Al-Nahrain University

College of Science

Department of Biotechnology

Evaluating the Effects of Probiotics and Vinegars on Bacteria Isolated from Burns and Wounds Infections

A Thesis

Submitted to the College of Science / Al-Nahrain University as a partial fulfillment of the requirements for the Degree of Master of Science in Biotechnology

By

MUSTAFA ATTIYAH HADID

B.Sc. Biotechnology / College of Science / Al-Nahrain University (2013)

Supervised by

Dr. Abdulwahid B. Al-Shaibani

(Professor)

December 2015 Safer 1437

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا
عَلَّمْتَنَا إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ

صَدَقَ اللهُ العَظِيم

سورة البقرة الآية ﴿٣٢﴾

Supervisor (s) Certification

I, certify that this thesis entitled **"Evaluating the Effects of Probiotics and Vinegars on Bacteria Isolated from Burns and Wounds Infections"** was prepared by **"Mustafa Attiyah Hadid"** under my supervision at the College of Science / Al-Nahrain University as a partial fulfillment of the requirements for the degree of Master of Science in Biotechnology.

Signature:

Name: **Dr. Abdulwahid B. Al-Shaibani**

Scientific Degree: **Professor**

Date:

In view of available recommendation, I forward this thesis for debate by examining committee.

Signature:

Name: **Dr. Hameed M. Jasim**

Scientific Degree: **Professor**

Title: **Head of Biotechnology Department**

Date:

Committee Certification

We, the examining committee certify that we have read this thesis entitled **"Evaluating the Effects of Probiotics and Vinegars on Bacteria Isolated from Burns and Wounds Infections"** and examined the student **"Mustafa Attiyah Hadid"** in its contents and that in our opinion, it is accepted for the Degree of Master of Science in Biotechnology.

Signature:

Name: **Dr. Saleh A. Wohaieb**

Scientific Degree: **Professor**

Date:

(Chairman)

Signature:

Name: **Dr. Jehan Abdul Sattar**

Scientific Degree: **Professor**

Date:

(Member)

Signature:

Name: **Dr. Zaid Akram Thabit**

Scientific Degree: **Lecturer**

Date:

(Member)

Signature:

Name: **Dr. Abdulwahid B. Al-Shaibani**

Scientific Degree: **Professor**

Date:

(Member/ **Supervisor**)

I, herby certify upon the decision of the examining committee.

Signature:

Name: **Dr. Hadi M. A. Abood**

Scientific Degree: **Assistant Professor**

Title: **Dean of College of Science**

Date:

Dedication

To the spirit of my father

Mustafa

Acknowledgment

At the beginning, Praise to great Allah the Lord of the Universe who gave me the faith and strength to accomplish this work.

The Prayers and the peace are upon our Prophet the master of all the creatures Mohammad, and his relative and companions.

Special thanks with the respect and appreciation to my supervisor Prof. Dr. Abdulwahid B. Al-Shaibani for his continuous efforts, guidance, valuable advices and for providing all required facilities to conduct this work.

I would like also to thank Prof. Dr. surgeon Sadeq Al-Mukhtar for his useful appreciable advices, help and support among most part of the work.

Grateful thanks are conveyed to the head and staff of Biotechnology Department / College of Science / Al-Nahrain University especially assistant instructors Rana Adnan and Nora Adel for their help and assistance throughout the study.

Deep thanks are devoted to Mr. Abdulkhaleq Abbas head of the Food Control Department at the "Central Public Health Laboratory" of Ministry of Health, and his staff especially Dr. Hayder Shannoon for all kinds of assistance in analyzing various samples for microbiological evaluation.

I am thankful to Dr. Waleed Al-Azawi for his help in data statistical analysis.

I feel that I am deeply thankful to my friends and colleagues Hanan, Yasameen, Mohammad, Hani, Mahmood, Rasool, Husham and Mohammad.

Finally, I am sincerely grateful to all my family, especially my mother, for their kindness, patience and support throughout this study.

Mustafa

Summary

This study aimed to isolate bacterial causative of burn and wound infections and treat them by vinegars and probiotics. For such purpose, a total of 58 swab samples were obtained from patients of both gender and various ages who referred to two teaching hospitals in Baghdad suffering from burn and wound infections of different severity and location of injuries in their body. Only 43 (74.2%) of the samples were positive for giving bacterial growth.

The samples were cultured on MacConkey agar and blood agar as initial step for cultivation of bacteria. Results showed that 64 bacterial isolates were obtained.

After identification by cultural, microscopic, biochemical characterizations and Vitek 2 system they were found to be belonging to Gram positive and negative bacteria of the following 10 species: *Pseudomonas aeruginosa* (28), *Staphylococcus aureus* (12), *Klebsiella pneumoniae* (10), *Staphylococcus epidermidis* (6), *Escherichia coli* (3), *Pseudomonas putida* (1), *Pseudomonas alcaligenes* (1), *Enterobacter cloacae* (1), *Acinetobacter baumannii* (1), and *Stenotrophomonas maltophilia* (1).

After subjection of these isolates to the susceptibility test toward ten of the more abundantly-used antibiotics as well as against two antibiotics that are given to the patients in the hospital where the samples had been collected, results declared that amikacin and imipenem were the most effective antibiotics against both Gram positive and negative bacteria. Among the above-mentioned pathogenic isolates, *Pseudomonas* spp were the most resistance species followed by *Enterobacter cloacae* and *Acinetobacter baumannii*.

As probiotic, two types were used *Lactobacillus reuteri* alone and mixture of (*Lactobacillus reuteri*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus* and *Bifidobacterium longum*) propagated, independently, in MRS medium. Then their fermentation products were concentrated and tested for inhibitory activity against burn and wound infections bacterial isolates.

Results showed that only the three-fold concentrated fermentation product of *Lactobacillus reuteri* possessed observable activity, while other probiotic fermentation product did not do so.

When various types of apple vinegar were used as antibacterial agents against the bacterial isolates causing burn and wound infections, the obtained results revealed great ability for these vinegars in eliminating the pathogenic bacteria.

Distinctly, the chemically-synthesized type apple vinegar gave the highest antibacterial activity followed by the commercial apple type then the homemade apple vinegar.

Upon using combinations of *Lb. reuteri* three-fold concentrated fermentation product and apple vinegar in different ratios, results showed that synergistic effect was recorded in most of the ratios with the exception of the effect against *Acinetobacter baumannii* when the vinegar alone gave better effect. However, highest antibacterial activity against pathogenic bacterial isolates was recorded by the ratio of 1:3 *Lb. reuteri* three-fold concentrated fermentation product to chemically-synthesized apple vinegar.

List of Contents

Chapter One: Introduction and Literature Review

Chapter Two: Materials and Methods

Chapter Three: Results and Discussion

Chapter Four: Conclusions and Recommendations

List of Tables

List of Figures

3.7	Inhibition zone against *P. aeruginosa* isolated from burn wound caused by A) *Lb. reuteri* three-fold concentrated fermentation product and chemically synthesized apple vinegar at ratio 1:3 B) chemically synthesized apple vinegar alone C) *Lb. reuteri* three-fold concentrated fermentation product alone	60

List of Abbreviations

Abbreviation	**Meaning**
CoNS	Coagulase-negative *Staphylococci*
MRSA	Methicillin-resistant *Staphylococcus aureus*
MRS	de Man, Rogosa and Sharpe
TSI	Triple sugar iron
V_E	Commercial apple vinegar
V_H	Homemade apple vinegar
V_C	Chemically synthesized apple vinegar

Chapter One

Introduction and Literature Review

1. Introduction and Literature Review

1.1 Introduction:

More than 200 different species of bacteria normally live on the skin (Benbow, 2010) and an open wound provides a moist, warm and nutritious environment perfect for microbial colonization and proliferation (Young, 2012).

When one or more microorganisms multiply in the wound, local and systemic responses occur in the host, which can lead to infection and a subsequent delay in healing (Angel *et al*., 2011).

Cutting (2010) stated that maintaining the bacteria at a level at which the host is in control is an important part of avoiding wound infection.

In 2001, Bowler *et al*. found that when chronic wounds are poorly perfused they are more susceptible to infection, as blood delivers oxygen, nutrients and immune cells, thus providing little opportunity for microorganisms to colonize and proliferate.

Burn wounds are a major focus for infection, as they become readily colonized with several species of potentially pathogenic microorganisms, including *Pseudomonas aeruginosa* and *Staphylococcus* sp. (Mayhall, 2003).

Systemic sepsis resulting from invasive infection remains the leading cause of death among thermally injured patients (Martineau and Dosch, 2007).

The skin forms a protective barrier against invasion by bacteria, fungi and viruses and any breach in this barrier provides easy access for microbial invasion (Liwimbi and Komolafe, 2007).

Intact skin is the perfect defense to bacterial invasion, but damage to the skin allows bacteria, fungi and yeasts to enter (Young, 2012).

Evidence supporting the presence of biofilms on the surface of chronic human wounds and burns has been reported (Rashid *et al*. 2000).

Mah and O'Toole (2001) declared that biofilms are harder to eradicate than their planktonic counterparts, as sessile bacteria more effectively resist adverse

environments by forming aggregates, adapting phenotypes, and/ or generating metabolic changes to evade a hostile milieu and host immune responses.

Bjarnsholt (2013) stated that where the bacteria succeed in forming a biofilm within the human host, the infection often turns out to be untreatable and will develop into a chronic state.

Probiotics used as a term to describe the use of live microorganisms as food supplements improving the intestinal microbial balance of the host (Salminen *et al*., 1999).

The use of probiotics as an alternative therapy for treatment and prevention of bacterial infections has been reported (Bomba *et al*., 2006).

Gorbach (2000) pointed out there is a growing interest in probiotics as a safe therapeutic agent through their ability to alleviate food allergies, enhance nonspecific and specific immune responses, suppress intestinal infections, and anti-carcinogenic activity (Grajek *et al*., 2005).

According to Doron and Gorbach, (2006), probiotics possess many mechanisms to exert their beneficial effects; they prevent colonization, cellular adhesion, invasion by pathogenic organisms, they have antimicrobial activity, and they modulate the host immune response.

Vinegar is a sour liquid comprised mainly of acetic acid, typically 4-18% acetic acid by mass, which is prepared in households by the fermentation of many fruits, the solution is also commercially available. It is cheap and easily found in markets (Ismael, 2013).

Vinegar has been shown to be effective in the prevention and control of microbial contamination in intra-canal treatment of apical periodontitis in teeth (Estrela *et al*., 2004).

Due to the importance and wide spread of burn wound infections caused by pathogenic bacteria, this study was designed for the aim of comparing the antimicrobial effect of various types of apple vinegar, probiotics or their

combination in order to select and apply the most efficient treatment. To achieve this such aim, the following steps had been used:

- Isolation and identification of pathogenic bacteria causing burn and wound infections.
- Selecting some types of probiotic bacteria or their products.
- Application of probiotics fermentation product against infective bacteria causing burn and wound infections.
- Application of various types of apple vinegar.

1.2 Literature Review:

1.2 .1 Burns and wounds:

An effective management of wounds, especially chronic wounds, in the health care setting can have an impact in the population health, reducing morbidity and improving function and quality of life. Wounds presented by patients vary from one setting to another, ranging from acute surgical wounds, traumatic wounds (such as those that occur following an accident), burn wounds or chronic wounds (such as diabetic foot) (Bessa *et al*., 2015).

Wounds are contaminated with microorganisms that are part of the saprophytic microflora of the skin and the type and quantity of these microorganisms vary from one wound to another (Cooper and Lawrence, 1996).

Origin, body location, size and duration of the wound, are considered as important factors that should be taken into account in the wound management because of their impact on wound colonization and infection (White *et al*., 2001).

In 2004, Edwards and Harding described non-healing wounds as biologically characterized by prolonged inflammation, defective reepithelialization, and impaired matrix remodeling. In addition, in all chronic wounds there is an interaction between patient and the bacteria present in the wound.

Burns are one of the most common and devastating forms of trauma. Patients with serious thermal injury require immediate specialized care in order to minimize morbidity and mortality (Church *et al*., 2006).

Abston *et al.* (2000) defined burn 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.

Burn wounds are a suitable site for multiplication of bacteria and are more persistent richer sources of infection than surgical wounds, mainly because of the larger area involved and longer duration of patient stay in the hospital (Agnihotri *et al.,* 2004).

Infection is a major cause of morbidity and mortality in hospitalized burn patients (Manus *et al*., 1994).

In 2010, Keen *et al*., illustrated that use of topical and systemic antimicrobials and enhanced infection control practices have replaced β-hemolytic streptococci with *S aureus* and gram-negative pathogens such as *P. aeruginosa*, *Klebsiella pneumoniae*, and *A. baumannii* a s major pathogens in burn wound infections.

Wound infection by antibiotic resistant organisms such as *Pseudomonas aeruginosa*, *Acinetobacter* spp and *Klebsiella* spp should be considered as a potential risk, and their sensitivity pattern should be identified. The existence of hospital pathogens and multiple drug resistant (MDR) organisms in burn wards must be defined (Greenhalgh *et al*., 2007).

As a result of significant improvement of surgical treatments and intensive care in burn wards, it seems that infection is the direct cause of nearly 75% of deaths following burn (Agnihotri *et al*., 2004).

The rate of nosocomial infections is higher in burn patients due to various factors like nature of burn injury itself, immunocompromised status of the patient (Pruitt *et al*., 1998), age of the patient, extent of injury, and depth of burn in combination with microbial factors such as type and number of organisms, enzyme and toxin production, colonization of the burn wound site, systemic dissemination of the colonizing organisms (Pruitt *et al*., 1984).

Magnet *et al.* (2013) reported that the larger area of tissue is exposed for a longer time that renders patients prone to invasive bacterial sepsis. In extensive burns when the organisms proliferate in the eschar, and when the density exceeds 100,000 organisms per gram of tissues, they spread to the blood and cause a lethal bacteremia. Therapy of burn wound infections is therefore aimed at keeping the organism's burden below 100,000 per gram of tissues which increases the chances of successful skin grafting.

The denatured protein of the burn eschar provides nutrition for the organisms. A vascularity of the burned tissue places the organisms beyond the reach of host defense mechanisms and systemically administered antibiotics (Order *et al*., 1965). In addition, cross-infection results between different burn patients due to overcrowding in burn wards (Gupta *et al*., 1993).

Also thermal destruction of the skin barrier and concomitant depression of local and systemic host cellular and humeral immune responses are pivotal factors contributing to infectious complication in patients with severe burn (Luterman *et al*., 1986).

Burn wound infections are largely hospital acquired and the infecting pathogens differ from one hospital to another (Ogunsola *et al*., 1998).

The burn wound represents a susceptible site for opportunistic colonization by organisms of endogenous and exogenous origin; 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 (Mooney and Gamelli, 1989).

Monafo and Freedman (1987) declared that burn wound surfaces are sterile immediately following thermal injury; 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.

Topical antimicrobials decrease microbial overgrowth but seldom prevent further colonization with other potentially invasive bacteria and fungi (Monafo and Freedman, 1987).

These are derived from the patient's gastrointestinal and upper respiratory tract and the hospital environment (Hansbrough, 1987). Following colonization, these organisms start penetrating the viable tissue depending on their invasive capacity, local wound factors and the degree of the patient's immunosuppression (Manson *et al*., 1992).

If sub-eschar tissue is invaded, disseminated infection is likely to occur, and the causative infective microorganisms in any burn facility change with time (Forbes *et al*., 1998).

Individual organisms are brought into the burns ward on the wounds of new patients. These organisms then persist in the resident flora of the burn treatment facility for a variable period of time, only to be replaced by newly arriving microorganisms (Magnet *et al*., 2013).

Factors that are associated with improved outcome and prevention of infection likely include early burn eschar excision, topical and prophylactic antibiotics, and aggressive infection-control measures (Kasten *et al*,.2011).

Introduction of new topical agents and systemic antibiotics influence the flora of the wound (National Committee for Clinical Laboratory Standards, 1993).

1.2.2 Human skin:

Skin is one of the largest organs in the human body in terms of size and weight. The average adult skin surface area is 1.5 to 2.0 square meters. An intact human skin surface is vital to the preservation of body fluid homeostasis, thermoregulation, and the host's protection against infection. The skin also has immunological, neurosensory, and metabolic functions such as vitamin D metabolism (Church *et al.*, 2006).

The skin is derived from ectoderm and mesoderm and has two anatomic layers: the epidermis or outermost nonvascular layer consists of several layers of epidermal cells that vary in thickness over various body surfaces, and the dermis or corium is largely made of collagen and contains the microcirculation, a complex vascular plexus of arterioles, venules, and capillaries. The two skin layers are bound together by a complex mechanism that is essential for normal function (Church *et al*., 2006).

Figure 1.1 provides a schematic representation of the skin layers in relation to the depth of burn injury (Roth and Hughes, 2004).

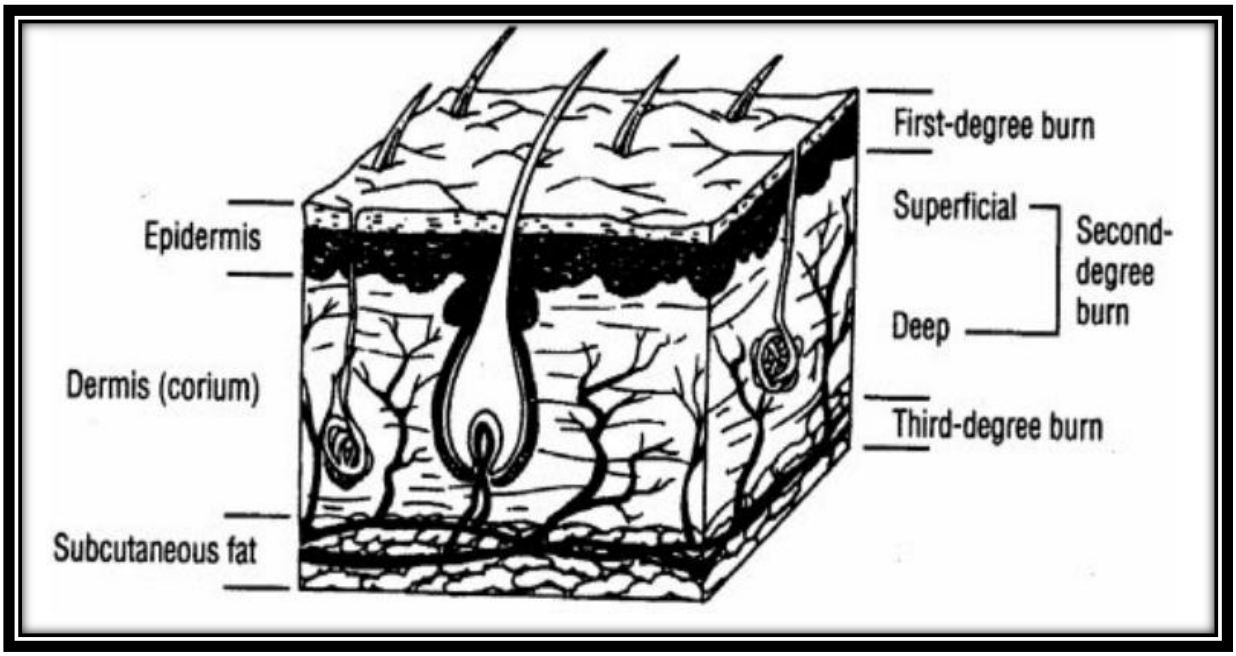

Figure (1.1): Basic skin anatomy, showing the depth of injury for first-, second-, and third-degree burns (Roth and Hughes, 2004).

In 2011, Al-Muhammadi and Azeez stated that burns are classified according to increasing depth as epidermal, first-degree; superficial and deep partial-thickness, second degree; full-thickness third-degree; and full thickness with underlying structure fourth-degree.

Thermal injury creates a breach in the surface of the skin. A basic knowledge of skin anatomy and physiology is required to understand emergency burn assessment and approaches to burn care (Wysocki 2002).

Thermal burns are caused by intense external sources of heat, such as flames, scalding liquids, or steam. Burns resulting from an impaired driving crash are most likely thermal burns.

As the body the first line of defense, the skin is continuously subjected to potentially harmful environmental agents, including solid matter, liquids, gases, sunlight, and microorganisms. The skin also serves as immunological barrier (Abston *et al*., 2000).

Al-Muhammadi and Azeez (2011) declared that burns are the only truly quantifiable form of trauma, and 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. They added that the effects of thermal injury are both local and systemic infections. The term systemic inflammatory response syndrome (SIRS) summarizes these conditions. SIRS with infection (i.e. sepsis syndrome) is a major factor determining morbidity and mortality in thermally injured patients.

Yang *et al*. (2007) stated that response to thermal injury includes cellular protection mechanisms, inflammation, hypermetabolism, prolonged catabolism, organ dysfunction and immunosuppression.

In 2006, Church *et al.* illustrated that the single most important factor in predicting burn-related mortality, need for specialized care, and the type and likelihood of complications is the overall size of the burn as a proportion of the patient's total body surface area (TBSA).

Severe burn injury is characterized by a marked hypermetabolic response and hypermetabolism and even more markedly by loss of lean body mass. This hypermetabolic response is accompanied by a progressive decline of host defenses via immunological abnormalities (Marvaki *et al*., 2001).

1.2.3 Microbial infections of burn and wound:

Wound infections can be defined as the presence of replicating organisms within a wound with subsequent host injury (Dow *et al*., 1999).

Microbial colonization of wounds is characterized by the presence of multiplying microorganisms on the surface of a wound, but with no immune response from the host (Edwards and Harding, 2004) and with no associated clinical signs and symptoms (Bessa *et al*., 2015).

Microorganisms, such as bacteria, rapidly colonize open skin wounds after burn injury. Such colonization originates from the patient’s endogenous skin and gastrointestinal and respiratory flora (Barret and Herndon 2003).

Microorganisms may also be transferred to a patient’s skin surface via contact with contaminated external environmental surfaces, water, clothes, air, and the soiled hands of health care workers (Weber *et al*., 1997).

Immediately following injury, Gram-positive bacteria from the patient's endogenous skin flora or the external environment predominantly colonize the burn wound (Barret and Herndon 2003).

Endogenous Gram-negative bacteria from the patient's gastrointestinal flora also rapidly colonize the burn wound surface in the first few days after injury (Ramzy *et al*., 2000).

Microorganisms transmitted from the hospital environment tend to be more resistant to antimicrobial agents than those originating from the patient's normal flora (Clark *et al*., 2003).

Church *et al*. (2006) pointed out that table (1.1), bacteria and fungi are the most common pathogens of burn wounds. These microbes form multi-species biofilms on burn wounds within 48 – 72 h of injury. Moreover, Gram-positive bacteria are some of the first to colonize burns, followed quickly by gram-negative. Fungal infection tends to occur in the later stages after the majority of bacteria have been eliminated by topical antibiotics. They listed in table (1.2) the microorganisms that originate from the patient's own skin, gut and respiratory flora, as well as from contact with contaminated health care environments and workers.

Table 1.1: Microorganisms causing invasive burn wound infection (Church *et al.*, 2006).

Group	Species
Gram-positive organisms	*Staphylococcus aureus* Methicillin-resistant *S. aureus* Coagulase-negative staphylococci *Enterococcus* spp. Vancomycin-resistant enterococci
Gram-negative organisms	*Pseudomonas aeruginosa*

	Escherichia coli *Klebsiella pneumoniae* *Serratia marcescens* *Enterobacter* spp. *Proteus* spp. *Acinetobacter* spp. *Bacteroides* spp.
Fungi	*Candida* spp. *Aspergillus* spp. *Fusarium* spp. *Alternaria* spp. *Rhizopus* spp. *Mucor* spp.
Viruses	Herpes simplex virus Cytomegalovirus Varicella-zoster virus

Prior to the antibiotic era, *Streptococcus pyogenes* (group A beta-hemolytic streptococci) was the predominant pathogen implicated in burn wound infections and was a major cause of death in severely burned patients (Bang *et al*., 1999).

Lilly *et al.* in 1979 reported that *Staphylococcus aureus* became the principal etiological agent of burn wound infections shortly after the introduction of penicillin G in the early 1950s, which resulted in the virtual elimination of *Streptococcus pyogenes* as a cause of infection in thermally injured patients (Phillips *et al*., 1989).

Methicillin-resistant *Staphylococcus aureus* (MRSA) can be difficult to eradicate because they often can colonize a host for a long time before causing an

infection; until symptoms of infection emerge, MRSA remains undetected and untreated (Zetola *et al*., 2005).

Although *Staphylococcus aureus* remains a common cause of early burn wound infection, *Pseudomonas aeruginosa* from the patient's endogenous gastrointestinal flora and/or an environmental source is the most common cause of burn wound infections in many centers (Altoparlak *et al*., 2004).

The incidence of infections due to less commonly encountered microbes, including other gram-positive and gram-negative bacteria, fungi, and viruses, has also increased steadily in subsequent decades (Dalamaga *et al*., 2003).

The emergence worldwide of antimicrobial resistance among a wide variety of human bacterial and fungal burn wound pathogens, particularly nosocomial isolates, limits the available therapeutic options for effective treatment of burn wound infections (Gales *et al.*, 2001).

MRSA, methicillin-resistant coagulase-negative staphylococci, vancomycin-resistant enterococci, and multiply resistant gram-negative bacteria that possess several types of beta-lactamases, including extended spectrum beta-lactamases, *ampC* beta-lactamases, and metallobeta-lactamases, have been emerging as serious pathogens in hospitalized patients (Embil *et al*., 2001).

1.2.4 Formation of biofilms:

Biofilms are complex communities of surface-attached aggregates of microorganisms embedded in a self-secreted extracellular polysaccharide matrix, or slime. A wide range of natural and artificial environments and provide their constituent microbial cells with a plethora of protected dynamic microenvironments (Stoodley *et al*., 2002).

Once mature, biofilms act as efficient barriers against antimicrobial agents and the host immune system, resulting in persistent colonization and/or infection at the site of biofilms formation (Edwards and Harding, 2004).

In animals with experimentally inflicted partial thickness cutaneous burns, mature biofilms develop in 48 to72 h; while in vitro experiments with

Pseudomonas aeruginosa strains recovered from human burn wounds demonstrate that mature biofilms can form in about 10 h (Harrison-Balestra *et al.*, 2003).

Harrison-Balestra *et al*. (2003) stated that factors delaying the formation of biofilms in vivo may be related to the need for microbial nutrient replenishment, exposure to killing by the immune system, and immediate wound cleansing. Bacteria within a biofilm typically undergo a phenotypic change whereby microbial virulence factor production is altered and metabolic rate and motility are reduced (Edwards and Harding 2004).

Harrison-Balestra *et al.* (2003) suggested channels formed within the protective environment of the biofilm facilitate the transport of nutrients and microbial waste products. Intercellular signaling molecules produced by bacteria within the biofilm are able to traverse these channels and influence the overall growth pattern and behavior of the biofilm in response to various host and environmental factors (Mack *et al*., 2004).

1.2.5 Virulence factors and tissue invasion:

The risk of invasive burn and wound infection is influenced by the extent and depth of the injury, various host factors, and the quantity and virulence of the microbial flora colonizing the wound. Common burn wound pathogens such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* produce a number of virulence factors that are important in the pathogenesis of invasive infection (Church *et al*., 2006).

Pseudomonas aeruginosa produces a number of cell-associated (adhesins, alginate, pili, flagella, and lipopolysaccharide) and extracellular (elastase, exoenzyme S, exotoxin A, hemolysins, iron-binding proteins, leukocidins, and proteases) virulence factors that mediate a number of processes, including adhesion, nutrient acquisition, immune system evasion, leukocyte killing, tissue destruction, and bloodstream invasion (Tredget *et al*., 2004).

Laupland *et al*. (2005) declared that *Pseudomonas aeruginosa* also carries many intrinsic and acquired antimicrobial resistance traits that make infected burn wounds difficult to treat.

In 2004, Foster mentions that *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.

Resistance to methicillin in *Staphylococcus aureus*, and more recently emergence of resistance to glycopeptides and oxazolidinones, also complicate the treatment of burn wound infections and sepsis caused by this highly virulent organism (Meka *et al*., 2004).

1.2.6 Probiotics:

Probiotics is a term derived from the Greek, meaning "for life". Nobel laureate Elie Metchnikoff formulated the probiotic concept approximately 100 years ago. He proposed that consumption of certain 'lactic bacilli' would be beneficial to humans by maximizing health-promoting activities of the gastrointestinal microbiota and minimizing their potentially harmful effects (Metchnikoff, 1907; Casas and Dobrogosz, 2000).

Lilley and Stillwell (1965) defined probiotics as substances secreted by one microorganism to stimulate the growth of another microorganism, as opposite to an antibiotic.

Parker (1974), who described probiotics as organisms and substances which contribute to intestinal microbial balance, was the first to include microorganisms into the definition which is in the sense that it is used today.

Later, Fuller (1989) recognized probiotics as "a live microbial feed supplement which beneficially affects the host (humans or animals) by improving its intestinal microbial balance". Furthermore, the probiotics concept was broadened, as "a viable mono- or mixed- culture of microorganisms which applied to animal or

man, beneficially affects the host by improving the properties of the indigenous microflora" (Havenaar and Huisin't Veld, 1992).

The definition of probiotics continues to be improved, and was made official by the 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" (Corcionivosch *et al*., 2010).

Probiotics may produce their effects with viable as well as nonviable bacteria, suggesting that metabolic or secreted factors or structural or cellular components may mediate their immunomodulatory activities (Brochers *et al*., 2009).

Giahi *et al*. (2012) mentioned that dead bacteria and bacterial molecular components may also exhibit probiotic properties.

Furthermore, "postbiotic" is a term used to define the secreted probiotic-derived compounds that have beneficial effects on the host (Cicenia *et al*., 2014).

In 2013, Fontana *et al*., declared that *Bifidobacterium* and *Lactobacillus* strains are the most widely used probiotic bacteria which exhibited health-promoting properties, such as the maintenance of the gut barrier function and the local and systemic modulation of the host immune system (Collado *et al*., 2009).

A number of health benefits have been claimed for probiotic bacteria and are also being recommended as a preventive approach to maintain their beneficial effects on humans including:

- The balance of intestinal microflora (Shah, 2007).
- Stabilization of intestinal microflora, excluding colonization of enteropathogenic bacteria by adhesion to the intestinal wall and competition for nutrients, (Denev, 2006).
- Reduction of lactose intolerance (de Vrese *et al*., 2001).
- Prevention of antibiotic-induced diarrhoea (Pochapin, 2000).
- Prevention of colon cancer (Wollowski *et al*., 2001).
- Stimulation of the immune system (Isolauri *et al*., 2001).

Probiotic microorganisms should express high tolerance to acid and bile and ability to adhere to intestinal surfaces in order to survive and colonize in the gastro-intestinal tract (GIT). However, *in vivo* testing is expensive, time consuming and requires approval by ethical committees. Hence, reliable *in vitro* methods for selection of promising strains have been used by researchers (Jacobsen *et al*., 1999).

Ammor *et al.* (2007) indicated that probiotics have a unique feature which is their antibiotic resistance expression and transferability as there is a great concern over possible spread of resistance determinants to human pathogenic and opportunistic bacteria.

The application of probiotics provides a potential alternative strategy to the use of antibiotics (Hou *et al*., 2015).

In 2010, Rattanachaikunsopon and Phumkhachorn indicated that *Lactobacilli* are highly competitive largely due to their applications in the production of fermented food. They can also produce antimicrobial substances including bacteriocins that have ability to inhibit pathogenic and food spoilage bacteria. These compounds have shown to exert specific antagonistic properties against Gram-negative and Gram-positive pathogens.

Adhesion of lactic acid bacteria (LAB) to mucosal surfaces has been studied in vitro using Caco-2 cells (Duary *et al*., 2011).

1.2.7 Mechanism of action of probiotics:

The major probiotic mechanisms of action include 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 (Plaza-Diaz *et al*., 2012).

1.2.7.1 Enhancement of the epithelial barrier:

The intestinal barrier is a major defense mechanism used to maintain epithelial integrity and to protect the organism from the environment (Plaza-Diaz *et al*., 2012).

Once this barrier function is disrupted, bacterial and food antigens can reach the submucosa and can induce inflammatory responses, which may result in intestinal disorders, such as inflammatory bowel disease (Sartor, 2006).

Several studies have indicated that enhancing the expression of genes involved in tight junction signaling is a possible mechanism to reinforce intestinal barrier integrity (Anderson *et al*., 2010).

In 2012, Hummel *et al*. found that lactobacilli modulate the regulation of several genes encoding adherence junction proteins, such as E-cadherin and β-catenin, in a T84 cell barrier model. Moreover, incubation of intestinal cells with lactobacilli differentially influences the phosphorylation of adherence junction proteins and the abundance of protein kinase C (PKC) isoforms, such as PKC, thereby positively modulating epithelial barrier function.

1.2.7.2 Adherence and colonization to gut:

Adhesion to intestinal mucosa is regarded as a prerequisite for colonization and is important for the interaction between probiotic strains and the host (Juntunen *et al*., 2001).

Adhesion is also important for modulation of the immune system (Perdigon *et al*., 2002) and antagonism against pathogens (Hirano *et al*., 2003).

As stated by Lin *et al*. (2008), probiotic microorganisms have the ability to adhere to the epithelial cells due to their anti–adhesive effects which block adherence of the pathogens.

These effects which might be due to competitive exclusion for the same receptor by the probiotics and the pathogens, are secretion of proteins that destroy

the receptor, induction of bio-surfactants, establishing a biofilm, and production of receptor analogues (Oelschlaeger, 2010).

1.2.7.3 Competitive exclusion of pathogenic microorganisms:

Competitive exclusion by intestinal bacteria is based on a bacterium-to-bacterium interaction mediated by competition for available nutrients and for mucosal adhesion sites. To gain a competitive advantage, bacteria can also modify their environment to make it less suitable for their competitors (Plaza-Diaz *et al*., 2012).

The production of antimicrobial substances, such as lactic and acetic acid, is one example of this type of environmental modification (Schiffrin and Blum 2002).

Another way of competition is by limiting the resources by probiotic bacteria, it was found by Weinberg (1997) that almost all bacteria need iron as an essential element with the exception of Lactobacillus which didn't need iron in their natural habitat.

In a study performed in Italy, *Lactobacillius acidophilus* and *Lb. delbrueckii* were found to be able to bind ferric hydroxide at their surface making it unavailable to pathogenic microorganisms. This mechanism is of crucial advantage in competition with other microorganisms which depend on iron (Elli *et al*., 2000).

1.2.7.4 Anti–invasive effects of probiotics:

Hess *et al.* (2004) declared that not only adhesion but also invasion of epithelial cells is an important property for full pathogenicity of many gut pathogens; the ability to inhibit bacterial invasion of gut epithelial cells by pathogens is rather wide spread among probiotics.

Number of researchers confirmed that some probiotics (like Lactobacillus and Bifidobacterium strain Bb12) had the ability to secrete factors which interfere

with the invasion of host epithelial cells by *Salmonella typhimurium* (Botes *et al*., 2008).

1.2.7.5 Production of antimicrobial substance:

Organic acids, in particular acetic acid and lactic acid, have a strong inhibitory effect against Gram-negative bacteria, and they have been considered the main antimicrobial compounds responsible for the inhibitory activity of probiotics against pathogens (Alakomi *et al*., 2000; Makras *et al*., 2006).

The undissociated form of the organic acid enters the bacterial cell and dissociates inside its cytoplasm. The eventual lowering of the intracellular pH or the intracellular accumulation of the ionized form of the organic acid can lead to the death of the pathogen (Ouwehand 1998).

In 2009, Wohlgemuth *et al*. reported that lactic acid bacteria including *Lb. plantarum* and *Lb. acidophilus* had the ability to inhibit growth of Gram positive and Gram negative bacteria. This is due to their ability to produce organic acids (lactic acid and acetic acid), hydrogen peroxide, bacteriocin-like substances, and possibly bio-surfactants (Bierbaum and Sahl, 2009).

1.2.7.6 Immunomodulatory effects of probiotics:

Probiotic bacteria can exert an immunomodulatory effect. These bacteria have the ability to interact with epithelial and dendritic cells and with monocytes/macrophages and lymphocytes (Plaza-Diaz *et al*., 2012).

Isolauri *et al.* (1995) mentioned that many probiotic strains were able to stimulate production of immunoglobulin A (IgA) that helps in maintaining humoral immunity of the intestine by binding to the antigen and limiting their access to the epithelium.

1.2.8 *Lactobacillus* as a probiotic:

Lactobacilli are often considered to be commensal or beneficial participants in human microbial ecology and considerable research is being carried out on the effects for the use of lactobacilli as additives in both human and animal diets (Hummel *et al*., 2007).

Shah (2007) mentioned that the probiotic activity of lactic acid bacteria (Lactobacilli, Streptococci and Bifidobacteria) has been emphasized.

1.2.8.1 *Lactobacillus reuteri*:

Lactobacillus reuteri is an obligatorily heterofermentative lactic acid bacteria, a microaerophilic, and is a common inhabitant of the gastrointestinal tract of humans (Kawai *et al*., 2010) and animals such as pigs, turkeys, chickens, and monkeys (Jonsson *et al*., 2001).

Gines *et al.* (2000) approved that some species of *Lb. reuteri* produce the enzyme invertase, which is used in converting sugar from sucrose (Kaplan and Bakir, 1998).

In addition, *Lb. reuteri* produces a large amount of glucan and fructan exopolysaccharides, which are considered probiotics (Hijum *et al*., 2001). Number of researchers investigated these prebiotics with regards to antitumour activity (Roos and Katan, 2000), immunomodulation (Schiffrin *et al*., 1995), and cholesterol reduction (Roberfroid, 1993).

One of the proposed mechanisms of action that *Lb. reuteri* uses is the production of the antimicrobial compound 3-hydroxypropionaldehyde (3-HPA), also referred to as reuterin (Talarico *et al.*, 1988).

Reuterin is produced as an intermediate step in the conversion of glycerol to 1,3- propanediol, a pathway proposed to regenerate NAD+ from NADH and to contribute to improved growth yield (Luthi-Peng *et al.*, 2002).

Mitsouka (1992) estimated *Lb. reuteri* as the most important representative of Lactobacillus microflora in humans and numerous animals. Sarra *et al.* (1979) found out *Lb. reuteri* even as dominant heterofermentative species of Lactobacilli in calves' intestine.

1.2.8.2 *Lactobacillus acidophilus*:

Lactobacillus acidophilus is a well-known and well-studied probiotic microorganism. However, different strains undoubtedly vary in their efficiency and probiotic potentialsm (Ng *et al*., 2009).

1.2.8.3 *Lactobacillus rhamnosus*:

Lactobacillus rhamnosus was identified as a potential probiotic strain because of its resistance to acid and bile, good growth characteristics and adhesion capacity to the intestinal epithelial layer (Doron *et al*., 2005).

1.2.9 *Bifidobacterium longum*:

According to a study, Bifidobacteria constitute over 95% of the intestinal flora in breast-fed infants (Yoshioka *et al*., 1991).

Bifidobacterium is producing lactic acid and acetic acid as the main products of glucose utilization (Ishibashi *et a*l., 1997).

1.2.10 Acetic acid (vinegar):

1.2.10.1 Definition of acetic acid and apple vinegar:

Acetic acid is an organic compound with a chemical formula of CH3CO2H (also written as CH3COOH or C2H4O2). It is a colorless liquid with a distinctive sour taste and pungent smell. It is classified as a weak acid but corrosive when concentrated.

Morales *et al.* (2002) mentioned other constituents of vinegar include vitamins, minerals, salts, amino acids, poly phenolic compounds and nonvolatile organic acids (Natera *et al*., 2003).

Vinegar, from the French *vin aigre*, meaning "sour wine," can be made from almost any fermentable carbohydrate source. Initially, yeasts ferment the natural food sugars to alcohol. Next, acetic acid bacteria (*Acetobacter*) convert the alcohol to acetic acid (Johnston and Gaas 2006).

Apple cider vinegar is a product with increasing interest in the recent years, because of proposed health benefits brought about by a diet containing vinegar, besides its wide culinary usability.

Vinegar is sometimes the result of failed cider fermentation or poor storage conditions for cider (Heikefelt, 2011).

Cider and vinegar are both produced by fermentation. During alcoholic fermentation, yeasts utilize sugar in apple juice to produce ethanol, an anaerobic process that results in cider.

The production of vinegar involves an additional aerobic fermentation step, where acetic acid bacteria convert ethanol in cider into acetic acid (Heikefelt, 2011).

1.2.10.2 Mode of action of vinegar:

In 1973, Leveen declared the mode of action of acetic acid is through the acidification of wound which increases the pO_2 and reduces the histotoxicity of ammonia which may be present. This acidification of a wound is, however, relatively short lived.

1.2.10.3 Vinegar as antimicrobial agent:

As early as in 1916, elimination of *Pseudomonas* in superficial war wounds with the application of 1% acetic acid was reported. Again in 1968, a 5% solution of acetic acid was shown to be effective at eliminating *Pseudomonas aeruginosa* from infected wounds (Hansson and Faergemann, 1995).

Hansson and Faergemann (1995) indicated that gauze dressings soaked with acetic acid were effective in decreasing the number of *Staphylococcus aureus* and Gram- negative rods with patients have venous leg ulcers.

In 1992, Milner mentioned absence of pain or discomfort as adverse effect for using acetic acid, upon using 5% acetic acid in treatment of 9 patients, none of them showed discomfort, two wounds lost *Pseudomonas*, species within 2 days, four within one week, and only one patients had grown bacteria after three weeks. Following eradication of *Pseudomonas*, the wounds were found to heal rapidly.

Sloss *et al.* (1993) proved that *Pseudomonas* cultured can be inhibited by acetic acid in vitro. Drosou *et al.* (2003) found that cytotoxic effects of acetic acid in vitro but clinically no such effects have been found.

Some studies have suggested that it is possible that application of acetic acid may confer other benefits on the healing process as well as the removal of bacteria. When the effect of acetic acid on reepithelization was conducted on animal and human models, no negative impact on wound healing was detected (Kjolseth *et al*., 1994).

Although acetic acid was initially delaying the reepithelization, but after the eighth day, this effect disappeared and tensile wound strength was not influenced (Lineaweaver *et al*., 1985).

Chapter Two

Materials and Methods

2. Materials and Methods:

2.1 Materials:

2.1.1 Apparatus and equipments

The following apparatus and equipments were used in this study:

Table 2.1: Apparatus and equipments

Apparatus or equipment	Company (Origin)
Anaerobic jar	Rodwell (England)
Autoclave	Express (Germany)
Compound light microscope	Olympus (Japan)
Deep-Freeze	GLF (Germany)
Digital balance	Ohans (France)
Incubator	GallenKamp (England)
Laminar air flow	Memmert (Germany)
Magnetic stirrer	GallenKamp (England)
Micropipette	Witey (Germany)
pH-meter	Radiometer (Denmark)
Refrigerator	Concord (France)
UV/VIS spectrophotometer	BUCK (USA)
Vacuum oven	MTI (USA)
Vitek2	Biomereiux (France)
Vortex	Giffin(England)
Water bath	GallenKamp (England)
Water distiller	GLF (Germany)

2.1.2 Biological and chemical materials:

The following chemicals and biological materials were used in this study:

Table 2.2: Biological and chemical materials

Material	Company (Origin)
Ethanol 70%	Locally produced (Iraq)
Peptone	Himedia (India)
Methyl red	
Agar	
NaOH	BDH (England)
NaCl	
HCl	

2.1.3 Therapeutic agents:

The following therapeutic agents were used in this study:

Table 2.3: Therapeutic agents

Agent	Source
Apple vinegar	Locally produced (Iraq) and Imported (Turkey)
Probiotics (*Lactobacillus* spp and *Bifidobacterium longum*)	Nature's way (USA)
Probiotic (*Lactobacillus reuteri*)	BioGaia (Sweden)

2.1.4 Reagents, solution and kit (Ready-to-use):

a) Reagents: (BHD / England)

- KOH, α-naphthol.
- Kovacs.
- Oxidase.
- Catalase.

b) Turbidity standard solution:

- McFarland No. 0.5 (1×10^{8}). (Pro-Lab Diagnostics / Canada).

c) Kit:

- Gram stain (Fluka / Switzerland).
- Vitek 2 system: (bioMérieux / France)

GN ID Card: gram-negative fermenting and non-fermenting.

GP ID Card: Gram-positive cocci and non-spore-forming bacilli.

2.1.5 Antibiotic discs: (Bioanalyse / Turkey):

Table 2.4: Antibiotic discs

Antibiotic	Symbol	Concentration (µg)
Amikacin	AK	30
Amoxicillin	AMX	30
Ampicillin	AP	30
Cefixime	CFM	30
Cefotaxime	CTX	30
Chloramphenicol	C	30
Ciprofloxacin	CIP	30
Clindamycin	DA	30
Erythromycin	E	15
Imipenem	IPM	10
Tetracycline	TE	30
Vancomycin	VA	30

2.1.6 Culture media:

a) Ready-to-use media:

The following media were prepared and sterilized as mentioned on their containers by the manufacturing companies:

Table 2.5: Ready-to-use media

Medium	**Company (Origin)**
Brain heart infusion broth	Difco (USA)
Cetrimide agar	Biolife (Italy)
de Man, Rogosa and Sharpe (MRS) agar	Himedia (India)
de Man, Rogosa and Sharpe (MRS) broth	
Eosin Methylene blue agar (EMB)	
MacConkey agar	
Manitol salt agar	
Triple sugar iron agar	
Muller-Hinton agar	Oxoid (England)
Methyl red – Voges Proskauer (MR-VP) broth	
Stuarts medium	
Nutrient broth	
Simmon's citrate agar	

b) Laboratory-prepared media:

Blood agar and peptone water were freshly prepared and sterilized as will be explained in item (2.2.6).

2.2 Methods

2.2.1 Samples collection and cultivation:

Samples were collected from 58 patients of various ages and genders suffering from burn and wound injuries who referred to Al-Kindy Teaching Hospital and Al-Yarmook Teaching Hospital from November 2014 to February 2015.

The samples were taken from wounds by sterile disposable cotton swabs and kept in the transport medium (Stuart transport medium). They were, then, cultured onto MacConkey agar and blood agar plates before incubating at 37°C for 24 hrs. After incubation, grown bacterial colonies were subjected for identification as illustrated in item (2.2.7).

A special form (Appendix 1) was designed and to be filled with the name, sex, age, date of sampling, degree of injury and previous treatment of each patient.

2.2.2 Sterilizing methods: (Bailey *et al*., 1990)

Two methods of sterilization were used:

a) Moist-heat sterilization (Autoclaving):

Microbial culture media, solutions, and reagents were sterilized by autoclaving at 121°C (15 Ib\ inch²) for 15 min unless otherwise stated.

b) Dry-heat sterilization (oven):

Electric oven was used to sterilize glassware at 180 °C for 3 hrs.

2.2.3 Preparation of solutions:

a) Normal saline:

It was prepared by dissolving 0.85 g NaCl in 100 ml of distilled water and sterilized by autoclaving.

b) Sodium Hydroxide solution (NaOH):

It was prepared by dissolving 0.4 g NaOH (0.1 N) in 100 ml of distilled water.

2.2.4 Probiotic solutions:

Two oral probiotic solutions were used in this study:

a) Liquid probiotic solution:

It was prepared by inoculating 30 drops of the stock oral probiotic solution in 5-7 ml of MRS. For each 5 drops the probiotic solution contains 1×10^8 CFU/ml of *Lactobacillus reuteri* cells as stated by the manufacturer.

b) Tablet probiotic solution:

It was prepared by dissolving one chewable tablet in 5-7 ml of sterilized tap water, then; 1 ml of the solution was transferred to a test tube containing 9 ml of MRS broth. Each tablet contained a mixture of *Lactobacillus acidophilus, Lactobacillus reuteri, Lactobacillus rhamnosus,* and *Bifidobacterium longum* in concentration of 1×10^9 CFU/ml for each species as stated by the manufacturer.

2.2.5 Activation of probiotics:

Probiotic solutions were activated separately by transferring 1 ml of each to 5-7 ml of MRS broth before incubating anaerobically in a candle jar at 37 C° for 2 days for each of *Lactobacillus reuteri* and the mixture.

2.2.6 Preparing fresh media:

a) Blood agar: (Atlas *et al*., 1995).

It was prepared by dissolving 40 g of blood base agar in 1000 ml of distilled water and autoclaved. After cooling to 50°C, 5% of human blood was added, mixed well and distributed into sterilized Petri-dishes.

b) Peptone water: (Mackie and McCartney, 1996).

This medium was prepared by dissolving 5 g of peptone in 100 ml of distilled water. It was distributed in test tubes (5ml each) and sterilized by autoclaving, then stored at 4°C until use.

2.2.7 Identification of bacterial isolates:

Suspected bacterial isolates (Item 2.2.1) were primarily identified by microscopic and cultural examinations, then by the biochemical tests for final identification as follows:

A) Cultural examination: (Garrity, 2005)

Colonies grown on cultural media were described according to their shape, size, margin, color, and odor.

B) Microscopic examination: (Forbes *et al*., 2007).

Gram staining was used to describe shape, Gram reaction and grouping of isolate cells.

C) Biochemical tests: (Holt *et al.*, 1994; Garrity, 2005)

a) Indole test:

Peptone water test tubes prepared in item (2.2.6.b) were inoculated with the fresh culture of the bacterial isolates, separately, before incubated at 37 °C for 24 hrs. A portion of 0.5 ml Kovac's reagent was added for each test tube. Appearance of red ring at the top of broth indicates a positive result.

b) Methyl-red test:

Suspected colonies were inoculated in MR-VP broth and incubated at 37°C for 24 hrs. After incubation, 3-4 drops of methyl red reagent were added. Converting media color after incubation to red is a positive result.

c) Voges-Proskauer test:

Suspected colonies were inoculated in MR-VP broth and incubated at 37°C for 24 hrs, and then two drops of VP1 and four of VP2 were added. Appearance of red color after 30 min indicates a positive result.

d) Citrate test (Simmon's Citrate slant):

Simmon's Citrate slant were inoculated with the suspected bacterial isolates by streaking on the slant and incubated at 37 °C for 24 hrs. Appearance of growth and changing medium color from green to blue indicate a positive result.

e) Oxidase test:

This test was done by using a moisten paper with few drops of oxidase reagent. Cells from suspected isolates were picked up with a

sterile wooden stick and smeared on the moisten paper. A positive result was detected by development of a violet or purple color within 10 seconds.

f) Catalase test:

A single colony of suspected bacterial isolates was placed onto a clean glass microscope slide with a sterile toothpick, and then a drop of hydrogen peroxide was placed onto the colony. Production of gaseous bubbles indicates the presence of catalase enzyme.

g) Coagulase test:

Two forms of the coagulase test have been applied to detect the enzymes: the tube test and the slide test. In the slide test, bacteria were transferred to a slide containing a small amount of plasma. Agglutination of the cells on the slide within one to two minutes indicates the presence of bound coagulase. The tube test is performed by adding the test organism to rabbit plasma in a test tube. Coagulation of the plasma (including any thickening or formation of fibrin threads) within 24 hours indicates a positive reaction. The plasma is typically examined for clotting (without shaking) after about 4 hours because it is possible for coagulation to take place early and revert to liquid within24 hours.

h) Triple sugar iron agar test (TSI):

Triple sugar iron agar slants were inoculated with the suspected bacterial isolates, and then incubated at 37 °C for 24 hrs. The result was read as follows:

No change / No change → No change / No change

Alkaline/Alkaline →Red/ Red (lactose and sucrose nonfermenter).

Alkaline/ No change → Red/ Red (glucose, lactose and sucrose nonfermenter).

Alkaline /Acid → Red /Yellow (glucose fermentation only)

Acid /Acid → Yellow/Yellow (glucose, lactose and/or sucrose ferment).

H_2S production → Black precipitation

Gas production → Bubbles formation.

2.2.8 Identification of bacteria by VITEK 2 system:

The VITEK 2 which is recently installed at the Central Health Laboratories/Ministry of Health is an automated microbiology system utilizing growth-based technology.

A sterile swab sample used to transfer a sufficient number of colonies of a pure culture and to suspend them into 3 ml of normal saline (NaCl 0.45%, pH 5-7). Then turbidity adjusted by a turbidity meter called the DensiCheck to match 0.5 – 0.6 McFarland which is the proper inoculum density for Gram-negative and Gram-positive bacteria as stated by the manufacturer, (Appendages 3.1to 3.10).

2.2.9 Maintenance of bacterial isolates:

Culture of bacterial isolates were maintained according to Johnson *et al*., (1988) as follows:

a) Short – term storage (few weeks):

Bacterial isolates were maintained for few weeks by culturing on plates of brain heart agar, and incubated at 37°C for overnight. The plates were then tightly wrapped with parafilm and stored at 4 °C.

b) Medium – term storage (few months):

Bacterial isolates were maintained as stab culture for few months by inoculation in small screw capped bottles containing (5 – 8) ml of sterile brain heart agar (as slants), then incubated at 37°C for 24 hrs. The bottles were then tightly wrapped with parafilm and stored at 4°C.

c) **Long time storage:** (Boonaert and Rouxhet, 2000)

Test tube containing 10 ml sterile brain heart broth was inoculated with the bacterial isolate, and incubated at 37°C for 24 hrs. After incubation, sterile glycerol (20%) was added and mixed by vortex before freezing at (– 20) °C.

2.2.10 Antibiotic susceptibility test:

Antibiotic susceptibility tests were performed by using Kirby Bauer's disc diffusion method according to the Manual on Antimicrobial Susceptibility Testing (2004). Results were compared with Clinical Laboratory Standards Institute (CLSI) (2012).

The inoculums were prepared by suspending the colonies of isolates, grown for18-24 hrs on agar plate, in the saline solution to match the 0.5 McFarland turbidity standard.

A sterile cotton swab was dipped into the suspension and pressed firmly on the inside wall of the tube to remove excess inoculums from the swab.

The Muller-Hinton agar plates were inoculated by streaking the swab all over the entire agar surface.

The inoculated plates were then placed at room temperature for 3 to 5 min to allow adsorption of excess moisture. After that, the antibiotic discs were placed and pressed gently on the inoculated plates with forceps to ensure contact with the agar.

Then the inoculated plates were incubated at 37 ◦C for 18-24 hrs. After incubation, diameters (mm) of the inhibition zones were measured and compared with the standards of the CLSI.

2.2.11 Apple vinegar samples:

Three types of apple vinegar samples were used in this study:

a) Commercial apple vinegar (V_E):

It is commercial imported vinegar that produced in large-scale fermentor then sold as a brand at the local retail markets in sealed 500- ml bottles.

b) Homemade apple vinegar (V_H):

It was obtained from the local shops as home-made raw apple vinegar filled in various shapes and sizes of containers. It was pasteurized before use.

c) Chemically-synthesized apple vinegar (V_C):

It was made by diluting the concentrated glacial acetic acid and fortified with apple flavor. It is usually sold as a commercial brand in sealed bottles of 470 ml apple vinegar.

2.2.12 Measurement of Titratable Acidity:

According to Garner *et al.* (2008), pH of vinegar sample (2 ml) was measured by the pH-meter and the value is recorded. After that, 50 ml distilled water was added to the vinegar sample, the sample was titrated with 0.1 N NaOH to an end point of 8.2, then, pH was measured and the milliliters (ml) of NaOH used was recorded. Finally, titratable acidity was calculated by using the following formula:

$$\% \text{ Titratable acidity} = N \times V_1 \times \text{Eq.wt} / V_2 \times 10$$

Where
N = normality of titrant
V_1 = volume of titrant
Eq.wt = equivalent weight of predominant acid
V_2 = volume of sample

2.2.13 Detecting antibacterial activity against bacterial isolates:

Agar well bioassay was employed for testing the antibacterial activity of the agents (probiotics and vinegar). The probiotic bacterial isolates were prepared by inoculating 2% of the inoculum of *Lb. reuteri* (6×10^8) or the mixture of isolates (1×10^9 for each) in MRS broth of pH 6. Then incubated, anaerobically by a candle jar, at 37°C for 2 days (for the mixture) and 2 days for the *Lactobacillus reuteri* isolate (this process was repeated three times to increase the intensity of bacterial cells) (Lewus *et al*., 1991).

The fermentation product of each probiotic were concentrated as follows: One hundred ml of the unconcentrated fermentation product were concentrated to (50 ml) by putting in the vacuum oven at (40-45) °C to make the one-fold concentrated fermentation product. The experiment was repeated on the one-fold concentrated fermentation product to obtain the two-fold concentrated

fermentation product (25 ml), and same thing was done for the two-fold concentrated fermentation product to obtain the three-fold concentrated fermentation product (12.5 ml). All fermentation products (the unconcentrated or any obtained concentrated fermentation product) were tested for their antibacterial activity against the pathogenic bacterial isolates as well as their pH values were measured by using pH meter.

Wells of 5 mm diameter were made by a cork borer in the Muller-Hinton agar plates that were already spread with the pathogenic bacterial isolate cultures. The wells were filled with 0.1 ml of each of the probiotics unconcentrated fermentation product, one-fold concentrated, two-fold concentrated and three-fold concentrated or each of vinegar types individually. Then combinations between each probiotic fermentation product (unconcentrated and concentrated) are made with each of three types of apple vinegar at three different ratios as follow: probiotic 2:2 vinegar, probiotic 3:1 vinegar and probiotic 1:3 vinegar before incubation at 37°C for 24 hrs. The antibacterial activity was estimated by measuring the inhibition zone diameters (in mm) around the wells by ruler.

Controls contained only distilled water. The antibacterial assay for each of the probiotics, vinegars or their combinations against all microorganisms tested was performed in duplicates.

2.2.14 Statistical analysis:

Statistical analysis was performed using the SPSS System software. ANOVA table with Tukey's multiple comparisons test was used to determine differences between multiple groups. A *P*-value of <0.05 was considered significant.

Chapter Three

Results and Discussion

3. Results and Discussion

3.1 Isolation of bacteria:

A total of 58 swab samples were collected from patients suffering from different wound injuries. Majority of the samples (51 swabs) were taken from patients of burn injury. The other 7 swabs were taken from wounds other than burn injury and they had been excluded since they gave no bacterial growth when propagated on the isolation medium.

Out of the 51burn injury samples, 43 (84.3%) of them showed bacterial occurrence which causing wound infection in the patients suffering from burns. In this regard, Mooney and Gamelli (1989) pointed out that burn wound infections are one of the most important and potentially serious complications that occur in the acute period following injury. Raja and Singha (2007) mentioned that the type and the amount of microorganisms on and in the injured tissues influence the wound healing process as well as infectious complications considered as major causes of morbidity and mortality.

Our study showed high prevalence of bacterial infections among burn patients which agrees with results of earlier study of Ekrami and Kalantar (2007) from Iran. The reasons for this high prevalence may be due to factors associated with the acquisition of nosocomial pathogens in patients with recurrent or long-term hospitalization, complicating illnesses, prior administration of antimicrobial agents, or the immunosuppressive effects of burn trauma (Alwan *et al*., 2011).

Samples infected with only one type of pathogenic bacteria were detected in 21 (48.84%) of 43 patients, while 22 (51.16%) of the patients were infected with more than one type of bacteria (Polymicrobial infection).

From the 43 samples that gave positive bacterial growth, 64 bacterial isolates were obtained which were subjected to the identification.

3.2 Identification of bacterial isolates:

The sixty-four bacterial isolates were identified, primarily, to the genus by the cultural and microscopic examinations, and then to the species by the biochemical tests, their numbers and percentages are listed in table (3.2).

Final identification was confirmed by using VITEK 2 system. Results obtained in this regard as follows:

3.2.1 Cultural characterization:

Identification of the 64 suspected bacterial isolates were performed at first depending on the characteristics of colonies grown on the surface of MacConkey and blood agar. Some suspected bacterial isolates were allowed to grow on the selective media that are more specific for their species as EMB agar, mannitol salt agar and cetrimide agar.

Regardless to lactose fermenting ability, 46(71.8%) isolates were able to grow on MacConkey agar with various shapes and morphologies, 30 (46.8 %) appeared pale or colorless non-lactose fermentor which suspected to belong to *Pseudomonas* (Garrity *et al*., 2005). From the 16 (25%) remaining isolates 10 (15.6%) isolates were found to be lactose fermentor, dome- shaped, 3-4 mm in diameter after overnight incubation with a mucoid aspect and sometimes stickiness, these results come in accordance with the corresponding characteristics that mentioned by Garrity *et al*. (2005) for *Klebsiella*, 3 (4.6%) isolates grew well and produced rose pink to red colonies and were lactose fermentor which indicates that they may belong to *Escherichia* (Garrity *et al*., 2005). 1(1.5%) isolate was smooth, sometimes mucoid, pale yellow to grayish-white, isolate with such characteristics suspected to belong to *Acinetobacter*. 1(1.5%) isolate grew as tiny pinpoints distinct non-lactose fermenting colorless colony which suspected to belong to *Stenotrophomonas*. While one isolate showed late lactose fermentation which suspected to belong to *Enterobacter*.

All 64 isolates obtained from burn wound infections were able to grow on Blood agar with different shapes and morphologies of the colonies, 28 (43.7%)

isolates display beta hemolysis, a metallic sheen, and blue or green pigment and grape-like odor, isolates with such characteristics suspected to belong to *Pseudomonas aeruginosa* (Garrity *et al*., 2005). 12 (18.7%) isolates were large, creamy white and form beta hemolytic colonies according to Garrity *et al*. (2005) *Staphylococcus* has such characteristics. On the other hand, 6 (9.3%) isolates formed non-hemolytic creamy white colonies which suspected to belong to Coagulase Negative *Staphylococci* (Gillespie and Hawkey, 2006).

There were 10 (15.6%) of the isolates grey, round, shiny, non-hemolytic and mucoid colonies, isolates with such characteristics suspected to belong to *Klebsiella*, while 3 (4.6%) isolates were grey, moist non-hemolytic colonies which suspected to belong to *Escherichia* (Garrity *et al*., 2005). 2 (3.1%) isolates did not possess distinctive colony morphology or odor; they suspected to belong to *Pseudomonas*. 1(1.5%) colony appeared yellow or green on blood agar which suspected to belong to *Stenotrophomonas* (Garrity *et al*., 2005). 1(1.5%) isolate was 2-3 mm in diameter not pigmented and comparable to those of Enterobacteria, isolate with such characteristics may belong to *Acinetobacter* (Garrity *et al*., 2005).1(1.5%) isolate appeared as round colony in 2-3 mm in diameter non-haemolytic which suspected to belong to *Enterobacter* (Garrity *et al*., 2005).

Isolates that suspected to belong to Gram-negative bacteria were subcultured on Eosin Methylene Blue (EMB). There were 3 (4.6%) isolates showed dark center and greenish metallic sheen colonies which suspected to belong to *E. coli*, while 11(17.1%) isolates, 10 (15.6%) *Klebsiella* spp and 1 *Enterobacter* spp (1.5%) grew with purple dark centered mucoid colonies due to lactose fermentation and acid production. Other isolates, remain their normal color or take on the coloration of the medium they suspected to belong to *Pseudomonas* (30; 46.8%), *Acinetobacter* and *Stenotrophomonas* which represented by one isolate (Leboffe and Pierce, 2011).

Isolates that suspected to belong to genus *Staphylococcus* were subcultured on mannitol salt agar and *Pseudomonas* on cetrimide agar for further

identifications. On mannitol salt agar which considered selective and differential medium for the genus *Staphylococcus* (Benson, 2001), 18(28.1%) isolates had the ability to grow, 12(18.7%) isolates had the ability to ferment mannitol sugar and form large golden colonies surrounded by wide yellow zones and turned the color of the medium from pink to yellow, 6 (9.3%) other isolates were mannitol non fermentor which appeared as small pink colonies. Isolates grew, but produce no color change are suspected to be Coagulase Negative Staphylococcus (CoNS), while the development of yellow halos around the bacterial growth is presumptive evidence that the organism is a pathogenic *Staphylococcus* (usually *S. aureus*) (Leboffe and Pierce, 2011).

For further identification 28 (43.7%) isolates that suspected to belong to *Pseudomonas aeruginosa* were subcultured on cetrimide agar which is a selective media for the particular specie (Brown and Lowbury, 1965); isolates showed a blue-green to green pigment.

3.2.2 Microscopic characterization:

The results showed that 30 (46.8%) of the isolates appeared Gram negative, straight or slightly curved rods and motile which suspected to belong to *Pseudomonas* (Forbes *et al*., 2007). 18 (28%) of the isolates were cocci arranged in irregular clusters, uniformly positive for gram stain reaction, non-motile and non-spore forming, AL-Kazaz (2006) described *Staphylococci* with same characteristics.10 (15.6%) isolates were Gram-negative, rod-shaped and encapsulated in pairs or short chains under microscope, isolates with such characteristics may belong to *Klebsiella*, while 3(4.6%) isolates appeared Gram-negative, rod-shaped and motile which suspected to belong to *Escherichia* (Forbes *et al*., 2007).1(1.5%) isolate appeared as coccobacilli, or straight rods with rounded ends which suspected to belong to *Enterobacter* (Forbes *et al*., 2007). 1(1.5%) isolate was Gram-negative and had the appearance of plump cocci, or coccobacilli which suspected to belong to *Acinetobacter* (Forbes *et al*., 2007).1(1.5%) isolate

was Gram-negative, short to medium size and straight rods which suspected to belong to *Stenotrophomonas* (Forbes *et al*., 2007).

3.2.3 Biochemical characterization:

Results of the biochemical tests used for identification of bacterial isolates to the species are as shown in table (3.1).

Table 3.1: Results of the biochemical tests of bacterial isolates that obtained from burn wound infection patients.

Bacterial isolate	**Biochemical test**								
	IND	**MRVP**	**CIT**	**CAT**	**OXI**	**CoA**	**TSI**	**Gas**	**H_2S**
Pseudomonas aeruginosa	-	- -	+	+	+	0	r/r	-	-
Staphylococcus aureus	-	- -	-	+	-	+	y/y	-	-
Klebsiella pneumoniae	-	- +	+	+	-	0	y/y	+	-
Coagulase negative *Staphylococci* (CoNS)	-	- -	-	+	-	-	y/y	-	+
Escherichia coli	+	+ -	-	+	-	0	y/y	+	-
Enterobacter cloacae	-	- +	+	+	-	0	y/y	+	-
Acinetobacter baumannii	-	- -	+	+	-	0	r/r	-	-
Stenotrophomonas maltophilia	-	- -	V	+	-	0	r/r	-	-
P. Putida	-	- -	+	+	-	0	r/r	-	-
P. alcaligenes	-	- -	+	+	+	0	r/r	-	-

IND; Indole, MRVP; Methyl red Voges-Proskauer, CIT; Simmon citrate, CAT; Catalase, OXI; Oxidase, CoA; Coagulase, TSI; Triple Sugar Iron, Gas; Gas production, (-); negative, (+); positive, r; red, y; yellow, v; 16 to 84% strains positive, 0; not performed.

Group of 28 isolates gave negative results for indole, methyl red, Voges-Proskauer, gas production and H_2S precipitation, while they gave positive results for citrate utilization, catalase and oxidase, and they showed red color in both slant and butt for triple sugar iron test according to Holt *et al.* (1994) and Garrity *et al*. (2005) these isolates suspected to belong to *P. aeruginosa*. One isolate showed the same biochemical results as above which suspected to belong to *Pseudomonas alcaligenes*, while another isolate also showed the same results except for oxidase which gave a negative result, this isolate suspected to belong to *Pseudomonas putida* despite that Holt *et al.* (1994) and Garrity *et al*. (2005) mentioned that *P. putida* gave oxidase positive result.

Group of 18 isolates gave negative results for indole, methyl red, Voges-Proskauer, citrate utilization, oxidase, gas production and H_2S precipitation while gave positive results for coagulase and catalase and gave yellow color for both slant and butt which suspected to belong to *S. aureus* except 6 isolates were coagulase negative and were positive for H_2S precipitation which identified as Coagulase Negative *Staphylococci* (CoNS) (Holt *et al.*, 1994; Garrity *et al*., 2005).

There were 10 isolates that gave negative results for indole, methyl red, oxidase and H_2S precipitation while gave positive results for Voges-Proskauer, citrate, catalase and gas production and they gave yellow color for both slant and butt, these isolates identified as *Klebsiella pneumoniae* (Holt *et al.*, 1994; Garrity *et al*., 2005).

Three isolate gave positive results for indole, methyl red, catalase and gas production as well as they gave yellow color for slant and butt in TSI test, while they gave negative results for Voges-Proskauer, citrate, oxidase and H_2S precipitation, these isolates identified as *E. coli* (Holt *et al.*, 1994; Garrity *et al*., 2005).

One isolate identified as *Enterobacter cloacae* since it gave positive results for Voges-Proskauer, citrate, catalase and gas production while showed negative

results for indole, methyl red, oxidase and H_2S precipitation and gave yellow color for slant and butt in TSI test (Holt *et al.*, 1994; Garrity *et al*., 2005).

Tow isolates gave negative results for indole, methyl red, Voges-Proskauer, oxidase, gas production and H_2S precipitation while they gave red color for both slant and butt and one of them was positive for catalase and citrate which suspected to be *Acinetobacter baumannii* and the other one was positive for catalase and gave variant results for citrate hence it suspected to be *Stenotrophomonas maltophilia* (Holt *et al.*, 1994; Garrity *et al*., 2005).

The results of the cultural, microscopic and biochemical identification suggest that the 64 bacterial isolates were belonging to 10 different species.

After that, bacterial species subjected to Vitek 2 system for confirmation.

Table 3.2: Bacterial species obtained from burn wound infections

Bacterial isolate	**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	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
P. alcaligenes	0	1	1	1.5
P. putida	0	1	1	1.5

As shown in figure 3.1, Gram-negative bacteria were the predominant pathogens in the burn wound infections represented by 46 (72%) isolates while

Gram positive bacteria represented by only 18 (28%) isolates, similar findings were also recorded by various studies such as Ekrami and Kalantar (2007); Alwan *et al*. (2011); Magnet *et al*. (2013). But in studies of Liwimbi and Komolafe (2007) and Alebachew *et al*. (2012), Gram-positive are found to be the predominant organisms in the burn wound infections.

The results of this study indicate that *P. aeruginosa* was the commonest isolate (43%) followed by *Staphylococcus aureus*, this come in accordance with many previous studies which declared that *P. aeruginosa* followed by *Staphylococcus aureus* are the commonest isolates in burn wound infections (Agnihotri *et al*., 2004; Ekrami and Kalantar, 2007; Alwan *et al*., 201; Magnet *et al*., 2013).

In study of Manjula *et al.* (2007) who reported that *Pseudomonas* species was the commonest pathogen isolated (51.5%) from burn wound followed by *Acinetobacter* species (14.28%), *S. aureus* (11.15%), *Klebsiella* species (9.23%) and *Proteus* species (2.3%).

Other study found that *S. aureus* was the most common isolate while the second common isolate was *P. mirabilis* followed by *Streptococci* spp (Liwimbi and Komolafe, 2007).

Our study showed *P. aeruginosa* as a common cause of nosocomial infection, similar to earlier study of Ekrami and Kalantar (2007). Other studies also showed that nosocomial infection caused by *P. aeruginosa* was the major danger in burn patients (Rastegar *et al.*, 1998; Rastegar *et al.*, 2005).

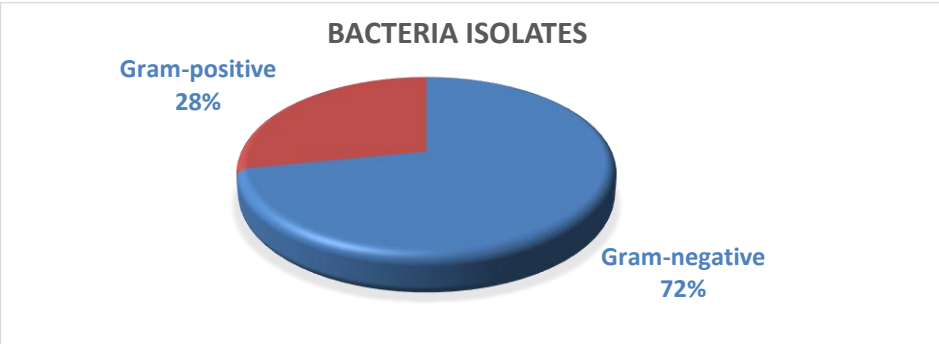

Figure 3.1: The distribution of Gram-negative and Gram-positive bacteria in burn wound infections.

3.2.4 Identification of bacterial species by VITEK system:

To ensure identification of the bacterial species, VITEK 2 system was used through its GN (Gram-negative) and GP (Gram-positive) cards. By this system of identification, 64 bacterial isolates were found to be belonging to ten different species. The results of VITEK 2 system agreed with the obtained results of the biochemical tests that applied for the pathogenic isolates that shown in table 3.1.

Their probabilities and confidences are shown in table (3.3), while results of tests included in VITEK 2 system for each of the bacterial species are shown in appendages 3.1 to 3.10.

Aziz *et al*. (2014) referred to the probability and confidence of identification of Vitek 2 system as the accuracy of the Vitek 2 system.

Garcia-Garrote *et al*. (2000) pointed out that the VITEK 2 system is an easy-to-handle system that provides a rapid (during 4 to 15 h) and reasonably accurate means for the identification of bacterial species.

One of the most important advantages of the VITEK 2 system is the significant reduction in handling time, which will have a positive impact on the work flow of the clinical microbiology laboratory. However, the system needs further improvement in its accuracy of identification, interpretation of results and database.

Table 3.3: Results of identification of burn wound bacterial isolates by the VITEK 2 system

Bacterial species	**Probability**	**Confidence**	**Biochemical details**
Pseudomonas aeruginosa	99%	Excellent identification	Appendix 3.1
Staphylococcus aureus	89%	Good identification	Appendix 3.2
Klebsiella pneumoniae	87%	Acceptable identification	Appendix 3.3

Staphylococcus epidermidis	99%	Excellent identification	Appendix 3.4
Escherichia coli	95%	Very good identification	Appendix 3.5
Enterobacter cloacae	97%	Excellent identification	Appendix 3.6
Acinetobacter baumannii	99%	Excellent identification	Appendix 3.7
Stenotrophomonas maltophilia	99%	Excellent identification	Appendix 3.8
P. alcaligenes	97%	Low discrimination	Appendix 3.9
P. Putida	98%	Low discrimination	Appendix 3.10

3.3 Antibiotic susceptibility of pathogenic bacteria:

Bacterial species were examined for their susceptibility towards 10 different antibiotics by using disc diffusion method and the obtained results are illustrated in table (3.4).

All isolates were found to be sensitive to amikacin, which was closed to that result obtained by Paterson and Yu (1999) who reported high sensitivity to amikacin among bacterial isolates that isolated from different microbial infections.

Umadevi *et al*. (2011) also declared that the members of *Enterobacteriaceae* were found to be susceptible to amikacin. Results also showed that most the bacterial isolates were completely sensitive to imipenem.

In this regard, Livemore *et al*. (2001) found that imipenem have strong activity against most Enterobacteriaceae bacteria included in their study.

In contrast, all bacterial isolates were resistant to the β-lactam group (other than imipenem) antibiotics used with the exception *Staphylococcus epidermidis* (CoNS) isolates which were moderately sensitive to these antibiotics. This may be related to isolates-possessing of β-lactamase enzymes which are able to inactivate

these antibiotics through cleaving the β-lactam ring of the drug (Levinson and Jawetz, 2000).

Highest resistance for antibiotics used in this study has been observed in *Pseudomonas* species which were sensitive towards only two antibiotics imipenem and amikacin.

In 2002, Lambert demonstrate that low antibiotics susceptibility of *Pseudomonas* is due to; low permeability of its cell membrane, the genetic capacity to express a wide repertoire of resistance mechanisms, resistant through mutation in chromosomal genes which regulate resistance genes, and to the acquired additional resistance genes from other organisms via plasmids, transposons and bacteriophages.

The most sensitive bacterial isolates toward most of the antibiotics used in this study were those belonging to the CoNS which were sensitive to all antibiotics used except towards tetracycline which show no activity on CoNS. With the exception of their resistance to tetracycline CoNS isolates were sensitive to all other antibiotic used. In addition, amikacin and vancomycin were the most efficient antibiotics against these isolates when they were highly sensitive to these antibiotics.

In earlier study carried out by Srivastava *et al*. (2014), vancomycin and amikacin were found to be the most effective antibiotics towards CoNS.

Regarding *S. aureus* isolates, all were totally (100%) sensitive to amikacin, but highly resistance to tetracycline. In a study performed by Resn (2013), *S. aureus* which obtained from diabetic foot infections were found to be sensitive to amikacin.

Among the Enterobacteriaceae species used in this study, *Enterobacter cloacae* isolates, in general, were the highly resistant to most of the antibiotics but sensitive to only three of them (amikacin, imipenem and ciprofloxacin). *Klebsiella pneumoniae* isolates were highly sensitive to amikacin, imipenem, and ciprofloxacin and moderately sensitive to chloramphenicol but completely

resistance to the others. In this regard, Sohely*et al*. (2009) found that *Enterobacter* spp and *Klebsiella* spp were completely sensitive against imipenem and less to amikacin.

On the other hand, *E. coli* isolates were completely sensitive against amikacin, imipenem and chloramphenicol, while moderately sensitive to ciprofloxacin but complete resistance to the others. Iona *et al*. (2010) declared that isolates belonged to *E. coli* were highly sensitive toward amikacin and imipenem.

Wazait *et al*. (2003) referred the resistance of *E. coli* to some antibiotics to its ability for easily acquire resistance factor from the environment and easily resist penicillin derivatives drugs such as ampicillin. *Stenotrophomonas maltophilia* antibiotic-sensitivity patterns in this study were observed toward amikacin, chloramphenicol, vancomycin tetracycline, and ciprofloxacin.

Interestingly, as a remarkable result, *Stenotrophomonas maltophilia* was the only bacteria among all others that showed resistance toward the antibiotic imipenem.

In this regard, Quinn (1998) demonstrates that *Stenotrophomonas maltophilia* is intrinsically resistant to multiple antibiotics and disinfectants, while Alonso and Martinez (1997) referred such acquired resistance of this bacteria to the antibiotic efflux which may be behaves as the intrinsic and resistant contributing factors in this regard. Indeed, antibiotic efflux mechanisms are increasingly recognized as a major factor in the intrinsic and acquired resistance of a number of significant human pathogens (Nikaido, 1996).

According to the results obtained in this study, *Acinetobacter baumannii* was susceptible to amikacin, imipenem and tetracycline. Isolate of this species were the most resistant after those of *Pseudomonas*.

Espinal *et al.,* (2012) declared that the low sensitivity of *A. baumannii* toward antibiotics may be due to Biofilm formation which enables the bacteria to survive. Biofilms can alter the metabolism of microorganism, then reducing their sensitivity to antibiotics. A slower metabolism contributes to the prevention of

bacteria from uptaking an antibiotic. Plus, they also provide a physical barrier against larger molecules and may prevent desiccation of the bacteria (Yeom*et al.*, 2013). Another reason was highlighted by Higgins *et al*. (2013) who demonstrate that the resistance of *A. baumannii* may be related to β–lactamase since *A. baumannii* is known to produce at least one β–lactamase enzyme.

Table 3.4: Susceptibility percentage (%) of burn wound infection causative bacteria toward antibiotics

Isolate	**Tested isolate**	**AP**	**AK**	**TE**	**DA**	**IPM**	**E**	**C**	**VA**	**CIP**	**CFM**
Pseudomonas aeruginosa	15	0	86	0	0	94	0	0	0	0	0
Staphylococcus aureus	12	0	100	0	8	83	41	75	66	25	0
Klebsiella pneumoniae	10	0	90	0	0	90	0	50	0	80	0
Staphylococcus epidermidis	6	50	100	16	50	50	50	83	100	66	66
Escherichia 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.

3.4 Effect of antibiotics used in hospital on some pathogenic bacteria:

From this part of the study and the following steps, one isolate is selected randomly from each group of similar species except *Pseudomonas aeruginosa* since they were the highest isolated species two isolates were selected and they will be represented as *P. aeruginosa*1 and *P. aeruginosa*2.

Augmentin, cefotaxime and amoxicillin are given to the patients who attending the hospitals when the samples collected as part of the treatment to eradicate the infection causatives.

Due to the unavailability and difficulty in purchasing the experimental augmentin, only the last two antibiotics were used in the study.

Results in table (3.5) ensured that the pathogenic bacterial isolates included in this study were totally resistant to cefotaxime and amoxicillin while *Stenotrophomonas maltophilia* and *Staphylococcus epidermidis* were very slightly affected by the last antibiotic.

Furthermore, the 5 and 8 mm inhibition zones given against these two bacterial were uncomparable with any of those zones given by all probiotic bacteria, apple vinegars or their combinations. This highly resistance of pathogenic bacteria toward used drug may be due to the overuse of antibiotics in treatment of the patients that leads to elevate resistance of the pathogens to antibiotics (Rice *et al*., 1990). Peterson (2002) pointed out that using antibiotics for much longer time as well as their oral route of administration also affect their rate of absorption into blood stream.

Table 3.5: Susceptibility pattern toward amoxicillin and cefotaxime of some pathogenic burn infection causing bacteria

Isolate	**Tested isolate**	**Susceptibility and inhibition zone (mm)**	
		Amoxicillin	**Cefotaxime**
Pseudomonas aeruginosa	2	R	R

Klebsiella pneumoniae	1	R	R
Enterobacter cloacae	1	R	R
Acinetobacter baumannii	1	R	R
Stenotrophomonas maltophilia	1	R(5)	R
Staphylococcus aureus	1	R	R
Staphylococcus epidermidis	1	R(8)	R
P. alcaligenes	1	R	R
P. putida	1	R	R
E. coli	1	R	R

3.5 Antibacterial activity of probiotics against pathogenic bacteria:

3.5.1 *Lactobacillus reuteri*:

As a probiotic, among all the unconcentrated and concentrated fermentation product, the three-fold concentrated fermentation product of *Lb. reuteri* was the only concentration to excreted an inhibitory effect ($p < 0.05$) against the pathogenic bacteria included in this study as illustrated in table 3.6. While the unconcentrated fermentation product, one-fold concentration fermentation product and two-fold concentration fermentation product gave no inhibitory activity against pathogenic isolates ($p > 0.05$).

Table 3.6: Inhibitory effect of *Lb. reuteri* probiotic of the unconcentrated and concentrated fermentation product

Bacterial species	**Inhibition zone (mm)**			
	Unconcentrated fermentation product (pH= 6.1)	One-fold concentrated fermentation product (pH= 5.8)	Two-fold concentrated fermentation product (pH= 5.8)	Three-fold concentrated fermentation product (pH= 5.6)
*Pseudomonas aeruginosa*1	0	0	0	18
*P.aeruginosa*2	0	0	0	15.5

Klebsiella pneumoniae	0	0	0	14.5
Enterobacter cloacae	0	0	0	13.5
Acinetobacter baumannii	0	0	0	7
Stenotrophomonas maltophilia	0	0	0	14.7
Staphylococcus aureus	0	0	0	17.2
Staphylococcus epidermidis	0	0	0	15.5
P. alcaligenes	0	0	0	14.5
P. putida	0	0	0	13.5
E. coli	0	0	0	14

Jacobsen *et al*. (1999) found that the antagonist activity of lactic acid bacteria might be referred to its ability in producing organic acids (which lower the pH) and bacteriocins, in addition to competition on the nutrients with the pathogenic bacteria.

El-Ziney and Debevere (1998) pointed out that this antibacterial activity could account for production of reuterin which *Lb. reuteri* is a known producer of it.

Moreover, Cleusix *et al*. (2007) reported for the first time the antimicrobial activity of reuterin produced by *Lb. reuteri* on various intestinal bacteria.

Among all, *P. aeruginosa*1 was the most affected isolate with highest recorded inhibition zone of 18 mm (Fig.3.2). Adversely, the least effective inhibitory effect was recorded against *Acinetobacter baumannii* with an inhibition zone 7 mm.

In this regard, results achieved by Alexandre *et al*. (2014) showed that *Lactobacillus* spp was able to inhibit the growth of *P. aeruginosa* in parallel with an increase in acid concentration and pH decrease that caused by acid production.

Figure 3.2: Inhibition zone given by *Lactobacillus reuteri* three-fold concentrated fermentation product against *Pseudomonas aeruginosa* isolated from burn wound infections.

3.5.2 Mixed probiotic (*Lactobacillus acidophilus, Lb. reuteri, Lb. rhamnosus,* and *Bifidobacterium longum*):

When the inhibitory effect tested for the mixture of four different species of probiotics against burn wound causative bacteria, results showed that no inhibitory effect recorded ($p > 0.05$) at the unconcentrated fermentation product or any obtained concentration against all the pathogenic bacteria isolated from burn wound infections.

In this regard, Chapman *et al*., (2012) mentioned that probiotic species may inhibit each other when incubated together *in vitro*. Furthermore, they declared that inhibition was observed for all combinations of probiotics species that had been

used in their study, suggesting that when used as a mixture there may be inhibition between probiotics, potentially reducing efficacy of the mixture.

3.6 Antibacterial activity of apple vinegar:

Titratable acidity and pH values of the three types of apple vinegar used in this study are shown in table 3.7 below.

Table 3.7: Titratable acidity and pH values of apple vinegar used in treatment of pathogenic bacteria.

Type of vinegar	Titratable acidity (%)	pH
Commercial apple vinegar (V_E).	5.05 %	3.17
Homemade apple vinegar (V_H).	4.71%	3.42
Chemically-synthesized apple vinegar (V_C).	7.14%	2.7

Results of the inhibitory effect of the three types of vinegar against causative bacteria of burn wound infections declared that ability to inhibit the growth of pathogenic bacteria varied broadly among bacterial species (Fig.3.3).

Application of commercial apple vinegar (V_E) lead to the highest inhibitory effect against *Staphylococcus epidermidis* when the formed inhibition zone reached 24.5 mm, while the lowest recorded inhibition zone was 16 mm against *Stenotrophomonas maltophilia*. However, the homemade apple vinegar (V_H) exerted its lowest activity with same inhibition zone (16 mm) against *Stenotrophomonas maltophilia* also, while its highest activity (20 mm) was recorded against each of *P. aeruginosa*2 and *S. aureus*. When the chemically synthesized apple vinegar (V_C) was tested for its inhibitory effect, growth of *Staphylococcus epidermidis* isolate was the most affected with an inhibition zone of 24.7 mm, while the most resistant isolate were those belonging to *Klebsiella pneumoniae* with 16.5 mm.

Statistical analysis showed that there are no significant differences (p <0.05) between treatments of the effect of V_C and V_E types of vinegar against tested pathogenic bacteria (appendix 3.11), while both were significantly different from the vinegar type V_H.

Against most of the pathogenic bacterial isolates, vinegar type V_C exhibited more effective inhibitory activity compared to the other two types especially against *Stenotrophomonas maltophilia* when the inhibition zone reached 19 mm while 16 mm for each of the other types. Adversely, V_C showed less inhibitory effect than V_E especially against *P. aeruginosa*1, *P. aeruginosa*2 and *Klebsiella pneumoniae*.

The inhibitory effect of vinegar may due to its main active compound acetic acid which is considered as a preservative.

According to Malicki *et al.* (2006) the antibacterial effect of organic acids is mainly caused by its undissociated forms. They passively diffuse through the bacterial cell wall and internalizing into neutral pH dissociating into anions and protons. Release of the protons causes the internal pH to decrease which exert inhibitory effects on the bacteria (Ricke, 2003).

Various researchers have proved the antibacterial activity of vinegar; Lingham *et al.* (2012) reported that vinegar when applied as an antibacterial agent was shown to be effective in reducing spoilage bacteria that isolated from catfish.

Study of Medina *et al.* (2007) found that vinegar of 5% acetic acid concentration possessed a bactericidal effect against *S. aureus*; *E. coli*, *Salmonella enteritidis, Listeria monocytogenes* and *Yersinia* spp.

Aljamali (2012) declared that several types of vinegar including apple vinegar have an antimicrobial activity against *P. aeruginosa* and *S. aureus.*

Results from study of Yang *et al.* (2009) suggested that vinegar (acetic acid) exhibits the most antimicrobial efficacy, followed by lemon juice (citric acid) and baking soda (sodium bicarbonate).

On the other hand, Hindi (2013) demonstrated that apple vinegar has a low antibacterial activity against bacteria isolated from clinical samples.

Other study declared that acetic acid solutions with low concentration have only slight effect of inhibiting growth of *S. aureus* and *P. aeruginosa* (Rund, 1996).

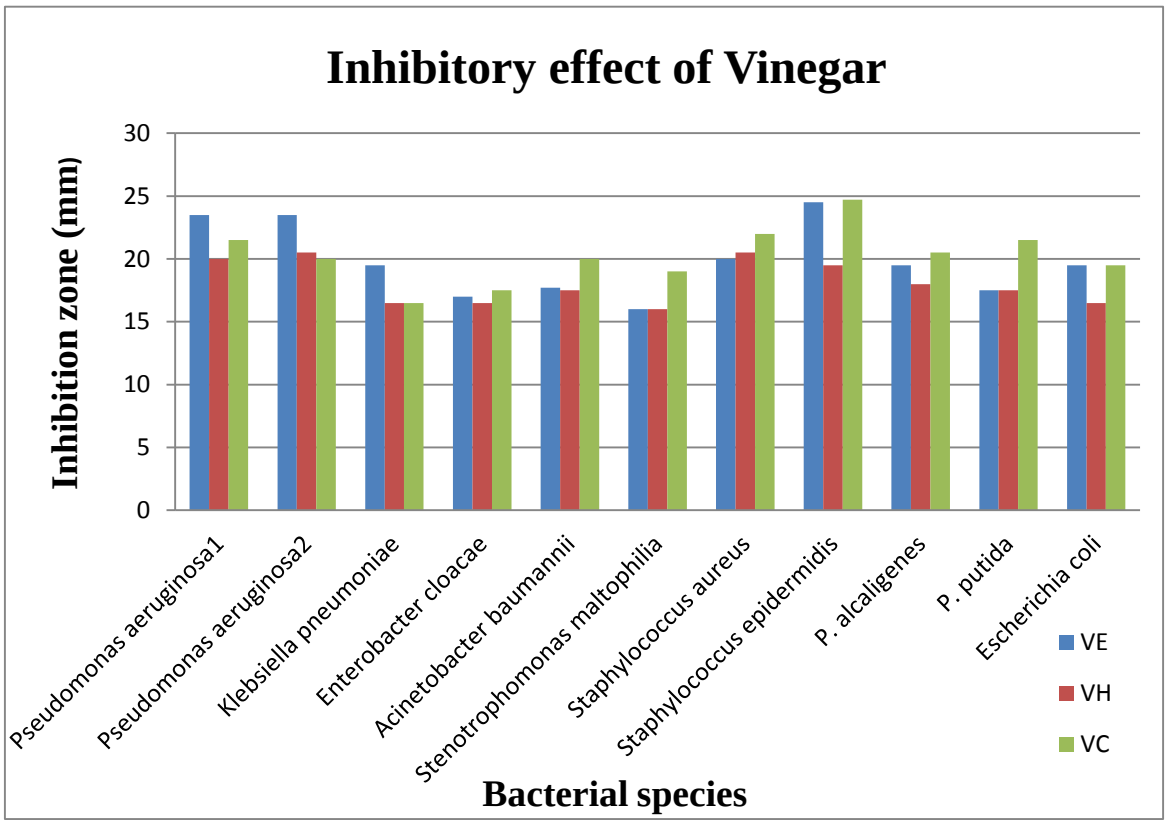

Figure 3.3: Inhibitory effect of three different types of apple vinegar on pathogenic bacteria isolated from burn wound infected patients.

3.7 Antibacterial activity of *Lb. reuteri* three-fold concentrated fermentation product and apple vinegars combination:

When each type of vinegar, combined in three different ratios with the three-fold concentrated fermentation product of probiotic bacteria *Lb. reuteri* (since it was the only probiotic that gave an inhibitory effect) as follow: *Lb. reuteri* three-fold concentrated fermentation product 2:2 vinegar, three-fold concentrated

fermentation product 3:1 vinegar and three-fold concentrated fermentation product 1: 3 vinegar.

Results illustrated in figures (3.4, 3.5 and 3.6), show that the combinations of three-fold concentrated fermentation product of *Lb. reuteri* and apple vinegars were, generally, more effective against pathogenic bacteria than each antibacterial agent individually.

Among the three types of vinegars, chemically synthesized apple vinegar (V_C) was the most effective type when combined with *Lb. reuteri* three-fold concentrated fermentation product, followed by the commercial apple vinegar (V_E) then by the homemade apple vinegar (V_H).

Effect of each three types of apple vinegar were significantly different ($p < 0.05$) from each other when they combined with *Lb. reuteri* three-fold concentrated fermentation product in ratio of *Lb. reuteri* 2:2 vinegar, but no significant differences were recorded between the effect of combination of (*Lb. reuteri* three-fold concentrated fermentation product-V_C) and (*Lb. reuteri* three-fold concentrated fermentation product-V_E), while both were significantly different from the effect of (*Lb. reuteri* three-fold concentrated fermentation product-V_H) in ratio of *Lb. reuteri* 1:3 vinegar (appendix 3.11).

At ratio of *Lb. reuteri* three-fold concentrated fermentation product3:1 vinegar no significant differences were recorded between the effect of combination of (*Lb. reuteri* three-fold concentrated fermentation product–V_E) and (*Lb. reuteri* three-fold concentrated fermentation product – V_H), while both were significantly different from the effect of (*Lb. reuteri* three-fold concentrated fermentation product – V_C).

The most effective antibacterial activity was obtained in ratio of *Lb. reuteri* three-fold concentrated fermentation product 1:3 vinegar followed by *Lb. reuteri* three-fold concentrated fermentation product 2:2 vinegar then *Lb. reuteri* three-fold concentrated fermentation product 3:1 vinegar for all types of vinegar.

The combination ratio of *Lb. reuteri* three-fold concentrated fermentation product 1 to 3 V_E vinegar gave the most efficient antibacterial activity against pathogenic bacterial isolates, with the exception of *P. aeruginosa*1 and *E. coli* isolates which were highly affected also by this combination but in the ratios of 2:2 for the first and 3:1 for the second. The highest inhibition zone (29 mm) was recorded against *P. aeruginosa*1 by the ratio of *Lb. reuteri* three-fold concentrated fermentation product 2:2 vinegar, while the lowest was 17 mm against each of *Klebsiella pneumoniae* and *Stenotrophomonas maltophilia* in combination ratios of 3:1 for the first and 2:2 for the second bacteria.

Upon using the 1:3 combination ratio of homemade apple vinegar (V_H) with *Lb. reuteri* three-fold concentrated fermentation product, the most effective antibacterial activity was obtained, especially against *Staphylococcus epidermidis* when the synergistic effect between *Lb. reuteri* three-fold concentrated fermentation product and vinegar reached 25 mm compared to 15.5 by *Lb. reuteri* three-fold concentrated fermentation product alone and 19.5 mm by vinegar alone. On the other hand, the lowest antibacterial activity of this combination (16 mm) by each of the 2:2 and 3:1 ratio against *Stenotrophomonas maltophilia*.

Staphylococcus epidermidis was also highly affected by the combination ratio of *Lb. reuteri* three-fold concentrated fermentation product 1:3 V_C vinegar with inhibition zone of 27.5mm.

In this combination against *Acinetobacter baumannii*, the vinegar alone gave better effect when the inhibition zone recorded was 20 mm to 18, 18 and18.5 mm for 2:2, 3:1 and 1:3 ratios, respectively.

Such synergistic effect may be to the synergistic effect between acetic acid (from vinegar) and lactic acid produced by *Lb. reuteri*.

In this regard, Schnurer and Magnusson (2005) mentioned that acetic acid and propionic acid showed a synergistic effect with lactic acid when they used as food preservative.

Furthermore, reuterin that produced from *Lb. reuteri* may have a synergistic effect with the acetic acid present in apple vinegar.

El-Ziney *et al*. (1999) also recorded a synergistic effect between reuterin and organic acid when the reuterin activity had been improved against *Listeria monocytogenes* and *E. coli* O157.

In a study by Arqués (2004), reuterin showed a synergistic effect against *Listeria monocytogenes* and a slight effect on *S. aureus* when combined with the antibacterial peptide nisin.

In 2015, Bjarnsholt *et al*. proved that acetic acid when combined with the antibiotic tobramycin showed an enhanced effect as antimicrobial agent against *P. aeruginosa* isolated from wound infections.

The Study of Sabir *et al*. (2007) declared a successful eradication of multidrug resistant *Pseudomonas* causing Lumbar osteomyelitis using a combination of acetic acid and systemic antibiotics.

Entani *et al*. (1998) mentioned that the combined use of vinegar and sodium chloride, with using an appropriate treatment temperature, was found to be markedly effective for the prevention of bacterial food poisoning.

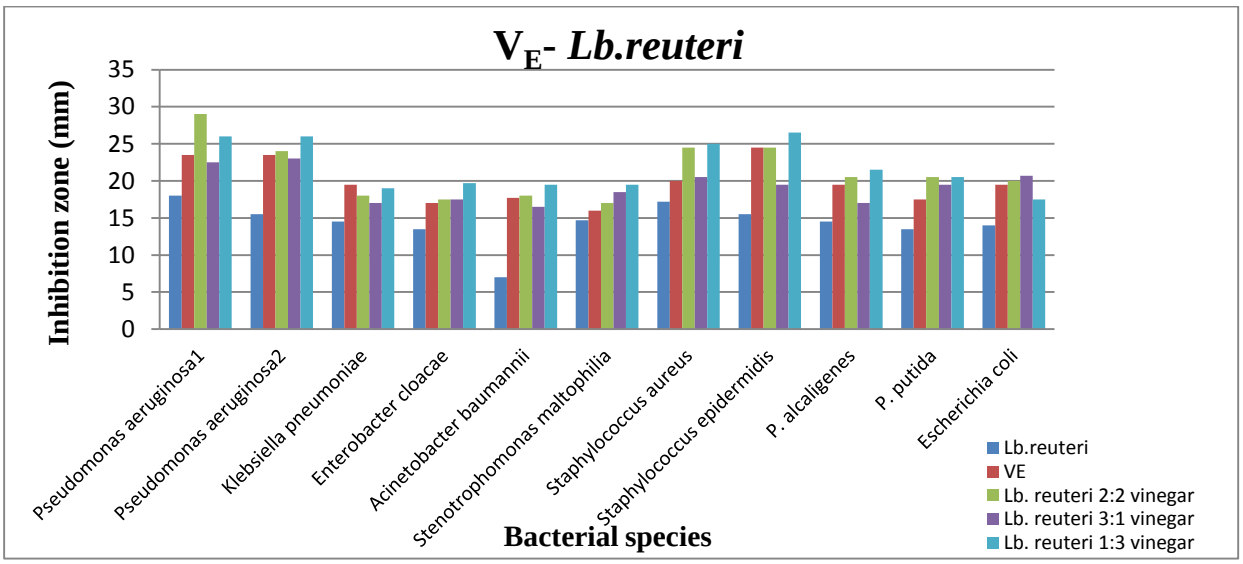

Figure 3.4 Inhibition zones against burn wound bacterial species formed *Lb. reuteri* three-fold concentrated fermentation product alone, commercial apple vinegar (V_E) alone and their mixture.

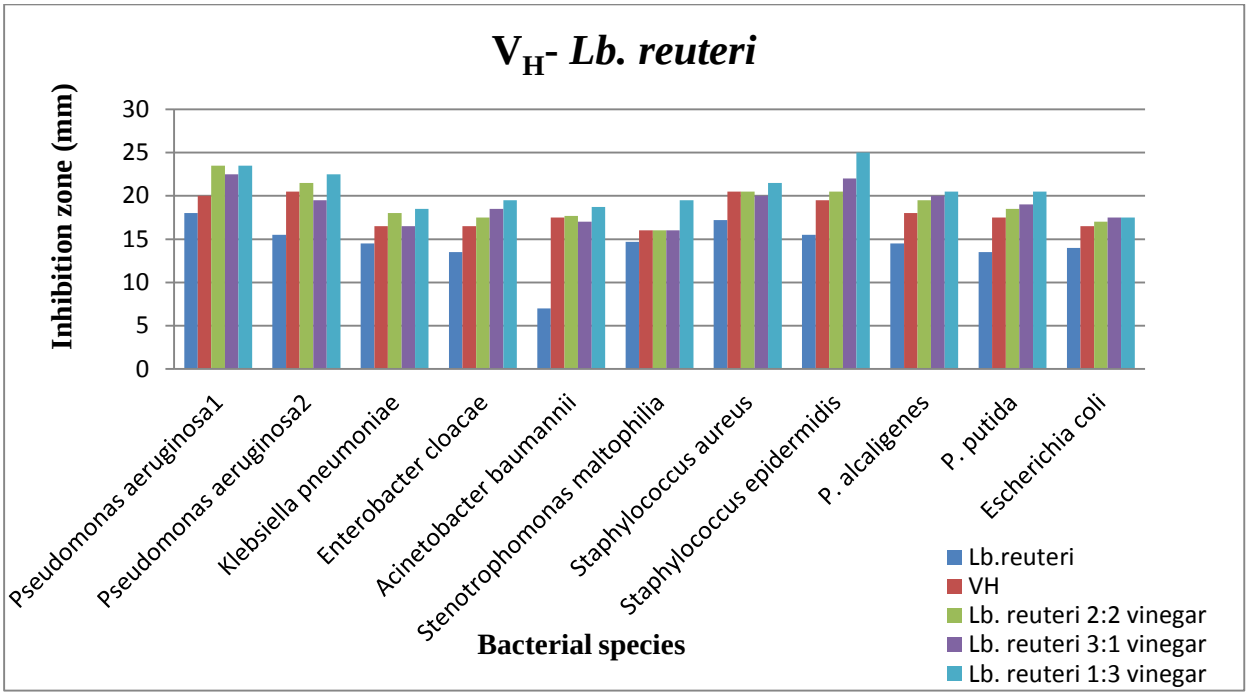

Figure 3.5 Inhibition zones against burn wound bacterial species formed by *Lb. reuteri* three-fold concentrated fermentation product alone, homemade apple vinegar (V_H) alone and their mixture.

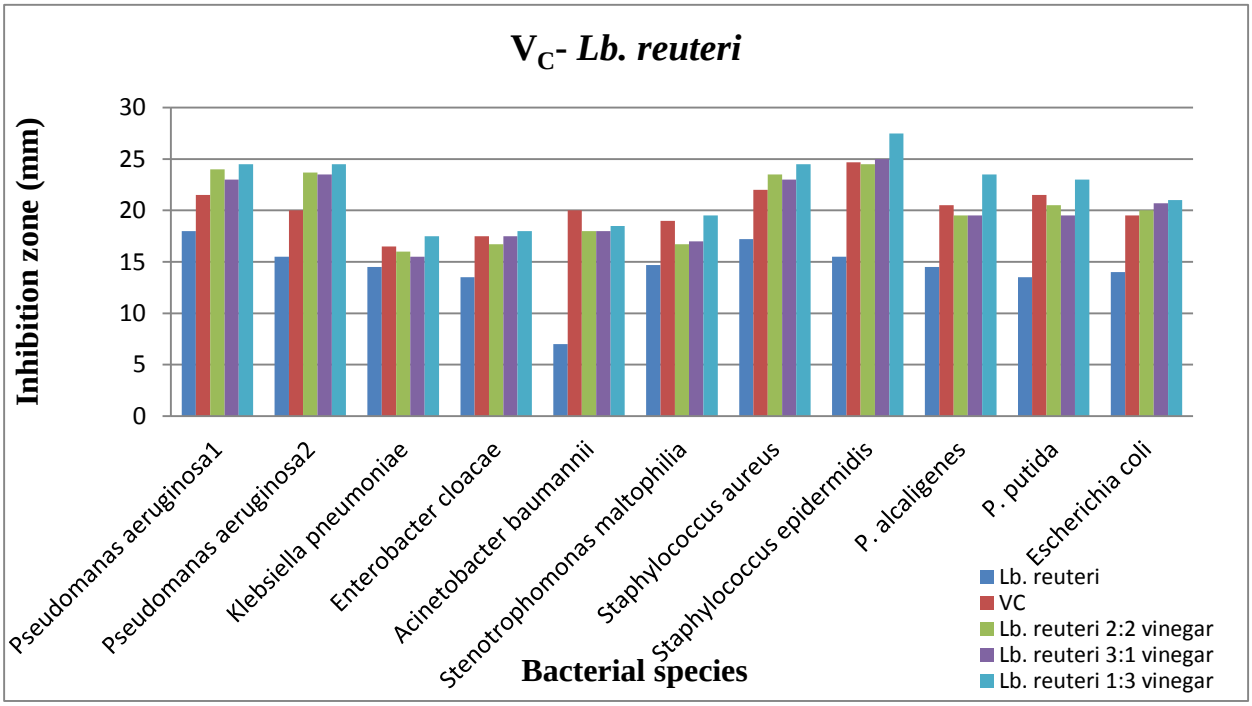

Figure 3.6 Inhibition zone against burn wound bacterial species formed by *Lb. reuteri* three-fold concentrated fermentation product alone, chemically synthesized apple vinegar (V_C) alone and their mixture.

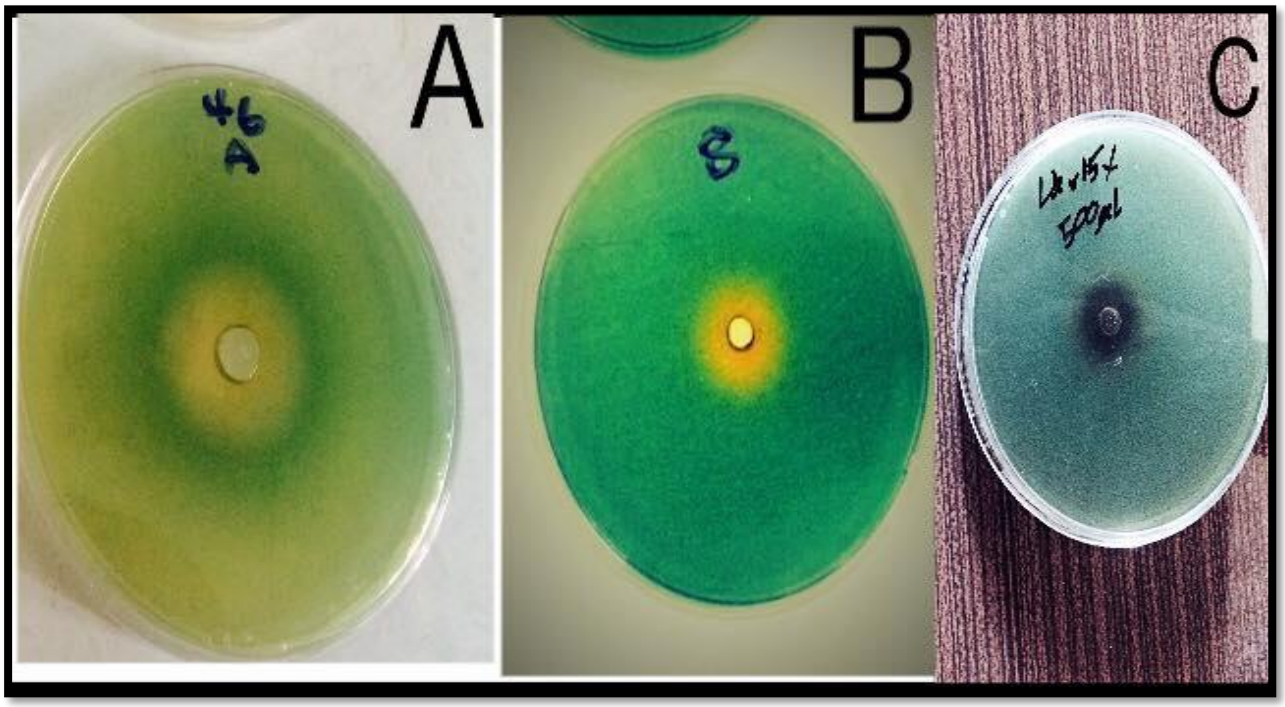

Figure 3.7 Inhibition zone against *P. aeruginosa* isolated from burn wound caused by (A) combination of *Lb. reuteri* three-fold concentrated fermentation product and chemically-synthesized apple vinegar at ratio 1:3, (B) chemically-synthesized apple vinegar alone and (C) *Lb. reuteri* three-fold concentrated fermentation product alone.

Chapter Four

Conclusions and Recommendations

4. Conclusions and Recommendations

4.1 Conclusions:

1- Polymicrobial pattern of infection was recorded in more than half of the burn wound patients.

2- *Pseudomonas aeruginosa* as a Gram negative and *Staphylococcus aureus* as a Gram positive bacteria were the most common pathogenic causatives.

3- As a probiotic, *Lactobacillus reuteri* exhibited inhibitory effect against bacteria causing burn wound infections, while a mixture of *Lb. acidophilus*, *Lb. reuteri, Lb. rhamnosus,* and *Bifidobacterium longum* did not do so.

4- Among the various types of apple vinegar used, the chemically-synthesized apple vinegar was the most effective in inhibiting growth of bacteria isolated from burn wound infections.

5- A synergistic effect was found between three-fold concentrated fermentation product of *Lb. reuteri* and apple vinegar when the highest antibacterial activity was achieved against burn wound infections bacteria in vitro.

4.2 Recommendations:

1- More studies are needed on the other types of nosocomial infections that caused by anaerobic bacteria and fungi.

2- Utilization of antibiotic should be periodically changed based on monitoring of antibiotic resistance trends.

3- More *in vivo* studies are needed on probiotics against pathogens isolated from burn wound infections.

4- Develop more therapeutic and prophylactic commercial medicines by combination of *Lb. reuteri* fermentation product or any other species of *Lactobacillus* and vinegar.

References

(A)

Abston, S.; Blakeney, P. and Desai, M. (2000). Acute Burn Management. Resident Orientation Manual. Galveston Shriners Burn Hospital and University of Texas Medical Branch Blocker Burn Unit.

Agnihotri, N.; Gupta, V. and Joshi, M. (2004). Aerobic bacterial isolate from burn wound infections and their antibiotics: a five-year study. J. Burns., 30: 241-3.

Alakomi, H. L.; Skytta, E.; Saarela, M.; MattilaSandholm, T.; Latva-Kala, K. and Helander, I. M. (2000). Lactic acid permeabilizes Gram-negative bacteria by disrupting the outer membrane. Appl Environ Microbiol., 66:2001–2005.

Alebachew, T.; Yismaw, G.; Derabe, A. and Sisay, A. (2012). *Staphylococcus aureus* burn wound infection among patients attending Yekatit 12 hospital burn unit, Addis Ababa, Ethiopia, Ethiop. J. Health Sci., 22(3): 209-213.

Alexandre, Y.; Le Berre1, R.; Barbier, G. and Le Blay, G. (2014). Screening of *Lactobacillus* spp. for the prevention of *Pseudomonas aeruginosa* pulmonary infections. BMC Microbiology. 14:107.

Aljamali, N. M. (2012). Antimicrobial Activity of Different Types of Vinegar. Magazine of Alkufa University for Biolog. 4(2).

Al-Muhammadi, M. O. and Azeez, H. A. (2011). Some physiological changes in burn patients. Medical Journal of Babylon., 8(3): 303 – 319.

Alonso, A. and Martinez, J. L. (1997). Multiple antibiotic resistance in *Stenotrophomonas maltophilia*. Antimicrob Agents Chemother. 41: 1140-1142.

Altoparlak, U.; Erol, S.; Akcay, M. N.; Celebi, F. and Kadanali, A. (2004). The time-related changes of antimicrobial resistance patterns and predominant bacterial profiles of burn wounds and body flora of burned patients. Burns. 30:660 - 664.

Alwan, M. J.; Lafta, I. J. and Hamzah, A. M. (2011). Bacterial isolation from burn wound infections and studying antimicrobial susceptibility. Kufa Journal for Veterinary Medical Sciences, 2(1): 121-131.

Ammor, M.S.; Florez, A.B. and Mayo, B. (2007). Antibiotic resistance of non-enterococcal lactic acid bacteria and Bifidobacteria. Food Microbiology., 24(6): 559-570.

Anderson, R. C.; Cookson, A. L.; McNabb, W. C.; Park, Z.; McCann, M. J.; Kelly, W. J. and Roy, N. C. (2010). *Lactobacillus plantarum* MB452 enhances the function of the intestinal barrier by increasing the expression levels of genes involved in tight junction formation. BMC. Microbiol. 10:316.

Angel, D. E.; Lloyd, P.; Carville, K. and Santamaria, N. (2011). The clinical efficacy of two semi-quantitative wound swabbing techniques in identifying the causative organism(s). Int Wound J; 8:176–185.

Arqués, J. L.; Fernandez, J.; Gaya, P.; Nunez, M.; Rodriguez, E. and Medina, M. (2004). Antimicrobial activity of reuterin in combination with nisin against food-borne pathogens. Int J Food Microbiol.95(2):225-9.

Atlas, R. N.; Brown, A. E. and Paks, L. G. (1995). Laboratory Manual of Experimental Microbiology. (1st Ed.). Mosby. USA.

Aziz, Z. S.; Al-Muhanna, A. Sh.; Salman, A. J. and Alzuhairi, A. M. (2014) *Klebsiella* and *Raoultella* biotyping and probability of identification by Vitek-2 system. IJIRSET 3(4): 11289-11294.

(B)

Bailey, S.; Baron, E. and Finegold, S. (1990). Diagnosis Microbiology (8th Ed.). The C.V. Mosby. USA.

Bang, R. L.; Gang, R. K.; Sanyal, S. C.; Mokaddas, E. M. and Lari, A. R. (1999). Beta-haemolytic *Streptococcus* infection in burns. Burns. 25:242 – 6.

Barret, J. P. and Herndon, D. N. (2003). Effects of burn wound excision on bacterial colonization and invasion. Plast. Reconstr. Surg., 111:744 - 750.

Benbow, M. (2010). Wound swabs and chronic wounds. Practice Nurse 39(9):27–30.

Bessa, L. J.; Fazii, P.; Di Giulio, M. and Cellini, L. (2015). Bacterial isolates from infected wounds and their antibiotic susceptibility pattern: some remarks about wound infection. Int Wound J 2015; 12:47–52.

Benson, J. M. (2001). Microbiological Applications: Laboratory Manual in General Microbiology (8th Ed.). McGraw Hill.

Bierbaum, G. and Sahl, H. G. (2009). Lantibiotics: mode of action, biosynthesis and bioengineering. Curr. Pharm. Biotechnol., 10: 2-18.

Bjarnsholt, T. (2013). The role of bacterial biofilms in chronic infections. APMIS Suppl. 136:1-51.

Bjarnsholt,T.; Alhede,M.; Jensen,P. O.; Nielsen,A. K.; Johansen, H. K.; Homøe,P.; Hoiby, N.; Givskov, M. and Kirketerp-Moller, K. (2015). Anti-biofilm properties of acetic acid. Adv Wound Care. 4(7): 363–372.

Bomba, A.; Jonecová, Z,; Koščová, J.; Nemcová, R.; Gancarčíková, S.; Mudroňová, D.; Sciranková, Ľ.; Buleca, V.; Lazar, G.; Pošivák, J.; Kašteľ, R. and Marecová, M. (2006). The improvement of probiotics efficacy by synergistically acting components of natural origin: a review. Biologica Bratislava. 61(6): 729 – 734.

Boonaert, C. J. P. and Rouxhet, P. G. (2000). Surface of lactic acid bacteria: relationship between chemical composition and physiochemical properties. Appl. Environ. Microbiol. 66(6): 2548 - 2554.

Botes, M.; Loos, B.; Van Reenen, C. A. and Dicks, L. M. T. (2008). Adhesion of probiotic strains *Enterococcus mundtii* st4sa and lactobacillus plantarum 423 to caco-2 cells under conditions simulating the intestinal tract and the presence of antibiotics and anti-inflammatory medicaments. Arch. Microbiol., 190: 573 - 584.

Bowler, P. G.; Duerden, I. and Armstrong, D. G. (2001). Wound microbiology and associated approaches to wound management. Clin Microbiol Rev. 14(2): 244–269.

Brochors, A. T.; Selmi, C.; Meyers, F. J.; Kean, C. L. and Gershwin, M. E. (2009). Probiotics and immunity. J. Gastroenterol. 44:26-46.

Brown, V. I. and Lowbury, E. J. L. (1965). Use of an improved cetrimide agar medium and other culture methods for *Pseudomonas aeruginosa*. J. Clin. Path. (18): 752-756.

(C)

Chapman, C. M. C.; Gibson, G. R. and Rowland, I. (2012). *In vitro* evaluation of single- and multi-strain probiotics: Inter-species inhibition between probiotic strains, and inhibition of pathogens. Anaerobe, Vol. 10. PMID: 22677262.

Church, D.; Elsayed, S.; Reid, O.; Winston, B. and Lindsay, R. (2006). Burn wound infections. Clin. Microbiol. Rev., 19 (2), 403 – 434.

Cicenia, A.; Scirocco, A.; Carabotti, M.; Pallota, L.; Mariganani, M. and Severi, C. (2014). Postbiotic activities of Lactobacillus-derived factors. J. Clin. Gastroenterol. 1:18-22.

Clark, N. M.; Patterson, J. and Lynch III, J. P. (2003). Antimicrobial resistance among gram-negative organisms in the intensive care unit. Curr. Opin. Crit. Care .9:413 - 423.

Cleusix, V.; Lacroix, C.; Vollenweider, S.; Duboux, M. and Le Blay, G. (2007). Inhibitory activity spectrum of reuterin produced by *Lactobacillus reuteri* against intestinal bacteria. BMC Microbiol 12:7-101.

Clinical Laboratory Standards Institute (CLSI). (2012). Performance Standards for Antimicrobial Disk Susceptibility Tests. Approved standard, 11th Ed.

Cooper, R. and Lawrence, J. C. (1996). The isolation and identification of bacteria from wounds. J Wound Care. 5:335–40.

Collado, M. C.; Isolauri, E.; Salminen, S. and Sanz, Y. (2009). The impact of probiotics on gut health. Curr. Drug. Metab. 10(1):68-78.

Corcionivoschi, N.; Drinceeanu, D.; Stef, L.; Luca, J.; Julean, C. and Mingyrat, O. (2010). Probiotics identification and ways of action. Innovative Romanian Food Biotechnology. 6:1-11.

Cutting, K. F. (2010). Addressing the challenge of wound cleansing in the modern era. Br J Nurs (Tissue Viability Supplement). 19(11): S24–S29.

(D)

Dalamaga, M.; Karmaniolas, K.; Chavelas, C.; Liatis, S.; Matekovits, H. and Migdalis, I. (2003). *Stenotrophomonasmaltophilia*: A serious and rare complication in patients suffering from burns. Burns. 29:711-713.

deVrese, M.; Stegelmann, A.; Richter, B.; Fenselau, S.; Laue, C. and Schrezenmeir, J. (2001). Probiotics - compensation for lactase insufficiency. American Journal of Clinical Nutrition. 73(2 Suppl.): 421 -S429.

Denev, S. A. (2006). Role of Lactobacilli in gastrointestinal ecosystem. Bulgarian Journal of Agricultural Science. 12(1): 63- 114.

Doron, S. and Gorbach, S. L. (2006). Probiotics: their role in the treatment and prevention of disease. Expert. Rev. Anti. Infect. Ther. 4(2): 261 – 275.

Doron, S.; Snydman, D. R. and Gorbach, S. L. (2005). *Lactobacillus* GG: Bacteriology and clinical applications. Gastroenterology Clinics of North America., 34:483-498.

Dow, G.; Browne, A. and Sibbaild, R. G. (1999). Infection in chronic wounds: controversies in diagnosis and treatment. Ostomy/ Wound Manage. 45: 23-40.

Drosou, A.; Flabella, A. and Kirsner, R. (2003). Antiseptics on Wounds- an area of Controversy. Wounds. 15:149.

Duary, R.K.; Singh, Y.R.; Batish, V. K. and Grover, S. (2011). Assessing the adhesion of putative indigenous probiotic lactobacilli to human colonic epithelial cells. Indian Journal of Medical Research. 134(5): 664-671.

(E)

Edwards, R. and Harding, K. G. (2004). Bacteria and wound healing. Curr. Opin. Infect. Dis., 17:91–96.

Ekrami, A. and Kalantar, E. (2007). Bacterial infections in burn patients at a burn hospital in Iran. Indian J. Med. Res. 126: 541-544.

Elli, M.; Zink, R.; Rytz, A.; Reniero, R. and Morelli, L. (2000). Iron requirement of *Lactobacillus spp* in completely chemically defined growth media. J. Appl. Microbiol., 88: 695 – 703.

El-Ziney, M. and Debevere, J. (1998). The effect of reuterin on *Listeria monocytogenes* and *Escherichia coli* O157:H7 in milk and cottage cheese. J. Food Prot. 61:1275–1280.

El-Ziney, M.G.; van den Tempel, T.; Debevere, J. and Jakobsen, M. (1999). Application of reuterin produced by *Lactobacillus reuteri* 12002 for meat decontamination and preservation. J. Food Prot. 62(3): 257-261.

Embil, J. M. ; McLeod, J. A. ; Al-Barrak, A. M. ; Thompson, G. M. ; Aoki, F. Y. ; Witwicki, E. J. ; Stranc, M. F. ; Kabani, A. M. ; Nicoll, D. R. and Nicolle, L. E.

(2001). An outbreak of methicillin resistant *Staphylococcus aureus* on a burn unit: Potential role of contaminated hydrotherapy equipment. Burns. 27: 681–688.

Entani, E.; Asai, M.; Tsujihata, S.; Tsukamoto, Y. and Ohta, M. (1998). Antibacterial Action of Vinegar against Food-Borne Pathogenic Bacteria Including *Escherichia coli* O157:H7. Journal of Food Protection. 7: 929-1086.

Espinal, P.; Marti, S. and Vila, J. (2012). Effect of biofilm formation on the survival of *Acinetobacter baumannii* on dry surfaces. The Journal of Hospital Infection 80(1): 56-60.

Estrela, C.; Holland, R.; Bernabe, P. F.; de Souza, V. and Estrela, C. R. (2004). Antimicrobial potential of medicaments used in healing process in dogs' teeth with apical periodontitis. Braz Dent J, 15: 181 -185.

(F)

Fontana, L.; Bermudez-Brito, M.; Plaza-Diaz, J.; Muñoz-Quezada, S. and Gil, A. (2013). Sources, isolation, characterization and evaluation of probiotics. Br J Nutr. 109 Suppl. 2: S35–S50.

Forbes, B. A.; Sahm, D. F. and Weissfeld, A. S. (1998). Bailley and Scott Diagnostic Microbiology. 10th ed., Mosby.

Forbes, B. A.; Sahm, D. F. and Weissfeld, A. S. (2007). Bailey & Scott's Diagnostic Microbiology 12th Edition: Mosby Elsevier, St. Louis. USA.

Foster, T. J. (2004). The *Staphylococcus aureus* "superbug." J. Clin. Investig.114:1693 –1696.

Fuller, R. (1989). Probiotics in man and animals. Journal of Applied Bacteriology. 66, 365-378.

(G)

Gales, A. C.; Jones, R. N.; Turnidge, J.; Rennie, R. and Ramphal, R. (2001). Characterization of *Pseudomonas aeruginosa* isolates: occurrence rates, antimicrobial susceptibility patterns, and molecular typing in the global SENTRY antimicrobial surveillance program, 1997–1999. Clin. Infect. Dis., 32(Suppl. 2): S146–S155.

Garcia-Garrote, F.; Cercenado, E. and Bouza, E. (2000). Evaluation of a New System, VITEK 2, for Identification and Antimicrobial Susceptibility Testing of *Enterococci*. J Clin Microbiol.38(6): 2108–2111.

Garner, D.; Crisosto, C. H.; Wiley, P. and Crisosto, G. M. (2008). Measurement of pH and Titratable Acidity. Central Valley Postharvest Newsletter. 17(2): 2.

Garrity, G. (2005) Bergey's Manual of Systematic Bacteriology. (2nd Ed.). Vol.2. Williams and Wilkins, Baltimore. London.

Giahi, L.; Aumueller, E.; Elmadfa, I. and Haslberger, A. G. (2012) Regulation of TLR4, p38 MAPkinase, IκB and miRNAs by inactivated strains of lactobacilli in human dendritic cells. Benef Microbes. 3: 91–98.

Gillespie, S. H. and Hawkey, P. M. (2006). Principles and Practice of Clinical Bacteriology 2nd Ed. John Wiley and Sons. England.

Ginés, S.C.; Maldonado, M.C.; Valdez, G.F. (2000). Purification and characterization of invertase from *Lactobacillus reuteri* CRL 1100. Current Microbiol. 40: 181-184.

Gorbach, S. L. (2000). Probiotics and gastrointestinal health. Am. J. Gastroenterol. 95: S1 – S4.

Grajek, W.; Olejnik, A. and Sip, A. (2005). Probiotics, prebiotics and antioxidants as functional foods. Acta Biochimica polonica. 52(3): 665 – 671.

Greenhalgh, D.G.; Saffle, J.R.; Holmes 4th, J. H.; Gamelli, R.L.; Palmieri, T. L. and Horton, J.W. (2007). American Burn Association, Consensus Conference to Define Sepsis and Infection in Burns. J. Burn Care Res., 28(7):776 - 90.

Gupta, M.; Gupta, O. K.; Yaduvansh , R. K. and Upadhyahy , J. (1993). Burn epidemiology: The pink city scene. Burns. 19: 47-51.

(H)

Hansbrough, J.F. (1987). Burn Wound Sepsis. J. Intensive Care Med. 2: 313-327.

Hansson, C. and Faergemann, J. (1995). The Effect of Antiseptic Solutions on Microorganisms in Venous Leg Ulcers. Acta. Derm. Venereol. 75:31- 3.

Harrison-Balestra, C.; Cazzaniga, A. L.; Davis, S. C. and Mertz. P. M. (2003). A wound-isolated *Pseudomonas aeruginosa* grows a biofilm in vitro within10 hours and is visualized by light microscopy. Dermatol. Surg., 29:631–635.

Havenaar, R. and Huisin't Veld, J. H. J. (1992). Probiotics: a general review. In Wood, B. J. B. and Holzapfel, W.H. (Ed.), The Lactic Acid Bacteria (pp. 151-170). New York: Elsevier Applied Science.

Heikefelt, C. (2011). Chemical and Sensory Analyses of Juice, Cider and Vinegar Produced Different Apple Cultivars.

Hess, P.; Altenhöfer, A.; Khan, A. S.; Daryab, N.; Kim, K. S.; Hacker, J. and Oelschlaeger, T. A. (2004). A Salmonella fim homologue in *Citrobacter freundii* mediates invasion in vitro and crossing of the blood brain barrier in the rat pup model. Infect. Immun., 72: 5298 – 5307.

Higgins, P.; Perez-Llarena, F.; Znder, E.; Fernandez, A.; Bou, G. and Seifert, H. (2013). OXA-235, a novel Class D beta-lactamase Involved in Resistance to Carbapenems in *Acinetobacter baumannii*. Antimicrobial agents and chemotherapy.

Hijum, S.A.F.T.; Bonting, K.; Maarel, M.J.E.C.; Dijkhuizen, L. (2001). Purification of a novel fructosyltransferase from *Lactobacillus reuteri* strain 121 and characterization of the levan produced. FEMS Microbiol. Lett., 205, 323-328.

Hindi, N. K. K. (2013). In vitro Antibacterial Activity of Aquatic Garlic Extract, Apple Vinegar and Apple Vinegar - Garlic Extract combination. American J Phytomedicine and Clinic Therapeutics; (1): 042-051.

Hirano, J.; Yoshida, T.; Sugiyama, T.; Koide, N.; Mori, I. and Yokochi, T. (2003). The effect of *Lactobacillus rhamnosus* on enterohemorrhagic *Escherichia coli* infection of human intestinal cells in vitro. Microbiol Immunol. 47:405–409.

Holt, J.; Krieg, N.; Sneath, P. and Williams, S. (1994). Bergey's Manual of determinative Bacteriology (9th Ed.). Williams and Wilkins. Maryland. USA.

Holt, J.G.; Krieg, N.R.; Sneath, P.H.A.; Staley, J.T. and Williams, S.T. (1994). Facultatively Anaerobic Gram-Nagative Rods. In: Bergey's Mannual of Systematic Bacteriology, Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T.

Hou, C.; Zeng, X.; Yang, F.; Liu, H. and Qiao, S. (2015). Study and use of the probiotic *Lactobacillus reuteri* in pigs. Journal of Animal Science and Biotechnology. 6-14.

Hummel, A.S.; Hertel, C.; Holzapfel, W.H. and Franz, C. (2007). Antibiotic resistance of starter and probiotic strains of Lactic acid bacteria. Applied and Environmental Microbiology. 73(3): 730-739.

Hummel, S.; Veltman, K.; Cichon, C.; Sonnenborn, U. and Schmidt, M. A. (2012). Differential targeting of the E-cadherin/β-catenin complex by gram-positive probiotic Lactobacilli improves epithelial barrier function Appl. Environ. Microbiol; 78:1140–1147.

(I)

Iona, M.; Mootilia, M.; Mihaela, Z.; Mariana, N. and Laura, V. (2010). Antibiotic sensitivity of *Escherichia coli* strains isolated from infected skin wounds. Farmacia. 58(5): 637.

Ishibashi, N.; Yaeshima, T. and Hayasawa, H. (1997). Bifidobacteria: Their Significance in Human Intestinal Health. Mal. J. Nutr., 3: 149-159.

Ismael, N. F. (2013). "Vinegar" as Antibacterial Biofilm formed by *Streptococcus pyogenes* Isolated from Recurrent Tonsillitis Patients, in vitro. Jordan Journal of Biological Sciences. 6(3): 191- 197.

Isolauri, E.; Joensuu, J.; Suomalainen, H.; Luomala, M. and Vesikari, T. (1995). Improved immunogenicity of oral D × RRV reassortant rotavirus vaccine by *Lactobacillus casei* GG. Vaccine. 13: 310-312.

Isolauri, E.; Sütas, Y.; Kankaanpää, P.; Arvilommi, H. and Salminen, S. (2001) Probiotics: effects of immunity. American Journal of Clinical Nutrition., 73 (2 Suppl.): S444-S450.

(J)

Jacobsen, C. N.; Nielsen, V. R.; Hayford, A. E.; Møller, P. L.; Michaelsen, K. F.; Pærregaard, A.; Sandström, B.; Tvede, M. and Jakobsen, M. (1999). Screening of probiotic activities of forty-seven strains of *Lactobacillus* sp. by in-vitro techniques and evaluation of colonization ability of five selected strains in human. Applied and Environmental Microbiology. 65: 4949-4956.

Jacobsen, C.N.; Nielsen, V.R.; Hayford, A.E.; Moller, P.L.; Michaelsen, K.F.; Paerregaard, A.; Sandstrom, B.; Tvede, M. and Jakobsen, M. (1999) Screening of probiotic activities of forty-seven strains of Lactobacillus sp. by in-vitro techniques and evaluation of colonization ability of five selected strains in human. Applied and Environmental Microbiology 65: 4949-4956.

Johnson, J. R.; Moseley, S. L.; Roberts, P.L. and Stamm, W. E. (1988). Aerobactin and other virulence factor genes, among strains of *Escherichia coli*

causing urosepsis: association with patient characteristics. Infect. Immune. 56: 405 – 412.

Johnston, C. S and Gaas, C. A. (2006). Vinegar: Medicinal Uses and Antiglycemic Effect. Med. Gen. Med., 8(2):61.

Jonsson, H.; Ström, E.; Roos, S. (2001). Addition of mucin to the growth medium triggers mucus-binding activity in different strains of *Lactobacillus reuteri* in vitro. FEMS Microbiol. Lett., 204, 19-22.

Juntunen, M.; Kirjavainen, P. V.; Ouwehand, A. C.; Salminen, S. J. and Isolauri, E. (2001). Adherence of probiotic bacteria to human intestinal mucus in healthy infants and during rotavirus infection. Clin. Diag. Lab. Immunol., 8: 293–296.

(K)

Kaplan, O. and Bakir, U. (1998). The effect of chemical crosslinking of invertase with dimethyl suberimidate on its pH stability. World J. Microbiol., and Biotechnol., 14: 277-280.

Kasten, K. R.; Makley, A. T. and Kagan, R. J. (2011). Update on the critical care management of severe burns. J Intensive Care Med. 26(4):223-36.

Kawai, Y.; Ishii, Y.; Uemura, K.; Kitazawa, H.; Saito, T.; Itoh, T. (2001). *Lactobacillus reuteri* LA6 and *Lactobacillus gasseri* LA39 isolated from faeces of the same human infant produce identical cyclic bacteriocin. Food Microbiol. 18: 407-415.

Keen, E. F 3rd.; Robinson, B. J. and Hospenthal, D. R. (2010). Incidence and bacteriology of burn infections at a military burn center. *Burns*.36(4):461-8.

Kjolseth, D.; Frank, J. and Barker, J. (1994). Comparison of the Effects of Commonly Used Wound Agents on Epithelialization and Neovascularization. J. Am. Coll. Surg., 179:305-12.

(L)

Lambert, P. A. (2002). Mechanisms of antibiotic resistance in *Pseudomonas aeruginosa*. J R Soc Med. 95 (41): 22–26.

Laupland, K. B.; Parkins, M. D.; Church, D. L.; Gregson,D. B.; Louie, T. J.; Conly, J. M.; Elsayed, S. and Pitout, J. D. (2005). Population-based epidemiological study of infections caused by carbapenem-resistant *Pseudomonas aeruginosa* in the calgary health region: importance of metallo- betalactamase (MBL)-producing strains. J. Infect. Dis., 192:1606–1612.

Leboffe, M. J. and Pierce, B. E. (2011). A photographic Atlas for The Microbiology Laboratory. (4th Ed.). 65-95. Morton Publishing. Colorado.

Leveen, H. (1973). Chemical Acidification of Wounds. Annals of Surgery. 6: 745 - 753.

Levinson, W. and Jawetz, E. (2000). Medical Microbiology and Immunology (4th Ed). Appleton and Lange.

Lewus, C. B.; Kaiser, A. and Montville, T. J. (1991). Inhibition of food-borne bacterial pathogens by bacteriocins from lactic acid bacteria isolated from meat. J. Appl. Environ. Microbiol. 57: 1683 – 1688.

Lilley, D. M. and Stillwell, R. H. (1965). Probiotics: Growth promoting factor produced by microorganisms. Science. 147: 747-748.

Lilly, H. A.; Lowbury, E. J.; Wilkins, M. D. and Cason, J. S. (1979). Staphylococcal sepsis in a burns unit. J. Hyg., (London) 83:429 – 435.

Lin, C. K.; Tsai, H. C.; Lin, P. P.; Tsen, H. Y. and Tssai C. C. (2008). *Lactobacillus acidophilus* LAP5 able to inhibit the *Salmonella choleraesuis* invasion to the human Caco-2 epithelial cell. Anaerobe. 14: 251 – 255.

Lineaweaver, W.; Howard, R.; Soucy, D.; McMorris, S.; Freeman, J and Crain, C. (1985). Topical Antimicrobial Toxicity. Arch Surg., 120(3): 267-70.

Lingham, T.; Besong, S.; Ozbay, G. and Lee, JL. (2012). Antimicrobial Activity of Vinegar on Bacterial Species Isolated from Retail and Local Channel Catfish (*Ictalurus punctatus*). J Food Process Technol. S11-001.

Livemore, D.; Oakton, K.; Carter, M. and Warner, M. (2001). Activity of Etrapenem (MK-0862) versus Enterobacteriaceae with potent lactamases. Antimicrob. Agents Chemother. 45(2):31-7.

Liwimbi, O. M. and Komolafe, I. O. O. (2007). Epidemiology and bacterial colonization of burn injuries in Blantyre. Malawi Medical Journal. 19(1): 25-27.

Luterman, A.; Dasco, C. C. and Curreri, P. W. (1986). Infection in Burn Patients. Am. J. Med., 81: 45-52.

Luthi-Peng, Q., Dileme, F. B. & Puhan, Z. (2002). Effect of glucose on glycerol bioconversion by *Lactobacillus reuteri*. Appl Microbiol Biotechnol 59, 289–296.

(M)

Mack, D.; Becker, P.; Chatterjee, I.; Dobinsky, S.; Knobloch, J. K.; Peters, G.; Rohde, H. and Herrmann, M. (2004). Mechanisms of Biofilm Formation in *Staphylococcus epidermidis* and *Staphylococcus aureus*: Functional Molecules, Regulatory Circuits, and Adaptive Responses. Int. J. Med. Microbiol. 294:203–212.

Mackie, A. and McCartney, C. (1996). Practical Medical Microbiology (14th Ed.). In: J. G. Collee, A. G. Fraser, B. P. Marmion, and A. Simmons. (Eds). Churchill. Livingstone.

Magnet, M. H.; Arongozeb; Khan, G. M. and Ahmed, Z. (2013). Isolation and identification of different bacteria from different types of burn wound infections and study their antimicrobial sensitivity pattern. International
Journal of Research in Applied Natural and Social Sciences. 1(3): 125-132.

Mah, T.-F.C. and O'Toole, G.A. (2001). Mechanism of biofilm resistance to antimicrobial agents. Trends Microbiol 9: 34–39.

Makras, L.; Triantafyllou, V.; Fayol-Messaoudi, D.; Adriany, T.; Zoumpopoulou, G.; Tsakalidou, E.; Servin, A. and DeVuyst, L. (2006) Kinetic analysis of the antibacterial activity of probiotic lactobacilli towards *Salmonella enterica* serovar

typhimurium reveals a role for lactic acid and other inhibitory compounds. Res. Microbiol.157:241–247.

Malicki, A.; Zawadzki, W.; Bruzewicz, S.; Graczyk, S. and Czerski, A. (2004). Effect of formic and propionic acid mixture on *Escherichia coli* in fish meal stored at 12ºC. Pakistan J Nutrition. 3: 353–356.

Manjula, M.; Priya, D. and Varsha, G. (2007). Bacterial isolates from burn wound infections and antibiograms: eight-year study. Ind J Plastic Surg. 40:22-25.

Manson, W. L.; Pernot, P. C.; Fidler, V. and Sauer, E. W. (1992). Colonization of burns and duration of hospital stay of severely burned patients. J. Hosp. Infect., 22: 55-63.

Manus, A.; Mason, A.; Manus, W. and Pruitt, B. (1994). A decade of reduced gram negative infections and mortality improved isolation of burned patients. Arch. Surg., 129: 1306 - 9.

Martineau, L. and Dosch, H. M. (2007). Biofilm reduction by a new burn gel that targets nociception. J Appl Microbiol. 103(2):297-304.

Marvaki, C.; Joannovich, I.; Kiritsi, E.; Iordanou, P. and Iconomo, T. (2001).The effecttiveeness of early enteral nutrition in burn patients. Annals of Burns and Fire Disasters. 16 (4).

Mayhall, C. G. (2003). The epidemiology of burn wound infections: then and now. Clin Infect Dis 37: 543–550.

Medina, E.; Romero, C.; Brenes, M. and De Castro, A. (2007). Antimicrobial activity of olive oil, vinegar and various beverages against food borne pathogens. Journal of Food Protection. 7(5): 1194-9.

Meka, V. G.; Pillai, S. K.; Sakoulas, G.; Wennersten, C.; Venkataraman, L.; DeGirolami, P. C. ; Eliopoulos, G. M. ; Moellering, Jr., R. C. and Gold, H. S. (2004). Linezolid resistance in sequential *Staphylococcus aureus* isolates associated with a T2500A mutation in the 23SrRNA gene and loss of a single copy of rRNA. J. Infect. Dis., 190:311–317.

Metchnikoff, E. (1907). The prolongation of life: Optimistic study (P. C. Mitchell, Trans.). London: Heinemann.

Milner, S. (1992). Acetic Acid to Treat *Pseudomonas aeruginosa* in Superficial Wounds and Burns. The Lancet. 340:61.

Mitsouka, T. (1992). The human gastrointestinal tract. In B. Wood (ed.), The lactic acid bacteria in health and disease. Elsevier Appl. Science, Vol. 1, 69-14.

Monafo, W. W. and Freedman, B. (1987). Topical Therapy for Burns. Surg Clin North Am. 67: 133-145.

Mooney, D. P. and Gamelli, R. L. (1989). Sepsis Following Thermal Injury. Comp. Ther. 15: 22 29.

Mooney, D. P. and Gamelli, R. L. (1989). Sepsis following thermal injury. Comp Ther. 15:22 – 29.

Morales, M.; Tesfay, W.; Garcia-Parrilla, M.; Casas, J and Troncoso, A. (2002). Evolution of the Aroma Profile of Sherry Wine Vinegars During An experimental Aging in Wood. J. Agric. Food Chem., 50: 3173-3178.

Murray, C. and Hospenthal, D.R. (2008). "Burn Wound Infections" Available at : http://emedicine.medscape.com/article/213595-overview.

Natera, R.; Castro, R.; Garcia-Moreno, M.; Hernandez, M. and Garcia-Barroso, C. (2003). Chemometric Studies of Vinegars from Different Raw Materials and Process of Production. J. Agric. Food Chem., 51: 3345-3351.

(N)

National Committee for Clinical Laboratory Standards. (1993). Methods for determining bactericidal activity of antimicrobial agents. Tentative Guidelines, M26-T NCCLS. Villanova, PA.

Ng, S.C.; Hart, A.L.; Stagg, A.J. and Knight, S.C. (2009) Mechanisms of action of probiotics: recent advances. Inflammatory Bowel Diseases 15: 300-310.

Nikaido, H. (1996). Multidrug efflux pumps of gram-negative bacteria. J. Bacteriol. 178(20):5853-9.

(O)

Oelschlaeger, T. A. (2010). Mechanisms of probiotic actions – A review. Int. J. Med. Microbiol., 300(1): 57 – 62.

Ogunsola, F. T.; Oduyebo, O.; Iregbu, K. C.; Coker, A. O. and Adetunji, A. (1998). A review of nosocomial infections at LUTH: Problems and strategies for improvement. J. Nigerian infection Control Association., 1: 14-20.

Order, S. E.; Mason, A. D.; Switzer, W. F. and Moncrief, J. A. (1965). Arterial Vascular Occlusion and Devitalization of Burn Wounds. Ann Surg. 161: 502-508.

Ouwehand, A. C. (1998). Antimicrobial components from lactic acid bacteria; in Salminen S, von Wright A (ed.): Lactic Acid Bacteria: Microbiology and Functional Aspects. New York, Dekker, pp 139–159.

(P)

Parker, R. B. (1974). Probiotics, the other half of the antibiotic story. Animal Nutrition and Health. 29: 4-8.

Paterson, D. and Yu, V. (1999). Editorial Response: Extended-spectrum β-lactamases: a cell for improved detection and control. Clin Infect Dis. 29: 1419-1422.

Perdigon, G.; Maldonado Galdeano, C.; Valdez, J. C. and Medici, M. (2002). Interaction of lactic acid bacteria with the gut immune system. Eur. J. Clin. Nutr. 56: S21–S26.

Peterson, D. (2002). Looking for Risk Factors for the Acquisition of Antibiotic Resistance: A 21st Century Approach. 34: 1564-1567.

Phillips, L. G.; Heggers, J. P.; Robson, M. C.; Boertman, J. A.; Meltzer, T. and Smith, D. J. Jr. (1989). The effect of endogenous skin bacteria on burn wound infection. Ann. Plast. Surg., 23:35 - 38.

Plaza-Diaz, J.; Munoz-Quezada, S.; Gomez-Llorente, C. and Gil A. (2012). Probiotic mechanisms of action. Ann Nutr Metab. 61:160-174.

Pochapin, M. (2000). The effect of probiotics on *Clostridium difficile diarrhoea*. American Journal of Gastroenterology. 95(suppl.): S11-S13.

Pruitt, B. A.; Colonel, M. C. and McManus, A. D. (1984). Opportunistic infections in severely burnt patients. Am. J. Med., 76: 164-154.

Pruitt, B. A.; McManus, A. T.; Kim, S. H. and Goodwin, C. W. (1998). Burn Wound Infections: Current Status. World J Surg. 22:135-145.

(Q)

Quinn, J. P. (1998). Clinical problems posed by multiresistant nonfermenting gram-negative pathogens. Clin Infect Dis. 27:117-124.

(R)

Raja, N. S. and Singh, N. N. (2007). Antimicrobial susceptibility pattern of clinical isolates of *Pseudomonas aeruginosa* in tertiary care hospital. J Microbiol Immunol Infect. 40(1): 45-49.

Ramzy, P. I.; Wolf, S. E.; Irtun, O.; Hart, D. W.; Thompson, J. C. and Herndon. D. N. (2000). Gut epithelial apoptosis after severe burn: Effects of gut hypoperfusion. J. Am. Coll. Surg., 190(3): 281 - 287.

Rashid, M. H.; Rumbaugh, K.; Passador, L.; Davies, D.G.; Hamood, A.N.; Iglewski, B.H. and Kornberg, A. (2000) Polyphosphate kinase is essential for biofilm development, quorum sensing, and virulence of *Pseudomonas aeruginosa*. Proc Natl Acad Sci USA 97: 9636–9641.

Rastegar, A.; Alaghehbandan, R. and Akhlaghi, L. (2005). Burn wound infection and antimicrobial resistance in Tehran, Iran: An increasing problem. Ann Burn Fire Disasters; 18: 1115-8.

Rastegar, A.; Bahrami, H. and Alaghehbandan, R. (1998). *Pseudomonas* infection in Tohid burn centre, Iran. Burns. 24: 637-41.

Rattanachaikunsopon, P. and Phumkhachorn P. (2010). Lactic acid bacteria: their antimicrobial compounds and their uses in food production. Annals of Biological Research. 1(4): 218-228.

Resn, N. S. (2013). A comparative Study on Treatment of Diabetic Foot Infection by Acetic Acid, Rifampcin and Probiotics. MSc. Thesis. Al-Nahrain University.

Rice, L.; Willey, S.; Papanicolason, G.; Merdeiros, A.; Eliopoulos, G.; Moellering, R. and Jacoby, G. (1990). Outbreak of ceftazidime resistance caused by extended-spectrum of β- lactamase at a Massachusetts chronic-care facility. Antimicrob Agents Chemother. 34(11):2193-9.

Ricke, S. C. (2003). Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. Poultry Sci. 82: 632–639.

Roberfroid, M. (1993) Dietary fiber, inulin, and oligofructose: A review comparing their physiological effects. Critical Reviews in Food Science and Nutrition. 33 (2):103-148.

Roos, N. M.; Katan, M. B. (2000). Effects of probiotic bacteria on diarrhea, lipid metabolism, and carcinogenesis: a review of papers published between 1988 and 1998. American Journal of Clinical Nutrition. 71 (2): 405-411.

Roth, J. J. and Hughes. W. B. (2004). The essential burn unit handbook. Quality Medical Publishing, St. Louis, Mo.

Rund, C. (1996). Nonconventional topical therapies for wound care. Ostomy Wound Manage. 42: 22-24.

(S)

Sabir, N.; Rafi, S.; Hashmi, I. and Farooqi, B. J. (2007). Lumbar osteomyelitis with *Pseudomonas*. J Pak Med Assoc. 57(10): 517-8.

Salminen, S.; Ouwehand, A.; Benno, Y. and Lee, Y. K. (1999). Probiotics: how should they be defined? Trends in food science and technology. 10: 107-110.

Sarra, P.G.; Magri, M.; Bottazzi, V.; Dellaglio, F. and Bosi, E. (1979). Frequenza di bacilli lattici eterofermentanti nelle feci di vitelli lattanti. Arch. Vet. Ital., 30: 16-21.

Sartor, R. B. (2006). Mechanisms of disease: Pathogenesis of Crohn's disease and ulcerative colitis. Nat. Clin. Pract. Gastroenterol. Hepatol. 3:390–407.

Schiffrin, E. J. and Blum, S. (2002). Interactions between the microbiota and the intestinal mucosa. Eur. J. Clin. Nutr. 56:S60–S64.

Schiffrin, E.J.; Rochat, F.; Link-Amster, H.; Aeschlimann, J.M. and Donnet-Hughes, A. (1995). Immunomodulation of human blood cells following the ingestion of lactic acid bacteria. J. Dairy Sci., 78 (3): 491-497.

Schnurer, J. and Magnusson, J. (2005). Antifungal lactic acid bacteria as biopreservatives. Trends Food Sci. Technol. 16:70–78.

Shah, N.P. (2007). Functional cultures and health benefits. International Dairy Journal. 17(11): 1262-1277.

Sloss, J.; Cumberland, N. and Milner, S. (1993). Acetic Acid Used for Elimination of *Pseudomonas aeruginosa* from Burn and Soft Tissue Wounds. J. R. Army Med. Corps.139:139.

Sohely, S.; Farhana, A.; Fahmida and Ahmed, A. S. (2009). Antimicrobial sensitivity pattern of uropathogens in children. Bangladesh J Med Microbiol. 03 (01): 18-22.

Srivastava, J.; Srivastava, A. K.; Tandan, P.; Singh, B. N. and Yadav, R. (2014). Conjunctival microflora and their antibiotic susceptibility in north Indians prior to cataract surgery. Int. J. Curr. Microbiol. App. Sci. 3(9): 254-259.

Staley and S.T. Williams (Eds.). (9^{th} ed.) William and Wilkins, Baltimore, USA.

Stoodley, P.; Sauer, K.; Davies, D. G. and Costerton. J. W. (2002). Biofilms as complex differentiated communities. Annul. Rev. Microbiol., 56:187209.

(T)

Talarico, T. L., Casas, I. A., Chung, T. C. & Dobrogosz, W. J. (1988). Production and isolation of reuterin, a growth inhibitor produced by *Lactobacillus reuteri*. Antimicrob Agents Chemother 32, 1854–1858.

Tredget, E. E.; Shankowsky, H. A.; Rennie, R.; Burrell, R. E. and Logsetty, S. (2004). Pseudomonas infections in the thermally injured patient. Burns. 30: 3–26.

(U)

Umadevi, S.; Shailesh, K.; Noyal, M.; Joshy, M.; Kandhakumari, G.; Sreenivasan, S.; Sruthi, R. and Selvaraj, S. (2011). Microbiological Study of Diabetic Foot Infections. Indian Journal of Medical Specialties.

(W)

Wazait, H.; Patel, H.; Veer, V. Kelsey, M. and Vander Meulen, J. (2003). Catheter Associated Urinary Tract Infections: Prevalence of Uropathogenes and pattern of antimicrobial resistance in a UK Hospital. Britain Journal of Urology. 91: 806-809.
Weber, J. M.; Sheridan, R. L.; Pasternack, M. S. and Tompkins, R. G. (1997). Nosocomial infections in pediatric patients with burns. Am. J. Infect. Control. 25: 195 - 201.

Weinberg, E. D. (1997). The Lactobacillus anomaly: total iron abstinence. Perspect. Biol. Med., 40: 578 – 583.

White, R. J.; Cooper, R. and Kingsley, A. (2001). Wound colonization and infection: the role of topical antimicrobials. Br J Nurs. 1 0:563–78.

Wohlgemuth, S.; Loh, G. and Blaut, M. (2009). Recent developments and perspectives in the investigation of probiotic effects. Int. J. Med. Microbiol. 300 (1): 3-10.

Wollowski, I.; Rechkemmer, G. and Pool-Zobel, B.L. (2001) Protective role of probiotics and prebiotics in colon cancer. American Journal of Clinical Nutrition. 73(2 Suppl): 451S-455S.

Wysocki, A. B. (2002). Evaluating and managing open skin wounds: colonization versus infection. AACN Clin. Issues. 13:382 - 397.

(Y)

Yang, E.; Maguire, T.; Yarmush, M. L.; Berthiaume, F. and Androulakis, I. P. (2007). Bioinformatics analysis of the early inflammatory response in a rat thermal injury model. BMC Bioinformatics. 8(10).

Yang, H.; Kendall, P. A.; Medeiros, L. and Sofos, J.N. (2009). Inactivation of *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella typhimurium* with compounds available in households. J Food Prot. 72(6):1201 -8.

Yeom, J.; Shin, J.; Yang, J.; Kim, J. and Hwang, G. (2013). H NMR-Based Metabolite Profiling of Planktonic and Biofilm Cells in *Acinetobacter baumannii*. PolS one. 8(3): 1656-2.

Yoshioka, H.; Fujita, K.; Sakata, H.; Murono, K. and Iseki, K. (1991). Development of the normal intestinal flora and its clinical significance in infants and children. Bifidobacteria and Microflora 10(1): 11-17.

Young, L. (2012). Identifying infection in chronic wounds. Wound Practice and Research: Journal of the Australian Wound Management Association. 20(1): 38 – 44.

(Z)

Zetola, N.; Francis, J.S.; Nuermberger, E. L. and Bishai, W. R. (2005). Community-acquired meticillin-resistant *Staphylococcus aureus*: an emerging threat. Lancet Infect Dis. 5: 275 - 286.

Appendix 1.1

Form of infection

Burn and Wound Patients

Name of patient: ..... **اسم المريض:** .....

Sex: ..... **الجنس:** .....

Age: [ ] **Years** **العمر:** [ ] **سنة**

Date of injury: ..... **تاريخ الاصابة:** .....

Date of sampling: ..... **تاريخ اخذ العينة:** .....

Degree of injury: ..... **درجة الاصابة:** .....

Previous treatment: ..... **العلاج السابق:** .....

Appendix 3.1: Results of Vitek2 tests for *Pseudomonas aeruginosa*

Test	**Result**	**Test**	**Result**	**Test**	**Result**	**Test**	**Result**	**Test**	**Result**	**Test**	**Result**
APPA	-	ADO	-	PyrA	-	IARL	-	dCEL	-	BGAL	-
H2S	-	BNAG	-	AGLTp	-	dGLU	+	GGT	+	OFF	-
BGLU	-	dMAL	-	dMAN	+	dMNE	+	BXYL	-	BAIap	+
ProA	+	LIP	+	PLE	-	TyrA	-	URE	-	dSOR	-
SAC	-	dTAG	-	dTRE	+	CIT	+	MNT	+	5KG	-
ILATk	+	AGLU	-	SUCT	+	NAGA	-	AGAL	-	PHOS	-
GlyA	-	ODC	-	LDC	-	IHISa	-	CMT	+	BGUR	-
O129R	+	GGAA	-	IMLTa	+	ELLM	-	ILATa	-		

Appendix 3.2: Results of Vitek2 tests for *Staphylococcus aureus*

Test	**Result**	**Test**	**Result**	**Test**	**Result**	**Test**	**Result**	**Test**	**Result**	**Test**	**Result**
AMY	-	PIPLC	-	AspA	-	AGAL	-	POLYB	+	BACI	+
APPA	-	CDEX	-	BGURr	-	URE	+	dMAL	+	PUL	-
LeuA	-	ProA	-	dSOR	-	NAG	-	MBdG	+	ADH2s	-
AlaA	-	TyrA	-	LAC	-	dMNE	+	dTRE	+		
dRIB	-	ILATk	+	dMAN	+	SAC	+	AGLU	-		
NOVO	-	NC6.5	+	SAL	-	BGAL	-	PHOS	+		
dRAF	-	O129R	-	ADH1	+	AMAN	-	BGUR	-		
OPTO	+	dXYL	-	BGAR	-	PyrA	+	dGAL	+		

Appendix 3.3: Results of Vitek2 tests for *Klebsiella pneumoniae*

Test	**Result**	**Test**	**Result**	**Test**	**Result**	**Test**	**Result**	**Test**	**Result**	**Test**	**Result**
APPA	-	ADO	+	PyrA	+	IARL	-	dCEL	+	BGAL	-
H2S	-	BNAG	-	AGLTp	-	dGLU	+	GGT	-	OFF	+
BGLU	+	dMAL	+	dMAN	+	dMNE	+	BXYL	-	BAIap	-
ProA	-	LIP	-	PLE	+	TyrA	-	URE	-	dSOR	+
SAC	+	dTAG	-	dTRE	+	CIT	+	MNT	-	5KG	-
ILATk	+	AGLU	-	SUCT	-	NAGA	-	AGAL	(-)	PHOS	+
GlyA	-	ODC	-	LDC	-	IHISa	+	CMT	+	BGUR	_
O129R	+	GGAA	-	IMLTa	-	ELLM	-	ILATa	-		

Appendix 3.4: Results of Vitek2 tests for *Staphylococcus epidermidis*

Test	**Result**	**Test**	**Result**	**Test**	**Result**	**Test**	**Result**	**Test**	**Result**	**Test**	**Result**
AMY	-	PIPLC	-	AspA	-	AGAL	-	POLYB	+	BACI	+
APPA	-	CDEX	-	BGURr	-	URE	(-)	dMAL	+	PUL	-
LeuA	-	ProA	-	dSOR	-	NAG	-	MBdG	-	ADH2s	-
AlaA	-	TyrA	-	LAC	-	dMNE	-	dTRE	-		
dRIB	-	ILATk	+	dMAN	-	SAC	+	AGLU	-		
NOVO	-	NC6.5	+	SAL	-	BGAL	-	PHOS	-		
dRAF	-	O129R	+	ADH1	+	AMAN	-	BGUR	-		
OPTO	+	dXYL	-	BGAR	-	PyrA	-	dGAL	-		

Appendix 3.5: Results of Vitek2 tests for *Escherichia coli*

Test	**Result**	**Test**	**Result**	**Test**	**Result**	**Test**	**Result**	**Test**	**Result**	**Test**	**Result**
APPA	-	ADO	-	PyrA	-	IARL	-	dCEL	-	BGAL	+
H2S	-	BNAG	-	AGLTp	-	dGLU	+	GGT	-	OFF	+
BGLU	-	dMAL	+	dMAN	+	dMNE	+	BXYL	-	BAIap	-
ProA	-	LIP	-	PLE	-	TyrA	-	URE	-	dSOR	+
SAC	-	dTAG	-	dTRE	+	CIT	-	MNT	-	5KG	-
ILATk	+	AGLU	-	SUCT	+	NAGA	-	AGAL	-	PHOS	-
GlyA	-	ODC	-	LDC	+	IHISa	-	CMT	+	BGUR	+
O129R	+	GGAA	-	IMLTa	-	ELLM	-	ILATa	-		

Appendix 3.6: Results of Vitek2 tests for *Enterobacter cloacae*

Test	**Result**	**Test**	**Result**	**Test**	**Result**	**Test**	**Result**	**Test**	**Result**	**Test**	**Result**
APPA	-	ADO	-	PyrA	-	IARL	-	dCEL	+	BGAL	+
H2S	-	BNAG	+	AGLTp	-	dGLU	+	GGT	+	OFF	+
BGLU	+	dMAL	+	dMAN	+	dMNE	+	BXYL	+	BAIap	-
ProA	-	LIP	-	PLE	+	TyrA	+	URE	-	dSOR	+
SAC	+	dTAG	-	dTRE	+	CIT	+	MNT	+	5KG	-
ILATk	+	AGLU	-	SUCT	+	NAGA	-	AGAL	+	PHOS	-
GlyA	-	ODC	+	LDC	-	IHISa	-	CMT	+	BGUR	-
O129R	+	GGAA	-	IMLTa	-	ELLM	-	ILATa	-		

Appendix 3.7: Results of Vitek2 tests for *Acinetobacter baumannii*

Test	Result	Test	Result	Test	Result	Test	Result	Test	Result	Test	Result
APPA	-	ADO	-	PyrA	-	IARL	-	dCEL	+	BGAL	-
H2S	-	BNAG	-	AGLTp	+	dGLU	+	GGT	-	OFF	-
BGLU	-	dMAL	-	dMAN	-	dMNE	+	BXYL	-	BAIap	-
ProA	-	LIP	-	PLE	-	TyrA	+	URE	-	dSOR	-
SAC	-	dTAG	-	dTRE	-	CIT	+	MNT	+	5KG	-
ILATk	+	AGLU	-	SUCT	+	NAGA	-	AGAL	-	PHOS	-
GlyA	-	ODC	-	LDC	-	IHISa	-	CMT	+	BGUR	-
O129R	+	GGAA	-	IMLTa	-	ELLM	-	ILATa	-		

Appendix 3.8: Results of Vitek2 tests for *Stenotrophomonas maltophilia*

Test	**Result**	**Test**	**Result**	**Test**	**Result**	**Test**	**Result**	**Test**	**Result**	**Test**	**Result**
APPA	+	ADO	-	PyrA	-	IARL	-	dCEL	-	BGAL	-
H2S	-	BNAG	+	AGLTp	-	dGLU	-	GGT	+	OFF	-
BGLU	+	dMAL	-	dMAN	-	dMNE	-	BXYL	-	BAIap	-
ProA	+	LIP	+	PLE	-	TyrA	-	URE	-	dSOR	-
SAC	-	dTAG	-	dTRE	-	CIT	+	MNT	-	5KG	-
ILATk	+	AGLU	+	SUCT	+	NAGA	-	AGAL	-	PHOS	+
GlyA	-	ODC	-	LDC	-	IHISa	-	CMT	-	BGUR	-
O129R	-	GGAA	-	IMLTa	-	ELLM	-	ILATa	-		

Appendix 3.9: Results of Vitek2 tests for *Pseudomonas alcaligenes*

Test	Result	Test	Result	Test	Result	Test	Result	Test	Result	Test	Result
APPA	-	ADO	-	PyrA	-	IARL	-	dCEL	-	BGAL	-
H2S	-	BNAG	-	AGLTp	-	dGLU	-	GGT	-	OFF	-
BGLU	-	dMAL	-	dMAN	-	dMNE	-	BXYL	-	BAIap	+
ProA	+	LIP	-	PLE	-	TyrA	+	URE	-	dSOR	-
SAC	-	dTAG	-	dTRE	-	CIT	-	MNT	-	5KG	-
ILATk	+	AGLU	-	SUCT	-	NAGA	-	AGAL	-	PHOS	-
GlyA	-	ODC	-	LDC	-	IHISa	-	CMT	+	BGUR	-
O129R	-	GGAA	-	IMLTa	+	ELLM	-	ILATa	+		

Appendix 3.10: Results of Vitek2 tests for *Pseudomonas putida*

Test	**Result**	**Test**	**Result**	**Test**	**Result**	**Test**	**Result**	**Test**	**Result**	**Test**	**Result**
APPA	-	ADO	-	PyrA	-	IARL	-	dCEL	-	BGAL	-
H2S	-	BNAG	-	AGLTp	-	dGLU	+	GGT	+	OFF	-
BGLU	-	dMAL	-	dMAN	-	dMNE	+	BXYL	-	BAIap	+
ProA	+	LIP	(+)	PLE	-	TyrA	+	URE	-	dSOR	-
SAC	-	dTAG	-	dTRE	-	CIT	+	MNT	+	5KG	-
ILATk	+	AGLU	-	SUCT	+	NAGA	-	AGAL	-	PHOS	-
GlyA	-	ODC	-	LDC	-	IHISa	+	CMT	+	BGUR	-
O129R	+	GGAA	-	IMLTa	+	ELLM	-	ILATa	+		

Appendix 3.11:

Mean values of inhibition zones against burn wound bacteria by different apple vinegar types alone and their combination with *Lb. reuteri* three-fold concentrated fermentation product in three different ratios for each

Type of vinegar	Number of treatments	Treatment			
		Vinegar alone	Ratio 2 to 2	Ratio 3 to 1	Ratio 1 to 3
V_E	22	19.864[a]	20.863[a]	18.681[b]	21.931[a]
V_H	22	18.114[b]	19.159[c]	18.954[b]	20.659[b]
V_C	22	20.296[a]	20.381[b]	20.204[a]	22.022[a]

* Numbers with the same letter are not significant at level *p*-value <0.05

الخلاصة

تهدف هذه الدراسة لعزل البكتريا المسببة لالتهابات الحروق و الجروح ومعالجتها بواسطة الخل و المعززات الحيوية. لهذا الغرض تم جمع 58 مسحة من مرضى من كلا الجنسين و بمختلف الاعمار يرقدون في مستشفيين تعليميين في بغداد يعانون من التهابات الحروق و الجروح متنوعة في حدتها و في موقع الاصابة. فقط 43 (74.2%) من النماذج كانت موجبة في اعطاء نمو بكتيري.

النماذج زرعت على MacConkey agar و blood agar كخطوة اولية لزرع البكتريا. اظهرت النتائج ان 64 عزلة حُصلت و بعد التشخيص بواسطة الزرع و المجهر و الفحوص البايوكيميائية و بنظام ال Vitek 2 قد وجد انها تنتمي الى البكتريا الموجبة و السالبة لصبغة الغرام للأنواع التالية:

Pseudomonas aeruginosa (28) و *Staphylococcus aureus* (12) و *Klebsiella pneumoniae* (10) و *Staphylococcus epidermidis* (6) و *Escherichia coli* (3) و *Pseudomonas putida* (1) و *Pseudomonas alcaligenes* (1) و *Enterobacter cloacae* (1) و *Acinetobacter baumannii* (1) و *Stenotrophomonas maltophilia* (1).

اختبرت حساسية العزلات اتجاه عشر مضادات حيوية شائعة الاستخدام بالاضافة الى مضادين حيويين يعطيين للمرضى في المستشفى كجزء من العلاج , اظهرت النتائج ان ال amikacin و imipenem كانا الاكثر فعالية ضد البكتريا الموجبة و السالبة لصبغة الغرام. عزلات *Pseudomonas* كانت الاكثر مقاومة ثم *Enterobacter cloacae* و *Acinetobacter baumannii*.

نوعين من المعززات الحيوية استخدمت *Lactobacillus reuteri* , و مزيج من *Lb. reuteri* و *Lb. acidophilus* و *Lb. rhamnosus* و *Bifidobacterium longum* كمعززات حيوية زرعت بشكل مستقل على الوسط MRS. بعد ذلك نواتج تخمر هذه العزلات تم تركيزها و فحص فعاليتها التثبيطية ضد عزلات بكتريا التهابات الحروق و الجروح.

اظهرت النتائج ان المركزالثالث لناتج تخمر *Lactobacillus reuteri* كان المركز الوحيد الذي يحتوي فعالية مضاد بكتيري , بينما نواتج تخمر المعززات الحيوية الاخرى لا تمتلك مثل هذه الفعالية.

اظهرت النتائج ان انواع خل التفاح التي تم استعمالها كمضاد بكتيري ضد العزلات البكتيرية المسببة لالتهابات الحروق و الجروح تمتلك قابلية في القضاء على البكتريا المرضية. خل التفاح المصنّع كيميائيا اعطى اعلى فعالية مضاد بكتيري تلاه خل التفاح التجاري وبعدهما كان خل التفاح المصنّع منزلياً.

عند فحص خليط من المركز الثالث لناتج تخمر *Lb. reuteri* و خل التفاح بنسب مختلفة اظهرت النتائج تأثير مؤازر سُجل في معظم النسب بإستثناء التأثير ضد *Acinetobacter baumannii*. اعلى فعالية مضاد بكتيري ضد العزلات البكتريّة المرضية قد سُجلت بواسطة النسبة 3:1 المركز الثالث لناتج تخمر *Lb. reuteri* الى خل التفاح المصنّع كيميائيا.

جامعة النهرين

كلية العلوم

قسم التقانة الاحيائية

تقييم تأثيرات المعززات الحيوية و الخل على البكتريا المعزولة من التهابات الحروق و الجروح

رسالة

مقدمة الى مجلس كلية العلوم / جامعة النهرين كجزء من متطلبات نيل درجة الماجستير في العلوم/ التقانة الاحيائية

من قبل

مصطفى عطية حديد

بكالوريوس تقانة احيائية / جامعة النهرين (2013)

بأشراف

الدكتور عبد الواحد باقر الشيباني

(استاذ)

صفر 1437

كانون الاول 2015