ORIGINAL ARTICLE

# APPLICATION OF PROBIOTIC ANTISEPSIS FOR PURULENT COMPLICATIONS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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## ABSTRACT
**The aim:** To improve the results of surgical treatment of patients with type 2 diabetes and purulent-necrotic wounds by using probiotic antiseptics.
**Materials and methods:** 66 patients with type 2 diabetes and purulent-necrotic complications took part in this study. Probiotic antiseptics were used for local treatment in the experimental group (n=31), and traditional antiseptics were used in the control group (n=35). The levels of pro-inflammatory markers in the blood (IL-6, TNF-a, CRP) were studied; microscopic material was taken to study the type of cytogram during bandaging, before wound treatment with antiseptics or debridement on admission to the hospital (1st day), on the 3rd day and on the 7th day.
**Results:** Analysis of dynamic changes in pro-inflammatory markers between the first and seventh days proved that only in the experimental group there was a statistically significant difference (IL-6 (P=0.004), TNF-a (P=0.001), CRP (P=0.018)). Detection of regenerative-inflammatory and regenerative cytogram types on the 7th day in the experimental group had a statistically significant difference compared to the control group (p=0.002 and p<0.001, respectively).
**Conclusions:** the use of probiotic antisepsis accelerates wound healing in patients with type 2 diabetes and purulent-necrotic complications.

**KEY WORDS:** Diabetes Mellitus 2 type, *Bacillus subtilis*, Interleukin-6, TNF-alpha, C-Reactive Protein

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## INTRODUCTION

Every year, the number of patients with type 2 diabetes mellitus (T2DM) in the world increases by 5–7%, and every 12–15 years it doubles. According to WHO statistical materials, in 2005 there were 200 million patients with diabetes in the world, in 2013 – more than 382 million, in 2014 – 422 million, in 2019 – 462 million, in 2021 – 532 million, in 2045 they predict their growth to 700 million people [1]. T2DM has now reached the scale of a pandemic.

According to the data of the Public Health Center of Ukraine, the incidence of T2DM has doubled over the past 10 years. In 2016, more than 1.2 million people in Ukraine suffered from T2DM causes more than 3% of primary disability of the working population of Ukraine [2].

Along with the increase in T2DM incidence, the mortality rate from its complications also increases. According to WHO data, the mortality rate increased by 3% between 2000 and 2019 [3].

The constant increase in the number of patients with T2DM leads to an increase in the number of its complications. One of its most frequent complications is diabetic foot syndrome (DFS), which develops in 6-11% of patients, and 40-70% of them require surgical treatment with the use of antibacterial drugs. 30% of hospitalizations of patients with T2DM are related to DFS [4].

In 40% of patients, a recurrence of purulent-necrotic processes of the lower extremities occurs within the first year after treatment, and in 60% – within three years [5].

In patients with purulent-necrotic processes of soft tissues, aerobic gram-positive cocci are the most common causative agents, among which *Staphylococcus aureus* and streptococci are most often isolated. Patients with a long course and relapse have a combined flora that often acquires antibiotic-resistant properties [6].

In patients with purulent-necrotic wounds of soft tissues, a “circulus vitiosus” is formed, which is caused by a violation of wound healing in diabetes, as well as an increase in the level of blood glucose in the presence of an inflammatory focus [7].

Analysis of procalcitonin, interleukin-6, and fibrinogen levels is used to diagnose complications of purulent-necrotic soft tissue processes in T2DM [8].

Taking into account the trends in the incidence of T2DM and the development of its purulent complications, the issue of finding and developing new antimicrobial agents for the treatment of this category of patients has become extremely urgent.

## THE AIM

The purpose of the work was to improve the results of surgical treatment of patients with T2DM and purulent-necrotic wounds by using probiotic antiseptics.

## MATERIALS AND METHODS

This study was conducted at the clinical base of the Department of General Surgery No. 2 of the Bogomolets National Medical University in the Kyiv City Clinical Hospital No. 3. The criteria for inclusion in the experiment were: men and women, aged from 18 to 75 years, the presence of purulent-necrotic wounds of various localization and (on the background) T2DM. All included patients (n=66) were randomly divided (randomized) into two groups by the method of closed envelopes. The research group included 31 patients, where probiotic antiseptics were used as a local treatment, and the control group - 35 patients - with the use of traditional antiseptics (Table I).

The above data indicate that the control and experimental groups are comparable to each other ($p>0.05$).

The use of probiotic antiseptics included the use of a combination of antiseptics based on lactic acid bacteria (LAC), which are pathogenic for humans.

To treat the skin around the wound, a gel was used, the composition of which included: ethyl alcohol 60.0%, 2-propanol 5.0%, *Bacillus megaterium*<5%, *Bacillus subtilis* 5.0%, enzymes 5-10%. A gel was used to clean the wound, which included: purified water,

**Fig. 1.** Dynamics of macroscopic changes in the wound of Patient S., 54 years old: 1a. State before admission to the hospital; 1b. The 3rd day in the hospital; 1c. The 7th day in the hospital

**Fig. 2.** Dynamics of macroscopic changes in the wound of Patient S. 58 years old: 2a. State before admission to the hospital; 2b. The 3rd day in the hospital; The 7th day in the hospital

**Table I.** Characteristics of patients included in the experiment

| Indicator | Group distribution | | |
|---|---|---|---|
| | Control (CG) | Experimental (EG) | p |
| Age, years | 53,5±5,7 | 54,8±5,3 | 0,658 # |
| Female/male, abs. (%) | 18(51,4 %) / 17(48,6%) | 16(51,6 %) / 15(48,4%) | 0,913 ## |
| Upper extremity lesions, abs (%) | 26 (74,3%) | 25 (80,7%) | 0,936 # |
| Lower extremity lesions, abs. (%) | 6 (17,1%) | 5 (16,1%) | 0,873 # |
| Other body parts, abs. (%) | 3 (8,6%) | 2 (3,2%) | 0,921 # |
| Duration of type 2 diabetes, years | 10,3 | 9,8 | 0,737 # |
| Glycosylated hemoglobin levels, % | 6,7±0,23 | 6,9±0,26 | 0,862 # |
| Prior administration of antibacterial agents, days | 57,1±6,9 | 56,25±4,8 | 0,811 ## |
| The number of co-morbid conditions, n | 2,4 | 2,6 | 0,683 # |
| IL-6 level, pg/ml | 37,2±6,1 | 48,1±10,6 | 0,767 # |
| TNF-a level, pg/ml | 14,5±0,97 | 13,9±1,4 | 0,138 # |
| CRP level, mg/l | 48,5±5,9 | 61,3±9,2 | 0,375 # |

Note: # – Student's T –test, ## – Wilcoxon T-test.

**Table II.** Average values of the levels of pro-inflammatory markers

| | The first day | | | The third day | | | The seventh day | | | p# | p## |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Group | CG | EG | p | CG | EG | p | CG | EG | p | | |
| IL-6 | 37,2 | 48,1 | 0,767 | 26,6 | 24,0 | 0,610 | 27,5 | 23,9 | 0,031 | 0,317 | 0,004 |
| TNF-a | 14,5 | 13,9 | 0,138 | 13,7 | 15,1 | 0,904 | 14,1 | 10,3 | 0,525 | 0,803 | 0,001 |
| CRP | 48,5 | 61,3 | 0,375 | 47,2 | 49,9 | 0,428 | 40,1 | 36,9 | 0,048 | 0,119 | 0,018 |

Note: # – comparison of indicators between the first day and the seventh in the control group;
## – comparison of indicators between the first day and the seventh in the experimental group.

**Table III.** Changes in cytogram types

| Cytogram type (%) | The first day | | | The third day | | | The seventh day | | |
|---|---|---|---|---|---|---|---|---|---|
| | CG | EG | p | CG | EG | p | CG | EG | p |
| Necrotic | 12,5 | 6,25 | <0,001 | 0 | 0 | - | 0 | 0 | - |
| Degenerative-inflammatory | 50 | 43,75 | 0,865 | 43,75 | 12,5 | 0,045 | 0 | 0 | - |
| Inflammatory | 37,5 | 50 | 0,572 | 56,25 | 56,25 | 0,597 | 28,125 | 0 | <0,001 |
| Inflammatory and regenerative | 0 | 0 | - | 0 | 25 | <0,001 | 62,5 | 37,5 | 0,083 |
| Regenerative-inflammatory | 0 | 0 | - | 0 | 6,25 | <0,001 | 9,375 | 43,75 | 0,002 |
| Regenerative | 0 | 0 | - | 0 | 0 | - | 0 | 18,75 | <0,001 |

Note: comparison of changes in cytogram types was performed using Fisher's F-test.

anionic surfactant 5-15%, amphoteric surfactant, nonionic surfactant, ethoxylated alcohols, sodium chloride, enzymes, *Bacillus megaterium*, *Bacillus subtilis*, citric acid, preservative. For the final stage of wound treatment, 2-3 doses of spray were used, which included *Bacillus subtilis* > $5*10^7$ CFU/ml, *Bacillus megaterium* > $5*10^7$ CFU/ml, didecyldimethylammonium chloride 0.1%.

The main mechanism of probiotic antiseptics is to create antagonism in the wound between mycobacteria and pathogenic microorganisms, which caused and maintained inflammation. The creation of colonies of *Bacillus subtilis* when using the spray forms a biofilm, which also has protective properties against the penetration of other pathogenic microorganisms into the wound and prevents their reproduction in the wound.

The control group used antiseptics based on octenidine dihydrochloride (0.001%) and 2-phenoxyethanol (2%), or antiseptics based on decamethoxine.

All patients received the treatment of concomitant pathology with the participation of specialists, symptomatic treatment and empiric (at the beginning of treatment) antibiotic therapy, and etiological - after


**Fig. 3.** Dynamics of macroscopic changes in the wound of Patient S., 52 years old: 3a. When admitted to the hospital; 3b. The 3rd day in the hospital; 3c. The 7th day in the hospital, secondary surgical treatment, autodermoplasty according to Thirsch; 3d. The 7th day in the hospital, secondary surgical treatment, autodermoplasty according to Thirsch

determining antibiotic sensitivity, daily debridement with wound dressing and control of laboratory indicators with the determination of cytogram types.

To study the levels of pro-inflammatory markers, blood was taken from the peripheral vein on an empty stomach, and microscopic material was taken for cytogram-type research during bandaging, before wound treatment with antiseptics or debridement. The material was collected from patients during admission (1st day) to the hospital, on the 3rd day, and on the 7th day.

Wound healing processes were analyzed based on the types of cytograms according to Steinberg: type I – "necrotic", type II – "degenerative-inflammatory", type III – "inflammatory", IV – "inflammatory-regenerative", V – "regenerative-inflammatory", VI - "regenerative".

The statistical analysis of the levels of pro-inflammatory markers and the study of their results were carried out in IBM SPSS Statistics Base (version 28). Statistically significant results were considered when a value of $p<0.05$ was obtained. Quantitative data were presented as arithmetic mean ± standard deviation (SD). The Chi-square test was used to check the normality of the data distribution. The criteria for normal distribution were $p>0.05$. When obtaining data that corresponded to a normal distribution, the Student's T-test for related and unrelated samples was used to compare the data. When data that differed from a normal distribution were obtained, the Wilcoxon W-test for related and unrelated samples was used to analyze the data. The analysis of cytogram types and their changes was carried out using Fisher's exact method, which made it possible to compare the frequency of symptoms.

Modern principles of bioethics were followed during the conduct of this experiment. Commission of the Bogomolets National Medical University issues of bioethics approved this research design. The conducted research did not carry risks of deterioration of the patients' health, the research was conducted with the inclusion of bioethical norms and biostatistical standards. Before the start of the study, all patients signed an informed consent to participate in this study and further use and publication of their data. All used medicinal products are registered and authorized for use in Ukraine.

## RESULTS

The increased glycosylated hemoglobin level testified to the long course of T2DM and the insufficient level of glycaemia correction, which forms the background for the purulent-necrotic complications of T2DM development. The increased level of IL-6, the level of CRP, and the level of TNF-a significantly exceeded normal values, which indicates a pronounced inflammatory process.

After analyzing the obtained data, it was found that on the 7th day there was a statistically significant difference between the levels of Interleukin-6 and C-reactive protein between the control and experimental groups (27.5 and 23.9 ($p=0.031$) respectively). The level of tumor necrosis factor, in turn, did not have a statistically significant difference in the control and experimental groups (40.1 and 36.9 ($p=0.048$), respectively). In both groups, a unidirectional positive trend towards a decrease in pro-inflammatory cytokines was observed, which, in our opinion, is due to the effectiveness of the prescribed treatment. However this effect in the experimental group became statistically significant only on the 7th day, which is directly related to the duration of the wound process, with a decrease the degree of systemic inflammation against the background of local wound treatment (Table II).

In the control group, the average level of IL-6 on the first day was 37.2, on the seventh day – 27.5 ($p=0.317$), TNF-a on the first day was 14.5, on the seventh day – 14.1 (p=0.803), CRP on the first day – 48.5, on the seventh day – 40.1 (p=0.119). In the experimental group, the average level of IL-6 on the first day was 48.1, on the seventh day – 23.9 (p=0.004), TNF-a on the first day was 13.9, on the seventh day – 10.36 (p=0.001), CRP on the first day – 61.3, on the seventh day – 36.9 (p=0.018) (Table II).

During the analysis of dynamic changes in the indicators of pro-inflammatory markers between the first and seventh days, we found a statistically significant difference only in the experimental group. In comparison, there was no statistically significant difference between the first and seventh day in the control group. The obtained result testified to the higher effectiveness of the use of probiotic antiseptics in patients with purulent-necrotic wounds of various localization in T2DM.

The obtained data show that there was a statistically significant difference between the first type of cytogram (p<0.001) in the control and the experimental group of patients upon admission to the hospital, and there was no statistically significant difference between the detection of the second and third types of cytograms (p=0.865 and p=0.572, respectively) (Table III).

On the third day, a statistically significant difference was found between the second, fourth, and fifth types of cytogram (p=0.045, p<0.001, p<0.001, respectively). The obtained result shows that on the 3rd day, the majority of patients with active inflammatory processes remained in the control group, while patients with the regenerative phase of inflammation began to appear in the experimental group.

On the seventh day, there were no patients with the first and second types of cytograms, but in the control group, there were still patients with the third type of cytogram, which indicates a longer period of transition to the regenerative phase of the inflammatory process. Analysis of the results of detection of the fourth type of cytogram on the seventh day had no statistically significant difference (p=0.083). When analyzing the fifth and sixth types of cytograms, a statistically significant difference was obtained (p=0.002 and p<0.001, respectively) (Table III).

Having analyzed the above data, it can be concluded that the healing processes occurred faster in patients from the experimental group, which testified to the effectiveness of the use of probiotic antiseptics in comparison with chemical antiseptics.

The data obtained by us indicate a higher efficiency, acceleration of regenerative processes in the wound in patients who were treated with the use of probiotic antiseptics, compared to patients treated with traditional chemical antiseptics.

## DISCUSSION

At the moment, there is no "gold standard" for the purulent-necrotic wounds treatment in patients with T2DM. The magnitude of pathogenetic mechanisms, the severity and duration of diabetes, irrational antibiotic therapy, frequent relapses or incomplete recovery lead to the need for repeated treatment, which in turn cause the continuation of the pathological process with higher resistance to the prescribed treatment, the appearance of antibiotic-resistant strains of bacteria in wounds.

The main methods of local treatment of patients with purulent-necrotic wounds in T2DM are surgical treatment with the use of antiseptics, hydrogel and collagen-based bandages, the use of VAC therapy, phototherapy, ultrasonic cavitation, and laser wound cleaning [9].

In addition to local treatment, hypoglycemic, antibacterial, anti-inflammatory therapy, epithelial growth factors, and endothelial growth factor are used [9]. Higher levels and risks of antibiotic resistance in patients with foot infection in T2DM are being described each year, and during the COVID-19 pandemic, this rate has exceeded previous levels [10]. Tao YW and others in their study describe the positive effect of the use of systemic probiotics in patients with T2DM, which led to the normalization of the intestinal microflora and improved response to insulin therapy [11].

Probiotic antiseptics have been successfully used in patients with wounds in critical ischemia with detection of multiresistant pathogens such as *Enterococcus Faecalis, Klebsiella pneumonia, Proteus mirabilis*. In this study, no bacterial growth was detected after 21 days of use [12]. S. Martínez-Pizarro describes the positive results of treatment of patients with wounds caused by *A. baumannii, Pseudomonas aeruginosa* [13]. There are also a number of publications in the medical literature that describe the results of treatment of patients with atopic dermatitis, acne, psoriasis, and erysipelas with probiotics based on *S. thermophiles, V. filiformis, B. longum, B. subtillis* [14-16].

Regarding the use of a probiotic antiseptic based on *Lc. chungangensis*, a study was conducted on mice with T2DM and purulent wounds and results were obtained, indicating a positive effect in reducing IL-4, IL-6, IL-10, and TNF-α, growth factors (TGF-β1 , VEGF, PDGF, and FGF), and chemokines (CCL2 and CXCL4) [17].

Regarding the use of probiotic antiseptics based on *B. Subtilis in vitro*, a positive effect on the formation of biofilms and the displacement of *Pseudomonas aeruginosa, Escherichia coli, Salmonella enterica* from the nutrient medium was proven [18].

CRP, IL-6, TNF-α, and Procalcitonin are recommended for diagnosing the level of inflammation [19, 20].

Therefore, one of the promising areas of probiotics use is the development of probiotic antiseptics for the treatment of long-term purulent-necrotic lesions of soft tissues in patients with T2DM.

## CONCLUSIONS

1. Local use of probiotic antiseptics leads to faster statistically significant positive dynamics of changes in systemic inflammation indicators in patients with purulent-necrotic wounds in T2DM. (p=0.001).
2. Probiotic antiseptic accelerates the healing of wounds in these patients, compared to the use of chemical antiseptics. (p<0.001).
3. Implementation of the latest methods and treatment schemes into clinical practice requires detailed study, but has great potential for development. Probiotic antiseptics can be a new direction in local treatment of patients with purulent-necrotic wounds in T2DM.

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**Conflict of interest:**
*The Authors declare no conflict of interest.*

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