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MAJOR ARTICLE

Remediation of Mucorales-contaminated Healthcare Linens at a Laundry Facility Following an Investigation of a Case Cluster of Hospital-acquired Mucormycosis

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Background. In an investigation of hospital-acquired mucormycosis cases among transplant recipients, healthcare linens (HCLs) delivered to our center were found to be contaminated with Mucorales. We describe an investigation and remediation of Mucorales contamination at the laundry supplying our center.

Methods. We performed monthly RODAC cultures of HCLs upon hospital arrival, and conducted site inspections and surveillance cultures at the laundry facility. Remediation was designed and implemented by infection prevention and facility leadership teams.

Results. Prior to remediation, 20% of HCLs were culture-positive for Mucorales upon hospital arrival. Laundry facility layout and processes were consistent with industry standards. Significant step-ups in Mucorales and mold culture-positivity of HCLs were detected at the post-dryer step (0% to 12% [$P = .04$] and 5% to 29% [$P = .01$], respectively). Further increases to 17% and 40% culture-positivity, respectively, were noted during pre-transport holding. Site inspection revealed heavy Mucorales-positive lint accumulation in rooftop air intake and exhaust vents that cooled driers; intake and exhaust vents that were facing each other; rooftop and plant-wide lint accumulation, including in the pre-transport clean room; uncovered carts with freshly-laundered HCLs. Following environmental remediation, quality assurance measures and education directed toward these sources, Mucorales culture-positivity of newly-delivered HCLs was reduced to 0.3% ($P = .0001$); area of lint-contaminated rooftop decreased from 918 m^2 to 0 m^2 on satellite images.

Conclusions. Targeted laundry facility interventions guided by site inspections and step-wise culturing significantly reduced Mucorales-contaminated HCLs delivered to our hospital. Collaboration between infection prevention and laundry facility teams was crucial to successful remediation.

Keywords. mucormycosis; Mucorales; healthcare linens; microbiologic surveillance; outbreak.

Mucormycoses are infections caused by fungi of the order Mucorales, which typically occur in persons with defects in immune function and other host defenses. Mucorales are widely distributed in the environment, including in soil, vegetation and compost, and spores are easily aerosolized and dispersed. Mucormycosis manifests as respiratory tract or disseminated infections (which generally stem from spore inhalation and carry high mortality rates), cutaneous infections (which stem from direct inoculation of spores and can be difficult to eradicate despite surgery and antifungal therapy), and gastrointestinal infections due to ingestion of spore-contaminated medication [1]. Healthcare outbreaks of mucormycosis are well recognized and ascribed to contaminated healthcare supplies and environmental reservoirs [2–4]. Outbreaks may be caused by a single or diverse Mucorales species.

Several investigations have established epidemiologic links between mucormycosis cases and patient exposure to healthcare linens (HCLs), and detected heavy Mucorales contamination of HCLs and HCL carts during nosocomial outbreaks [5–7]. In these studies, disease-causing environmental strains were not conclusively identified by surveillance cultures and phylogenetic analyses of whole genome sequence data; however, cases were no longer reported after offsite HCL facilities were changed [5, 7] or HCL cart cleaning protocols were revised [6]. In the Mucorales on Unclean Linen Discovery (MOULD) study, we demonstrated that freshly laundered HCLs were contaminated by Mucorales upon delivery to 47% (7/15) of major transplant and cancer centers in the United States [8]. At 20% of centers, >10% of arriving HCL articles were contaminated. The Centers for Disease Control and Prevention (CDC) recognize HCLs as potential sources of pathogenic fungi and bacteria, but deem the general risk posed by HCLs in healthcare settings to be low [9]. We have called for collaboration between medical and industry groups to better

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understand and mitigate risks that may be posed to patients by laundering, storage, and use of HCLs, in particular among immunosuppressed populations [8]. At present, there are no US federal regulations for HCL processing facilities. Optional third party accreditations are offered by trade organizations like the Healthcare Laundry Accreditation Council (HLAC) and the Textile Rental Services Association (TRSA), which certify facilities as providing "hygienically clean" HCLs based on self-reporting and nonevidence based definitions [10, 11]. In the MOULD study, HLAC or TRSA accreditation of a hospital's HCL agency was not associated with the likelihood of receiving items that were free from Mucorales [8].

Over an 11-month period from May 2015 through April 2016, we diagnosed 4 solid organ transplant (SOT) recipients at our center with likely healthcare-associated mucormycosis [12]. Patients were housed exclusively in 1 of 2 hospitals separated by a walkway traversing a city block, and they were infected with *Rhizopus microsporus* (n = 2), *R. arrhizus* var *delemar* (*R. delemar*, n = 1), or *Lichtheimia corymbifera* (n = 1). By October 2015, HCLs were identified as the likely source by the Infection Prevention (IP) team. Surveillance cultures of freshly laundered HCLs and carts immediately upon delivery to the medical center and at the offsite HCL processing facility supplying the center demonstrated extensive contamination by *Rhizopus*, *Lichtheimia* and other Mucorales. In contrast, Mucorales or other fungi were rarely recovered from cultures of hospital environments and supplies that were not associated with HCLs. Comprehensive core protein phylogenetic and global genome feature analyses of 72 clinical and environmental Mucorales strains revealed that *R. microsporus* infecting 2 patients in separate hospitals seven months apart were highly similar, suggesting a common source exposure [12]. The strains were most closely related to an HCL strain from the offsite facility, which was virtually identical in core genome but distinct by whole genome size and global protein content. All other clinical and environmental Mucorales strains were genetically distinct. No further healthcare-associated mucormycosis have been diagnosed in our program following multi-faceted IP interventions, which included temporary introduction of isavuconazole as antifungal prophylaxis and dedicated gamma-irradiated HCLs for SOT recipients pending investigation and remediation of potential sources of Mucorales-contaminated HCL at the agency. A detailed description of the epidemiologic investigation of cases and the multi-faceted IP interventions initiated in their aftermath both at the hospital and the agency will be presented in a future report. Several studies of mucormycosis outbreaks have given cursory descriptions of microbiologic surveillance and remediation at laundry facilities [5–7]. However, these studies have not provided detailed, step-by-step analyses of laundering processes. In one study, changes to an HCL cart cleaning protocol were discussed [6], but otherwise details on remediation methods were lacking. In the present report, we describe our systematic investigation of potential sources of HCL Mucorales contamination at the offsite laundry facility between October 2016 and January 2017, and our subsequent collaborative efforts to remediate causes of contamination.

METHODS

In-Hospital HCL Surveillance

We performed monthly cultures of freshly laundered HCLs directly upon arrival at our center from October 2016 to October 2019, using previously described methods [8]. Briefly, single Replicate Organism Detection and Counting (RODAC) agar plates (25 cm^2) with malt extract, lecithin, and Tween 80 were stamped 10 times at different locations on a given HCL article. Seven articles of 7 types of HCL were sampled (bath blanket, thermal blanket, fitted sheet, flat sheet, pillowcase, washcloth, patient gown). Therefore, 49 articles of HCL were cultured each month. RODAC plates were immediately sealed and incubated at 35°C.

HCL Facility Surveillance

A dedicated team (A. J. S., M. H. N.) made 5 site visits in October 2016 through January 2017. On the last 4 visits, they performed cultures at different stations of the laundering process. Articles of HCL were cultured using the 10-stamp RODAC method immediately post-washing/pressing, post-dryer removal, post-ironing/folding, pre-transport (i.e., awaiting delivery to the hospital), and upon hospital delivery. Cultures were incubated as above. Percentages of plates contaminated with fungi were compared between stations using Fisher exact test (significant $P < .05$).

Feedback and Remediation

Surveillance culture results and findings from visual inspections upon site visits were shared with HCL facility leadership during several meetings. A jointly devised remediation plan was initiated in February 2017. Subsequent monthly culture data from HCLs arriving at the hospital were shared with the HCL facility. All available Google Earth Pro (v. 7.3.2.5776, Google LLC) images were used to estimate area of lint accumulation on the facility's roof.

RESULTS

Baseline In-Hospital HCL Surveillance

Before remediation was initiated at the laundry agency in February 2017, 20% (19/95) of freshly laundered HCLs were culture-positive for Mucorales immediately upon arrival at our center (Figure 1).

Inspection of the Laundry Agency and HCL Processing

HCL facility layout was consistent with industry standards, including separation between soiled and clean areas (Figure 2).

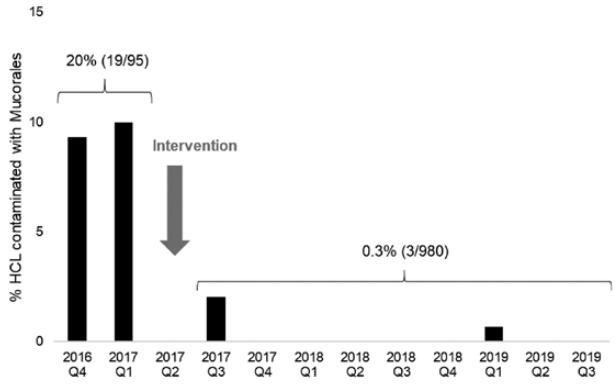

Figure 1. Cultures of healthcare linens immediately upon arrival at our center from the laundry facility. Percentage of articles that were culture-positive for Mucorales is on the *y*-axis. Timeline (by quarters) is on the *x*-axis. Remedial interventions were completed in second quarter 2017 (red arrow). Note the highly significant reduction in HCL Mucorales contamination after remediation ($P = .0001$). Abbreviation: HCL, healthcare linens.

Stepwise laundering processes and workflow were aligned with CDC and HLAC guidelines [11]. Soiled HCLs were brought into the facility in trucks, sorted, and deposited in tunnel washers. Trucks were sprayed with a disinfectant and swept to dry. Carts underwent automatic washing in a tunnel that was connected to the clean area, where they were sprayed with disinfectant and hand wiped dry. Following washing, HCLs were transferred to a press station for excess water removal and then moved by an electric lift to the dryer. The drying cycle lasted for approximately 20 minutes at 170°C. Following a 2-minute cool-down in the dryer using air brought in from the roof, HCLs were released onto a conveyor belt. At the end of the belt, articles of HCL were placed into clean bags. Bags were lifted to the ceiling and transported to the sorting area via a monorail system. Articles were manually sorted, pressed by flatwork ironers, and folded by an automated machine. Fully laundered and folded HCLs were placed into carts in a holding area where they awaited uploading onto trucks for transport to customers.

The first site visit by investigators in October 2016, which was scheduled a week in advance, revealed a clean, state-of-the-art plant. As had been noted during an initial investigation by IP staff immediately after the mucormycosis case cluster was recognized in fall 2015, the roof of the facility had considerable lint accumulation, especially surrounding the air ventilation (vent) system. The intake vents, which delivered unfiltered air into driers, were in close proximity to and facing exhaust vents, which carried air expelled from driers (Figure 3, top). Openings and internal surfaces of intake and exhaust air vents were covered with thick layers of lint; swab cultures of lint grew confluent Mucorales (*Syncephalastrum* spp.) and other molds (*Aspergillus niger*, *Curvularia* spp.) by 24 hours. The subsequent 4 site visits between November 2016 and January 2017 were unannounced. The plant had significant lint accumulation on the ceiling, indoor vents, and press and fold machines. At all visits, carts holding laundered and folded HCLs were uncovered as they awaited transport.

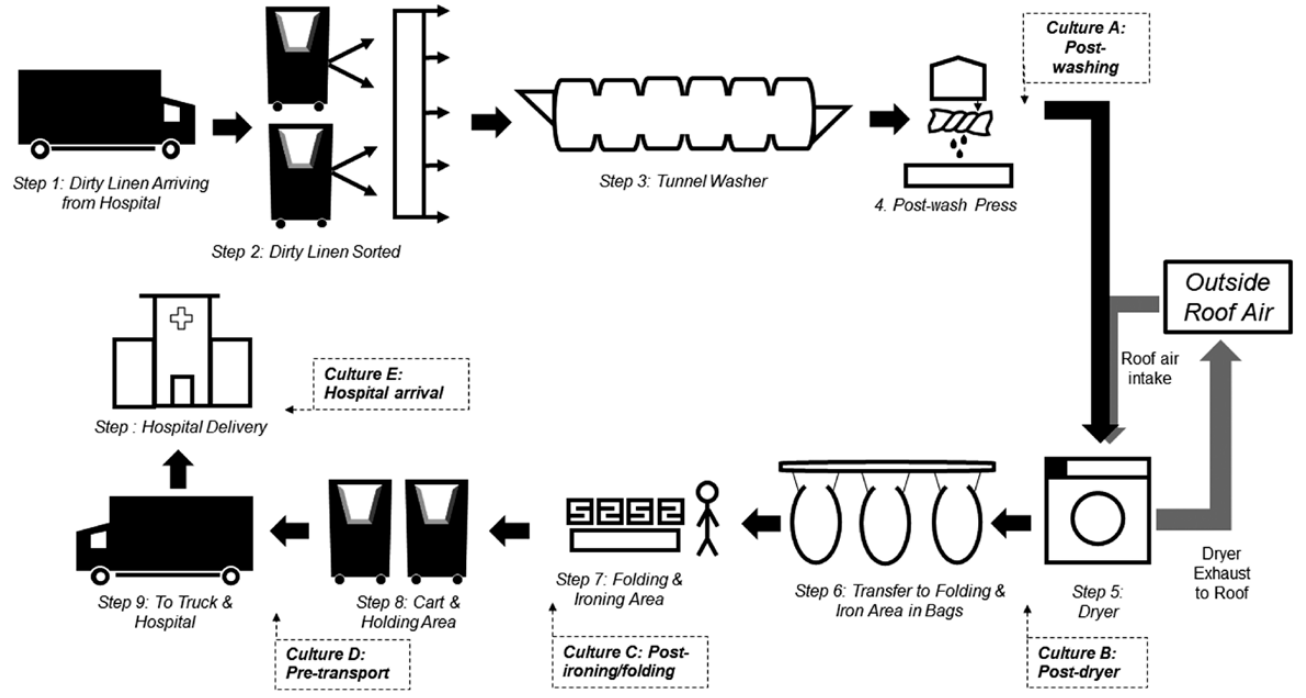

Figure 2. Step-wise healthcare linen processing at the offsite facility. Steps at which surveillance cultures were performed are shown within dashed boxes (culture A–E). Note that outside roof air was brought into driers via intake vents to cool down HCLs upon completion of the drying cycle (step 4). Air from driers was then recirculated to the roof via exhaust vents. Abbreviation: HCL, healthcare linens.

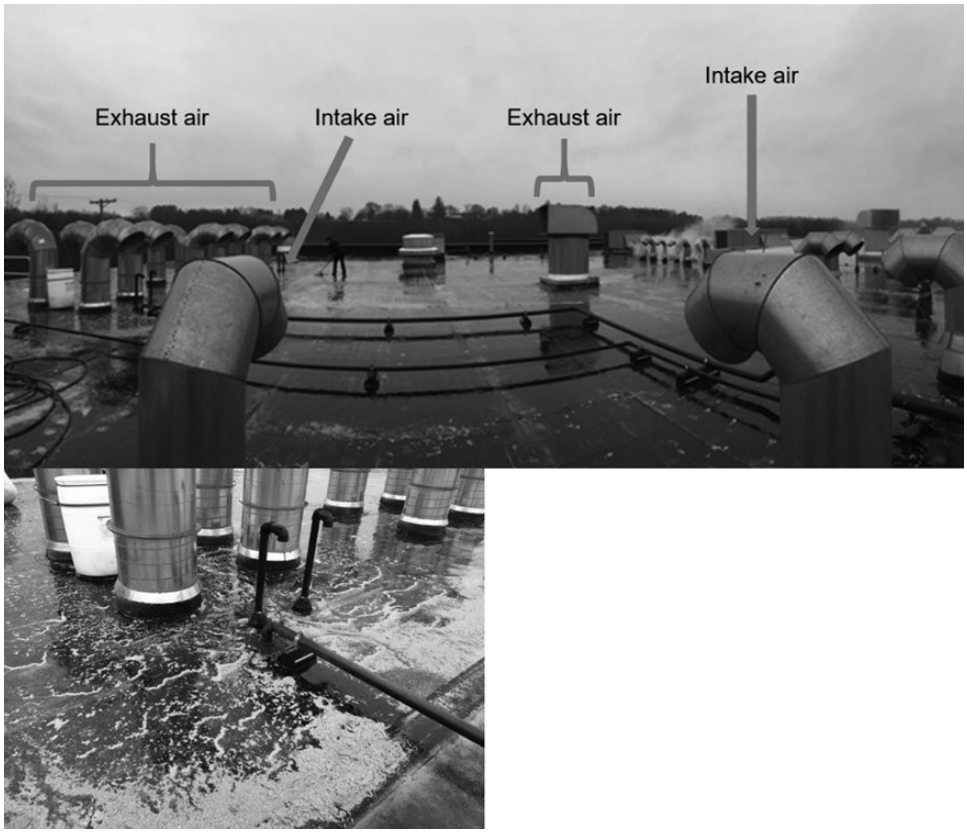

Figure 3. Layout of the laundry facility roof. Intake and exhaust air vents were facing one another (*top*). There was significant lint accumulation on the roof (appearing white in the photos), in particular surrounding intake and exhaust vents (*bottom*).

Surveillance and Remediation at the Laundry Facility

During the 4 unannounced visits, fungal cultures were systematically obtained from HCLs at 4 stations indicated in Figure 2 and immediately upon hospital delivery. There was a significant step-up in HCL fungal positivity from post-washing/pressing to post-dryer stations ($P = .01$ and .04 for all molds and *Rhizopus* spp., respectively); there was an additional nonsignificant increase in positivity from post-ironing/folding to pre-transport stations (Figure 4).

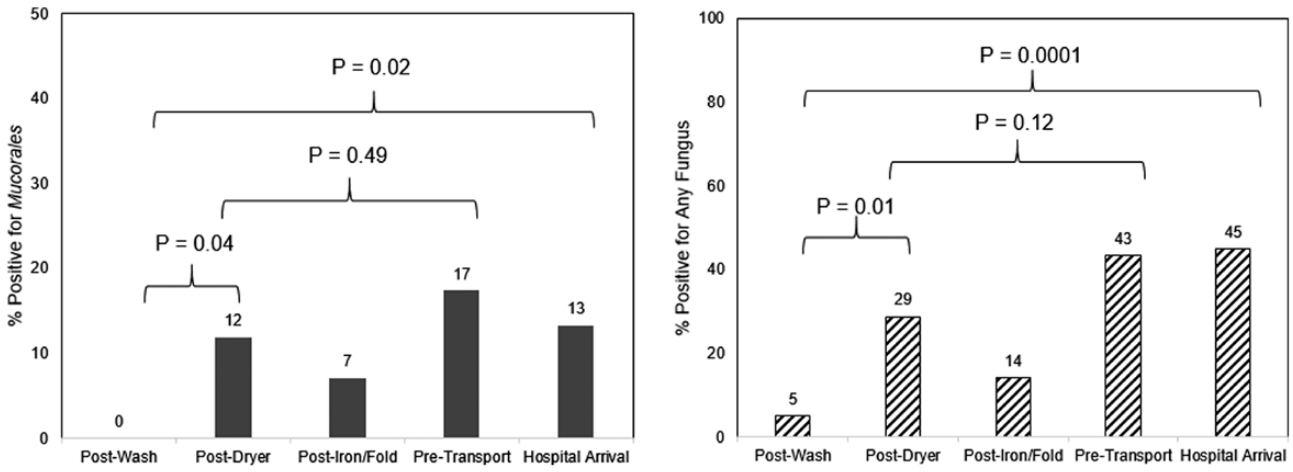

Figure 4. Fungal culture positivity of healthcare linens at steps in the laundering process. Results are shown for *Rhizopus* spp. (*left*) and any mold (*right*). Percentages of articles that were culture-positive at each step are presented on the *y*-axis as cumulative data over 4 unannounced visits to the facility. There were significant step-up in culture positivity between post-wash and post-dryer steps. Culture positivity was significantly higher immediately upon arrival at our hospital than at the post-wash step.

Molds and *Rhizopus* spp. were recovered from 45% and 13% of HCLs immediately upon delivery to our center, respectively, compared to 5% and 0% of HCLs at the post-wash station ($P = .0001$ and .02, respectively).

Remediation focused on the drying step, where HCLs were likely to be contaminated by Mucorales and other molds via intake air vents, and on the pre-transport step, where HCLs were stored in uncovered carts in an environment with generalized lint accumulation. Interventions were targeted to intake and exhaust vents, lint control on the roof and throughout the plant, and protection of HCLs held in carts prior to transport to healthcare facilities. Interventions included (1) placement of a large filter device around exhaust vents to catch lint; (2) movement of air intake vents away from exhaust vents; (3) frequent lint removal on the roof; (4) enhanced environmental cleaning with increased attention to and frequency of lint removal from floors, walls, and ceiling; (5) placement of plastic coverings over carts with freshly laundered HCLs; and (6) education on and assessments of adherence to HLAC and CDC guidelines [9, 11]. Costs of these undertakings were borne by the linen agency. In addition, the facility hired an additional employee for daily cleaning of equipment in contact with HCLs, 2 employees for overall facility cleaning, and an employee to perform daily audits on cleaning processes.

Post-Remediation Evaluations

Beginning 4 months after remediation strategies were instituted, we resumed monthly surveillance cultures of HCLs immediately upon arrival at our center [8]. Quarterly pooled HCL fungal culture results pre- and post-remediation are summarized in Figure 1. Over 27 months post-remediation through third quarter 2019, overall mean culture positivity of HCLs was 0.3% (3/980; $P = .0001$ vs pre-remediation 20% [19/95]). There were only 2 dates on which any HCLs were contaminated with Mucorales (*Rhizopus* spp.). On both dates, culture positivity was below the 10% threshold generally used to define HCLs as "hygienically clean" [8]. Periodic cultures of newly-delivered HCLs in 2020 were also negative for fungi (data not shown).

Prior to remediation, the estimated area of lint contamination around intake and exhaust vents on the laundry facility roof increased over 4 years from 0 to 918 m^2 (Figure 5). The area of contamination was reduced to 0 m^2 4 months post-remediation, the last time that a satellite image was available.

DISCUSSION

Here we report on our investigation of an offsite laundry agency servicing our center that had high levels of Mucorales contamination of HCLs. We pinpointed air vents, storage of washed and folded HCLs in uncovered carts, and lint accumulation on the roof and within the plant as likely sources of contamination. Environmental remediation, quality assurance measures, and

Figure 5. Satellite images of the laundry facility roof showing areas of lint accumulation, 2010–2017. Google Earth Pro (v. 7.3.2.5776, Google LLC) images were used to estimate area of lint accumulation on the facility's roof. Areas of accumulation appear in white, as marked below by dashed splines. Estimated areas of contamination appear beneath each image. Google Earth displays satellite and aerial photographs that are taken at specific locations on different dates and under different lighting and weather conditions. Photographs are sourced from different providers, such as state agencies, geological survey organizations, and commercial imagery providers. Images are typically updated every 1–3 years (https://blog.google/products/maps/how-do-satellite-images-work/). We used all publicly available images available at the time of submission. No images were available after September 2017.

education directed toward these sources was associated with marked and sustained reductions in Mucorales-contaminated HCLs delivered to our center. As of this writing, no further hospital-acquired mucormycosis cases have been diagnosed for 4 years following a multifaceted IP intervention that included the offsite remediation. To our knowledge, this is the first detailed description of a plant inspection, systematic step-by-step process evaluation, and successful remediation of microbial contamination at an HCL processing facility. The study is particularly notable for productive collaboration between a healthcare system-based IP team and a commercial laundry, and for long-term follow-up of the effectiveness of remediation efforts. As increased attention is paid to HCLs as potential sources of nosocomial outbreaks of infections by fungi and bacteria, our approach and experience may serve as a model for other IP programs and laundry facilities.

Three previously published investigations and attempted environmental remediations at HCL laundry facilities linked to nosocomial mucormycosis outbreaks have yielded mixed results. Surveillance cultures at laundries in 2 studies revealed Mucorales contamination of HCLs, environmental surfaces, equipment, air samples, and/or HCL carts [5, 7]. Specific sources of contamination were not identified in either study, but investigators suspected steps in laundering, handling, or storage between the end of laundering and hospital delivery, or a combination of process failures in laundering, plant hygiene, and transportation [5, 7]. Remediation was attempted at one of the facilities, but specific details were not provided and efforts were unsuccessful [5]. There was no mention of attempted remediation in the other study [7]. In both instances, no further mucormycosis cases were reported after hospitals changed HCL agencies. In the third study, the offsite facility was inspected, but surveillance cultures were not collected [6]. However, there was significant Mucorales contamination of HCL carts at the hospital. Contamination was significantly reduced and mucormycosis cases were no longer observed after regular and more rigorous cleaning of carts was implemented at the laundry agency. A review of outbreaks of fungal and bacterial infections attributed to HCLs found that contaminated washing equipment was the most commonly implicated source (accounting for 58% of outbreaks), followed by poor hygiene during storage of HCLs at hospital or laundry (33%), and transport from laundry to hospital (8%) [13]. As we demonstrated, the key to successful investigation of a laundry facility for microbial contamination in the setting of a nosocomial outbreak is systematic inspection of the plant and microbiologic sampling at each step of delivery, handling, and laundering processes.

Investigation of the laundry facility roof revealed air vents and lint collection as potential sources of fungal contamination. HLAC stipulates that hot and dry laundry in driers should undergo sufficient cool-down to enable personnel to handle articles safely [11]. We concluded that HCL contamination was most plausible at cool-down, because temperatures during drying were too high to sustain Mucorales growth. It is common practice for large commercial laundries to cool linens through intake of outside air. We found that unfiltered air was driven through an unclean intake ventilation system that was covered by thick layers of lint, cultures of which revealed heavy fungal growth. Substantial lint accumulation was also evident within exhaust air vents, which directly faced intake vents. Therefore, potential loops of recirculation were established from roof to driers to roof, within a fungus-contaminated ventilation system. Serial satellite images of the roof showed progressive accumulation of lint over a 4-year period prior to our mucormycosis case cluster. The clean HCL processing room, where freshly laundered and folded articles awaited transport to the hospital, also had ample accumulation of lint and dust, including on floor, ceiling, walls, and equipment. Carts holding HCLs in the processing room were not covered, allowing exposure to the environment. Our study, other investigations, and HLAC standards reiterate that lint and dirt control are crucial to hygienic HCL processing [5–7, 11]. Lint is a collection of fibers recovered from textiles like cotton or linen, which is enriched for cellulose that can serve as a nutrient source for Mucorales and certain other fungi [14, 15]. In warm and moist environments, lint provides an ideal medium for Mucorales to proliferate.

Findings of our site investigation, including results of surveillance cultures and plant inspection, were shared with laundry agency leadership. They implemented jointly agreed-upon, multifaceted remedial interventions, as detailed in the Results section. TRSA and HLAC certification guidelines encourage processing facilities to partner with IP staff and other hospital representatives to assure delivery of "hygienically clean" HCL that are "free of pathogens in sufficient numbers to cause human illness" [10, 11]. There are no scientifically validated definitions of "sufficient numbers" or pathogens that pose greatest risk to hospitalized patients [8]. Routine culturing of HCLs at hospitals or laundry facilities is not mandated in the United States, nor is it recommended by CDC [9]. Nevertheless, IP programs should understand HCL laundering processes relevant to their center, and the general state of HCL hygiene. Particular focus should be paid to HCL delivery to immunosuppressed patients.

Our study has several limitations. We did not perform stepwise culturing at the HCL facility post-remediation, nor did we culture HCLs at the hospital before they were returned to the laundry facility. However, step-ups in Mucorales culture-positivity of HCLs at the facility implicated distinct stages of the laundering process as likely sites of contamination, and long-term reductions in HCL contamination at our center speak to the effectiveness of remediation. Periodic surveillance cultures of newly delivered HCLs continue to be performed. Satellite images of the agency roof after September 2017 were not available to document sustained clearance of lint. Finally, we cannot definitively conclude that successful HCL facility remediation was responsible for the

subsequent absence of hospital-acquired mucormycosis in our program. Multifaceted IP interventions implemented in the aftermath of cases also included use of isavuconazole as Mucorales-active antifungal prophylaxis and gamma-irradiated HCLs in SOT recipients; [16] these practices were discontinued in 2018 and 2020, respectively. Periodic surveillance cultures of newly-delivered HCLs continue to be performed at our center.

In conclusion, our targeted remediation at a commercial laundry facility achieved significant and sustained reductions in Mucorales contamination of HCLs. The successful collaboration between a hospital epidemiology team and laundry leadership demonstrates the value of strong partnerships based on shared values for patient safety. We renew our previous call for increased collaboration between hospital epidemiologist, IP practitioners, clinicians, hospital administrators, industry leaders, and public health officials to develop reasonable standards for producing, testing, and certifying hygienically clean HCLs that balance patient safety, workflow, and costs [8].

Notes

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Potential conflicts of interest. C. J. C. has been awarded investigator-initiated research grants from Astellas, Merck, Melinta, and Cidara for studies unrelated to this project, served on advisory boards or consulted for Astellas, Merck, the Medicines Company, Cidara, Scynexis, Shionogi, Qpex, and Needham & Company, and spoken at symposia sponsored by Merck and T2Biosystems. M. H. N. has been awarded investigator-initiated research grants from Astellas, Merck, Mayne Pharma, and Pulmocide for studies unrelated to this project and has served on advisory boards or consulted for Astellas, Mayne Pharma, and Pulmocide. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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