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Original Article

Effectiveness of an ultraviolet-C disinfection system for reduction of healthcare-associated pathogens

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KEYWORDS
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Abstract *Background:* Healthcare-associated infections caused by multidrug-resistant (MDR) pathogens are significantly associated with increased mortality and morbidity. Environmental cleaning can reduce transmission of these pathogens but is often inadequate. Adjunctive methods are warranted to enhance the effectiveness of disinfection particularly in hospital settings where healthcare-associated infections are of major concern.
Methods: We conducted a study to examine the effectiveness of a mobile, automatic device, Hyper Light Disinfection Robot (model: Hyper Light P3), which utilized ultraviolet-C (UV-C) to kill MDR-*Pseudomonas aeruginosa*, MDR- *Acinetobacter baumannii*, methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecium (*VRE), *Mycobacterium abscessus* and *Aspergillus fumigatus*. The performance of this device in disinfecting hospital rooms previously admitted by patients harboring MRSA and VRE was also assessed.
Results: Except for VRE and *M. abscessus*, more than 3 $\log_{10}$ reduction of vegetative bacteria colonies was observed after UV-C irradiation of 5 min at a distance of 3 m from the device. At the distance of 1 m, substantial and comparable reduction of colonies was observed across all tested microorganisms regardless of exposure time. The killing effect was less pronounced for *A. fumigatus* particularly at the distance of 2–3 m. In uncleaned hospital rooms, there was significant reduction in the number of bacteria colonies sampled from different surfaces after UV-C irradiation for 15 min.
Conclusions: UV-C disinfection system was effective in killing MDR pathogens. Further study is warranted to confirm its effectiveness as an adjunctive method in disinfecting hospital environment.

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Introduction

Healthcare-associated infections caused by multidrug-resistant pathogens are significantly associated with increased mortality, morbidity and excessive healthcare costs.[1] Thorough cleaning of hospital environment is crucial in limiting transmission of pathogens and reducing healthcare-associated infections. However, up to one half of room surfaces were found to be inappropriately cleaned by traditional manual methods of disinfection using various assessment tools (e.g., visual observation, adenosine triphosphate bioluminescence, aerobic colony counts).[2–5] Despite several interventions such as education, improving cleaning technique, performance feedback have been used to improve cleaning effectiveness, the effects were limited.[3–7] Therefore, in addition to traditional interventions, some novel, no touch methods are warranted to improve terminal room disinfection. The ultraviolet (UV) disinfection system is among one of the novel technologies that have been intensely investigated as an alternative to conventional disinfection procedures for killing pathogenic and spoilage microorganisms in hospital settings. Currently, most UV disinfection devices primarily utilize ultraviolet-C (UV-C) radiation with wavelengths between 200 and 270 nm. At particular wavelengths such as 254 nm, UV-C light is able to destroy the molecular bonds and disrupt DNA or RNA via pyrimidine dimerization, causing death of a variety of environmental microorganisms.[8] The UV-C device offers several advantages compared with standard room disinfection using traditional disinfectants. These include germicidal activity against broad-spectrum organisms, shorter time requirement for vegetative bacteria, safety and eco-friendly without hazardous residual, saving costs such as labor and consumables, and relatively simple way to set up and operate in healthcare facilities.[9,10] Additionally, disinfection of the surfaces that are frequently missed by cleaning staff or that are already cleaned manually but still contaminated due to difficult retention of disinfectant (eg. edges of tables).[9] However, different UV-C devices varied in their performance and required time in inactivating microorganisms.[7,11–13] Moreover, the effectiveness of UV-C devices in eradicating nosocomial pathogens other than bacteria, such as fungus and mycobacteria, had been scarcely addressed.

In previous study, a 6 log pulsed UV-C system (Mediland Enterprise Corporation, Taoyuan, Taiwan, R.O.C) has been reported to be effective in reducing several bacteria by pulsed UV-C irradiation including methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, vancomycin-resistant *Enterococcus faecium* (VRE) and *Clostridium difficile*, by more than 4 $\log_{10}$ reduction (up to 6 $\log_{10}$ reduction) within 5–15 min at a distance of 2.7 m.[14] The Hyper Light Disinfection Robot, model: Hyper Light P3 (Mediland Enterprise Corporation, Taoyuan, Taiwan, R.O.C) is a mobile, automatic device, which is made for environmental disinfection by UV-C irradiation (254 nm). The primary objective of this study was to assess the effectiveness of the Hyper Light P3 in reduction of the most frequently encountered multidrug-resistant clinical isolates, namely *P. aeruginosa* (MDRPA), *Acinetobacter baumannii* (MDRAB), MRSA, VRE, *Mycobacterium abscessus* and *Aspergillus fumigatus*, on solid and liquid media. The impact of using this device in disinfection for patient rooms in hospital setting was also evaluated.

Methods

Setting

The study was conducted at National Taiwan University Hospital, a 2400-bed acute care medical center in Taipei, northern Taiwan, during the period October 2015 to March 2016.

Preparation of bacteria

Stored clinical strains of multidrug-resistant *P. aeruginosa* (MDRPA), *multidrug-resistant A. baumannii* (MDRAB), VRE and ATCC strains of methicillin-resistant *S.aureus* (MRSA, ATCC 33592), and *M. abscessus* (ATCC 19977) were used for in vitro study. All bacteria were subjected to identification and susceptibility testing in accordance with Clinical Laboratories Standards Institute (CLSI) guidelines.[15] One ml of each isolates suspended in phosphate buffered saline were spread on sterilized petri dish to cover the whole surface.

Preparation of fungal spores

Standard strains of *A. fumigatus* (ATCC 204305) were used for this experiment. A sample of the pure culture of each fungal species were streaked onto the slanted surface of sabouraud dextrose agar medium and incubated for 3 days at 35 °C. Fungal spores were collected by washing with sterile deionized water containing 0.05% Tween 80. Spores in suspension were counted microscopically (×40 magnification) using a hemocytometer and the viability of the spores were confirmed by culturing aliquots of a 250 μl serially diluted suspension onto sabouraud dextrose agar plates. To prevent microbial growth, fungal stock suspensions were stored at 4 °C for all experiments.

The Hyper Light P3 device

The device is 1.9 m tall and measures 0.72 m at the widest portion of the base. The device was designed for manipulation by a single operator. It was operated remotely outside the room and included multi-motion sensors, which turn off the device automatically to prevent potential injury if the door is accidentally opened. There was one petri dish placed on the table in the laboratory in each UV-C irradiation cycle. The height from petri dish to ground was 78 cm. The device delivers a dose of 2750 μW/cm2 at a distance of 1 m. The device was wheeled into different strategic position that was 1 m from the petri dish. Then, the device was wheeled to a distance of 2 and 3 m from other sets of petri dishes with the same number of colonies, respectively. The above experiments were repeated with 5, 10 and 15 min of exposure time. Baseline petri dishes were left untreated outside of the room (i.e., positive controls). This protocol was repeated for each tested pathogens.

Quantification of colonies

A total of 9 ml of phosphate buffered saline was used to wash thoroughly the bacteria or fungi on petri dish. The studied bacteria were plated on trypticase soy agar containing 5% sheep blood and incubated at 37 °C for 16 h; whereas the fungi under investigation were cultured at 35 °C for 48 h. Colony-forming units (CFU) were enumerated by serially diluting and plating suspensions on selective agar.

Disinfection of environmental surfaces in patient rooms

The efficacy of the Hyper Light P3 device was assessed in three uncleaned rooms previously admitted by patients harboring MRSA, VRE and other nosocomial pathogens with at least a 7-day hospitalization. Swabs premoistened with saline were used to collect cultures from seven high-touch surfaces (e.g., bedside table, telephone and bedrail) in each room before and after use of the Hyper Light P3 device. The device was placed in three different locations in the room (living area, central of the room and in front of restroom) and high-touch surfaces such as bedrails, bedside table and switch were as close as possible to the device to get optimal exposure to UV-C device (Fig. 1). The operator leaved the room and closed the door. Then he used remote control to start the machine, which ran for 5 min at each site (total 15 min) and stopped automatically. The device was wheeled to another location by one person between UV-C radiation cycles. An approximately 10 × 10-cm area was cultured before UV-C disinfection and adjacent areas of the same size were cultured after disinfection. Each swab was used to inoculate on selective media for culture. Enumeration of the total colony counts was performed as described above.

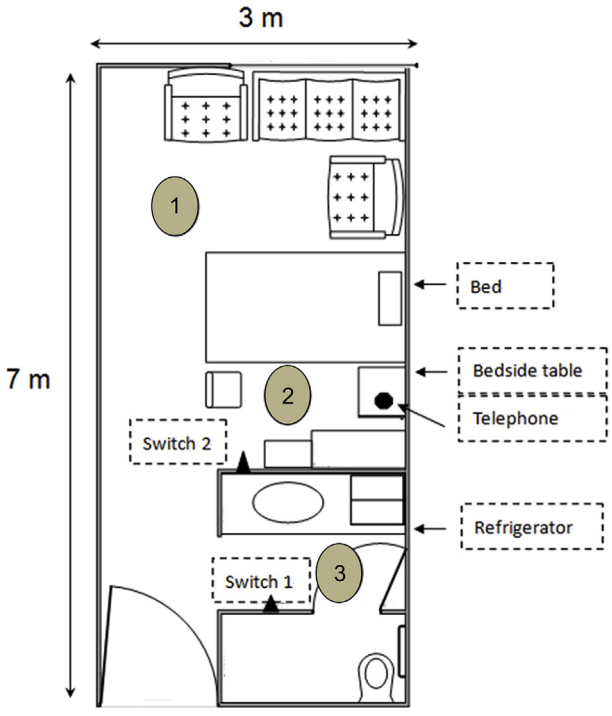

Figure 1. Schema of representative patient's room showed the positioning of the Hyper Light P3 device and area of sampling. The UV-C device was placed in three different locations (number 1, 2, and 3, which indicated living area, central of the room and in front of restroom, respectively) in the room and ran for 5 min at each site (total 15 min). We operated the device outside the room by remote control. The device was wheeled to another location by one person between UV-C irradiation cycles.

Statistics

Statistical analysis was performed at a two-sided significance level of 0.05, using GraphPad Prism 6 (GraphPad Software Inc., La Jolla, CA, USA). Median colony counts before and after application of UV-C irradiation was analyzed using Wilcoxon signed rank test. Percent reduction and $\log_{10}$ CFU reductions of colonies were calculated as follows:

$$\text{Percent reduction} = \frac{(B - A)}{B} X100$$

$$\text{Log}_{10}\text{ CFU reduction} = \text{Log}_{10}(B - A)\text{ CFU}$$

Where:

B = Number of viable microorganisms.
A = Number of viable microorganisms after UV-C irradiation.

Results

In vitro effectiveness of the Hyper Light P3 device in killing bacteria, mycobacteria and fungi

Overall, the killing efficacy of the Hyper Light P3 device was greater when the distance of petri dishes to UV-C device was shorter (1 m ≥ 2 m ≥ 3 m) and the exposure time was longer (15 min ≥ 10 min ≥ 5 min) (Table 1). The total $\log_{10}$ reduction of CFU/cm^2 is illustrated in Fig. 2. In the most stringent condition (5 min exposure at a distance of 3 m), more than 3 $\log_{10}$ vegetative bacteria could be killed by UV-C, except for VRE and *M. abscessus*, the reduction of which decreased to 1.5—2.5 $\log_{10}$ CFU. Substantial and comparable reduction of colonies was observed across all tested bacteria regardless of exposure time at the distance of 1 m. The killing effect was less pronounced for *A. fumigatus* particularly at the distance of 2—3 m.

Efficacy of the Hyper Light P3 device in disinfection of patient rooms

UV-C decontamination of surfaces in three hospital rooms that had been occupied by VRE and MRSA carrier was examined, and the samples collected from various environmental surfaces before and after UV-C irradiation were incubated for 24 h and 48 h, respectively (Table 2). A total of twenty high-touch surfaces were sampled. Various bacteria colony counts sampled from different surfaces were

Table 1 Number of colony forming units of bacteria or fungi recovered after UV-C irradiation for specified time at different distance from the Hyper Light P3 device.

	Colony-forming units					
	MRSA	MDRAB	MDRPA	VRE	*M. abscessus*	*A. fumigatus*
5 min						
Control[a]	1.5×10^7	2.4×10^7	1.8×10^7	2.8×10^7	7.2×10^7	3.0×10^6
1 m	0	0	0	0	0	1000
2 m	600	0	200	1600	4600	300,000
3 m	4800	4200	3000	800,000	200,000	700,000
10 min						
Control[a]	7×10^8	3.5×10^7	8.2×10^8	3.4×10^8	9.2×10^8	3.0×10^6
1 m	0	0	0	0	0	600
2 m	400	0	0	0	800	240,000
3 m	2600	400	600	600	16,000	380,000
15 min						
Control[a]	1.02×10^9	1.08×10^8	2.6×10^7	1.6×10^7	5.0×10^7	3.0×10^6
1 m	0	0	0	0	0	200
2 m	0	0	0	0	0	2200
3 m	800	0	200	200	400	140,000

[a] Control means bacteria or fungus growth on baseline agar plates which were left outside of the room without UV-C irradiation.
MRSA: methicillin-resistant *Staphylococcus aureus*; MDRAB: multidrug-resistant *Acinetobacter baumanni*; MDRPA: multidrug-resistant *Pseudomonas aeruginosa* (MDRPA); VRE: vancomycin-resistant *Enterococcus faecium*; *M. abscessus*: *Mycobacterium abscessus*; *A. fumigatus*: *Aspergillus fumigatus*.

found, whereas five (25%) and one (5%) surfaces showed no bacteria growth before UV-C irradiation. Most reduction rates of total bacteria colony counts sampled from different surfaces in 3 patients' room after UV-C irradiation were 100%, except that of bedrail, bedside table and telephone (ranging from 0% to 98%) (Table 2). Significant reduction in the median number of total bacteria colony counts after UV-C irradiation of 15 min was demonstrated after 24 h incubation (35 CFUs vs 0 CFUs, $p = 0.0005$) and 48 h incubation (165 CFUs vs. 0 CFUs, $p < 0.0001$) of the samples respectively (Table 3).

Discussion

The present study demonstrated that the Hyper Light P3 device was effective in killing healthcare-associated multidrug-resistant bacteria (MRSA, MDRAB, MDRPA, VRE), as well as *M. abscessus* and *A. fumigatus* in vitro. Significant reduction of bacteria on different surfaces in uncleaned hospital rooms previously occupied by carriers of MRSA, VRE and other nosocomial pathogens was also observed.

Previous experimental studies have shown that the duration of irradiation exposure was a crucial determinant of the performance of the UV-C device.[9,13,16,17] Under experimental setting, more than 3—4 $\log_{10}$ vegetative organisms could be inactivated within 15—93 min by UV-C irradiation.[9,13,16,17] Main targeted organisms included MRSA, VRE and MDRAB. Nerandzic et al. revealed that using two different UV-C disinfection devices, the Pathogon (Steris Corporation, Mentor, Ohio, USA) and the Tru-D (Lumalier Corporation, Memphis, TN, USA) devices at fixed distance (1.22 m), the killing efficacy decreased mildly for MRSA from more than 4 $\log_{10}$ CFU/cm^2 to approximately 3 $\log_{10}$ CFU/cm^2 and for VRE from more than 5 $\log_{10}$ CFU/cm^2 to approximately 4 $\log_{10}$ CFU/cm^2 after UV-C irradiation of 20—40 min, compared with 10 min.[13] Cadnum et al. also demonstrated the similar effectiveness of two different UV-C disinfection devices, Optimum-UV (Clorox Company, Oakland, CA, USA) and the Tru-D devices for MRSA at similar setting (at a distance of 1.22 m, within 5—40 min).[18] Our study showed that the Hyper Light P3 device could reduce not only MRSA, VRE, but also MDRAB, MDRPA and *M. abscessus* by more than 4 $\log_{10}$ CFU/cm^2 after UV-C irradiation with a shorter duration (5 min) at a distance of 2 m.

Previous studies also revealed the shorter distance from agar plate to the UV-C device, the greater killing efficacy the device could achieve.[9,13] Nerandzic et al. reported that at the distance of 1.22 m from the devices, UV-C irradiation of 41 min could reduce MRSA by more than 4 $\log_{10}$ CFU/cm^2, VRE by more than 5 $\log_{10}$ CFU/cm^2. However, when the distance increased to 3.05 m from the device with the same exposure time, the killing efficacy of the device decreased to less than or equal to 3 $\log_{10}$ CFU/cm^2 for both MRSA and VRE.[13] The Hyper Light P3 device could reduce all studied vegetative bacteria including MRSA and VRE by more than 4 $\log_{10}$ CFU/cm^2 within 10 min in average, even at a distance as far as 3 m.

The novelty of the present study is the demonstration of the impact of the UV-C disinfection system against growth of nontuberculous mycobacteria (NTM) and Aspergillus under experimental setting, which was rarely addressed in previous studies. NTM have been increasingly reported to cause iatrogenic bloodstream infection, catheter-related infection, skin and soft tissue infections, surgical site infection as well as outbreaks in the hospitals.[19—21] These emerging pathogens are very hard to be eliminated from the hospital environment with traditional disinfectants and the disease they caused are also difficult to cure.[21] Besides

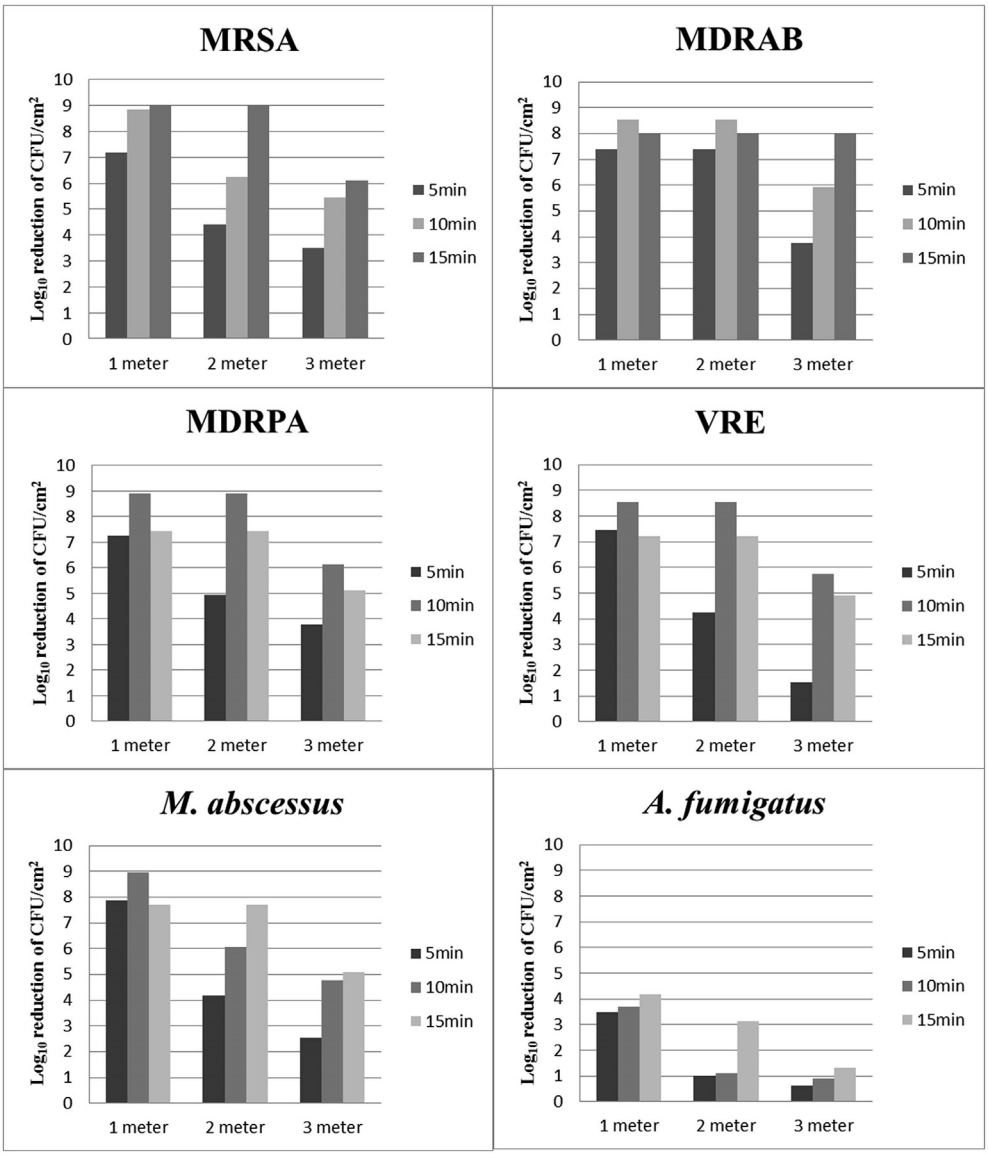

Figure 2. The effectiveness of the Hyper Light P3 device on reducing various strains of bacteria or fungi expressed in $\log_{10}$ reduction of CFU/cm^2. Abbreviations: CFU, colony-forming units; MRSA, methicillin-resistant *Staphylococcus aureus*; MDRAB, multidrug-resistant *Acinetobacter baumanni*; MDRPA, multidrug-resistant *Pseudomonas aeruginosa* (MDRPA); VRE, vancomycin-resistant *Enterococcus faecium*; *M. abscessus*, *Mycobacterium abscessus*; *A. fumigatus*, *Aspergillus fumigatus*

water supply system, contaminated aqueous solutions and inadequate high-level disinfection of medical devices such as bronchoscopy and endoscopy,[22] NTM infections may be caused by potentially contaminated room environment or indirect person-to-person transmission, especially in cystic fibrosis patients.[23–25] A few reports showed that UV irradiation might be potentially used in reducing numbers of water-borne NTM in potable water distribution systems and controlling airborne NTM and fungal spores with room air cleaners.[26,27] Aspergillosis is the second common cause of invasive fungal infections in healthcare settings, causing morbidity and mortality particularly in immunocompromised patients including those whom with hematologic malignancy and hematopoietic stem cell transplant recipients.[28,29] In spite of special interventions (e.g., installation of high-efficiency particulate air (HEPA) filters or use of laminar air flow rooms), healthcare-associated aspergillosis continues to occur.[30] Our study showed that *A. fumigatus*, the most prevalent *Aspergillus* spp. causing human diseases, could be reduced by more than 3 $\log_{10}$ CFU/cm^2 after being irradiated by UV-C within 5—15 min at the distance of 1 m in vitro. It is worth further investigation whether the device could be applied in disinfection of hospital rooms occupied by immunocompromised hosts where aspergillosis is a great concern (e.g., bone marrow transplant units).

Although nearly 100% reduction of bacteria colonies could be reached on most of the tested surfaces by using the Hyper Light P3 device for room decontamination before manual cleaning, the UV-C device should be used as an adjunctive method of enhanced terminal room disinfection to standard housekeeping cleaning instead of replacing it in

Table 2 Reduction in bacteria colony counts on different surfaces in 3 patient rooms after UV-C irradiation.

Room	Site of sampling	No. of CFU after 24 h incubation			No. of CFU after 48 h incubation		
		Before UV-C	After UV-C	Reduction (%)	Before UV-C	After UV-C	Reduction (%)
A	Left bedrail	20	0	100	60	0	100
	Right bedrail	0	0	NA	1020	0	100
	Top of bedside table	50	0	100	250	0	100
	Telephone	280	0	100	1120	1	100
	Door knob of refrigerator	170	0	100	180	0	100
	Switch 1	120	0	100	220	0	100
	Switch 2	0	0	NA	0	0	NA
B	Left bedrail	10	0	100	80	110	0
	Right bedrail	0	10	0	130	30	77
	Top of bedside table	490	10	98	4370	550	87
	Telephone	10	0	100	70	20	71
	Switch 1	100	0	100	130	0	100
	Switch 2	30	0	100	80	0	100
C	Left bedrail	60	0	100	180	0	100
	Right bedrail	0	0	NA	30	0	100
	Top of bedside table	1700	90	95	3740	520	86
	Telephone	0	30	0	230	80	65
	Door knob of refrigerator	40	0	100	320	0	100
	Switch 1	20	0	100	20	0	100
	Switch 2	140	0	100	150	0	100

No.: number; CFU: colony-forming units; NA: not available.

Table 3 Analytical data and comparison of bacteria colony counts on different surfaces in 3 patients' rooms before and after UV-C irradiation with incubation for 24 and 48 h.

UV-C irradiation	Incubation time (hours)	No. of samples	Median CFU (IQR)	Min	Max	P value
Before UV-C	24	20	35 (2.5—135)	0	1700	0.0005
After UV-C	24	20	0 (0)	0	90	
Before UV-C	48	20	165 (72.5—302.5)	0	4370	<0.0001
After UV-C	48	20	0 (0—27.5)	0	550	

No.: number; CFU: colony-forming units; IQR: interquartile range; Min: minimum; Max: maximum.

clinical practice for several reasons. First, in consistent with previous studies, the effectiveness of the Hyper Light P3 device was lowered on shadowed sites, such as bedrail, bedside table and telephone.[7,9,16,17] Second, the UV-C device may not be feasible to use in double or triple room because other patients who are still admitted (especially disabled) in the same room will get harm from UV exposure.[10,16] Therefore, it may be more practical to use the UV-C device for final disinfection of single room, rather than for daily cleaning. Third, the device might not be applicable in crowded wards or large open space due to the consideration of safety and efficacy of UV-C device. Lastly, UV-C irradiation does not remove dust and stains, and the killing efficacy may be reduced if dirt and debris exist.[13,31]

Our study had several limitations to be noted. We did not evaluate the impact of the Hyper Light P3 device on other important nosocomial pathogens such as *C. difficile* or carbapenem-resistant Enterobacteriaceae (CRE) in experimental setting. Previous studies have shown that UV-C irradiation has good efficacy to inactivate *C. difficile or* CRE.[9,32] However, longer exposure time (usually more than 40 min) and larger accumulative doses of UV-C irradiation might be required to kill spore-forming microorganism or to achieve the sporicidal activity.[9,13,16,18] In addition, it was performed in three single rooms in only one hospital. Therefore, the results may not be generalizable to other settings. Furthermore, in contrary with previous studies,[9,16,31] we only demonstrated the killing effects of UV-C irradiation on all organisms on various surfaces in three hospital rooms instead of focusing on targeted multidrug-resistant pathogens. As such, we were unable to evaluate the effectiveness of UV-C disinfection for particular pathogen of interest.

In conclusion, the Hyper Light Disinfection Robot (model: Hyper Light P3) was effective in killing a number of multidrug-resistant bacteria, mycobacteria and fungi that are commonly encountered at hospital environment. A larger scale of clinical study is warranted to confirm its effectiveness as an adjunct to standard cleaning in reduction of nosocomial pathogens in healthcare settings.

Conflicts of interest statement

All authors declare no conflicts of interest.

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