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# Evaluation of a shoe sole UVC device to reduce pathogen colonization on floors, surfaces and patients

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## SUMMARY

**Background:** An ultraviolet C (UVC) decontamination device that delivers germicidal UVC radiation to the soles of shoes has become available recently.

**Aim:** To demonstrate that shoe soles can be vectors for healthcare-associated infection, and to investigate if a UVC shoe sole decontamination device would decrease this risk effectively.

**Methodology:** Three bacterial strains (*Staphylococcus aureus*, *Enterococcus faecalis* and *Escherichia coli*) and a non-toxigenic strain of *Clostridium difficile* were spiked on to standardized rubber-soled shoe soles and then selected at random for UVC exposure or no UVC exposure. Experiments were performed to test the efficacy of the UVC device to decontaminate shoe soles and flooring. *E. faecalis* was spiked on to shoes to assess colonization of a simulated healthcare environment and patient.

**Results:** The UVC device decreased shoe sole contamination significantly for all tested bacterial species, and decreased floor contamination significantly for all floor types and species tested ( $P < 0.01$  for all experiments). The  $\log_{10}$  reduction was the highest for *E. coli* (mean  $\pm$  standard deviation  $2.6 \pm 0.79$ ), followed by *E. faecalis* ( $2.19 \pm 0.68$ ), *S. aureus* ( $1.74 \pm 0.88$ ) and *C. difficile* ( $0.42 \pm 0.54$ ) ( $P < 0.0001$  for all analyses). Exposure of shoe soles to the UVC device decreased contamination significantly (mean  $\log_{10}$  reduction  $2.79 \pm 1.25$ ;  $P < 0.0001$ ). Proportions of samples from furniture, bed and patient dummy samples decreased from 96–100% positive in controls to 5–8% positive in UVC device experiments ( $P < 0.0001$  for all analyses).

**Conclusion:** A UVC decontamination device was shown to reduce the colony-forming unit counts of relevant pathogenic organisms from shoe soles with subsequent decreased colonization of floors, healthcare equipment, furniture, beds and a patient dummy.

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## Introduction

Multi-drug-resistant organisms (MDROs) including *Clostridium difficile* are present on the shoe soles of healthcare workers and people living in the community [1]. Studies in healthcare settings and non-healthcare community settings

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have demonstrated the presence of methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE) and multi-drug-resistant Gram-negative bacteria on shoe soles [2,3]. Toxigenic *C. difficile* strains were shown on shoe sole samples from non-healthcare homes in Houston, Texas, USA [4]. A number of studies have also investigated transmission dynamics between shoe soles or floor surfaces and patient colonization, with most studies demonstrating colonization potential via aerosolization, direct contact or indirect methods [5–8]. Ultraviolet C (UVC) devices are used in hospitals to decontaminate the hospital environment [9,10].

Recently, a shoe sole UVC decontamination device has become available (HealthySole Plus, Healthy Sole, Inc, Minden, NV, USA). This ETL/CETL/CE-listed stand-on device delivers 8 s of UVC radiation to shoe soles triggered by four infra-red sensors located in the base of the unit corresponding to the front and back of shoe soles. The objectives of this experimental study were to study whether shoe soles can be vectors for environmental colonization with healthcare-associated pathogens, and whether a UVC shoe sole decontamination device can decrease the risk of colonization effectively.

## Materials and methods

### Bacterial strains

American Type Culture Collection (ATCC) or clinical strains of *S. aureus* (SA168), *Enterococcus faecalis* (EF312) and *Escherichia coli* (ATCC 25922) were used along with a non-toxigenic strain of *C. difficile* (ATCC70057) and an environmental strain of an *Enterococcus* spp. [11,12]. Isolates were stored in Cryocare vials (Key Scientific Products, Round Rock, TX, USA) at  $-80^{\circ}\text{C}$ . Isolates from the stock vials were sub-cultured on blood agar plates and/or MacConkey agar plates (*E. coli*) for purity at least twice at  $37^{\circ}\text{C}$ . All strains were incubated aerobically for 24 h, except *C. difficile* which was incubated anaerobically for 72 h for spore preparation. An inoculum of approximately  $10^{6-7}$  colony-forming units (cfu)/mL was used in every experiment. Following incubation, inoculum was prepared in phosphate buffered saline for *E. faecalis*, *E. coli* and *S. aureus*, and in de-ionized water and Tween 20 for *C. difficile*. Pre-exposure inoculum concentration was measured by direct agar plating on specific agar media (blood agar for *S. aureus* and *C. difficile*, enterococcus agar for *E. faecalis* and MacConkey agar for *E. coli*). For the clinical trials, 100  $\mu\text{L}$  of 10-fold diluted inert dye methylene blue was added to the inoculum for exposure tracing on the transmission surfaces. Colony counts were obtained from plates containing 30–300 colonies.

### Inoculation of shoe soles and experimental design

To inoculate shoe soles, a working stock of each micro-organism was prepared. Fifty to 200 microlitres of each stock was spiked on to a designated area of a standardized rubber-soled shoe sole (Comfort Clogs, Marcus Uniforms, Milwaukee, WI, USA) and incubated for 20–60 min. Shoes were then allocated at random (1:1) to UVC exposure or no UVC exposure by a separate investigator (IH or JA) to blinded investigators performing the experiments. Three separate sets of experiments were performed to test the efficacy of the UVC device to

decontaminate shoe soles and prevent subsequent colonization of the environment of simulated patients (Figure 1).

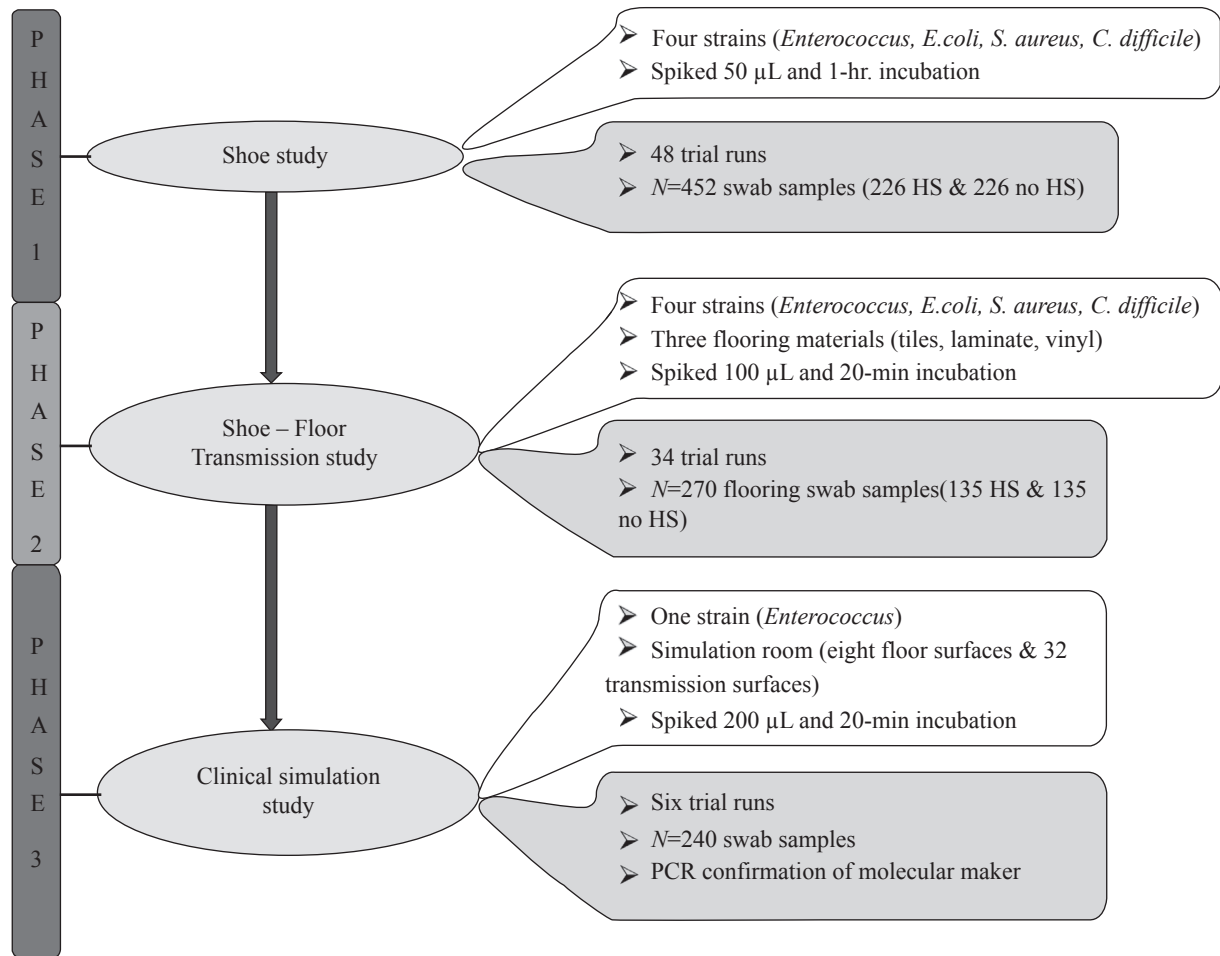
In the first set of experiments, the spiked areas of shoe soles were swabbed, serially diluted, plated on to selective media, and incubated at  $37^{\circ}\text{C}$ . For each species, cfu counts of shoe soles exposed to UVC and shoes soles not exposed to UVC were compared.

In the second set of experiments, the transfer of each bacterial species from shoe soles to three types of flooring (laminated, tile and vinyl) was assessed. Spiked shoes assigned at random to UVC exposure were rubbed over the flooring systematically for 1 min. Standardized areas of flooring were swabbed, serially diluted, and plated on to selective media. Flooring cfu counts were compared for each species and flooring type based on shoe exposure to the UVC device.

In the third set of experiments, a 25–30-min simulation of human traffic flow within a hospital patient room was mimicked. The mock room consisted of a patient dummy, bed, furniture (counter top, foot plate of water dispenser, waste bin, intravenous pole and chair) and laminated flooring. Using a standardized, pre-arranged script that mimicked human traffic by healthcare personnel, environmental services and family members, environmental contamination of flooring, furniture, bed and patient was assessed. For these experiments, shoes were spiked with an environmental, well-characterized environmental *Enterococcus* spp. (VRE\_MMC7), and then assigned at random (1:1/experiment) to UVC decontamination. Investigators then entered the room using the spiked shoes and followed the assigned script. A variety of surface swabs were taken from the patient dummy, furniture and floor after each 20-min simulation. Swabs were serially diluted and plated on to selective enterococcus agar plates. cfu counts were compared for each species and room area based on shoe exposure to the UVC device. All enterococci grown on surface swabs were sequenced to ensure no other enterococcal contamination (description below). The room was cleaned with 70% isopropanol or bleach at the end of each simulation. Random swabs from five or six representative surfaces were taken after 1 h of cleaning and 1 h before the start of the experiment to determine cleaning efficacy, and to further ensure no other enterococcal contamination. Shoes were clean without visible dirt, and were decontaminated with bleach between the experiments. All experiments included positive and negative controls. This study was considered exempt from review by the Institutional Review Board at the University of Houston.

### *Enterococcus C2/C3* gene sequencing

Van C2/C3 specific gene sequence marker for the environmental enterococcus isolate was used to confirm transmission of the enterococci from shoe soles to environmental surfaces and the mock patient during the simulation study. One hundred experimental samples were polymerase chain reaction (PCR) amplified to detect vanC2/C3 marker to confirm the same clone of the strain. Detection was performed by PCR using vanC2/C3 specific primers (forward primer 5'CGGGGAAGATGGCAGTAT3' and reverse primer 5'CGCAGGGACGGTGATTTT3') with minor modification according to a published protocol [13]. PCR products were analysed on a 2% agarose gel with 0.5 Tris-borate-EDTA buffer. A 1 kb DNA ladder (Thermo Scientific, Waltham, MA, USA) was used as the molecular size marker. The gels were stained with ethidium bromide and photographed



**Figure 1.** Experimental set-up. *E. coli*, *Escherichia coli*; *S. aureus*, *Staphylococcus aureus*; *C. difficile*, *Clostridium difficile*; PCR, polymerase chain reaction; HS, HealthySole.

under UV light. Amplification of *vanC2/C3* specific gene produced distinct bands corresponding to a molecular size of 484 base pairs (bp).

### Statistical analysis

Average  $\log_{10}$  reductions in cfu counts are presented as mean  $\pm$  standard deviation unless otherwise stated. Changes in cfu count based on shoe sole exposure to the UVC device were assessed by Student's *t*-test. In the simulated clinical trial, the number of environmental swabs that grew enterococci based on shoe sole exposure to the UVC device was assessed by Pearson's Chi-squared test. SAS Version 9.4 (SAS Institute, Cary, NC, USA) was used for all analyses. A *P*-value  $<0.05$  was considered to indicate significance.

### Results

To test shoe sole decontamination, 100 shoes (50 pairs) were tested for *S. aureus*, *E. faecalis* and *E. coli*, and 152 shoes (76 pairs) were tested for *C. difficile*. The UVC device decreased shoe sole contamination significantly for all tested bacterial species ( $P<0.01$  for each species). In shoe soles exposed to the UVC device, the  $\log_{10}$  reduction was highest for *E. coli* ( $2.81\pm 0.80$ ), followed by *S. aureus* ( $2.67\pm 0.81$ ), *E. faecalis*

( $2.10\pm 0.62$ ) and *C. difficile* ( $0.42\pm 0.68$ ). Results of the flooring experiments are shown in Table I. All experiments were performed with 20 swabs for each floor surface, except for *C. difficile* experiments which were performed with 30 swabs for each floor surface. Shoe sole exposure to the UVC device decreased floor contamination significantly for all floor types and species tested ( $P<0.01$  for all experiments).  $\log_{10}$  reduction was highest for *E. coli* ( $2.6\pm 0.79$ ), followed by *E. faecalis* ( $2.19\pm 0.68$ ), *S. aureus* ( $1.74\pm 0.88$ ) and *C. difficile* ( $0.42\pm 0.54$ ) ( $P<0.0001$  for all analyses).

Two hundred and forty samples were collected during the clinical simulation experiments, including from the patient dummy (48 samples), furniture (84 samples), floor (60 samples) and bed (48 samples). Exposure of shoe soles to the UVC device decreased contamination significantly compared with control shoes (mean  $\log_{10}$  reduction  $2.79\pm 1.25$ ;  $P<0.0001$ ). A significant reduction in contamination was observed from all sampling types (Table II). Proportions of samples from furniture, bed and patient samples decreased from 96–100% positive in control shoes to 5–8% positive in experiments where the UVC device was used (Figure 2;  $P<0.0001$  for all analyses). Sequencing of all 100 samples for the *vanC2/C3* specific gene produced distinct bands at 484 bp molecular size suggesting no contamination from other sources during the experiments (Figure 3).

**Table I**

Mean log<sub>10</sub> difference in colony-forming unit (cfu) counts on three different types of flooring after exposure to shoe soles exposed to an ultraviolet C (UVC) device compared with control shoes

Species	Flooring	No. of floor samples tested	Mean log <sub>10</sub> difference in cfu count on flooring (UVC device vs control)	P-value
<i>Staphylococcus aureus</i>	Vinyl	20	1.85±0.55	<0.0001
	Tile	20	1.56±1.33	0.017
	Laminate	20	1.8±0.57	<0.0001
<i>Escherichia coli</i>	Vinyl	20	2.63±0.79	<0.0001
	Tile	20	2.69±0.88	<0.0001
	Laminate	20	2.48±0.62	<0.0001
<i>Enterococcus faecalis</i>	Vinyl	20	2.16±0.85	<0.0001
	Tile	20	2.11±0.61	<0.0001
	Laminate	20	2.29±0.60	<0.0001
<i>Clostridium difficile</i>	Vinyl	30	0.34±0.33	0.0093
	Tile	30	0.55±0.56	0.013
	Laminate	30	0.43±0.45	0.016

Values are mean±standard deviation.

**Table II**

Mean log<sub>10</sub> difference in colony-forming unit (cfu) counts during clinical simulation study after exposure to shoe soles exposed to the ultraviolet C (UVC) device compared with control shoes

	No. of samples tested	Mean log <sub>10</sub> difference in cfu count on flooring (UVC device vs control)	P-value
Patient	48	2.59±0.84	<0.0001
Furniture	84	2.76±0.85	<0.0001
Floor	60	2.56±1.33	<0.0001
Bed	48	3.31±0.6	<0.0001

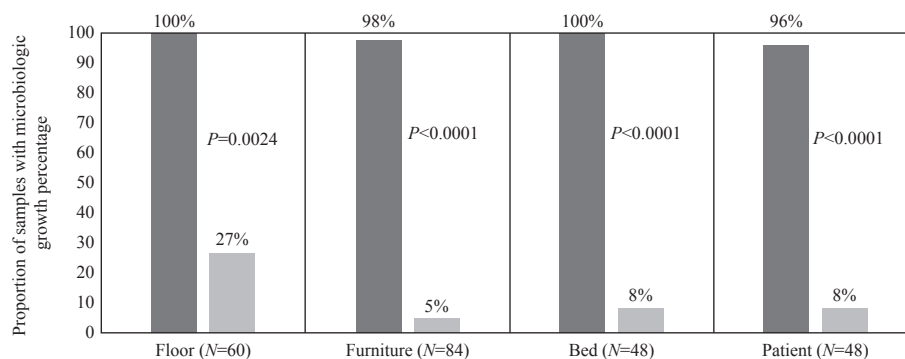
Values are mean±standard deviation.

## Discussion

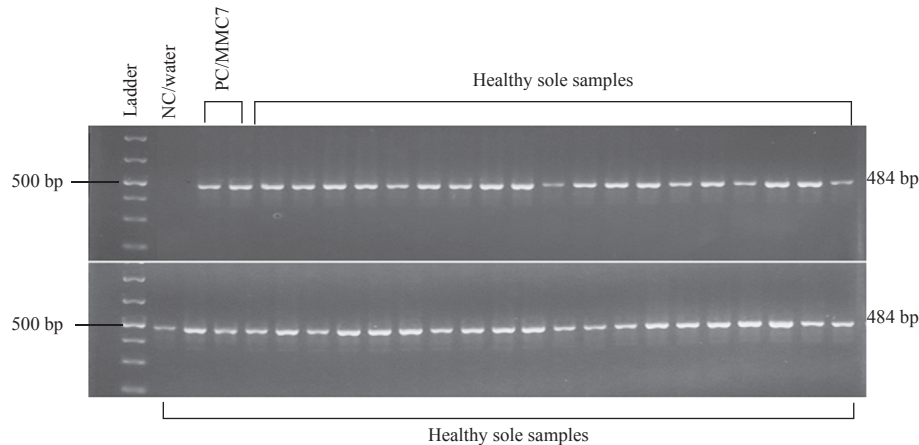
Shoe soles contain a high bioburden of microbiological organisms [1]. Studies have demonstrated that shoe soles can colonize patients with microbiological pathogens via direct or indirect transmission pathways. However, very few effective strategies have been available to decontaminate shoe soles. In

this study, a UVC device specifically designed to decontaminate shoe soles was tested for its ability to decrease cfu counts on spiked shoe soles and subsequent transmission to floors or a simulated hospital room. Using relevant healthcare-associated pathogens, standardized procedures to spike shoe soles, a variety of flooring types, and a standardized script to mimic hospital conditions, this study demonstrated that experiments performed with shoes exposed to the UVC device had significantly lower cfu counts on shoe soles, flooring and patient care areas. These results demonstrate that a UVC device targeting shoe soles can decrease subsequent environmental bioburden and patient colonization. The strengths of this study include a large number of samples tested, a rigorous study design including blinding of research personnel to the intervention, multiple species tested, and PCR confirmation to ensure no environmental contamination during the simulated clinical trial.

Floors in hospital patient rooms are frequently contaminated with pathogens. In a study of five hospitals, high-touch objects (blood pressure cuffs and call buttons) were often in contact with the floor with subsequent transfer of pathogens to hands [14]. A number of UVC devices have been tested to



**Figure 2.** Randomized, blinded, clinical simulation study of shoe soles exposed to the ultraviolet C device (light grey bars) vs control shoes (dark grey bars). Proportions represent number of samples with microbiological growth from each sampling area.



**Figure 3.** Polymerase chain reaction (PCR) amplification of *vanC2/C3* gene positive *Enterococcus* spp. from experimental isolates. All isolates produced PCR product at 484 base pairs (bp) suggesting no contamination from other sources during the experiment. NC, negative control; PC, positive control.

decontaminate the hospital environment, including UVC devices with UV-reflective paint [15] or UVC specific to vectors such as stethoscopes [16,17]. As shoe soles likely have a much higher microbial burden than most other sites, a UVC device that decontaminates shoe soles effectively is likely to represent an important addition to these other UVC disinfection strategies. These results suggest that confirmation of these results in healthcare areas should focus on floor contamination, healthcare equipment, furniture and beds, along with patient colonization. This study focused on the contribution of shoe sole contamination using standardized, controlled experiments. The contribution of shoe soles in conjunction with hand hygiene, environmental services, personal protective gear and other infection control procedures will need to be tested in the real-world setting.

This study has certain limitations. It used a standardized inoculum and representative healthcare-associated pathogens using standardized procedures. Further tests may be warranted on different shoe or flooring types or pathogens. A non-pathogenic *Enterococcus* spp. was used in the simulation clinical trial to avoid any possibility of exposure of study investigators to infection. Although similar results are likely, clinical trials on the prevention of pathogenic organism colonization or infection are warranted. Personnel trained in operation of the UVC device following standardized protocols for all experiments. Whether the same care and ubiquitous use in a busy healthcare setting could be accomplished will require validation. However, the UVC device is equipped with an 8-s timer that allows the user to know that the UVC device has completed its cycle; this should enhance compliance in the real-world setting. Finally, a clinical trial is required to determine the ability of the UVC device to prevent contamination and infection in a real-world setting.

In conclusion, a UVC decontamination device was shown to reduce cfu counts of relevant pathogenic organisms from shoe soles with a subsequent decrease in colonization of floors, healthcare equipment, furniture, beds and a patient dummy.

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### Conflict of interest statement

KWG: Research support paid to the University of Houston from HealthySole. All other authors declare no conflicts of interest relevant to this article.

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