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Short Communication

Airborne Microorganisms from Waste Containers

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Abstract: In physician's offices and biomedical labs, biological waste is handled every day. This waste is disposed of in waste containers designed for holding red autoclave bags. The containers used in these environments are closed hands-free containers, often with a step pedal. While these containers protect the user from surface-borne microorganisms, the containers may allow airborne microorganisms to escape via the open/close mechanism because of the air current produced upon open/close cycles. In this study, the air current was shown to be sufficient to allow airborne escape of microorganisms held in the container, including *Aspergillus niger*. However, bacterial cultures, such as *Escherichia coli* and *Lactococcus lactis* did not escape. This may be due to the choice of bacterial cultures and the absence of solid waste, such as dust or other particulate matter in the waste containers, that such strains of bacteria could travel on during aerosolization. We compared these results to those obtained using a re-designed receptacle, which mimimizes air currents, and detected no escaping microorganisms. This study highlights one potential source of airborne contamination in labs, hospitals, and other environments that dispose of biological waste.

Key words: Biological waste containers, Biological safety, Sterilization, Airborne microorganism, Air quality

Recent well-publicized cases of deadly infections traced back to hospitals, physician's offices, and nursing homes have amplified public concern about airborne microorganisms. Some of these concerns have highlighted sanitary issues, exposed overuse of antibiotics, and raised a number of questions about the sources of the contamination. The primary pathogenic microorganisms that have received press attention with regards to airborne transmission include *Aspergillus flavus, Gram-negative bacilli, Neisseria meningitidis, Serratia marcescens, Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae,* and *Tubercle bacilli*[1, 2]. These are microorganisms that reside in skin, hair, clothes, and the indoor environment. They can be found in the air ducts of buildings that have not been maintained. These organisms can be highly dangerous, especially in a hospital setting, but also in clinical laboratories and even research labs. Therefore, reducing the exposure of individuals to these disease-causing pathogens is of concern to the medical profession. This increased awareness led to several research studies focused on identifying more effective air filtration devices[3, 4] and other remediation approaches[5–7]. However, very few studies have focused on identifying the bioaerosol source or determining how these microorganisms move through the environment[8], both key factors when considering indoor air quality and limiting exposure of individuals to hazardous bioaerosols. Bioaerosols are likely to result from any number of environmental perturbations, including biofilm

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disruption (such as from the bottom of a sink), airflow modifications (such as would result from the changing of bedsheets), or improper waste disposal, among other possible sources.

Many studies on indoor air quality have focused on farming or food production environments[9–15]. Only a few studies have reported on clinical or research laboratory environments. For example, recent work from Kim *et al.*[1] examined airborne contaminants in five general hospitals in Korea. They tested for 14 different varieties of bacteria and fungal cultures at various locations (including the lobby, the intensive care unit, and the surgical ward) in the hospitals. They found that in the main lobby of the hospitals, the levels of airborne microorganism contamination were the highest, followed by the surgical ward and the intensive care unit, with the lowest levels of contamination reported. The bacterial strains they detected in significant concentrations were *Staphylococcus* spp., *Micrococcus* spp., *Corynebacterium* spp., and *Bacillus* spp. In terms of fungal strains, they identified *Cladosporium* spp., *Penicillium* spp., and *Aspergillus*. In fact, they found that the indoor concentration of airborne bacteria and fungi was higher than the outdoor concentration. Many of the species identified were not identified in the outdoor areas surrounding the hospital, indicating that some of the microorganisms were arising from inside the hospital itself. While not all of the microorganisms identified were pathogenic, some strains of these genre are disease causing, and their significant circulation in the medical environment should be a concern. It was specifically stated in the Kim *et al.* study that the cleanliness of the hospital was not in question; but that the movement of individuals and the ventilation efficiency may assist in spreading airborne contamination.

A second recent study[16] also examined bioaerosols in the hospital setting. This study focused primarily on the microbial diversity in hospital rooms. They hypothesized that aerosol-based bacteria were arising from biofilms in the sink drains. They found that some of the microorganisms detected were both in the air and in the sink drains, such as *Pseudomonas aeruginosa*, *Cladosporium* sp., *Aspergillus* sp. and *Chryseobacterium* sp. However, they found that some of the airborne species identified (*Staphyloccus epidermis, Staphylococcus hominis, Micrococcus luteus, Bacillus* sp.) were not identified in the sink drains, indicating that they were arising from other sites in the hospital room, such as from bedsheets or waste containers. Other environmental studies, such as those performed in Di Giulio *et al.*[17], examined the native air quality in university laboratories, and found that many of the natural organisms from the outdoor environments would enter the building, however, the transport of specific microorganisms commonly used in bacteriological laboratories was not examined in depth.

One potential source of bioaerosols that is not usually cited in studies is the waste container used to dispose of biological hazardous waste. Items such as swabs, gloves, dressings, bed pads, tubing, instrument covers, etc. are often disposed of in a biohazard waste container. In addition, upon bed changes, the used bedding occasionally can be placed in one of these containers. With the exception of sharps containers, many of these containers are simply modified garbage cans. They are hands-free to prevent the user from needing to touch the container, which limits direct hand-to-mouth transmission of pathogens. In addition, users often wash their hands after using a waste disposal unit, and follow good laboratory practices with regards to disposal (using practices such as turning gloves inside out during disposal).

In a hands-free closed container, the opening and closing motion of the lid creates an air current. This air current is palpable if you stand in front of a heavy-duty metal step pedal (hands-free) waste container, such as those used in some offices. From a fluid dynamics perspective, when the lid of a container is opened, a transient flow condition is introduced. This transient flow will result in a three-dimensional air vortex. This air vortex will be split, with some of the air (and particulates/droplets traveling in the air) transported away from the opening of the container, while some will be directed back into the container. The pattern and flow of the air vortex will be heavily dependent upon the container dimensions, the shape and weight of the container lid, and the velocity of lid opening[18]. We hypothesize that this air vortex may allow aerosols to form from within the container, potentially contributing to the airborne microorganism population. We expect that microorganisms can travel via dry aerosilization or through microdroplet dispersion that would be transmitted through an open/close cycle of this type of container. In the present study, we examine the potential for creating an aerosol of airborne microorganisms, including agar and broth-grown bacteria and fungal cultures, from a typical biohazard waste container. We compare this waste container to a waste container designed to limit the escape of aerosols by a simple action of twisting the inner liner bag closed upon lid closing, eliminating the vortex effect observed with a traditional step-pedal waste container.

All chemicals used in media preparation were obtained

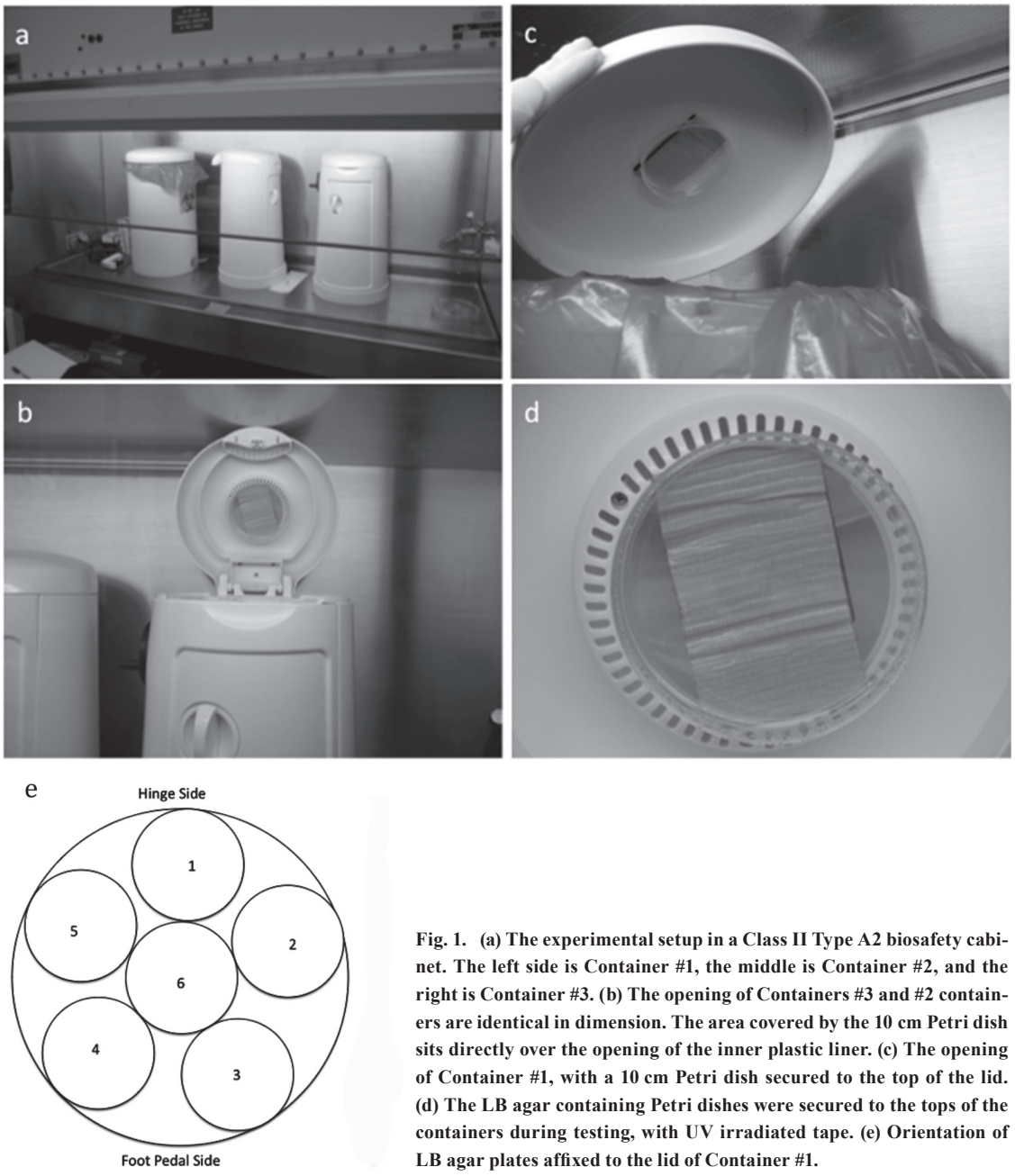

Fig. 1. (a) The experimental setup in a Class II Type A2 biosafety cabinet. The left side is Container #1, the middle is Container #2, and the right is Container #3. (b) The opening of Containers #3 and #2 containers are identical in dimension. The area covered by the 10 cm Petri dish sits directly over the opening of the inner plastic liner. (c) The opening of Container #1, with a 10 cm Petri dish secured to the top of the lid. (d) The LB agar containing Petri dishes were secured to the tops of the containers during testing, with UV irradiated tape. (e) Orientation of LB agar plates affixed to the lid of Container #1.

from Fisher Scientific (Pittsburgh, PA), unless otherwise noted. Luria-Bertani (LB) agar (10 g bacto-tryptone, 5 g yeast extract, 10 g NaCl, 15 g agar) was added to 1 L of ddH_2O and autoclaved at 121 °C for 15 min. Agar plates were prepared by pouring ~15 ml of the molten LB agar into each 10-cm bacteriological grade polystyrene Petri dish (VWR, West Chester, PA). No special agarose additives were used. LB broth (5 g yeast extract, 10 g peptone, 10 g NaCl) was added to 1 L of double distilled water (ddH_2O) and autoclaved at 121 °C for 15 minutes. Liquid cultures were prepared by adding 5 ml of the LB broth to 15 ml polypropylene centrifuge tubes (BD Biosciences, Bedford, MA). All media were stored at 4 °C prior to use. These media are basic formulations that are not specific to all of the organisms tested in this study, but the formulations have been used in routine culture and maintenance of the organisms we examined.

Three waste containers were used for testing. In Fig. 1a, the left container is a traditional step pedal (hands-free) waste container designed for red autoclave bag use (denoted as "Container #1"). The middle container is an experimental control waste container (to contain no intentionally

Table 1. Number of microbiological cultures used in the experiments

Experiment	*E. coli* (10 cm)	*L. lactis* (10 cm)	*E. coli* (15 ml)	*L. lactis* (15 ml)	*E. coli* (liquid, in ml)	*L. lactis* (liquid, in ml)	*A. niger* (10 cm)
1	8	8	5	5	10	10	
2 *A. niger* added	8	8	5	5	10	10	2
3 container load increased	8	8	5	5	10	10	

10 cm refers to agar plates seeded with microorganisms. 15 ml refers to closed tubes of liquid microbiological cultures. Liquid in ml refers to open liquid that was introduced directly to the containers. Therefore, in experiment 1, eight (8) 10 cm plates of ***E. coli*** growing on LB agar were placed in the waste container, as well as the listed numbers of ***L. lactis*** and other culture types.

seeded microorganisms), marketed as the Arm & Hammer diaper pail from Munchkin Products (North Hills, CA, USA) (denoted as "Container #2"). The third container, on the right, is similar to the middle container, but redesigned to possess a mechanism that allows for slow opening of the aperture of the waste bag (denoted as "Container #3"). This container is currently in the prototype stage of development and not available on the market. Container #1 was 55 cm tall and 30 cm in diameter, with a waste bag opening of 30 cm. Containers #2 and #3 were of identical dimensions: 56 cm tall and 25 cm in diameter, with a 13 cm opening into the bag liner[19–23]. The bags used in the study were either red autoclave bags (VWR, West Chester, PA, USA) or the bags designed for the commercially available Container #2.

Container #1 was purchased directly from Discount Office Supplies (Columbus, WI, USA) (Product Number SAF9683WH). This container was lined with a typical lab red autoclavable "biohazard" bag. Container #2 is typically used to dispose of soiled baby diapers and possesses a rack and pinion gear that will twist the inner bag shut upon lid closing; the inner bag will remain shut when the lid opens[19–23]. Container #3 was designed to twist the inner liner bag closed upon lid closing; but upon opening, the bag will untwist via a mechanism controlled by a handcrank on the side of the pail. The handcrank simulated what would be an automatic opening of the inner bag upon opening the lid with a foot pedal. This prototype design can eventually lead to a foot pedal (hands-free) controlled motion.

Three experiments were performed during December 2010 and January 2011. To prepare the containers for testing, the three containers were set up adjacent to one another in a Class II Type A2 biosafety cabinet (Nuaire, Plymouth, MN) (Fig. 1a), with a minimum inflow velocity of 100 ft/min. The airflow in the biosafety cabinet was allowed to run continuously during the experimental analysis. The pails were wiped down, inside and out with 70% ethanol, then UV irradiated inside and out for 48 h to sterilize.

In the first experimental design, Containers #1 and #3 were loaded with equal amounts of the microbiological cultures shown in Table 1. *E. coli* refers to *Escherichia coli* [strain DH5α (Invitrogen, Grand Island, NY)] a Gram-negative bacterium that is commonly used in laboratory genetic engineering. *L. lactis* refers to *Lactococcus lactis* bacteria (ATCC 7962), a strain of Gram-positive bacteria that was formerly included in the genus Streptococcus Group N1. As a control, Container #2 was kept free of microbiological cultures.

To prepare the cultures for deposition into Containers #1 and #3, 10 cm dishes loaded with LB agar were streaked with glycerol stock cultures of the microorganisms and allowed to grow overnight at 37 °C in a humidified incubator. The 15 ml tube cultures were seeded in LB broth (Fisher Scientific) and allowed to grow overnight in a 37 °C shaker. In addition, 20 ml of LB broth infected with *E. coli* and *L. lactis* (10 ml each, grown overnight at 37 °C) were poured directly into the containers. Following introduction of the cultures to the pails, the tops and outer rims of the containers were wiped thoroughly with ethanol and allowed to rest overnight. The light in the biosafety cabinet and the airflow were left on during this 24 h period. The room was approximately 29 °C during the 24 h period.

To test for microorganisms that were possibly aerosolized in the air currents from the opening and closing of the containers, a typical daily cycle was performed. We estimated that, on average, a waste container is opened roughly every 30 minutes over the course of a 10-h day. Using asceptic conditions, while the airflow in the biosafety cabinet was running, we cycled through 20 openings and closings of each pail. To collect any microorganisms that were traveling with the air currents, we secured sterile LB agar Petri dishes to the tops of the pails (Figs. 1b-d). Each waste container received one Petri dish securely fastened to the center of the top of the lid. Following the open/close cycles, the Petri dish lids were replaced, and the dishes were asceptically removed from the tops of the contain-

ers and placed in a 37 °C humidified incubator. Any Petri dish that was removed in a non-asceptic fashion (i.e., the interior touched part of the waste container) was not considered valid for testing. In addition, sterile swabs were used to sample the outer portions of all three containers, including the tops of the lids, rims of the containers, and the outer circumference. These swabs were then streaked onto LB agar plates.

Following the first experimental design, two modifications were made. The first modification (Table 1) was to add an additional microorganism to the containers, in the form of *A. niger,* in addition to fresh cultures of *E. coli* and *L. lactis*. *A. niger* refers to *Aspergillus niger* (ATCC 6275), a common Aspergillus species of fungi. The *A. niger* was cultured on standard LB plates for several days prior to the start of the experiment to ensure sporulation under these moderately inhibitory growth conditions. In Containers #2 and #3, the diameter of the Petri dish covered 60% of the plastic liner opening. To cover approximately the same relative area on the lid of Container #1, we adhered six 10 cm Petri dishes (coverage = 65% of total) to the inside of the lid (Fig. 1e). Testing was carried out in an identical manner as described above.

The second modification (Experiment 3) was to increase the volume of the waste being added to the bags. We sterilized 3 autoclave bags filled with unused LB agar plates, tied them securely shut, and UV irradiated the surface overnight. These were then added to the bottoms of the plastic liners in each container. The purpose of this was to fill the volume without increasing the microbial load. Fresh bacterial cultures were then added to the containers. This increased the volume being held by the container to –75% capacity. Experimental testing was then carried out as in Experiment 1, with the LB agar plate configuration as shown in Fig. 1e.

The microorganisms tested were *Escherichia coli, Lactococcus lactis, and Aspergillus niger*. The strains chosen here are laboratory strains commonly used for genetic engineering; they were chosen on the basis of availability and general safety. Only the second experiment contained *A. niger*, but all experiments received cultures of *E. coli* and *L. lactis*. *A. niger* was limited to only the second experiment, and this experiment supported our hypothesis: *A. niger* growth was detected during the microorganism aerosil analysis. The third experiment was specifically designed to determine if container overloading would impact bacterial aerosol formation, therefore *A. niger* would have confounded our results through competitive growth.

The first experiment failed to support our hypothesis.

Table 2. Cultures from Experiment 2 of waste container testing

Plate #	E. coli Container #1/#2/#3	*L. lactis* Container #1/#2/ #3	*A. niger* Container #1/#2/#3
1	–/o/o	–/o/o	–/o/o
2	–/o/o	–/o/o	+/o/o
3	–/o/o	–/o/o	+/o/o
4	–/o/o	–/o/o	–/o/o
5	–/o/o	–/o/o	–/o/o
6 (or center of container lid)	–/–/–	–/–/–	+/–/–
Inner Lid of Container	+/–/–	–/–/–	+/–/–
Outer Surface of Container	–/–/–	–/–/–	–/–/–

Cultures were collected from Petri dishes containing LB agar. (−) indicates no growth, (+) indicates positive growth. The plate numbers refer to the numbering scheme in Figure 1. The containers were also swabbed for potential microorganism currents settling on the surfaces. (o = Not applicable, due to container lid geometry: only 1 Petri dish fits at the tops of Containers #2 and #3, and is consistent with the placement of Plate #6).

No growth was observed in the Petri dishes after 24 h of incubation post-experiment for any of the waste containers tested. We attributed this to a number of potential causes, including: minimal Petri dish lid coverage on Container #1 (only one Petri dish was affixed to the center, covering –15% of the area), microorganism choice (the organisms chosen were readily available and safe (considered biosafety level 1)), and the volume of waste filling the inner lining of the bags (less than 25% of the bag filled with our waste load, meaning that the air current would need to travel at least 40 cm to the top of the lids as the open/close cycles are performed). To overcome all but the last of these deficiencies, we redesigned our testing setup.

This second experimental design confirmed our hypothesis. Cultures from Container #1 were positive for *A. niger* 24 h after the open/close cycle testing (Table 2). Specifically, out of the six LB agar plates, plate numbers 2, 3, and 6 (Fig. 1e) all were positive for growth. These cultures were allowed to continue to grow for 36 more h, at which point the positive growth was 100% confirmed.

In addition to the growth observed from the open/close cycles with Container #1, the containers were swabbed with sterile swabs for examination of any microorganisms that traveled in the air current and attached to other surfaces. Of these, only one exhibited positive growth. The swab from the inside of the inner lid from Container #1 tested positive for *A. niger* growth and bacterial growth (either *E. coli* or *L. lactis*, species not confirmed). The outer surfaces of the containers appeared to not collect any airborne microorganisms in this experiment. Even after

60 h, all of these plates were negative for microbiological growth. This is an interesting observation, and one that was unexpected. We were expecting the microorganisms (in this case, *A. niger*) to be able to travel through the air and settle on the surrounding surfaces. This was not observed, an observation likely attributable to the biosafety cabinet fans that were left running during the experimental testing. The fans likely altered the trajectory of the airborne microorganisms.

Containers #2 and #3 were tested in an identical fashion to the Container #1, and no growth was observed from any of the LB agar plates cultured, either from swabs of the inner lid or from the LB agar plate affixed to the top of the lid. It should be noted that in Containers #2 and #3 the LB agar plate during testing was in physical contact with the inner bag liner (due to the container design and experimental setup). While no growth was expected from our negative control (Container #2), Container #3 also appears to limit airborne microorganisms by virtue of the bag twisting closed upon the lid closing.

While the second experimental design confirmed that certain microorganisms (*A. niger*) would be able to escape in an airborne fashion from a traditional hands-free biosafety container, only a limited bacterial aerosol was collected by our experiment. Therefore, the volume of waste in the containers was increased, holding bacterial load constant. To do so, we added pre-sterilized waste to the bottoms of the bags in each case. Fresh bacterial cultures were added to the containers (Table 1), and testing proceeded in a similar manner to Fig. 1e, using the larger surface area coverage for collection. The containers were ~75% full. However, even with this setup, no bacterial growth was observed on the test LB agar plates, indicating that the bacterial cultures chosen for this experiment were not aerosolized from our waste containers.

Container #3 prevented the escape of bioaerosols from the waste container. This effect is attributable to multiple factors. A primary factor is that the open/close mechanism is slow. This slow mechanism likely leads to a smaller vortex effect. In addition, the waste bag itself is closed upon the open/close cycle. The waste bag is slowly untwisted either during or after opening, limiting the potential for significant air currents. Therefore, this type of container may be more effective at containing bioaerosols, specifically those organisms, such as *A. niger*, that are easily aerosolized. We believe that the failure of *A. niger* to escape Container #3 is primarily attributable to the lack of significant air current from the inner contents of the waste bag. The waste bag was twisted closed and opened slowly upon the opening of the container. This delayed opening of the waste container bag limits the air current arising from the inside of the container, thus limiting the aerosolization of *A. niger* spores.

We surmise that the failure of the bacterial cultures to become bioaerosols from Container #1 is due to two primary factors. The first is the nature of the bacteria chosen for the study. The organisms used are readily available and well-controlled, however, they may become aerosolized only when attached to other particulate matter, such as skin or dust. Both the *E. coli* strain and *L. lactis* are currently used as test organisms for genetic engineering undergraduate labs at Lehigh University. We did not have access to many of the bacterial strains identified in the Kim *et al.*[1] and Gilbert *et al.*[16] studies. In addition, we did not have access to particularly contagious organisms, such as Methicillin Resistant *Staphylococcus aureus* (MRSA) or *Streptococcal pyogenes*. Several studies have shown that MRSA can become airborne[24–27]. MRSA has been isolated from sinks, floors, and sheets in hospitals where particular emphasis was placed on the recurrent airborne nature of the bacteria, resulting from movement in the rooms. This movement can be likened to the air currents produced by opening and closing a biohazard waste container (as well as other air perturbations, such as the changing of sheets). Therefore, we believe that our experiments represent a preliminary analysis that lays the groundwork for a more comprehensive study with airborne microorganisms of a significant type and variety. It is the goal of the authors to raise awareness of this potential source and to seed new research ideas in this area.

The testing space airflow was the second limiting factor for this study. The study was performed in an aseptic environment. The only sterile field that was available during the 6 weeks we worked on this project was a biosafety cabinet. The biological safety cabinet used in the study has a 100 feet/minute intake velocity, with a recirculation ratio of 70% (30% is exhausted to the room). Thus, the airspace within the cabinet is surrounded by negative pressure. This negative pressure in the biosafety cabinet may have altered our microbiological sampling by increasing the resistance to droplet flow. As noted in a 2006 paper from Morawska[8], the fate of droplets in the indoor environment is key to the spread of microorganisms. In our study, the airflow was left on during testing and between tests, which may have removed any microorganisms that were escaping beyond our LB agar plates affixed to the tops of the inner lids. This removal also could have limited any collection that might have been positive for microorganisms

on the outside of the adjacent pails. Finally, given that our samples were primarily in the solid state − i.e., on agar plates, we did not have true droplet flow. Droplet analysis will be the subject of future research.

However, even with the limited biological organisms available and our chosen testing site, we have successfully demonstrated that the traditional hands-free waste container is capable of producing aerosols of microorganisms. The aerosolization of *A. niger* is a significant finding, and we believe that this effect will translate to additional microorganisms, both fungal and bacterial, depending upon the aerosolization parameters and the contents of a waste container (i.e., if the container also contains particulate matter). Given the increase in hospital infections and the many safeguards that have been taken, it makes sense to examine our waste handling as a potential contributing factor in these cases. Our study demonstrates that the current waste handling procedures may not be suitable for handling waste safely, especially in the case of easily aerosolized organisms. The handling of linens and other items in clinical environments is also of consideration, as soiled linens are often placed in containers similar to those examined here. The next step will be to expand upon this study with pathogenic organisms in an environment similar to a hospital room; that would be considered a sterile field with regards to airflow. In addition, we will further develop the design of a hands-free biohazard waste container that limits the escape of airborne microorganisms during the simple act of opening and closing the container. Finally, we will design a fluid dynamics model that could simulate the vortex effect expected during the open/close cycle of a traditional step-pedal waste container; this will accompany future research to build a model-based hypothesis.

This study demonstrates that biohazardous waste disposal is potentially a source of airborne contamination in laboratories and clinical settings. While significantly more work is required to fully demonstrate that hazardous microorganisms can be caught in a bioaerosol during the open/close cycles of a standard biohazardous waste disposal container, the question of how waste handling contributes to bioaerosol transport in indoor environments is highly related to clinical indoor air quality and the prevention of disease. Bioaerosols are often transported on a solid medium, such as dust, soil, skin, etc. While our study did not specifically examine these bioaerosol dynamics, they warrant a future investigation, as many bacteria may only become aerosolized when attached to particulates. In future work, we will investigate the aerosolization of pathogenic microorganisms in an airflow setting similar to that experienced in a typical clinical or research environment. In conclusion, we encourage an exploration of waste disposal techniques and how these might impact airborne microorganism transport in facilities that handle biological waste.

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