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Histopathological Assessments on Kidney and Spleen of Experimentally Infected Male Rats by Staphylococcus aureus: Antibiotics versus Probiotics

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Abstract:

Background: *Staphylococcus aureus* (*S. aureus*) stands as a prominent contributor to infections and fatalities on a global scale. Antibiotic resistance added a layer of complexity to the management of *S. aureus* infections. Presently, there is no viable vaccination option. Probiotics have been identified as possible agents that can help overcome antibiotic resistance. **Aim**: In the current study aimed to investigate the histological changes in organs such as the kidney and spleen in male rats after infection with 10 µL *S. aureus* (1.0 × 107 CFU/mL) intraperitoneally and treated with a daily dose of 1 ml of (floratil) yeast suspension, daily oral dose of (1 ml) of probiotic colloid for 7 days, and compared with the group inoculated intraperitoneally injections of 1 mL of an antibiotic suspension (linezolid tablets) for 7 days. **Results:** the animals treated with probiotics showed more significant histopathological alterations and recovery as compared to antibiotics-treated groups in response to restoring renal and splenic tissue from the histological effects caused by bacterial infection; this study shows the protective effects of antibiotics, probiotics, and yeasts in maintaining kidney and spleen tissue structures after *S. aureus* infection histologically and to compare between them. **Conclusion:** this study investigated the protective effects of probiotics on renal and splenic tissues against Staphylococcus aureus to the extent that they are more effective than antibiotics in recovery of histological damage.

Keywords: Pathogenic bacteria, Histological features, Linezolid, Probiotics, Enterotoxin.

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Introduction:

Staphylococcus aureus, a prevalent pathogenic bacterium, significantly contributes to global morbidity and mortality. This microorganism has the potential to inducer various illnesses, including moderately severe skin infections, as well as deadly pneumonia and sepsis [1,2,3]. The bacteria can also be obtained from animals, particularly in the cattle industry [4,5]. *Staphylococcus aureus* causes feline otitis, especially during the colder months [6]. Additionally, these bacteria are responsible for severe animal ailments, such as suppurative disease, arthritis, mastitis, and urinary tract infections [7] due to virulence factors like extracellular toxins and enzyme secretion [8]. Though there has been consistent interest in the virulence of *S. aureus* since its recognition as a significant pathogen in the late nineteenth century, recent discoveries have intensified research endeavors aimed at comprehending the mechanisms underlying *S. aureus* virulence [9].

A staphylococcal infection's success is influenced by its ability to escape host defenses [10]. To achieve this, the bacterium exits the bloodstream with a formidable concentration of humoral and cellular mechanisms of immune defenses and infiltrating organs and tissues, thereby giving rise to encapsulated abscesses [1]. The organs and tissues invasion by these bacteria through the circulatory system necessitates not only immune evasion only; but also relies on adhesion and various structural mechanisms [11]. Surface-anchored proteins from the family of microbial surface components recognizing adhesive matrix molecules can play a crucial role in facilitating different aspects of these processes [12].

Staphylococcus aureus evades innate immune defenses during the early stages of infection, but its interaction with the acquired immune system becomes crucial after 1-2 weeks post-infection [13]. Also, resistance to antibiotics by these microorganisms can lead to therapeutic failure, higher medical costs, and mortality in humans and animals [14,15]. Most chemical drugs cause nephrotoxicity through pathogenic effects such as tubular toxicity, crystal formation, inflammation, and glomerular hemodynamic changes [16]; renal damage occurs due to the antioxidant system [17]. Antibiotic resistance and the challenge of discovering a vaccine for *S. aureus* have led to a renewed focus on developing alternative therapeutic options for bacterial infections [18]. Alternatively, the probiotic strategies may also be explored as anti-virulence treatments against infections by *S. aureus* [19 ,20, 21]. Probiotics are live bacteria that improve host health and function; adding beneficial bacteria to the diet can improve nutrient absorption, digestion, and the immune system, leading to better health and disease resistance [22]. Moreover, probiotics are recognized for enhancing and fortifying the immune system [23]. The current study aimed to investigate the histological alterations produced by *staphylococcus aureus* oxidative damage induced in kidney and spleen tissues and to compare the ameliorative effects of antibiotics and probiotics in these tissues.

Materials and Methods:

Ethical approval:

The local ethics committee of the Veterinary Medicine College of Kerbala University (UOK.VET.MI.2022.054) accepted the study protocol, subject information, and consent form of patients for sample collection.

***Staphylococcus aureus* Isolation and Characterization:**

Twenty bacterial isolates were obtained from Al-Hussein Medical City Hospital in Kerbala Province, were cultured on blood agar and mannitol salt agar, which is a particular media for these bacteria, and observed catalase production as well as Gram staining from various types of clinical regions, including wounds, pimples, skin infections, and urine [24,6].

Animal Design, Nutrition and Physical Conditions:

The experimental animals (N = 25) utilized in the current research were all of an age of eight weeks male white western rats weighing around (200-250g) that were obtained from a local animal house at the Veterinary Medicine College, University of Kerbala. The rats were housed in appropriate metal cages individually in a well-ventilated area under controlled room temperature, and a consistent 12-hour light/12-hour dark cycle was maintained in the animal house at the college. Animals were free to consume tap water and food provided in water bottles. They underwent a two-week acclimatization period in the research environment before engaging in the current study to ensure their overall health.

Experimental design:

The animals were segregated into five groups (n=5) each; with the initial group functioning as a negative control and receiving intraperitoneal injections of normal saline, the other four groups received i.p. injections of a challenge dose of bacterial suspension of 10 µL *Staphylococcus aureus* (1.0 × 107 CFU/mL) to establish an infection. After 48 hours, the second group received no therapy, whereas the third group received daily a dose of 1 ml of (floratil) yeast suspension (one sachet 100 mg mixed with 100 mL of distal water) orally for 7 days. The fourth group was given 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 25ºC (room temperature) for 7 days, the last group inoculated intraperitoneally injections of 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 in a daily basis for 7 days. Upon completion of the experiment, rats from all groups of the current study underwent euthanasia via cervical dislocation, and visceral organs such as the kidney and spleen were collected for histopathological investigations.

Histopathological Analysis:

After the animals were euthanized and dissected, the kidney and spleen were aseptically taken and immediately immersed in a 10% (v/v) neutral-buffered formalin solution for fixation. Histological portions were processed into graded alcohol series, clarified with Xylene, and embedded with paraffin and samples were cut into 4 mm thick sections [25]. The slides were stained using the hematoxylin-eosin routine method. Microscopical imaging with histological diagnosis and scoring was applied to all slides by a certified pathologist (from the Department of Pathology, Veterinary Medicine College, Kerbala University, Iraq) using an optical microscope with a specified digital camera (Optika, Germany).

Results:

A light microscope examination revealed a range of histological alterations for the main organs, kidney and spleen in the infected and treated animals, compared to the standard group.

All kidney and spleen tissue sections evaluated from the control negative group showed regular histological features. As opposed to the challenge group, severe pathological alterations were observed: severe glomerular atrophy, severe tubular epithelial degeneration, manifested by star-shaped lumen with severe peri glomerular and interstitial tissue polymorph nucleated inflammatory cells infiltration (figure 1), significant white pulp liquefactive necrosis and splenic depletion, severe granulomatous inflammation in white pulp with remarkable inflammatory cells infiltration and

hemosiderosis in red pulp and sever megakaryocytes infiltration in red pulp(figure 2) Otherwise, treated groups showed noticeable improvements in the examined organs.
Treatment with probiotics and antibiotics resulted in tissue damage recovery and regeneration of red pulp, transparent red and white pulp regions, and marginal zones in the group treated with the antibiotic (Figure 3). Still, internal damage was not noticeable as the white pulp increased, and the peripheral zone was noted. Following probiotic treatment, WBC infiltration was also slightly observed in the red pulp region (Figure 4). The histological alterations in the animals treated with yeast were less significant (figure 5).

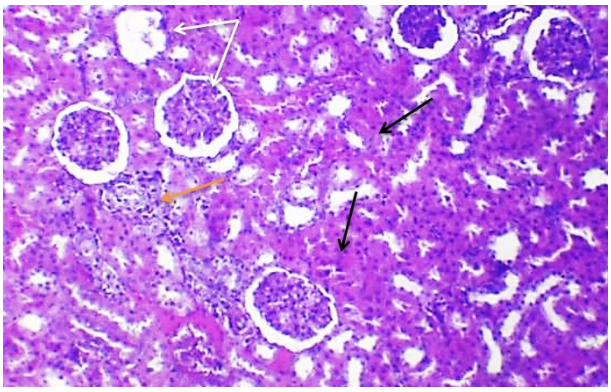

Figure (1): Photomicrograph of renal tissue section for a challenge group animal reveals remarkable histopathological changes, significant glomerular atrophy (white arrow), severe tubular epithelial degeneration, manifested by a star-shaped lumen (black arrow) with considerable infiltration of interstitial inflammatory cells, (highlighted by yellow arrow). (H&E stain, 10X magnification).

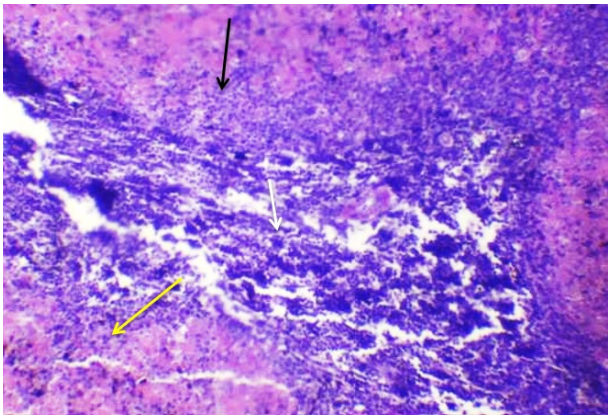

Figure (2): Photomicrograph of spleen for a challenge group animal showing severe histopathological alterations, significant white pulp liquefactive necrosis and splenic depletion (white arrow), severe granulomatous inflammation in white pulp (indicated by black arrow) with remarkable inflammatory cells infiltration in red pulp (showed in yellow arrow). (H and E,10 X).

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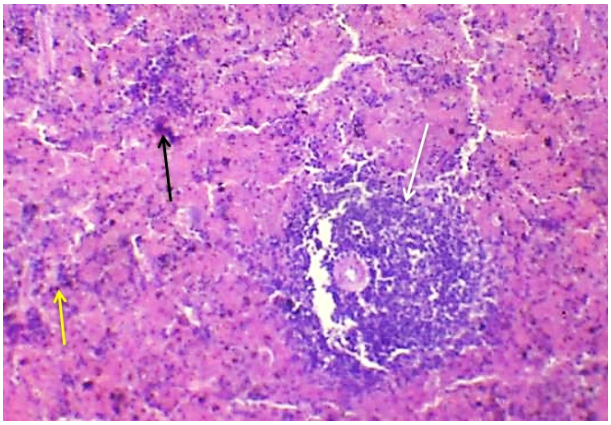

Figure (3): Photomicrograph of spleen for Antibiotic animal showing significant reverse histological alterations, significant lymphoid follicle with central arteriole with mild lymphocytic hyperplasia (white arrow), less hemosiderosis in the red pulp (black arrow) with slight infiltration of inflammatory cells in the red pulp (indicated yellow arrow). (H and E,10 X).

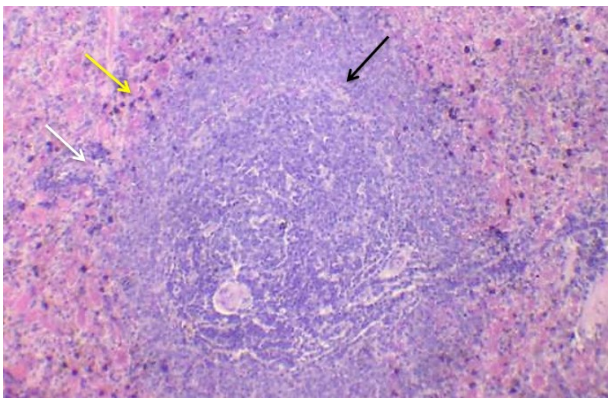

Figure (4): Photomicrograph of the spleen of the animal treated with Probiotics after infection with *S. aureus*, showing follicular hyperplasia (referred to by black arrow) with mild hemosiderosis (yellow arrow) and slight infiltration of inflammatory cells in the red pulp (revealed in white arrow). (H and E,10 X).

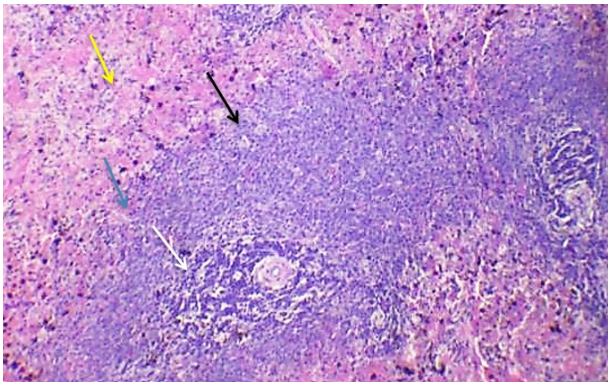

Figure (5): Photomicrograph of the spleen of a treated animal with yeast, showing significant follicular hyperplasia (black arrow) with mild hemosiderosis (yellow arrow) and white pulp necrosis (white arrow). (H and E,10 X).

The morphology of the renal epithelial cells was restored. Still, the renal glomeruli remained reversed to normal with considerable tubular vacuolation in the probiotics (Figure 6), yeast-treated groups (Figure 7), the cortex and medulla were visible and intact compared to the challenged group; however, the Antibiotic group is identical to that of the regular group (Figure 8).

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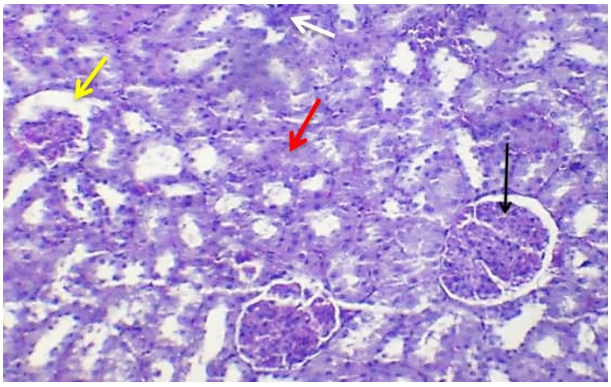

Figure (6): Photomicrograph of kidney section of a male rat treated with Probiotics for a one-week post-infection with *S. aureus* reveals slight reversible alterations, some normal glomerular histology (black arrow), less others are atrophied (yellow arrow), renal tubular degeneration (red arrow), slight infiltration of inflammatory cells interstitially (indicated by white arrow). (H and E,10 X).

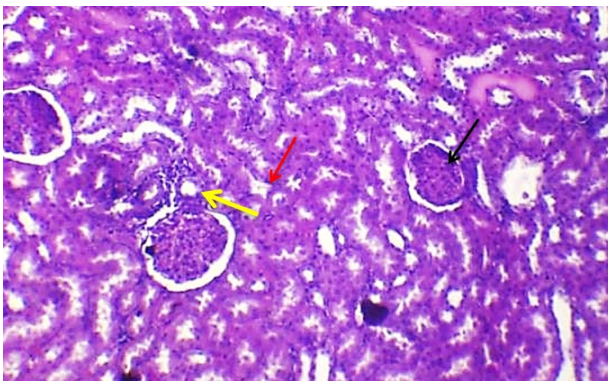

Figure (7): Photomicrograph of kidney section of a male rat treated with yeast after experimental infection with *S. aureus* reveals slight reversible alterations, represented by normal glomerular histology (black arrow), renal tubular epithelial degeneration manifested by lumen with star-shaped lumen (red arrow), slight interstitial infiltration of inflammatory cells (marked by yellow arrow). (H and E,10 X).

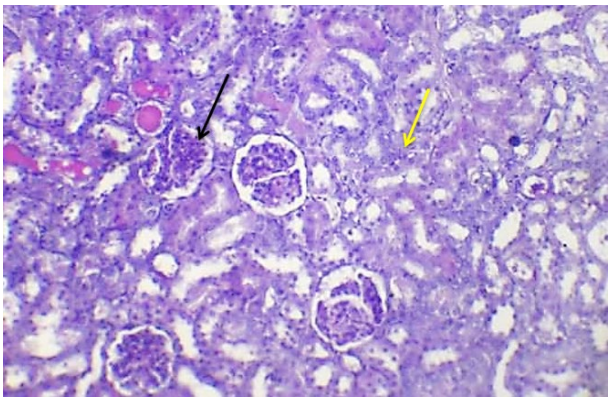

Figure (8): Photomicrograph of renal tissue section for an antibiotic-treated group animal showing marked reversible changes, close to normal glomeruli (black arrow), mild tubular epithelial degeneration (yellow arrow). (H and E,10 X).

Discussion:

The findings of the current investigation unveiled notable pathological alterations characterized by pronounced suppurative inflammation observed under microscopic examination; the affected organs in the *S. aureus*-challenged group exhibited a substantial presence of polymorphonuclear leukocytes (PMNs), predominantly neutrophils, confirming agreement of this observation with other researchers, who shown that, Infections caused by *S. aureus* often manifest as acute pyogenic

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conditions, spreading to adjacent deeper tissues and giving rise to multiple abscesses and pyogranulomas; accompanied by extensive suppurative inflammation [26,27].

Polymorphonuclear leukocytes (PMNs), particularly neutrophils, were considered the primary cellular defense against MRSA infections [28]. As a result of tissue injury, circulating (PMNs) exited the vascular system and reached the infection site; this was supported by a study by Kolaczkowska and Kubes [26]. Septicemia and endotoxemia caused severe blood vessel constrictions and organ degeneration, demonstrating that *S. aureus* cell wall-derived protein A hemolysins played a role in inducing the biosynthesis of inflammatory cytokines by macrophages and/or monocytes [29]. So, that explained the correlations with our findings.

Degeneration was observed in the kidney and spleen of challenge group animals, most likely due to the cytotoxicity induced by *S. aureus* compounds; these explanations agreed with the findings of [30], who examined the activity of the cytotoxicity of toxin generated by *S. aureus* as evidenced by histopathological abnormalities. Histopathology and tissue injury of the spleen were seen, including red and white pulp degradation, an expanded marginal zone, an increase in follicle count, and granulomatous inflammation, which were also mentioned in earlier studies [31]. Furthermore, animals given *S. aureus* developed focal hemorrhages between the white pulps, lymphoid depletion, and necrobiosis. Congestion in the blood arteries and sinusoids and localized hemosiderosis in the red pulps were also found; this indicates that the spleen has entered the acute phase of the illness, confirmed by [32], who concluded that the *S. aureus* causes increasing in the production of reactive oxygen species (cellular oxidative stress on tissue organs). Histopathology of the kidney of *an S. aureus-infected* rat revealed significant changes represented by marked pyelonephritis, and these alterations were agreed upon by [33].

Nevertheless, in our study presentations, the oral administration of probiotics and intraperitoneal treatment with antibiotics revealed excellent therapeutic action in the kidney and spleen of *S. aureus*-infected rats. Unfortunately, antibiotic treatment has led to the rise of drug-resistant bacterium types, making the disease challenging to cure [34]. Consequently, our evaluation involved two types of probiotic species: *lactobacilli* and yeast. Lactobacilli bacteria can produce various antimicrobial compounds, such as natural acids (acidic and lactic acids), hydrogen peroxide, carbon dioxide, diacetyl, ethanol, and peptides (bacteriocins); these compounds are valuable not only in the reduction of foodborne pathogens and bacterial damage during storage and consumption but also, offer broader utility [35]. Probiotics employ various methods to compete within the ecological niches, and these mechanisms bolster the host's resistance to pathogen colonization [36]. Bacteriocins are believed to be able to eliminate their targets while sparing the producer due to the presence of immunity proteins; however, numerous mechanisms resemble those found in contemporary antimicrobial agents [37]. In the current study, probiotics play a central role in helping to maintain the integrity of renal and splenic tissues, improving damaged tissue, and keeping healthy tissue, as indicated by previous studies [38]. The histopathological sections in groups of animals inoculated with antibiotics showed moderate tissue recovery, as reviewed in previous studies [39,40].

Conclusions:

This study examined probiotics and antibiotics' capacity to ameliorate the histological injuries in the kidneys and spleens produced by staphylococcus aureus infection. Furthermore, it was investigated that probiotics have protective effects on renal and splenic tissues against Staphylococcus aureus to the extent that they are more effective than antibiotics in histological damage recovery.

To enhance this approach, further clinical trials involving diverse organs and infection models are planned for the near future**.**

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Additionally, we acknowledge the dedication of the Pathology staff in facilitating the experiment at the pathology laboratory, College of Veterinary Medicine/Kerbala University, and for their diligent efforts in processing samples and conducting the histopathology procedure.

Conflict of interest:
The authors declare that there is no conflict of interest.

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