Original Article **OPEN ACCESS**

Therapeutic efficacy of *Lactobacillus acidophilus* against bacterial isolates from burn wounds

Mohammed Sh. Jebur

Institute of Medical Technology, Baghdad, Iraq.

Citation: Jebur MS. Therapeutic efficacy of *Lactobacillus acidophilus* against some bacterial isolates burn wound cases. **North Am J Med Sci** 2010; 2: 586-591.
Doi: 10.4297/najms.2010.2586
Availability: www.najms.org
ISSN: 1947 – 2714

Abstract

Background: Probiotics are live microorganisms which are mainly strains of *Lactobacillus* spp., *Bifidobacterium* spp. When administered in adequate amounts, these microorganisms offer a health benefit for the host. Probiotic organisms are also available commercially in milk, sour milk, ice cream and other foods. **Aims**: To identify bacterial species isolated from burn wounds, and also to evaluate (*In-vitro*) the therapeutic efficacy of *Lacto. acidophilus* against these bacterial isolates. To compare this activity to other antibacterial agents which are used medically in the treatment of burn wound cases. **Materials and Methods**: Burn wound swabs were obtained from 50 patients who had been admitted to hospitals in Baghdad during August to November 2009. These swabs were inoculated onto enriched and differential culture media. Subcultures were performed on selective media. The necessary biochemical tests were conducted and the organisms identified using standard procedures. Susceptibility of isolated pathogens to local isolates *Lacto. Acidophilus* (with $1x10^8$ cells/mL) and 10 commonly used burn wounds antibiotics was examined using standard susceptibility testing. **Results**: Ninety different organisms were isolated. Gram-positive cocci accounted for 16 (17.7%) and gram-negative bacilli for 74 (82.2%) bacterial isolates. *Pseudomonas aeruginosa* 30(33.3%) were the most commonly isolated organisms, followed by *Escherichia coli*, *Enterobacter spp.*, *Klebsiella spp.*, *Proteus spp.*(22.2,20,4.4,2.2%), respectively. *Staphylococcus aureus* isolates were performed in 8(8.8%). However, the incidence of *Staphylococcus epidermidis* was 2 (2.2%), while ß-haemolytic *Streptococci* was 4(4.4%). In susceptibility testing, *Lacto. acidophilus* had coverage against 90 (100%) of 74 gram-negative and 16 of gram-positive bacteria tested. The coverage of the remaining 10 antibacterial agents used was different in their activity (resistance or sensitivity), which ranged between 50-100%. **Conclusion**: The results of the study concluded that *lactobacillus acidophilus* concentration of $1X10^8$ cells/mL had a high activity to inhibit the growth *in-vitro* of all pathogenic gram-positive and gram-negative bacteria, which cause burn wound infections. This indicated the therapeutic efficacy of *lactobacillus acidophilus* bacteria.

Keywords: *Lactobacillus acidophilus*, probiotic, antibacterial agents, burn wound infections

Correspondence to: Professor Mohammed Sh. Jebur, Institute of Medical Technology, Institute of Medical Technology, Foundation of Technical Education, Babalmuaathem Square, Baghdad, Iraq. Tel.: (00964) 416-9844, Fax: (00964) 415-0010, Email: d_mohamed_1959@Yahoo.com

Introduction

Probiotics are defined as living microorganisms, principally bacteria, which are safe for human consumption. This definition has been approved by the United Nations Food and Agriculture Organization (FAO) and World Health Organization (WHO) [1].When ingested in sufficient quantities, probiotics have beneficial effects for human health, beyond basic nutrition. These effects may result from suppression of pathogens and stimulation of probiotic growth that contributes to the nutrition and health of the gut [2].

The major strains, *Lactobacillus(lacto.)acidophilus*, *Lactobacillus casei* and various *Bifidobacterium* species, are the most dominant bacteria in the small and large intestine of humans that can inhibit the growth of pathogenic microorganisms, through production of organic acids and bacteriocins [3]. Theresa, several strains of lactic

acid bacteria (LAB) were reported to display stimulatory properties on cells of the innate immune system *in-vitro*. These include macrophages and natural (NK) cells that induce adjuvant activity at the mucosal surface and improve phagocytosis by increasing the proportion of lymphocytes and NK cell [4]. Also, probiotic bacteria DNA can suppress systemic inflammatory responses to pathogenic bacterial DNA [5]. The explanation of therapeutic efficacy of probiotic bacteria may be clear through its ability to modulate epithelial barrier function [6], with possible interaction with toll-like receptor 2 (TLR-2) [7]. TLR-2 recognizes bacterial lipoteichoic acid, zymosan and other different medical methods.

Through many studies, it is believed that LAB could protect sites of bacterial invasion from its colonization of pathogenic agents by preventing the attachments of these pathogens to sites. LAB produces substances which inhibit their multiplications, by competing with other microorganisms for nutritional requirements. This might inhibit the multiplication of these agents by excreting substances, mainly hydrogen peroxide (H_2O_2), lactic acid and bacteriocin-like substances [8-10].

Burns are one of the most common and devastating forms of trauma, and occur in both children and adults [11]. Burns induce an immunosuppressive state that predisposes burn patients to infectious complications [12]. These injuries destroy the physical skin barrier that normally prevents the invasion of microorganisms and consequently, provides novel sites for bacterial colonization, infection and clinical sepsis [13]. Major injury due to trauma and burns has been demonstrated to increase susceptibility to infectious complications and related multiple organ failure primarily as a result of a suppressed immune system [14]. The skin has a complex flora; infections can result when there is a breakdown in the integrity of the skin or when the immune defense is compromised. The microorganisms from the burned wound invade the unaffected tissue and local sepsis develops; if they invade the lymphatic and vascular system, systemic sepsis develops [15].

Many types of bacteria have the ability to produce skin infections. *Staphylococcus(Staph.) aureus* is the most common cause of skin infections. About 20% of the population ares long-term carriers of *Staphylococcus aureus* [16]. Common burn wound pathogens such as gram-positive organisms are *Staphylococcus aureus,* Methicillin-resistant *Staphylococcus aureus*, Coagulase-negative *Staphylococci, Enterococcus spp., Streptococcus spp.*, and Vancomycin-resistant *Enterococci*, while gram-negative organisms are *Pseudomonas(Psu.) aeruginosa, Escherichia(E.) coli, Klebsiella spp., Serratia marcescens, Enterobacter spp., Proteus spp., Acinetobacter spp.,* and *Bacteroides spp* [17- 20].

This study was conducted to identify and diagnose the bacterial species isolated from burn wounds, and also to evaluate the therapeutic efficacy of *Lactobacillus acidophilus* against these bacterial isolates. The activity of these isolates was also compared to other antibacterial agents which are used medically in the treatment of burn wound cases.

Materials and Methods

Patients and specimen collection

Fifty burn wound swabs were collected from patients who suffered from burn infection of second to third degree with a duration of 10-15 days. These patients were admitted to hospitals in Baghdad during August to November 2009. Burn swabs were collected aseptically and transported immediately in sterile test tubes containing brain/heart infusion broth to laboratory. These swabs were cultured on Blood agar, Nutrient agar, MacConkey agar, and Manitol Salt agar, which incubated aerobically and anaerobically for 24-36 hours at 37˚C [21].

Bacterial isolates

Diagnosis of bacterial isolates was performed [22-24] through colony morphology of bacterial isolates, microscopic gram stain investigation and biochemical tests [23, 24].

Lactobacillus acidophilus

Lactobacillus acidophilus local isolate was obtained from stock culture (of yogurt) collections from the Food, Science and Biological Technology Department, Agriculture College of Baghdad University. This isolate was recultured on De Man Rogosa Sharpe agar (MRS), incubated anaerobically with a gas generating kit(5-10%CO_2 of atmosphere) at 37^{o}C for 24 hours [24]. Many tests were carried out to ensure Lact. acidophilus isolates [25-27]

Antibacterial Activity

Antibacterial activity of *Lactobacillus acidophilus* was tested against a variety of bacterial strains that became isolated from burn wounds by using Agar-Well Diffusion method [28]. *Lactobacillus acidophilus* suspension was performed for use in susceptibility testing with 1% concentration from liquid culture of bacteria that contain $1X10^8$ cells/mL [29]. The antibacterial agents (from Bioanalyse-Turkey) were Amikacin (AK) 30µg, Ampicillin (AM) 10µg, Cefotaxim (CTX) 30µg, Chloramphenicol (CHPC) 30µg, Ciprofloxacin(CIP) 5µg, Lincomycin (LN) 2µg, Gentamycin (CN) 10µg, Tetracycline (TE) 30µg, Azithromcin (AZM) 15µg, and Trimethoprim-Sulphamethoxazole (SXT) 1.25/23.75 [30]. Inhibition zones were recorded on Mullor-Hinton agar [31, 28].

Statistical analysis

SPSS programs were used to determine statistically significant differences of variables. Chi-square test (Kruskal Wallis Test) was used to explain the significant differences between bacterial isolates and antimicrobial susceptibility of agents. Probability value (p-value) of less than 0.05 was considered statistically significant, while p-value of more than 0.05 was considered statistically not

significant. Mean value and percent were used for the incidence of bacterial distributions [32].

Results

On evaluation of the burn wound swabs, there were only 39 (78%) cases that showed positive bacterial culture with statistical differences ($p<0.0001$) to negative bacterial culture(Fig. 1 and Table1). Thirty (76.9%) swabs showed mixed growth colony with highly statistical differences ($p<0.0001$) than single bacterial growth, 9 (23%)(Table 1).

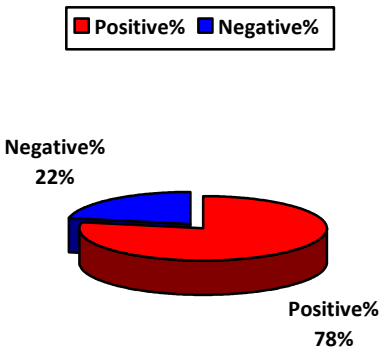

Fig. 1 Distribution percentage of bacterial swabs.

Table 1 Distribution of positive bacterial swabs (50) cases and cultures.

	Negative	Positive	Positive culture	
			Mixed cultures	Single cultures
Culture Total	11(22%) 50	39(78%)*	30(76.9%)** 39	9(23.1%)

* Significant differences (P<0.0001). Chi-Square=15.68 , df=1.
** Significant differences (P<0.0001). Chi-Square=11.308 , df=1.

Table 2 Distribution of (90) bacterial isolates.

Isolates	No. of isolates	Percentage (%)
*Pseudomonas aeruginosa**	30	33.3%
Escherichia coli	20	22.2
Enterobacter spp.	18	20
Klebsiella spp.	4	4.4
Proteus spp.	2	2.2
*Staphylococcus aureus**	8	8.8
ß-haemolytic *Streptococci*	6	6.6
Staph. epidermidis	2	2.2
Total	90	100%

* Significant differences (P<0.0001). Chi-Square=65.378 , df=7.

Ninety isolates were recorded, so that the frequencies of gram-negative isolates were more than gram-positive 74(82.2%) and 16(17.7%), respectively($p<0.0001$). Table 2 shows the frequency of gram-negative isolates of burn wound swabs. *Pseudomonas aeruginosa* had a predominant microorganism of 30(33.3%) with highly statistical differences ($p<0.0001$), followed by *Escherichia (E.) coli* 20 (22.2%), *Enterobacter spp.*18 (20%), *Klebsiella spp.* 4(4.4%), and *Proteus spp*. 2(2.2%). Gram-positive isolates showed lower frequency than gram-negative isolates, with high statistical differences ($p<0.0001$), where *Staphylococcus(Staph.) aureus was* 8 (8.8%), followed by ß-haemolytic *Streptococci* and *Staph. Epidermidis of* 6 (6.6%) and 2 (2.2%), respectively. (Table 2).

Results of susceptibility tests for bacterial isolates to *lactobacillus acidophilus* and antibacterial agents are shown in Table 3. *Pseudomonas aeruginosa* was seen to have a high ability to resist all antibacterial agents with different percentages and there were high significant differences ($p<0.0001$). At the same time, it was highly sensitive (100%) to *lactobacillus Acidophilus*, Ciprofloxacin and Amikacin ($p<0.05$). *Escherichia coli* isolates showed sensitive profile to *lactobacillus acidophilus* (100%), Amikacin (95%), Ciprofloxacin (90%), Azithromycin (95%), and were resistant to the rest of the agents. *Enterobacter spp.* isolates were resistant to all antibacterial agents, which were highly sensitive to *lactobacillus acidophilus*, Ciprofloxacin, Azithromycin (100%, 94.4%, and 100%, respectively). Three isolates of *Klebsiella spp.* were sensitive to *lactobacillus acidophilus*, Lincomycin, Cefotaxim and Trimethoprim-sulphamethoxazol with 75% percent for all**.** *Proteus spp*. isolates recorded only sensitivity (100%) to *Lactobacillus acidophilus*, Cefotaxim, Amikacin, Ciprofloxacin and Gentamycin.

Staphylococcus aureus was the main gram-positive pathogenic bacteria of burn wound infections, which appear highly sensitive with significant differences ($p<0.0001$) to isolates of *Lactobacillus acidophilus,* Amikacin, Ampicillin, Ciprofloxacin, Gentamycin, Tetracycline and Azithromycin with 100% percent. Its resistance to other antibacterial agents was variable as shown in Table 3. These results of susceptibility resembled those obtained from isolates of *Staphylococcus epidermidis*. Thereas, ß-haemolytic *Streptococci* isolates showed 100% sensitivity to *lactobacillus acidophilus,* Gentamycin, Ciprofloxacin, Lincomicin and Azithromycin.

The results concluded that *lactobacillus acidophilus* concentration of $1X10^8$ cells/mL showed a high activity to inhibit the bacterial growth *in-vitro* of all pathogenic gram- positive and gram-negative bacteria, which cause burn wound infections. This indicates the therapeutic efficacy of *lactobacillus acidophilus* bacteria.

Discussion

The high percentage 39 (78%) from positive bacterial culture of burn wound swab samples might be attributed to the fact that burn wounds appear in high incidence as compared to other forms of trauma. This may be due to extensive skin barrier disruption as well as alteration of cellular and humoral immune responses [12-14, 30].

Table 3 Antibacterial susceptibility of *lactobacillus acidophilus* isolates and antibacterial agents.

Antibacterial agents	*Ps. aeruginosa**	*E. coli*	*Enterobacter spp.*	*Klebsiella spp.*	*Proteus spp.*	*Staphy. aureus*	*ß-haemolytic Streptococci*	*Staph. epidermidis*
*lacto.acidophilus**	S(100%)	S(100%)	S(100%)	S(75%)	S(100%)	S(100%)	S(100%)	S(100%)
AK 30µg	S(100%)	S(95%)	R(77.7%)	R(100%)	S(100%)	S(100%)	R(50%)	S(100%)
AM 10µg	R(93.3%)	R(80%)	R(88.8%)	R(100%)	R(100%)	S(100%)	R(100%)	S(100%)
CTX 30µg	R(66.6%)	R(80%)	R(72.2%)	R(75%)	R(50%)	R(62.5%)	R(66.6%)	R(62.5%)
CHPC 30µg	R(90%)	R(95%)	R(94.4%)	R(100%)	R(100%)	R(87.5%)	R(83.3%)	R(87.5%)
CIP 5µg	S(100%)	S(90%)	S(94.4%)	R(50%)	S(100%)	S(100%)	S(83.3%)	S(100%)
LN 2µg	R(83.3%)	R(85%)	R(77.7%)	S(75%)	R(50%)	R(62.5%)	S(100%)	R(62.5%)
CN 10µg	R(100%)	R(95%)	R(94.4%)	R(100%)	S(100%)	S(100%)	S(83.3%)	S(100%)
TE 30µg	R(93.3%)	R(90%)	R(88.8%)	R(100%)	R(100%)	S(100%)	R(66.6%)	S(100%)
AZM 15µg	R(66.6%)	S(80%)	S(100%)	R(50%)	R(50%)	S(100%)	S(100%)	S(100%)
SXT1.25/23.75.	R(86.6%)	R(90%)	R(83.3%)	S(75%)	R(100%)	R(87.5%)	R(83.3%)	R(87.5%)

*:Significant differences(P<0.001); Chi-Square=31.098; df=10; (Kruskal Wallis Test); S: sensitive isolates; R: resistant isolates.

In addition, these results explain that the contaminated environments of hospital wards may become a source of these pathogenic microorganisms to burn patients [33]. These findings encourage invasion of burn wound pathogenic bacteria.

With predominant gram-negative bacteria 74(87.2%) to 16 (17.7%) gram-positive bacteria, it is believed that these results are due to an opportunistic nature and the ability to produce pus-containing toxins. These toxins cause septicemia and interaction with patient's immunity, which leads to immunosuppressive cases [24, 35, 13, 15].

The reasons for the high incidence of *Pseudomonas aeruginosa* isolates might be due to factors associated with acquisition of nosocomial pathogens with recurrent long-term hospitalization that complicates illnesses, prior or random administration of antibacterial agents and immunosuppressive effects of burn wound infection [36, 13]. *Escherichia coli*, *Enterobacter spp., Klebsiella spp.* and *Proteus spp*. isolates were segregated with low frequencies and different percents due to their acquisition of nosocomial properties and migration of these agents from gastrointestinal, urinary and respiratory tracts to burn wounds and immunosuppressant activities [14, 37, 38].

Results of gram-positive bacteria which were isolated from burn wound swabs showed different percents of *Staphylococcus aureus*, ß-haemolytic *Streptococci*, and *Staphylococcus epidermidis* 8(8.8%), 6(6.6%) and 2(2.2%), respectively. This indicates their ability to produce skin infections[18] with a diverse array of virulence factors that facilitate adherence of host tissues, including coagulase, protein A, leukocidins, haemolysins and superantigens [14, 15, 39, 40].

Based on susceptibility testing, the study compared *Lactobacillus acidophilus* to many antibacterial agents that could be used locally or systemically in burn wound cases. Table 3 showed the susceptibility of bacterial isolates which have multidrug resistance to several antibacterial agents used with different percentages [39-42]. All isolates appeared highly sensitive to *Lactobacillus acidophilus* with significant differences ($p<0.05$). These results explain the antibacterial activity of *Lactobacilli* through interaction with TLR-2 which recognizes bacterial lipoproteins, lipoteichoic acid, zymosan and other different medical methods[7], production of several antibacterial materials such as hydrogen peroxide, lactic acid or substances such as antibiotics [9]. In addition, *Lactobacilli* have a high ability to inhibit pathogenic growth and multiplication through competition with other pathogenic microorganisms for nutritional requirements [8-10].

Burn wounds induce an immunosuppressant state that predisposes the invasion by opportunistic pathogenic bacteria such as those isolated in a recent study [12]. This invasion provides novel sites for bacterial colonization and clinical sepsis [3].

Besides the antibacterial activities of *Lactobacilli*, it was reported to display stimulatory properties on cells of innate immune system *in-vitro*, including macrophages and natural killer cells[4]. Probiotic *Lactobacilli* DNA can suppress systemic inflammatory responses to pathogenic bacterial DNA [5]. These immunological effects of *Lactobacilli* were essential in burn wound cases to inhibit or reduce bacterial complication that may lead to the death of the patient [37].

Conclusion

This study indicates the antibacterial efficacy and immunological properties of *Lactobacillus acidophilus* through investigation of burn wound pathogenic agents with susceptibility testing. We recommend the medical importance of *Lactobacilli* species in clinical applications for burn wound infections[44].

Acknowledgements

We are grateful to the Institute of Medical Technology, Baghdad-Iraq, for providing laboratory requirements and support of the work. We also thank Prof Dr. Jassim Karhoot for comments on the manuscript.

References

1. Food and Health Agriculture Organization of United Nations and World Health Organization. Guidelines

for the evaluation of probiotics in food. Joint FAO/WHO Working Group Report on Drafting Guidelines for the Evaluation of Probiotics in Food, 2002. (Accessed August 31, 2009, at fttp://www.fao.org/es/esn/food/wgreport2.pdf).
2. Naidu AS, Bidlack WR, Clemens RA. Probiotic spectra of Lactic Acid Bacteria(LAB). Crit. Rev Food Sci Nutr 1999; 38:13-126.
3. Mazza G. Functional Food, Biochemical and Processing Aspects. Taylor and Francis Gp. LLC. Boca Raton, FL; 1998:357-374.
4. Mc-Cracken BJ, Gaskins HR. Probiotics and the immune system. In: Tannok GW(ed.): probiotics: a critical review. Norfolk, Va: Horizon Scientific Press; 1999: 85-111.
5. Jijon H, Backer J, Diaz H, *et al*. DNA from probiotic bacteria modulates murine and human epithelial and immune function. Gastroenterology 2004; 126:1358-1373.
6. Madsen K, Cornish A, Soper P, *et al.* Probiotic bacteria enhance murine and human intestinal epithelial barrier function. Gastroenterology 2001; 121: 580-591.
7. Kalliomaki M, Walker WA. Physiologic and pathologic interactions of bacteria with gastrointestinal epithelium. Gastroenterol Clin North Am 2005; 34:383-399.
8. Andreu A, Stapleton AE, Fennel CL, Hillier SL, Stam WE. Hama agglutination, adherence and surface properties of vaginal Lactobacillus species. J Infect Dis 1995; 171:1237-1240.
9. Vallor AC, Antonio MAD, Hawes SE, Hiller SL. Factors associated with acquisition of, or persistent colonization by vaginal *Lactobacilli*, role of hydrogen peroxide production. J Infect Dis 2001; 184:1431-1436.
10. Cadieux P, Burton J, Braunstein I, Bruce AW, et al. *Lactobacillus* strains and vaginal ecology. JAMA 2002; 287:1940-1941.
11. Church D, Elsayed S, Rrid O, Winston B, Lindsay R. Burn wound infections. Clin Microbiol Rev 2006; 19(2):403-434.
12. Brigham PA, McLoughlin E. Burn incidence and medical care use in the United States: estimate, tends and data sources. J Burn Care Rehabil 1996; 17:95-107.
13. Vindenes H, Bjerknes R. Microbial colonization of large wounds. Burns 1995; 21:575-579.
14. Atiyeh BS, Al-Amm CA. Immunology of burn injury. An overview. Ann Burns Fire Disasters 2001;14(2):
15. Komolafe OO, James T, Kolongolera L. Bacteriology of burn at the Queen Elizabeth Central Hospital, Blantyre, Malaw. Burn 2003; 29:235-238.
16. Kluytmans J, Van Belkum A, Verbrugh H. Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. Clin Microbiol Rev 1997; 10:505-520.
17. Agnihotri N, Gupla V, Joshi RM. Aerobic bacterial isolates from burn wound infections and their antibiograms-a five year study. Burns 2004; 30:241-243.
18. Bang RL, Gang RK, Sanyal SC. Mokaddas E.M. and Lari A. R. Beta-haemolytic *Streptococcus* infection in Burns. Burns 1999; 25:242-246.
19. Revathi G, Puri J, Jain BK. Bacteriology of burns. Burns 1998; 24:374-349.
20. Sheridan RI, Schuiz JT, Weber JM, Ryan CM, Pasternack M.S, Tompkins RG. Cutaneous infections complicating burns. Burns 2000; 26:621-624.
21. Collee J, Fraser AG, Marmian BP, Simmon SA. Mackie and McCarthy Practical Microbiology. 4th ed. Churchill and Livingston, Inc. USA; 1996.
22. Baron EG, Peterson LR, Finegold SM. Bailey and Scott's Diagnostic Microbiology. 9th ed. C. V. Mosby Company; 1995.
23. Benson HJ. Microbiological application: Laboratory manual in general microbiology. 17th ed. W. B. McGraw Hill; 1998.
24. MacFaddin JF. Biochemical test for identification of medical bacteria. 3th ed. Williams and Wilkins-Baltimore. New York; 2000.
25. McGinnis MR. Laboratory handbook of Medical Mycology. Academic press. New York; 1980.
26. De Man JC, Rogosa M, Sharpe M E. A medium for the cultivation of lactobacilli. J Appl Bact 1960; 23 (1): 130-135.
27. Forbes BA, Saham DF, Weissfeld AS. Diagnostic Microbiology. 10th ed. Mosby. Inc. USA; 2002.
28. NCCL. Antibiotic susceptibility methods. Clinical Laboratory Standards Institute (CLSI); 2000.
29. Schillinger U, Luck FK. Antibacterial activity o*f Lactobacillus sake* isolated from meat. Appl Environ Microbiol 2000; 55(8):1901-1906.
30. Balakit Huda AW. Study of some clinical, bacteriological and immunological aspects of patients with burn injury. MSc thesis. College of Medicine. University of Babylon; 2006.
31. Perez C, Pauli M, Bezerque P. An antibiotic assay by the agar-well diffusion method. J Aotabiologiae 1990; 15:113-115.
32. Sanyal SC, Mokaddas EM, Gang RX, Bang RL. Microbiology of septicemia in burn patients. Ann Burns Fire Disasters 1998;11(1).
33. Torregrossa MV, Valentino L, Cucchiara P, Masellis M, Sucameli M. Prevention of hospital-acquired infections in the Palermo burns center. Annals of Burns and Fire Disasters 2000; 13(3). Cited by Huda A.W. Balakit. Study of some clinical, Bacteriological and Immunological Aspects of Patients with Burn injury. MSc thesis. College of Medicine. University of Babylon; 2006.
34. Mousa HA. Aerobic, anaerobic and fungal burn wound infections. J Hosp Infec 1997; 37:317-323.
35. Maitra A. Environmental diseases, In Kumar, V.; Cotran R. and Robbins S. Robbins Basic Pathology, 7th ed. Saunders, an imprint of Elsevier Science, London; 2003.
36. Rastegar LA, Alaghehbandan R, Akhlaghi L. Burn wound infections and antimicrobial resistance in

Tehran, Iran: an increasing problem. Ann Burns Fire Disasters 2005; 18(2).
37. Herek O, Ozturkk H, Ozyurt M, Albay A, Cetinkursun S. Effects of treatment with immunoglobulin on bacterial translocation in burn wound infection. Ann Burns Fire Disasters 2000; 13(1).
38. Bowler AG, Duerden BI, Armstrong DG. Wound Microbiology and associated approaches to wound management. Clin Microbiol Rev 2001; 14:244-269.
39. De Macedo JLS, Rosa SC, Castro C. Sepsis in burned patients. Revista da Sociedade Brasileria de Medicina Tropical 2003; 36(6):647-652.
40. Foster TJ. The *Staphylococcus aureus* "superbug" . J Clin Investig 2004; 114:1693-1696.
41. Richard P, Floch RL, Chamoux C, Pannier M. *Pseudomonas aeruginosa* outbreak in a burn unit: role of antimicrobials in the emergence of multiply resistant strains. J Infect Dis 1994; 170:377-383.
42. Brooks GF, Butel JS, Morse SA. Jawetz, Melnick and Aldelberg's Medical Microbiology. 23th ed. Lange Medical Books, McGraw-Hill; 2004.
43. Kehinde AO, Ademola SA, Okesola AO. Pattern of bacterial pathogens in burn wound infections in Ibadan, Nijeria. Ann Burns Fire Disasters 2004; 17(1):12-15.
44. Shihd M, Malik A. Resistance to aminoglycoside modifying enzymes in *Pseudomonas aeruginosa* isolates from burns patients. Indian J Med Res 2005; 122: 324-329.