	3	0	25	44491	21	44489	44408	7	44411	64279	64907	65265	65022	65023	64395	65021
2024 PT4	4	18	22	25	0	44482	22	44480	44409	20	44412	64288	64916	65274	65031	65032	64404	65030
2024 PT5	5	44484	44488	44491	44482	0	44486	6	91	44486	92	64717	63904	64260	64015	64016	64833	64020
2024 PT6	6	24	18	21	22	44486	0	44484	44403	26	44406	64282	64907	65265	65022	65023	64398	65021
Ice 1	7	44482	44486	44489	44480	6	44484	0	87	44484	88	64713	63900	64256	64011	64012	64829	64016
Ice 2	8	44411	44405	44408	44409	91	44403	87	0	44413	5	64634	63821	64177	63932	63933	64750	63937
Ice 3	9	4	8	7	20	44486	26	44484	44413	0	44416	64282	64910	65268	65025	65026	64398	65024
Water	10	44414	44408	44411	44412	92	44406	88	5	44416	0	64637	63824	64180	63935	63936	64753	63940
2020 PT1	11	64280	64276	64279	64288	64717	64282	64713	64634	64282	64637	0	3198	3797	3355	3356	166	3355
2020 PT1	12	64908	64904	64907	64916	63904	64907	63900	63821	64910	63824	3198	0	605	163	164	3350	163
2020 PT1	13	65266	65262	65265	65274	64260	65265	64256	64177	65268	64180	3797	605	0	444	445	3647	450
2020 PT2	14	65023	65019	65022	65031	64015	65022	64011	63932	65025	63935	3355	163	444	0	1	3205	6
2020 PT2	15	65024	65020	65023	65032	64016	65023	64012	63933	65026	63936	3356	164	445	1	0	3206	7
2020 PT3	16	64396	64392	64395	64404	64833	64398	64829	64750	64398	64753	166	3350	3647	3205	3206	0	3205
2020 PT3	17	65022	65018	65021	65030	64020	65021	64016	63937	65024	63940	3355	163	450	6	7	3205	0

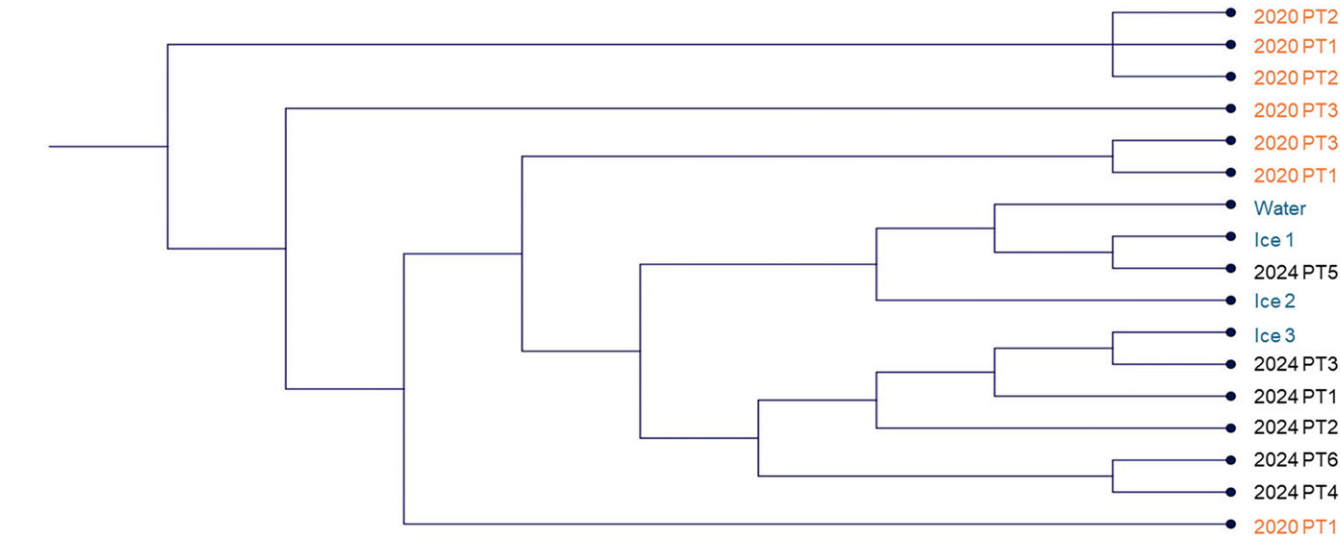


Figure 4. Genetic relatedness of clinical and environmental isolates. Clinical isolates from 2024, but not 2020, were closely related to environmental isolates collected during the same time in 2024, as demonstrated by whole genome Single Nucleotide Polymorphism (SNP) analysis. 2020 patient cultures grew multiple genotypes of *M. mucogenicum* from a single BAL procedure. A) SNP matrix showing the number of SNP differences between isolates. B) Phylogenetic tree based on the SNP matrix showing the clustering of genetically related isolates.

Table 2. Laboratory cost analysis

Cost type	Cost	Unit	Spent per isolate
CLS technologist	\$80	hour	3
Reagents:			
Middlebrook 7H11/7H11 selective biplate	\$6	plate	1
MALDI-TOF	\$0.50	spots	2
Total cost per isolate			\$247.00
Total cost incurred			\$88,426.00

them to be contaminants (44.2%) or not acknowledging the abnormal result (53.2%) in the EMR. Clinical outcomes of other pseudo-outbreaks due to NTM have resulted in unnecessary antibiotic use, complications from antibiotic use, unnecessary respiratory isolation, and prolonged hospital stays.^{13,15}

The largest impact to our institution from the pseudo-outbreak was on our microbiology laboratory and IP team, given that it cost nearly \$90,000 in microbiologist time and supplies, and nearly 100 hours of IP time, which may have diverted resources away from more clinically significant laboratory and quality improvement work. Additional institutional impacts included the \$10,000 cost of replacing the ice machine, cost and radiation from repeat imaging, patient anxiety regarding abnormal results, time spent by pulmonologist explaining abnormal results, and unnecessary ID consultations.

It is notable that in nearly all cases, growth of *M. mucogenicum* was initially seen on nocardia cultures, despite most bronchoscopies being performed for non-infectious workup such as cancer evaluation. Growth of rapidly growing NTM in BYCE medium and not in AFB cultures has been described in prior outbreak investigation of environmental sources of contamination.⁷ BYCE is less selective and contains more growth factors compared with conventional AFB media, which likely promoted growth of *M. mucogenicum* in our pseudo-outbreak. One study showed that a selective rapidly growing mycobacteria medium, which does not require decontamination of clinical specimens, isolated 100% of *M. mucogenicum* specimens compared with only 6.3% isolated by conventional AFB cultures.¹⁸ Diagnostic stewardship of nocardia and AFB cultures may have limited the pseudo-outbreak. Recent national guidelines have focused on diagnostic stewardship and recommend against routinely sending respiratory cultures when clinical suspicion for infection is low.¹⁹

The importance of NTM surveillance, in-person IP observations, and leadership engagement must be emphasized because our outbreak continued for four years after the initial investigation and recommendations against using non-sterile ice and water in 2020. We found that pulmonologists performing bronchoscopy at our institution were not aware of sterile saline syringes being placed in non-sterile ice tubs and denied this practice when interviewed in 2020 and in 2024. Current Centers for Disease Control guidelines do not recommend routine NTM surveillance, and only two states (Tennessee and Oregon) require hospital reporting of NTM infections.^{20,21} This lack of standardized monitoring may allow similar pseudo-outbreaks and true outbreaks to remain undetected. Some researchers have advocated for routine NTM surveillance as part of a water management program at healthcare facilities.^{2,14,22}

Limitations of our study include its retrospective design and reliance on EMR data which may have resulted in incomplete

capture of patient outcomes. We could not account for potential additional workup and treatment for *M. mucogenicum* that patients may have received at outside institutions. We were also unable to estimate the pseudo-outbreak’s effect on teams outside of Infection Prevention and Microbiology and therefore likely underestimated the event’s true institutional impact.

This large, four-year pseudo-outbreak of *M. mucogenicum* highlights several critical points for infection prevention and diagnostic stewardship. In line with other studies, our study underscores the risk of using non-sterile water and ice during bronchoscopy and shows that ice machines can serve as persistent reservoirs of NTM. Although such pseudo-outbreaks may have limited clinical impact, they impose substantial financial and operational burden on the healthcare system. Our findings also emphasize the need for robust surveillance and vigilance in infection control practices. Combining infection prevention interventions through staff education and ongoing surveillance of environmental mycobacterial species recovery on routine culture is essential for preventing similar pseudo-outbreaks.

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Competing of interests. All authors report no conflicts of interest relevant to this article.

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