Comparison of methods to control floor contamination in an animal research facility

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The authors evaluated the effectiveness of adhesive mats, contamination control flooring, and shoe covers in decreasing the presence of microbial agents on animal holding room floors and footwear. Swab samples taken from animal holding room floors after the use of each product were compared with samples taken from rooms after no products were used. Swab samples were also taken from the heels and soles of the footwear of animal care staff before and after use of each product. The use of contamination control flooring or shoe covers significantly reduced the amount of organic material (as indicated by ATP levels measured by a luminometer) present on floors. Bacterial and ATP contamination of footwear was significantly lower after the use of shoe covers than after the use of adhesive mats or contamination control flooring, and the use of shoe covers led to a greater decrease in contamination before and after use than did use of either of the other two products. Although shoe covers were superior to both adhesive mats and contamination control flooring for decreasing contamination of animal room floors and footwear, facilities must take into account the contamination control standards required, the cost of the product, and the labor and time associated with product use when deciding which contamination control practices to implement.

A variety of products and practices are available to mitigate introduction of unwanted microbial agents in animal research facilities. To minimize floor contamination introduced on footwear, facilities may require use of dedicated footwear, direct disinfection of footwear or use of products designed to eliminate or decrease floor contamination such as adhesive mats, disinfectant mats, shoe covers and contamination control flooring. The few published studies regarding the efficacy of these products to control contamination of footwear have yielded mixed results, however.

Use of adhesive mats to control contamination of footwear has been described primarily in hospital environments[1–3]. Although one study indicated that use of adhesive mats may reduce footwear contamination, it is also suggested that mats lose their adhesive capacity quickly and may then be a potential source of footwear contamination[4]. Another study indicated that the use of adhesive mats may be of no benefit[5]. Studies evaluating the efficacy of disinfection baths and mats in animal hospitals and laboratory animal facilities suggest that they are effective in reducing footwear contamination[6–9], although one study that was carried out in an equine hospital did not find any benefits of disinfection mat use[10].

Most studies evaluating the effectiveness of shoe covers also have taken place in hospital environments. One study indicated that shoe covers might reduce bacterial contamination of operating room floors[11], but another indicated that shoe covers do not affect bacterial counts on operating room floors[12]. When shoe covers were used by health care providers

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treating bone marrow recipients, there was no decrease in time to antibiotic use or duration of treatment of the patients[13]. A few studies regarding the efficacy of shoe covers in laboratory animal facilities have been published. One study indicated that using shoe covers may reduce floor contamination in laboratory animal housing facilities[6]. In contrast, a different study indicated that use of shoe covers is of questionable value and may compromise bioexclusion[14]. In light of the results of these and other studies, the efficacy of shoe cover use remains inconclusive[15–17].

Publications regarding the use of contamination control flooring are limited. One published study comparing the efficacy of contamination control flooring and adhesive mats in a pharmaceutical facility used for microbiological testing purposes indicated that contamination control flooring was more effective at controlling footwear- and wheel-borne microorganism contamination of critical environments[18]. Another study showed the effectiveness of contamination control flooring for decreasing airborne contamination[19]. No studies of contamination control flooring use in facilities housing laboratory animals have been published to our knowledge.

Currently, we do not routinely use practices to control footwear contamination in our rodent housing facilities except in select areas where a higher level of protection is desired, including rooms where mice and rats need to be maintained free of all excluded rodent pathogens such as *Helicobacter* spp. and mouse norovirus. Reasons for not using footwear contamination control practices include product costs, time required for maintenance and use, and lack of published data related to product efficacy. In addition to the scarcity of information available about the use of shoe covers, adhesive mats and contaminated control flooring for reducing floor contamination in laboratory animal facilities, there is a lack of reliable reports comparing the efficacy of these products. Here we compare the efficacy of contamination control flooring, adhesive mats and shoe covers in reducing the contamination of flooring and the footwear of personnel in our animal facility.

METHODS

Animal facility

This study was carried out in a facility that houses mice and rats used for biomedical research[6]. The facility is arranged in suites, each consisting of an anteroom with corresponding animal holding and associated procedure rooms **(Fig. 1)**. Individual suites house either mice or rats in individually ventilated caging (Allentown, Inc., Allentown, NJ). Cages are changed in laminar flow cage changing stations (Allegard Dual Access Small Animal Cage Changing and Transfer Station, Nuaire, Plymouth, MN).

Animal care staff members wear dedicated safety toe footwear when working in the facility. Investigators and their staff members are not required to wear dedicated footwear. Animal room floors in the facility are swept and mopped at the end of each day, except on weekends or holidays. At the time of the study, cleaning personnel used Ful-Trole 64 disinfectant cleaner (850 parts per million or 2 oz per 1 gal water; Multi-Clean, Shoreview, MN) for floor sanitation.

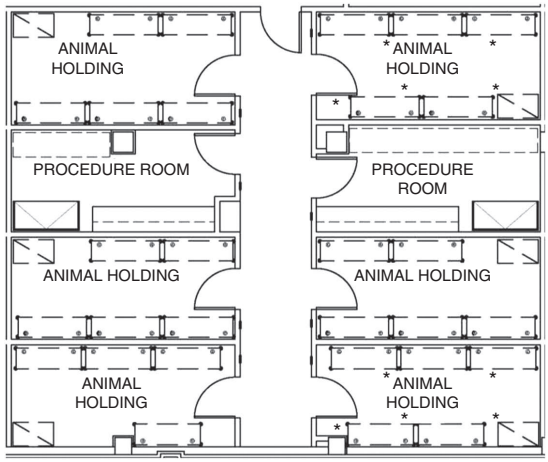

FIGURE 1 | Diagram of animal facility suite. Asterisks denote sampling locations.

Floor contamination control products

Adhesive mats (Clean Room Sticky Mats, American Floor Mats, Rockville, MD), measuring 24 in × 36 in, feature a tacky surface that sticks to debris on the bottom of footwear. The mat has multiple sheets that can be removed as necessary. Mats were placed ~3 ft inside the suite adjacent to the entrance door.

Shoe covers (Verona Safety Supply, Inc., Madison, WI) were made of water-resistant, but not leak-proof, polypropylene and spunbound with a non-slip tread. Shoe covers were supplied on a rack outside each suite entrance. Individuals entering the suite were instructed to place shoe covers over their footwear as they entered the suite and then disinfect their gloves. Individuals were required to discard their shoe covers at the suite entrance just before exiting the suite.

Contamination control flooring (Dycem Limited, Warwick, RI) was installed just inside the entrance to each of the three suites after the studies involving adhesive mats and shoe covers were completed. The manufacturer claims that this product will attract and collect more than 99% of particles (0.2–100 μm) from the contact surface of shoes or wheels via electromagnetic forces. In addition, the product contains a silver-based, proprietary antimicrobial agent that kills microorganisms that come into contact with its surface and may remove up to 75% of airborne contaminants (http://www.dycem-cc.com/biomaster.php). A 6 ft × 10 ft

section of the flooring, consisting of a mat with a polymeric covering, was adhered to the floor 3–4 ft inside the suite and adjacent to the door. We cleaned the flooring approximately 30 min before sample collection, in accordance with manufacturer recommendations, to minimize the possibility of previous contamination affecting study results and to assure the flooring was dry before samples were collected.

We carried out a total of four separate sampling trials, one testing each contamination control product and one in which no products were used, that were separated by at least 30 d. Each trial included six animal holding rooms in three suites (two rooms per suite). For each trial involving a product, all individuals entering any of the three suites used the product being tested beginning 2 weeks before sample collection. We collected samples from the floors of each of the six animal holding rooms using collection swabs (PocketSwab Plus, Charm Sciences, Lawrence, MA). We sampled five locations, measuring 3 in × 3 in, in each room for a total of 30 samples in each trial. Samples were collected by swabbing the entire sampling location in a circular motion.

In addition to collecting samples from animal holding room floors, we also obtained samples from the footwear of animal care staff members (n = 10) before and after the use of each product using collection swabs (PocketSwab Plus, Charm Sciences, Lawrence, MA). Each participant wore the same shoes while using each of the three products. We identified a specific location, with a diameter of ~1 in, on the center of the heel and on the sole of each participant's right shoe from which to collect the samples. For the adhesive mat and contamination control flooring trials, each participant stepped on the mat with his or her right foot, making sure the entire foot came into contact with the mat. Participants were only allowed to step on the contamination control flooring in locations that we visually identified as clean and not having been stepped on by another participant. Samples were collected from the heel and sole immediately after foot placement and before the participant took any additional steps. After use by a participant, adhesive mat sheets were removed (providing an unused sheet for the next participant). For the shoe cover trial, individuals placed a shoe cover on their right foot and the heel and sole were sampled immediately. The shoe cover was not allowed to contact the floor prior to sampling. After use by a participant, shoe covers were discarded.

Contamination measures

We evaluated microbiological load in the samples by culturing the swab samples on Replicate Organism Detection and Counting (RODAC) plates (Pharmacal Research Laboratories, Inc., Naugatuck, CT) containing trypticase soy agar and polysorbate 80 (refs. 20–23). We incubated the plates at 37 °C for 48 h and then counted colony-forming units (CFU) on each plate.

We evaluated the amount of organic debris in the samples using an ATP-based monitoring system (PocketSwab Plus, Charm Sciences, Inc., Lawrence, MA)[20,22–28]. We analyzed the swabs using a luminometer (novaLUM, Charm Sciences, Inc., Lawrence, MA) in accordance with the manufacturer's instructions. The amount of ATP present in each of the samples was measured in relative light units (RLU).

Statistical analysis

We used statistical software (Prism version 5.0; GraphPad Software, Inc., La Jolla, CA) to carry out our statistical analyses. We compared the contamination of the animal room floors, as measured by bacterial load or amount of ATP, between the trials using repeated measures analysis of variance (ANOVA). We calculated the mean number and percent change in bacterial load and amount of ATP at the two different locations on participants' footwear before and after use of each product use, and we carried out a two-tailed, paired t-test for statistical comparisons. Differences were considered significant when $P < 0.05$.

RESULTS

There were no significant differences in bacterial counts on RODAC plates swabbed with samples from the animal holding room floors with use of any of the products. Mean bacterial counts were lowest in the shoe covers trial (2.20 ± 0.4828 CFU) and highest in the control trial (3.367 ± 0.5435 CFU). Bacterial counts were lower in the contamination control flooring trial (2.633 ± 0.7482 CFU) than the adhesive mats trial (3.1 ± 0.5082 CFU; **Fig. 2a**).

Mean organic loads on the animal holding room floors were significantly lower when contamination control flooring or shoe covers were used (16,980 ± 3,070 RLU and 15,869 ± 4,074 RLU, respectively) than

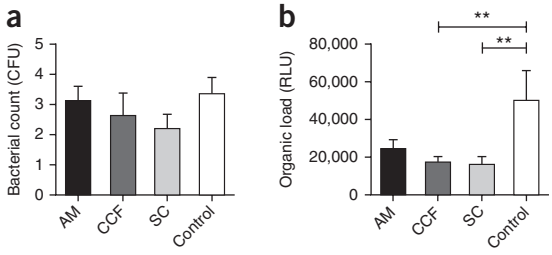

FIGURE 2 | Contamination of animal room floors. (**a**) There were no significant differences in bacterial counts (CFU) in the animal holding rooms with use of any of the products. (**b**) Use of contamination control flooring and of shoe covers significantly reduced organic loads (RLU) in the animal holding rooms compared with use of no products (**P < 0.05). AM, adhesive mats; CCF, contamination control flooring; SC, shoe covers. Error bars represent ± s.e.

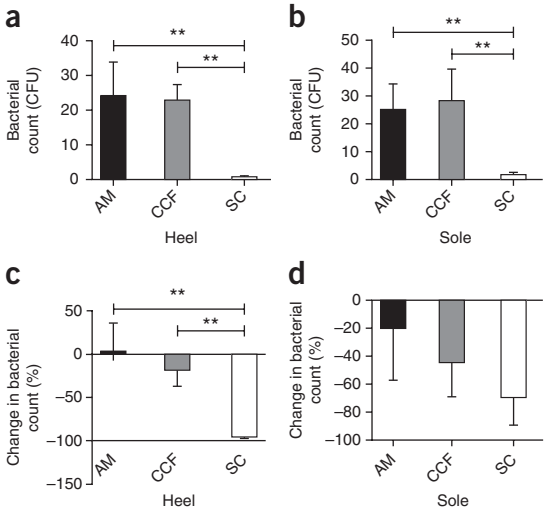

FIGURE 3 | Bacterial contamination (CFU) of footwear. Mean bacterial counts from the heels (**a**) and soles (**b**) of footwear were significantly lower after the use of shoe covers than after the use of adhesive mats or contamination control flooring (**$P < 0.05$). (**c**) The decrease in bacterial load on the heels of footwear was significantly greater when shoe covers were used than when adhesive mats or contamination control flooring were used (**$P < 0.05$). (**d**) There was no significant effect of using any of the products on the bacterial load on the soles of footwear. AM, adhesive mats; CCF, contamination control flooring; SC, shoe covers. Error bars represent ± s.e.

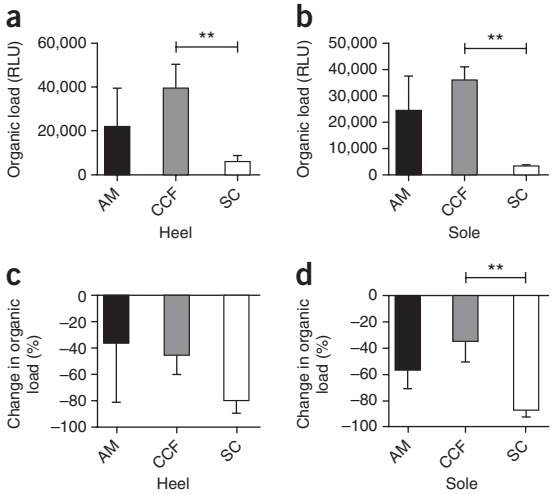

FIGURE 4 | Organic debris (RLU) on footwear. Mean organic load on the heels (**a**) and soles (**b**) of footwear were significantly lower after the use of shoe covers than after the use of contamination control flooring (**$P < 0.05$). (**c**) There was no significant effect of using any of the products on the organic load on the heels of footwear. (**d**) The decrease in organic load on the soles of footwear was significantly greater after the use of shoe covers than after the use of contamination control flooring (**$P < 0.05$). AM, adhesive mats; CCF, contamination control flooring; SC, shoe covers. Error bars represent ± s.e.

when no products were used (50,035 ± 15,732 RLU; $P < 0.05$; **Fig. 2b**). The mean amount of ATP was slightly lower in the shoe covers trial than in the contamination control flooring trial. Contamination control flooring and shoe covers were more effective than adhesive mats (24,077 ± 4,961 RLU) at decreasing organic load on animal holding room floors.

Mean bacterial counts on RODAC plates swabbed with samples from the heels (**Fig. 3a**) and soles (**Fig. 3b**) of footwear were significantly lower after the use of shoe covers (0.8 ± 0.2906 CFU and 1.5 ± 0.8596 CFU, respectively) than after the use of adhesive mats (24.2 ± 9.713 CFU and 24.9 ± 9.320 CFU, respectively) or contamination control flooring (22.9 ± 4.677 CFU and 28.1 ± 11.34 CFU, respectively; $P < 0.05$). There was no significant difference in the mean bacterial counts on heels or soles of footwear after use of adhesive mats versus contamination control flooring. These results suggest that the use of shoe covers results in less bacterial contamination of footwear than does use of adhesive mats or contamination control flooring.

We evaluated the percent change in bacterial load on the heels and soles of footwear before and after use of each product. The decrease in bacterial load on the heels of footwear was significantly greater when shoe covers were used (–94.85 ± 2.013%) than when adhesive mats (2.881 ± 32.93%) or contamination control flooring (–18.35 ± 18.83%) were used ($P < 0.05$; **Fig. 3c**). There was no significant decrease in bacterial load on the soles of footwear after using any of the products (**Fig. 3d**), though the decrease in bacterial load was slightly greater after shoe cover use (–69.17 ± 20.12%) than after use of adhesive mats (–19.26 ± 37.87%) or contamination control flooring (–44.37 ± 24.47%). There was no significant difference in the decrease in bacterial load on the heels and soles of footwear after use of adhesive mats versus contamination control flooring, but use of adhesive mats resulted in the smallest decreases in bacterial load on both parts of footwear. These results suggest that shoe covers are the most effective of the products we tested for decreasing bacterial contamination on footwear and that contamination control flooring is only slightly more effective than adhesive mats.

Mean organic loads on the heels (**Fig. 4a**) and soles (**Fig. 4b**) of footwear were significantly lower after the use of shoe covers (6,117 ± 3,003 RLU and 2,950 ± 625.4 RLU, respectively) than after the use of contamination control flooring (39,729 ± 11,147 RLU and 35,767 ± 5,077 RLU, respectively; $P < 0.05$). There were no significant differences in mean organic loads on either part of footwear after the use of adhesive mats versus contamination control flooring, though organic loads on the heels and soles of footwear were slightly lower after use of adhesive mats (22,037 ± 17,697 RLU and

24,064 ± 13,279 RLU, respectively) than after use of the contamination control flooring.

We evaluated the percent change in organic load on the heels and soles of footwear before and after use of each product. There was no significant effect of using any of the products on the organic load on the heels of footwear, though shoe cover use resulted in the largest decrease in organic load (–80.18 ± 9.539%; **Fig. 4c**). The decrease in organic load on the soles of footwear was significantly greater after the use of shoe covers (–86.85 ± 5.628%) than after the use of contamination control flooring (–34.65 ± 15.89%; $P < 0.05$; **Fig. 4d**). Use of adhesive mats resulted in a greater decrease in organic load on both the heels and soles of footwear (–35.69 ± 45.6% and –56.37 ± 14.73%, respectively) than did use of contamination control flooring (–45.17 ± 14.69% and –34.65 ± 15.89%, respectively). These results suggest that shoe covers are more effective than contamination control flooring for decreasing the organic debris on footwear and that adhesive mats are slightly more effective than contamination control flooring for decreasing the organic load on footwear.

DISCUSSION

We designed this study to evaluate the efficacy of three products to reduce microbial contamination of footwear and floors in laboratory animal facilities: adhesive mats, shoe covers and contamination control flooring. We included adhesive mats and shoe covers because these products are commonly used in institutions that house laboratory animals. We also considered including additional products, such as disinfectant mats. One study indicated that disinfectant mats were as effective as shoe covers in preventing contamination of animal holding room floors[6]. We decided against including disinfectant mats in this study, however, because the mats often become soiled, resulting in chemical residues on facility floors[6].

None of the products we evaluated led to significantly less bacterial contamination of animal holding room floors. Contamination control flooring and shoe covers were superior to adhesive mats, however, in reducing organic debris on animal holding room floors. Shoe covers were superior to both adhesive mats and contamination control flooring for decreasing contamination of footwear. Use of adhesive mats and use of contamination control flooring led to similar decreases in the amount of bacterial organisms and organic debris on footwear of animal care staff.

We anticipated that the use of shoe covers would lead to the lowest bacterial and organic contamination of the animal holding room floors and of footwear. Shoe covers are placed over potentially contaminated footwear and presumably provide a physical barrier to transmission of microbes and organic debris from footwear to the floor. In contrast, adhesive mats and contamination control flooring are intended to remove potential contaminants from the footwear before it comes in contact with the floor.

In our study, adhesive mats and contamination control flooring were of comparable efficacy for the removal of bacterial and organic debris from footwear, whereas the use of contamination control flooring, but not adhesive mats, resulted in significantly less organic debris on animal holding room floors compared with the use of no products. This difference in efficacy between adhesive mats and contamination control flooring in reducing organic debris on animal room floors may be attributed to the fact that contamination control flooring attracts airborne particles and microbes, decreasing airborne contamination[19]. Exposure to animal allergens is an occupational health concern in facilities housing rodents[29]. Because contamination control flooring can decrease airborne contamination, it may potentially decrease airborne animal allergens as well. This possibility warrants further study.

We decided to use bacterial load to evaluate contamination because we found no evidence in the literature to indicate an advantage in using fungal, parasitic or viral contamination as a means to evaluate disinfection efficacy[6]. One limitation to this approach is that other microbial agents also may be of concern to facilities housing laboratory rodents. In addition, we evaluated contamination by measuring ATP, which is commonly used in laboratory animal facilities as a rapid indicator of cleaning and sanitization efficacy[20,22–28]. One potential drawback to ATP evaluation is that disinfectants used in laboratory animal facilities often leave residues on surfaces that may interfere with ATP detection[23]. Because each measure has its drawbacks, we felt that using both methods to evaluate contamination would give us the best overall assessment of contamination and product efficacy.

When interpreting the results of our evaluation of footwear contamination, one must consider the amount of organic debris on footwear prior to product use. The participants in our study wore the same shoes throughout the study but not all shoes sampled were dedicated specifically for facility use. It is possible that the soles of some shoes were grossly contaminated, overwhelming the microparticle attraction capabilities of the adhesive mats and contamination control flooring. These products may be more efficacious in facilities where employees wear shoes dedicated for facility use.

The use of shoe covers does have potential drawbacks compared with the use of adhesive mats or contamination control flooring. Shoe cover use requires time; on average, it takes an individual 15-28 s to don shoe covers[6,14]. Individuals may contaminate their hands or gloves when placing shoe covers over their footwear, and contaminated gloves in turn can act as

fomites during routine husbandry procedures. In our personal experience, shoe covers do not form a tight seal around all footwear, perhaps depending on the type of footwear used or the size and style of available shoe covers. As a result, shoe covers may produce a 'bellows' effect during walking, which may cause pathogenic agents on shoes to become aerosolized. One study showed that the type of shoe covers used potentially affects aerosolization of pathogenic agents[16]. Another study indicated that shoe cover use resulted in no contamination of rodent caging in animal holding rooms[14]. Additional studies are warranted to determine whether potentially pathogenic agents on shoes may become aerosolized with shoe covers use, as this may present a contamination risk to research animals.

Use of adhesive mats is relatively straightforward and cost-effective compared with use of the other products tested but also presents potential drawbacks. Sheets become soiled over time and must be periodically removed, to prevent buildup of debris from footwear, which takes up employee time. A study in our facility found that the time required to replace an adhesive mat was ~60 s (K.P.A., unpublished observations). Additionally, adhesive mats may adhere to the wheels of equipment moved across its surface, forcing caretaking staff to spend additional time separating the adhered mat and wheels. Finally, employees may contaminate their gloves or other personal protective equipment during sheet removal.

There are advantages and disadvantages to use of contamination control flooring as well. Compared with the other two products, contamination control flooring has a much higher initial cost, which depends on the number of installation locations and surface area required, and requires professional installation. Once the product is installed, however, it requires little maintenance: It can be cleaned with a sponge mop followed by use of a squeegee to wipe the surface, and standard floor cleaning agents can be used. Minimal additional time (~5 min) is required for cleaning contamination control flooring during daily routine floor maintenance (data not shown). In addition, according to the manufacturer, if properly maintained the product may last up to 4 or more years after installation.

Although shoe covers were superior to both adhesive mats and contamination control flooring for decreasing contamination of animal room floors and footwear, each of these products has different costs and benefits. Facilities must take into account the acceptable level of contamination, labor and time associated with product use, and purchase price when deciding what products to use. Additional studies are warranted to further evaluate the efficacy of these and other commercially available products for preventing footwear and floor contamination in laboratory animal facilities.

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COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests. A representative of Dycem Limited installed the contamination control flooring in the animal holding suites used in the study. Dycem Limited received no compensation from the Medical College of Wisconsin for the products or services rendered.

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