The Comparison between the Effect of Probiotics and Antibiotics against Enterotoxin A Produced by *Staphylococcus aureus* Microbial and Histopathological Study

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Abstract

Background: *Staphylococcus aureus* is a significant pathogen that causes nosocomial infections as well as community-acquired diseases; the spectrum of *S. aureus* infections ranges from pimples and furuncles to toxic shock syndrome and sepsis. Probiotics, which are defined as "live microorganisms that, when taken in suitable proportions, impart a health benefit on the host beyond basic nourishment," have attracted increasing public and scientific interest in recent years; probiotics are crucial for preserving the integrity of the gut mucosal barrier and preventing the expansion of potentially harmful bacteria. **Objectives:** The aim of this study was to evaluate the efficiency of probiotics to protect against the Staphylococcal enterotoxin A (SEA) of *S. aureus* on different organs of rats comparing with the effect of antibiotics. **Materials and Methods:** Twenty-five isolates were obtained from a variety of clinical locations. The gene sea was detected by using specific primer, 20 rats were used in this study and randomly divided into 5 groups. A complete necropsy was performed for all the animals. **Results:** The results of the *sea* gene showed that 16 out of 25 isolates harbored this gene. And the photomicrograph of liver and intestine, after treatment by probiotics (bacteria and yeast), revealed normal hepatocytes and newly formed villi with mild thickness and intact a muscular layer, respectively. At the same time, after treatment with antibiotics, the results showed remarkable reversible changes of hepatic architecture, areas of hepatocytes with normal arrangements and reversible changes, and these were represented by a significant villi length and the areas of necrosis in a muscular layer in liver and intestine, respectively. **Conclusion:** The conclusion revealed, probiotics of both types (bacteria and yeast) showed curable effect in the organs under study that were altered by SEA and gave efficient effect as much as antibiotics, and also suggested the usage of probiotics instead of antibiotics for better health.

Keywords: Antibiotics, histopathological changes, probiotics, staphylococcal-enterotoxin-A, *Staphylococcus aureus*

Introduction

Due to its toxin-mediated virulence, invasiveness, and antibiotic resistance, *Staphylococcus aureus* is a significant pathogen that causes nosocomial infections as well as community-acquired diseases; the spectrum of *S. aureus* infections ranges from pimples and furuncles to toxic shock syndrome and sepsis.[1] The majority of *S. aureus* infections depend on a variety of virulence factors, whereas some, such Staphylococcal food poisoning, only require a single type of virulence factors; *S. aureus* is also a significant cause of mastitis.[2] These bacteria are one of the harmful microorganisms since it has mechanisms for damaging the host's tissue and promoting colonization; moreover, it is considered as a major causative agent of foodborne diseases and contributed to high food poisoning incidence globally.[3] Staphylococcal strains can produce a wide range of enterotoxins; the literature that is now available demonstrates that their nomenclature (A, B, C, D, E, G, H, I, J, K, L, M, N, O, P, Q, R, and U) is dynamic as new

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staphylococcal enterotoxins (SEs) are continually being discovered.[4] About 95% of food poisoning cases around the world are believed to be caused by the first five (A–E) classical enterotoxins.[5] A very modest concentration of SEs, ranging from 20 ng to less than 1 µg, is required to produce a staphylococcal food poisoning symptom; enterotoxins are mainly encountered by those who consume milk and dairy products.[6] One of the most significant public health issues in the upcoming decade is going to be antibiotic resistance.[7] Since *S. aureus* can acquire plasmids or transposons encoded with antimicrobial-resistant genes from other species and genera, the advent of multidrug-resistant *S. aureus* is also a major concern for public health, one of the main issues in healthcare settings is the emergence of antimicrobial-resistant *S. aureus*, such as methicillin-resistant *S. aureus* (MRSA).[8] Probiotics, which are defined as "live microorganisms that, when taken in suitable proportions, impart a health benefit on the host beyond basic nourishment," have attracted increasing public and scientific interest in recent years; probiotics are crucial for preserving the integrity of the gut mucosal barrier and preventing the expansion of potentially harmful bacteria.[9] One of the most popular probiotics in the fermented food and beverage sectors is lactic acid bacteria (LAB), which are plentiful in the natural animal and human gastrointestinal flora. Numerous fermented milk products are already produced commercially using *Lactobacillus casei*, and several studies have shown that *L. casei* can prevent bacterial infections in mice when given orally.[10] Numerous of these probiotics are LAB, and anaerobic *bifidobacteria* have been shown to be effective in the treatment of diarrheal illnesses and altered intestinal microbiota.[11]

Materials and Methods

1- ***S. aureus*** **isolation and identification:** Twenty-five isolates were acquired by cultivating on blood agar and mannitol salt agar, which is a selective media for these bacteria, and assessing the catalase production, as well as Gram staining, from a variety of clinical locations, including wounds, pimples, skin infections, and urine.[12]

2- **Isolation of bacterial DNA and conditions of PCR:** Using the particular extraction kit for bacteria (Add bio, Korea), the entire DNA was extracted, and the process was carried out in accordance with the manufacturer's instructions. Additionally, the final volume of the reaction (20 µL), consisting of 5 µL of the master mix kit, 5 µL of DNA, 3 µL for each of the forward and reverse primers, and lastly 4 µL of DNase-free water, was used for the PCR stages. The amplification settings were 94°C for 5 min of denaturation, 35 cycles of amplification at 57°C for 2 min of annealing, 72°C for 1 min of extension, and 72°C for 7 min of final extension. The amplification was completed using a thermal cycler (Bioneer, Korea). The amplified amplicon was detected using a UV eliminator device. Table 1 shows the primer used in this study.

Animals

All of the experimental animals ($N = 20$) used in this study were 8-week-old male white western rats approximately weighing (250 g) were purchased from a local animal house at Veterinary Medicine College, University of Kerbala. The rats were kept in a standard caging maintained in a well-aired room, at constant temperature in the college animal house. Animals were permitted to consume tap water provided via water bottles and food freely.

Experimental protocol

These animals were randomly divided into five groups as

1. The first group ($n = 4$) was served as a negative control.
2. The second group ($n = 4$) was challenged with bacteria. The rats were intraperitoneally inoculated with 10 µL of bacterial suspension, containing 1.0×10^7 CFU/m, and left for the end of the experiment (positive control).
3. The third group ($n = 4$) was also intraperitoneally inoculated with 10 µL (1.0×10^7 CFU/m) of bacterial suspensions, then rats received an oral daily dose of 1 mL of (floratil) yeast suspension (one sachet 100 mg mixed with 100 mL of distal water) for 7 days.
4. The fourth group ($n = 4$) was injected intraperitoneally with 10 µL (1.0×10^7 CFU/m) of bacterial suspensions, then rats received orally a daily dose of 1 mL of vitalactic B probiotic colloid (one capsule containing 50 mg of *Lactobacilli* culture dissolved in 100 mL of distal water at room temperature) for 7 days.
5. The fifth group ($n = 4$) was injected intraperitoneally with 10 µL (1.0×10^7 CFU/m) of bacterial suspensions; rats received a daily intraperitoneal injection with 1 mL of an antibiotic suspension (linezolid tablets) (one tablet containing 600 mg of linezolid dissolved in 100 mL of distal water at room temperature) for 7 days as well.

At the end of experimental period, samples were taken from male rats that were sacrificed for histopathological examination.

Table 1: Primer used to investigation of enterotoxin-A in *Staphylococcus aureus*

Gene	Primer	Size of amplicon	Reference
sea	F 5′TAAGGAGGTGGTGCCTATGG3′ R 5′CATCGAAACCAGCCA3′	180 bp	[13]

Histopathological study

A complete necropsy was performed for all the animals. Organs were examined for gross lesions; pieces of intestine and liver were collected. Histopathology was performed for sections of the small intestine and liver, fixed in 10% neutral formalin. Sections underwent processes of slides preparations, according to Li *et al*.[14] Tissues obtained were dehydrated into graded ethanol series, followed by xylene clarification, before being embedded in paraffin wax. Several 4-mm thick sections using a microtome were cut from each sample and being stained with hematoxylin and eosin. Slides were examined using a light microscope and being photographed with a digital camera.

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients; verbal and analytical approval before the sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee by the number (UOK.VET.MI.2022.054).

Results

The detection of the presence of *sea* gene among enterotoxin-encoding genes *S. aureus* isolates was assessed by using specific primers (the result) which were illustrated in Figure 1.

The results of liver and intestinal sections of the third group of rats, which intraperitoneally inoculated with 10 µL of the isolates containing a bacterial suspension, and then were received an oral daily dose of floratil yeast suspension, were illustrated in Figures 2 and 3.

The results obtained for liver and intestine of the fourth group of rats that injected intraperitoneally with 10 µL of the isolates containing of bacterial suspensions, and then received orally a daily dose of vitalactic B probiotic colloid of *Lactobacilli* which were revealed in Figures 4 and 5.

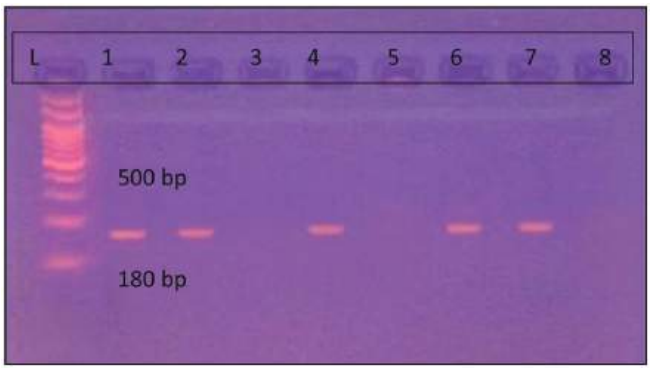

Figure 1: Gel electrophoresis for *sea* gene, L = ladder, isolates 1, 2, 4, 6, and 7 were positives, while 3, 5, and 8 were negatives

The obtaining results of the second group (positive control) of liver and intestine were shown in Figures 6A and B and 7A and B, respectively.

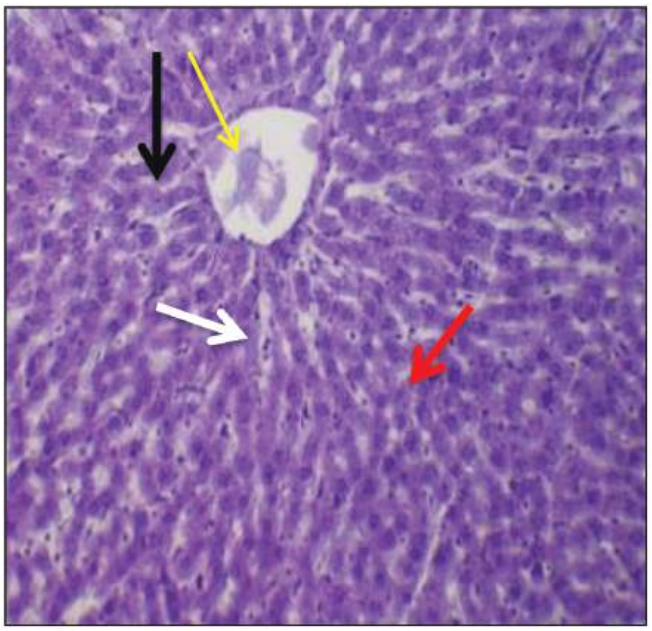

Figure 2: Photomicrograph of liver of a male rat treated with floratil probiotic for 1 week postinfection with *S. aureus* revealed significant hepatocytes cords (black arrow) radiated around mild congested central vein (yellow arrow), with mild hepatic degeneration (red arrow, slight interstitial infiltration of inflammatory cells (white arrow) (H and E, 10×)

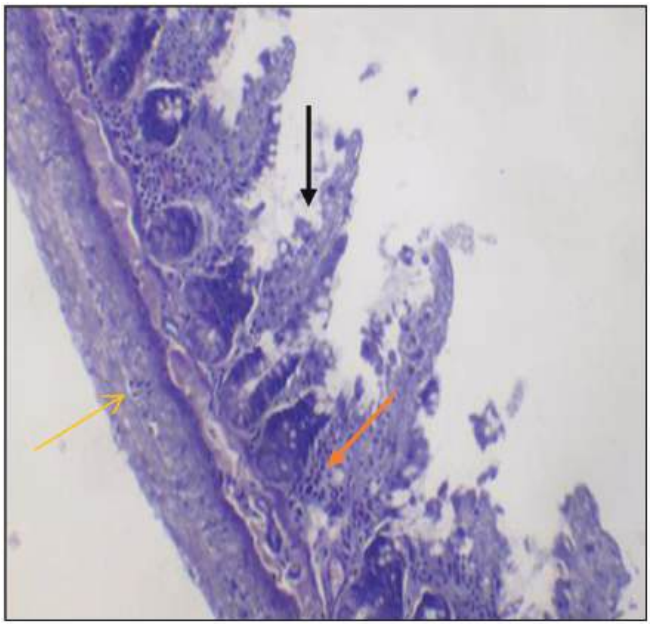

Figure 3: Photomicrograph of intestinal section of a male rat treated with floratil probiotic for 1 week postinfection with *S. aureus* showed newly formed villi (black arrow) with mild thickness, intact muscular layer (yellow arrow), with slight infiltration of inflammatory cells in submucosa (red arrow) (H and E, 4×)

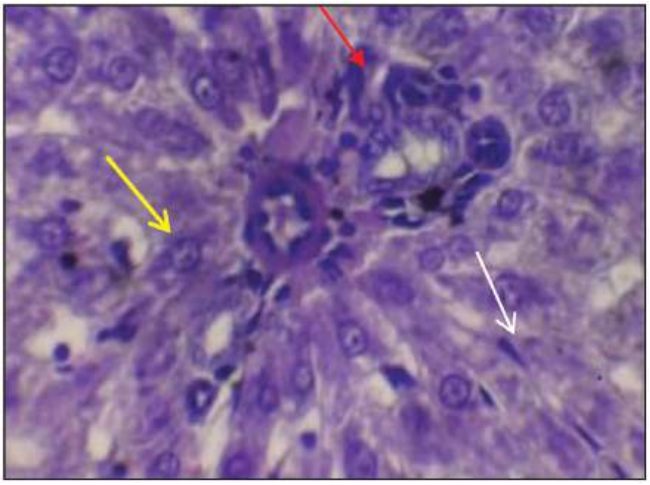

Figure 4: Photomicrograph of liver of a male rat treated with vitalactic B probiotic for 1 week postinfection with *S. aureus* revealed mild portal inflammatory cells infiltration (red arrow) normal hepatocytes (yellow arrow), with slight interstitial infiltration of inflammatory cells (white arrow) (H and E, 40×)

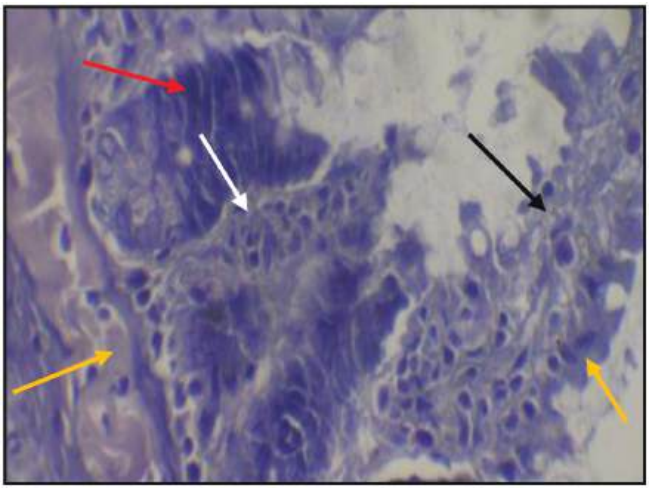

Figure 5: Photomicrograph of intestinal section of a male rat treated with vitalactic probiotic for 1 week postinfection with *S. aureus*, showed newly formed villi (black arrow) with intact mucosal epithelium and muscular layer (yellow arrow), with slight infiltration of inflammatory cells in submucosa (white arrow) and normal goblet cells (red arrow) (H and E, 10×)

Figure 8A and B as well as Figure 9A and B for the first group (negative control) is illustrated later.

The fifth group of animals was injected intraperitoneally with isolates containing 1.0×10^7 CFU/m bacterial suspensions, rats were received a daily intraperitoneal injection with an antibiotic suspension (linezolid tablets) as shown in Figures 10A and B and 11A and B].

Discussion

In a recent study, it was found that 16 out of 25 isolates harbored *sea* gene (62%), and when compared with other studies, 30 of the 62 *S. aureus* strains coding for toxins was detected; *sea* was the most prevalent virulence gene (24.1%).[15] *S. aureus* can produce a wide variety of virulence factors and about 70.9% were found to contain sea genes; and among 79.5% of the strains which carried in *sea* genes, and this rate is higher to the result reported by di Giannatale *et al.*[16]

Staphylococcus aureus is a common organism in nature, and in the food industry, food handlers and food contact surfaces are two of the primary causes of *S. aureus* contamination; the epidemics of food-borne illnesses linked to the use of milk and dairy products are brought on by this bacterium.[17] Infections with *S. aureus* are frequently acute pyogenic, spread to deeper tissues nearby, or metastasis to other locations affecting other organs, leading to disseminated or deep-seated infections that constitute a serious illness.[18]

It was discovered that Staphylococcal enterotoxin A (SEA) could cross the intestinal mucosal barrier with the aid of absorptive epithelial cells through endocytosis, but as far as we are aware, the role of SEA on intestinal injury and the underlying toxic mechanism remains unknown. SE subunits contribute to intestinal injury during gastrointestinal infection.[19] SE-food poisoning causes inflammatory changes throughout the gastrointestinal tract with severe lesions in the jejunum and ileum, likely as a result of the cytotoxic effects of *S. aureus* products. Degeneration was observed in the liver, kidney, intestine, and stomach.[18]

Rats exposed to SEA underwent discernible morphological alterations in the kidney, small intestine, and stomach. None of the rats' mesenteric lymph nodes, liver, spleen, or large intestine showed any alterations.[20] Intestinal epithelium destruction, goblet cell loss, random lack of nuclei staining, blatant inflammatory cell infiltration, villous blunting, crypt distortion, and overall tissue architecture were all present in the small intestines of mice treated with SEA; additionally, the ratio of villus height to crypt depth was used to quantify the degree of injury to the tissues of the duodenum, jejunum, and ileum. In comparison to the control group, administration of SEA significantly decreased the ratio of villus/crypt (V/C) of the duodenum, jejunum, and ileum to 43.1%, 30.9%, and 36.4%, respectively.[19]

Antibacterial drug degradation by enzymes, modification of bacterial proteins that are antimicrobial targets, and changes in membrane permeability are some of the factors that contribute to antimicrobial resistance in bacteria; plasmid, integron, or transposons can also play a role in the transmission of antibiotic resistance; the bacterial enzyme beta-lactamase, which is the most significant mechanism for resistance to cephalosporins and penicillin mediates the hydrolysis reaction; so by obtaining a genomic island known as the staphylococcal chromosomal cassette (mec), *S. aureus* becomes resistant to methicillin

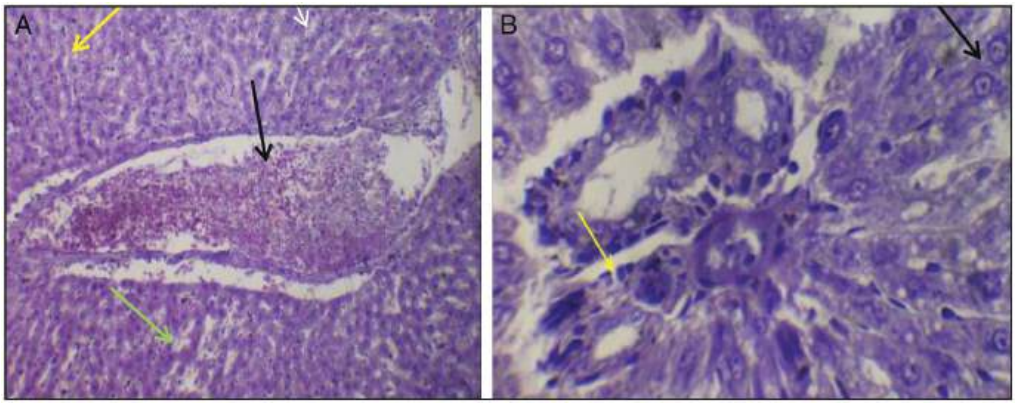

Figure 6 : (A) Photomicrograph of liver from *S. aureus*-infected male rat revealed severe pathological alterations, severe congestion of central vein (black arrow), significant hepatocytes degeneration (yellow arrow), with remarkable necrosis (white arrow), severe interstitial inflammatory cells infiltration (green arrow) (H and E, 10×). (B) Photomicrograph of liver from *S. aureus*-infected male rat revealed severe pathological alterations, significant infiltration of inflammatory cells (MNCs) in portal area (black arrow), significant hepatocytes degeneration (yellow arrow) (H and E, 40×)

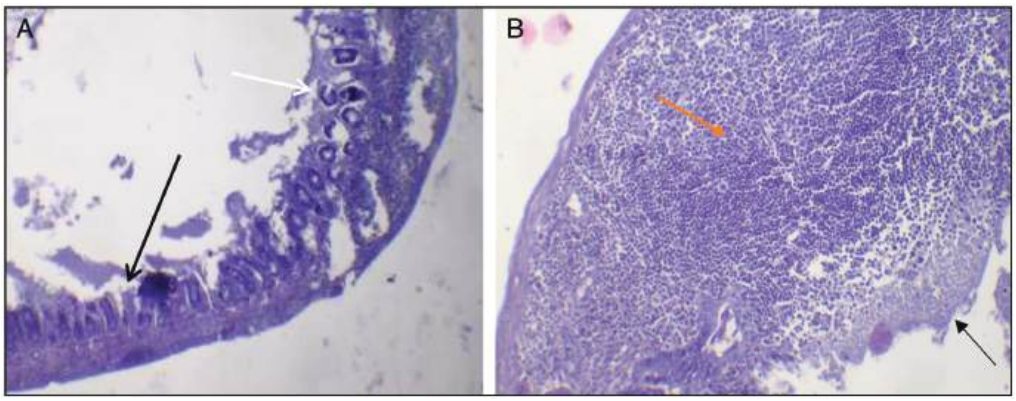

Figure 7: (A) Photomicrograph of intestinal transverse section from *S. aureus*-infected male rat revealed severe pathological alterations, significant damage and desquamation (loss) of villi epithelial mucosa (black arrow), significant atrophy of crypts (white arrow) (H and E, 4×). (B) Photomicrograph of intestinal transverse section from *Staphylococcus aureus*-infected male rat revealed severe pathological alterations, significant damage and desquamation of villi epithelial mucosa (black arrow), and significant and severe infiltration of inflammatory cells infiltration (granulocytes) in submucosa (red arrow) (H and E, 10×)

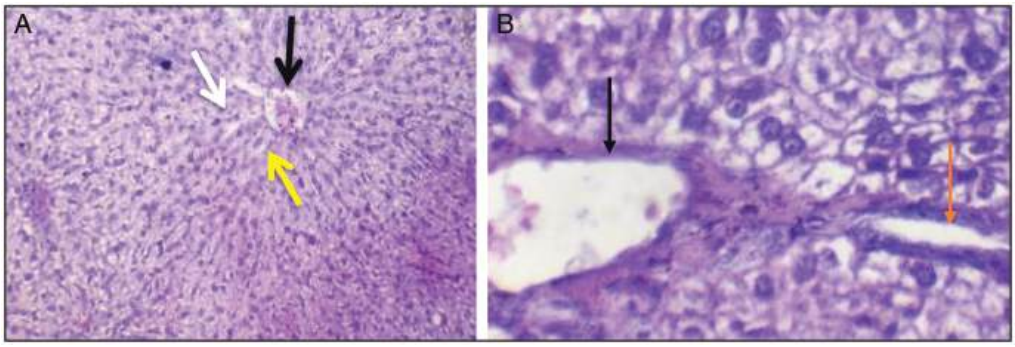

Figure 8: (A) Photomicrograph of hepatic lobule of control animal showed the normal hepatic architecture, significant hepatocytes cords (white arrow) radiating around normal central vein (black arrow) and normal sinusoidal spaces between the hepatic cords (yellow arrow) (H and E, 10×). (B) Photomicrograph of liver of control animal showed the normal portal area architecture and normal bile ductile (white arrow) with slight dilated portal vein (black arrow) (H and E, 40×)

and other lactam drugs.[21] It is crucial to remember that the majority of *S. aureus* isolates linked with antibiotic-related diarrhea are methicillin-resistant (MRSA).[22] Methicillin is a semisynthetic—lactam that is insensitive to—lactamases; resistance to this antibiotic is associated with the mecA gene, which encodes the penicillin-binding protein PBP2a; this protein allows the synthesis of the cell wall even at lethal concentrations of—lactams, but MRSA

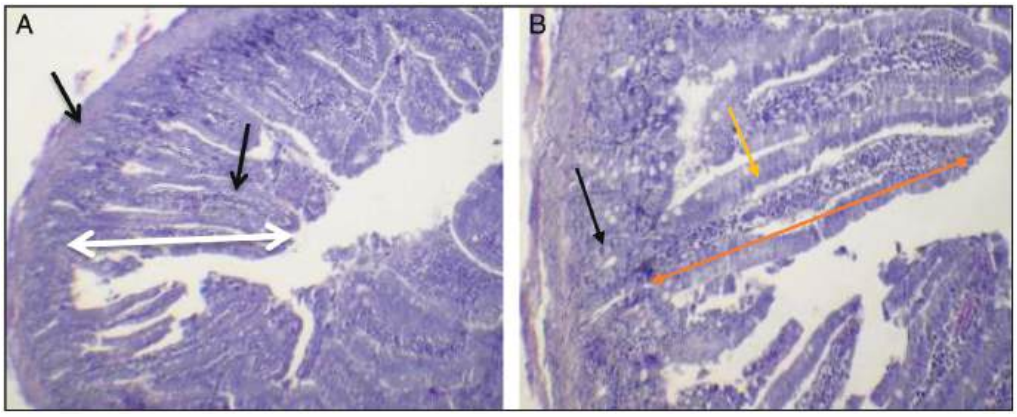

Figure 9: (A) Photomicrograph of transverse section of control animal intestine showed the normal intestinal wall histological architecture, normal and significant villi shape and length (white arrow), normal mucosa and submucosa (black arrow) (H and E, 4×). (B) Photomicrograph of transverse section of control animal intestine showed the normal duodenal wall histological architecture, normal and significant villi shape and length (red arrow), normal mucosal epithelia (yellow arrow), and normal crypts epithelium (black arrow) (H and E, 10×)

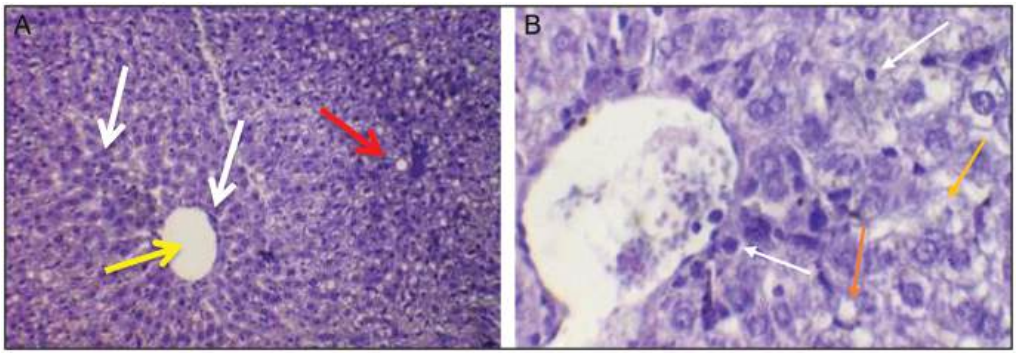

Figure 10: (A) Photomicrograph of liver section of a male rat treated with an antibiotic for 1 week postinfection with *S. aureus* revealed remarkable reversible changes of hepatic architecture, areas of hepatocytes normal arrangements (black arrow) radiated around mild congested central vein (yellow arrow), with significant hepatic vacuolation (red arrow), perivascular and interstitial infiltration of inflammatory cells (white arrow) (H and E, 10×). (B) Photomicrograph of liver section of a male rat treated with an Antibiotic for 1 week postinfection with *S. aureus* revealed remarkable hepatocytes degeneration (black arrow), areas of necrosis (yellow arrow), with significant hepatic vacuolation (red arrow), perivascular and interstitial infiltration of inflammatory cells (white arrow) (H and E, 40×).

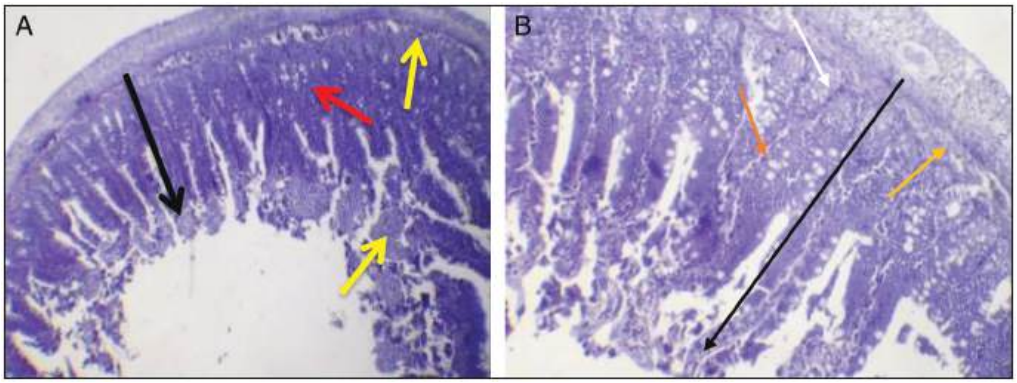

Figure 11: (A) Photomicrograph of transverse section of intestine of a male rat treated with an antibiotic for 1 week postinfection with S. aureus revealed remarkable reversible changes, represented by the significant villi length (black arrow), areas of necrosis (yellow arrow), with severe mucosal vacuolation (red arrow) (H and E, 4×). (B) Photomicrograph of transverse section of intestine of a male rat treated with an antibiotic for 1 week postinfection with S. aureus showed remarkable reversible changes, represented by the significant villi length (black arrow), areas of necrosis in muscular layer (yellow arrow), with severe mucosal vacuolation (red arrow) and submucosal inflammation (white arrow) (H and E, 10×).

strains are resistant to all—lactam antibiotics because of their high affinity for this protein.[23] Also, Kader *et al.*[24] showed that methicillin and oxacillin disc resistance was present in 88.24% of the staphylococcal isolates.

Natural food preservatives are now preferred over artificial ones by all parties involved in the food safety industry since they have fewer negative effects on human health or the nutritional value of foods.[25] The preservative activity of LAB in foods has a strong antagonistic effect against food spoilage and pathogenic microorganisms is mainly attributed to competitive exclusion for essential nutrients or adhesion sites of mucous cells, immune modulation, redox modification, accumulation of D-amino acids, and production of lactic acid. LAB are very important in converting agricultural products into safe, delicious, and shelf-stable foods for human consumption.[26] Since a probiotic approach is not antibiotic-based like the other ways that have been used to decolonize *S. aureus*, it would have various advantages over those methods and would not have the drawback of requiring considerable antibiotic use to decolonize a significant portion of people or patients, the risk of infection with *Clostridium difficile* and other pathogens that benefit from a thorough eradication of the intestinal microbiota makes the use of antibiotics, specifically for the decontamination of the intestine, extremely hazardous.[27] Consequently, the probiotics strategy provides pathogen-targeted eradication, which is not achievable with antibiotics; moreover, probiotics continuously create the material that is active, allowing for a maintained presence that is impossible to obtain even with numerous medication administrations; probiotics may be beneficial in the fight against *S. aureus* colonization and as quorum-sensing inhibitors in the treatment of various bacterial infections; They also shed new light on the relative importance of several bacterial colonization sites as well as the function of staphylococcal quorum-sensing in intestine colonization.[28]

Conclusion

It was noted from the results obtained in this study that probiotics of both types (bacteria and yeast) showed curable effect in the organs under study after being treated by SEA and gave efficient effect as much as antibiotics and suggested the usage of probiotics instead of antibiotics for better health.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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