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Evaluation of pulsed xenon ultraviolet irradiation of continuous spectrum for efficacy against multidrug-resistant nosocomial strains

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ABSTRACT

The present study demonstrated the evaluation results of pulsed xenon ultraviolet irradiation of continuous spectrum for efficacy against multi-drug resistant strains with high epidemic potential and resistance against main groups of chemical disinfectants (MRSA, VRE, A. baumannii, P. aeruginosa, Proteus mirabilis, S. aureus). Microbial concentration on plastic and metal surfaces was at least 10^7 CFU/cm^2. The samples were placed horizontally and vertically at 1.2-2 meters' distance from the irradiation source and treated for 5, 10 or 20 minutes. After 5 minutes' treatment the microbial reduction was at least 4.9 lg for all the pathogens and surface materials. The presence of bioburden on samples did not affect the disinfection efficacy. The decontamination efficacy for metal plates reduced insignificantly.

Keywords: MRSA, VRE, bacteria, decontamination, hospital strains, multi-drug resistance, efficacy, pulsed light, ultraviolet

INTRODUCTION

In recent decades increasing attention is paid to the development of prevention measures against Healthcare-associated infections (HAIs), caused by nosocomial bacterial strains with multiple drug resistance and resistance against main groups of chemical disinfectants. Most important current infectious agents of HAIs – Methicillin-resistant *Staphylococcus aureus* (MRSA) and Vancomycin-resistant *Enterococcus* (**VRE**) – are able to induce various clinical forms of infections: pneumonia, bacteremia, urinary tract infection, meningitis, osteomyelitis and others. According to present knowledge [1] MRSA induces yearly 170,000 infections in Europe, about 5,000 of them with fatal outcome. The additional cost of treating patients with MRSA is about €380 million yearly. In the USA this value reaches about $9.7 billion a year [2]. The detection frequency of VRE caused HAIs is about 40% in European countries [3] depending on the country and measures of infection prevention used at hospitals.

One of most common measures of HAIs prevention is manual cleaning and surfaces disinfection of hospital rooms and surfaces using chemical disinfectants solutions. However this disinfection method has a number of major drawbacks [4]: impossibility to clean the hard-to-reach spots, including places higher than human reach, strong dependence of the disinfection efficacy on the personnel strictly following the procedure of solution preparation and use, strong dependence of the disinfection efficacy on the exposure time of surfaces to the disinfectant, manpower input.

Presently is used an advanced method of air and surfaces decontamination of hospital rooms and objects, based on exposing the objects to high-intensity continuous spectrum pulses generated in xenon gas gap by high voltage application [5-7].

At the moment exists a vast database of microbiological and clinical studies, speaking for the high efficiency of the method; however there is no experimental base of bactericidal doses required for air and surfaces decontamination from hospital pathogens [5-7].

This study's purpose is to determine the bactericidal doses of pulsed UV irradiation as well as conditions required for rooms' decontamination with at least 99.9% efficiency for all current HAI pathogens.

MATERIAL AND METHODS

BACTERIAL STRAINS

S. aureus strain 907 and the following multi-drug resistant strains: P. aeruginosa, MRSA, vancomycin-resistant E. faecium (VRE), A. baumannii, as well as epidemic P. mirabilis were used throughout this study. The chosen pathogen strains were studied for resistance against chemical disinfectants of different groups. For controlling the decontamination efficacy were used E. coli strain 1257 and S. aureus strain 907. The tests demonstrated the resistance of non-fermenting enterobacteria (P. aeruginosa and A. baumannii) against disinfectants of all groups.

24-hours' suspension of the chosen strain was prepared as per industry-specific opacity standard No. 20 (9 lg) on the basis of normal saline or saline with 40% of sheep blood serum (6 ml of saline, 4 ml of blood serum). The cultures were incubated in blood agar. With a micropipette 0.02 ml of bacterial suspension were applied to the bottom of a sterile Petri dish or metal plate of equal size. Microdrops were evenly distributed throughout the surface of the samples and dried. The testing was performed within 1 hour since the application of microdrops with pathogens.

After treating the bacterial suspension at the bottom of Petri dish with UV irradiation, 10 ml of sterile saline were added to the dish and thoroughly stirred in circular motions. Then 10 ml of molten and cooled to 45°C growth medium were added. After that the dishes were covered and left to solidify. This procedure is precise as it allows for accounting occasional viable colonies.

The culture from metal plates was washed to a sterile drape after UV treatment and then shaken in a bottle with beads in 10 ml of sterile saline. 0.1 ml of the resulting suspension was inoculated into thick selective media.

UV IRRADIATION SOURCE

As UV irradiation source was used a pulsed xenon lamp of Yanex-2 unit. The electrical output of the lamp was 1000 W.

The U-shaped pulsed xenon lamp generated high intensity light pulses with 120 µs half-amplitude duration and 2.5 Hz frequency.

Irradiation in 200-400 nm range was registered by a back-thinned type CCD spectrometer with high quantum efficiency and high UV sensitivity "AvaSpec-ULS2048-USB2". Intensity of the lamp irradiation was measured by calibrated UV sensor "TOCON-probe", detecting irradiation in 220…275 nm range (at half-height) with maximum spectral sensitivity at 255 nm. The lamp irradiation spectrum is shown in Figure 1. Average bactericidal flux in 200 – 300 nm range was 42 W.

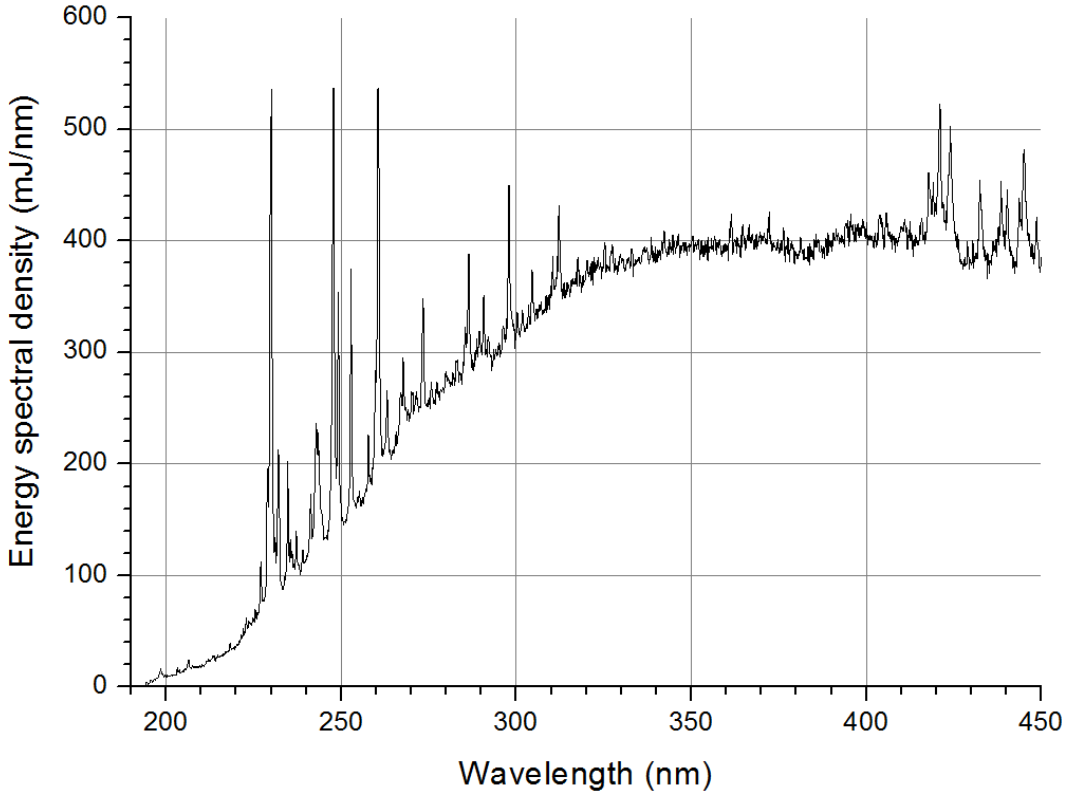

Figure 1. Irradiation spectrum of pulsed xenon unit "Yanex-2"

Thus, the irradiance at 2 meters' distance is 1 W/m^2.

METHODS

The samples with initial contamination of at least 10^7 CFU/cm^2 were placed vertically and horizontally in respect to irradiation source. For vertically placed samples the distance to Yanex-2 was 2 m, and the exposure time was 5, 10, and 20 minutes. For horizontal treatment the samples were placed on 80 cm high table at 1.2-1.7 meters' distance from the lamp; the exposure time was 5 and 10 minutes. The centre of the light emitting lamp part was at 100 cm height. 3-4 replications of tests were performed for each exposure time, followed by control of cultures' viability.

RESULTS AND DISCUSSION

The 5 minutes' irradiation of vertically placed plastic and metal samples by means of Yanex-2 reduced the contamination level at least 5 lg (Table 1). The 20 minutes' exposure to UV light led to sterilization in all performed tests (data not shown in the table). The results of 5 minutes' irradiation of vertically placed samples with 2 meters' distance are shown in Figure 2.

Table 1. Evaluation of disinfection efficacy of contaminated samples, located vertically at 2 meters' distance from the irradiation source

Test conditions	Surviving pathogens (efficacy), CFU/cm^2 (lg)		
	0 min	5 min (336 J/m^2)	10 min (672 J/m^2)
MRSA			
Petri dish w/o bioburden	5 x 10^8	28 (7.2)	0 (8.7)
Petri dish with bioburden		50 (7)	1 (8.7)
Metal plate w/o bioburden		67 (6.8)	-
Metal plate with bioburden		1.83 x 10^3 (5.4)	-
VRE			
Petri dish w/o bioburden	1.9 x 10^7	21 (5.9)	15 (6.1)
Petri dish with bioburden	2.3 x 10^7	19 (6)	20 (6)
Metal plate w/o bioburden	1.83 x 10^7	910 (4.3)	440 (4.6)
Metal plate with bioburden		600 (4.4)	610 (4.4)
P. aeruginosa			
Petri dish w/o bioburden	1.65 x 10^7	16 (6)	1 (7.2)
Petri dish with bioburden		9 (6.2)	1 (7.2)
Metal plate w/o bioburden	1.5 x 10^7	100 (5.1)	25 (5.7)
P. mirabilis			
Petri dish w/o bioburden	5 x 10^8	6 (7.9)	-
Petri dish with bioburden		2 (8.3)	-
Metal plate w/o bioburden	6 x 10^8	730 (5.9)	-
Metal plate with bioburden		730 (5.9)	-
A. baumannii			
Petri dish w/o bioburden	2.32 x 10^7	20 (6)	15 (6.1)
Petri dish with bioburden	1.6 x 10^7	35 (5.6)	27 (5.7)
S. aureus			
Petri dish w/o bioburden	7 x 10^8	32 (7.3)	-
Petri dish with bioburden		48 (7.1)	0 (8.8)

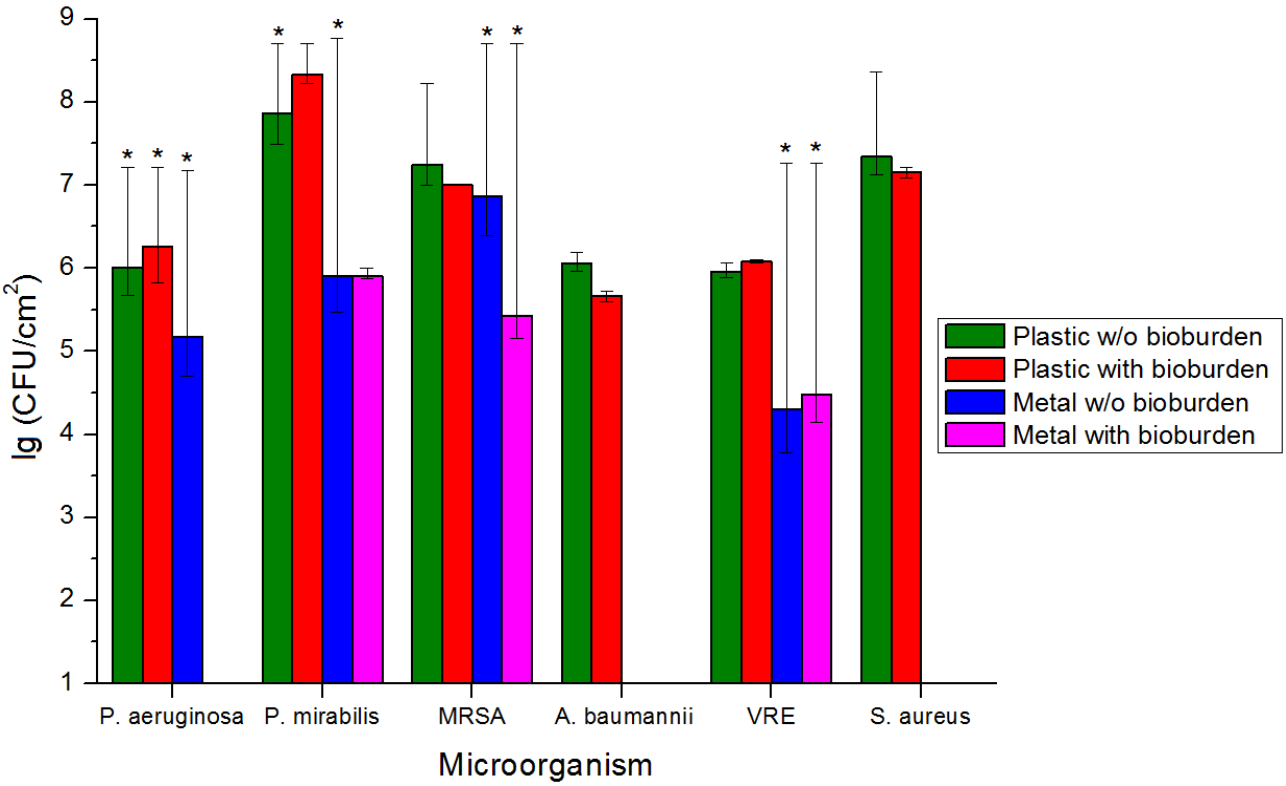

Figure 2. Evaluation of disinfection efficacy of contaminated samples with and without bioburden (*) – demonstrates 100% decontamination of at least one sample

The irradiation of horizontally placed metal plates provided 4.9-7.6 lg disinfection efficacy depending on the bacterial strain and distance to the irradiation source (Table 2).

Table 2. Evaluation of disinfection efficacy of horizontally placed metal plates, contaminated with hospital pathogens strains

Mean distance to sample, m	Presence of bioburden	Treatment time (UV dose), min (J/m^2)	Control, CFU/cm^2	Surviving pathogens (efficacy), CFU/cm^2 (lg)
MRSA				
1.6	w/o bioburden	5 (62,6)	1.15×10^7	100 (5)
1.7	with bioburden	5 (52,5)	1.4×10^7	160 (4.9)
1.4	w/o bioburden	10 (184)	1.15×10^7	100 (5)
1.5	with bioburden	10 (151)	1.4×10^7	83 (5.2)
P. aeruginosa				
1.4	w/o bioburden	5 (92)	4.6×10^7	0 (7.6)
1.5	with bioburden	5 (75,5)	4.7×10^7	33 (6.1)
1.2	w/o bioburden	10 (286)	4.6×10^7	0 (7.6)
1.3	with bioburden	10 (228)	4.7×10^7	0 (7.6)

Regardless of the samples orientation against the irradiation source, the disinfection efficacy is essentially independent of the bioburden presence and only slightly dependent on the surface material. The comparison with mercury based units shows a significantly higher decontamination efficacy of pulsed irradiation sources for equal doses [8-10]. Thus, the 99.9% efficient disinfection of the surfaces contaminated by S. aureus with bioburden shall require a dose of at the most 32 J/m^2 for a pulsed xenon lamp, and of 1,500 J/m^2 for a mercury vapor lamp [9]. Disinfection of a number of samples with 100% efficacy (Fig. 2) shows the absence of the effect of maximum efficiency achievement, characteristic of mercury based irradiation sources. This effect means that the disinfection efficacy does not grow once the initial microbial load reduces by 2-4 lg even if the UV dose is increased by 10 times [9, 10].

The achieved high values of decontamination efficacy for various samples, contaminated by drug-resistant pathogens strains with high epidemic potential, result from simultaneous impact of continuous spectrum UV Irradiation on almost all cell structures. Such multichannel impact ensures a sharp decrease of bactericidal doses [11-19]. Under the influence of pulsed UV light, the cells repair process either runs significantly more slowly [20] or completely stops [14, 21]. The study of the impact of pulsed UV continuous spectrum irradiation on cell membrane shows the complete destruction of the latter, including the loss of regulatory and barrier functions as well as cytoplasm efflux [7, 22].

CONCLUSIONS

The performed tests demonstrated high bactericidal efficacy of pulsed UV irradiation of continuous spectrum against the strains with multiple drug resistance and resistance against main groups of chemical disinfectants. The disinfection efficiency against both gram-negative and gram-positive microorganisms is only slightly dependent on the presence of bioburden and makes 4-8 lg. The material of the treated surface affects the decontamination efficacy in a minor way. The comparative studies with the test and hospital strains of S. aureus (907) and MRSA showed identical values of decontamination efficacy ≈7-7.5 lg. The obtained threshold bacterial doses are significantly lower than the corresponding values for devices with mercury based lamps, which allows for dramatically reducing the time of disinfection procedures. The experimental results permit to claim that Yanex-2 is a highly efficient and easy-to-use device for rapid surfaces and objects disinfection in hospital environment.

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