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EFFECT OF BIOSURFACTANT PRODUCED FROM STREPTOCOCCUS THERMOPHILUS AGAINST STAPHYLOCOCCUS AUREUS AND SOME PHYSIOLOGICAL PARAMETERS IN WHITE RATS MALE

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ABSTRACT:
The bacteria Streptococcus thermophilus belongs to the group of lactic acid bacteria, where the research aims to identify the role of Biosurfactant produced from the bacteria Streptococcus thermophiles for its importance in limiting the growth of Staphylococcus aureus and its effect on some of the physiological parameters of the white rats male ، Streptococcus thermophilus were isolated from local white cheese, and then 120 clinical samples (Wounds, Burns, Blood) were collected. Biosurfactant was extracted from Streptococcus thermophilus and its inhibitory activity was evaluated against Staphylococcus aureus. As well as, its resistance to some antibiotic effects was studied and the effect of Biosurfactant on some physiological parameters in white rats male.، 22 infected samples were obtained, distributed between (11,8, and 3) for each of the (Wound, Burn, and Blood) samples, respectively. The results also showed resistance of bacteria S. aureus to some of the antibiotics used in the study, the results of the statistical analysis to evaluate the inhibitory activity of the Biosurfactant towards S. aureus showed a significant increase at a significant level ($P < 0.05$). Besides, the effect of Biosurfactant on some physiological parameters in white rats male by a significant increase ($P < 0.05$) in the number of red blood cells RBCs, hemoglobin concentration Hb, and packed cell volume PCV in the treatment group T compared to the control group C. It can be concluded from this study the ability of Biosurfactant to eliminate S. aureus isolated from various clinical samples and its effect on the physiological parameters of white rats male.

Keywords: Biosurfactant, Streptococcus thermophilus, Staphylococcus aureus, physiological parameters

INTRODUCTION

Streptococcus thermophilus belongs to the group of lactic acid bacteria, which is described as gram-positive bacteria, spherical in shape, arranged in pairs or chains, facultatively anaerobic, the optimum temperature for its growth is 37 °C, and it can grow at high temperatures [1,2]. These bacteria possess the specifications of probiotics and are therefore widely used in this field, as well as have a long history of safe use as a primer to the yoghurt industry [3]. Lactic acid bacteria, including S. thermophilus, produce many substances that inhibit microbial growth and have an inhibitory effect on the growth of pathogenic microbes and those that cause food spoilage, among which are lactic acid, acetic acid, formic acid, ethanol, hydrogen peroxide, diacetyl, bacteriocin, and fatty acids [4,5,6].These primer bacteria are effective in the prevention and treatment of some diseases caused by pathogenic microbes by several mechanisms including the production of many inhibitory substances. Besides, their ability to inhibit colonization of pathogenic bacteria and treatment of gastrointestinal infections, especially those caused by Clostridium difficile and Helicobacter pylori [7], also it has a role in reducing problems with lactose intolerance [8]. S. thermophilus is one of the most important lactic acid bacteria used in fermented milk primers, as it is used in the manufacture of yoghurt and some types of cheese [9,10]. In addition to its role in inhibiting diarrhea-causing bacteria ([11]. S. thermophilus or its bacteriocin are used as probiotics and inhibiting the bacteria causing spoilage such as Clostridium sporogenous and Clostridium tyrobutyricum. Bacteriocin produced from this bacterium, called Thermophilin, which has a wide efficacy against pathogenic bacterial species such as Listeria monocytogenes, Salmonella typhimurium, Escherichia coli, and Yersinia pseudotuberculosis, Yersinia enterocolitica [12,13]. S. thermophilus also produce a Biosurfactant that have inhibitory activity against bacteria, fungi, and viruses [14]. Furthermore, Biosurfactant is known as biomaterials that reduce surface tension produced from some microbes, they act as anti-microbial and anti-adhesion substances and help get rid of some microorganisms, where these materials contain multiple compositions such as Polysaccharide – 1protein complex, fatty acids, phospholipids, Lipolipid, and glycolipid. The biosurfactant has a role in inhibiting the adhesion of pathogens causing urinary tract infections and is used as a preventive agent to prevent the adhesion of carcinogenic bacteria [15]. Staphylococcus aureus is gram-positive bacteria, non-moving and not-forming spores, it is part of the natural flora of a human. However, it is one of the germs that can cause serious infections when it occurs malfunction or infection to the human skin or disorders of the immune system such as meningitis and pneumonia, Endocarditis, and Rheumatoid Arthritis [16]. Carriers of S. aureus are estimated to 30%, it is among the germs that can cause serious infections when a dysfunction or disorders in the host's body's immune defenses, this percentage increases in people working in hospitals [17]. Infections with this type of bacteria occur when it enters the tissues, through wounds, or by scratches, or by touching the surface of the skin tissue of the host. As they cause disorders and dysfunction in this tissue, by secreting many enzymes, including Lipase, and Hyaluronidase that helps bacteria spread and break down the base material of the connective tissue [18]. The most important pathological conditions that these bacteria cause in humans are Urinary Tract Infection (UTI) and Toxic Lens Syndrome after applying lenses, endocarditis, bacteremia, skin abscesses, and congenital heart valve infection [19].As well as, the hands of contaminated medical personnel in hospitals play an important role in the transmission and spread of Methicillin-resistant staphylococcus aureus MRSA infection to medical devices and units, especially catheters and dialysis machines [20]. Staphylococcus aureus showed high resistance too many of the antibiotics used in the treatment of infections resulting from these bacteria and that the attempts made by many researchers to alter the antibiotics or to produce new types of them met with limited success. All types of staphylococcus aureus showed high resistance to antibiotics such as Erythromycin and Tetracycline and penicillin [21,22], pointed out in a study of 151 isolates of Staphylococcus aureus which tested their sensitivity to Erythromycin, 53 isolates were resistant to this antibiotic, while 25 isolates were stimulant-resistant to Erythromycin and Streptomycin, while their sensitivity to Quinolone antibiotic was contradicted [23].

This bacterium was chosen because of its widespread and its responsibility for various severe infections among hospitalized patients. In addition to the random use of antibiotics stimulated the resistance of bacteria to many of them, and due to the lack of local studies on the Biosurfactant production from bacteria Streptococcus thermophilus isolated from Iraqi white cheese. As well as, the importance of these therapeutic and preventive bacteria and their role in inhibiting bacterial pathogens, including bacteria Staphylococcus aureus in samples taken from various clinical injuries and determining the proportion of its resistance to antibodies because of its great importance to the patient's health. Therefore, this study was carried out to investigate the production of Biosurfactant from S. thermophilus and its inhibitory effect on the growth of Staphylococcus aureus and its effect on some physiological parameters for white rat's male.

MATERIALS AND METHODS:

1- Bacterial isolates:

- **Streptococcus thermophilus:** bacteria S. thermophiles were isolated from local white cheese made by the traditional method and from non-thermally processed milk, selected from a group of isolates on the (De Man, Rogosa and Sharpe) MRS medium, which was confirmed as being identified using the following cultural and biochemical tests mentioned in [1].
- **Pathological bacterial isolates:** Staphylococcus aureus was isolated from various clinical infections, where 120 different samples of the human body (Wounds, Burns, Blood) were collected from adult patients for both sexes from patients of Imam AL-Hussain Teaching Hospital in city of Karbala. The samples were cultured on the blood agar medium, then they were transferred to the selective medium, after which the isolates were identified depending on the microscopic and cultural characteristics, where several biochemical tests were carried out according to [24] procedure. In addition, APi Staph kit was used, as well as using Vitek 2 compact system to confirm the identification.

2- Sensitivity test for antibiotics:

The Disk diffusion method mentioned in [25] was adopted to conduct an antibiotic sensitivity test using Mueller Hinton agar medium and included each of Amoxicillin /clavulanic acid (30μg) Ceftazidime (30μg). As well as, Norfloxacin (30μg), Gentamicin (10μg), Penicillin G (10 μg), Choloramphinicol (10μg) Amoxicillin (25μg), Ampicillin (10μg), Cefotaxime (30 μg) (Bioanalysis, Turkey) and the results were compared with the standard Tables mentioned in [26] to determine the diameter of the inhibition zone.

3- Extraction of Biosurfactant from Streptococcus thermophilus:

Biosurfactant was extracted using the method mentioned in [15], the liquid MRS medium was inoculated with an S. thermophilus farm at age 24 hours, and incubated at 37 ° C for 18 hours. Then it was centrifuged at 10,000 rpm for 5 minutes and washed twice and the cells were re-suspended with phosphate-buffered saline PBS solution and then left at room temperature for two hours on the magnetic motor, centrifuged to get rid of cell residue. Finally, the liquid was filtered through 0.22 μm Millipore filters to obtain a Biosurfactant, the Lyophilizer was used to dry the Biosurfactant and the leachate for a purpose of obtaining a powder and then store at 4 °C until use.

4- Determination the inhibitory activity of Biosurfactant produced from S. thermophilus:

The well diffusion method [27] was used to estimate the efficiency in inhibiting the growth of pathogenic bacteria under test.

5- Effect of Biosurfactant produced from S. thermophilus on some physiological parameters in white rats:

In this study, 14 male rats (Rattus rattus) were used, whose weight was between 250-300 g, they were obtained from the Faculty of Medicine, University of Kufa, and distributed into two groups and placed in special cages. All laboratory conditions were prepared with aeration, lighting, and temperature from 20-30 ° C, and rats were given a feed of a granular type obtained from specialized sources of animal feed. The animals were randomly divided into two equal groups by seven animals in each group, and were treated as follows;

- **Control group C:** where normal drinking water was drunk throughout the three-week trial period.
- **Treatment group T**: which Biosurfactant extract was drinking throughout the three-week trial period

SAMPLES COLLECTION:

The animals were anesthetized using chloroform, where 5 ml of blood withdrawn from the heart directly (heart puncture) after 24 hours of the last dose of the Biosurfactant. Additionally, 2 ml of blood was put in a container that contains an ethylene diamine tetra Acetic acid (EDTA) for measuring blood parameters, and 3 ml of the remaining blood was put in an anticoagulant-free tube left for 15-20 minutes at the laboratory temperature. Finally, the serum was separated using a centrifuge at a speed of 3000 rpm for 15 minutes, and the serums were kept in a refrigerator temperature 4 ° C for measuring some biochemical parameters.

- **Physiological parameters of blood:**

1. **RBC:** the total number of Red Blood Cells was calculated using the Number Chamber Hemocytometer described by [28].
2. **Hemoglobin (Hb):** Hemoglobin concentration was calculated using the Cyanomethemoglobin method described by [28].
3. **Packed cell volume (%) (PCV):** Packed cell volume was measured using a capillary tube method [29].

Statistical analysis: The results were statistically analyzed using t-test and one-way analysis of variance, Duncan's

Multiple Range Test (DMRT) method was also used to test the multiple ranges according to the statistical package for the social sciences (SPSS.V. 22), and the standard deviation values were also calculated [30].

RESULTS AND DISCUSSION:

- **Streptococcus thermophiles: S. thermophiles** were obtained from the local Iraqi white cheese manufactured in the traditional method and from non-thermally processed milk, selected from a group of isolates on the MRS medium., which confirmed that it was identified by following the cultural and biochemical tests mentioned in [1].
- **Isolation and identification of Staphylococcus aureus:**

120 different clinical samples were collected and isolated from people with different clinical infections of both sexes and different ages from patients of Al-Hussein Hospital in Karbala city. Furthermore, samples were collected from different infections from the human body for each of the samples (Wounds, Burns, Blood), and 22(18.33%) infected samples were obtained, distributed between (11,8 and 3) for each of the samples of Burns, Wounds, and Blood, respectively. Staphylococcus aureus was identified according to their macroscopic properties by observing the shape of their cells, their regularity, the method they are arranged, and stained with a gram stain, where it was observed as similar to grape-like shape, gram-positive [31]. In order to confirm the accurate identification of bacteria S. aureus, an API Staph kit and Vitek 2 compact system was used. However, the Wound samples recorded the highest percentage with S. aureus bacteria reached 11(50%), followed by Burns samples amounted to 8 (36.36%), and the lowest percentage was for Blood samples reached 3 (13.64%) of the total, as shown in Table (1).

Table 1. The isolates number of Staphylococcus aureus and their percentages according to the source of the infection.

Samples source	Samples number	Bacterial isolates number	Percentage %
Wounds	40	11	50 %
Burns	40	8	36.36 %
Blood	40	3	13.64 %
Total	120	22	100 %

The results of the number of S. aureus isolates showed a difference in the isolation percentages from the different pathological Samples due to the variation in the number of pathological Samples under study. Hospital infection is among the most complex health problems facing doctors who deal with serious conditions today, despite the use of modern technologies in performing surgical operations in hospitals, and the good care shown by the medical staff towards burns patients. Anyhow, hospital infection has become a widespread problem worldwide and of greater importance than public health problems, causing increased human and economic damage has caused additional suffering for the sick, and prolonging their stay in the hospital as well as increasing the cost of treatment [32,33]. Thus, these infections are a reason for the high level of morbidity and increased mortality, like Pseudomonas aeruginosa and Staphylococcus aureus are at the forefront of the bacteria that cause these infections [34]. It was found that P. aeruginosa to be a major cause of the burn infections that claimed the lives of many burn patients [35], and bacteria S. aureus ranked the second in burn infections [36], while S. aureus was a major cause of post-infection, followed by P. aeruginosa [37]. The cause of these infections is either endogenous pathogens, which are represented by the normal flora present on the skin, intestine, and respiratory system for people was in the hospital, or exogenous, such as its transmission from medical staff or visitors, or surgical instruments, air, water, food, and floors [38]. As for bacteria S. aureus in the burns infection, the results of this study were agreed with [36] in the burn unit of a teaching hospital in Istanbul, where the percentage of S. aureus was 25%. As for the wounds infection of post-operation, S. aureus was the most isolation by 57% followed by P. aeruginosa of 42.4%, this result was consistent with [37], stated that the main cause of post-operation wound infections was S. aureus, followed by P. aeruginosa.

- **Antibiotic sensitivity test:**

Results show that bacteria S. aureus are resistant to antagonists of Ampicillin, Ceftazidime, Amoxicillin, Cefotaxime, and Choloramphinicol, moreover, bacteria S. aureus showed high sensitivity to Amoxicillin/clavulanic acid, Penicillin G, Gentamicin and Norfloxacin [39]. Studies have shown that the cause of bacterial resistance to different antibacterial is due to several reasons, including a change in the permeability of the cytoplasmic membrane or a change in the target site on which the antagonist works on. In addition to the production of bacteria for β-lactamase enzymes, that makes it resistant to most types of penicillin's [40,41,42]. Studies have shown that the indiscriminate use of antibiotics without completing the treatment period leads to an increase in the resistance problem by bacteria [43]. To treat these diseases, many antibacterial are used, but the increased use of these substances, which is often random and for a long time, has led to the emergence of side effects that harm to the individual health on one hand and the emergence of strains resistant to antibacterial on the other [44,45]. Due to the large random use of most antibiotics, including a β-Lactam antibiotic in hospitals and societies, it is the cause of the emergence of problems of resistant strains, the inefficiency of treatment, and the emergence of strains that were distinguished as being resistant to antibiotics. as shown in Table (2).

Table 2. Bacteria S. aureus resistance to different types of antibiotics

Antibiotics	S. aureus
Ceftazidime (30µg)	R
Amoxicillin /clavulanic acid (30µg)	S
Chloramphenicol (10µg)	R
Penicillin G (10u)	S

Amoxicillin (25µg)	R
Ampicillin (10µg)	R
Cefotaxime (30 µg)	R
Gentamicin (10µg)	S

R: Resistance, S: Sensitive

- **Determination of the inhibitory activity of Biosurfactant produced from S. thermophilus:**

The inhibitory activity of Biosurfactant for bacteria S. thermophilus against pathogenic bacteria S. aureus isolated from various clinical infections was estimated to ensure it has an inhibitory effect. As four different concentrations were taken from Biosurfactant (12.5, 25, 50, 100) mg/ml against isolated S. aureus for different clinical infection to know the inhibition efficacy for each concentration and according to the type of sample. Through the obtained results, the concentration of 100 mg/ml of the Biosurfactant gave the highest average of inhibition zone diameters 26 mm, while the concentration 12.5 mg/ml gave the lowest average of inhibition diameters 12 mm for bacteria S. aureus isolated from wound samples. Followed by burn samples, where the concentration 100 mg/ml gave the highest average of inhibition diameters 24 mm, and the lowest concentration 12.5 mg/ml gave the lowest average of inhibition diameters 10 mm. While the blood samples gave the lowest average of inhibition diameters 20 mm for the concentration of 100 mg/ml and 8 mm and the concentration 12.5 mg/ml, compared to the control treatment, which contains the sterile liquid MRS medium. The results of the statistical analysis indicate the presence of statistically significant differences at the probability level ($P<0.05$) when comparing the inhibition diameters of each of the concentrations used according to the sample type, as shown in Table (3).

Table 3. The average inhibition zone diameters for Biosurfactant produced from bacteria S. Thermophilus against S. aureus isolated from various clinical infection

Sample type	Concentrations				P. value
	12.5 mg\ ml	25 mg\ ml	50 mg\ ml	100 mg\ ml	
	Mean ±S D	Mean ±S D	Mean ±S D	Mean ±S D	
Blood	8.0 ± 1.0a	14.0 ± 2.0b	16.0 ± 1.7b	20.0 ± 2.0c	0.000*
Burns	10.0 ± 2.0a	14.0 ± 1.0b	18.0 ± 2.0c	24.0 ± 2.0d	0.000*
Wounds	14.0 ± 1.0a	18.0 ± 2.0b	22.0 ±2.0c	28.0 ± 2.0d	0.000*

* Similar small letters indicate that there were no significant differences at the probability level $p \leq 0.05$ between single-row groups
*Various small letters indicate significant differences at the probability level $p \leq 0.05$ between single row groups

The above results indicated that the increase in the inhibitory activity of Biosurfactant increases when its concentration increase and this study is consistent with [46,47]. As the inhibitory activity of lactic acid filtrates increases clearly when the concentration increases and this study is consistent with increasing the inhibitory activity of the Biosurfactant produced from bacteria Bifidobacterium spp. against the gram-positive and negative bacteria and the yeasts with increasing its concentration [48]. It also agrees with the opinions of researchers about the inhibitory activity of S. thermophilus bacteria, where [3] indicated that S. thermophilus bacteria have an inhibitory effect against E. coli, P. fluorescens, Klebsiella pneumonia, and S. aureus. [11] indicated the ability of bacteria S. thermophilus on inhibition of the gram-positive and negative pathogenic bacteria, it was observed from the study results that the Biosurfactant that produced from the S. thermophilus isolated from the local Iraqi white cheese has the inhibitory activity against all pathogenic bacteria under study due to it contains inhibitory material.

- **Effect of Biosurfactant produced from S. thermophilus on some physiological parameters in white rat's male:**

The results of the statistical analysis in Table (4) showed a significant increase at the probability level ($P < 0.05$) in the average number of RBC, Hb concentration, Packed cell volume PCV in the treatment group compared with the control group. Finally, the increase in the animals treated with the Biosurfactant extracted from the lactic acid bacteria can be attributed to the improvements in digestion and absorption accompanied by increased red blood cells.

Table 4. Effect of Biosurfactant produced by S. thermophiles on some physiological parameters in white rat's male:

Test	Group	Mean	Std. Deviation	P-value
RBC 106 \ ml	C	6.15	0.30	0.000**
	TI	7.86	0.45	
Hb (g\d)	C	14.11	0.67	0.000**
	TI	16.89	0.62	
PCV (%)	C	42.16	0.93	0.020*
	TI	46.59	1.96	

*significant at $P < 0.05$ **significant at $P < 0.001$

CONCLUSIONS:

It can be concluded from this study the ability of Biosurfactant produced from Streptococcus thermophilus isolated from local cheese to eliminate the Staphylococcus aureus isolated from Wounds, Burns, and Blood and its effect on the physiological parameters of white rat's male.

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REFERENCES:

1. Hardie, J.M, and Whiley, R.A. (1995). The Genus Streptococcus. In: The Genera of Lactic Acid Bacteria. Edited by Wood, B.J. and Holzapfel, W.H.
2. Robinson, R. K., Tamime, A. Y. And Wszolek, M. (2002). Microbiology of Ferment Milks. In: Dairy Microbiology Handbook, 3th ed, edited by Robinson, R. K. Wiley – Interscience, inc.
3. Akpinar, A., Yerlikaya, O., and Kiliç, S. (2011). Antimicrobial activity and antibiotic resistance of Lactobacillus delbrueckii ssp. Bulgaricus and Streptococcus thermophilus strains isolated from Turkish homemade yoghurts. African J. of Microbiol Res.
4. Šušković J, Kos B, Beganović J, Leboš Pavunc, A, Habjanič K, Matošić S. (2010). Antimicrobial activity - The most important property of probiotic and starter lactic acid bacteria. Food Technol. Biotechnol. 48(3): 296-307.
5. Vuyst, L. De, and Leroy, F. (2010) Bacteriocins from Lactic Acid Bacteria: Production, Purification, and Food Applications J Mol Microbiol Biotechnol.13:194–199.
6. Ukeyima, M. T., Enujiugha, V. N., and Sanni, T. A. (2010). Current applications of probiotic foods in Africa. African J. Of Biotechnol. 9 (4): 394-401.
7. Petti S, Tarsitani G. And Simonetti D'Arca A. (2008). Antibacterial activity of yoghurt against viridans streptococci in vitro. Arch. Oral Biol. 53: 985-990.
8. FAO and WHO. (2002). Guidelines for the Evaluation of Probiotics in Food Drafting Guidelines for the Evaluation of Probiotics in Food London Ontario, Canada.
9. Parente, E. And Cogan, T. M. (2004). Starter cultures: general aspects. In Cheese, Chemistry, Physics, and Microbiology, Third Edition ed. Fox, P. F., mcsweeney, P. L. H., Cogan, T. M. And Guinee, T. P. Pp. 123–148. London: Elsevier Academic Press.
10. Delcour, J., Ferain, T., and Hols, P. (1996). "Advances in the genetics of thermophilic lactic acid bacteria". Curropinbiotechnol 70, 497–504.
11. Nurhajati, J., Chrysanti, I., Indrawati, I. and Syaftika, N. (2008). Antibacterial Activity of L. Bulgaricus and S. Thermophilus Soygurt Cultures., Proc ASEAN Congr Trop Med Parasitol. 3:51-8.
12. Aslam, M., Shahid, M., Rehman, F. U., Naveed, N. H., Batool, A. I., Sharif, S. And Asia, A. (2011). Purification and characterization of bacteriocin isolated from Streptococcus thermophilus. African J. Of Microbiol. Resear. 5(18): 2642-2648.
13. Hassam, A. N, and Frank, J.F. (2001). Starter cultures and their use In Applied Dairy Microbiology. 2ed Edit by Marth, E . Hand steele, L. J, Marcel Dekker, In c.U.S. A.
14. Rodrigues, L., Van Der Mei, H., Banat, I.M., Teixeira, J. and Oliveira, R. (2006). Inhibition of microbial adhesion to silicone rubber treated with biosurfactant from Streptococcus thermophilus, FEMS Immunology & Medical Microbiol. 46(1):107-112.
15. Rodrigues, L.R., Jos´e, A., Henny, C., van der M. And Ros´ario O. (2006). Isolation and partial characterization of a biosurfactant produced by Streptococcus thermophilus. Biointerfaces. 53: 105–112.
16. Ifeanyichukwu, I . ; chika, E.; Emmanuel , N . , Anthonia , O. ; Ngozi, A. And Esther, U . I . (2015). Community-acquired methicillin-resistant Staphylococcus aureus (Ca – MRSA) carriage amongst tertiary School Students. American Journal of Science and Technology, 2 (1) : 18 – 21.
17. Tong, S. ; Chen , L. ; and Fowler , V. (2012). Colonization Pathogenicity, Host Susceptibility and Therapeutics for Staphylococcus aureus: what is the Clinical Relevance. Semin . Immunopathol. 34(2) : 185 – 200.
18. Janstova , B . ; Necidova , L . and Janstova , B . (2012) . Comparing the growth of S . aureus and Production of staphylococcal enterotoxin C in Sheep's and goats milk . J . Microbiology. Biotechuology and food Sciences. I (February special issue) : 758 – 768.
19. MCGrath, E. J ; and Asmar , B. I. (2011). Nosocomial infection and efflux pump contributes to the reduced Susceptibility of Laboratory – derived Staphylococcus aureus mutants to tigecysscline. Antimicrob Agents chemother. 49:65-71 .
20. Shakibaie , M.R. ; Golkari , X. and Salajegheh , Gh. (2014). Antimicrobial Susceptibility, Virulence factors and biofilm formation among Staphylococcus aureus isolate from hospital infections in Kerman, Iran. Journal of Microbiology and Infection Diseases 4(4) : 152 – 158 .
21. KLOSS, W.E. (1990). Systematic and the natural history of Staphylococci I.J APPl . Bacterial symp.suool 69.252.
22. Jenssen,w.D.S. ,thakker-varia ,D. T. ,Dubin, and M. p.,Weinstein. (1987). prevalence of macrolides-lincosamides – streptogramin B resistance and ermgeneclass among clinical strains of Staphylocci and Streptococci . Antimicrob . Agents chemotherapy.
23. Kloos, W, E.and T.L Bannerman (1994). Update on the clinical significance of coagulase-negative Staphylococci. clin microbial Rev7:117- 140
24. Forbes, B.A., Saham, D.F, and weissfeld, A.S. (2002). Diagnostic Microbiology. 10thed. Mosby. Inc. U.S.A.
25. Vandepitte, J.; Verhaegen, J.; Engbaek, K.; Rohner, P.; Piot, P. and Heuck, C. C. (2003). Basic laboratory procedures in clinical Bacteriology. 2nd ed. World Health Organization Geneva. PP. 109-120.
26. CLSI (2012). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement. CLSI document M 100-S22. Wayne, PA: Clinical and Laboratory Standards Institute.
27. Gupta, U., Radramma, Rati, E.R. and Joseph, R. (1998). Nutritional Quality of lactic acid fermented bitter gourd and fenugreek leaves. Int. J. Food Sci. And Nutr. 94(2):101108.
28. Coles, E. H. (1980). Veterinary Clinical Pathology. 3ed ed. W. B. Sanders. Co. Philadelphia. Pp: 190- 192.
29. Hillman, R. S., and Ault, K. A. (2002). Hematology in Clinical Practice. 3rd ed., McGraw-Hill Co, New York
30. Seltman, H. J. (2012) 'Experimental design and analysis', Online at: http://www stat. Cmu. Edu/, hseltman/309/Book/Book. Pdf.
31. Morello, J.K.; Mizer, H. E and Granato, P.A. (2006). Laboratory Manual And work Book in Microbiology Application To Patient Care. 8thed. Mc Graw Hill.
32. Malik, A.; Hasani, S.E.; Shahid, M.; Khan, H.M. and Ahmad, A.J. (2003). Nosocomial Klebsiella infection in neonates in a tertiary care hospital: protein profile by SDS – page and klebocin typing as epidemiological markers. Indian Journal of Medical Microbiology. 21(2) : 82-86.
33. Schwaber, M.J.; Cosgrove, S.E.; Gold, H.S.; Kaye, S. and Carmeli, Y. (2004). Fluoroquinolones protective against cephalosporin resistance in gram-negative nosocomial pathogens. Centers for Disease Control and prevention. 10(1):1-11
34. Jain, A., and Singh, K. (2007). Recent advances in the management of nosocomial infections. JK Science (9) 1:3-8.
35. Rastegar, L.A.R.; Alaghehbandan, R. and Akhlaghi, L. (2005). Burn wound infections and antimicrobial resistance in Tehran, Iran: an increasing problem. Annals of Burns and Fire Disasters. XVIII (2): 1-9.
36. Oncul, O.; Yüksel, F.; Altunay, H.; Acikel, C.; Celikoz, B. and Cavuslu, S. (2002). The evaluation of nosocomial

infection during 1-year – a period in the burn unit of a training hospital in Istanbul, Turkey. Burns. 28:738-744.

37. Isibor, J.O.; Oseni, A.; Eyaufe, A.; Osagie, R. and Turay, A. (2008). Incidence of aerobic bacteria and Candida albi cans in postoperative wound infections. African Journal of Microbiology Research. 2:288-291.
38. Prescott, L.M.; Harley, J.P., and Klein, D.A. (2005). Microbiology. (6th ed .) McGraw – Hill. U.S.A.
39. Wilson, C. L. & Droby, G.G. (2000). Microbial food contamination. Boca Raton, FL. USA: CRC Press.
40. Parveen, S.S., and Thyothsna, K. (2011). Methicillin resistance among isolates of Staphylococcus aureus antibiotic sensitivity pattern and phage typing.Ann. Biol. Res., 2(4): 57-61
41. Chamber, H.F. (1997). Methicillin resistance in staphylococci genetics and mechanisms of resistance. Clin. Microbiol. Rev. 10: 781 – 791.
42. Cross, J.T., and Campbell, G. (1999). Drug – resistance pathogen in the community in hospital-acquired Pneumonia. Clin. Chest. Med., 20 (3): 499-506.
43. Heffernan, H. ; Bakker, S. (2011). Annual survey of methicillin-resistant Staphylococcus aureus (MRSA). Institute of environmental science & research Ltd (ESR) ; Wellington .2013.
44. Lai, S.; Themblay, J. & Deziel, E. (2009). Swarming motility a multicellular behavior conferring antimicrobial resistance. Environmental microbiology. 11:126-136.
45. SCENIHR (Scientific Committee on Emerging and New Identified Health Risk) (2010). A research strategy to address the knowledge gaps on the antimicrobial resistance effects of biocides, 17 march 2010.
46. Sreekumar, O., and Hosono, A. (2000). "Immediate effect of Lactobacillus acidophilus on the intestinal flora and fecal enzymes of rats and the in vitro inhibition of Escherichia coli in coculture " J.Dairy Sci. 83:931- 939.
47. Almjalawi B. S. A; (2016). Study of the Effectiveness of Biosurfactant Extracted from Bacteria Bifidobacterium spp. In the Inhibition of Biofilm of Pathogens Isolated from Cardiac Catheterization Patients and Its Effect in Phagocytosis, Thesis. College of Education for Pure Sciences Department of Biology, University of Karbala.
48. Almjalawi, B. S. A., Al-Awade, H. A. R. K., Al-Hamil, A. R. H., Study the Inhibition Activity of Bifidobacterium Spp. Filtrate Against Some Pathogenic Bacteria Isolated from Patients with Cardiac Catheterization In-Vitro. International Journal of Research and Development in Pharmacy & Life Sciences (Ijrdpl), 5, 2099-2106.