ORIGINAL ARTICLES

BACTERICIDAL EFFECTIVENESS OF HIGH-INTENSITY PULSED BROADBAND IRRADIATION IN TREATING INFECTED WOUNDS

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Abstract
The study aimed to investigate the bactericidal efficacy of high-intensity pulsed broadband irradiation in the treatment of infected wounds. An experimental study was conducted on 90 mature male Wistar rats. An infected wound model was created by contaminating with *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Candida albicans*. Animals in Group 1 received high-intensity pulsed broadband irradiation. Animals in Group 2 received traditional UV irradiation. Animals in Group 3 had their wounds cleaned with 0.1% chlorhexidine solution. By the 3rd day of treatment, animals that received pulsed high-intensity broadband irradiation showed a significant reduction in contamination by *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* compared to Group 3. By the 7th day of treatment, half or the majority of animals in Groups 1 and 2 showed complete decontamination of wounds concerning *Staphylococcus aureus* and *Klebsiella pneumoniae*. Most animals in Group 1 showed complete wound clearance of *Pseudomonas aeruginosa*. By the 10th day, nearly all animals in Group 1 demonstrated complete decontamination of wounds. Statistical analysis revealed a significant difference in the reduction of wound contamination with Staphylococcus aureus and Klebsiella pneumoniae by the 10th day in Groups 1 and 2 compared to Group 3. Thus, the use of high-intensity pulsed broadband irradiation of wounds reduces the degree of pathogenic microorganism contamination in a shorter time frame.

Keywords: infected wound, ultraviolet irradiation, high-intensity pulsed broad-spectrum irradiation, local wound treatment, bacteriological control.

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БАКТЕРИЦИДНАЯ ЭФФЕКТИВНОСТЬ ИСПОЛЬЗОВАНИЯ ВЫСОКОИНТЕНСИВНОГО ИМПУЛЬСНОГО ШИРОКОПОЛОСНОГО ОБЛУЧЕНИЯ ПРИ ЛЕЧЕНИИ ИНФИЦИРОВАННЫХ РАН

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Резюме

Целью исследования явилось изучение бактерицидной эффективности высокоинтенсивного импульсного широкополосного облучения при лечении инфицированных ран. Проведено экспериментальное исследование на 90 половозрелых крысах-самцах линии Wistar (3 группы). Моделировали инфицированную рану контаминированием *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Candida albicans*. Животным 1-й группы проводили высокоинтенсивное импульсное широкополосное облучение. Животным 2-й группы проводили традиционное УФ облучение. Животным 3-й группы проводили туалет раны раствором хлоргексидина 0,1%. Проведенное исследование показало, что к 3-му дню лечения у животных, которым проводили импульсное высокоинтенсивное широкополосное облучение ран, имело место существенное уменьшение контаминации *Staphylococcus aureus*, *Klebsiella pneumoniae* и *Pseudomonas aeruginosa* по сравнению с 3-й группой. К 7-му дню лечения в 1-й и во 2-й группах у большинства животных наблюдали полную деконтаминацию ран в отношении *Staphylococcus aureus* и *Klebsiella pneumoniae*. У большинства животных 1-й группы выявлено полное очищение ран от *Pseudomonas aeruginosa*. К 10-му дню практически у всех животных 1-й группы отмечена полная деконтаминация ран. Статистический анализ показал, что к 10-му дню лечения у животных 1-й и 2-й групп по отношению к *Staphylococcus aureus* и *Klebsiella pneumoniae* выявлена существенная разница в снижении степени контаминации ран по сравнению с результатами у животных 3-й группы. Таким образом, применение импульсного высокоинтенсивного широкополосного облучения ран снижает степень контаминации патогенных микроорганизмов в более ранние сроки.

Ключевые слова: инфицированная рана, ультрафиолетовое облучение, импульсное высокоинтенсивное широкополосное облучение, местное лечение ран, бактериологический контроль.

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Introduction

In modern surgery, an important issue is the prevention and treatment of infections. In the first half of the 20th century, owing to the scientific works of A. Fleming, H.W. Florey and E.B. Chain, a new era in medicine began, which was marked by the emergence of antibiotics, for the discovery of which the scientists were awarded the Nobel Prize. Undoubtedly, the first and most important link in the treatment of infections, including wound infection, is antibacterial therapy. However the widespread use of antibacterial drugs has led to the evolution of microorganisms and the emergence of new types of pathogens with resistance to antibiotics.

According to WHO, there is an increase in antibacterial resistance to antibiotics. For instance, 50% of *Escherichia coli* strains are resistant to methicillin, *Staphylococcus aureus* (MRSA) and *Klebsiella pneumonia* – to third-generation cephalosporins and fluoroquinolones [1, 2]. Many types of microorganisms, including fungi, produce a protective extracellular polymer matrix, the so-called biofilms, which are quite difficult for modern systemic antimicrobial drugs to penetrate. There is a need to prescribe high doses of antimicrobial drugs, which can increase the risk of side effects [3, 4, 5].

The literature contains many works devoted to the issue of antibacterial resistance and the search for new drugs for the treatment of infections with antibacterial resistance [2, 6, 7, 8, 9, 10, 11]. Nevertheless, the issue of treating wound infections remains relevant. At the same time, many authors emphasize additional treatment methods that can achieve complete decontamination of wounds or sufficiently reduce their contamination. These methods include the effect of exogenous nitric oxide, vacuum therapy, hydrosurgical treatment of wounds, the use of ultrasonic cavitation, and photodynamic therapy [12, 13, 14, 15].

Light technologies are a set of developing methods in wound treatment. At the same time, low-frequency laser therapy and photodynamic therapy are currently widely used to treat wound infections [16, 17, 18, 19, 20]. The ultraviolet range includes electromagnetic radiation with wavelengths from 100 to 400 nm, which is divided into four independent spectral regions.

The wavelength range from 315 to 400 nm is defined as UVA, the range from 280 to 315 nm as UVB, radiation with wavelengths from 200 to 280 nm is classified as the UVC range, and the region from 100 to 200 nm is classified as vacuum ultraviolet. Short-wave UV radiation in the UV-C and UV-B ranges has a pronounced bactericidal effect with maximum efficiency at wavelengths from 250 to 270 nm and allows for the inactivation of various types of microorganisms, including antibiotic-resistant strains of pathogenic bacteria [21, 22, 23].

A large selection of methods of physical impact using light technologies on wound infection with increasing antibacterial resistance determine the relevance of improving phototherapy methods and selecting optimal effective modes of their use.

The aim of the study was to study the bactericidal effectiveness of high-intensity pulsed broadband irradiation in the treatment of infected wounds.

Materials and Methods

An experimental study approved by the Interuniversity Ethics Committee (extract from protocol No. 06-23 dated 15/06/23) was conducted. The experiment was performed on mature male Wistar rats weighing 220-250 g in the vivarium of the Russian University of Medicine of the Ministry of Health of the Russian Federation. All animals were quarantined for 2 weeks.

Manipulations on animals were performed under general anesthesia. Pre-medication with a 2% xylazine solution was performed. Then general anesthesia was performed with a solution of zoletil 100.

After achieving anesthesia, an infected wound was modeled under aseptic conditions. A skin incision 20x20 mm was made in the withers area. A trigger in the form of a gauze ball with a suspension of cultures from control strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Candida albicans* in equal volumes and dilutions, containing 10^9 microbial bodies in 1 ml, was introduced into the wound. The wound was sutured with a polypropylene thread by applying two interrupted sutures. In the postoperative period, all animals had access to liquid for drinking and received standard nutrition. One day after wound modeling, the sutures were removed. Then all animals were randomly divided into three Groups of thirty individuals.

Animals of the 1st Group (n=30) in the postoperative period after removal of sutures underwent daily wound cleaning with 0.1% chlorhexidine solution followed by high-intensity pulsed broadband irradiation and application of a dressing with 0.1% chlorhexidine solution to the wound. Irradiation was carried out for 10 days.

Animals of the 2nd Group (n=30) after daily wound cleaning with 0.1% chlorhexidine solution underwent traditional UV irradiation with application of a dressing with 0.1% chlorhexidine solution to the wound. Irradiation was carried out for 10 days.

Animals of the 3rd Group (n=30) underwent daily wound cleaning with 0.1% chlorhexidine solution and application of a dressing with 0.1% chlorhexidine solution to the wound.

High-intensity pulsed broadband irradiation was performed using a device developed by the Research Institute of Power Engineering of Bauman Moscow State Technical University. The operating principle of the device is based on pulsed irradiation of affected areas with high-intensity optical radiation of a continuous spectrum generated by a small-sized pulsed xenon lamp of the PPS 5/60 type. The lamp operates in a pulse-periodic mode with a pulse frequency of 5 Hz and an average electric power of 100 W. The average radiation power of the lamp in the UV-C range of the spectrum (200-280 nm) was 3 W, the pulsed power of UV-C radiation was 24 kW.

The device had three modes: mode 1 – irradiation cycle duration of 10 s (50 pulses); mode 2 – 20 s (100 pulses); mode 3 – 40 s (200 pulses). We selected the following wound treatment technique depending on the degree of contamination and the stage of the wound process: during the first five days of treatment, mode 3 was used (200 pulses with an irradiation cycle duration of 40 s) with an irradiation distance of 5 cm from the wound, starting from the sixth day of treatment. Mode 2 was used for the next five days (100 pulses with a cycle duration of 20 s) at a distance of 10 cm.

Traditional UV irradiation was carried out using the IUVQ-01 "Solnyshko" – a UV quartz irradiator based on a mercury bactericidal lamp of the ACBU-7 type with an electric power of 7 W. The radiation power in the UV-C range was 1.2 W. Irradiation was carried out daily for 10 days for 3 minutes from a distance of 10 cm from the irradiator to the wound.

To assess the bactericidal effectiveness of high-intensity pulsed broadband radiation and traditional UV irradiation in the treatment of infected wounds, a bacteriological study was carried out on the day of suture removal (before treatment), on the 3rd, 7th, 10th, 14th and 21st days from the start of treatment.

The analysis of the degree of contamination and the dynamics of decontamination of wounds with various microflora was performed. For this purpose, at each control period, the number of animals with different degrees of contamination of four types of microorganisms (*Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Candida albicans*) 10^8, 10^6 and $<10^4$ on the wound surface was taken into account. Samples were seeded using the sector seeding method (according to Gold-Rodoman) on Petri dishes with blood agar, as well as with Endo and Saburo media.

The study was conducted in the bacteriological laboratory of the A.S. Loginov Moscow Medical Scientific Center of the Healthcare Department of the City of Moscow. The results are presented as a percentage. Comparative assessment of qualitative features within and between Groups was performed using the Pearson χ^2 criterion, for which conjugation tables were preliminarily constructed and evaluated. A feature was considered statistically different at $p<0.05$. Multiple comparisons were performed using the Bonferroni correction. For comparisons between Groups, $k=0.05/3=0.0167$.

Results

Before the treatment, bacteriological examination of the wound surface showed that there was no statistically significant difference between the study Groups in the degree of microflora contamination. Almost all animals were contaminated with *Staphylococcus aureus* 10^8 CFU. Wound contamination with *Pseudomonas aeruginosa* (10^8 CFU) was noted in 90% of animals in the three study Groups.

By the 3rd day of treatment, statistically significant decrease in the degree of wound contamination with *Staphylococcus aureus* was revealed in animals of the 1st Group compared to the 2nd and 3rd Groups (Table 1). Positive dynamics were observed in all Groups in relation to the reduction in the degree of wound contamination with *Klebsiella pneumoniae* on the 3rd day. By the 3rd day of control, a significant difference in the treatment results was found between the 1st and 3rd Groups (p=0.0025, χ^2=14.3 and p=0.01, χ^2=11, respectively) in relation to the contamination of wounds with *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Positive dynamics in the form of decontamination and a decrease in the degree of contamination of the wound surface with *Candida albicans* were also noted. No statistical difference was found between the Groups during this period in terms of *Candida albicans* contamination (p=0.33, χ^2=4.58).

By the 7th day of treatment, positive dynamics were observed in relation to the reduction of the degree of contamination of all microorganisms (Table 2). Moreover, it was noted that there was no contamination of 10^8 CFU in any Group. Analysis of the contamination of wounds with *Staphylococcus aureus* by this time showed that there was a significant difference in the results of decontamination and reduction of the degree of contamination between the 1st and 3rd Groups (p<0.0001, χ^2=41.14), and a difference was also revealed between the 2nd and 3rd Groups (p<0.0001, χ^2=29.14).

At the same time, the contamination of *Klebsiella pneumoniae* was reduced in all Groups of animals (Table 2). The rates of *Pseudomonas aeruginosa* contamination of wounds in animals of the 1st Group by the 7th day of treatment differed from those in the 2nd and 3rd Groups (p=0.01, χ^2=8.93, p<0.0001, χ^2=25.84, respectively). There was a positive trend with respect to *Candida albicans*; in all Groups, complete decontamination of wounds with *Candida albicans* was observed in most animals. However, no statistically significant difference was found between the Groups by this time (Table 2).

Таблица 1
Контаминация ран различной микрофлорой у животных в трех группах на 3-й день лечения
Table 1
Contamination of wounds by various microflora in animals across three groups on day 3 of treatment

Микрофлора Microflora	КОЕ CFU	Группы / Groups						P
		1		2		3		
		Абс. Abs.	%	Абс. Abs.	%	Абс. Abs.	%	
Staphylococcus aureus	10^8	2	6,67	6	20	11	36,67	p=0,0014, χ^2=21,64; P1-2 p=0,043, χ^2=8,13; P1-3 p=0,0003, χ^2=18,6; P2-3 p=0,25, χ^2=4,07
	10^6	7	23,33	13	43,33	13	43,33	
	$<10^4$	13	43,33	9	30	6	20	
	Нет роста No growth	8	26,67	2	6,67	0		
Klebsiella pneumoniae	10^8	0		1	3,33	6	20	p=0,0071, χ^2=17,65; P1-2 p=0,26, χ^2=4; P1-3 p=0,0025, χ^2=14,3; P2-3 p=0,13, χ^2=5,5
	10^6	4	13,33	8	26,67	10	33,33	
	$<10^4$	12	40	13	43,33	10	33,33	
	Нет роста No growth	14	46,67	8	26,67	4	13,33	
Pseudomonas aeruginosa	10^8	5	16,67	9	30	14	46,67	p=0,034, χ^2=13,48 P1-2 p=0,28, χ^2=3,82; P1-3 p=0,01, χ^2=11; P2-3 p=0,17, χ^2=4,95
	10^6	8	26,67	6	20	9	30	
	$<10^4$	8	26,67	11	36,67	6	20	
	Нет роста No growth	9	30	4	13,33	1	3,33	
Candida albicans	10^8	0		0		0		p=0,33, χ^2=4,58
	10^6	2	6,67	4	13,33	5	16,67	
	$<10^4$	6	20	9	30	11	36,67	
	Нет роста No growth	22	73,33	17	56,67	14	46,67	

By the 10th day of treatment, the *Staphylococcus aureus* contamination differed significantly between the Groups (Table 3). The number of animals with wound decontamination with *Staphylococcus aureus* was significantly lower in Group 1 compared to Groups 2 and 3 ($p=0.01$, $\chi^2=6.4$ and $p<0.0001$, $\chi^2=22.33$, respectively). In Group 2, the results differed significantly compared to Group 3 ($p=0.01$, $\chi^2=9.13$). The decrease in the degree of *Klebsiella pneumoniae* contamination among animals in Group 1 was significantly greater than in Groups 2 and 3 ($p=0.01$, $\chi^2=9.23$ and $p<0.0001$, $\chi^2=25.71$, respectively).

Also, the wound cleansing indices in the 2nd Group differed significantly from those in the 3rd Group ($p=0.0002$, $\chi^2=17.01$). When analyzing the wound contamination with *Pseudomonas aeruginosa* by the 10th day of treatment, there was a statistically significant difference between the Groups ($p=0.0001$, $\chi^2=29.03$). The results in the 1st Group were significantly better compared to the 3rd Group ($p=0.0001$, $\chi^2=23.81$). By the 3rd day of control, decontamination of *Candida albicans* wounds was detected in all animals of the 1st and 2nd Groups, and only 2 (6.67%) animals of the 3rd Group had a contamination of 10^4 CFU.

On the 14th day of treatment, positive dynamics were observed compared to the previous days of control. In all animals of the 1st Group, complete decontamination of wounds was observed in relation to all microorganisms. In the 2nd Group, 27 animals showed complete decontamination in relation to *Staphylococcus aureus* and *Klebsiella pneumoniae*, and in 26 animals in relation to *Pseudomonas aeruginosa*. In the 3rd Group, positive dynamics were also observed, in most rats, complete cleansing of wounds was detected.

Таблица 2
Контаминация ран различной микрофлорой у животных в трех группах на 7-й день лечения
Table 2
Contamination of wounds by various microflora in animals across three groups on day 7 of treatment

Микрофлора Microflora	КОЕ CFU	Группы Groups 1 Абс. Abs.	1 %	2 Абс. Abs.	2 %	3 Абс. Abs.	3 %	P
Staphylococcus aureus	10^8	0		0		0		p<0,0001, χ^2=54,62; P1-2 p=0,051, χ^2=5,8; P1-3 p<0,0001, χ^2=41,14; P2-3 p<0,0001, χ^2=29,14
	10^6	0		4	13,33	24	80	
	$<10^4$	8	26,67	11	36,67	0		
	Нет роста No growth	22	73,33	15	50	6	20	
Klebsiella pneumoniae	10^8	0		0		6	20	p<0,0001, χ^2=48,82; P1-2 p=0,35, χ^2=0,88; P1-3 p<0,0001, χ^2=32,87; P2-3 p<0,0001, χ^2=28,68
	10^6	0		0		10	33,33	
	$<10^4$	5	16,67	8	26,67	10	33,33	
	Нет роста No growth	25	83,33	22	73,33	4	13,33	
Pseudomonas aeruginosa	10^8	0		0		2	6,67	p<0,0001, χ^2=27,91 P1-2 p=0,01, χ^2=8,93; P1-3 p<0,0001, χ^2=25,84; P2-3 p=0,047, χ^2=7,94
	10^6	0		7	23,33	14	46,67	
	$<10^4$	9	30	10	33,33	9	30	
	Нет роста No growth	21	70	13	43,33	5	16,67	
Candida albicans	10^8	0		0		0		p=0,052, χ^2=5,88
	10^6	0		0		0		
	$<10^4$	1	3,33	2	6,67	5	16,67	
	Нет роста No growth	29	96,67	28	93,33	25	83,33	

Таблица 3
Контаминация ран различной микрофлорой у животных в трех группах на 10-й день лечения
Table 3
Contamination of wounds by various microflora in animals across three groups on day 10 of treatment

Микрофлора Microflora	КОЕ CFU	Группы Groups						P
		1		2		3		
		Абс. Abs.	%	Абс. Abs.	%	Абс. Abs.	%	
Staphylococcus aureus	10^8	0		0		0		p<0,0001, χ^2=26,86; P1-2 p=0,01, χ^2=6,4; P1-3 p<0,0001, χ^2=22,33; P2-3 p=0,01, χ^2=9,13
	10^6	0		0		5	16,67	
	<10^4	1	3,33	8	26,67	13	43,33	
	Нет роста No growth	29	96,67	22	73,33	12	40	
Klebsiella pneumoniae	10^8	0		0		0		p<0,0001, χ^2=35,46; P1-2 p=0,01, χ^2=9,23; P1-3 p<0,0001, χ^2=25,71; P2-3 p=0,0002, χ^2=17,01
	10^6	0		0		5	16,67	
	<10^4	0		3	10	13	43,33	
	Нет роста No growth	30	100	27	90	12	40	
Pseudomonas aeruginosa	10^8	0		0		1	3,33	p=0,0001, χ^2=29,03; P1-2 p=0,01, χ^2=8,93; P1-3 p=0,0001, χ^2=23,81; P2-3 p=0,026, χ^2=9,25
	10^6	0		1	3,33	9	30	
	<10^4	0		7	23,33	6	20	
	Нет роста No growth	30	100	22	73,33	14	46,67	
Candida albicans	10^8	0		0		0		p=0,12, χ^2=4,09
	10^6	0		0		0		
	<10^4	0		0		2	6,67	
	Нет роста No growth	30	100	30	100	28	93,33	

By the 21st day, among the animals of the 2nd Group, only 1 animal showed growth of *Staphylococcus aureus*, in the remaining animals, complete decontamination of wounds was detected. In the 3rd Group, 1 animal had contamination of 10^6 CFU, 5 animals had growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* of 10^4 CFU, and 2 animals had growth of *Klebsiella pneumoniae* of 10^4 CFU.

Discussion

There are scientific studies devoted to the effectiveness of pulsed high-intensity optical irradiation in experiments. Thus, some authors note the bactericidal effect of pulsed high-intensity optical irradiation for the treatment of linear wounds in experimental animals. It is worth noting that the modeled wounds were not subjected to initial infection, they were practically aseptic [24].

There is also information on the clinical effectiveness of using high-intensity optical irradiation in experiments *in vitro* and *in vivo* [25, 26]. The authors of the study claim that high-intensity optical irradiation has pronounced bactericidal and wound-healing effects and reliably provides higher rates of wound healing compared to the use of only a typical antibacterial and wound-healing agent – Levomekole (methyluracilum, chloramphenicol) ointment [25, 26]. It should be emphasized that the modeled wounds were infected with one microorganism in low contamination of 10^3 CFU. At the same time, there is no data on wound contamination control and, moreover, antibacterial therapy was started immediately after the application of pathogenic flora to the wound.

In our study, experimental infection of the wound was performed with four pathogenic strains in equal volumes and dilutions containing 10^9 microbial bodies in 1 ml.

Bacteriological control was carried out, which confirmed pathogenic infection of the wounds, after which the animals were divided into three Groups depending on the treatment method. We conducted a comparative analysis of the effectiveness of decontamination and reduction of the degree of contamination of infected wounds between pulsed high-intensity broadband irradiation, traditional ultraviolet irradiation and drug local treatment. Bacteriological control was performed during the treatment. It was revealed that pulsed high-intensity broadband irradiation has a higher antibacterial activity compared to traditional irradiation and local treatment.

Conclusion

Thus, the conducted bacteriological study showed that against the background of the treatment, positive dynamics were obtained at each control period in the form of a decrease in the degree of contamination or complete decontamination of wounds in all Groups. Moreover, by the 3rd day of treatment against the background of pulsed high-intensity broadband irradiation of wounds, there was a statistically significant decrease in the degree of contamination of wounds with *Staphylococcus aureus, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa* compared to the Group where traditional local treatment of wounds with an antiseptic was carried out ($p = 0.0003$, $\chi^2 = 18.6$, $p = 0.0025$, $\chi^2 = 14.3$ and $p = 0.01$, $\chi^2 = 11$, respectively).

By the 3rd day of control, no significant difference was found in the results of treatment of infected wounds between the Group of animals that underwent traditional ultraviolet irradiation of wounds and the Group of animals that received local drug therapy.

On the 7th day, the dynamics of treatment results between the Groups differed statistically significantly. In the 1st and 2nd Groups, as a result of treatment, most animals achieved complete decontamination of wounds with respect to *Staphylococcus aureus* and *Klebsiella pneumoniae*. Most animals treated with pulsed high-intensity broadband irradiation showed complete cleansing of wounds from *Pseudomonas aeruginosa*.

Compared with the previous control periods, by the 10th day, almost all animals in the 1st Group had complete decontamination of wounds from all types of microflora. During this period, in the 2nd Group, most animals showed a decrease in the degree of bacterial contamination, as well as complete cleansing of wounds. Statistical analysis showed that with pulsed high-intensity broadband or traditional UV irradiation of wounds on the 10th day, a reliable difference in the effectiveness of treatment with respect to *Staphylococcus aureus* and *Klebsiella pneumoniae* was revealed compared with local drug therapy.

Consequently, the use of pulsed high-intensity broadband irradiation of wounds by the claimed method reduces contamination of pathogenic microorganisms, both gram-negative and gram-positive, at an earlier time, in contrast to traditional drug methods of local treatment and traditional ultraviolet irradiation of wounds.

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