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FULL PAPER

Assessing the safety of probiotic spray as an antibiotic alternative: A clinical trial at velayat burn injuries hospital

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It is crucial to find antibiotic alternatives and explore new chemicals and treatments. Probiotics can produce a range of antimicrobial compounds, making them a viable substitute for antibiotics in preventing and treating bacterial infections. Probiotics can produce various antimicrobial compounds such as organic acids, bacteriocins, hydrogen peroxide, diacetyl, and acetaldehyde. This experimental study aimed to assess the safety of a probiotic spray on participants receiving treatment at Velayat Burn Injuries Hospital in Rasht. A clinical trial was conducted to evaluate the safety of probiotic spray on healthy volunteers. The study involved ten healthy volunteers who visited Velayat Burn Injuries Hospital in 2023. Throughout the trial period, a standard checklist was used to review patient demographic information, prospective side effects, and assessments of local and systemic adverse events. According to the research, the probiotic spray that was under investigation showed no signs of causing any local or systemic reactions. The study suggests that it could be a possible option for treating second-degree burn wounds as it aids in promoting wound healing. In addition, showed use of probiotics as an alternative to antibiotics is a safer treatment option with fewer complications.

KEYWORDS
Probiotics; safety, clinical trial, wounds and injuries, drug side effects, volunteers

Introduction

Burn injuries are a major cause of disability and death among patients. They not only endanger the patient's life and health but also have the potential to cause complications and long-term damage to various internal systems. Burn injuries harm the skin, which is the first

line of defense of the body. This creates an environment that promotes the growth of bacteria and can lead to organ failure, immune system dysfunction, and eventually some of the most important medical disorders. These complications are so severe that the World Health Organization (WHO) has recognized them as a global public health concern, and the fourth most common type of trauma worldwide [1,2].

Silver sulfadiazine is used as a primary treatment for burn wounds worldwide, including in Iran since 1968 due to its unique properties and benefits [3]. It is a broad-spectrum antimicrobial agent that effectively prevents and treats infections in burn wounds. In addition, silver sulfadiazine has anti-inflammatory properties that help reduce pain, swelling, and redness associated with burn injuries. However, the drug has some significant drawbacks. For instance, using silver sulfadiazine dressings may lead to a false scab formation due to adhesion to the wound surface, thereby delaying the healing process [4]. The other potential side effects include:

- Silver Toxicity
- Leukopenia
- Increased pigmentation
- Skin discoloration
- Drug resistance
- Toxic epidermal necrolysis
- Neutropenia
- Failure of the skin to return to its original state
- Delayed separation of scar tissue from the skin
- The possibility of secondary fungal infections.
- Moreover, individuals who are allergic to sulfonamide medicines should not use this ointment, and it should not be used in pregnant women as it might cause jaundice and kernicterus in babies [5, 6].

As biotechnology has progressed, researchers have turned their attention to using natural antimicrobial metabolites to hinder the growth of pathogenic microorganisms. These naturally occurring compounds can effectively replace chemical preservatives. Probiotic bacteria produce several antimicrobial substances that can serve as substitutes or complementary agents to antibiotics and drugs such as silver sulfadiazine. This is especially true for bacterial infections linked to biofilm, which have received less attention from researchers [7,8].

Probiotics are living bacteria that are not harmful and are beneficial to the host's health. They are essential and can be found in various pharmaceutical products. Studies on probiotics' effects on cell regeneration and differentiation, which are significant areas in evolutionary biology, have revealed that these microbes can stimulate both specific and non-specific immune systems. They impact multiple levels of the immune system by increasing cytokines and immunoglobulin levels, boosting mononuclear cell proliferation, activating macrophages, increasing natural killer cell activity, and enhancing immunity against pathogenic bacteria [9].

Probiotic bacteria have been found to promote the proliferation of immune cells and the production of pro-inflammatory cytokines, including Tumor Necrosis Factor-alpha (TNF-) and Interleukin-6. In addition, probiotics can affect the proliferation of lymphocytes and the cytokines production by T-cells. Significantly, probiotics possess these immune-boosting properties without inducing harmful inflammatory reactions [10].

This study aimed to investigate the safety of a spray containing metabolites extracted from probiotic bacteria and its potential side effects on volunteers referred to Velayat Burn Injuries Hospital in Rasht, in 2023.

Methods

A double-blind clinical trial was conducted on ten qualified volunteers who were referred to the Velayat Burn Injuries Hospital in Rasht

during the spring and summer in 2023. The study has been registered in the ethics committee of Guilan University of Medical Sciences under the number IR.gums.REC.1401.608 and in the Iranian Registry of Clinical Trials under the number IRCT20210524051384N9.

During the study, the volunteers gave written consent to receive probiotic spray twice a day, at a certain distance from the designated area, and a rate of 100 to 300 micrograms per puff. The spray was shaken daily before use.

The medical specialist closely monitored the volunteers for any potential side effects. These included local reactions such as burning sensation, itching, pruritus, skin inflammation, skin sensitivity to touch, skin rashes, erythema, hives and skin atrophy, and systemic reactions such as swollen throat, lymphadenopathy, blood sugar changes, cardiovascular disease, electrolyte imbalances, allergic reactions, or kidney complications.

The monitoring took place at five different stages, specifically on days 1, 7, 14, 21, and 28.

The sample size was determined to be 10 individuals based on a 90% test power, 95% confidence level, and 10% probability of error.

Statistical analysis

The data were analyzed using a one-way ANOVA test in SPSS version 23.0 (Chicago, IL); significance was defined as $p < 0.05$.

Results

Ten individuals participated in the study over the spring and summer seasons in 2023. Seven female volunteers and three male volunteers aged 18 to 60 years were chosen for the study's safety assessment. Local and systemic adverse events were monitored and measured using a standard checklist in six stages (days 1, 2, 7, 14, 21, and 28). The findings revealed that the probiotic spray being investigated had no specific negative effects (Table 1). Figure 1 shows how we monitor and measure adverse events related to our topical probiotic spray.
After conducting this study, we can hope for positive outcomes from further research on topical probiotic solutions. Probiotics may become a safer and more effective alternative to antibiotics, with fewer adverse effects.

Discussion

As biotechnology has advanced, researchers have focused on utilizing the natural growth-inhibiting metabolites of infections as an alternative to chemical preservatives. Probiotics have been shown in many studies to effectively promote wound healing in humans and animals. Probiotics possess anti-inflammatory properties that assist the immune system, and their ability to aid in tissue healing reduces wound size and inflammation. Lactobacilli is one of the most important probiotics for the management of infections and wound healing because of its powerful anti-inflammatory properties. However, there is still a lack of evidence to support the use of probiotics in treating burn victims. Therefore, this study aimed to investigate the topical application of supernatant lactobacilli for the treatment of second-degree burn sites [11-13].

Harris Jabbar Al-Mutakharri and his colleagues conducted a study to investigate the impact of Lactobacillus acidophilus bacteriocin on biofilms formed by antibiotic-resistant bacteria, including *Pseudomonas aeruginosa* strain P7. As per the researchers, both planktonic (free-floating) and biofilm forms of these bacteria are susceptible to Lactobacillus acidophilus bacteriocin [14].

In this study, the authors investigated the side effects of probiotic spray on volunteers who were referred to Velayat Burn Injuries Hospital in Rasht in 2023, following the success of the laboratory phase in previous studies [15-19].

In 2020, Hadrup N. *et al.* conducted a study to evaluate the effectiveness of 1% silver sulfadiazine cream in treating grade 2 burns in their country. The cream is a combination of silver and sulfadiazine, which has been used for over a century as a topical antibiotic for second-degree burns. According to the study, the cream has several benefits such as reducing bacterial activity and minimizing the risk of infection in the wound. However, it also has significant drawbacks, such as being expensive and difficult to access, adhering to the wound surface, causing toxic effects on keratinocyte regeneration, delaying wound healing, changing skin color and transparency, causing methemoglobinemia with a prevalence of 5 to 15%, and leading to false scarring, which can result in errors in burn depth assessment. In addition, it requires frequent dressing changes throughout the day, has low penetration power in burn scars, poor antibiotic activity against *Pseudomonas aeruginosa*, exacerbation of acute hemolytic anemia in people with favism, and allergy development in people sensitive to sulfonamide compounds. Due to these adverse effects, the researchers concluded that it is better to use newer medications and approaches for the burns treatment [20].

In 2019, Ebrahimi *et al.* investigated that using 1% silver sulfadiazine ointment can cause side effects. These side effects include a delay in wound healing and discoloration of the area surrounding the wound [21].

In 2020, Wasef *et al.* discovered that silver nanoparticles served as effective antibacterial agents against both antibiotic-resistant negative and positive-gram bacteria. However, the physical and chemical processes required to create these nanoparticles are expensive, time-consuming, and potentially harmful to the environment. Therefore, they suggested using plant extracts that contain silver nanoparticles to treat burn wounds instead [22].

During the evaluation of probiotic spray on healthy volunteers, the current study discovered that the spray is cost-effective and has no local or systemic negative effects. This is in comparison to the other market items, such as silver sulfadiazine cream, which has multiple problems, as outlined in the preceding paragraphs and supported by various studies conducted over the years. Therefore, the probiotic spray is considered a crucial feature.

It is suggested that patients with burns be treated with this probiotic product, which has no side effects, to check its effectiveness and therapeutic results.

One of the limitations of the current study is related to the implementation stage, specifically in the selection of samples. During the process of determining entry and exit, certain limitations arise, which include a population limitation for age groups (18 to 65 years old) and a gender limitation for pregnant women.

TABLE 1 Demographic and safety assessment of ten participants

	Adverse Events		**Age**			
sex	systemic	local	30 -18	40 - 31	50 - 41	**60 - 51**
Female	Negative	Negative	1	3	1	**2**
Male	Negative	Negative	0	2	0	**1**

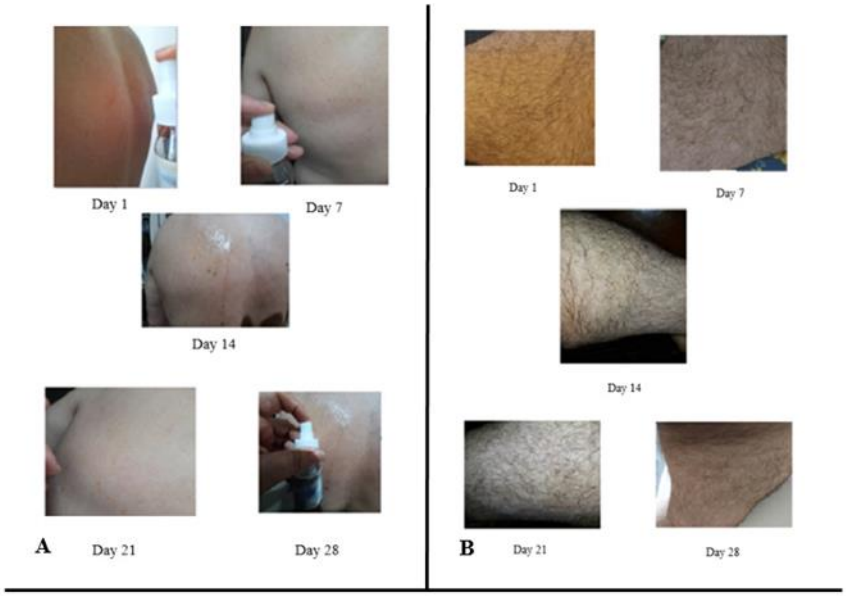

FIGURE 1 Monitoring and measuring adverse events of topical probiotic spray

Conclusion

Based on the findings of the current study on the effectiveness of using a spray containing probiotics for wound healing, further research in this field using topical probiotic products can yield favorable results. By replacing antibiotics with probiotic products, we can reduce the risk of infection and promote faster healing with greater safety and efficacy.

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Ethical Approval

This study was approved by the Guilan University of Medical Sciences Ethical Committee (approval ID: IR.gums.REC.1401.608).

Competing interests:

The authors declare no conflict of interest.

Authors' contributions:

Mohammad Abootaleb and Narjes Mohammadi Bandari, were involved in the design and manufacturing of medicine. They also contributed to the study design, data collection, analysis, and drafting of the manuscript. Mohammad karimli, Alireza Feizkhah, Sanaz Maesumi, Mozhdeh Esmaeilzadeh, and Parisa Bagheri assisted in patient recruitment, data collection, and interpretation of results. Mohammadreza Mobayen oversaw the entire study, provided guidance in study design and data analysis, and critically reviewed and revised the manuscript. All authors have reviewed and approved the final version of the manuscript.

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