European International Journal of Multidisciplinary Research and Management Studies (ISSN: 2750-8587)

Received: 20 April 2026 | Accepted: 05 May 2026 | Published: 31 May 2026

DOI: 10.55640/eijmrms-06-05-09

RESEARCH ARTICLE

The Role of Bacteriological Studies in Determining the Effectiveness of Comprehensive Treatment of Experimental Alloxan Diabetic Purulent-Necrotic Wounds

Ergashev U.Y.
Tashkent State Medical University, 100109 Tashkent, Uzbekistan, Farabi street 2, Uzbekistan

Zokhirov A.R.
Tashkent State Medical University, 100109 Tashkent, Uzbekistan, Farabi street 2, Uzbekistan

VOLUME: Vol.06 Issue05 2026

PAGE: 45-52

Abstract

The article conducted bacteriological studies of smears from purulent-necrotic wounds to determine the effectiveness of complex treatment using ozone- and detoxification therapy for purulent-necrotic wounds on the legs of experimental rats against the background of alloxan diabetes.

KEYWORDS

Alloxan diabetic purulent-necrotic model, ozone therapy, Reomannisol, bacteremia, contamination.

INTRODUCTION

Due to the purulent-necrotic complicated form of diabetes mellitus, the cost of treating the infection is significant, and as the number of patients suffering from it increases, the costs of their treatment also increase [1]. A purulent-necrotic lesion of the lower extremity due to diabetes mellitus can spread through direct infection or through the bloodstream (haematogenous). The purulent-necrotic process is dominated by the entry of infection into the wound, the most important of which are Staphylococcus aureus, Pseudomonas auriginosa, Escherichia coli [9].

In Uzbekistan, according to statistics from the Republican Scientific and Practical Medical Center of Endocrinology, more than 277,000 people struggle with diabetes mellitus and its complications, of whom more than 3,000 are minors [3].

According to data from the Russian Federation, the cost of treating patients with purulent-necrotic complicated forms of diabetes mellitus is 3.62 million rubles per patient, half of which is spent on primary care, while the remainder is spent on primary and repeated amputations and prosthetics [4.5].

Furthermore, the average duration of inpatient treatment for ulcers, minor amputations, and major amputations alone is 13.3, 20.5, and 59.6 days. The average annual cost per patient is 3,368 USD (ulcer only), 10,468 USD (minor amputation), and 30,131 USD (major amputation) [6,7]. The long duration of treatment for the purulent-necrotic form of diabetes mellitus, high disability and mortality rates, long hospitalizations, and high medical costs not only significantly affect the patient's quality of life and physical and mental health but also lead to significant economic losses for society [8,9,10].

Objective of the study: To determine the effectiveness of the local drug in combination with ozonated physiological solution on the bactericidal and bacteriostatic processes of bacteria in purulent-necrotic wounds of the lower extremities caused by experimental alloxan diabetes.

METHODS

Experimental studies were conducted on 195 white male rats weighing 150-200 g, kept in the vivarium of the Tashkent State Medical University. All procedures and animal manipulations were performed using general pain relief in accordance with the "Rules for performing work using experimental animals," adhering to the humanitarian principles outlined in the European Community Guidelines (86/609/EEC) and the Helsinki Declaration. Experimental animals were divided into 4 groups: Group 1 - unchanged group (intakt); Group 2 (control group) - creation of an experimental model of a purulent-necrotic leg against the background of alloxan diabetes; Group 3 (comparison group) - traditional complex treatment (ozonotherapy + Reosorbilakt) against the background of creating an experimental model of a diabetic purulent-necrotic leg; Group 4 (main group) – an experimental model of an alloxan-induced diabetic purulent-necrotic foot, combined with complex treatment with ozone therapy and rheomannisol. After injecting sodium thiopental at a dose of 80 mg/g into the abdominal cavity relative to the body weight of each rat, after general anesthesia, the dorsal part of the rat's hind legs (only one of the left or right hind legs is selected) is treated with an antiseptic, and 0.25 ml of a 10% calcium chloride solution ($CaCl2$) is injected subcutaneously into the leg using an insulin syringe. Before this manipulation, 20.0 ml of a physiological solution is poured into a Petri dish, into which 6-7 feces of the same rat are placed. Once the stools have swollen, they are crushed to form a suspension. The resulting solution is passed through a gauze napkin (so that during the injection, the needle of the syringe is not obstructed by fecal fibers and tissue detritus). Take 1 ml of the prepared suspension and inject it subcutaneously into the area where calcium chloride was administered. After 72 hours, we open the purulent-necrotic wound and see its condition. To prepare the ozonated physiological solution, we ozonate 200.0 ml of the physiological solution at a concentration of 3-4 mg/l at a rate of 0.25 l/min for 10 minutes in the "Medozons Beauty" apparatus (MEDOZONS LLC, Russia). The purulent-necrotic area of each rat leg is washed 3 times a day with an ozonated physiological solution for 15–20 minutes. A smear from the purulent-necrotic wound was taken from the control group of rats in the 3 groups selected for the study on days 1, 3, 7, 10, 14, and 21. The comparison group rats were examined on days 1, 3, 7, 10, and 14, while the primary group was examined on days 1, 3, 7, and 10. In the bacteriological method, it is necessary to examine a smear from rats from a purulent-necrotic wound by placing it in a nutrient medium for 30 minutes to 1 hour. According to Gold's method, in a nutrient medium, a smear on a swab placed inside a sugar broth provides a favorable environment for bacteria, in which their strains reproduce. Facultative-anaerobic and aerobic bacteria grow and reproduce well in 5% blood agar, herbaceous-saline agar, and Saburo media. Colony counts in 1 ml of medium are measured in colony-forming units (CFU), i.e., in CFU/tampon or CFU/ml, and the degree of colonization is determined.

RESULTS AND DISCUSSION

As a result of bacteriological and bacterioscopic studies, the following strains were obtained in all rats (see Table 1).

The quantitative indicator of microorganisms is of great importance for assessing the course of infections in the wound area and wound healing, as well as the effectiveness of treatment. The number of microorganisms in 1 gram of tissue taken from the wound area is estimated by the number of colony-forming units (CFU) in 1 ml of test solution according to the generally accepted method. The count of the number of microorganisms is determined by the number of colonies in 1 ml of the smear.

We also studied how to treat Pseudomonas aeruginosa and Escherichia coli bacteria with ozone in a saturated nutrient medium. The obtained data showed that after treatment with the ozonated physiological solution for 30 seconds, it had a pronounced bactericidal effect, i.e., 90-95% of the bacteria were killed. We have an idea that if we increase the treatment time of the wound with ozonated saline, it will be completely cleared of bacterial strains (see Table 2).

It can be seen that salty and liquid environments reduce the effectiveness of ozone in ozonated physiological solution; conversely, organic substances in a medium with a high and sufficient nutrient content do not reduce the effectiveness of ozone, but increase it, and the bactericidal effect on bacteria is high. This serves as the basis for the local application of an ozonated physiological solution against bacteria in diabetic purulent-necrotic wounds.

Table 1

Distribution of bacterial strains in groups

Aerobic bacteria	Quantity of strain	%
Control group		
Monoculture		
Staphylococcus aureus	10	17.55
Escherichia coli	15	26.32
Pseudomonas aeruginosa	19	33.33
Escherichia inadivae	4	7.0
Proteus mirabilis	3	5.27
Streptococcus anginosus	6	10.53
Total	57	100.00
Comparison group		
Monoculture		
Staphylococcus aureus	8	15.68
Escherichia coli	16	31.37
Pseudomonas aeruginosa	18	35.29
Escherichia inadivae	3	5.88
Proteus mirabilis	2	3.92
Streptococcus anginosus	2	3.92
Staphylococcus epidermis	2	3.92
Total	51	100.00
Main group		
Staphylococcus aureus	5	13.88
Escherichia coli	11	30.55
Pseudomonas aeruginosa	15	41.66
Escherichia inadivae	2	5.55
Proteus mirabilis	1	2.77
Streptococcus anginosus	1	2.77

Enterococcus faecalis	1	2.77
Total	36	100.00

Table 2.

Antibacterial effect of ozonated physiological solution on Pseudomonas aeruginosa and Escherichia colibacteria

Processing method	Time, min.	Survival rate, %
Ozonated hypertonic solution	30 seconds	100.00
	1 minute	95.75
	2 minutes	94.25
	5 minutes	90.50
	10 minutes	90.50
Ozonated physiological solution	30 seconds	85.00
	1 minute	76.00
	2 minutes	48.20
	5 minutes	39.75
	10 minutes	24.20
Ozonated physiological solution mixed in a saturated nutrient medium	30 seconds	15.00
	1 minute	2.00
	2 minutes	0.50
	5 minutes	0.00
	10 minutes	0.00
Ozonated physiological solution mixed in a liquid nutrient medium	30 seconds	70.10
	1 minute	38.15
	2 minutes	28.50
	5 minutes	17.20
	10 minutes	10.65

At the first stage of the experiment, taking into account the final results, 10-minute washing with an ozonated physiological solution (concentration 6.5 mg/l) reduced any microflora from the guaranteed 107 CFU/ml to 105 CFU/ml. When treating the purulent-necrotic area with ozonated distilled water, the number of microbes decreased from 107

CFU/ml to 103 CFU/ml. When treated with an ozonated physiological solution (concentration 6.5 mg/l) for 10 minutes using a saturated food broth, complete destruction of microbes is noted.

It was found that the nature of the experiments varied, and that "biological protection," i.e., the ability of nutrients and blood plasma to exert a strong oxidizing effect on double bonds, gave us a clear idea of the rapid decomposition of ozone into atomic oxygen. This has a strong bactericidal effect on microbes. Due to the difficulty of breaking bivalent ozone bonds in saline solutions and liquid media, the formation of atomic oxygen slows down and the effect on microbes decreases (see Table 2).

In the first experiment, the ozonated physiological solution exerted a direct oxidizing effect on the bacterial cell membrane and cell organelles, and a bacteriostatic process occurred on them. When treated with an ozonated distilled aqueous solution, it exerted a bactericidal effect on the bacterial cell membrane. When the nutrient was treated with an ozonated physiological solution, it was found that the bacteria were completely destroyed.

However, it is necessary to strive for maximum removal of tissue detritus, blood clots, fibrin filaments, and purulent exudate from the area of the purulent-necrotic wound, as well as tissue devitalization. It was also found that the ozonated physiological solution reduces the oxidative and bactericidal effects. This reduces the effectiveness of the local application of the ozonated physiological solution.

In such cases, the decrease in the effective effect of ozone is due to the loss of its energy for the oxidation of organic substances present in the substrate. Taking all the above circumstances into account, it was shown in which medium to dissolve ozone to achieve its maximum bactericidal effect, how to know the exposure time, how to maximize the cleaning of the wound from necrotic tissues, and how to apply it.

In all groups, we detected high contamination of 2 bacterial strains, namely Pseudomonas aeruginosa and Escherichia coli , and observed bactericidal and bacteriostatic effects of the ozonated physiological solution in combination with Reosorbilakt and Remannisol only against these two types of bacteria.

Pseudomonas aeruginosa in the control group was 3.3±0.85x106 CFU/ml on the 1st day of the experiment, and on the 3rd day, it increased by 1.06 times ($p<0.001$) compared to the 1st day; on the 7th day 1.22 times ($p<0.001$); 1.14 times on the 10th and 14th days ($p<0.001$); We found that it decreased by 1.65 times ($p<0.001$), and by 2.2 times ($p<0.001$) on the 21st day. Escherichia coli on the 1st day of the experiment was 4.3±0.96x106 CFU/ml, and on the 3rd day increased by 1.11 times ($p<0.001$); on the 7th day 1.07 times ($p<0.001$); On the 10th day, it decreased by 1.23 times ($p<0.001$), on the 14th day by 1.43 times ($p<0.001$), and on the 21st day by 1.79 times ($p<0.001$) (see Table 3).

Table 3.

Dynamics of changes in bacterial decrease in the control group.

Control group						
Bacterial culture	Day 1	Day 3	Day 7	Day 10	Day 14	Day 21
	Colony-forming unit, CFU/ml					
Pseudomonas aeruginosa	$3.3 \pm 0.85 \times 10^{6}$	$3.5 \pm 0.72 \times 10^{5}$	$2.7 \pm 0.65 \times 10^{4}$	$2.9 \pm 0{,}5 \times 10^{4}$	$2.0 \pm 0.44 \times 10^{4}$	$1.5 \pm 0.2 \times 10^{2}$
Escherichia coli	$4.3 \pm 0.96 \times 10^{6}$	$4.8 \pm 0.8 \times 10^{4}$	$4.0 \pm 0.75 \times 10^{3}$	$3.5 \pm 0.52 \times 10^{3}$	$3.0 \pm 0.32 \times 10^{2}$	$2.4 \pm 0.25 \times 10^{2}$

In the comparison group, Pseudomonas aeruginosa on the 1st day of treatment increased 1.27 times compared to the period before treatment ($p<0.001$); on the 3rd day by 1.13 times compared to the 1st day ($p<0.001$); On the 7th day, 1.82 times ($p<0.001$); 2.1 times on the 10th and 14th days ($p<0.001$); 3.5 times ($p<0.001$). Escherichia coli on the 1st day of treatment decreased by 1.11 times ($p<0.001$) compared to the period before treatment, and on the 3rd day by 1.6 times ($p<0.001$); on the 7th day 1.9 times ($p<0.001$); On the 10th day, it decreased by 2.67 times ($p<0.001$), and on the 14th day, by 3.63 times ($p<0.001$). On the 21st day, no bacterial strain was detected (see Table 4).

Table 4.

Dynamics of changes in bacterial decrease in the comparison group.

Comparison group							
Bacterial culture	Before treatment	After treatment					
		Day 1	Day 3	Day 7	Day 10	Day 14	Day 21
	Colony-forming unit, CFU/ml						
Pseudomonas aeruginosa	$4.2±0.9 x10^6$	$3.3±0.8 x10^6$	$2.9±0.77 x10^5$	$2,3±1,3x 10^4$	$2.0±0.36 x10^2$	$1.2±0.23 x10^1$	-
Escherichia coli	$4.0±1.6 x10^5$	$3.6±1.2 x10^4$	$2.5±0.84 x10^3$	$2.1±0.47 x10^3$	$1.5±0.33 x10^2$	$1.1±0.24 x10^1$	-

In the main group Pseudomonas aeruginosa 1.57 times compared to the 1st day of treatment ($p<0.001$); on the 3rd day by 1.86 times compared to the 1st day ($p<0.001$); On the 7th day, it decreased by 3.41 times ($p<0.001$). Escherichia coli decreased by 1.34 times ($p<0.001$) on the 1st day of treatment compared to the day of admission, and by 2.78 times ($p<0.001$) on the 3rd day; On the 7th day, we observed a decrease of 4.87 times ($p<0.001$). Against the background of local application of ozonated saline and reduction of general intoxication with Rheomannisol, no bacteria were detected on days 10, 14, or 21 (see Table 5).

Table 5.

Dynamics of changes in bacterial decline in the main group.

Main group							
Bacterial culture	Treatment cha	After treatment					
		Day 1	Day 3	Day 7	Day 10	Day 14	Day 21
	Colony-forming unit, CFU/ml						
Pseudomonas	4.1	2.6 ±0.91x10	2.2 ±0.67x10	1.2 ±0.37x10	-	-	-

aeruginosa	$\pm 1.2x10^6$	5	4	2			
Escherichia coli	3.9 $\pm 0.92x10^5$	2.9± $0.64x10^4$	1.4±0.45 $x10^2$	0.8 $\pm 0.11x10^2$	-	-	-

A comparison of treatment methods in the comparison and main groups showed significant improvements in soft tissue injuries of the lower extremities, including local ozone therapy and intra-abdominal administration of Reosrbilakt. Ozonated saline improved tissue oxygenation and exerted a bactericidal effect on wound infections, while Reosorbilakt improved microcirculation and contributed to the improvement of endogenous intoxication. The restoration of soft tissues and skin of the lower extremities during treatment with ozone therapy and Reomannsiol had a positive effect compared to the comparison group. Due to its properties to improve microcirculation and activate tissue regeneration, rheomannisol initiated recovery processes earlier than Reosorbilakt. The positive dynamics in the main group accelerated by the 7th day.

CONCLUSION

On the 7th day of the growth dynamics of microbes in the purulent-necrotic area, which determines wound healing, the bacterial strain dropped to 2.0x104 levels and 1.4x103 levels, while on the 10th day, no bacterial strain was detected, indicating that the microbes had an effective effect on CFU/ml.

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