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# TREATMENT OF BACTERIA CAUSING BURN WOUND INFECTIONS BY *LACTOBACILLUS REUTERI* AS A PROBIOTIC

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## ABSTRACT

This study aimed to investigate the effect of *Lactobacillus reuteri* on pathogenic bacteria isolated from burn wound infections. A total of 51 swabs were obtained from patients of both sexes who admitted to Al-Kindy Teaching Hospital suffering from invasive burn wound infections for the period of November2014 to February 2015.The most common detected pathogenic species were *Pseudomonas aeruginosa*(43.7%) followed by *Staphylococcus aureus* (18.7%), *Klebsiella pneumoniae* (15.6%), coagulase-negative *Staphylococci* (9.3%), *E. coli* (4.6%), and each of *Ps. putida*, *Ps. alcaligenes*, *Enterobacter cloacae*, *Acinetobacter baumannii*, and *Stenotrophomonas maltophilia* (1.5%). Antibiotic susceptibility of isolates towards 10 commonly used antibiotics showed that all isolates were sensitive to Amikacin and Imipenem except of *Stenotrophomonas maltophilia* which was resistance to Imipenem. Adversely, all isolates were resistant to the penicillin group with the exception of coagulase-negative *Staphylococci* which were moderately sensitive to Ampicillin. When used as a probiotic, *Lactobacillus reuteri* excreted antibacterial activity against the burn wound infections bacteria. The maximum inhibition zone caused by *Lb.reuteri* concentrated filtrate was achieved against *Ps. aeruginosa*1 when it reached 18 mm followed by *S. aureus*17.2, while the minimum inhibition zone (7 mm) was against *Acinetobacter baumannii*.

**KEYWORDS:** Burn wound infections, *Lactobacillus reuteri*, Probiotic.

## INTRODUCTION

Burns can be defined as an injury to the skin that damages or destroy skin cells and tissue. It

is generally caused when skin makes contact with flames, chemicals, electricity, or radiation.[1] As they become readily colonized with several species of potentially pathogenic micro-organisms, including *Pseudomonas aeruginosa* and *Staphylococcus* sp., burn wounds are a major focus for infection.[2] Because of the larger area involved and longer duration of patient stay in the hospital burn wounds are a suitable site for multiplication of bacteria and are more persistent richer sources of infection than surgical wounds.[3] The infections in burn wounds are largely hospital acquired and from one hospital to another there is a difference in the infecting pathogens.[4] A susceptible site for opportunistic colonization by organisms of endogenous and exogenous origin is provided by burn wounds; thermal injury destroys the skin barrier that normally prevents invasion by microorganisms. This makes the burn wound the most frequent origin of sepsis in these patients.[5] Following thermal injury, burn wound surfaces are sterile immediately; these wounds eventually become colonized with microorganisms, Gram-positive bacteria that survive the thermal insult, such as *S. aureus* located deep within sweat glands and hair follicles, heavily colonize the burn wound surface within first 48 h.[6] The severity of any burn injury is related to the size and depth of the burn, and to the part of the body that has been burned.[7] Gram-positive bacteria from the patient's endogenous skin flora or the external environment predominantly colonize the burn wound immediately after the injury.[8] While from the patient's gastrointestinal flora endogenous Gram-negative bacteria also rapidly colonize the burn wound surface in the first few days after injury.[9] A number of virulence factors that are important in the pathogenesis of invasive infection are produced by common burn wound pathogens such as *Pseudomonas aeruginosa* and *Staphylococcus aureus.*[10] *Pseudomonas aeruginosa* produces a number of cell-associated and extracellular virulence factors that mediate a number of processes, including adhesion, nutrient acquisition, immune system evasion, leukocyte killing, tissue destruction, and bloodstream invasion.[11] *Staphylococcus aureus* also has a diverse array of virulence factors that facilitate adherence to host tissues, immune system evasion, and destruction of host cells and tissues, including coagulase, protein A, leukocidins, hemolysins, and superantigens.[12] Probiotics have been described by Food and Agriculture Organization (FAO) and the World Health Organization (WHO) as "live microorganisms which when administrated in adequate amounts confer a health benefit on the host".[13] As another definition of probiotics, they can be defined as substances secreted by one microorganism to stimulate the growth of another microorganism, as opposite to an antibiotic.[14] Enhancement of the epithelial barrier, increased adhesion to intestinal mucosa and concomitant inhibition of pathogen adhesion, competitive exclusion of pathogenic microorganisms, production of

anti-microorganism substances and modulation of the immune system, are some of the probiotics mechanisms.[15] *Lactobacilli* can produce antimicrobial substances including bacteriocins that have ability to inhibit pathogenic and food spoilage bacteria. These compounds possess specific antagonistic properties against Gram-negative and Gram-positive pathogens.[16] *Lactobacillus reuteri* is a common inhabitant of the gastrointestinal tract of humans and an obligatorily heterofermentative lactic acid bacteria.[17] Glucan and fructan exopolysaccharides are considered probiotics that produced in large amount by *Lactobacillus reuteri*.[18]

The aim of the present study was to test the ability of *Lactobacillus reuteri* as a probiotic to possess any antimicrobial activity toward bacteria that cause burn wound infections.

## MATERIALS AND METHODS

**Cultural Media:** Media used for bacterial isolation and identification are ordinary media such as Blood agar and special media such as MacConkey agar, Cetrmide agar, Manitol salt agar and Eosin methylene blue agar.

**Sample Collection:** 51 swab samples were taken from burn wound patients from both sex and various ages who admitted to burn unit in Al-Kindy Teaching Hospital from November 2014 to February 2015. The swab samples then transported to Microbiology Lab in Department of Biotechnology\ College of Science at Al-Nahrain University. At first for detecting any bacterial growth all samples were cultured on Blood agar and MacConkey agar and incubated aerobically at 37 $^{o}$C overnight. After that bacterial isolates were subcultured on selective media for further identification such as Cetrmide agar, Manitol salt agar and Eosin methylene blue agar. After cultural identification[19] bacterial isolates were identified by biochemical methods[20, 21] and as final identification Vitek 2 system was used.[22]

**Probiotics solution:** Probiotic filtrate preparation was achieved by inoculating 2% of *Lactobacillus reuteri* in MRS broth and incubated anaerobically by candle jar at 37 $^{o}$C for 6 days.[23] To make one-fold concentrated filtrate, 100 ml of unconcentrated filtrate was put in vacuum oven at 45-50 $^{o}$C the same procedure repeated to obtain tow-fold concentrated filtrate (25 ml) and three- fold concentrated filtrate (12.5 ml).

Antibiotic susceptibility test was performed on Muller-Hinton agar using the standard disk diffusion methods according to Manual on Antimicrobial Susceptibility Testing (2004). The

antibiotics used were as follow: Amikacin (AK30), Ampicillin (AP30), Cefixime (CFM30), Chloramphenicol (C30), Ciprofloxacin (CIP30), Clindamycin (DA30), Erythromycin (E15), Imipenem (IPM10), Tetracycline (TE30) and Vancomycin (VA30). By applying ordinary ruler the inhibition zone of antibiotics were measured by mm.

**RESULTS**

From a total of 51 swab samples bacterial growth was found in 43 indicating that 84.3% of burn patients have invasive burn wound infections and only 8 (15.6%) gave negative bacterial growth. From 43 swab samples that gave positive bacterial growth 64 bacterial isolates belonging to 10 different species are isolated, they are shown in table (1). The results showed that *Pseudomonas aeruginosa* was the commonest isolates (43.7%) followed by *Staphylococcus aureus* (18.7%) and then by *Klebsiella pneumoniae* (15.6%), Coagulase-negative *Staphylococci* (9.3%), *E. coli* (4.6%), *P. putida*, *P. alcaligenes*, *Stenotrophomonas maltophilia*, *Acinetobacter baumannii* and *Enterobacter cloacae* gave only one isolate for each (1.5%). According to the results of this work the predominant pathogens of burn wound infections found to be Gram negative bacteria (72%) while Gram positive were represented by 28% isolates.

*Pseudomonas* species were the most resistant isolates since they were susceptible toward only amikacin and imipenem followed by *Enterobacter cloacae* that showed susceptibility towards only three antibiotics amikacin, imipenem and ciprofloxacin and *Acinetobacter baumannii* that was sensitive towards only amikacin, imipenem and tetracycline. On the other hand, CoNS were the most sensitive isolates, they showed extreme sensitivity toward amikacin and vancomycin and less sensitivity towards chloramphenicol and moderately sensitive toward each of ampicillin clindamycin, imipenem, and erythromycin, while showed a little more sensitivity toward ciprofloxacin and cefixime and they resist tetracycline. Table (2) showed the results of antibiotic susceptibility test of the bacterial isolates.

The data of the present work indicated that *Lactobacillus reuteri* has an antibacterial effect on burn wound infectious bacteria. When filtrate of *Lb. reuteri* tested for antibacterial activity the unconcentrated filtrate showed no effect on bacterial isolates the same results obtained with one-fold concentrated filtrate and tow-fold concentrated filtrate but at three-fold concentrated filtrate growth of pathogenic isolates where inhibited. The highest antibacterial activity of *Lactobacillus reuteri* filtrate was observed against *P.aeruginosa*1 when it reached 18 mm followed by *Staphylococcus aureus* when the recorded inhibition zone was17 mm. In

contrast, the lowest inhibition zone was obtained against *Acinetobacter baumannii* (7 mm). The inhibitory effect of *Lactobacillus reuteri* concentrated filtrate against bacterial isolates is illustrated in Figure (1).

**Table 1: Bacterial species and their frequency that isolated from burn wound infections**

| Bacterial isolates | Occurrence of isolate in samples | | Total number | Percentage % |
|---|---|---|---|---|
| | Single | Mixed | | |
| *Pseudomonas aeruginosa* | 13 | 15 | 28 | 43.7 |
| *Staphylococcus aureus* | 3 | 9 | 12 | 18.7 |
| *Klebsiella pneumoniae* | 0 | 10 | 10 | 15.6 |
| *Staphylococcus epidermidis* (CoNS) | 3 | 3 | 6 | 9.3 |
| *Escherichia coli* | 0 | 3 | 3 | 4.6 |
| *Enterobacter cloacae* | 0 | 1 | 1 | 1.5 |
| *Acinetobacter baumannii* | 1 | 0 | 1 | 1.5 |
| *Stenotrophomonas maltophilia* | 1 | 0 | 1 | 1.5 |
| *Ps. alcaligenes* | 0 | 1 | 1 | 1.5 |
| *Ps. putida* | 0 | 1 | 1 | 1.5 |

**Table (2): Susceptibility percentage (%) toward antibiotics of burn wound infections causative bacteria**

| Isolate | Number of tested isolate | AP | AK | TE | DA | IPM | E | C | VA | CIP | CFM |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *P.aeruginosa* | 15 | 0 | 85.7 | 0 | 0 | 89.2 | 0 | 0 | 0 | 0 | 0 |
| *S. aureus* | 12 | 0 | 100 | 0 | 8.3 | 83 | 41.6 | 75 | 66.6 | 25 | 0 |
| *Klebsiella pneumoniae* | 11 | 0 | 90 | 0 | 0 | 90 | 0 | 50 | 0 | 80 | 0 |
| CoNS | 6 | 50 | 100 | 16 | 50 | 50 | 50 | 83 | 100 | 66 | 66 |
| *E. coli* | 3 | 0 | 100 | 0 | 0 | 100 | 0 | 100 | 0 | 66 | 0 |
| *Enterobacter cloacae* | 1 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 0 | 100 | 0 |
| *Stenotrophomonas maltophilia* | 1 | 0 | 100 | 100 | 0 | 0 | 0 | 100 | 100 | 100 | 0 |
| *Acinetobacter baumannii* | 1 | 0 | 100 | 100 | 0 | 100 | 0 | 0 | 0 | 0 | 0 |
| *P. putida* | 1 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 0 | 0 | 0 |
| *P. alcaligenes* | 1 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 0 | 0 | 0 |

AP: Ampicillin; AK: Amikacin; TE: Tetracycline; DA: Clindamycin; IPM: Imipenem; E: Erythromycin; C: Chloramphenicol; VA: Vancomycin; CIP: Ciprofloxacin and CFM: Cefixime.

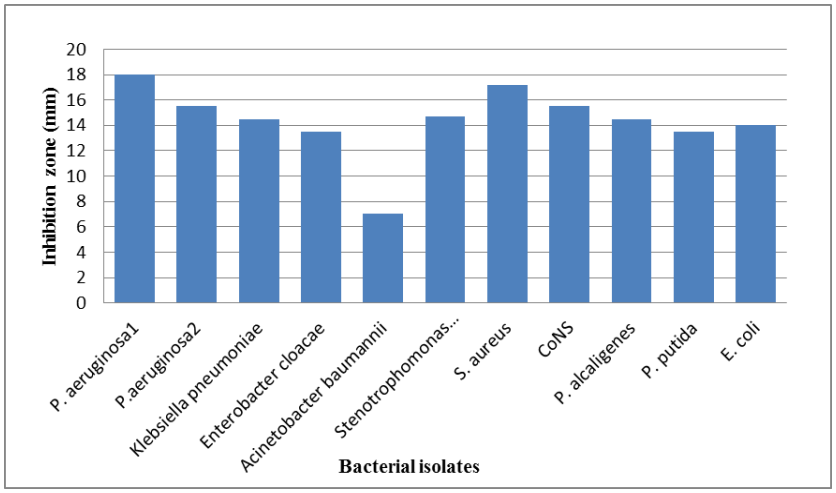


**Figure 1: Inhibitory effect of concentrated filtrate of *Lactobacillus reuteri* on bacteria causing burn wound infections.**

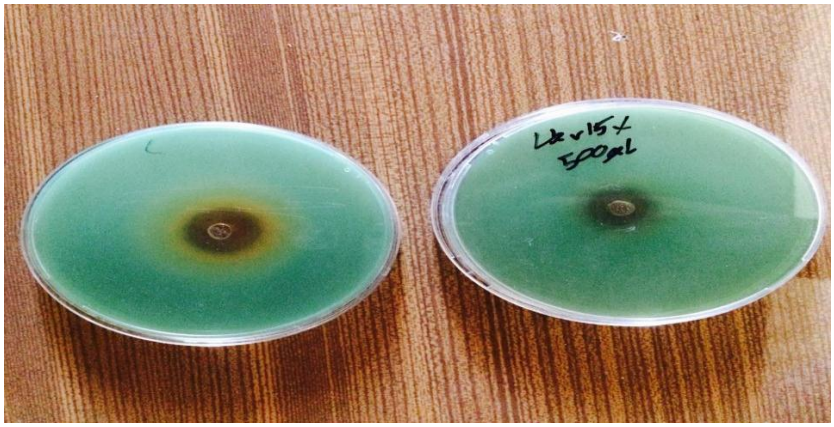

**Figure (2): inhibitory effect of concentrated filtrate of *Lactobacillus reuteri* against *P. aeruginosa* that isolated from burn wound infections**.

## DISCUSSION

The fact that 43 out of a total 51 swab samples that gave bacterial growth indicate that 84.3% of the patients have developed an invasive burn wound infections which come in accordance with the foundation of Mooney and Gamelli[5] who illustrated that in the acute period following burn injury the burn wound infections are the most critical and potentially serious complications that take place. The data of this work showed that the rate of isolation of gram-negative organism was more than gram-positive; these results are consistent with those reported by Magnet *et al.*[24] who reported that the rate of isolation Gram negative bacteria from burn wound infections is more than Gram positive bacteria. According to the results, *Pseudomonas aeruginosa* was the commonest isolate (43.7%) followed by *S. aureus* (18.7%) similar finding recorded by Magnet *et al.*[24] and Alwan *et al.*[25] on their study. Most of the

isolates were mixed with other bacterial species and some of them showed a resistant pattern towards many antibiotics which indicates that there is a high contamination rate in burn wound in our hospitals.

Sensitivity of all bacterial isolates towards amikacin was reported by this study followed by imipenem which was effective against all isolates except *Stenotrophomonas maltophilia*. The results agree with the foundation of Paterson and Yu[26] who reported high sensitivity to amikacin among bacterial isolates of their study.

Most of bacterial isolates showed resistance to antibiotics used in this study especially *P. aeruginosa* who was sensitive toward only 2 types of antibiotics. This high resistance to antibiotics may come from the fact that *P. aeruginosa* have developed resistance through mutation in chromosomal genes which regulate resistance genes in addition to low permeability of its cell wall as Lambert mentioned in 2002.[27] He mentioned another reason that is *P. aeruginosa* can acquire additional resistance genes from other organisms via plasmids, transposons and bacteriophages.

Among all used antibiotics ampicillin was the worst in inhibiting bacterial isolates; it was moderately effective against only CoNS (50%) while showed no effect against all other pathogens. In this regard, Levinson and Jawetz[28] declared that penicillin group drug can be inactivated by bacterial isolates through β-lactamase enzymes which cleave the β-lactam ring of the drug.

Our results explained that *Lactobacillus reuteri* possesses an antibacterial activity against burn wound infections bacteria. The ability of producing reuterin may accounts for this antibacterial activity.[29] Furthermore Jacobsen *et al*.[30] mentioned several mechanisms that make *Lactobacillus reuteri* able to inhibit pathogenic bacteria such as lowering the pH by producing organic acids, competing with pathogenic bacteria for nutrients and production of bacteriocins.

The results of this work showed that no inhibitory effect was observed with the usage of neither unconcentrated filtrate, one-fold concentrated filtrate nor tow-fold concentrated filtrate such results can be explained by that the low concentration of the probiotic isolate leads to low concentration of active compounds that responsible for the antibacterial activity.[31] On the other hand, three-fold concentrated filtrate of *Lactobacillus reuteri* showed

an excellent antibacterial activity especially against *P.aeruginosa*1 when the inhibition zone reached 18 mm this support the idea of Alexandre *et al*.[32] who proved that *P.aeruginosa* growth can be inhibited by increasing acid concentration that produced by *Lactobacillus* spp. Adversely, *Acinetobacter baumannii* showed the highest resistance toward concentrated filtrate of *Lactobacillus reuteri* when the inhibition zone was 7 mm which is the lowest value recorded in this study.

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