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ANTIMICROBIAL EFFECTS OF LACTOBACILLUS RHAMNOSUS AND LACTOBACILLUS BUCHNERI FILTRATES ON BACTERIAL BURN INFECTIONS

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Abstract: This study was done for evaluation the antimicrobial effects of Lactobacillus rhamnosus and Lactobacillus buchnerri filtrates on the main causes of burn infections with studying the histopathology of this burn. One hundred thirty-five swabs were taken from 135 burn cases from patients come to clinics in Baghdad. These swabs were cultured on primary media, then biochemical tests were done for identification of these bacteria. Lactobacillus filtrates was prepared. The antimicrobial susceptibility test for this filtrate was done by using agar well diffusion, also antibiotic susceptibility test was done for many antibiotics against bacterial isolates. The slices revealed the epithelium layer, the top layer of skin, and the dermal layer, the middle layer, both of which were destroyed. The presence of bacteria is confirmed by the significant infiltration of leukocytes. This was consistent with what was seen visually, namely the ulceration of the burn region and the presence of pus. The current results showed that Pseudomonas aeruginosa was the most prevalent isolates from infected burns followed by E. coli. The results of antibiotic sensitivity test showed that most isolates were MDR to most antibiotics, While the Lactobacillus filtrates showed a high antimicrobial effectivity against all bacteria.
In conclusion, Pseudomonas aeruginosa was most predominant bacteria isolated from burns, also other bacteria were isolated in significant percentages which showed high susceptibility to lactobacillus filtrates.

Keywords: Lactobacillus, Burns, Bacteria

Introduction

Generally speaking, microbes are the most common type of probiotics, and they are classified as healthy for human ingestion. The FAO and WHO of the United Nations have both given their stamp of approval to this definition [1]. When consumed in large enough doses, probiotics have health benefits that extend beyond those seen with regular food consumption. Potential mechanisms underlying these outcomes include the inhibition of bacteria and the promotion of microbial development, both of which improve the gut's nutrient supply and general health [2].

Some of the most common bacteria in a human's small and large intestines are those that produce organic acids and bacteriocins, which can halt the development of harmful microbes [3]. These bacteria include L. acidophilus, L. casei, as well as different Bifidobacterium species. Theresa, it has been shown in-vitro that a number of

different types of lactic acid bacteria (LAB) exhibit stimulatory characteristics on cells of the natural immunity, these cells, which include macrophages as well as NK cells, increase the number of lymphocytes and NK cells, which in turn improves phagocytosis [4].

Furthermore, the DNA of beneficial microbes can dampen the body's inflammation reaction to DNA from pathogens [5]. Modulating epithelium barrier function [6] and possibly interacting with TLR-2 [7] may be the key to understanding the medicinal effectiveness of probiotic microbes. TLR-2 can identify lipoteichoic acid, zymosan, and other medicinal treatments produced by microbes.

Numerous studies have led researchers to think that LAB may be able to prevent the growth of sites of bacterial infiltration by harmful agents by stopping the union of these pathogens to the sites. By outcompeting other microbes for food sources, LAB compounds prevent their own reproduction. It is possible that substances such as H2O2, lactic acid, as well as bacteriocin-like substances that are excreted could prevent the proliferation of these agents [8-10].

One of the most prevalent and life-altering types of stress, burns affect people of all ages and can be caused by many different factors [11]. Patients who have suffered burns are at a higher risk for developing contagious problems due to the immunocompromised state this condition causes [12]. These wounds compromise the skin's ability to function as a physical barrier, allowing bacteria and other microbes to invade and multiply; as a result, new sites for colonization, infection, and clinical sepsis are created [13].

Major injuries and burns have been shown to inhibit the immune system, making patients more vulnerable to viral problems and associated multi-organ failure [14].

Damage to the skin or a weakened immune system can lead to illnesses caused by the skin's complicated microbiota. Local sepsis occurs when bacteria from the burn site spread to healthy tissue; if they spread to the capillary and arterial systems, systemic sepsis sets in [15].

Infections of the epidermis can be caused by a wide variety of microorganisms. The bacterium S. aureus is a leading source of cutaneous diseases. Nearly 20% of people are chronic S. aureus carriers [16]. Common burn wound pathogens such as grampositive organisms are MRSA, S. aureus, C-N Staphylococci, Streptococcus spp., Enterococcus spp., while gram-negative organisms are E. coli, Pseudomonas aeruginosa, Serratia marcescens, Klebsiella spp. as well as Proteus spp. [17-20].

This study was done for evaluation the antimicrobial effects of Lactobacillus rhamnosus and Lactobacillus buchnerri filtrates on the main causes of burn infections.

Materials and Methods:

One hundred thirty-five swabs were taken from 135 burn cases from patients come to clinics in Baghdad. These swabs were cultured on primary media, then biochemical tests were done for identification of these bacteria [16]. After that the bacteria were confirmed by using VITEK.

Four samples of fresh traditional dairy products (yogurt and cheese) total were chosen at random from various traditional marketplaces for isolation of Lactobacillus rhamnosus and Lactobacillus buchnerri. All the samples were made on the same day that they were selected. In order to prevent perishing, the gathered samples were delivered right away to the lab in a cool storage case at about 4°C. To suspend the bacterial content, samples of cheese and yogurt were combined. Then, 18 mL of ordinary saline (1:10 w/v) and 2 g of each sample were combined, and the mixture was agitated for 10 minutes at 600 rpm. In order to disperse the residue in PBS, 20 mL of phosphate-buffered saline (PBS) was added to the solid phase, which contained bacteria, after 50 milliliters of the sample had been spun for at least 5 minutes at 1000 rpm. The resulting fluid was then spun for 15 minutes at 400 rpm after being kept at 37°C for 2 hours. Finally, the serially diluted samples were distributed on MRS

agar (Merck, Germany) and kept at 37°C for 72 hours in an atmosphere holding 10% CO2 after removing the cells from the solution. Following the incubation period, the cells underwent biochemical analysis. Gram-positive rods that exhibited a negative catalase reaction and lacked motility were subjected to purification and subsequently preserved at a temperature of 4°C. In order to validate the outcomes of the biochemical characterization, the VITEK system was employed to analyze all of the isolates.

The bacterial colony that was free of contaminants was introduced into MRS broth and subjected to incubation at a temperature of 37 °C for a duration of 24 hours. Following a 24-hour incubation period, the specimen underwent centrifugation at 8000g for a duration of 10 minutes at a temperature of 4°C. The Cell-free supernatant (CFS) was obtained and subjected to filtration using a sterilized 0.22 µm filter (Millipore, USA). The resulting sterile CFS was subsequently preserved at a temperature of 4 °C for subsequent analysis.

Using a modified version of the agar well diffusion method published by Touré et al. [24]. Specifically, 2 L of an overnight culture of each LAB strain (final content 7 log CFU/mL) was observed on MRS agar plates. After air drying the plates for 30 minutes at room temperature, they were placed in anaerobic vessels with gas pack and kept at 37 degrees Celsius for 18 hours. Plates were incubated aerobically at 37°C. After 48 hours of incubation, we measured the distance from the LAB colonies' periphery to the periphery of the clean zones. Zones of inhibition greater than 20 mm, between 10 and 20 mm, and below 10 mm were classified as intense, moderate, and mild inhibitions, respectively. Each round of testing was done in duplicate.

Among the antimicrobial drugs used were 10 mg of Gentamycin (CN), 10 mg of Ampicillin (AM), 30 mg of Tetracycline (TE), 30 mg of Cefotaxim (CTX), 30 mg of Chloramphenicol (CHPC), 15 mg of Azithromcin (AZM), 5 mg of Ciprofloxacin (CIP), and 1.25 mg of Sulfamethoxazole (SXT). The formation of inhibition zones on MullerHinton agar has been documented [17,18].

To make skin slices that were formalin-fixed and embedded in paraffin, the standard procedure described by Zgair and Chhibber was adhered to. Hematoxylin and eosin were used to stain sections of the skin after the skin pieces were produced.

Statistical analysis by using SPSS.

Results:

The current results showed that Pseudomonas aeruginosa was the most prevalent isolates from infected burns followed by E. coli (Table 1, Figure1).

Table 1. Percentages of bacterial isolates from infected burns

Isolates	**No. of isolates**	**Percentages**
S. aureus	10	7.4
S. epidermidis	2	1.5
Pseudomonas aeruginosa	52	38.5**
Klebsiella pneumonia	5	3.7
E. coli	26	19.25*
Total	95	70.4%

P≤ 0.003

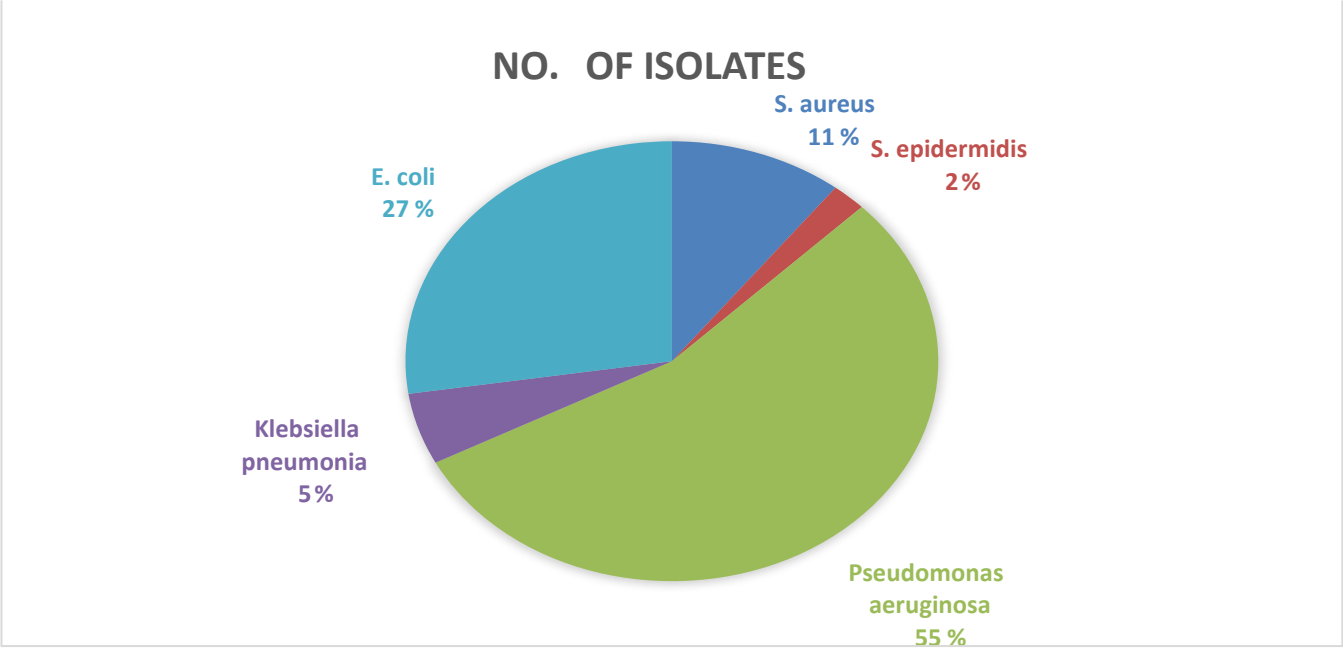

Figure 1. Percentages of bacterial isolates from infected burns

The results of antibiotic sensitivity test showed that most isolates were MDR to most antibiotics (Table 2). While the Lactobacillus filtrates showed a high antimicrobial effectivity against all bacteria (Table 2, Figure 2).

Table 2. Number and percentages of resistance (R) to antibiotics

Antibiotic	S. aureus	S. epidermidis	Pseudomonas aeruginosa	Klebsiella pneumonia	E. coli
Lactobacillus filtrate	0 (0%)	0 (0%)	2 (3.8%)	1 (20%)	0 (100%)
Gentamycin	10 (100%)	2 (100%)	52 (100%)	5 (100%)	10 (38.5%)
Ampicillin	8 (80%)	2 (100%)	52 (100%)	5 (100%)	26 (100%)
Tetracycline	9 (9%)	2 (100%)	52 (100%)	5 (100%)	26 (100%)
Cefotaxime	10 (100%)	2 (100%)	52 (100%)	5 (100%)	26 (100%)

Chloramphenicol	6 (60%)	1 (50%)	52 (100%)	4 (80%)	26 (100%)
Ciprofloxacin	4 (40%)	1 (50%)	52 (100%)	4 (80%)	26 (100%)
Azithromycin	6 (60%)	2 (100%)	52 (100%)	2 (40%)	26 (100%)
Trimethoprim-Sulphamethoxazole	10 (100%)	2 (100%)	52 (100%)	5 (100%)	13 (50%)

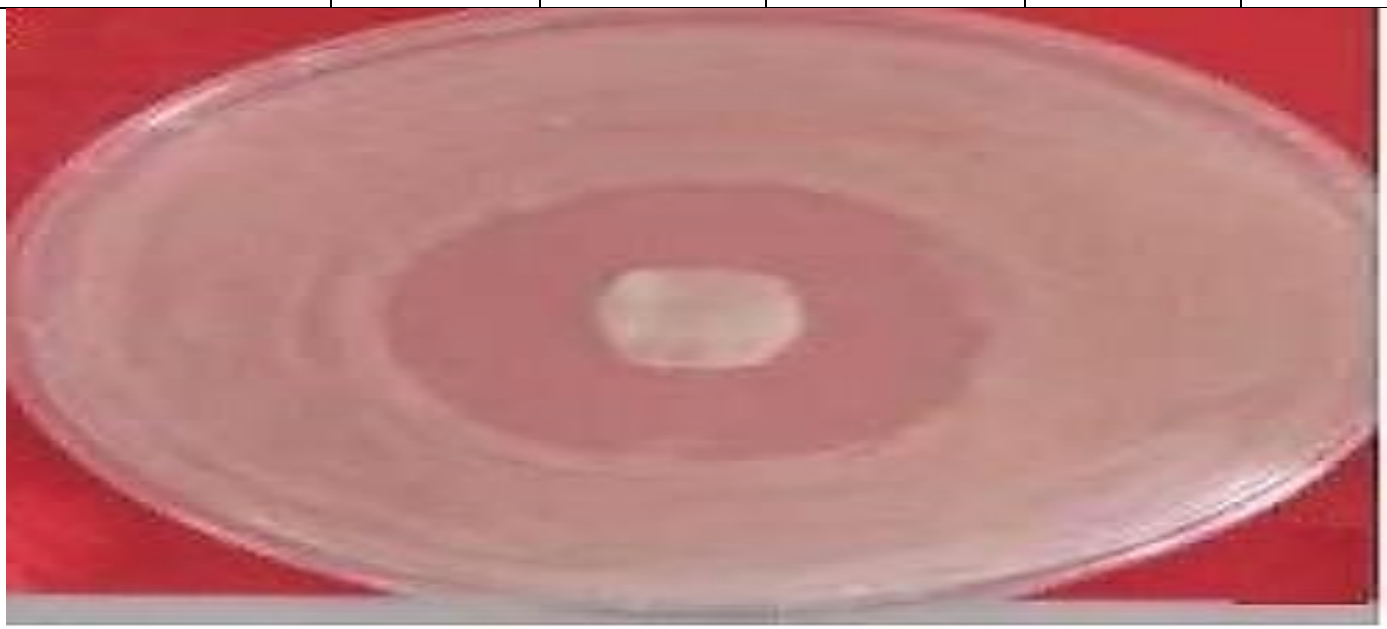

Figure 2. Antimicrobial activity of Lactobacillus filtrates

The slices revealed the epithelium layer, the top layer of skin, and the dermal layer, the middle layer, both of which were destroyed (Fig. 1). The presence of bacteria is confirmed by the significant infiltration of leukocytes (Fig. 2). This was consistent with what was seen visually, namely the ulceration of the burn region and the presence of pus.

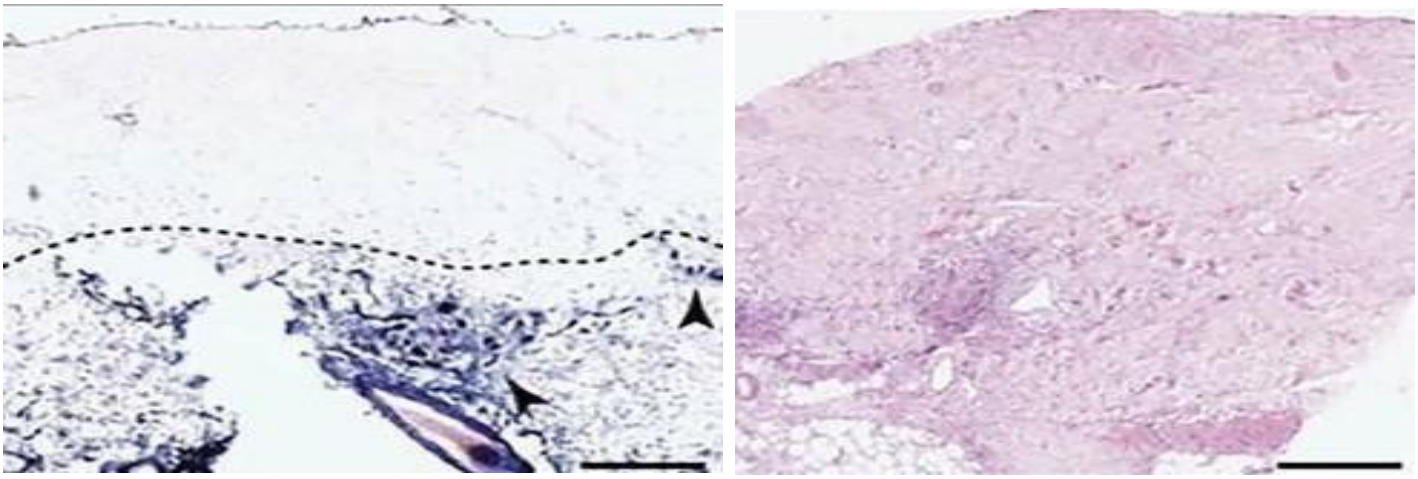

Figure 1. shows dermal layer, the middle layer, both of which were destroyed, H&E stain.

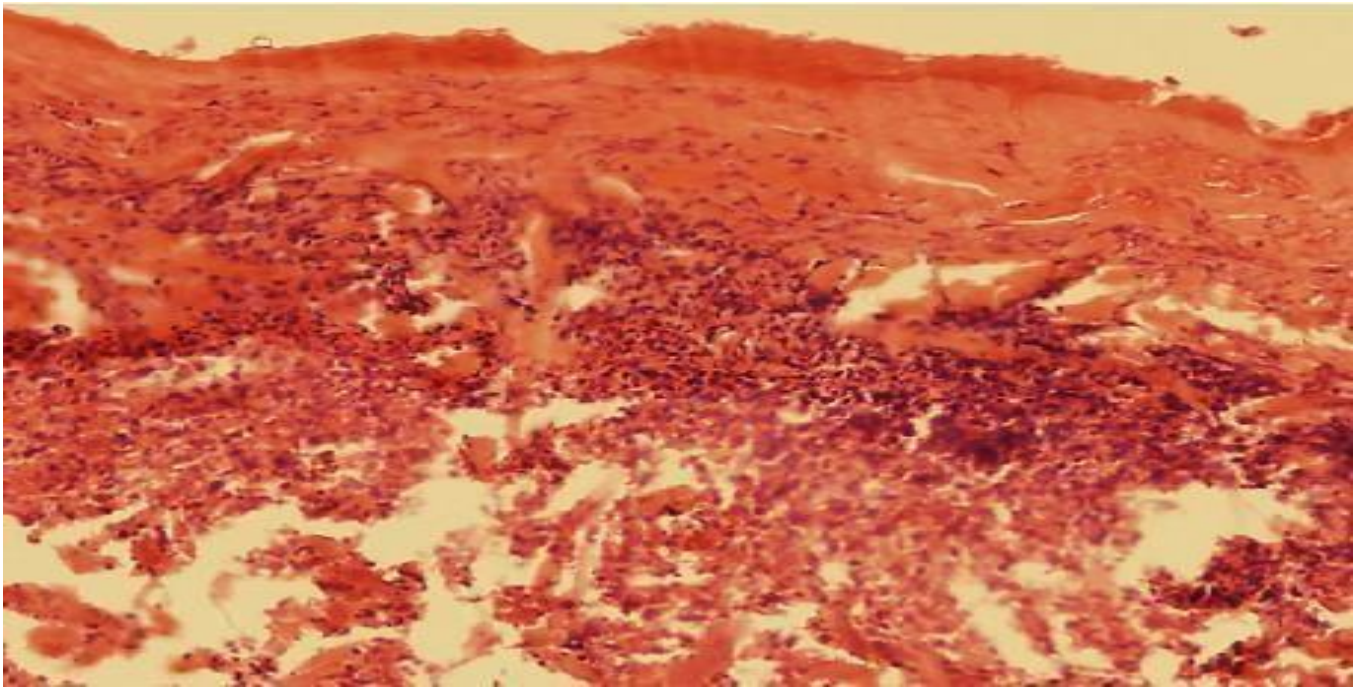

Figure 2. shows burn histology with infiltration of leukocytes, H&E stain.

Discussions:

There may be a correlation between the frequency with which burn injuries occur and the high rate at which bacteria are successfully cultured from biopsy samples taken from such injuries. Possible causes include changes in cellular and humoral immunological responses [12-14,19] and widespread breakdown of the skin's protective layer.

These findings also shed light on how these harmful microbes may be transmitted to burn victims through the polluted surroundings of hospital wards [20]. These results promote the spread of microbes that cause burn site infections.

These outcomes are typically associated with gram-negative bacteria because of their invasive character and their capacity to create pus-containing poisons. In instances of immunosuppression, these poisons are responsible for septicemia and their association with the patient's immune system [21].

Pseudomonas aeruginosa have high frequency may be due to inhibitory effects of burn site infection, previous or random application of antimicrobial drugs, or a combination of these factors, and the acquisition of nosocomial pathogens associated with prolonged hospitalization for chronic conditions [13]. Isolates of E. coli and Klebsiella spp. were found to be genetically distinct at low frequency and in varying percentages owing to the bacteria's acquired nosocomial characteristics and their ability to spread from the gastric, urine, and pulmonary passages to the skin, causing burns and exhibiting immunosuppressive activities [14,22].

The percentages of S. aureus and S. epidermidis recovered from burn site samples were not consistent. This suggests that they can cause diseases of the skin [18] using a wide variety of virulence factors, such as coagulase, leukocidins, haemolysins, protein A, as well as superantigens to firmly attach to the host's tissues [14,15].

Multidrug-resistant bacterial samples' susceptibilities to the spectrum of antibacterial has been studied by many authors [23–26]. Each strain showed a high degree of sensitivity to Lactobacillus filtrates, with variations being statistically significant ($p \leq 0.05$). These findings account for the antimicrobial activity of Lactobacilli by demonstrating how they interact with TLR-2, a receptor that recognizes bacterial lipoproteins, zymosan, lipoteichoic acid, as well as other medical methods [7], and how they produce a variety of antimicrobial materials, including hydrogen peroxide, lactic acid, and antibiotics [9]. In addition, Lactobacilli can effectively prevent the spread of pathogens by starving them of the nutrients they need to thrive [8-10].

In addition to inhibiting the growth of bacteria, Lactobacilli has been shown to stimulate macrophages and natural killer cells in vitro [4]. Systemic inflammation reactions triggered by harmful bacterial DNA can be mitigated by DNA from probiotic Lactobacilli [5]. These immune results of Lactobacilli were crucial in instances of burn wounds to prevent or lessen bacterial complications that could have led to the patient's death [27].

The members of Enterobacteriaceae exhibited a rising trend in resistance towards the majority of antimicrobials utilized in this study. The beta-lactam group and folate pathway inhibitors were particularly affected, owing to the production of betalactamase enzyme and dihydropteroate synthases, which were encoded by blaTEM, blaTEM-1, blaSHV-1, blaCTX-M, sul1, sul2, and sul3 genes, respectively. Anca et al. [28] conducted a review on the presence of aac(3)-IIIa, aac(6')-II, and aac(6’)-Ieaph(2”) as a cause for aminoglycoside modifying enzymes [29].

Multidrug resistance can be observed in Staphylococcus sp. due to the presence of virulence factors. The horizontal transmission of antimicrobial resistance genes is favored by the ability of microorganisms to generate biofilms, as evidenced by their sequence and experimental indications. Furthermore, the phenomenon of microbial exchange between individuals and their environment, including the interactions among humans, animals, and the surrounding ecosystem, has been discussed by Ciro César et al. [30].

William et al. have shown that various resistant microorganisms have emerged as the malevolent agents responsible for causing infections in burn patients. These microorganisms include methicillin-resistant Staphylococcus aureus, S. epidermidis, Pseudomonas spp., and Klebsiella spp. Advancements in antimicrobial therapies and the introduction of novel antimicrobial categories have expanded the clinician's arsenal of therapeutic options [31].

Conclusion:

Pseudomonas aeruginosa was most predominant bacteria isolated from burns, also other bacteria were isolated in significant percentages which showed high susceptibility to lactobacillus filtrates.

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