

Use Of A Portable Far-UV System For Bacterial Decontamination

A report compiled from findings related to laboratory based testing of the Sterilray device in the Department of Medical Microbiology, School of Medicine, University of Manchester.

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Summary

The Sterilray™ Disinfection Wand is a novel system that emits Ultraviolet light (Far-UV) over a range of wavelengths from a hand held device. It is backed with several bold claims as an alternative to standard cleaning procedures for infection control within hospitals. Organisms responsible for nosocomial infections and therefore requiring eradication in hospitals include *Escherichia coli*, Vancomycin resistant *Enterococcus*, Methicillin resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Bacillus pumilus* is considered a model spore forming organism which can show UV resistance. Preparations of these organisms were made on Columbia agar plates, glass, fabric and Polyflor™ Polysafe anti slip flooring and irradiated with Far-UV from the Sterilray™ system. Environmental surfaces and objects within the hospital were tested using this system with swabs taken before and after Far -UV irradiation determining its 'in practice' efficiency. Effective sterilisation was observed for all organisms on agar plates and reductions in viable cells were observed on glass and flooring surfaces. No reduction was observed on fabric surfaces. Further testing on these surfaces is required before accurate conclusions can be made about the efficacy of the Sterilray™ device. Organisms found naturally on environmental surfaces were mostly destroyed and this system was shown to eradicate fungal spores. Results suggest that the Sterilray™ Disinfection Wand could be an efficient disinfection tool, especially since more powerful settings are yet to be trialled.

Background

Microorganisms are responsible for many millions of cases of disease each year and nosocomial infections have taken on greater significance in recent times. This is due to the increased prevalence of multidrug resistant 'superbugs' such as Methicillin resistant *Staphylococcus aureus* (MRSA) and Vancomycin resistant *Enterococcus* (VRE), which are problematic worldwide (Balkhy *et al* 2007, Fridkin *et al* 2003, Lee *et al* 2007, Tiemersma *et al* 2004). These organisms are very difficult to treat once patients have become infected as they are increasingly resistant to the narrowing number of drugs available for treatment (Yasliani *et al* 2009). The overuse of antibiotics over the last few decades has applied an evolutionary stress upon bacteria and certain bacterial strains have accumulated antibiotic resistance genes either via natural selection acting upon random mutation or by horizontal transfer from other bacteria.

Hospitals are often the centre of outbreaks because infected patients or healthcare workers can transfer infectious agents to surfaces and commonly used objects. Despite the best efforts of healthcare facilities to maintain a clean and safe environment, contact transfer of pathogenic microorganisms appears inevitable. Healthy individuals may also come into close contact with infected patients or contaminated surfaces and disease can spread rapidly.

Infection control guidelines have been proven to inhibit the spread of disease within hospitals. Standard principles set out by the National Institute for Health Clinical Excellence (NICE) involve hand hygiene and other methods of reducing the spread of infection when carrying out health care procedures on patients (NICE 2009). *Clostridium difficile* spores are tough and are difficult to destroy using the same procedures carried out to eradicate vegetative organisms. As a result, they can survive in the hospital environment for months if sufficient actions are not taken. Separate guidelines recommended by the Health Protection Agency (HPA) and adopted by the National Health Service are available on cleaning and hand hygiene along with recommendations for the prescription of antibiotics to susceptible individuals (HPA 2009). The strategy for antibiotic stewardship is difficult to determine since each individual will have different predisposing factors and susceptibility to the development of disease following drug treatment. Further widespread consultation among health care professionals is required to determine a universal strategy for antibiotic stewardship to prevent the development of resistance (Gould 2009).

Sporicidal disinfectants that are used in infection control contain high concentrations of chlorine (at least 1000ppm), which can be hazardous to health care workers and patients as well as damaging to the surfaces to which they are administered.

Dettenkofer and Block (2005) noted the potential for resistance to develop if there is overuse of disinfectants and this has already been discovered in methicillin-resistant *Staphylococcus haemolyticus* (Ben Saida *et al* 2008). Strict adherence to cleaning policies brings about effective disinfection of surfaces and allows safe use of hospital equipment and medical devices (Rutala and Weber 1999). However there is a clear need to develop alternative decontamination systems which work on a variety of surface types and are sufficiently effective for use in infection control.

Aim

To evaluate the efficacy of the Sterilray™ Disinfection Wand for biocidal activity against various pathogenic bacteria on different types of surface.

Objectives

1. Test the Sterilray™ Disinfection Wand against organisms that are typically responsible for hospital acquired infections on a variety of surface types including glass, fabric and flooring.
2. Establish whether this system is effective at eradicating spores on the surfaces mentioned above.
3. Determine the effect of this system on naturally occurring bacteria found on many different surfaces and objects within a hospital.
4. Verify whether the findings correlate with claims made by the manufacturer and, therefore, confirm whether this system could be considered an alternative to current infection control procedures within hospitals.

Materials

Bacterial strains

Bacillus pumilus ATCC 27142 (Raven Labs, USA).

Enterococcus faecalis (Vancomycin resistant *Enterococcus* (VRE)) NCTC 370

Escherichia coli, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (methicillin resistant (MRSA)) clinical isolates obtained from the Manchester Royal Infirmary bacteriology laboratories

Media

Columbia agar (Oxoid Ltd, UK)

Columbia agar with horse blood (CBA) (Oxoid Ltd, UK)

MacConkey agar (Oxoid Ltd, UK)

Cystine lactose electrolyte deficient (CLED) agar (Oxoid Ltd, UK)

Brain Heart Infusion (BHI) broth (Oxoid Ltd, UK)

Sterilray™ Disinfection Wand

The system is manufactured by Healthy Environment Innovations (HEI), USA produces single line emission in the Far-UV (F-UV) that has a wavelength shorter than 245nm. Experiments were carried out in the 'smooth' setting only, which has a power of 12.93mW cm⁻². Luminance remained constant since the distance between the irradiated surface and the lamp was always 65mm. The University of Manchester safety inspector found it to be safe to use. The maximum radiant dose that organisms were exposed to was 250mJ cm⁻² with an irradiation time of 66 seconds due to overheating issues with the lamp. The range of radiant doses the organisms were exposed to were 0, 20, 40, 60, 80, 100, 150, 200, and 250mJ cm⁻².

Additional equipment

ILT1700 radiometer (International Light Technologies, USA)

Rotamixer (Hook & Tucker Zenyx, UK)

U100H Sonicator (Ultrawave Ltd, UK)

Water bath

Spore strips (Raven Labs, USA)

Glass petri dish

Autoclave tape

P1000 Gilson pipette

P200 Gilson pipette

P20 Gilson pipette

P200 multichannel pipette

P20 multichannel pipette

Methods

Preparation of aerobic organisms

A 2 McFarland's standard solution was prepared for each organism; 100µl of each of these was added to separate 10ml BHI broths and incubated in a shaker at 37°C overnight. After this, broths were centrifuged at 4000rpm for 10 minutes and the pellet resuspended in 1ml of phosphate buffered saline (PBS).

Preparation of *B. pumilus* spores

Fifteen spore strips saturated with *B. pumilus* spores were left in 20ml of sterile distilled water for 24 hours. Twenty glass beads were added before this was sonicated for 2 hours. Once the strips had been reduced to a pulp, sterile forceps were used to compress this pulp and release as much liquid as possible. After the removal of the compressed pulp, the liquid underwent a heat shock process for 30 minutes at 80°C. The tubes were vortexed for 20 seconds before plating was carried out on Columbia agar using the Miles and Misra technique to ensure spores were isolated at a high concentration. Plates were incubated aerobically at 37°C overnight and examined for growth.

Columbia agar plates

A dilution series from 10⁰ to 10⁻⁷ was prepared for each organism in a microtitre plate. For all organisms, 10µl drops of each dilution were transferred in a line to Columbia agar plates as displayed in Figure 1. This was repeated twice more per plate. Plates were exposed to the allocated radiant doses which were measured

with a radiometer. Following aerobic incubation overnight at 37°C the organisms were enumerated using the Miles and Misra technique.

Glass Surface

Fragments of glass of area 1.5cm² were prepared from microscopy slides and sterilised in ethanol. Nine fragments per organism were required for each radiant dose to be applied and 10µl drops of undiluted bacteria were added to each fragment and left to dry. Following exposure to the pre determined radiant dose, 14µl of distilled water was added to each fragment and mixed to resuspend the irradiated cells. Only 10µl of this suspension was added to 90µl PBS to create a 10⁻¹ dilution from which a dilution series in PBS was created to 10⁻⁷. This series was plated on Columbia agar and incubated at 37°C overnight after which the organisms were enumerated using the Miles and Misra technique.

Fabric Surface

Fabric was obtained from a clean lab coat, cut into nine sets of 1cm² squares and autoclaved at 121°C for 20mins in a glass petri dish. Nine fabric squares were required per organism and 10µl drops of undiluted bacteria were added to each fragment and left to dry. Following exposure to the allocated radiant dose each fabric square was placed in 200µl PBS in a 1.5ml Eppendorf tube. These were vortexed for 30 seconds before 10µl of the solution was added to 90µl PBS to create a 10⁻¹ dilution from which a dilution series in PBS was created to 10⁻⁷. This dilution series was plated on Columbia agar, incubated at 37°C and enumerated using the Miles and Misra technique.

Flooring Surface

Polyflor™ Polysafe anti slip flooring was cut into 1.5 X 0.5cm fragments and autoclaved at 121°C for 20mins in a glass petri dish. Nine fragments per organism were required for each radiant dose to be applied and 10µl drops of undiluted bacteria were added to each fragment and left to dry.

Following exposure to the allocated radiant dose each flooring fragment was placed in 200µl PBS in a 1.5ml Eppendorf tube. These were vortexed for 30 seconds before 10µl of the solution was added to 90µl PBS to create a 10⁻¹

dilution from which a dilution series in PBS was created to 10^{-7} . This dilution series was plated on Columbia agar, incubated at 37°C and enumerated using the Miles and Misra technique.

Table 1. Environmental surfaces tested with the Sterilray™ disinfection wand and the locations of the samples taken

Environmental surface or object	Location of samples
Laboratory bench	4 areas
Floor	4 areas
Laboratory seat	4 seats
Sink	4 areas over 2 sinks
Toilet seat	4 areas over 2 seats
Telephone	4 areas over 2 telephones
Office desk	4 areas
Vowel keys on a computer keyboard	4 vowel keys
Door handle	4 door handles
Light switch	4 switches
Hand rail	4 different locations
Adjustable pipettes	4 adjustable pipettes
Soap dispenser	4 soap dispensers
Tap	4 taps
Incubator	3 areas

Environmental surfaces

A variety of surfaces were tested (shown in Table 1) to determine whether this system could eradicate bacteria from common locations and different surface types. All surfaces were exposed to 100mJ cm^{-2} measured with a timed exposure of 26 seconds. Swabs were taken before and after exposure and plated on CBA, MacConkey agar and CLED plates to aid the presumptive identification of species.

A 10 X 5cm template was used for the laboratory bench, flooring, a standard laboratory incubator, sink, seat, toilet seat and office desk and 7 diagonal crossings of the region were made with each swab. Care was taken to ensure that swabs carried out after irradiation were not taken from the same region as the swabs carried out before. This was to ensure that any difference in the numbers of bacterial colonies between before and after swabs was not due to removal of bacteria by previous swabbing.

Figure 4 shows the pattern used on light switches and computer keyboard keys. One side of the surface was swabbed before and the other side after irradiation for door handles, soap dispensers, taps, telephones, adjustable pipettes and hand rails. The incubator required cleaning to eradicate a known fungal contaminant and the Sterilray™ device was used in place of standard cleaning procedures allowing evaluation of its efficiency. However, only 3 samples were made and not the standard procedure of 4 for all other surfaces.

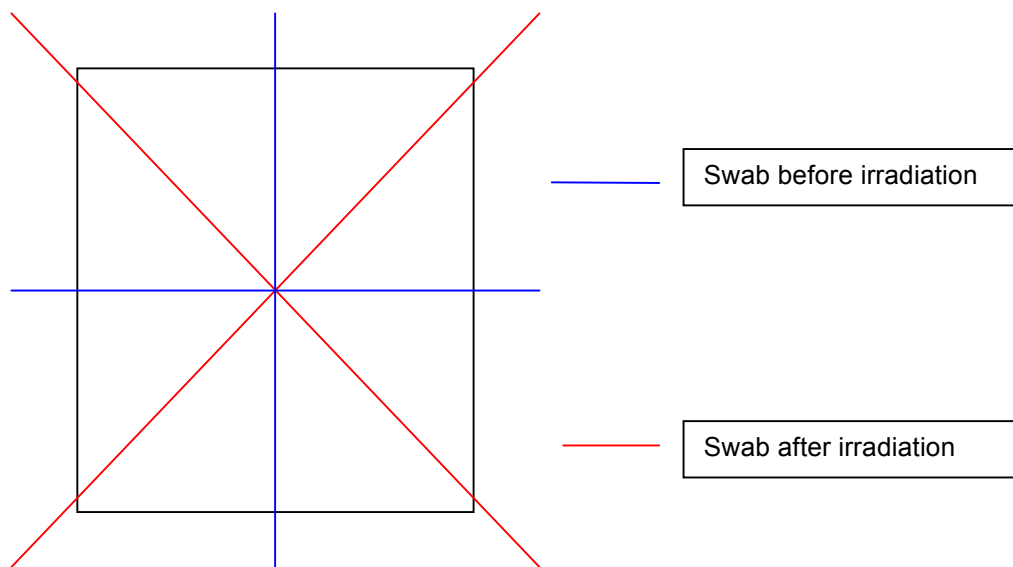


Figure 4. Swab patterns for light switches and computer keyboard keys

Results

Bacterial preparations on Columbia agar plates

The efficacy of this system against organisms irradiated on Columbia agar plates is shown in Figure 5. A ~10-log reduction in viable cells was observed for all vegetative cell types after exposure to a radiant dose of 250mJ cm⁻² apart from VRE which showed a 6-log reduction. However, a ~5-log reduction or greater was observed for all bacterial species tested after exposure to a radiant dose of 20mJ cm⁻². *B. pumilus* spores demonstrated an approximately 3-log reduction in viable cells after exposure to just 20mJ cm⁻² and this reduction did not differ after 250mJ cm⁻². The standard deviations were low as indicated by the small error bars, which suggests that accurate conclusions can be obtained from the data.

Bacterial preparations on glass

The efficiency of this system against bacteria irradiated on glass is shown in Figure 6. *P. aeruginosa* results were obtained using a wet preparation of bacteria on the glass since the drying process appeared to kill the cells.

An 8-log reduction in viable cells was observed for MRSA after exposure to a radiant dose of 150mJ cm⁻² with greater than a 3-log reduction following exposure to just 20mJ cm⁻². VRE showed a 3-log reduction by 20mJ cm⁻² and a total 5-log reduction after exposure to 250mJ cm⁻². Data for *E. coli* and *P. aeruginosa* are less clear but both show a general decrease in the number of cells that survive with a 2-log and 3-log reduction respectively. *B. pumilus* shows a dramatic decrease in viable cells following exposure of spores to Far-UV and by 40mJ cm⁻² a 5-log reduction is observed.

Bacterial preparations on fabric

The efficacy of this system on bacteria irradiated on fabric is shown in Figure 7. None of these organisms (including *B. pumilus* spores) show a significant reduction in viable cells following exposure to 250mJ cm⁻².

Bacterial preparations on Polyflor™ Polysafe anti slip flooring

The efficacy of this system on bacteria irradiated on flooring is shown in Figure 8. *E. coli* and MRSA did not show a significant reduction in viable cells that survive exposure up to 250mJ cm⁻² of Far-UV and the relatively low standard deviations suggest that this was an accurate trend. A 3.5-log reduction in viable cells was observed for VRE following exposure to 40mJ cm⁻² and this did not change after exposure to 250mJ cm⁻². Results for *P. aeruginosa* are not consistent although a 7-log reduction in viable cells was observed by 200mJ cm⁻². *B. pumilus* spores also show a 4.5-log reduction after exposure to 200mJ cm⁻².

Environmental surfaces and objects

Table 2 shows the effect of this system on organisms present naturally on common surfaces and objects found in hospitals. The results for individual surfaces are shown in Tables 3-16 in the appendix. In nearly all instances, the Sterilray™ Disinfection Wand reduced the number of organisms found and often completely disinfected a surface. There was also a large reduction in the number of cells found following exposure on more heavily contaminated surfaces such as toilet seats and taps.

A Mann Whitney U test was carried out on the collective data and the p value obtained was <0.001 indicating that the decrease in the number of viable cells observed after irradiation is a significant effect. The Sterilray™ system was shown to eradicate aerobic spore bearers (ASB) as well as fungus and fungal spores found on various surfaces but particularly within the contaminated incubator. The most frequent organisms to survive the irradiation process were the *Micrococcus* spp.

Table 2. Microorganisms found on hospital surfaces before and after exposure to 100mJ cm⁻² from a Sterilray™ Disinfection Wand.

Organism	Before Irradiation	After Irradiation
<i>Staphylococcus</i> spp.	337	24
<i>Streptococcus</i> spp.	160	11
<i>Micrococcus</i> spp.	159	31
<i>Enterobacteriaceae</i> spp.	84	1
<i>Aspergillus</i> spp.	79	6
ASB	26	3
<i>Penicillium</i> spp.	12	0
Fungus	10	0
<i>Pseudomonas</i> spp.	9	1
<i>Enterococcus</i> spp.	9	0
Yeast	7	0
<i>Bacillus</i> spp.	2	0
Unidentified	2	0
Total	896	77

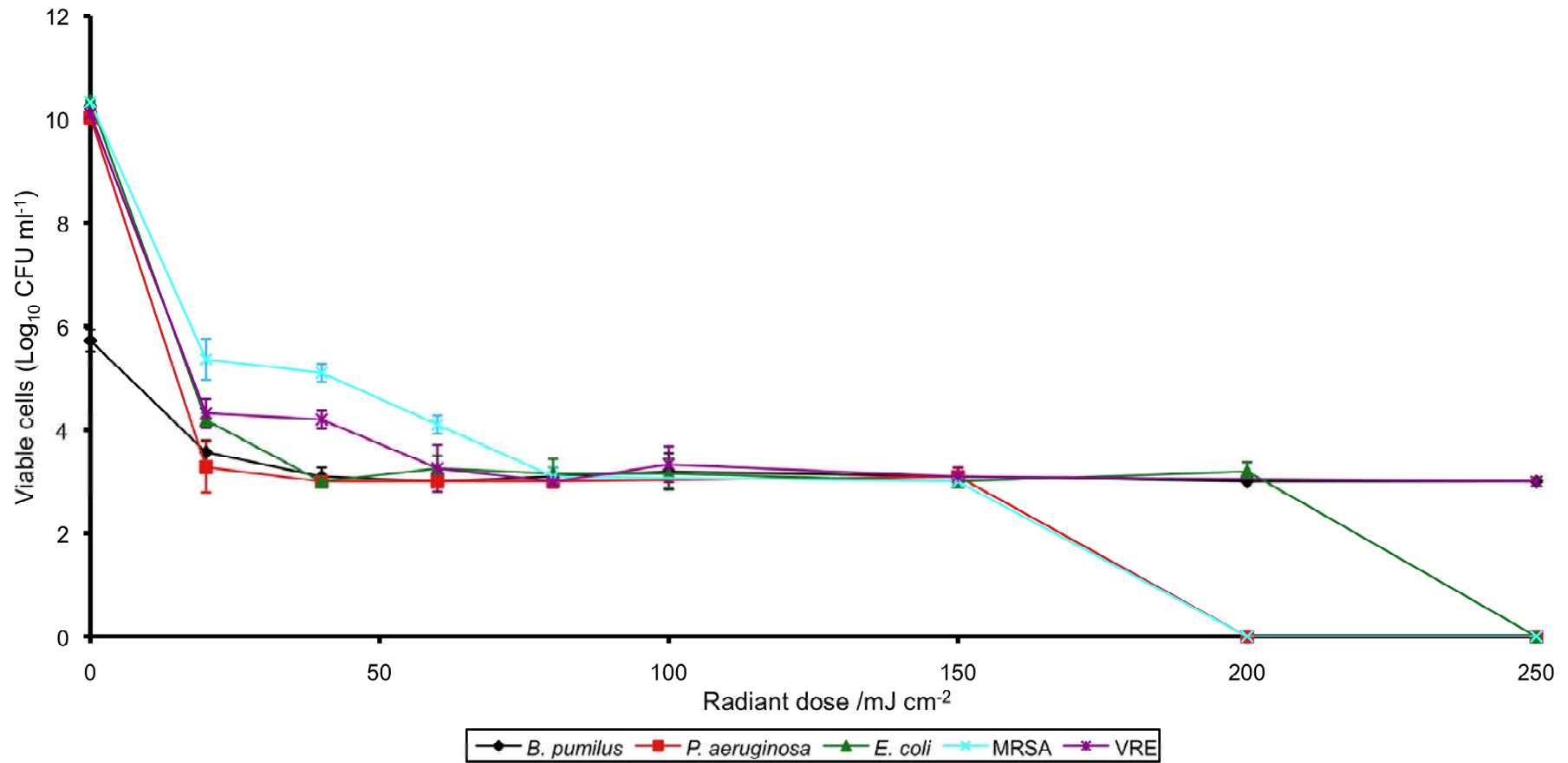


Figure 5. Viable cells observed after exposure of vegetative organisms and *B. pumilus* spores on Columbia agar plates to a range of radiant doses from a Sterilray™ Disinfection Wand. The error bars indicate the standard deviation at each data point.

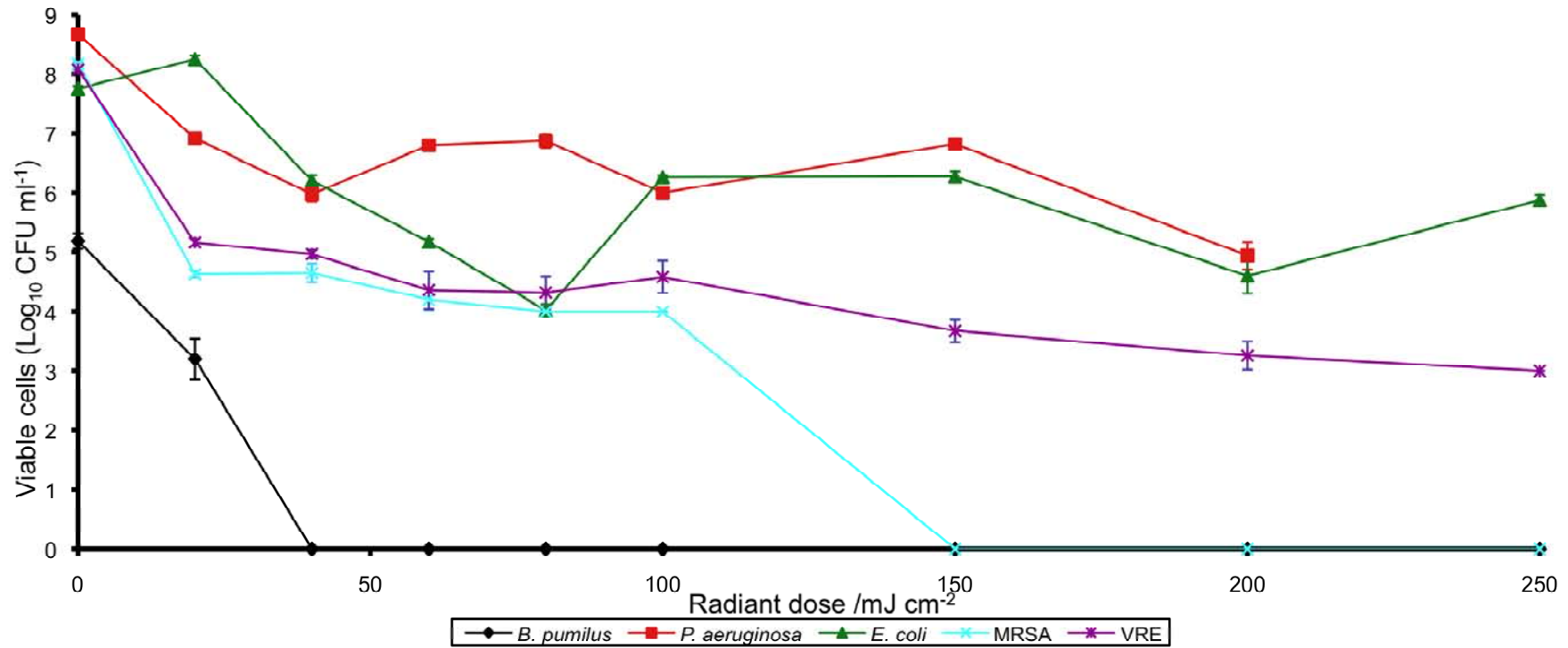


Figure 6. Viable cells observed after exposure of vegetative organisms and *B. pumilus* spores on glass fragments to a range of radiant doses from a SterilrayTM Disinfection Wand. The error bars indicate the standard deviation at each data point.

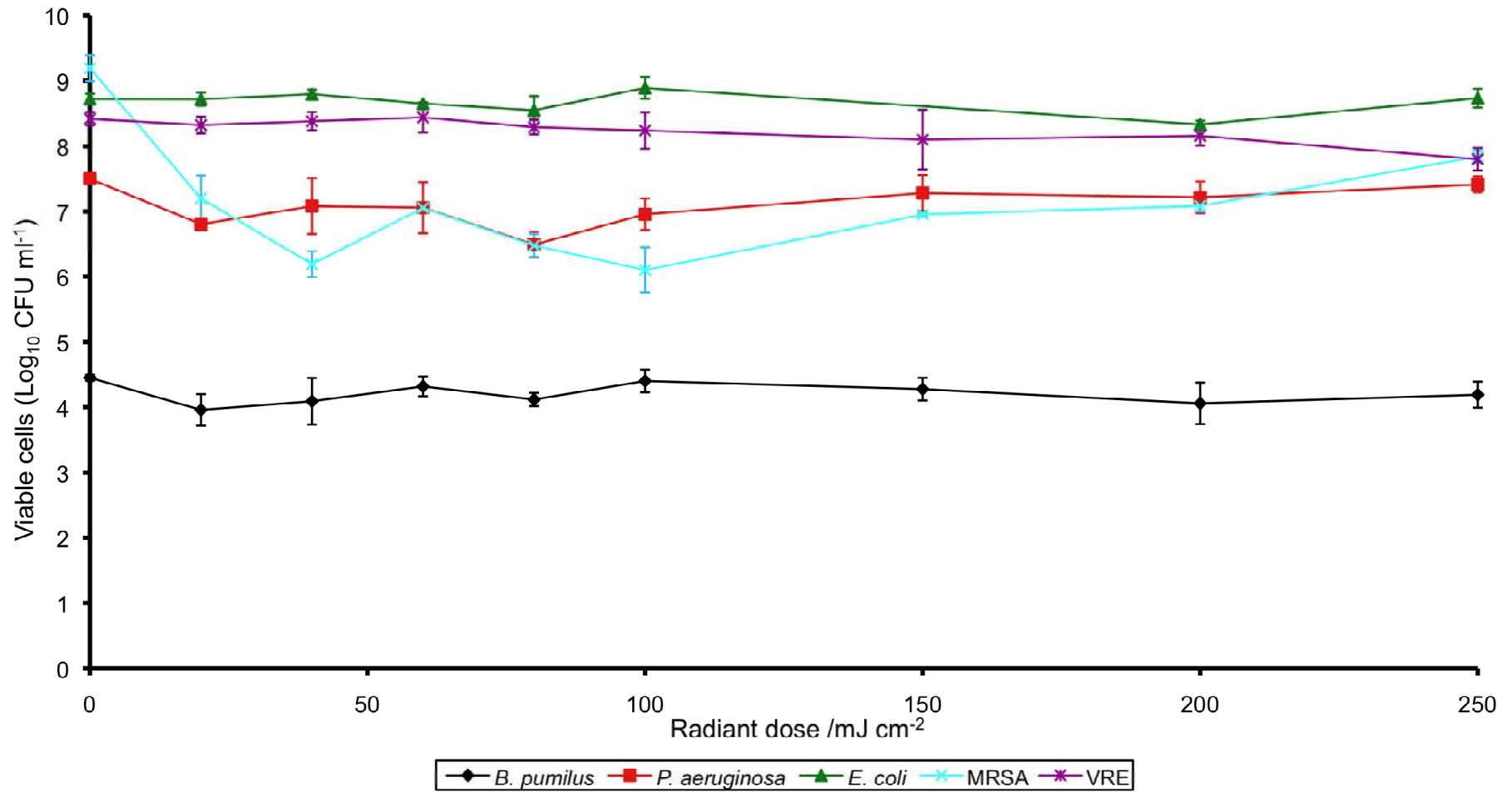


Figure 7. Viable cells observed after exposure of vegetative organisms and *B. pumilus* spores on a fabric surface to a range of radiant doses from a Sterilray™ Disinfection Wand. The error bars indicate the standard deviation at each data point.

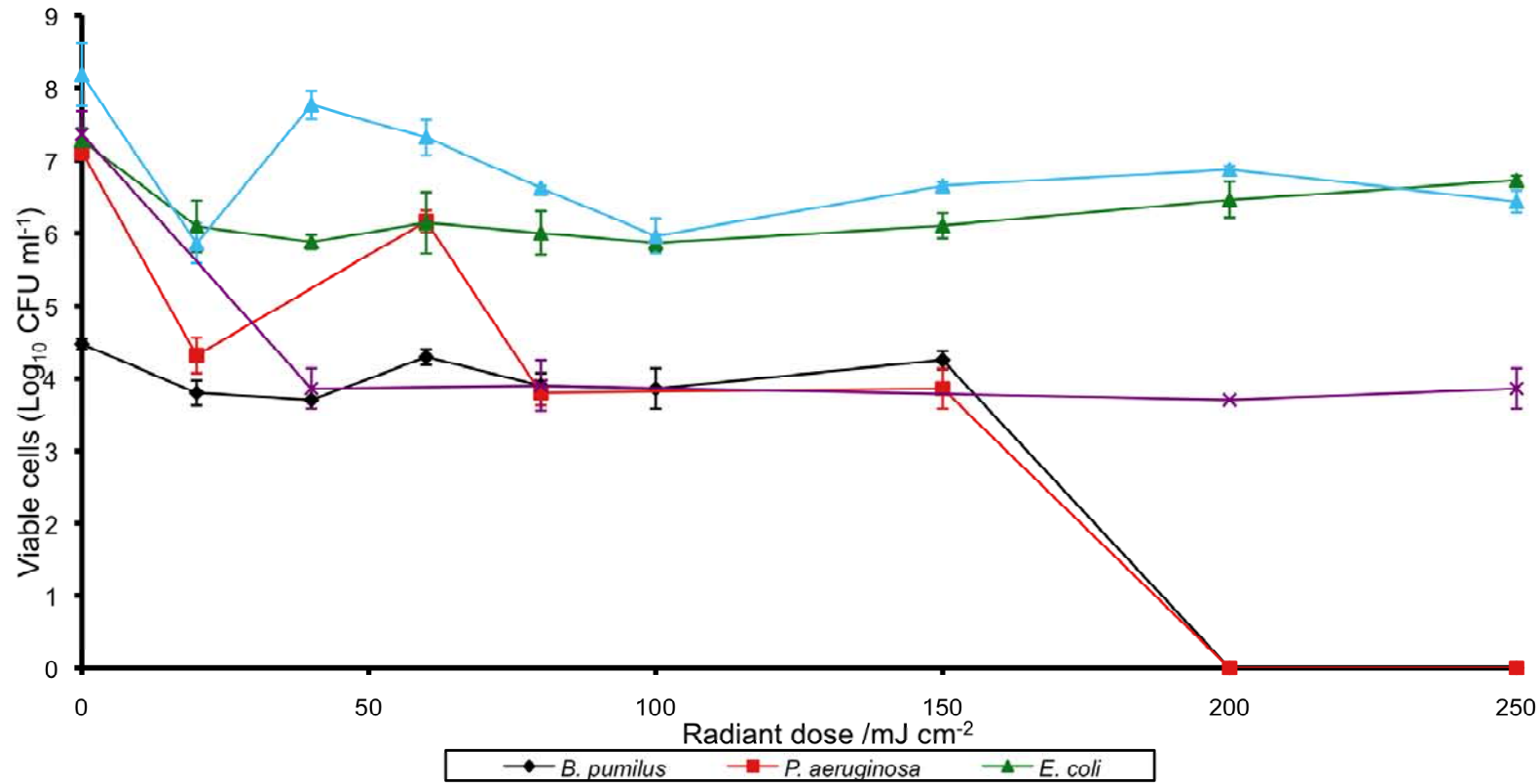


Figure 8. Viable cells observed after exposure of vegetative organisms and *B. pumilus* spores on Polyflor™ Polysafe anti slip flooring surface to a range of radiant doses from a Sterilray™ Disinfection Wand. The error bars indicate the standard deviation at each data point.

Conclusions

A thorough laboratory based evaluation has been carried out of the efficacy of the Sterilray Device against a range of bacteria under artificial conditions and against environmental contaminants. The data obtained are extremely encouraging and the device represents a strong candidate for use in clinical areas for the reduction of bacterial and fungal loads on solid surfaces.