Bacteriotherapy with *Lactobacillus plantarum* in burns

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ABSTRACT

Bacterial colonisation and infection remain the major causes of delayed healing and graft rejection following burns. Topical treatment is necessary to reduce the incidence of burn wound infection. Silver sulphadiazine (SD-Ag) is an often used microbicidal agent. However, this treatment produces adverse reactions and side-effects. On the basis of experimental data and clinical application of lactobacilli as probiotics, we performed this exploratory study to establish the effectiveness of bacteriotherapy with topical application of the innocuous bacteria *Lactobacillus plantarum* cultured in De Man, Rogosa and Sharpe medium to provide an alternative method for burn treatment using SD-Ag as a reference. These innocuous bacteria would compete with other bacteria that are wound pathogens and would modify the wound environment and promote tissue repair. Eighty burned patients from the Plastic Surgery and Burns Unit were grouped into infected (delayed) second- and third-degree and non infected (early) third-degree burns and treated with *L. plantarum* or SD-Ag. The proportion of patients with delayed second-degree burns was 0·71 for *L. plantarum* and 0·73 for SD-Ag (relative rate: −2·72%) with respect to the decrease in bacterial load ($<10^5$ bacteria/g of tissue), promotion of granulating tissue wound bed and healing. In early third-degree burns, the values were 0·75 for *L. plantarum* and 0·84 for SD-Ag (relative rate: −1·07%) in preventing wound infection and promotion of granulation tissue, 0·90 in graft taking for both treatments (relative rate: 0%) and 0·75 for *L. plantarum* and 0·77 for SD-Ag (relative rate: −2·60%) in healing. In delayed third-degree burns, values were 0·83 for *L. plantarum* and 0·71 for SD-Ag (relative rate: +16·90%) with respect to the decrease in the bacterial load ($<10^5$ bacteria/g of tissue) and providing a granulating tissue wound bed, 0·90 in graft taking for both treatments (relative rate: 0%) and 0·75 for *L. plantarum* and 0·64 for SD-Ag (relative rate: + 17·19%) in healing. Although the number of patients (between 12 and 15 per group) did not enable the application of a power statistical test, these results suggest that the *L. plantarum* treatment should be studied in greater depth and could be used as a valid alternative for the topical treatment of burns.

Key words: Bacteriotherapy • Burns • Infection • *Lactobacillus plantarum*

INTRODUCTION

The burn wound surface is sterile immediately following injury; however, it is repopulated quickly with gram-positive organisms from hair follicles, skin appendages and the environment during the first 48 hours. More virulent gram-negative organisms replace the gram-positive organisms after 5–7 days. Burns produce disruption of the mechanical integrity of the skin and generalised immune suppression that allows micro-organisms to multiply freely. Currently, the common pathogens isolated from burn are *Staphylococcus aureus*, found in 75% of wounds, *Pseudomonas aeruginosa* (25%), *Streptococcus pyogenes* (20%) and various coliforms (5%). Other streptococci, anaerobic organisms and fungi can also cause infections (1). The

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Key Points

- the burn wound surface is sterile immediately following injury; however, it is repopulated quickly with gram-positive organisms from hair follicles, skin appendages and the environment during the first 48 hours
- more virulent gram-negative organisms replace the gram positive organisms after 5–7 days
- burns produce disruption of the mechanical integrity of the skin and generalised immune suppression that allows micro-organisms to multiply freely

Key Points

- new research has shown that bacteria have elaborate chemical signalling systems that enable them to communicate within and between species to initiate biofilm formation and trigger virulence 6 factors
- when there are two or more strains inside a wound, bacterial interference can occur, and molecules secreted by one organism interfere with the production of virulence factors by another which may result in the suppression of virulence factors, and wound healing may occur
- bacteriotherapy is used in the treatment of infections in middle ear, bladder, gut and vagina in humans and in the wounds in animal models
- the suboptimal facilities that prevail in developing country hospitals added to insufficient funds, inadequate information, poor management and lack of personnel result in delays in wound treatment
- this study was carried out to determine whether the application of an L. plantarum culture in De Man, Rogosa and Sharpe (MRS) medium on the burn wound can provide an alternative to treat infected second-degree burns and non infected and infected third degree burns in comparison to the conventional SD-Ag treatment

above bacteria are producers of biofilms, a complex community embedded in a polysaccharide matrix. Bacteria growing in biofilms are extremely resistant to antibiotics, antiseptics and to the host immune response. Consequently, factors that aim to control bacterial burden must work on biofilm formation (1,2).

Wound bed preparation refers to the clearance of necrotic and/or sloughy materials from the bed of the wound to produce granulation tissue. In the case of infected or necrotic tissue, the wound bed must be prepared either using surgical debridement or by a progressive local treatment that eliminates dead tissue. Early excision and grafting cannot always be performed because of the medical condition of the patient or because of the lack of adequate donor sites.

When necessary, topical antimicrobial agents are used to prevent infection. Povidone iodine is used in the bath, for scalp washes and on the wound when infection appears (3). After 1960, topical antimicrobial agents containing silver revolutionised burn care, reducing morbidity and mortality, despite the side-effects associated with silver deposition on tissues (4,5). Silver sulphadiazine (SD-Ag) is the gold standard in topical burn treatment (1,4).

In most burn care units, antibiotics (erythromycin or flucloxacillin), used for the prevention of wound infection, are not routinely administered to burn patients because of their cost, the high incidence of resistance and the risk of adverse drug effects (1,3,6).

Alternative medical therapy such as natural medicines and old-fashioned treatments are resurfacing. Sugar, honey, carica papaya fruit and aromatherapy with aromatic oils have been effectively used as dressings and for the debriding and cleansing of infected wounds. Some of these medicines, however, may be toxic and are not recommended for use on broken skin (1,7,8).

Several studies show that there are often mixed populations of the same and/or different species of bacteria within a wound (8). New research has shown that bacteria have elaborate chemical signalling systems that enable them to communicate within and between species to initiate biofilm formation and trigger virulence factors. Intraspecies communication chemical signals have been identified as octapeptides in staphylococcal species and *N*-acyl-homoserine lactone molecules (AHL) in gram-negative bacteria and the interspecies signal, synthesised by an enzyme called LuxS is a furanosyl borate diester and may act inhibiting or stimulating other bacterial functions. *Lactobacillus plantarum* has LuxS. There are also bacteria producers of enzymes that specifically degrade AHL, hydrolysing one of the bonds in the conserved lactone ring present in every tested AHL or bacteria that stimulates its own groups' quorum-sensing signal-dependent virulence cascade, while cross-inhibiting that of all other groups (1,9,10).

When there are two or more strains inside a wound, bacterial interference can occur, and molecules secreted by one organism interfere with the production of virulence factors by another. This may result in the suppression of virulence factors, and wound healing may occur. Bacterial interference is an important concept for modern treatment regimes. Bacteriotherapy – using harmless bacteria to displace pathogenic organisms – is an alternative and promising way of fighting infections (11). Bacteriotherapy is used in the treatment of infections in middle ear, bladder (12,13), gut and vagina (14) in humans and in the wounds in animal models (15,16).

Early excision and grafting in burn wound treatment have become the norm. However, because of the lack of skin donors or skin substitutes together with the large number of patients and the limited economic resources in our hospitals, early excision and grafting cannot always be performed, which makes treatment of burn patients difficult. The suboptimal facilities that prevail in developing country hospitals added to insufficient funds, inadequate information, poor management and lack of personnel result in delays in wound treatment. Burn wound infections occur more frequently in countries with overcrowded burn units, fewer infection control barriers and less access to immediate wound debridement or antimicrobial therapies (17).

In a previous study, we investigated the interference of *L. plantarum* with *P. aeruginosa* in controlled experiments. We determined that *L. plantarum* inhibited in vitro the activity of *N*-acyl-homoserine lactone and bacterial growth as well as the formation of biofilm and elastase by *P. aeruginosa*. The in vivo application of *L. plantarun* to burns infected with the pathogen inhibited colonisation, modifying the inflammatory response and promoting tissue repair in a burn murine model (15).

This study was carried out to determine whether the application of an *L. plantarum*

culture in De Man, Rogosa and Sharpe (MRS) medium on the burn wound can provide an alternative to treat infected second-degree burns and non infected and infected third-degree burns in comparison to the conventional SD-Ag treatment.

MATERIALS AND METHODS

Patients

Eighty male and female burned patients from the Plastic Surgery and Burns Unit were included in the study. All patients were informed about the aim of the study and gave their consent. The study was approved by the Hospital Ethics Committee.

The hospital is one of the main state-supported reference health care centres in the northwestern area of Argentina. Patient enrolment was conducted so as to obtain two sufficiently homogeneous groups with respect to age, gender, bacterial load, localisation and type of burns (second and third degree and % of total body surface area (TBSA) as described in Table 1.

Injuries were classified as second-degree burns when involved from the upper to the deeper dermal layers with blister formation and a pink/white area and as third-degree burns when involving all dermal layers, which could also affect subcutaneous fat and/or muscle; as early when these patients underwent first excision when necessary, with an average of 1 day post burn (range 0–2 days) and clinical examination showed no signs of infection and as delayed when had their first surgery on an average of 5 days post burn (range 3–7 days) and wounds showed clinical evidence of infection with microbial counts of 10^6–10^8 bacteria/g tissue. Improper initial burn wound care was frequently observed in these groups. Quantitative bacterial counts below 10^5 cells per gram of tissue rarely indicate invasive infection, while those exceeding 10^5 cells per gram usually do.

Inclusion criteria: patients between 18 and 55 years old, both male and female, with no serious complications of the heart, liver, kidney or blood system and no systemic infection.

Exclusion criteria: patients who had diabetes, malignancy, autoimmune disease, a serious disease of the heart, liver or kidney or had a blood producing disorder, an inclination to bleed or bleeding disease; serious systemic infection; pregnant or breastfeeding women; those who had an allergic reaction to silver ions.

Table 1 Characteristics of the patients treated with *Lactobacillus plantarum* or SD-Ag

	Patient (*n*)	Degree and % of total body surface area (TBSA) mean (range)			Age in years (range)	Sites	Bacteria/g tissue (range)
		II D	III E	III D			
L. plantarum	14	12 (6–24)			35 (18–55)	Arms, hands, legs, abdomen, thorax	3×10^7 (3×10^6–8×10^7)
	12		10 (2–15)		35 (18–50)	Arms, leg, hands, neck	Non infected
	12			11 (2–15)	39 (22–58)	Arms, leg, hands, thorax	9×10^6 (6×10^6–10^8)
SD-Ag	15	16 (8–22)			30 (18–55)	Leg, thorax, abdomen, arms, hands, face, neck	8×10^6 (2×10^6–6×10^7)
	13		9 (4–12)		45 (19–59)	Leg, thorax, abdomen, hands	Non infected
	14			8 (2–15)	37 (18–60)	Thorax, abdomen, hands, leg, arms	10^7 (2×10^6–7×10^7)

II D, delayed second-degree burns; III D, delayed third-degree burns; III E, early third-degree burns; SD-Ag, silver sulphadiazine.

Key Points

- eighty male and female burned patients from the Plastic Surgery and Burns Unit were included in the study
- the hospital is one of the main state supported reference health care centres in the 8 northwestern area of Argentina
- inclusion criteria: patients between 18 and 55 years old, both male and female, with no serious complications of the heart, liver, kidney or blood system and no systemic infection
- exclusion criteria: patients who had diabetes, malignancy, autoimmune disease, a serious disease of the heart, liver or kidney or had a blood producing disorder, an inclination to bleed or bleeding disease; serious systemic infection; pregnant or breast feeding women; those who had an allergic reaction to silver ions

Key Points

- each patient was allocated to receive, in random order, treatment with L. plantarum or SD-Ag
- the wounds treated with L. plantarum were not treated with any traditional antimicrobial
- the primary objective of our study was to evaluate the antimicrobial efficacy in the infected burns and the generation of granulation tissue in infected and non infected lesions of both treatments. A secondary objective was the evaluation of the efficacy of the treatments in healing

Each patient was allocated to receive, in random order, treatment with *L. plantarum* or SD-Ag as described below.

Treatment

From the time of admission to the start of application of *L. plantarum* or SD-Ag, burn wounds were cleansed with saline. Microbiological studies by biopsy cultures of the wound were routinely performed. In second-degree burns, blisters were debrided. When necessary, separation of the eschar was performed by tangential excision.

Fourteen patients with delayed second-degree burns, 12 early third-degree burns and 12 delayed third-degree burns were treated with *L. plantarum*.

A whole culture of 10^5 *L. plantarum* American type culture collection (ATCC) 10 241/ml in the log phase grown in MRS broth (Oxoid, Basingstoke, UK) at 37°C was used. A volume of the culture large enough to cover the wound (1 ml/cm^2) was spread on a gauze pad and applied to the burn, which was then routinely bandaged. Moisture was optimum until the next day. This procedure was used once a day over a period of 10 days. New cultures were prepared every day. The wounds treated with *L. plantarum* were not treated with any traditional antimicrobial.

Fifteen patients with delayed second-degree burns, 13 early third-degree burns and 14 delayed third-degree burns were treated with 1% SD-Ag cream (Denver®; Farma Lab, Buenos Aires, Argentina). Patients received a daily antiseptic bath with chlorhexidine 0·05%, and then, the burn wounds received a 3-mm layer of SD-Ag cream every 24 hours for 10 days. The cream caused an exudate by wound maceration.

The assumption that early third-degree burns were not infected was only clinical based on the study of their appearance and corroborated by no infection detection during and after treatment.

The primary objective of our study was to evaluate the antimicrobial efficacy in the infected burns and the generation of granulation tissue in infected and non infected lesions of both treatments. A secondary objective was the evaluation of the efficacy of the treatments in healing.

Evaluation of the microbial burden

It was performed by biopsy culture methods. One to three biopsies, according to the wound size, were performed for each wound using for each biopsy 4-mm^3 tissue samples that included viable tissue from under the eschar. The samples were obtained for culture before (0 days) and at 10 days following treatment.

This technique, which requires local anaesthetic infiltration, relates microbial density to depth of eschar penetration and confirms tissue invasion.

To determine the colony forming unit (CFU)/g of tissue, the specimen was homogenised in Luria Bertani broth and serially diluted onto non selective and different selective media: MacConkey and Trypticase Soy Agar for gram-negative bacilli, Blood Agar and Mannitol Salt Agar for gram-positive cocci and MRS Agar for *L. plantarum*. They were pour plated for quantitative bacteriology. The inoculated plates were incubated at 37°C for 24 hours. The plates were read at the dilution containing 30 or more colonies per plate. The number of colonies was then multiplied by that portion of a 1-mL inoculum used to inoculate the plate. The results were an average of all specimens.

Wound bacterial pathogens were identified as *S. aureus*, *P. aeruginosa*, *Staphylococcus epidermidis*, *Enterobacter cloacae*, *Klebsiella pneumonia* and *Enterococcus faecalis*. After 10 days of treatment, lactobacillus cultures in MRS tested negative. *L. plantarum* did not survive in the wound, as previously observed in the wounds treated in the experimental mouse model (15).

Wounds with bacterial loads greater than 10^5 bacteria/g of tissue after 10 days of treatment were considered a reason to end the study.

Clinical evaluation

Clinical follow-up was performed for 10 days (range 7–13 days) to determine the apparition of active granulation tissue, which was recognised as bright red tissue in the bed of the wound.

In delayed second-degree burns, when granulation tissue was evident and the bacterial load was lower than 10^5/g of tissue, the area was covered with standard burn wound bandage and the observation period of wound healing was 30 days.

In third-degree burns, the wound was ready for skin graft when granulation tissue was evident at 10 days (range 7–13 days); in delayed third-degree burns when granulation tissue was evident and the bacterial load was lower than 10^5/g of tissue. Skin autografts as well as thin and intermediate split-thickness skin grafts were performed.

The area was covered with standard burn wound bandage, which remained untouched for 6 days, and graft taking was determined clinically up to 6–10 days after grafting. Graft evolution was assessed clinically up to 50 days.

The criteria to chose the cut-off 10^5 bacteria/g of tissue as reason to end the study is because bacterial load at a level of above 10^5 microorganisms per gram of tissue is generally accepted to diagnose infection and is an important factor in delayed healing in chronic wounds and no tendency to healing (6).

Changes in graft skin colour from light to dark (colour difference between the grafted skin and the area adjacent to the recipient site), existence of blisters and no adherence to the underlying wound bed were analysed.

Partial or total loss of skin graft by death of the graft, haemorrhage, blood clots and infection were evaluated clinically and considered as a reason for ending the study.

Statistics

Because of the low number of patients, we used an exploratory analysis considering the proportion of patients to quantify the efficacy of the treatments at different stages, less than 105 bacteria/g of tissue, granulation tissue, graft taking (only in third-degree burns) and completely healed burns, and we compared the efficacy of the treatments with *L. plantarum* and SD-Ag with relative rates. Relative rate values higher than +10% are considered statistically significant if sample numbers are above 30. In this study, samples numbers are between 12 and 15, so we considered this cut-off value (+10) as indicative of differences.

RESULTS

All the following results are summarised in Table 2.

In delayed second-degree and delayed third-degree burns, improper initial burn wound care was frequently observed before treatment and wounds showed clinical evidence of infection with microbial counts of 10^6–10^8 bacteria/g tissue, and all types of bacteria were isolated: *P. aeruginosa* (51%), *S. aureus* (38%), *S. epidermidis* (10%) and others such as *E. cloacae*, *K. pneumoniae* and *E. faecalis* (2%). *P. aeruginosa* and *S. aureus*, the most frequently isolated bacteria, produced biofilm when they were assayed by biofilm formation in 96-well polyvinyl chloride microtiter plates stained with 0·1% crystal violet technique.

In patients with delayed second-degree burns, no grafting was required for healing with either the *L. plantarum* or the SD-Ag treatment. In this group, the efficacy of treatment with *L. plantarum* and SD-Ag treatment with respect to decrease in the bacterial load (<105 bacteria/g of tissue), separation of necrotic tissue, providing a granulation wound bed and complete healing were 71% and 73%, respectively (relative rate: −2·72%).

In patients with early third-degree burns, the effectiveness of both treatments with regard to preventing infection, debriding and providing granulation tissue suitable for grafting in the wound bed were as follows: *L. plantarum*, 75%, and SD-Ag, 85% (relative rate: −1·07%); graft taking with *L. plantarum*, 90%, and with SD-Ag, 90% (relative rate: 0%); complete healing with *L. plantarum*, 75%, and with SD-Ag, 77% (relative rate: −2·60%).

Bacterial load higher than 10^5 bacteria/g of tissue was considered as a reason for ending the study.

In patients with delayed third-degree burns, both treatments decreased bacterial load (<105 bacteria/g of tissue) and promoted granulation tissue: *L. plantarum*, 83%, and SD-Ag, 71% (relative rate: +16·90%); graft taking with *L. plantarum*, 90%, and with SD-Ag, 90% (relative rate: 0%); complete wound healing with *L. plantarum*, 75%, and with SD-Ag, 65% (relative rate: +17·19%).

The percentage of graft taking between both treatments was similar (*L. plantarum*, 90%, and SD-Ag, 90%). In patients who suffered graft rejection, we observed certain complications of skin graft surgery such as death of the graft, haemorrhage, blood clots, etc.

In delayed second-degree burns treated with *L. plantarum*, 4 of 14 patients had a bacterial load of $7 \times 10^6 \pm 5{\cdot}2 \times 10^6$ with isolation of *P. aeruginosa* (47%), *S. aureus* (51%) and other bacteria (2%) after treatment. For SD-Ag treatment in 3 of 15 patients, we found $3{\cdot}4 \times 10^6 \pm 3 \times 10^6$ with isolation of *P. aeruginosa* (51%) and *S. aureus* (49%).

In delayed third-degree burns for *L. plantarum* treatment (2 of 12 patients), the bacterial load was $7 \times 10^6 \pm 2 \times 10^5$ with isolation of *P. aeruginosa* (52%), *S. aureus* (44%) and other bacteria (4%); for SD-Ag (4 of 14 patients), it was $5 \times 10^6 \pm 4{\cdot}4 \times 10^6$ with isolation of

Table 2 Comparison of patients treated with *Lactobacillus plantarum* or SD-Ag

Treatment	Proportion of patients (p)		Relative rates* (%)
	L. plantarum	SD-Ag	
II D			
No of patients	14	15	
Graft	No	No	
Bacteria/g tissue	$n = 14$, p = 0·71	$n = 15$, p = 0·73	−2·73
$<10^5$	$10 = 6 \times 10^3 \pm 5 \times 10^3$	$11 = 3{\cdot}6 \times 10^4 \pm 2{\cdot}3 \times 10^4$	
$>10^5$	$4 = 7 \times 10^6 \pm 5{\cdot}2 \times 10^6$	$4 = 3{\cdot}4 \times 10^6 \pm 3 \times 10^6$	
Granulation tissue (range 7–13 days)	$n = 14$, p = 0·71 (10/14)	$n = 15$, p = 0·73 (11/15)	−2·73
Healing (range 30–50 days)	$n = 14$, p = 0·71 (10/14)	$n = 15$, p = 0·73 (11/15)	−2·73
III E			
No of patients	12	13	
Granulation tissue (range 7–13 days)	$n = 12$, p = 0·75 (10/12)	$n = 13$, p = 0·84 (11/13)	−1·07
Graft taking (range 6–10 days)	$n = 10$, p = 0·90 (9/10)	$n = 11$, p = 0·90 (10/11)	0
Healing (range 30–50 days)	$n = 12$, p = 0·75 (9/12)	$n = 13$, p = 0·77 10/13	−2·60
III D			
No of patients	12	14	
Bacteria/g tissue*	$n = 12$, p = 0·83	$n = 14$, p = 0·71	+16·90
$<10^5$	$10 = 7 \times 10^3 \pm 4 \times 10^3$	$10 = 2{\cdot}5 \times 10^3 \pm 2 \times 10^3$	
$>10^5$	$2 = 7 \times 10^6 \pm 2 \times 10^5$	$4 = 5 \times 10^6 \pm 4{\cdot}4 \times 10^6$	
Granulation tissue (range 7–13 days)	$n = 12$, p = 0·83 (10/12)	$n = 14$, p = 0·71 (10/14)	+16·90
Graft taking (range 6–10 days)	$n = 10$, p = 0·90 (9/10)	$n = 10$, p = 0·90 (9/10)	0
Healing (range 30–50 days)	$n = 12$, p = 0·75 (9/12)	$n = 14$, p = 0·64 (9/14)	+17·19

II D, delayed second-degree burns; III D, delayed third-degree burns; III E, early third-degree burns; SD-Ag, silver sulphadiazine.
*Relative rates $= \frac{\mathrm{pL.plantarum} - \mathrm{pSD\_Ag}}{\mathrm{pL.plantarum}} \times 100$.

P. aeruginosa (57%), *S. aureus* (40%) and other bacteria (3%).

When the bacterial load was lower than 10^5 bacteria/g of tissue (around 10^3 bacteria), the bacteria isolated from *L. plantarum* treatment was *P. aeruginosa* (10%), *S. aureus* (30%), *S. epidermidis* (30%) and other bacteria (30%); for SD-Ag, they were *P. aeruginosa* (10%), *S. aureus* (30%), *S. epidermidis* (20%) and other bacteria (40%).

Patients had their grafts taken on the 6th postoperative day, which was confirmed on the 10th day. Skin graft taking was good in all cases with complete healing at 50 days, except in one patient in each group of third-degree early and delayed burns with both treatments. Grafts were lost after 10 days and were considered a reason to terminate the study. The skin graft failure was because of poor graft adherence to the recipient bed and tissue death.

Invasive infection by pathogens from the burn wound (sepsis), determined by clinical signs and symptoms and standard blood bacteriological assays, was not observed in any of the patients before, during or after either treatment.

Safety assessment

No differences between the two groups were found in routine blood test. No local allergic or systemic symptoms were found for either treatment.

Clinical observation showed that the quality of healing and epithelialisation was similar for both treatments with respect to elasticity, smoothness of appearance and handling.

Side-effects of *L. plantarum* treatment: five patients suffered tolerable pain when the *L. plantarum* culture was applied. Forty-eight hours after the end of the treatment with *L. plantarum*, this bacterium was not recovered from either peripheral blood or wound samples.

DISCUSSION

In our Plastic Surgery and Burns Unit, intra-hospital infections are frequent. Consequently, antimicrobial therapy is important in the treatment and prophylaxis of wound infections. In our unit, as in other burn care facilities, antibiotics are not routinely administered because of their cost and of the high degree of antibiotic resistance (1,3,6).

The largest group of micro-organisms was represented by *P. aeruginosa* (51%), *S. aureus* (38%), *S. epidermidis* (10%) and others (2%). These pathogenous bacteria are classical biofilm forming and, consequently, are protected against host defences and antibiotics (18).

Bacterial pathogens possess highly evolved mechanisms for infection and for adaptation to various host cells that protect them against the host's immune system. The progression of infectious diseases and their severity from onset to cure are constantly influenced by the interplay between the pathogen and the host. The interference of pathogens with members of the commensal flora is often overlooked, although commensal bacteria represent the third player in host–pathogen interactions (19).

New products and various new therapeutic approaches are now available because no completely satisfactory response has been obtained with current therapeutic agents. Consequently, novel treatments are being developed such as bacteria signalling molecule interference together with the increasing use of natural antimicrobial agents such as honey, papaya fruit and tea-tree oil (1).

Financial and logistic considerations require alternatives to the relatively expensive burn dressings available in developed countries. Our hospital in Tucumán, Argentina, where health care is free, cannot afford elements such as special dressings or expensive treatments for the care of burns.

The gold standard in topical burn treatment is SD-Ag, a useful antibacterial agent for burn wound treatment. Recent findings, however, indicate that the compound delays the wound-healing process and that silver may have serious cytotoxic activity on various host cells (4,20).

We compared the SD-Ag treatment, used mainly for the control of the microbial burden, with a new therapeutic approach, bacteriotherapy with *L. plantarum* cultured in MRS medium. This therapy was based on the encouraging results obtained in studies of the interference of an *L. plantarum* culture on the pathogenic capacity of *P. aeruginosa*. *L. plantarum* interfered in vitro with *N*-acyl-homoserine lactone, elastase and biofilm formation by *P. aeruginosa* and enhanced phagocyte activity and tissue repair in an experimental burn murine model (15). Another report described the successful use of *Lactobacillus fermentum* RC-14 and its secreted products to inhibit surgical implant infections caused by *S. aureus* (16). It seems probable that *L. plantarum* has the same activity because it proved effective in decreasing the amount of *S. aureus* and other burn-infecting micro-organisms.

Our decision to use *L. plantarum* in our experiments was based on the fact that there are no reports indicating the possible virulence activity of *L. plantarum* in experimental models (15) or in spontaneous infections (14). Although lactobacilli have an excellent overall safety record among probiotics, there are a few reported cases of infection in premature neonates with severe immune deficiencies caused mainly by *Lactobacillus rhamnosus* (21). Forty-eight hours after the end of the treatment with *L. plantarum*, this bacterium was not recovered from either peripheral blood or wound samples. *L. plantarum* is a commensal micro-organism that does not produce virulence factors and succumbs easily to the antimicrobial battery of host defences, particularly PMN (polymorphonuclear) activity (15).

In this work, the low number of patients prevented us from applying a statistical power and the relative rates of proportions only allowed us to suggest differences in the efficacy of the two treatments. We found that the values of the relative rates suggested no differences with respect to preventing infection, promoting granulation tissue, graft taking and healing wounds in early (non infected) third-degree burns.

In delayed second-degree burns, the *L. plantarum* treatment was as effective as the SD-Ag one concerning decrease in the bacterial load, promotion of the appearance of granulation tissue and wound healing. In delayed third-degree burns, the value of the relative rate ($>+10$) suggests a higher efficacy of the *L. plantarum* treatment if the number of samples were greater than 30.

The effect of the *L. plantarum* culture on the wounds observed in this study could be because of the fact that the cytokine pattern induced by *L. plantarum* in inflammatory cells is the opposite of those induced by pathogens like *P. aeruginosa* (22); *P. aeruginosa* induced higher levels of PGE_2 (prostagladin E_2) compared with the low levels induced by *L. plantarum* (23), and an *L. plantarum* mutant showed a great anti-inflammatory activity (24).

Acid pH has also been reported to contribute to the activation of the cells involved in the immune response and in tissue repair (25); the

Key Points

- the use of a L. plantarum culture in burns would be a valid alternative to other treatments in early and delayed third-degree burns as well as in delayed second-degree burns
- L. plantarum cultures can be easily prepared in any laboratory with minimum equipment. They are inexpensive and easy to apply
- L. plantarum treatment would mean a significant reduction in costs and inconvenience to the patient because it would ensure quality in burn care in a cost-effective manner
- this preliminary trial of this treatment suggests no differences between treatment with L. plantarum and with SD-Ag in infected second-degree and non infected third-degree burns
- as this is a first approach to the subject, further studies on the treatment and prophylaxis of burn infections with L. plantarum will be required before establishing the L. plantarum treatment in the context of current treatments

acidity of supernatants of *L. plantarum* cultured in MRS is an important factor in the interference with virulence factors from *P. aeruginosa* (15).

Although acidity may be the cause of the burning sensation in some patients, in no case did we observe the necrotic activity found with other antiseptic agents not currently in use such as acetic acid or sodium hypochlorite. It seems possible that the various components produced by *L. plantarum* could exert some effects on the protection of the tissue that would counteract the detrimental effect of acidity so that they could colonise the gut without causing tissue lesion or vertical or horizontal invasion, even in the case of gut surgery, when the oral administration of *L. plantarum* enhances healing remarkably (26).

CONCLUSIONS

The use of a *L. plantarum* culture in burns would be a valid alternative to other treatments in early and delayed third-degree burns as well as in delayed second-degree burns. *L. plantarum* cultures can be easily prepared in any laboratory with minimum equipment. They are inexpensive and easy to apply. Optimal management of burn patients is enormously expensive, and in our country, it is a great problem. *L. plantarum* treatment would mean a significant reduction in costs and inconvenience to the patient because it would ensure quality in burn care in a cost-effective manner.

This preliminary trial of this treatment suggests no differences between treatment with *L. plantarum* and with SD-Ag in infected second-degree and non infected third-degree burns. In infected third-degree burns, treatment with *L. plantarum* would show greater efficacy. As this is a first approach to the subject, further studies on the treatment and prophylaxis of burn infections with *L. plantarum* will be required before establishing the *L. plantarum* treatment in the context of current treatments.

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