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Reaerosolization of Spores from Flooring Surfaces To Assess the Risk of Dissemination and Transmission of Infections

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The aim of this study was to quantify reaerosolization of microorganisms caused by walking on contaminated flooring to assess the risk to individuals accessing areas contaminated with pathogenic organisms, for example, spores of *Bacillus anthracis*. Industrial carpet and polyvinyl chloride (PVC) floor coverings were contaminated with aerosolized spores of *Bacillus atrophaeus* by using an artist airbrush to produce deposition of $\sim 10^3$ to 10^4 CFU · cm^{-2}. Microbiological air samplers were used to quantify the particle size distribution of the aerosol generated when a person walked over the floorings in an environmental chamber. Results were expressed as reaerosolization factors (percent per square centimeter per liter), to represent the ratio of air concentration to surface concentration generated. Walking on carpet generated a statistically significantly higher reaerosolization factor value than did walking on PVC ($t = 20.42$; $P < 0.001$). Heavier walking produced a statistically significantly higher reaerosolization factor value than did lighter walking ($t = 12.421$; $P < 0.001$). Height also had a statistically significant effect on the reaerosolization factor, with higher rates of recovery of *B. atrophaeus* at lower levels, demonstrating a height-dependent gradient of particle reaerosolization. Particles in the respirable size range were recovered in all sampling scenarios (mass mean diameters ranged from 2.6 to 4.1 μm). The results of this study can be used to produce a risk assessment of the potential aerosol exposure of a person accessing areas with contaminated flooring in order to inform the choice of appropriate respiratory protective equipment and may aid in the selection of the most suitable flooring types for use in health care environments, to reduce aerosol transmission in the event of contamination.

After a deliberate release or natural contamination of an indoor environment with a suspected high-consequence biological pathogen (e.g., *Bacillus anthracis*), there is a requirement to undertake sampling to determine the agent and its concentration and distribution. Until sampling has been undertaken, the risks cannot be fully evaluated, and operations must be undertaken with a high degree of caution. Hence, to ensure that sampling personnel remain safe during the task, sampling is undertaken by operators wearing high levels of personal protective equipment (PPE), such as particle masks and Tyvek suits or even air-fed suits incorporating full-face respirators. The wearing of PPE to ensure that a complete barrier is made between the operator and the environment often means a hot, humid, and restrictive immediate working environment for the operator during sampling. Similarly, careful removal of contaminated PPE following sampling is a time-consuming process involving a thorough application of high-level disinfectants for a specific contact time, to ensure that no cross-contamination of the operators, or those assisting them, occurs during the removal of PPE. This significant PPE requirement and the training required to employ it safely reduce the number of personnel capable of carrying out sampling and increase the amount of staff training, time, and cost of the sampling exercise, thus ensuring that the release has a greater impact.

Floors are rarely identified as hand touch sites, but deposited material could be reaerosolized as people walk or move equipment across a floor. This reaerosolization potentially could create human exposure either via the respiratory route or by contamination of clothing followed by contact transmission. Although foot traffic has been shown to affect biological aerosol concentrations during sampling (1), the effect of human activity on the reaerosolization of bacteria from flooring has been only partially characterized (2). It has been postulated that normal human activities such as walking and sitting may contribute to 25% of indoor air particle dust concentrations (3). Human activities that resuspend house dust have been identified as a potential cause of the "personal cloud" effect, along with the observation that levels of personal exposure are often higher than indoor concentrations (4). Brauer et al. measured the personal exposure of a subject walking around a small dormitory room and found that the personal cloud was more pronounced when the person was walking than when the subject was sedentary (5). Ferro et al. found that pushing a vacuum cleaner, with the vacuum cleaner turned on or off, increased human exposure to coarse particulate matter ~20 times compared with background concentrations (6).

Therefore, there is evidence that human activities affect the reaerosolization of particles not only from an individual's own body and clothing but also from environmental surfaces such as floors. In the event of a malicious occurrence or an outbreak of disease leading to a contaminated environment, the perception of an exposure risk may lead to areas being evacuated. In such cases, entry may be restricted to personnel wearing personal protective equipment, until an accepted decontamination procedure has been undertaken and the area is made safe. In the event of a malicious event, it is to be assumed that a person entering a "hot

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zone" would be clean of the pathogen of interest. Therefore, reaerosolization from the contaminated environment would be a considerable means of transmission of infection to a person in such a situation.

The objective of this study was to quantify reaerosolization from two commonly used flooring surfaces contaminated with pathogens to better understand the risks of exposure to personnel, thus allowing more effective risk assessments to be undertaken.

MATERIALS AND METHODS

Bacterial preparations. A primary, high-spore-concentration solution of *Bacillus atrophaeus* (NCTC 10073) was prepared by the PHE Production Division under pharmaceutical conditions (7). Appropriate dilutions from these stock solutions were prepared in sterile distilled water to achieve the desired spore concentrations (ca. 4×10^6 CFU · ml^{-1}) for aerosolization and stored at 4°C. All assays were performed via serial dilution onto Trypticase soya agar (TSA; bioMérieux, France), followed by incubation for 18 to 24 h at 37°C.

Flooring materials. Polyvinyl chloride (PVC) flooring (Polyflor 2000 PUR) and carpet material (close weave of synthetic cut-pile fibers, 6 mm deep, on a 2-mm-deep compactly woven backing, in 50-cm by 50-cm tiles) were used in this study (NC Flooring, Frome, United Kingdom).

Artist airbrush. A Star 06XT299 airbrush (ca. 200-ml reservoir; RS Components, Corby, United Kingdom) was selected as the method of contamination of floor coverings, by spraying the spores suspended in water (ca. 10^6 spores/ml) onto the flooring material at a distance of 30 cm with an air pressure of 30 lb/in^2. The spores were deposited wet by impaction of the airbrush spray cone directly onto the vertical flooring surface. The volume dispensed by the airbrush was ca. 50 ml per min, but as not all of the sprayed volume landed on the flooring, the volume dispensed was not used as a measure of the amount of spores deposited on the surfaces. An artist airbrush was chosen on the basis of previous work by Levy et al. (8) employing a pen-sized model, scaled up to a larger size for speed of application. After a preliminary study, the Star airbrush was found to rapidly provide an even distribution of colonies on a TSA plate.

Surface samples. Water-soluble tape (catalog number 5414; 3M Industrial, MN, USA) was used to take surface samples by applying 5-cm lengths of tape to surfaces for ~40 s. A gloved finger was pressed onto the tape adhered to the flooring surfaces, ensuring that good contact was made with the fibers of the carpet and the surface of the PVC. Tape samples were transferred into 10 ml sterile distilled water, dissolved for 30 min at 37°C (±2°C), and mixed vigorously via vortexing before the assay was performed. Before reaerosolization studies began, tape was investigated as a surface sampling method. By directly seeding the water-soluble tape with a known amount of spores and assaying the recovered liquid, the impact of the tape on spore viability was validated. The sampling efficiency of the tape on carpet and PVC was also assessed by comparing rates of spore recovery by tape sampling of spray-contaminated flooring to those by direct immersion of sections of spray-contaminated flooring in 10 ml sterile distilled water. Following a single run, a single tape sample was taken within 10 min of the end of the activity, while room ventilation was switched on.

Environmental chamber. The study took place in an environmental chamber (4 m by 2.5 m by 2 m; 20 m^3), which had an optional flow of horizontal clean air supplied through a bank of HEPA filters, at a rate of 150 air changes/h. Air entered the chamber from grills on the rear right wall ca. 40 cm from the floor and was drawn out via a large bank of outlet grills on the entirety of the left wall. This enabled the room to be flushed with clean air to remove airborne organisms within 10 min, allowing a number of activities, and the sampling thereof, to be repeated over relatively short periods. Once the flooring surfaces had dried following contamination, they were affixed to the test chamber floor by using double-sided tape and used repeatedly for the duration of the study, which lasted 22 days for carpet and 19 days for PVC, from the first run to the last.

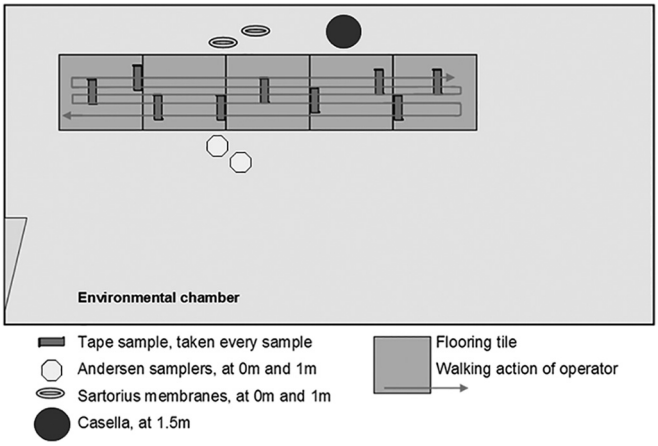

FIG 1 Sampling schematic of the environmental chamber and experimental setup.

Samplers. TSA plates were used for bacterial enumeration for all of these tests, and sampling times were 5 min. All samplers were positioned proximal to the contaminated flooring. See Fig. 1 for the sampling schematic.

Sartorius sampling heads, which have been shown to have a sampling efficiency of 99.999% for *B. atrophaeus* spores (9), were used. These sampling heads incorporate an 80-mm-diameter, 3-μm-pore-size Sartorius gelatin filter (Sartorius, United Kingdom) and were positioned 0 m and 1 m above the floor surface. The filters were connected to a vacuum pump to provide a flow rate of 50 liters min^{-1}. After sampling, the membrane filters were aseptically removed, placed onto TSA, and incubated at 37°C (±2°C) for 18 h. The Andersen fractionation sampler (10) consists of 6 metal stages through which air is consecutively drawn at a controlled rate of 28.3 liters min^{-1} (Thermo Scientific, United Kingdom). Two samplers were positioned at heights of 0 m and 1 m, respectively.

A low-volume Casella slit sampler (Casella, London, United Kingdom) was operated at 30 liters min^{-1} for 5 min at a height of 1.5 m, to sample the air that would be inhaled by an average adult at that height (11).

Sampling protocol. Flooring tiles of carpet ($n = 5$; 0.5 m by 0.5 m) and PVC ($n = 4$; 0.625 m by 0.5 m) were contaminated once at the beginning of the study with *B. atrophaeus* spores by using the Star airbrush within a class III biological safety cabinet. The floors were then left to dry, with the cabinet ventilation running, before being bagged for transport to the test chamber. The tiles were stuck firmly to the floor of the environmental chamber to form a continuous 2.5-m-long runway. For all activities within the test chamber, the operator wore a hooded Tyvek suit, an FFP3 (filtering face piece class 3) mask, latex gloves, and work boots.

The room ventilation system was operated prior to the tests and during the setup of the experiment to ensure the removal of any aerosol contamination generated during that process. Next, with ventilation switched off and samplers switched on, one run of activity was performed, as described below, and after 5 min, the samplers were switched off. The room was then flushed for 9 min. During this time, all samplers were reset, and a randomly selected surface sample was taken by using a 5-cm strip of water-soluble tape. With the ventilation turned off, the activity and 5-min sampling period were then repeated, with venting following each run. Background samples were taken at the start and again at least one more time during the course of activities, during which the operator was present in the room but not moving.

Light activity was defined as 30 s of walking; for carpet, 22.7 m was covered by an average of 76 steps, and for PVC, 20 m was covered by an average of 64 steps, per run. Heavy activity was defined as 30 s of walking, followed by 30 s of heavy-footed stamping across the flooring surface. For

TABLE 1 Percent reaerosolization factors for defined activities on different flooring surfaces at heights of 0, 1, and 1.5 m

Flooring and activity type (no. of samples)	Reaerosolization factor (% $cm^2 \cdot liter^{-1}$) (95% confidence interval)			
	At ht above contaminated flooring of:			
	0 m	1 m	1.5 m	Total
Carpet				
Heavy (10)	0.57 (0.47–0.67)	0.41 (0.35–0.48)	0.17 (0.16–0.19)	1.16 (1.00–1.32)
Light (8)	0.14 (0.11–0.16)	0.10 (0.08–0.12)	0.05 (0.04–0.07)	0.29 (0.24–0.35)
PVC				
Heavy (8)	0.0055 (0.0031–0.0079)	0.0033 (0.0023–0.0043)	0.0010 (0.004–0.0016)	0.0099 (0.0069–0.128)
Light (10)	0.00051 (0.00038–0.00065)	0.00037 (0.00027–0.00047)	0.00017 (0.0001–0.00025)	0.00105 (0.00081–0.00128)

carpet, 45 m was covered in an average of 153 steps, and for PVC, 35 m per run was covered in 138 steps. The operator performing each run was 175 cm tall, weighing ~75 kg.

Expression of results. The results are expressed as a reaerosolization factor, defined as the ratio of the microbial air concentration (CFU per liter) to the measured surface concentration (CFU per square centimeter), expressed as a percentage per square centimeter per liter.

Mass mean diameter. For calculation of the sizes of particle from the fractionation samplers (corrected for positive hole correlation), cumulative CFU percentages were plotted on a log-log scale against the calculated d-50 cutoffs (the particle size of 50% of the particles collected) for the Andersen stages (12). A linear regression was drawn to best fit the plots, using SigmaPlot 10.0. The mass mean diameter (MMD) was then calculated by reading off the corresponding particle size in micrometers on the *y* axis from 50% on the *x* axis.

Statistical analysis. Averages, tables, and graphs were produced by using Microsoft Excel 2010. Three-way analysis of variance (ANOVA) and Holm-Sidak pairwise multiple comparisons were performed to investigate interactions between groups by using SigmaPlot 10.0. For statistical tests, a confidence interval of 95% was always applied, and a P value of <0.05 was considered significant.

RESULTS

Use of water-soluble tape for surface sampling. Seeding experiments demonstrated that the tape itself did not impact the growth or viability of spores, with 97.59% (standard deviation [SD], 15.97%) of spores being recovered. Tape sampling of flooring that was spray contaminated with a solution containing 10^6 spores/ml gave 22.06% (SD, 11.27%) and 113.72% (SD, 35.62%) spore recoveries from carpet and PVC, respectively, compared to immersion of sections of flooring contaminated in the same manner.

Contamination of surfaces. Carpet tiles were contaminated with an average of 1.51×10^3 CFU spores $\cdot$ cm^{-2} (SD, 8.54×10^2 CFU $\cdot$ cm^{-2}), determined by using water-soluble tape for sampling. PVC flooring was contaminated with an average of 1.03×10^4 CFU spores $\cdot$ cm^{-2} (SD, 4.02×10^3 CFU $\cdot$ cm^{-2}), determined by using water-soluble tape for sampling. These numbers were used to calculate the reaerosolization factors, as described below, and represent the levels of contaminating organisms on the surfaces.

Reaerosolization factors. Table 1 shows the calculated reaerosolization factor values determined from the levels of contamination on each material, activity levels, and rates of recovery of *B. atrophaeus* spores at each sampling height per liter of air sampled. The highest mean reaerosolization factor values were recorded during heavy activity on carpet at ground level (mean reaerosolization factor, 0.573% $cm^2 \cdot liter^{-1}$), and the lowest reaerosolization factor value was recorded during light activity on PVC at a height of 1.5 m (mean reaerosolization factor, 0.000166% $cm^2 \cdot liter^{-1}$).

Statistical comparison. A statistically significantly higher aerosolization factor value was obtained with walking on carpet than with walking on PVC ($t = 20.42$; $P < 0.001$); heavy activity produced a statistically significantly higher aerosolization factor value than did light activity ($t = 12.421$; $P < 0.001$). Height also had a statistically significant effect on the aerosolization factor, with statistically significantly higher rates of recovery of *B. atrophaeus* at 0 m than at 1 m and 1.5 m, and at 1 m than at 1.5 m ($t = 8.441$ and $P < 0.001$ for 0 m versus 1.5 m, $t = 5.053$ and $P < 0.001$ for 1 m versus 1.5 m, and $t = 3.388$ and $P = 0.001$ for 0 m versus 1 m).

An evaluation was performed to establish the interaction between flooring type and activity level with respect to height. The effect of the interaction between flooring type and activity level was significant only at a height of 0 m ($P = 0.003$).

Particle size distribution. The MMDs and percent particle size distributions were measured by using Andersen air samplers at 0- and 1-m heights for carpet and PVC (Table 2 and Fig. 2 and 3). The MMDs for both heavy and light activities on carpet ranged from 2.56 to 2.59 μm, whereas PVC showed both a higher MMD and a larger range of MMDs (2.95 to 3.99 μm) associated with heavy and light activities and height.

The average percent particle counts per liter of air sampled for activities and heights for carpet show that the distribution of particles collected varies from that with PVC (Fig. 2 and 3). The majority of particles collected from the PVC air samples were found in the top stage of the Andersen air sampler, representing particles with an aerodynamic particle size of >7 μm. The air sample particles collected from the carpet samples showed a broader distribution of particle sizes recovered, with considerable proportions of the particles being recovered from each particle size range (compare Fig. 2 and 3).

TABLE 2 MMDs of particles recovered from Andersen air samplers under the conditions tested

Flooring	Activity type	Ht (m)	MMD (μm)
Carpet	Heavy	0	2.56
		1	2.59
	Light	0	2.55
		1	2.59
PVC	Heavy	0	3.10
		1	4.10
	Light	0	2.95
		1	3.99

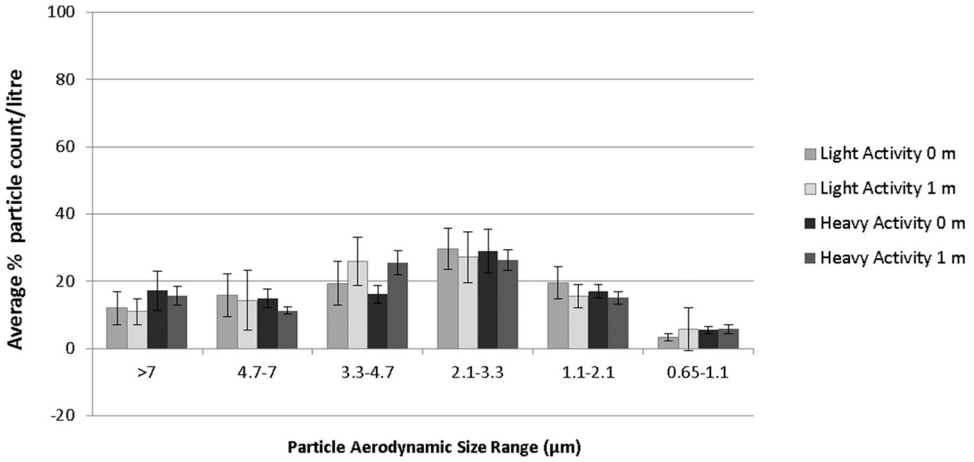

FIG 2 Average percent particle counts per liter recovered from Andersen air sampler stages after activities on carpet.

DISCUSSION

This study quantified the reaerosolization of spores on contaminated flooring caused by walking to assess the risks to personnel in biologically contaminated areas. The use of an artist airbrush has proven to be an effective and reproducible method for evenly contaminating flooring, supporting work by Levy et al., who found that even under unfavorable conditions, such as water sprayed onto hydrophobic polystyrene, airbrush deposition resulted in a satisfactory and homogeneous monolayer (8). Airbrush-generated spray caused liquid to build up in spots on the PVC surface but not carpet, which visibly absorbed all the spray. The mode of drying of the aerosol generated by the airbrush during deposition may affect the sizes of particles generated by reaerosolization activities. For example, the slower drying observed with PVC may give rise to spots of more concentrated spores, formed as the droplets dry and shrink on the surface. The reverse may be true for the fibers of the carpet, leading to a more even dispersal of spore concentrations across the carpet surface as the droplets are absorbed onto the carpet fibers. This may help explain the larger particle sizes generated from PVC; however, as the particle sizes generated during airbrush deposition are unknown, as are the potentially complex interactions between the surfaces and wet deposited spores, this link cannot be ascertained. Future work assessing the particle sizes generated by an airbrush may aid in assessing the nature of wet deposition of spores onto different materials.

This study also demonstrated that water-soluble tape gives rates of recovery that are comparable to, if not higher than, those of other surface-sampling methods such as the use of swabs (13, 14). Its ease of use and high rates of recovery, from carpet especially, indicate that water-soluble tape is a useful surface sampling tool for contaminated indoor areas. More work investigating its sampling efficiencies for other materials, and at different levels of contamination, would aid its characterization further.

It has been demonstrated that several variables affect the number of microbial aerosol particles reaerosolized from flooring and their size distribution. These variables include the level of activity, type of flooring, and distance from the source. The average total reaerosolization factor values for light and heavy activities for PVC were lower than those for carpet by factors of 117 and 276,

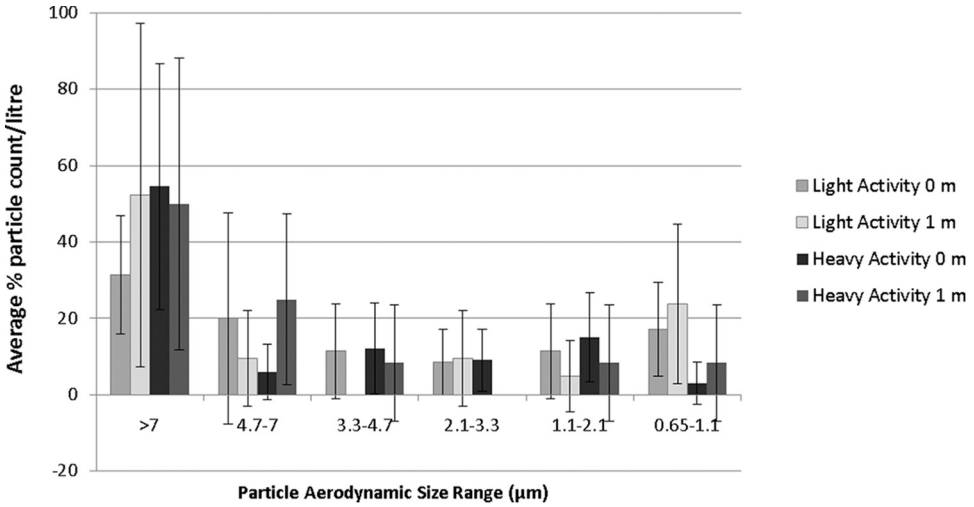

FIG 3 Average percent particle counts per liter recovered from Andersen air sampler stages after activities on PVC.

respectively. These findings agree with the findings of Buttner et al., who contaminated vinyl and carpet tiles with fungal spores (15). The results of the study by Buttner et al. suggested that the composition of the flooring surface plays a role in the dispersion of spores upon disturbance. Those authors postulated that the springy, relatively loose fibers of the carpet are deflected upon contact, resulting in greater propulsion of spores upon reexpansion. Our work supports this postulation, as the reaerosolization factor value at a height of 1.5 m was higher for carpet than for PVC by factors of 294 and 170 when light and heavy activities were performed, respectively.

The difficulty of accurately assessing the level of contamination on carpet can complicate surface results. The composition of carpet itself is a confounding factor; the vertical fibers increase the surface area-to-volume ratio (in comparison to PVC) and potentially allow spores to attach along the length of the fibers and the interstitial spaces. Activity, and a lack of activity, may redistribute spores either further into the bed of the carpet or outward toward the tips of the fibers. The most accurate method of assessing carpet contamination is removal of a portion of the material to assay for total bacterial counts (S. Paton, unpublished data); however, this was not possible for these experiments, as seeded carpet was reused for successive experiments. Successive analysis of repeat samples of water-soluble tape from different areas of the carpet did not reveal any downward or upward trend during each set of activities. This indicates that the placement and distribution of spores in carpet were maintained at similar levels over the time course of block experiments, with minimal losses due to reaerosolization. Furthermore, other studies indicated that the slow decline of organisms on naturally contaminated carpets occurred only after between 5 and 16 weeks under storage conditions (16); Kramer et al. indicated that clinically relevant bacteria can persist on dry surfaces for anywhere between 1 day and 30 months, with the survival time being dependent on the microbial species (17). This study provides an indication of the potential for microbial reaerosolization of respirable particles from two different flooring surfaces, at two different activity levels, and at a range of heights and, as such, can be used to determine the potential exposure of sampling personnel entering a contaminated area. Environmental sampling is a physical exercise, involving carrying potentially heavy equipment and bending to collect samples. The sampling process often necessitates that the operator be in close proximity to the source of contamination, and hence, any aerosols generated may be in the operator's breathing zone.

The estimated total exposure of sampling personnel per hour in an area contaminated with a known concentration of spores that are homogenously distributed has been calculated by using the following equation: potential exposure = flooring concentration $\cdot$ cm^{-2}/100 $\times$ reaerosolization factor $\times$ breathing rate h^{-1}.

Therefore, by using the flooring contamination levels in this study ($\sim 1 \times 10^3$ CFU $\cdot$ cm^{-2}), using average total reaerosolization factor values of 0.0099% for PVC and 1.16% for carpet, undertaking heavy activity, and estimating the breathing rate of sampling personnel to be 20 liters $\cdot$ min^{-1} (which is considered a reasonable estimate for activity of this type [18]), the levels of potential exposure are 1.2×10^2 CFU $\cdot$ h^{-1} for PVC and 1.4×10^4 CFU $\cdot$ h^{-1} for carpet. These parameters were chosen because they indicate a worst-case scenario: the person will be in the breathing zone of all heights measured at different times, and the activity performed will be fairly strenuous.

The inhalational ID1 (1% infectious dose) of *Bacillus anthracis* is estimated to be 160 spores (95% confidence interval, 100 to 250 spores) (19). The level of potential exposure for sampling personnel within these parameters, in the absence of respiratory protective equipment (RPE), would clearly pose an unacceptable risk. In all cases, a risk-adverse approach is recommended with regard to RPE in any such scenario, as this study investigated a relatively small set of parameters that could affect reaerosolization rates.

For example, relative humidity was measured to be between 40% and 60% during this study. It has been proposed that higher humidity (>60%) can raise the rates of reaerosolization of particles from carpet, due to the neutralization of static charges working to hold on to particles (2), but increases the capillary force between a smooth surface like PVC and small particles (20). This is a factor that could be investigated in future studies.

In addition, this study reflects one individual wearing one type of footwear and walking in a certain way over a certain length of two types of flooring contaminated with a single agent. To enhance our understanding of the reaerosolization process, the effects of other parameters on aerosolization, such as the contamination level, organism type, dissemination method (especially dry versus wet), interpersonal difference in activities, shoe type, and a range of flooring and environmental conditions, including contamination of operator clothing in the contaminated area, should be investigated. Furthermore, methods such as computational fluid dynamics could potentially be useful tools for interpretation and extrapolation of data. An understanding of the factors involved in reaerosolization will give a better understanding of the risks of reaerosolization from contaminated flooring materials. Additionally, following aerosolization of spores from a contaminated surface by activities on that surface, spores may land on the person performing that activity, be lost to the air-handling system in the area, or resettle onto the flooring surface. Further investigation into the dynamics of this movement of spores following reaerosolization activities may help shed some light on the complex interactions of spores in carpet.

In conclusion, under our experimental conditions, carpet provides a higher reaerosolization risk than does PVC in areas with high pedestrian traffic. The relevance of a greater reaerosolization risk with contaminated carpet could help inform the selection of floor coverings for use in health care settings, for example, in refurbishment or construction of new hospital wards. PVC would offer not only the easier-to-clean choice in high-spill areas proximal to patients but also the more logical choice in areas such as waiting rooms, where cleaning regimes may not be as stringent as those in patient areas. Thus, the number of hospital-acquired infections occurring due to the transfer of microorganisms from dirty floors could be reduced. The aerosols generated from a floor contaminated with $\sim 10^3$ to 10^4 CFU $\cdot$ cm^{-2} can reach breathing height, are within the respirable size range, and could transmit infection as they breach the proposed ID1 for *B. anthracis* under our experimental conditions. Further work examining the variables involved can inform future decontamination procedures.

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REFERENCES

1. **Greene VW, Vesley D, Bond RG, Michaelsen GS.** 1962. Microbiological contamination of hospital air. I. Quantitative studies. Appl Microbiol **10:** 561–566.
2. **Rosati JA, Thornburg J, Rodes C.** 2008. Resuspension of particulate matter from carpet due to human activity. Aerosol Sci Technol **42:**472–482. http://dx.doi.org/10.1080/02786820802187069.
3. **Wallace L.** 1996. Indoor particles: a review. J Air Waste Manag Assoc **46:**98–126. http://dx.doi.org/10.1080/10473289.1996.10467451.
4. **Long CM, Suh HH, Koutrakis P.** 2000. Characterization of indoor particle sources using continuous mass and size monitors. J Air Waste Manag Assoc **50:**1236–1250. http://dx.doi.org/10.1080/10473289.2000.10464154.
5. **Brauer M, Hirtle RD, Hall AC, Yip TR.** 1999. Monitoring personal fine particle exposure with a particle counter. J Expo Anal Environ Epidemiol **9:**228–236. http://dx.doi.org/10.1038/sj.jea.7500040.
6. **Ferro AR, Kopperud RJ, Hildemann LM.** 2004. Elevated personal exposure to particulate matter from human activities in a residence. J Expo Sci Environ Epidemiol **14:**S34–S40. http://dx.doi.org/10.1038/sj.jea.7500356.
7. **Sharp RJ, Scawen MD, Atkinson T.** 1989. Fermentation and downstream processing of Bacillus, p 255–292. *In* Harwood CR (ed), Bacillus. Springer, New York, NY.
8. **Levy C, Bornard I, Carlin F.** 2011. Deposition of Bacillus subtilis spores using an airbrush-spray or spots to study surface decontamination by pulsed light. J Microbiol Methods **84:**223–227. http://dx.doi.org/10.1016/j.mimet.2010.11.021.
9. **Parks SR, Bennett AM, Speight SE, Benbough JE.** 1996. An assessment of the Sartorius MD8 microbiological air sampler. J Appl Bacteriol **80:** 529–534. http://dx.doi.org/10.1111/j.1365-2672.1996.tb03252.x.
10. **Andersen AA.** 1958. New sampler for the collection, sizing, and enumeration of viable airborne particles. J Bacteriol **76:**471–484.
11. **Casewell MW, Fermie PG, Thomas C, Simmons NA.** 1984. Bacterial air counts obtained with a centrifugal (RCS) sampler and a slit sampler—the influence of aerosols. J Hosp Infect **5:**76–82. http://dx.doi.org/10.1016/0195-6701(84)90104-X.
12. **Buttner MP, Willeke K, Grinshpun SA.** 1997. Sampling and analysis of airborne micro-organisms, p 629–640. *In* Hurst CJ, Crawford RL, Garland JL, Lipson DA, Mills AL, Stetzenbach LD (ed), Manual of environmental microbiology, 3rd ed. ASM Press, Washington, DC.
13. **Lewandowski R, Kozlowska K, Szpakowska M, Stepinska M, Trafny EA.** 2010. Use of a foam spatula for sampling surfaces after bioaerosol deposition. Appl Environ Microbiol **76:**688–694. http://dx.doi.org/10.1128/AEM.01849-09.
14. **Brown GS, Betty RG, Brockmann JE, Lucero DA, Souza CA, Walsh KS, Boucher RM, Tezak M, Wilson MC, Rudolph T.** 2007. Evaluation of a wipe surface sample method for collection of Bacillus spores from nonporous surfaces. Appl Environ Microbiol **73:**706–710. http://dx.doi.org/10.1128/AEM.01082-06.
15. **Buttner MP, Cruz-Perez P, Stetzenbach LD, Garrett PJ, Luedtke AE.** 2002. Measurement of airborne fungal spore dispersal from three types of flooring materials. Aerobiologia **18:**1–11. http://dx.doi.org/10.1023/A:1014977900352.
16. **Anderson RL.** 1969. Biological evaluation of carpeting. Appl Microbiol **18:**180–187.
17. **Kramer A, Schwebke I, Kampf G.** 2006. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. BMC Infect Dis **6:**130. http://dx.doi.org/10.1186/1471-2334-6-130.
18. **ISO.** 2007. ISO/TS 16976-1. Respiratory protective devices—human factors. Part 1: metabolic rates and respiratory flow rates. ISO, Geneva, Switzerland.
19. **Toth DJA, Gundlapalli AV, Schell WA, Bulmahn K, Walton TE, Woods CW, Coghill C, Gallegos F, Samore MH, Adler FR.** 2013. Quantitative models of the dose-response and time course of inhalational anthrax in humans. PLoS Pathog **9:**e1003555. http://dx.doi.org/10.1371/journal.ppat.1003555.
20. **Ranade MB.** 1987. Adhesion and removal of fine particles on surfaces. Aerosol Sci Technol **7:**161–176. http://dx.doi.org/10.1080/02786828708959155.