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Germicidal Efficiency of 272 nm LED in Relation to the Hospital Strain of Klebsiella Pneumoniae

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RELEVANCE Currently, the use of ultraviolet (UV) radiation for the disinfection of objects and the treatment for infectious diseases is considered as a promising alternative to chemical biocides and antibiotics. Shortwave — UV-C and UV-B -light emitting diodes (LED) are a relatively new type of UV radiation sources and potentially able to meet the requirements of current medical technologies. However, their possibilities for the treatment of wounds and infectious diseases have not been practically researched to date, which determines the relevance of experiments aimed at studying the biocidal and therapeutic properties of short-wavelength UV LEDs.

PURPOSE OF STUDY To evaluate the bactericidal efficacy of 272 nm LED radiation against hospital strains of Klebsiella pneumoniae bacteria characterized by multidrug resistance.

MATERIAL AND METHODS The studies were carried out with an experimental sample of the LED apparatus for UV irradiation. In the irradiator of the device, 5 LEDs are installed with a maximum radiation at a wavelength of 272 nm and a total electrical power of 10 watts. The UV radiation dose achieved in one irradiation session (12 seconds) at a distance of 10 cm from the irradiator was 8 mJ/cm^2. In the experiments, a hospital strain of the bacterium K. pneumoniae, isolated from the patient's blood, was used. The strain was characterized by multidrug resistance. A daily culture suspension of K. pneumoniae with a concentration of 108 CFU/ml in a volume of 100 µl was transferred into a Petri dish with a diameter of 9 cm with meat-peptone agar and evenly distributed over a surface with a diameter of 8 cm. Petri dishes were irradiated from a distance of 10 cm from the irradiator. The change in the dose of UV irradiation from 4 to 80 mJ/cm^2 was carried out by varying the exposure time. Studies were carried out in 4 repetitions at each dose. After irradiation, the experimental and control (without irradiation) Petri dishes were placed in a thermostat at 37°C for 24 hours, then the grown colonies were counted. A total of 60 experiments were carried out.

RESULTS As a result of the research, it was shown that the LED device based on five 272 nm diodes provides deep and prompt disinfection of the surface from hospital strains of K. pneumoniae bacteria characterized by multidrug resistance. A dose of UV radiation of 8 mJ/cm^2 reduces surface contamination with K. pneumoniae bacteria by more than a million times (decontamination efficiency over 99.9999%). At doses less than 10 mJ/cm^2, the efficiency of the 272 nm LED device in terms of inactivation of K. pneumoniae bacteria is 3–4 times higher than the bactericidal efficiency of mercury lamps.

CONCLUSION The prospects of using UV devices based on LEDs with a maximum radiation at a wavelength of 272 nm in systems for the operational disinfection of massively contaminated surfaces, potentially including wound surfaces, have been shown.

Keywords: ultraviolet LED, bactericidal efficacy, surface contamination, hospital strain of Klebsiella pneumoniae

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CFU - colony forming unit
DNA - deoxyribonucleic acid
RCB - rechargeable battery
UV - ultraviolet
UV-B - ultraviolet radiation in the spectral range of 280–315 nm
UV-C - ultraviolet radiation in the spectral range of 200–280 nm

INTRODUCTION

Currently, leading medical specialists in this country and abroad are showing significant interest in new medical ultraviolet (UV) technologies which include deep UV light-emitting diode (LED) technologies [1–6]. The specific features of such technologies and technical means based on them — safety and environmental friendliness ("mercury-free"), compactness, the ability to control the spectral and temporal characteristics of radiation, etc. — stimulate interest in their research and development. However, their potential for the treatment of wounds and infectious diseases has been virtually unexplored to date, which determines the relevance of experiments aimed at studying the biocidal and therapeutic properties of deep UV LEDs.

The **aim** of this research was the microbiological assessment of the bactericidal effectiveness of 272 nm LED radiation against hospital strains of *Klebsiella pneumoniae* at high levels of surface contamination.

K. pneumoniae is an opportunistic, gram-negative microorganism and can cause various infections, including pneumonia, sepsis, urinary tract infections, liver abscesses, etc. It is a common cause of hospital-acquired infections, including those caused by strains resistant to antibiotics, even those used in extreme cases. There are few quantitative data on the sensitivity of *K. pneumoniae* to UV radiation; the dose characteristics of inactivation are limited and were mainly determined at a UV radiation wavelength of 254 nm and using mercury lamps [7–12].

Data on the sensitivity of *K. pneumoniae* to radiation from modern UV LEDs with a wavelength of 272 nm are currently not available in published sources.

MATERIALS AND METHODS

The experiments were carried out at the N.V. Sklifosovsky Research Institute for Emergency Medicine and the Izmerov Research Institute of Occupational Health.

The technical object of research was an experimental model of the LED UV irradiation device, developed at the Bauman Moscow State Technical University and in the future intended for rapid UV disinfection of microbiologically contaminated surfaces, treatment for wounds and infectious diseases.

The device is made in the form of a monoblock, including an irradiator and a built-in power source based on a rechargeable battery (RCB). The operating principle of the device is based on irradiating the surfaces of objects with an area of up to 100 cm^2 (in one session) with narrow-band UV radiation generated by deep UV LEDs. The emission spectrum of the LEDs used has a maximum at a wavelength of 272 nm and a half-width of ~12 nm (Fig. 1).

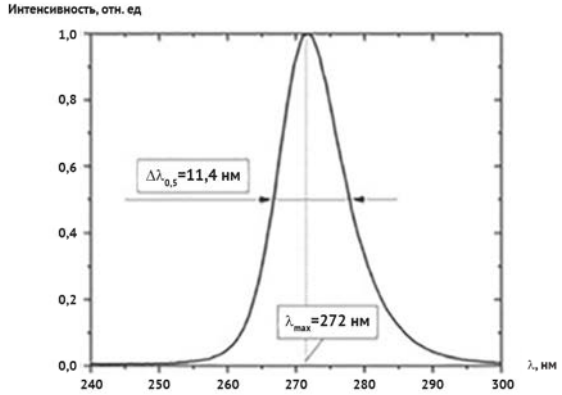

Fig. 1. Radiation spectrum of the device

The device's irradiator uses 5 light-emitting diodes with a total UV radiation power of 475 mW, placed on a panel with a diameter of 50 mm. The LEDs operate in continuous emission mode, the electrical power consumption of the device is ~10 W. Power is supplied from a built-in battery with a capacity of 1.5 A/h with a voltage of 18 V. The duration of continuous operation of the battery on one charge is 2 hours; weight of the device with battery – 850 g.

Spectral radiation characteristic measurements of the device were carried out using calibrated measuring instruments: a fiber optic spectrometer Solar S100 based on image sensor S8378-1024 (manufactured by Hamamatsu, Japan) with a cosine corrector and photodetector TOCON C8 (manufactured by SGLUX GmbH, Germany). For absolute calibration of the measuring instruments, we used a reference radiation source DH-3PLUS CAL (manufactured by Ocean Optics (Ocean Insight), USA) which includes deuterium and halogen lamps.

According to the measurements, in a plane located at a distance of 10 cm from the irradiator (13 cm from the LED panel), the diameter of the UV spot is ~ 12 cm at half intensity level. In a spot with a diameter of 10 cm, the unevenness of irradiation does not exceed 25%. The maximum irradiance in the UV region of the spectrum at a distance L=10 cm from the irradiator was ~0.67 mW/cm^2, while ~80% of the total emitted power was emitted in the UV-C range (Δλ=200–280 nm); the remaining 20% is in the UV-B region (Δλ=280–315 nm). In one irradiation session (with a session duration of ~12 s set in the device), a UV radiation dose of ~8 mJ/cm^2 is achieved in the center of the spot at a distance of 10 cm from the irradiator.

The microbiological test object of the research was *K. pneumoniae*. The experiments used a clinical strain of *K. pneumoniae* isolated from the blood of a patient who was being treated in the intensive care unit of the N.V. Sklifosovsky Research Institute for Emergency Medicine.

The strain was characterized by multiple drug resistance: it was a producer of extended-spectrum beta-lactamases, and resistant to antibiotics from the groups of cephalosporins, fluoroquinolones, aminoglycosides and carbapenems; remaining sensitive to tigecycline and colistin.

Blood was taken from the patient's peripheral vein in compliance with aseptic rules. For the research, 10 ml of blood was simultaneously collected into two vials: BactecTM Plus Aaerobic/F Culture Vials and BactecTM Plus Anaerobic/F Culture Vials. The obtained blood samples were placed in the laboratory into a Bactec FX blood culture analyzer (BD, USA), and after 18 hours of incubation, the contents of the bottles were plated on 5% blood agar. After obtaining bacterial growth on a solid nutrient medium, the microorganism was identified using a VITEK MS mass spectrometer (bioMérieux, France); and sensitivity to antibiotics was determined using a VITEK-2 Compact analyzer (bioMérieux, France).

A suspension of a daily culture of K. pneumoniae with a concentration of 10^8 microbial cells per 1 ml (0.5 McFarland) was prepared in a sterile physiological solution. Using a microsyringe, 100 µl of the suspension was transferred into a 90 mm Petri dish with meat peptone agar and evenly rubbed with a spatula over the surface of the agar, not reaching the walls of the dish at a distance of ~ 0.5 cm (to minimize wall effects). Thus, a surface with a diameter of ~8 cm and an area of ~50 cm^2 was seeded with 10^7 microbial cells (CFU). Accordingly, the initial surface density of contamination was ~$2 \cdot 10^5$ CFU/cm^2. The inoculated surfaces were dried at room temperature until completely dry.

Then the experimental Petri dishes with the seeded culture were irradiated with the LED device mounted in a special stand allowing the researchers to adjust the distance from the irradiator to the irradiated object. In the experiments performed, the distance from the irradiator to the contaminated surface was 10 cm (13 cm from the LED panel). The dose of UV radiation was changed by varying the duration of irradiation, which varied in the range from 6 to 120 seconds. The energy doses of UV radiation were 4–80 mJ/cm^2. The studies were repeated 4 times for each fixed duration of irradiation.

After completion of irradiation, experimental and control (without irradiation) Petri dishes were placed in a thermostat at 37°C for 24 hours. After incubation, the grown colonies were counted.

Experiments at the Izmerov Research Institute of Occupational Health were carried out using a similar method, but with the initial number of bacteria on the agar surface $N_0 = 7 \cdot 10^8$ CFU and in the range of applied doses from 22.5 to 136 mJ/cm^2. Additionally, experiments were carried out on irradiation of K. pneumoniae with a low-pressure mercury lamp (λ=254 nm) in the UV radiation dose range of 25–200 mJ/cm^2.

The effectiveness of disinfection was determined by calculating the logarithm of inactivation, equal to the decimal logarithm of the ratio of the initial number of microorganisms in the sample

N_0 to the number of microorganisms N_i that survived irradiation with a dose of D_i – $lg(N_0/N_i)$. The numerical value of the logarithm of inactivation shows by how many decimal orders the initial number of bacteria decreased after treatment with the given energy dose.

Another form of representing the bactericidal effectiveness of the device is the disinfection efficiency, expressed as a percentage and equal to the ratio of the number of inactivated (dead) bacteria N_i at a given dose D_i to the number of initially inoculated bacteria N_0

$$\eta_i = N_{ni}/N_0 \cdot 100\% = (1 - N_i/N_0) \cdot 100\%.$$

The number of nines in the numerical value η_i is equal to the integer number of the logarithm of inactivation.

The results of the calculations $lg(N_0/N_i)$ и N_i/N_0 were processed by the analysis of variance with corresponding calculations of the arithmetic mean value (X), standard deviation (σ_{n-1}), standard error of the mean value ($\sigma_{X\,i}$), maximum sampling error ($\pm\Delta_p$) and confidence interval [$X-\Delta_p$; $X+\Delta_p$] with confidence probability p=0.95.

$$\bar{X} = \frac{\sum_{i=1}^{n} X_i}{n};$$

$$\sigma_{n-1} = \sqrt{\frac{\sum_{i=1}^{n} (\bar{X} - X_i)^2}{n-1}};$$

$$\sigma_{\bar{X}_i} = \frac{\sigma_{n-1}}{\sqrt{n}};$$

$$\Delta_p = t_p \cdot \sigma_{\bar{X}_i}$$

where X_i — is the experimental value; n — number of experiments (n=4); t_p=2.353 — Student's T distribution with confidence level p=0.95, and number of experiments n=4.

EXPERIMENTAL RESULTS AND DISCUSSION

The table shows the result of counting the number of *K. pneumoniae* colonies after irradiation of Petri dishes with an inoculated culture by the LED apparatus (initial number of bacteria - 10^7 CFU) depending on the duration of irradiation and, accordingly, the energy dose of UV radiation. On control plates (without irradiation), continuous lawn growth of the *K. pneumoniae* culture was recorded.

Figure 2 presents the experimental results in the form of a survival curve of *K. pneumoniae* under UV irradiation by the device. The number of surviving bacteria is plotted along the ordinate axis in logarithmic coordinates, and the energy dose of UV radiation is plotted along the abscissa axis. The circles on the graph correspond to the values of the numbers of surviving bacteria averaged over four implementations at a given dose of UV irradiation, and the horizontal shelves correspond to the standard deviation.

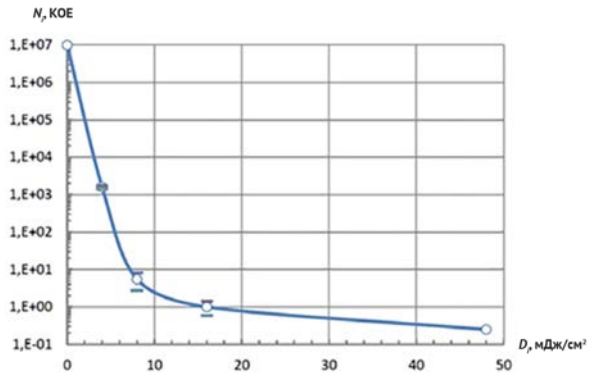

Fig. 2. The survival curve of K. pneumoniae bacteria under UV irradiation with an LED device. Horizontal shelves correspond to the standard deviation

At UV radiation doses equal to or greater than 48 mJ/cm^2, no surviving colonies were observed in all 4 irradiated Petri dishes. However, in the graphical interpretation of the experimental results, it is accepted that at a dose of D_i = 48 mJ/cm^2 the value of N_i = 0.25 CFU, implying that the probability of detecting a living bacterium at a given dose is less than 1/4 (zero result in four implementations).

As follows from the presented data, the LED device under study provides deep and rapid disinfection of surfaces contaminated with *K. pneumoniae* - already at doses of 8 mJ/cm^2; and after irradiation for 12 seconds, a reduction in surface contamination by more than 6 orders of magnitude is achieved, that is, more than million times (disinfection efficiency exceeds 99.9999%).

Increasing the duration of irradiation to 72 seconds (or, accordingly, the dose to 48 mJ/cm^2) leads to complete sterilization of the surface with an initial level of contamination of 10^7 CFU.

However, it should be noted, that with an increase in the energy dose to more than 8 mJ/cm^2, the rate of decrease in the level of infection (that is, the rate of inactivation) slows down, the survival curve tends to saturate or reach a "plateau". This ("two-phase") type of survival curve is determined by the heterogeneity of the irradiated bacterial population, that is, the presence of a fraction of *K. pneumoniae* that is resistant to the disinfectant factor (in this case, to UV radiation). Another possible reason for the formation of the "tail" of the survival curve is associated with factors of a non-biological nature, in particular, the mutual shading of bacteria at their high surface density, characteristic of the experimental conditions [7]. As the density of contamination increases, the effects of shading should increase and, therefore, the effectiveness of disinfection at a fixed dose will decrease.

If we rearrange the survival curve as a function of the dose of the relative number of surviving bacteria N_i/N_0 (рис. 3), then linear extrapolation of the section of the curve corresponding to high doses (that is, the "tail") to the y-axis will give the proportion of the resistant fraction in the original bacterial population γ. Under the conditions of the experiments, this fraction was $\gamma \approx 2 \cdot 10^{-7}$. The angle of inclination of the branches of the survival curve to the abscissa axis α' or α'' (Fig. 3) is proportional to the sensitivity of the bacterial fraction to the action of UV radiation, and its cotangent determines the dose of D_{90}, at which the population size of a given bacterial fraction decreases 10 times (disinfection efficiency is — 90%). The greater the angle of inclination, the more sensitive the bacterial fraction. Experimental points are approximated by the following function:

$$\frac{N_i}{N_0} = (1-\gamma) \cdot 10^{-\frac{D}{D'_{90}}} + \gamma \cdot 10^{-\frac{D}{D''_{90}}} \approx 10^{-\frac{D}{1{,}05}} + 2 \cdot 10^{-7} \cdot 10^{-\frac{D}{52}},$$

where $D'_{90} \approx 1.05$ mJ/cm^2, and $D''_{90} \approx 52$ mJ/cm^2 — are doses that reduce the size of the bacterial population by 10 times for the sensitive and resistant fractions of bacteria, respectively. The approximation function is presented in Fig. 3 with a solid thick line and demonstrates a fairly good agreement of the approximation with the experimental data (R^2 no less than 0.98). This function can be further used to predict the effectiveness of surface disinfection by the LED devices in question.

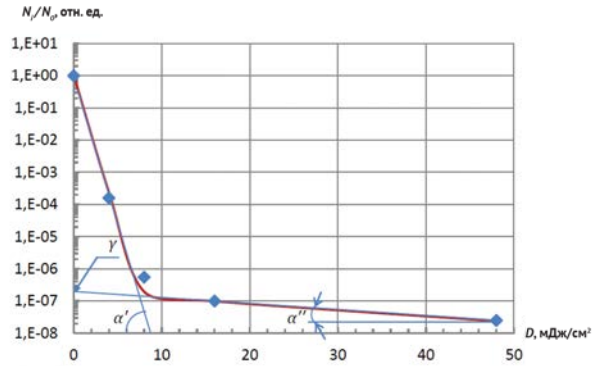

Fig. 3. Dependence of the relative number of surviving bacteria N_i/N_0 on the dose. Squares are an experiment; a solid thick line is an approximation

Figure 4 shows data on the effectiveness of inactivation of *K. Pneumoniae* by UV radiation, obtained in different experimental groups. The efficiency of inactivation is presented as the dependence of the logarithm of inactivation $lg(N_i/N_0)$ on the energy dose of UV radiation D.

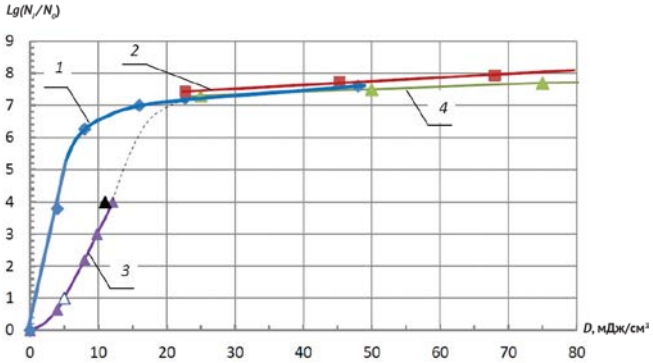

Fig. 4. Dependence of the logarithm of inactivation against K. pneumoniae bacteria on the energy dose of UV radiation.
Squares — wavelength λ=272±6 nm; triangles — wavelength λ=254±1 nm.

Curve 1 (blue squares) — $N_0=10^7$ CFU; experiments at the N.V. Sklifosovsky Research Institute of Emergency Medicine; curve 2 (red squares) — $N_0=7\times10^8$ CFU; experiments at the N.F. Izmerov Research Institute of Occupational Medicine; curve 3 (purple triangles) — data from [5]; curve 4 (green triangles) — $N_0=7\times10^8$ CFU; experiments at the N.F. Izmerov Research Institute of Occupational Medicine; white triangle — data from [3]; black triangle — data from [1, 2]; The dotted line is a possible (hypothetical) extrapolation of the experimental curve 3

Curve 1 demonstrates the results of these studies, carried out using the LED apparatus in the range of applied doses up to 80 mJ/cm^2 and the initial number of bacteria on the agar surface $N_0=10^7$ CFU. The values of the logarithm of inactivation are given up to a dose of 48 mJ/cm^2, starting from which no growth of microflora was observed in all four Petri dishes studied.

Curve 2 reflects the results of the experiments conducted at the N.F. Izmerov Research Institute of Occupational Health also using this LED apparatus, but with an initial number of bacteria on the agar surface equal to $7\cdot10^8$ CFU, and in the range of applied doses from 22.5 to 136 mJ/cm^2, while at doses exceeding 70 mJ/cm^2, single colonies were recorded in the irradiated Petri dishes. Comparison of these two curves indicates good agreement between the experimental results obtained in different laboratories under different initial conditions.

Figure 4 shows that up to values of the logarithm of inactivation $lg(N_0/N_i)$ ≈5.5, its dependence on the dose in semi-logarithmic coordinates is linear

$$lg(N_0/N_i) = k\cdot D_i = D_i/D_{90}$$

where k — is the inactivation constant (cm^2/mJ), numerically equal to D_{90}^{-1}.

At a dose of D_i = 4 mJ/cm^2, the value of the logarithm of inactivation, according to the measurements, is

$$lg(N_0/N_i) = 3.82\pm0.11.$$

From here, we obtain the dose value of UV irradiation D_{90}, which reduces the contamination of the surface with *K. pneumoniae* by 10 times,

$$D_{90} = D_i/lg(N_0/N_i) = 4/(3.82\pm0.11) = (1.05\pm0.03)\ \text{mJ/cm}^2$$

It is of interest to compare the obtained values of dose characteristics for the device based on LEDs with a central wavelength λ_{max} =272 nm and a half-width of the UV spectrum $\Delta\lambda_{0,5}$ =12 12 nm with the available literature data.

A research [7] provides a review of the available literature data for the period up to 2008 on the inactivation of bacteria important for bioprotection tasks by UV radiation with a wavelength λ = 254 nm (low-pressure mercury lamps). For *K. pneumoniae*, with reference to a study [8], a dose value is given that reduces the level of bacterial contamination by 4 decimal orders, that is, D_{-4lg}, equal to 11 mJ/cm^2. This experimental point is shown in the figure by the black triangle.

In a monograph [9], with reference to a research [10], for *K. pneumoniae* in water and wavelength of λ=254 nm, the dose value D_{90} = 4.2 mJ/cm² is given. These data are shown in Fig. 4 by the white triangle.

The most frequently cited work on the UV sensitivity of *K. pneumoniae* is the article by Giese N. and Darby J. [11]. In this research, the sensitivity of coliphage and a number of coliform bacteria, including Klebsiella, to narrow-band ($\Delta\lambda_{0.5}$ = 10 nm) UV radiation with central wavelengths of 254 nm, 280 nm and 301 nm was studied. The experiments used a medium-pressure mercury lamp with narrow bandpass filters. Suspensions of bacterial cells with initial concentrations of microorganisms at the level of 10^6–10^8 CFU/ml (in particular, the concentration of *K. pneumoniae* was $1.7 \cdot 10^6$ CFU/ml) were irradiated in Petri dishes. The results of these experiments with *K. pneumoniae* for a wavelength λ = 254 nm are shown in Fig. 4 in the form of curve 3. The same data are presented in the latest review [12] which reflects the current (as of 2021) state of knowledge about the effects of UV radiation on various microorganisms; the doses given are recommended for use in the design, application and testing of ultraviolet disinfection technologies and systems.

It should be noted that in a number of information sources (see, for example, in [13] and [14]), dose characteristics of *K. pneumoniae* are given with reference to [11], in particular, D_{90} = 12 mJ/cm², D_{-4lg} = 20 mJ/cm², which are absent in the cited work. In this regard, these data are not discussed here.

Curve 4 in Fig. 4 reflects the results of experiments on irradiation of K. pneumoniae with a low-pressure mercury lamp (λ = 254 nm) with an initial contamination of the agar surface $7 \cdot 10^8$ CFU (the experiments were performed at the Izmerov Research Institute of Occupational Health). The range of UV radiation doses studied was 25–200 mJ/cm². There was no growth of microflora in irradiated Petri dishes at doses of at least 150 mJ/cm².

Analysis of the presented experimental data shows the following:

1. Dose characteristics of *K. pneumoniae* inactivation given in various sources for mercury lamp radiation (λ=254 nm) are in fairly good agreement with each other. In particular, the dose of D_{90} varies among different authors in the range of 4.2–5.2 mJ/cm²; the doses required to reduce the bacterial population by 4 orders of magnitude are D_{-4lg} = 11–12 mJ/cm².

2. At low energy doses of UV radiation (D no more than 12 mJ/cm$_2$), the bactericidal efficiency of the LED device against *K. pneumoniae* is 3–4 orders of magnitude higher than the bactericidal efficiency of mercury lamps. Thus, when using a mercury lamp with a UV radiation dose of 10 mJ/cm2, bacterial contamination is reduced by 1000 times, while the use of the LED device is accompanied by a decrease in the initial contamination by more than 6 million times ($lg(N_0/N_i) \approx 6.8$).

3. At doses greater than 20 mJ/cm², there are no statistically significant differences in the effectiveness of disinfection of surfaces contaminated with *K. pneumoniae* by UV radiation with wavelengths of 254 and 272 nm.

The reasons for the significant difference in the inactivation efficiency of mercury lamps and LED devices at low energy doses are not entirely clear. A number of considerations can be made in the form of hypotheses that require further detailed research and experimental argumentation.

The first consideration is related to the possible difference in the UV sensitivity of the Klebsiella bacteria used in different experiments, due to genetic (different strains of bacteria) and phenotypic differences (different conditions of reproduction and growth, different age, etc.), as well as different conditions of UV irradiation (bacteria in water and on a surface).

Another explanation may be related to the different spectral characteristics of the UV sources used. Mercury lamps and LEDs create approximately the same irradiation on biological objects - milliwatts per square centimeter, so their main difference can only be associated with different wavelengths of radiation - low-pressure mercury lamps emit a narrow (Δλ~2 nm) spectral line at a wavelength λ~254 nm, while the LEDs used in the experiment generate narrow-band (Δλ~12 nm) UV irradiation centered at λ~272 nm. It is known [7, 9] that the main reason for the inactivation of the bacteria under the influence of radiation with λ~254 nm is the formation of thymine dimers in DNA (deoxyribonucleic acid), which subsequently leads to disturbances in the processes of DNA transcription and replication and the impossibility of cell division (reproduction). The maximum absorption spectrum of DNA thymine bases occurs at wavelengths λ_{max} ~ 265–266 nm [9], and this is in better agreement with the emission spectrum of the diode (λ_{max} ~ 272 nm) than with the spectrum of a low-pressure mercury lamp (λ_{max} ~ 254 nm).

But perhaps a more significant factor is the much better correspondence of the LED emission spectrum to the absorption spectrum of proteins, the maximum of which in the region λ more than 240 nm occurs at wavelengths λ_{max} ~ 280 nm, and is due to the absorption of aromatic amino acids included in their composition [9]. In the

spectral region λ~240–255 nm, the absorption of almost all proteins is low, and their photodestruction under the influence of radiation from the mercury lamp will be much less effective than under the influence of LED radiation. In this regard, the likelihood of the formation of not only pyrimidine dimerization, but also other DNA disorders in the cell, such as DNA–protein crosslinks, disruption of the transport properties of biomembranes, etc., increases when using the LED.

However, apparently, a more significant factor is the destruction of proteins (enzymes) involved in reparative intracellular processes. It is known that with low doses of UV radiation, the cell is able to eliminate defects that arise in DNA and, first of all, destroy thymine dimers, restoring the original genetic structure of DNA. This process is carried out with the help of special proteins (enzymes), the destruction of which leads to faster inactivation of bacteria under the influence of UV irradiation, that is, to an increase in their photosensitivity. On the survival curves, the effectiveness of repair processes is manifested in the form of the characteristic "shoulder" - a very low rate of inactivation at low doses of UV radiation (the larger the "shoulder", the more effectively the cell's repair system eliminates photodefects in DNA).

When studying the photosensitivity of Klebsiella bacteria in water under the influence of radiation with wavelengths of 254 nm [11], a statistically significant presence of the "shoulder" was noted (see Fig. 4, curve 3), indicating the functioning of repair systems. In our experiments, the presence of the "shoulder" on the survival curve of Klebsiella bacteria when exposed to LED irradiation with 272 nm was not observed (Fig. 4, curve 1), which indicates a low efficiency of repair processes (or their complete suppression). As a consequence, one can expect a significant increase in the sensitivity of bacteria to the action of such radiation.

At high doses of UV radiation, the role of the repair system becomes insignificant even if the proteins responsible for the restoration of DNA defects are not destroyed (its "saturation" occurs).

CONCLUSION

Currently, the use of UV radiation for the disinfection of objects and the treatment of localized infectious diseases is considered as a promising alternative to chemical biocides and antibiotics. The development and promotion of these technologies in sanitary and medical practice is largely determined by progress in the creation of effective sources of UV radiation that meet modern environmental, hygienic, economic and ergonomic requirements. Short-wave UVC and UVB light-emitting diodes are a relatively new type of UV radiation sources and have the potential to meet the requirements of current medical and environmental technologies. In this regard, the study of their functionality, in particular, biocidal action against various pathogenic microorganisms, is an urgent scientific and applied task.

In this research, the experimental study was conducted to evaluate the bactericidal effectiveness of the short-wave UV LED emitting in the spectral band of 272 ± 6 nm against hospital strains of *K. pneumoniae*. The experiments were performed at high levels of surface contamination -2. 10^5–10^7 CFU/cm^2. It was shown that the LED device with an electrical power consumption of 10 W provides deep and rapid disinfection of surfaces from hospital strains of *K. pneumoniae* characterized by multidrug resistance

The results obtained show the promise of using UV devices based on LEDs with a maximum radiation at a wavelength of 272 nm in systems for the rapid disinfection of massively contaminated surfaces, potentially including wound surfaces.

ВЫВОДЫ

1. A dose of UV radiation in the spectral band of 272±6 nm, 8 mJ/cm^2, reduces surface contamination with *K. pneumoniae* by more than a million times (disinfection efficiency is more than 99.9999%). The developed LED device with an electrical power consumption of 10 W provides this dose from a distance of 10 cm during an irradiation session of 12 seconds.

2. At doses less than 10 mJ/cm^2, the effectiveness of the 272 nm LED device in relation to the inactivation *of K. pneumoniae* is 3–4 orders of magnitude higher than the bactericidal effectiveness of mercury lamps; at doses of more than 20 mJ/cm^2, the bactericidal efficiencies of the mercury lamp and the 272 nm LED are equal.

3. The dose of D_{90} which reduces the initial contamination of the surface with K. pneumoniae by 10 times, for LEDs with a maximum emission at a wavelength of 272 nm is (1.05 ± 0.03) mJ/cm^2.

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