



Journal of Bioscience and Applied Research

JBAAR

WWW.JBAAR.ORG



## Studies on the antibacterial effect of probiotics and prebiotics against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Shaaban, M.T.<sup>1</sup>, Hussien, M.Z.<sup>2</sup>, Allam N.G.<sup>3</sup>, Moustafa H.H.<sup>1</sup>

1. Botany Dept., Fac. of Sci. Menoufia Univ., Egypt

2. Microbiology Dept., Fac. of Medicine. Tanta Univ., Egypt

3. Microbiology Dept., Fac. of Sci. Tanta Univ., Egypt

(Corresponding author e.mail. Dr.mohamedtawfik@yahoo.com)

### Abstract

The influences of *Lactobacillus acidophilus*, *Bifidobacterium bifidus* and *Streptococcus thermophiles* (probiotics mixture) were studied against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The ethanolic precipitate of the probiotics mixture revealed antimicrobial activity against *Staphylococcus aureus* and assayed qualitatively by GC-Mass spectrum as it partially identified as carbohydrate substances. Antimicrobial activities of the used probiotics and prebiotics against *S. aureus* were studied *in vitro* referring to commercial used antibiotics. To investigate the protective efficiency of probiotics mixture and their prebiotics against *S. aureus* infection in mice, a comparative studies have been conducted and the success of treatment or prevention had been measured through detection of Liver enzymes (Aspartate transaminase (AST) and Alanin transaminase (ALT) , bacterial colony count in target organs (liver and lung) , histological changes occurred in liver and intestine and immunoglobulin IgM titer. In conclusion it could be concluded that the mixed culture of probiotic strains could increase the protective effects against *S. aureus* infection.

**Keywords:** : Probiotics , *S. aureus* , IgM , AST , ALT

### 1 Introduction

Species of *Staphylococcus* bacteria are identified as one of the most important causes of acute disease in humans in many areas of the world. *Staphylococcus aureus* causes a wide range of infectious diseases, whether localized to

the skin or systemic diseases, in humans and animals (Vincze *et al.*, 2013). These include atopic dermatitis (AD), also known as eczema, boils (furuncles), carbuncles, impetigo, folliculitis, osteomyelitis, pneumonia, meningitis, food poisoning, mastitis and urinary tract infections (Ogston and Witte, 1984). Staphylococcal bacteremia leads to endocarditis and sepsis, diseases that, even under antibiotic therapy, are associated with high mortality (Klevens *et al.*, 2007). Furthermore, *Pseudomonas aeruginosa* causes several diseases, such as cutaneous diseases, especially folliculitis and dermatitis. In addition, *P. aeruginosa* causes systemic diseases, for example cystic fibrosis, or is associated with acquired immune deficiency syndrome patients (Elkin and Geddes, 2003). In the past two decades, *P. aeruginosa* has emerged as a significant pathogen, which causes between 10 - 20% of infections in hospitals. *P. aeruginosa* causes numerous infectious diseases to humans and animals, e.g. septicaemia, leg ulcers and burn wound infections (Buivydas *et al.*, 2013). The spread of multiple drug resistant bacteria indicates a growing need for new antimicrobial agents. Researchers are attempting to find successful solutions to overcome microbial infections, such as methicillin resistant *Staphylococcus aureus* (MRSA), which is an example of a multiple drug resistant bacterial species. MRSA strains have the ability to induce biofilms and this resulted in increase in their virulence (McCarthy *et al.*, 2015).

Probiotics have been defined by The Food Agricultural Organization/World Health Organization (FAO/WHO) as "live microorganisms which when administered in

adequate amounts confer a health benefit to the host" (Brown *et al.*, 2004). Probiotics are commonly consumed as part of fermented foods or cultured foods with specially added active live cultures; such as in some yogurts and fermented milk drinks, or as dietary supplements. Probiotics can be defined as mono or mixed culture of living microorganisms, which beneficially affect the human being by improving the balance of the indigenous microflora and in stimulating the immune system of the host, when consumed in an adequate amount as part of the food FAO/WHO (2001).

Lactic acid bacteria (Probiotics) are amongst the most important groups of microorganisms used in food industry (Sonamoto and Yokota 2011), many species are involved in the daily manufacturing of dairy products (Ayad *et al.*, 2004). Various commercial probiotic preparations are also available in market in the form of capsules, liquid/gel and powdered that claims for prevention of infectious diseases. Commercially available probiotic preparations include *Lactobacillus* alone or in combination with *bifidobacteria*, *Streptococcus* or *Saccharomyces*, showed beneficial effects (Saggiaro, 2004). The characteristics and the health potential benefits of probiotics are: (1) the capacity to adhere and colonize the intestinal mucosal cells, (2) the growth under intestinal conditions, (3) production of antimicrobial substances, (4) antagonism effects against pathogenic bacteria e.g. pathogenic *E. coli* strain (5) proven safety in human use, (6) lowering serum cholesterol, (7) bile acids tolerance, (8) reduction of duration or incidence of diarrhea, (9) enhancement of immune response, and (10) no pathogenic, toxic, allergic, mutagenic or carcinogenic reaction by the probiotic strains itself or its fermented products (Rasic *et al.*, 1992 and Elmer *et al.*, 1996). The characterization of Lactic Acid Bacteria (LAB) activities is of great importance to enhance their function at Industrial level. LAB produces a variety of antimicrobial compounds and effective substances such as lactic and acetic acids, prebiotics, antibiotics, bacteriocins (Abbas and Mahasneh, 2014). In the present work, the influences of *Lactobacillus acidophilus*, *Bifidobacterium bifidus* and *Streptococcus thermophiles* (probiotics mixture) were studied against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

## 2 Materials and Methods

**Test bacteria:** *Staphylococcus aureus* ATCC 25923 was cultured in mannitol salt agar medium (MSA) and *Pseudomonas aeruginosa* NCIB 8626 was cultured in Nutrient and *Pseudomonas* isolation agar (PIA). Both bacterial strains were kindly provided by Bacteriology Unit, Botany Department, Faculty of Science, Tanta University. The bacteria were cultured aerobically and maintained for 24 h at about 37°C.

### Source of probiotics

Samples of different commercial dairy products from local market were used to investigate antibacterial activity of their defined bacterial species, as following: ABT-3:

mixed culture containing *Lactobacillus acidophilus*, *Bifidobacterium bifidus* and *Streptococcus thermophilus*; (Juhina rayeb milk), La-1: *Lactobacillus acidophilus*; (Danon youghrt), Bb-1: *Bifidobacterium bifidus*; (Activia yoghurt) and Bb-2: *Bifidobacterium bifidus* plus inulin fibers as prebiotics (Lactel B .Active rayeb milk).

### Preparation of Probiotic sample

Samples of different commercial dairy products from local market containing defined probiotics isolates were prepared; the samples of dairy products were shaken vigorously to suspend the bacterial contents. Then, 10ml of each sample were dissolved into 10ml of normal saline 0.9% w/v. each 0.1 ml containing  $10^7$  CFU of the used probiotics (Silva *et al.*, 1999).

### Synergistic activity of antibiotics, probiotics and ethanolic precipitation against *Staphylococcus aureus* In vitro and in vivo.

The following types of antibiotics were used, Vancomycin, Streptomycin and Gentamycin, for antibacterial assay against *Staphylococcus aureus*. The antibiotic discs were applied according to the Bauer-Kirby technique (Bauer *et al.*, 1966). The inhibition zone diameters (IZDs) were measured after incubation of plates for 24 h at 37 °C in millimeters.

Agar well bioassay was employed for testing antibacterial activity of 4 types of tested Probiotics supernatant as described by (Sousa *et al.*, 2006). By a sterile swab, *Staphylococcus aureus* with a concentration of 1/2 McFarland ( $1.5 \times 10^8$  CFU/ml) was spread over a nutrient agar plate and incubated at 37°C for 24 hours, 10 µl of each supernatant of each probiotic was inoculated in wells and incubated at 37°C for 24 h. The inhibition zones were measured. Three replicates were carried out.

### The antimicrobial activity of different types of probiotics

ABT-3, La-1, Bb-1 and Bb-2 were tested against *Staphylococcus aureus*, The best antibacterial effect was obtained by mixture of probiotics Juhina rayeb milk (ABT). Over night incubation of broth culture of Juhina rayeb milk containing probiotics (ABT-3) prepared in MRS broth at 37°C, centrifuged at 3000 rpm for 30 min at 4°C, the protein fraction was precipitate using ammonium sulfates, and carbohydrate fraction was precipitated using ethanol. Antimicrobial activity of protein and carbohydrate fraction was investigated against *Staphylococcus aureus* in vitro and the inhibition zones were measured in mm.

### Determination of carbohydrates composition:

The total amount of carbohydrates in the ethanolic precipitate was determined by the phenol-sulfuric acid method described by (Dubois *et al.*, 1956). The carbohydrate composition was determined by Gas Chromatography Mass (GC-MS) using Agilent 6890 gas chromatograph equipped with an Agilent mass spectrometric detector, with a direct capillary interface and fused silica capillary column HP-5MS (30 m x 0.32 mm x 0.25 µm film thickness). Polysaccharides samples were injected under specific conditions. Wiely and Wiely

Nist mass spectral data base was used in the identification of the separated peaks.

**Determination of bacterial load in infected organs of mice** (Abdel-Barry *et al.*, 1997)

**Experimental Design:** The experiments were carried out on a total of 90 white albino 6-week-old male mice, obtained from the Animal House National Research Center (Giza, Egypt). They were divided into 11 groups. The groups were divided into two main divisions, local and systemic feeding or treatment in addition to six for controls (three for negative controls and three for positive controls).

#### **Burn wound infection model**

Burn wounds were induced by pressing the ends (1 cm<sup>2</sup> area) of two pre-heated brass rods against opposite sides of a raised dorsal skin fold for 10 s (Busch *et al.*, 2000). The brass rods were pre-heated in boiling water to about 95 °C and produced a third degree burn of 1–1.5 cm<sup>2</sup> area (1.5–2.2% of body surface area calculated according to Meeh's formula (Gilpin *et al.*, 1996). Immediately after burning the mice were resuscitated with an intraperitoneal injection of 1.5 ml sterile saline. After ten minutes, 100 µl of the *S. aureus* suspension (10<sup>7</sup> cells mL<sup>-1</sup>) was applied to the surface of the wound. Positive control group consisted of mice with infected burn wound with out any treatment of probiotics or antibiotics. Negative Control (normal food with out any treatment or burning).

#### **The experiment**

A dose of 0.1 ml containing 10<sup>7</sup> CFU of selected probiotics mixture (ABT) was administered to the correspondence groups, group 1 (G1) negative controls, group 2 (G2) positive controls, group 3 (G3) systemic probiotics (mice feeding by gavage "oral feeding"), group 4 (G4) systemic probiotics + local probiotics "spray" group 5 (G5) local probiotics group 6 (G6) systemic probiotics + systemic antibiotic (Vancomycin (V 30 µg)) group 7 (G7) systemic "probiotics+antibiotics"+ local probiotics+antibiotics" group 8 (G8) local probiotics+ local antibiotics group 9 (G9) systemic antibiotics group 10 (G10) local antibiotics + systemic antibiotics group 11 (G11) local antibiotics. The same dose of probiotic and other content were repeated daily to the mice in each group, for 9 days (Silva *et al.*, 2004).

**Blood and samples collection** (Van Herck, 1998).

Blood samples were obtained from mice through lateral tail vein; the clear sera were obtained by centrifugation at 5000 rpm for 5 minutes. The serums were used for some biochemical and immunological assay, Liver and intestine of each mouse were collected under aseptic condition and stored at -20°C until used for bacterial count and histological changes examination after experiments.

**Bacterial count in liver and lung** (Salem, 1997)

Mice were anesthetized with ether, killed and surface sterilized in a beaker containing 70% ethanol to wet them completely. Each mouse was dissected under aseptic conditions to obtain the target organs (lung and liver). Each organ was homogenized in 5 ml sterilized saline

solution. The homogenate was serially diluted and 0.1 ml was plated on mannitol salt agar (MSA) plates then incubated for 24 h at 37 °C and black colonies were counted in order to record the number of Colony Forming Unit per organ (CFU/organ) (Marshall, 1993).

All the tests were performed in triplicates and the graph was plotted with the mean values. Number of bacteria (CFU/organ) = (average number of bacterial colonies/ amount plated) x dilution

#### **Histopathological examination**

Sections for liver and lung were taken immediately after death or slaughter and fixed in 10 % buffered formalin, tissues were embedded in paraffin sectioned at 3µm and stained with hematoxylin and eosin according to Bancroft *et al.*, (1996).

#### **Assay of liver function enzymes**

At the end of the experimental time, blood samples were collected from retro-orbital venus plexus in sterile test tubes and centrifuged for serum separation at 1500 rpm for 15 min to estimate aspartate transaminase (AST) and alanin transaminase (ALT) according to the assay described by (Kaplan, 1996).

#### **Estimation of IgM titre in serum of mice by ELISA:**

The serum obtained from mice bled via the lateral tail vein were assayed for IgM antibody using radio-immunodiffusion diffco-plate of IgM for accurate quantitative immunoglobulin in biological fluids (Voller *et al.*, 1976).

#### **Statistical analysis:**

Statistical presentation and analysis of the results were conducted by calculating the means ± standard deviation, standard error, analysis of variance [ANOVA] and Duncan's multiple range tests using the software SPSS V17 (SPSS, 1999). The means were compared by least significant difference test at p 0.05.

### **3 Results**

#### **The antimicrobial activity of different types of probiotics against *Staphylococci aureus* in vitro**

ABT-3, La-1, Bb-1 and Bb-2 were tested against *S. aureus*, and it was clearly that the used probiotics had a weak inhibitory effect on *Staphylococci aureus*, and the zone of inhibition ranged from 6.5–8.5 mm. As illustrated in Table (1), Figures (1&2). The inhibition zones were measured in mm.

The obtained results revealed that the best antibacterial effect was obtained by mixture of probiotics Juhina rayeb milk (ABT)

The prepared sample of Juhina rayeb milk (ABT-3) centrifuged, then precipitated in the form of proteins by ammonium sulfates or precipitated in the form of exopolysaccharides by ethanol. Antimicrobial activity investigated against *S. aureus* in vitro. The inhibition zones were measured in mm. It is clearly that the prebiotics extracts (proteins, EPS) of Juhina rayeb milk

(ABT-3) had inhibitory effect on *S. aureus*, and the zone of inhibition ranged from 2.5-6 mm, and it is cleared that best result was obtained by exopolysaccharides fraction .

The ethanolic precipitate was partially identified as carbohydrates by UV-absorption spectra with a Shimadzu UV visible 240 double beam spectrophotometer as a clear peak was observed at 490 nm. polysaccharides were recorded according to modified phenol sulphoric acid (Dubois *et al.*, 1956).



Figure (1) Antimicrobial activity of probiotics against *S. aureus* in vitro

- Zone 1 Antibacterial activity of probiotics ABT against *S. aureus*
- Zone 2 Antibacterial activity of probiotics La-1 against *S. aureus*
- Zone 3 Antibacterial activity of probiotics Bb-2 against *S. aureus*
- Zone 4 Antibacterial activity of probiotics Bb-1 against *S. aureus*

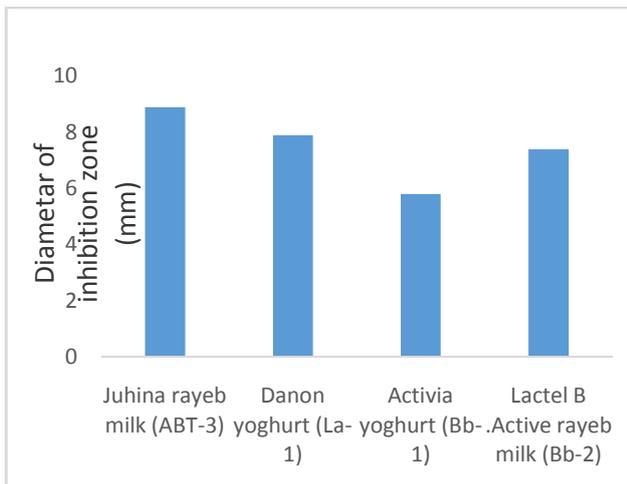


Figure (2) Antimicrobial activity of probiotics against *S. aureus* in vitro

Table (1). The antimicrobial activity of probiotics against *S.aureus* in vitro

Probiotic	Measurement of inhibition zone (mm)
Juhina rayeb milk (ABT-3)	8.9333 <sup>A</sup>
Danon yoghurt (La-1)	7.8667 <sup>B</sup>
Activia yoghurt (Bb-1)	5.8333 <sup>D</sup>
Lactel B .Active rayeb milk (Bb-2)	7.3667 <sup>C</sup>

The ethanolic precipitate was partially identified as carbohydrate and its composition was determined by gas chromatography (Agilent 6890 gas chromatograph (Fig.3,table 2).

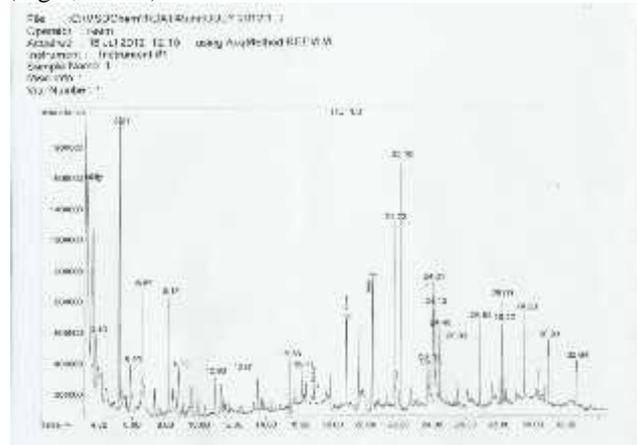


Figure (3) Compositional and structural characterisation of Exo polysaccharides By Gas Chromatography Mass ( GC –MS)

The results in Table( 3 ), illustrated that the total bacterial count of *S. aureus* in lung and livers was lower in the protected or treated groups with probiotics , prebiotics and antibiotics than other untreated groups (control) . The most effective extract was in group 6,7,8,9 and group 10 . The count of *S.aureus* colonies in lung markedly decreased, while no significant difference in count of *S.aureus* in the livers of mice .

**Table (2). Compositional and structural characterisation of carbohydrates By Gas Chromatography Mass ( GC – MS )**

R.T min.	Compound name
17.3	Fructose
19.2	Glucose
20.5	Sorbitol
22.10	Mannose

**Table ( 3 ) Effect of different treatments on bacterial load of *staphylococci aureus* in lung and livers of mice during experiment.**

Groups	No. of bacteria (CFU/organ)x10 <sup>3</sup>	
	Lung	Liver
Negative control_1-	0.000 <sup>G</sup>	0.000 <sup>E</sup>
2- positive control	97.000 <sup>A</sup>	41.333 <sup>A</sup>
3- Systemic treatment (probiotics)	81.667 <sup>B</sup>	36.333 <sup>B</sup>
4- Combination treatment (probiotics)	76.667 <sup>C</sup>	31.667 <sup>C</sup>
5- Local treatment(probiotics)	79.000 <sup>C</sup>	37.333 <sup>B</sup>
6- Systemic probiotic&antibiotic	57.667 <sup>E</sup>	30.000 <sup>C</sup>
7- Combination treatment (probiotic&antibiotic)	35.000 <sup>F</sup>	13.667 <sup>D</sup>
8- Local treatment (probiotic&antibiotic)	58.667 <sup>E</sup>	32.000 <sup>C</sup>
9-Systemic treatment(antibiotic)	60.333 <sup>E</sup>	32.333 <sup>C</sup>
10- Combination treatment (antibiotic)	59.667 <sup>E</sup>	31.000 <sup>C</sup>
11- local treatment (antibiotic)	73.333 <sup>D</sup>	40.333 <sup>A</sup>

F value (Lung) = 969.44\*\*

F value (Liver) = 257.34\*\*

\*\* means highly significant (Pr < .0001)

Means with the same letter are not significantly different.

As shown in Table( 4) the level of serum AST and ALT was significantly decreased among the protected and treated mice (G 6, 7, 8, 10 &11) compared to the control groups (G 2 ,3,4,5&9). The level of serum ALT, AST in negative control mice was ranged from 24.3 to 27.6 (IU/l). In conclusion all treatment decreased the level of

ALT ,AST comparing to infected controle .The best result was obtained with combination treatment ( probiotic &antibiotic).

Table ( 5 ) clarify that in the treated groups ,there was increase level of IgM titre in groups (3,4,5,6 ,7,8 ,9&10) compared with positive control groups

Table (4). AST and ALT in the serum of mice after some treatments (probiotics and antibiotics).

Groups	ALT (IU/L)	AST (IU/L)
Negative control_1-	27.6667 <sup>F</sup>	24.333 <sup>F</sup>
2- positive control	45.3333 <sup>A</sup>	43.333 <sup>A</sup>
3- Systemic treatment (probiotics)	38.0000 <sup>BC</sup>	37.667 <sup>BC</sup>
4- Combination treatment (probiotics)	36.6667 <sup>C</sup>	30.000 <sup>E</sup>
5- Local treatment(probiotics)	39.3333 <sup>B</sup>	38.000 <sup>B</sup>
6- Systemic probiotic&antibiotic	31.0000 <sup>DE</sup>	34.333 <sup>D</sup>
7- Combination treatment (probiotic&antibiotic)	28.0000 <sup>F</sup>	24.333 <sup>F</sup>
8- Local treatment (probiotic&antibiotic)	33.0000 <sup>D</sup>	30.333 <sup>E</sup>
9-Systemic treatment(antibiotic)	36.6667 <sup>C</sup>	35.333 <sup>CD</sup>
10- Combination treatment (antibiotic)	31.3333 <sup>DE</sup>	33.667 <sup>D</sup>
11- local treatment (antibiotic)	30.0000 <sup>E</sup>	36.000 <sup>BCD</sup>

F value (ALT) = 65.01\*\* , F value (AST) = 53.47\*\* \*\* means highly significant (Pr < .0001)  
Means with the same letter are not significantly different.

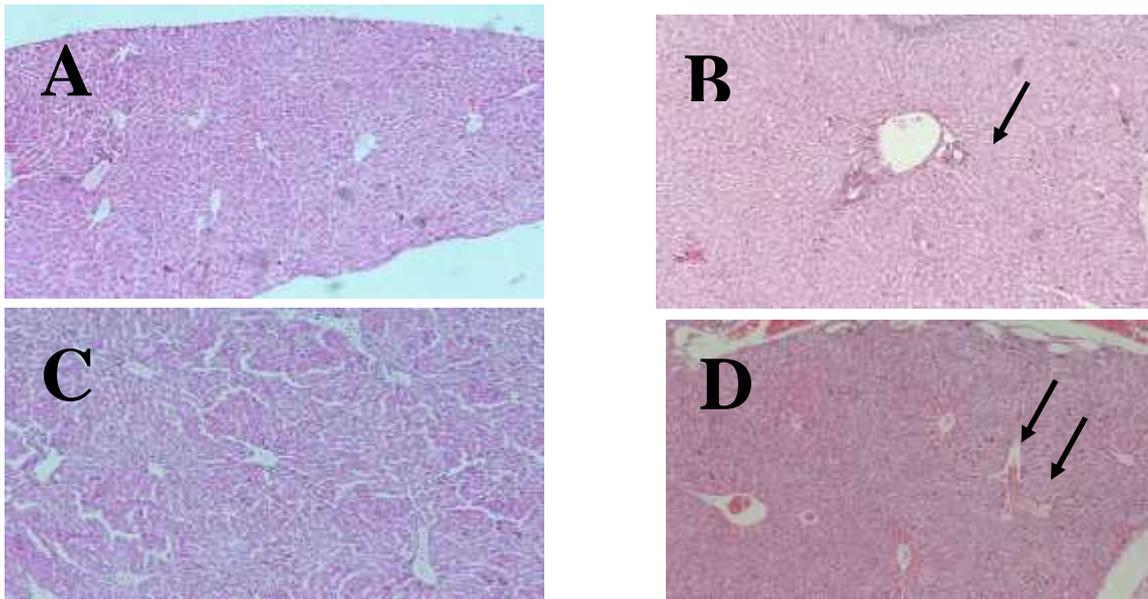
Table ( 5 ) Estimation of serum immunological studies(IgM) of mice during the experiment

Groups	IgM ( µg/ml)
Negative control_1-	28.000 <sup>G</sup>
2- positive control	62.000 <sup>A</sup>
3- Systemic treatment (probiotics)	56.667 <sup>B</sup>
4- Combination treatment (probiotics)	52.000 <sup>C</sup>
5- Local treatment(probiotics)	59.000 <sup>B</sup>
6- Systemic probiotic&antibiotic	43.667 <sup>E</sup>
7- Combination treatment (probiotic&antibiotic)	36.667 <sup>F</sup>
8- Local treatment (probiotic&antibiotic)	42.667 <sup>E</sup>
9-Systemic treatment(antibiotic)	54.000 <sup>C</sup>
10- Combination treatment (antibiotic)	48.333 <sup>D</sup>
11- local treatment (antibiotic)	58.000 <sup>B</sup>

F value (IgM) = 147.77\*\* , \*\* means highly significant (Pr < .0001) , Means with the same letter are not significantly different.

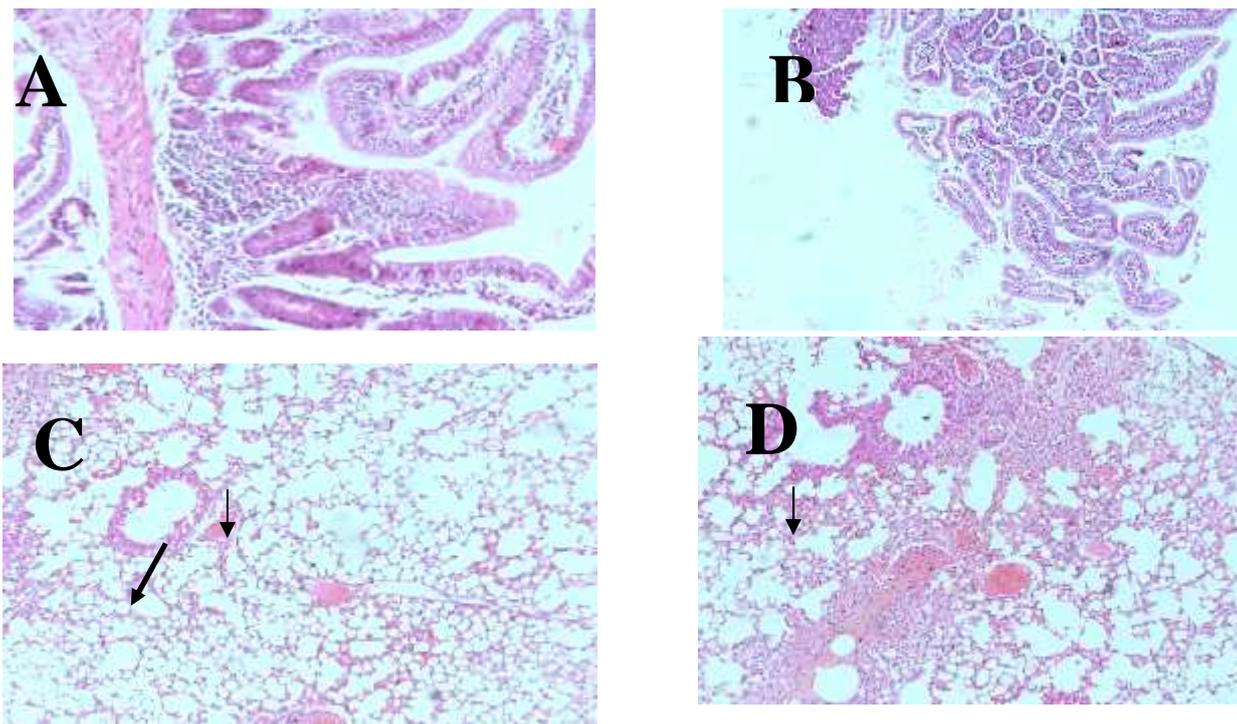
Histological examinations of lung and liver tissues at the end of protection and treatment experiment as shown in Figure (4) and Figure (5) showed that the best results have been noticed in groups used probiotics that

achieved no & few inflammatory degree in lung and liver respectively. In contrast, groups used antibiotics revealed more inflammatory cells and edema in both organs tissues compared to control groups .



**Figure (4) histological changes of liver of mice after some treatments (probiotics & antibiotics)**

(A)The normal appearance of the liver in the control group. (B) Few inflammatory cells around the central vein ( the arrow)( H&E,X 100).(C) Liver, no congestion , with near normal appearance of the liver parenchyma .(D) Some Dilated Congested Vesseles In The Liver After The Use Ofantibiotics ( marked by the arrow) (H&EX 100)



**Figure (5) histological changes of lung of mice after some treatments (probiotics & antibiotics)**

(A) Normal lung , no interstitial or intraalveolar(pneumonic) inflammatory infiltrate.(B) Lung with congested vessels, interstitial( the arrow) and intra alveolar inflammatory exudate.(C) Closer view of the lung ( thin arrow points to the inflammation, the thick arrow points to congested vessels).(D) Lung with decreased inflammatory response, minimal unremarkable residual interstitial inflammation and mildly congested vessels( the arrow points to mildly congested blood vessele).(H&E,X100)

#### 4 Discussion

This study aimed to discover a new approach to the treatment of *S.aureus* infection via combination therapy of probiotics and antibiotics. The synergistic activity of a new combination between them inhibited bacterial growth and reduced inflammation.

Successful probiotic bacteria are usually able to colonize the intestine, at least temporarily, by adhering to the intestinal mucosa. Adhesion of probiotic microorganisms to the intestinal mucosa is considered important for many of the observed probiotic health effects, such as antagonistic activity against enteropathogens, modulation of immune system (Ostad *et al*,2009) and increased healing of damaged gastric mucosa(Elliott *et al*,1998) .

Probiotics have the ability to decrease the incidence and duration of some types of diarrheal illnesses e.g., antibiotic associated, *Clostridium difficile*, traveler's and rotavirus. (FAO/WHO, 2002). A meta-analysis by Van Niel *et al.* concluded that "*Lactobacillus* is safe and effective as a treatment for children with acute infectious diarrhea." Van Niel and co-workers clearly demonstrated the relationship between *Lactobacillus* dose and reduction of diarrhea in children.

The polysaccharides synthesized by mixture of *Lactobacillus acidophilus*, *Bifidobacterium bifidus* and *Streptococcus thermophilus* (ABT-3) contained mannose. The monomer composition of the exopolysaccharides synthesized by *S. thermophilus* differed from the reports by other authors on this species (Cerning *et al.*, 1994).Also contain glucose and galactose. which supports by the results found by other authors ( Yang *et al.*, 2000). In which lactobacilli synthesized biopolymers whose compositions contained glucose and galactose. In the exopolysaccharides synthesized by *L. helveticus* the glucose:galactose ratio was 2:1.

In present study, the effects of probiotics mixture on the infection dynamics of *S.aureus*, was determined through measurement of histological changes in target organs, humeral immune response(IgM) ,some biochemical parameters ( ALT,AST) , in mice( *in vivo*).

We found that there was as significant effect on groups that treated with probiotics in combination with antibiotics . The meta-analysis considered nine studies involving various species of lactobacilli. It appears that people on antibiotic therapy can benefit from probiotic consumption. One negative side effect of antibiotics is that they kill beneficial, as well as undesirable, bacteria. Replenishing the flora with normal/beneficial bacteria during and after use seems to minimize intestinal disruption caused by antibiotic medications. ( Cremonini *et al*,2002 ) reviewed seven studies (881 total patients) covering probiotic mitigation of antibiotic-associated diarrhea. According to this analysis, probiotics (e.g., *Lactobacillus spp.*) can be used to prevent antibiotic-related diarrhea but do not appear to diminish existing diarrhea symptoms.

In recent human research, studies have looked at the role of synergistic activity of probiotics and antibiotics in the management of various infections (Doron *et al*,2008).

In present study we found that the level of the viable *S. aureus* was lower in the treated groups of mice than in the positive control groups. The difference was significant among the mice treated with ABT-3 (G 4 & G6,G7 &G 8). Silva *et al.*,2004 observed improved survival for mice pretreated with *Bifidobacterium longum* during challenge with *S.aureus.*, but without affecting numbers of the pathogen.

The activities of AST and ALT in mice of the control negative group and probiotic supplemented groups in the experiment were in harmony with that detected by (Sadiek and Bohm 2001), who demonstrated that the activities of AST and ALT were normally and nearly the same in control and probiotic-treated animals, thus indicating that probiotic had no side effects on the animal health. Concerning liver health, the main benefits of probiotics might occur through preventing the production and or uptake of lipopolysaccharides in the gut and therefore reducing levels of low-grade inflammation (Gratz *et al*,2010).

The levels of IgM in groups results demonstrated that probiotics treated groups has greater percentage increase over control. and the best result was obtained with combination treatment (local and systemic probiotic and antibiotic) Numerous reports suggested that probiotics and prebiotics can cause immunomodulatory effects that lead to enhanced resistance to enteric pathogens (Voller *et al.*, 1976).

The mechanisms of action have been studied and one method is thought to be due to the probiotic bacteria interfering with the invasion and adhesion of pathogens (Resta and Barrett 2003).

Most feeding dairy studies links between nutrients and microflora composition have been done with supplements such as viable bacteria (probiotics), but in this study we used commercial dairy products available in the Egyptian markets that analyzed microbiologically and nutritionally as a step to help the Egyptian dairy industry and governmental surveillance to improve the standards of dairy products

In conclusion, this study refers to the probiotics which have obvious curing effect on *Staphylococcus auerus* without any deleterious effect on animal health even when given in high doses. Also, it was found that using the mixed probiotic strains culture could increase the protective and treating effects against *staphylococcus auerus* infection and is more effective than using the individual probiotic strain, the results indicated that all protection groups used probiotics were weakly effective against *Staphylococcus auerus* infection also, the pretreatment with both antibiotics and probiotics significantly decreased the number of bacteria in investigated organs compared to untreated ones. The use

of probiotics in conjunction with antibiotics, will act to reduce the effects of the dysbiosis caused by the antibiotics, and maximise the benefits of the probiotic directly in the gut on competitive exclusion and immune stimulation. It is advisable however to stagger the administration of the antibiotic and probiotic such that the probiotic is administered at least three hours after the antibiotic dose, where possible, otherwise the antibiotic may reduce the efficacy of the probiotic microorganisms. It is important to note that the reverse is not true: probiotics will not cause a reduction in efficacy or effectiveness of the antibiotic. The administration of the probiotic for at least one week following the completion of the antibiotic course.

## 5 References

- Abbas, M. and Mahasneh, A. (2014): Isolation of *Lactobacillus* strain with probiotics a potential from camel's milk. Academic journals, Vol. 8(15): 1645-1655.
- Abdel-Barry J.A., Abdel-Hassan I.A., Al-Hakim M.H. (1997). Hypoglycemic and antihyperglycemic effects of *Trigonella foenum-graecum* leaf in normal and alloxan induced diabetic rats. J. Ethnopharmacol., 58:149-155.
- Ayad, E.H.E.; S. Nashat; N. Ei-Sadek; H. Metwaly and M. Ei-Soda (2004): Selection of wild lactic acid bacteria isolated from traditional Egyptian dairy products according to production and technological criteria. Food microbiology, 21: 715-725
- Bancroft, D., A. Stevens and R. Turner, (1996). Theory practical of histological Techniques, 4th Edn., Churchill Livingstone, Edinburgh, London, Melbourne.
- Bauer AW, Kirby WM, Sherris JC, Turck M. (1966) Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. Apr;45(4):493-496.
- Brown, A.C. and A. Valiere, (2004). Probiotics and Medical Nutrition Therapy. Nutr. Clin. Care 68-56:7, by a standardized single disk method, Am. J. Clin. Pathol. 45 (1966): 493-496.
- Buivydas, A., Pasanen, T., Senilo, A., Daugelavičius, R., Vaara, M., and Bamford, D. H. (2013). Clinical isolates of *Pseudomonas aeruginosa* from superficial skin infections have different physiological patterns. FEMS Microbiology Letters 343, 183-189.
- Busch NA, Zanzot EM, Loisel PM, Carter EA, Allaire JE, Yarmush ML, Warren HS (2000). A model of infected burn wounds using *Escherichia coli* O18:K1:H7 for the study of gram-negative bacteremia and sepsis. Infect. Immun.;68:3349-51
- Cerning J., Renard C., Thibault J., Bouillanne C., Landon M., Desmazeaud M. and Topisirovic L. (1994), Carbon source requirements for exopolysaccharide. Production by *Lactobacillus casei* CG11 and partial structure analysis of the polymer. Appl. Environ. Microbiol. 60, 3914D3919.
- Cremonini, F., S. Di Caro Nista, E., Bartolozzi, F. Capelli, G. Gasbarrini G. and Gasbarrini, A. (2002). Metaanalysis: the effect of probiotic administration on antibiotic associated diarrhea. Aliment Pharmacol. Ther., 16: 14611467.
- Doron S.I., Hibberd P.L., Gorbach S.L., (2008). Probiotics for Prevention of Antibiotic associated Diarrhea. J Clin Gastroenterol. 42(s2): 5863.
- Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smiths F (1956). Colorimetric method for determination of sugars and related substances. Anal. Chem., 3: 350-356.
- Elkin S, Geddes D (2003). "Pseudomonas infection in cystic fibrosis: the battle continues". Expert review of anti-infective therapy 1 (4): 609
- Elliott, C., Brzezinski J., Sheth, S., and Salvatoriello, R., (1998). Storymorphing in the affective reasoning paradigm: generating stories automatically for use with 'emotionally intelligent' multimedia agents. In Proceedings of the Second International Conference on Autonomous Agents. Minneapolis, MN: ACM. Press.
- Elmer WH, Johnson DA, Mink GI, (1996). Epidemiology and management of the diseases causal to asparagus decline. Plant Disease .80, 117-23.
- FAO/WHO, (2002). Guidelines for the evaluation of probiotics in food. Report of a joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food.
- FAO-WHO "Food and Agriculture Organization of the United Nations"- "World Health Organization" (2001): Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria.
- Gilpin DA (1996). Calculation of a new Meeh constant and experimental determination of burn size. Burns.;22:607-611.
- Gratz, S.W., H. Mykkanen and H.S. ElNezami, (2010) Probiotics and gut health: a special focus on liver disease World J. Gastroenterol., 16: 40310.
- Isolauri, E., Sutas, Y., Kankaanpää, P., Annilommi, H., and Salminen, S., (2001): Probiotics Effects on immunity. Am. J. Clin. Nutr., 73: 444-450.

Kaplan ,M.M.,(1996).Primary biliary cirrhosis. N Engl J Med;335:1570-80.

Klevens, R.M., Morrison, M.A., Nadle, J., Petit ,S., Gershman, K., (2007) Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. JAMA 298: 1763–1771.

McCarthy, H., Rudkin ,J.K., Black ,N.S., Gallagher ,L., O'Neill ,E., O'Gara ,J.P., (2015)Methicillin resistance and the biofilm phenotype in *Staphylococcus aureus*. Front. Cell. Infect. Microbiol., 28 January.

Ogston, A., and Witte, W. (1984). On abscesses. Reviews of Infectious Diseases 6, 122-128

Ostad, S.N., Salarian, A.A., Ghahramani, M.H., Fazeli, M.R., Samadi, N.and Jamalifar, H.,(2009). Live and heatinactivated *Lactobacilli* from feces inhibit *Salmonella typhi* and *Escherichia coli* adherence to caco2 cells. Folia Microbiologica 54 :157160

Rasic, J.L., Vujicic, I.F., Skrinjar, M., and Vulic, M. (1992).Assimilation of cholesterol by some cultures of lactic acid bacteria and bifidobacteria. Biotechnol. Lett. 14:39–44.

Resta-Lenert ,S., and Barrett, K.E., (2003). Live probiotics protect intestinal epithelial cells from the effects of infection with enteroinvasive *Escherichia coli* (EIEC). Gut. 52(7): 988-97.

Sadiq, A. and J. Boehm, (2001). Influence of pronifer as a probiotic on the rumen fluidand blood parameters of sheep fed different roughage concentrate based diets. Wiener Tierarztliche Monatschrift, 88: 410.

Saggiaro, A.(2004): Probiotics in the treatment of irritable bowel syndrome. J. Clin. Gastroenterol, 38(6): 104-106.

Salem M.L. (1997). Increase of the bacteriostatic function macrophages against early infection with gram positive listeria monocytogenes after treatment of mice with estradio. J Egypt Ger Soc Zool. 23:347378.

Silva, A.M., Bambirraa,E.A, Oliveira .A.L, Souza .P.P, Gomes .D.A, Vieira .E.C,and Nicoli. J.R.(1999).

Protective effect of bifidus milk on the experimental infection with *salmonella enteritidis* subsp. Typhimurium in conventional and gnotobiotic mice. J.Appl. Microbiol., 86:331-6.

Silva, A.M., Barbosa ,F.H., Duarte, R., Vieira L.Q., Arantes, R.M.,and Nicoli, J .R.(2004) Effect of Bifidobacterium longum ingestion on experimental salmonellosis in mice. J. Appl. Microbiol., 97: 2937

Sonomoto, K. and Yokota ,A. (2011): Lactic Acid Bacteria and Bifidobacteria: Current Progress in Advanced Research. Caister Academic Press. ISBN 978-1 904455-82.

Sousa A, Ferreira I, Calhella R, Andrade P, Valentao P, Seabra R, Estevinho L, Bentoa A and Pereira AJ (2006). Phenolics and antimicrobial activity of traditional stoned table olives 'alcaparra'. Bioorg med chem. 14:8533-8538.

Tietz NW (1976). Fundamentals of clinical chemistry WB saunders Co, Philadelphia. Tietz NW (1976). Fundamentals of Clinical Chemistry WB Saunders Tietz NW (1999). Text Book of Clinical Chemistry, 3rd eds. AACC

Van Herck H. (1998) Orbital Sinus Blood Sampling in Rats as Performed by Different Technicians: the Influence of Technique and Expertise. Lab Anim. 32, 377386.

Vincze S, Stamm I, Monecke S, Kopp PA, Semmler T,. (2013) Molecular Analysis of Human and Canine *Staphylococcus aureus* Strains Reveals Distinct Extended-Host-Spectrum Genotypes Independent of Their Methicillin Resistance. Appl Environ Microbiol 79: 655–662.

Voller, A., Bridwell, D.E., and Annbartlett, (1976). Enzyme immunoassay in diagnostic medicine theory and practice. Bulletin of World Health Organization 53:55-65.

Yang, PLiou, K.N., Mishchenko, M.I., and Gao, B. C. (2000) Efficient finite difference time-domain scheme for light scattering by dielectric particles: Application to aerosols. Appl. Opt., 39, 3727-3737.

