American Journal of Infection Control ■■ (2017) ■■-■■

Contents lists available at ScienceDirect

American Journal of Infection Control

journal homepage: www.ajicjournal.org

Major Article

Comparison of survivability of *Staphylococcus aureus* and spores of *Aspergillus niger* on commonly used floor materials

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Key Words:
Survivability
Staphylococcus aureus
Aspergillus niger
Spore
Floor materials
Carpet
Built environment

Background: The survivability of *Staphylococcus aureus* and spores of *Aspergillus niger* was compared on 5 common floor materials.

Methods: Floor materials were inoculated with a known concentration of *S aureus* and spores of *A niger* on day 0. Their survivability was measured on days, 2, 7, 14, and 28 by bulk rinsate method and enumerated using culture-based method.

Results: The difference in change of *S aureus* levels was statistically significant for all tested days ($P < .001$) for all floor materials. Vinyl composition tile (VCT) and porcelain tile (PT) had statistically similar survivability and differed statistically from carpets. On both VCT and PT, positive growth for *S aureus* occurred by day 2 (1-1.7 $\log_{10}$), declined slightly (0.1 to –0.2 $\log_{10}$) by day 7, and remained positive until day 28. However, *S aureus* was undetected by day 7 on both carpets. *A niger* spores were undetected on residential broadloom carpet and rubber-backed commercial carpet after day 2 but survived on VCT, PT, and wood until day 28.

Conclusions: Floor materials with hard and smooth surfaces, such as VCT and PT, can allow survival of *S aureus* and *A niger* for up to 4 weeks. It may imply that floor materials can play a major role in preserving microbial contaminants in the built environment.

Flooring material, relative to its contribution to indoor air quality, has been a contentious issue.[1,2] Indoor air quality continues to gain much interest recently because it has been identified as an important influence on human health, and most people spend >85% of their time indoors.[3,4] Many microbes responsible for health care–acquired infections, including norovirus and methicillin-resistant *Staphylococcus aureus* (MRSA), have been found on floors and other environmental surfaces.[5,6] Floor surfaces has been found to be enriched with microbial contaminants compared with other surfaces in built environment.[7] Bacterial levels recovered from hospital floors have been found to range from 3.3 colony forming units (CFU)/10 cm^2 to 488 CFU/10 cm^2.[8] Resuspension of microbes from floor can occur as a result of different activities, such as walking, vacuuming, cleaning, mopping, and so forth, which can contribute up to 10%-15% of total airborne bacteria in a room.[9,10]

Floors are generally composed of one of the following: wood, laminate, ceramic or porcelain tiles (PTs), carpet, vinyl, and linoleum. Most of the studies focusing on the microbiologic aspect of flooring have used the term floor without differentiating between various floor material types. Various other terms such as soft surface have been used for carpets, whereas hard or bare floor types are used for vinyl tiles.[11] Few researchers have compared microbial contamination on a variety of floor material types, such as carpets and vinyl tiles. Rylander et al reported higher surface bacterial levels on vinyl tiles compared with carpets.[12] However, Anderson et al reported higher counts of *Escherichia coli*, *S aureus*, and *Pseudomonas aeruginosa* on carpets.[13] Similarly, Foarde and Berry reported higher bacterial levels on carpet surfaces than on vinyl tiles in schools.[14] In another hospital study, researchers reported higher colonization of *S aureus* on vinyl floors compared with ceramic tile.[15] Harris et al found higher levels of pathogenic bacteria on vinyl surfaces than carpets, but generally lower numbers of bacterial genera on vinyl surfaces compared with carpets.[1] Environmental survivability of clinically relevant pathogens has been studied on fomites, especially in health care settings and food services, on surfaces such as glass, steel, laminate, clothes and upholstery, plastics, and carpets.[6,16-18] Kramer et al reviewed studies looking at persistence

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Conflicts of interest: None to report.

0196-6553/
http://dx.doi.org/10.1016/j.ajic.2017.02.014

of nosocomial pathogens on inanimate surfaces and found that test materials do not reveal consistent results.[18] Moreover, nontraditional surfaces, such as floors, have been studied sparingly.[19] The inconsistent results and lack of enough comparative studies for different floor materials make it challenging to draw any general or strong conclusions and necessitate comparative studies to better understand microbial survivability on different floor materials.

The aims of this study were to compare the survivability of microbes on most common floor materials. For this purpose, *S aureus* and *Aspergillus niger* were chosen as representative pathogens. American Association of Textile Chemists and Colorists methods 100-2004 and 174 were reviewed for selection of the test organism.[20] *S aureus* is one of the most frequently isolated pathogens in humans and is responsible for many infections. MRSA is a major cause of nosocomial infection. *S aureus* is capable of colonizing in healthy individuals without any symptoms, playing a role in community-acquired MRSA infection.[16,21]

Aspergillus spp is one of the most common fungal genera and is commonly found in the indoor environment. *Aspergillus* sp contamination has been studied mostly in relation to respiratory illnesses, such as allergy and asthma.[22] Additionally, nosocomial infection has been reported for *Aspergillus* spp in many cases, especially in immunocompromised patients.[23]

MATERIALS AND METHODS

Floor materials

Five common floor materials were chosen for the study. The choice of floor materials was based on the popularity of flooring materials. Carpet is popular and the main flooring choice for residential and commercial buildings. A wide estimate indicates almost 70% of U.S. houses have carpets.[24] Carpets accounted for almost 60% of total U.S. floor covering sold in the United States followed by vinyl (resilient), tile (ceramic/porcelain), and wood according to *Flooring Covering News* in 2015.[25]

The 5 floor material types were as follows:

1. Vinyl composition tile (VCT) (color: Lovorno Onyx, Allure tile, GripStrip resilient tile flooring);
2. Hardwood (wood): (color: Ash Gunstock);
3. Porcelain tile (MARAZZI Brazilian);
4. Residential broadloom carpet (BC) (Mohawk Thunderbolt cattail, nylon fiber) (the backing on these carpets is made of fabric and bonding agent); and
5. Rubber-backed commercial carpet (RCT): (Shaw carpet ecoworx product W5840, nylon fiber) (the backing on these carpets is made of thermoplastic polyolefin).[26]

All floor samples were cut to 5- × 5-cm square tiles. Our preliminary test of the floor materials had found these store-purchased floor materials to be already contaminated. Therefore, these floor samples were sterilized first by immersing in 70% ethyl alcohol for 20-30 minutes and air dried inside a biosafety cabinet for 24-48 hours.

Preparation of microorganisms

S aureus

Fresh culture of *S aureus* (ATCC 6538; ATCC, Manassas, VA) was prepared in Tryptic Soy Broth (Fisher Scientific, Pittsburgh, PA) and incubated at 37°C with 200 rpm for 18-24 hours in a shaking incubator. The tube was then centrifuged at 6,000 rpm for 10 minutes to collect the bacterial cells. The cells were finally resuspended in Nutrient Broth (Fisher Scientific). Proper dilution was made to obtain an approximate final concentration of 1.6×10^8 CFU/mL using a cell density meter (model CO8000; Biochrom UK, Cambridge, UK).

A niger

A niger (ATCC 9642; ATCC) spores were inoculated on Potato Dextrose Agar plate (Fisher Scientific). The plate was incubated in a dark place at 24°C. The fungal spores were harvested after 2 weeks of growth by scraping off the growth from the agar plate and collecting it in a 50-mL centrifuge tube (Fisher Scientific). The tube was vortexed for approximately 20 minutes, and spores were counted using a hematocytometer under a light microscope (Fisher Scientific) at 400× magnification. The final concentration prepared was 7.6×10^6 spores/mL.

Sample size

In the first experiment, 20 sets of each floor material type were inoculated on day 0, and then the samples were tested for microbial survivability on days 2, 7, 14, and 28. The same set of the experiments was repeated 2 more times. Therefore, a total of 300 floor samples were tested (5 floor materials × 4 samples of each floor material × 5 sampling days × 3 experiments = 300 floor samples in total).

Inoculation and survivability test

In the first experiment, a total of 20 sets of each sterilized floor material type were placed in a presterilized Petri dish (Fisher Scientific). On day 0, each of these 25-cm^2 floor samples was inoculated with either 0.25 or 0.5 mL suspensions of *S aureus* and *A niger* spores. From a bacterial suspension of approximately 1.6×10^8 CFU/mL concentration, RCT and BC were inoculated with 0.5 mL (or 8×10^7 CFU) of the suspension, whereas VCT, PT, and wood were inoculated with 0.25 mL (or 4×10^7 CFU).

A niger spore suspension of 7.6×10^6 spores/mL was used for inoculation. On day 0, both BC and RCT were inoculated with 0.5 mL (or 3.8×10^6 spores), and VCT, PT, and wood were inoculated with 0.25 mL (or 1.9×10^6 spores).

The inoculum was added on floor samples at 6 spots in an X pattern. The inoculated floor samples were then covered with a lid. They were placed at room temperature, 24°C, for subsequent days of testing. On day 0, right after inoculation, 4 of the inoculated floor samples from each floor material type were rinsed and washed with sterilized 1× phosphate buffer saline. Typical volume of phosphate buffer saline was 25 mL for RCT, VCT, and PT, whereas 30 mL was the typical volume for carpet and wood. The floor samples were submerged and shook vigorously with a sterilized forcep for 2 minutes. The floor samples were held with forceps, and the rinsate was collected in a sterile 50-mL centrifuge tube, mixed by vortexing for 1 minute, properly diluted, and plated on Tryptic Soy Agar (Fisher Scientific) and Potato Dextrose Agar for *S aureus* and *A niger*, respectively. Afterward, on days 2, 7, 14, and 28, 4 samples from each of the inoculated floor material types were collected and followed the same method to determine microbial survivability. All the counts were measured in CFU per 25 cm^2. An uninoculated and sterilized floor sample for each floor material types was rinsed and plated on day 0 to ensure sterility. The uninoculated floor samples on day 0 did not result in any growth, confirming the sterility of the floor materials. The temperature was kept at 22°C throughout the study period, whereas relative humidity remained in the range of 30%-70%, reflecting the typical indoor environment conditions. The collected data were log transformed and analyzed with STATA12 (StataCorp, College Station, TX) and MS Excel (Windows 2013; Microsoft, Redmond, WA).