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## INFLUENCE OF LED RADIATION ON THE *STAPHYLOCOCCUS AUREUS* SENSITIVITY TO ANTIBIOTICS

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We studied the influence of LED radiation of red + infrared (MEDOLIGHT Red device) and blue + infrared (MEDOLIGHT BluDoc device) ranges on clinical isolates *Staphylococcus aureus* and collection test strain *Staphylococcus aureus* ATCC 25923 sensitivity to antibiotics. The dependence of radiation on the studied objects depending on exposure, wavelength and frequency were defined. The result of photo modification on the state of microorganisms was increase of their sensitivity to some of the tested antibiotics. Based on the data obtained, we developed an algorithm and clinical recommendations for the use of LED radiation in the complex therapy of purulent-inflammatory diseases.

**Key words:** red, blue, infrared LED radiation, MEDOLIGHT devices, light frequency, *Staphylococcus aureus*, sensitivity to antibiotics.

## ВПЛИВ СВІТЛОДІОДНОГО ВИПРОМІНЮВАННЯ НА ЧУТЛИВІСТЬ *STAPHYLOCOCCUS AUREUS* ДО АНТИБІОТИКІВ

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Досліджено вплив світлодіодного випромінювання червоно-інфрачервоного та синьо-інфрачервоного діапазонів апаратів Medolight Red та Medolight BluDoc на чутливість до антибіотиків клінічних ізолятів *Staphylococcus aureus* та колекційного тест-штаму *Staphylococcus aureus* ATCC 25923. Визначено залежність впливу світла на досліджувані об'єкти від експозиції, довжини хвилі та частоти випромінювання. Наслідком фотомодифікації стану мікроорганізмів є підвищення їх чутливості до деяких протестованих антибіотиків.

На основі отриманих даних розроблено алгоритм та клінічні рекомендації використання світлодіодного випромінювання у комплексній терапії поверхневих гнійно-запальних захворювань.

**Ключові слова:** червоне, синє, інфрачервоне світлодіодне випромінювання, частота світлових імпульсів, апарати Medolight, *Staphylococcus aureus*, антибіотикорезистентність.

## ВЛИЯНИЕ СВЕТОДИОДНОГО ИЗЛУЧЕНИЯ НА ЧУВСТВИТЕЛЬНОСТЬ *STAPHYLOCOCCUS AUREUS* К АНТИБИОТИКАМ

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Исследовано влияние светодиодного излучения красно-инфракрасного и сине-инфракрасного диапазонов аппаратов Medolight Red и Medolight BluDос на чувствительность к антибиотикам клинических изолятов *Staphylococcus aureus* и коллекционного тест-штамма *Staphylococcus aureus* ATCC 25923. Определена зависимость влияния света на исследуемые объекты от экспозиции, длины волны и частоты излучения. Следствием фотомодификации состояния микроорганизмов является повышение их чувствительности некоторым из протестированных антибиотиков. На основе полученных данных разработан алгоритм и клинические рекомендации использования светодиодного излучения в комплексной терапии поверхностных гнойно-воспалительных заболеваний.

**Ключевые слова:** красное, синее и инфракрасное светодиодное излучение, частота импульсации света, аппараты Medolight, *Staphylococcus aureus*, антибиотикорезистентность.

### Introduction

The problem of resistance to antimicrobial drugs continues to deteriorate, this is one of the greatest threats to health protection and for its solution active actions in different fields are required [1,2]. Antibacterial drugs currently occupy one of the most important parts of the arsenal of drugs of medical institutions. From 25 % to 35 % of all hospitalized patients receive antibiotics according to various indications. At the same time, according to the WHO 50 % of antibiotics are appointed without reason [1,3].

*S. aureus* is one of the main pathogens of skin and soft tissue diseases – boils, abscesses, cellulitis, phlebitis, mastitis, causing pneumonia, osteomyelitis, meningitis, endocarditis and others. Clinical strains of *S. aureus* are the most common microorganisms which are found in surgical patients [4,5,6].

Despite the natural sensitivity of *Staphylococcus aureus* to most antibiotics (beta-lactams, aminoglycosides, fluoroquinolones, macrolides, lincosamides, glycopeptides, rifampicin, etc.), its resistance to them grows intensively and substantially extend. Consequently, results of infections treatment caused by *Staphylococcus aureus* complicated and worsen [5,7]. A significant concern evokes the emergence and spread of methicillin-resistant strains of *Staphylococcus aureus* (MRSA) that cause outbreaks of nosocomial and community-acquired infections [8].

Among the solutions of the problem, special attention deserves the use of non-drug methods, in particular radiation of the optical range, to increase sensitivity of microorganisms to existing drugs [9-14]. Due to proven positive influence on the human's body and almost complete absence of side effects and contraindications [10], LED light application is a promising method. It was also established that the microflora of the mouth can be affected by the light from halogen and LED sources, polarized and unpolarized. This reduces the number of bacterial colonies and their sizes, indicating to suppression or destruction of microbes [11]. But these data do not explain all the mechanisms

of antimicrobial effects of light, which depend on its spectral characteristics, power density, frequency of impulses and polarization. Also important is to clarify possibilities of changes of some of the properties of microorganisms, in particular their sensitivity to damaging factors, such as antibiotics.

Purpose of the work is to investigate direct effect and determine the optimum parameters of action of LED radiation of blue-red and red-infrared ranges on sensitivity to antibiotics of clinical isolates *Staphylococcus aureus* and test strain *Staphylococcus aureus* ATCC 25923.

### Materials and methods

We studied the effect of LED radiation of red and infra-red and blue and infrared ranges on sensitivity to antibiotics of clinical isolates of *Staphylococcus aureus* (n = 5) that were isolated from foci of inflammatory processes from surgical department patients of Uzhgorod CRH and collection test strain *Staphylococcus aureus* ATCC 25923.

Determining the impact of LED radiation on the sensitivity of microorganisms to antibiotics we used disco-diffusion method, according to current requirements of the Ministry of Health of Ukraine [15]. For this purpose, we took a pure 16–24-hour agar culture of microorganisms in a standardized liquid nutrient medium with concentration of  $1.5 \times 10^8$  colony forming units (CFU) per 1 ml (it meets the turbidity matching 0.5 Mcfarland standard) and reseeded on nutrient dense Mueller-Hinton medium in Petri dishes. Then we carried out exposure of microorganisms to LED radiation from a distance of 1 cm (Fig. 1), and then we superimposed discs with antibiotics and cultivated them in the incubator at 37 °C. Results were determined by measuring zones of microbial growth delay around the discs.

In order to exclude the impact of radiation on the growth properties of the nutrient media we carried LED radiation exposure of cultures that were in sterile Petri dishes separately and then replanted them in the culture medium and superimposed discs with antibiotics.



Fig. 1. Exposure of microorganisms to LED radiation (MEDOLIGHT Red and MEDOLIGHT BluDoc devices) in Petri dishes with nutrient medium

We used sources of LED radiation, red and infrared (IR) ( $640 \pm 30$  from  $880 \pm 30$  nm) and blue and infrared ( $470 \pm 30$  from  $940 \pm 30$  nm) ranges, respectively, MEDOLIGHT Red and MEDOLIGHT BluDoc, production of Bioptron AG by Zepter Group. Light power density of MEDOLIGHT Red device did not exceed  $5.35 \text{ mW/cm}^2$ , and of MEDOLIGHT BluDoc it was  $10.15\text{--}8.2 \text{ mW/cm}^2$  from a distance of  $0\text{--}1$  cm. By separate series we examined the effect of exposures to LED radiation with different wavelengths (5, 10, 15, 20 and 25 minutes) and frequencies (0, 10, 600, 3000 and 8000 Hz).

The results were compared with control series of not irradiated cultures. Also, in order to control the quality of microbiology investigations, in each series with clinical strains we used a collection test strain *Staphylococcus aureus* ATCC 25923 with known range values of microbial growth delays in areas of antibiotics influence.

We tested sensitivity of the research object to antibiotics of beta-lactam group, macrolides, fluoroquinolones, aminoglycosides, and vancomycin, which are included in the list of recommended antibiotics to determine sensitivity of *Staphylococcus spp.* [15].

## Results and discussion

We established that the LED radiation increased the sensitivity of clinical isolates of *S. aureus* and collection of test strain *S. aureus* ATCC 25923 to some antibiotics (compared with non-irradiated cultures). In this case, the effect of influence depended on the wavelength, exposure duration and frequency of radiation. The most pronounced sensitivity increase was observed when irradiation exposure of microorganisms lasted for 5 minutes.

Fig. 2 illustrates the effect of 5-minute exposure to MEDOLIGHT BluDoc LED device at different frequencies on sensitivity of *S. aureus* ATCC 25923 to ampicillin and other antibiotics.

Thus, 5-minute exposure test strain of *Staphylococcus aureus* ATCC 25923 to LED emission of blue + infrared range with frequency of 0 Hz (continuous emission) increased its sensitivity to ampicillin, in average, for 15.5%. Application of LED MEDOLIGHT BluDoc device radiation with exposure of 5 minutes and frequencies of 10, 600, 3000 and 8000 Hz lead to increased sensitivity of *Staphylococcus aureus* ATCC 25923 to ampicillin for 12–13,5%.

Fig. 3 shows increased sensitivity of *Staphylococcus aureus* ATCC 25923 to oxacillin after application of blue infrared range LED radiation.

Irradiation of *Staphylococcus aureus* ATCC 25923 by LED emission

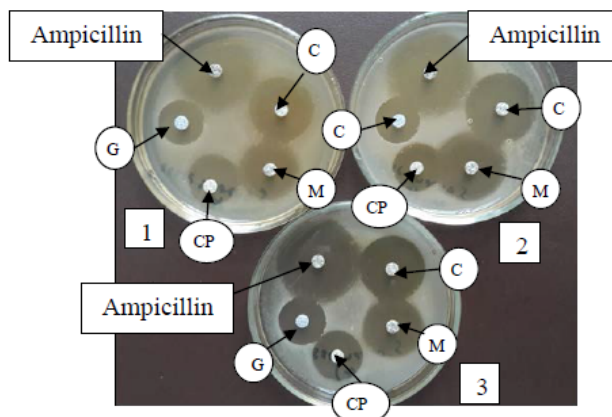


Fig. 2. Impact of LED irradiation of Medolight BluDoc device on sensitivity of *S. aureus* ATCC 25923 to antibiotics (exhibition 5 min)

1 — control; 2 — radiation with frequency of 0 Hz; 3 — radiation with frequency of 10 Hz; C — cefotaxime; M — meropenem; CP — cefepime; G — gentamicin

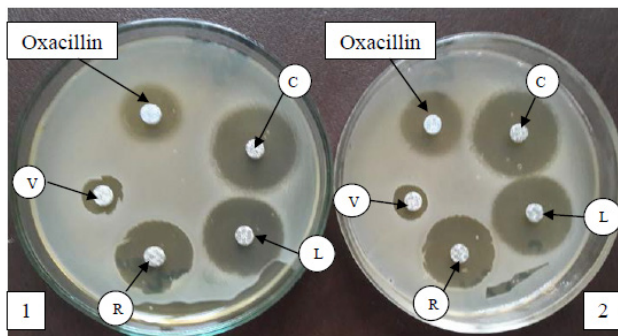


Fig. 3. LED irradiation effect of Medolight BluDoc device on antibiotics *S. aureus* ATCC 25923 (exhibition 5 min)

1 — control; 2 — radiation with frequency of 0 Hz; C — cefotaxime; L — levofloxacin; R — rifampicin; V — vancomycin

of blue + IR range with frequency of 0 Hz for 5 minutes increased its sensitivity to oxacillin by an average of 23.5%. After similar exposure with frequencies of 10, 600, 3000 and 8000 Hz we observed increase of sensitivity of these strains to oxacillin – by 17–22%.

Fig. 4 illustrates graphically the rate of sensitivity increase to ampicillin and oxacillin of *Staphylococcus aureus* ATCC 25923 under the influence of LED radiation of Medolight BluDoc device of different frequencies with 5 minutes exposure.

Table 1 shows statistically processed results of the impact of LED blue infrared radiation on sensitivity of *S. aureus* ATCC 25923 to ampicillin and oxacillin.

Using the LED irradiation of red + IR range led to a significant increase in sensitivity of studied strains *S. aureus* to levofloxacin (group fluoroquinolones), meropenem (group carbapenems) and tetracycline (group of the same name) (Fig. 5).

Thus, LED red + IR irradiation with exposure of 5 minutes at a frequency of 0 Hz resulted in increased sensitivity of *S. aureus* ATCC 25923 to levofloxacin by an average of 17%, tetracycline – by 27% and meropenem – by 22.47%. After similar exposure with frequencies of 10, 600, 3000 and 8000 Hz we observed an increase of sensitivity of *S. aureus* ATCC 25923 to levofloxacin by an average of 11–12,5%, to tetracycline – by 21–23% and to meropenem – by 14–16% ( Fig. 6).

In Table 2 we present statistically processed results of the effects of LED red + IR radiation on sensitivity of *S. aureus* ATCC 25923 to levofloxacin, tetracycline and meropenem.

It should also be noted that after application of LED radiation with 5 minutes' exposition, we observed increased sensitivity of the examined microflora also to such antibiotics as cefotaxime, rifampicin and meropenem for 6.5–12%, depending on the wavelength and frequency of light.

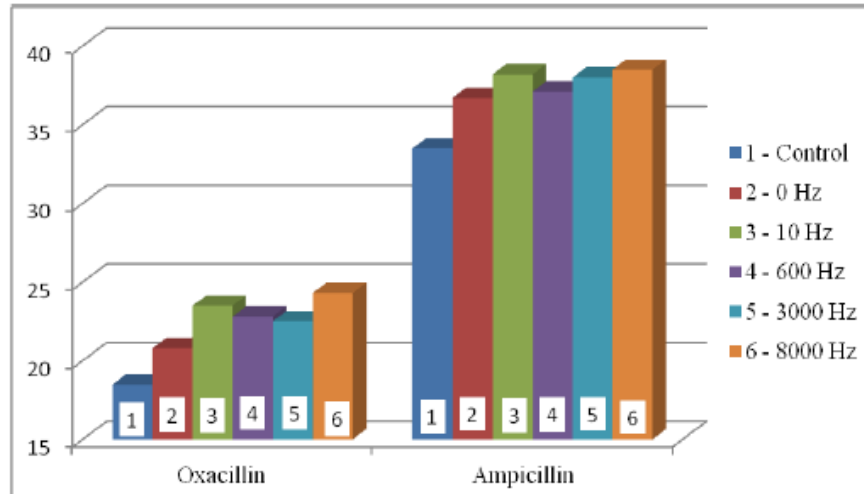


Fig. 4. Effect of blue + IR LED range emission on the value of stunted growth of *S. aureus* ATCC 25923  
On the vertical axis — diameter of zones of stunted growth, mm

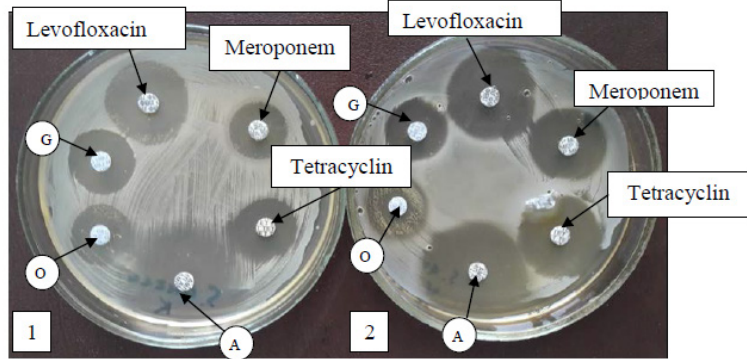


Fig. 5. Impact of LED radiation of Medolight Red device on sensitivity to antibiotics of *S. aureus* (5 min exposure)  
1 — control; 2 — radiation with frequencies of 0 Hz; A — ampicillin; O — oxacillin; G — gentamicin

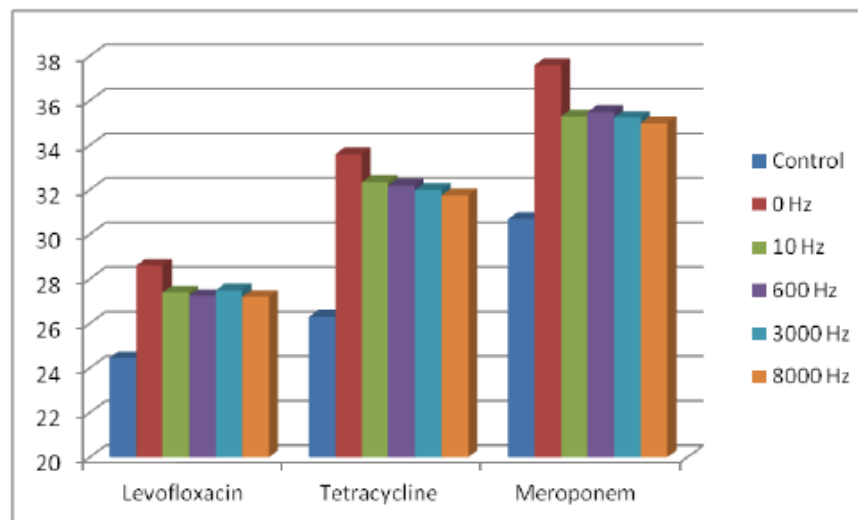


Fig. 6. Impact of LED radiation of red and infrared ranges on the size of areas of stunted growth of *S. aureus* ATCC 25923

Table 1

**Diameters of the stunted growth zones of control and irradiated  
by LED blue+IR culture of *S. aureus* ATCC 25923**

| Antibiotic | Control<br>(n=10) | Irradiation by LED radiation with exposure of 5 minutes |                           |                            |                             |                             |
|------------|-------------------|---|---------------------------|----------------------------|-----------------------------|-----------------------------|
|            |                   | Frequency 0 Hz<br>(n=10)                                | Frequency 10 Hz<br>(n=10) | Frequency 600 Hz<br>(n=10) | Frequency 3000 Hz<br>(n=10) | Frequency 8000 Hz<br>(n=10) |
| Ampicillin | 32,8±1,7          | 37,9±1,4<br>(P<0,001)                                   | 36,7±1,5<br>(P<0,001)     | 37,1±1,5<br>(P<0,001)      | 36,9±1,3<br>(P<0,001)       | 37,2±1,4<br>(P<0,001)       |
| Oxacillin  | 19,1±1,1          | 23,6±1,4<br>(P<0,001)                                   | 23,0±1,1<br>(P<0,001)     | 23,2±0,6<br>(P<0,001)      | 23,3±0,9<br>(P<0,001)       | 22,5±0,9<br>(P<0,001)       |

Table 2

**Diameters of the stunted growth zones of the control and irradiated samples  
by LED red and infrared radiation ranges of the culture *S. aureus* ATCC 25923**

| Antibiotic   | Control<br>(n=10) | Irradiation by LED radiation with exposure of 5 minutes |                           |                            |                             |                             |
|--------------|-------------------|---|---------------------------|----------------------------|-----------------------------|-----------------------------|
|              |                   | Frequency 0 Hz<br>(n=10)                                | Frequency 10 Hz<br>(n=10) | Frequency 600 Hz<br>(n=10) | Frequency 3000 Hz<br>(n=10) | Frequency 8000 Hz<br>(n=10) |
| Levofloxacin | 24,45±1,3         | 28,6,9±1,2<br>(P<0,001)                                 | 27,4±1,6<br>(P<0,01)      | 27,25±1,45<br>(P<0,001)    | 27,5±1,2<br>(P<0,001)       | 27,2±0,9<br>(P<0,001)       |
| Tetracycline | 26,3±2,1          | 33,6±1,95<br>(P<0,01)                                   | 32,4±2,1<br>(P<0,01)      | 32,2±2,4<br>(P<0,01)       | 32,0±2,0<br>(P<0,01)        | 31,75±2,45<br>(P<0,01)      |
| Meroponem    | 30,7±1,3          | 37,6±1,7<br>(P<0,001)                                   | 35,3±1,7<br>(P<0,001)     | 35,5±0,75<br>(P<0,001)     | 35,25±1,5<br>(P<0,001)      | 35±1,05<br>(P<0,001)        |

This regularity also concerned clinical isolates. 5-minute exposure to LED radiation increased sensitivity of all strains of *S. aureus*, cultured from locus of inflammatory diseases to ampicillin, oxacillin, cefotaxime, meroponem and rifampicin, and in some cases also to gentamicin and tetracycline.

Assessing the effects of irradiation of microorganisms in sterile cups, we noted the same regularities as after irradiation of bacteria in Petri caps with Hinton-Mueller agar, which allows to eliminate the impact of light on the properties of nutrient medium. Thus, the obtained results are related with the direct impact of photo modifying LED radiation on the examined flora.

Prolonged exposure of microorganisms to irradiation did not lead to the increase of their sensitivity, compared with 5-minute exposure. On the contrary, when irradiation lasted more than 15 minutes, sensitivity of all the investigated strains was within the statistical error compared with the control.

Taking in consideration almost complete absence of contraindications to the use of LED radiation, it can be used in the treatment of superficial suppurative inflammatory diseases caused by *Staphylococcus aureus*. This will allow to increase the effectiveness of antibiotics therapy for these infections.

## Conclusions

LED radiation of red+IR (640±30 and 880±30 nm) and blue+IR (470±30 and 880±30 nm) ranges makes photo modifying impact on the investigated strains of *Staphylococcus aureus*, which manifests itself in increase of their sensitivity to most tested antibiotics.

The degree of influence depends on the wavelength, duration and frequency of radiation impulses — the most pronounced increase of sensitivity was noted at the exposure of 5 minutes at frequency of 0 Hz (continuous radiation).

LED emission of blue-IR range led to the most pronounced increase of sensitivity to ampicillin and oxacillin, while at red and infrared radiation — to levofloxacin, tetracycline and meroponem.

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