HAEMATOLOGICAL ACTIVITY OF *Lactoba*cillus *casei* IN ENHANCING IMMUNITY AGAINST *Staphylococcus scuiri* IN RATS

BY

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LSC2009739

DEPARTMENT OF MICROBIOLOGY

UNIVERSITY OF BENIN

BENIN CITY.

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A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF DEGREE OF B.Sc. (HONS) IN MICROBIOLOGY, UNIVERSITY OF BENIN, BENIN CITY.

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CERTIFICATION

This is to certify that this project work was successfully carried out by **ROYAL OGHENEWEGBA UJAKPOR (MISS)**with matriculation number **LSC2009739,** of the department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria, under my supervision.

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(Project Supervisor)

APPROVAL

This project work was carried out by **ROYAL OGHENEWEGBA UJAKPOR (MISS)**with matriculation number **LSC2009739,** in partial fulfillment of the award of a Bachelor of Science, B.Sc. (Hons) degree in the Department of Microbiology, University of Benin, Benin City.

_____ _____

Prof. Mrs. F.I AKINNIBOSUN **Date**

(Head of Department)

DEDICATION

This report is primarily dedicated to the Most High God for His abundant blessings, mercy, and guidance throughout my university journey.

I am also dedicating this project to my late mum.

ACKNOWLEDGEMENTS

First and foremost, I wish to give my profound gratitude to God Almighty for His faithfulness, goodness and grace throughout my life and academic journey.

I want to sincerely acknowledge my supervisor **DR.MRS J.Z SAIDU** for her encouragement and insight towards the success of this work. I am grateful to the Head of Department, Microbiology, Prof. Mrs. F. I. Akinnibosun, and also my course adviser, Dr. (Mrs) R. Adams for their support and guidance throughout my academic years.

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I would like to express my deepest gratitude to several individuals and groups whose support and guidance have been instrumental in the completion of this project.

At the top of that list is my immediate family, I am immensely thankful to my dad, for his unwavering support and encouragement throughout my academic journey, to my ever supportive siblings, thanks for always supporting me without hesitation.

I am also thankful to my project group members for their collaboration and constructive suggestions, which have greatly enhanced the quality of this work.

Without the collective support of these individuals and groups, I would find it challenging to reach this milestone.

I say, remain blessed by God Almighty, I love you all, you all are the best!

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ABSTRACT

Probiotics usage in enhancing immunity has gained significant attention in biomedical research, particularly as global health challenge demands safer and more sustainable therapeutic intervention. This study aims at investigating the immunomodulatory effect of Lactobacillus casei in mitigating Staphylococcus sciuri infection. The study involves five groups each containing four rats in a cage, which comprises of Control group, a group challenged with only Staphylococcus sciuri, another group challenged with only Lactobacillus casei, a fourth group pre-treated with Lactobacillus casei before being challenged with Staphylococcus sciuri, the last group being challenged with Staphylococcus sciuri before being treated with antibiotics. Blood sample was obtained from the rat after sacrificing them and was taken for hematological reading. Results indicated that the control group had the highest White Blood Cell (WBC) of 19.2±2.00(103/ μL), while the lowest was observed in the Prophylactic group 9.2±2.00 (103/ μL), low count indicates the presence of infection. The heterotrophic bacterial count in stool sample shows significant differences, with Pre-treatment bacterial count ranging from 1.34 × 107 cfu/mL (probiotic group) to 2.34 × 10 8 cfu/mL (Antibiotic group). The improvement in White Blood Cell count particularly in the content of infection suggests an increased immune response. These findings support the hypothesis that Lactobacillus casei can function as a natural immunomodulator. Recommendations suggest that probiotics like Lactobacillus casei should be integrated into therapeutic regimens to enhance immune function especially in the face of infections caused by pathogens like Staphylococcus sciuri.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

The role of probiotics in enhancing immunity has gained significant attention in biomedical research, particularly as global health challenges demand safer and more sustainable therapeutic interventions. Among the numerous probiotics studied, *Lactobacillus casei* has been highlighted for its remarkable ability to regulate host immune responses and provide protection against pathogenic bacteria (La Fata *et al.,* 2018). *Lactobacillus casei*, a lactic acid bacterium commonly found in fermented dairy products, exhibits robust survival in the gastrointestinal tract due to its acid and bile tolerance (Leite*et al.*, 2015). This resilience enables it to exert immunomodulatory effects by enhancing phagocytic activity, modulating cytokine production, and reinforcing the intestinal epithelial barrier (Sánchez *et al.*, 2017).

In addition to its gastrointestinal benefits, *Lactobacillus casei* has been shown to influence systemic immunity, potentially reducing the severity of infections caused by various pathogens. The mechanisms of action involve stimulation of toll-like receptors (TLRs) on immune cells, leading to the production of pro-inflammatory and anti-inflammatory cytokines in a balanced manner (Nagpal *et al.*, 2012). Furthermore, this probiotic has demonstrated the ability to modulate T-cell responses, promoting immune homeostasis and enhancing resistance to infections (van Baarlen*et al.*, 2013).

Conversely, *Staphylococcus sciuri* is emerging as an opportunistic pathogen of significant concern. Once regarded as a commensal species found in the skin and mucosal surfaces of animals, it is now increasingly associated with zoonotic infections in humans. Its pathogenicity is heightened by its capacity to acquire and disseminate antibiotic resistance

genes, posing a serious threat to both veterinary and public health (Foster, 2019). *S. sciuri* has been implicated in cases of endocarditis, wound infections, and septicemia, with evidence suggesting its potential to evade host immune defenses, establish persistent infections, and compromise therapeutic outcomes (Becker *et al.*, 2020).

The interplay between beneficial microbes like *Lactobacillus casei* and pathogens such as *Staphylococcus sciuri* offers a promising area of study for developing alternative therapeutic strategies. Animal models, particularly Wistar rats, provide a controlled environment to explore these interactions. Wistar rats are widely used in immunological and hematological studies due to their genetic consistency and physiological similarities to humans. By leveraging such models, researchers can investigate the hematological and immunological effects of probiotics, offering insights into their potential applications in mitigating pathogenic infections.

Traditional antimicrobial treatments often disrupt the delicate balance of gut microbiota, leading to adverse effects such as secondary infections, reduced immunity, and metabolic dysregulation. As an alternative, probiotics offer a promising solution due to their ability to restore microbial balance, enhance immune function, and directly antagonize pathogenic bacteria through competitive exclusion and production of antimicrobial compounds (Ouwehand *et al.*, 2002).

Despite the growing interest in probiotic therapy, there is limited research on the specific effects of *Lactobacillus casei* in counteracting infections caused by *Staphylococcus sciuri*. The hematological and immunological responses elicited by this probiotic in the context of such infections remain underexplored. Understanding these interactions is critical for developing safe, natural, and cost-effective interventions that address the dual challenges of

infection control and antibiotic resistance. This study seeks to fill this gap by evaluating the therapeutic potential of *Lactobacillus casei* in Wistar rats infected with *Staphylococcus sciuri*.

1.2. AIM AND OBJECTIVES

To evaluate the hematological and immunomodulatory effects of *Lactobacillus casei* in Wistar rats infected with *Staphylococcus sciuri*.

The specific objectives of this study were to;

1. identify and enumerate*Lactobacillus casei* administration in Wistar rats.
2. investigate the immunomodulatory effect of *Lactobacillus casei* in mitigating *Staphylococcus sciuri* infection.
3. evaluate the potential of *Lactobacillus casei* as a probiotic intervention against pathogenic infections.
4. To investigate the microbial load from stool and organ samples of rat challenge with Lactobacillus casei and Staphylococcus scuiri

CHAPTER TWO

LITRERATURE REVIEW

The use of probiotics in promoting health and enhancing immune function has gained considerable attention due to their potential to act as natural therapeutic agents. Among the various probiotic species, *Lactobacillus casei* has emerged as a prominent candidate due to its immunomodulatory properties and antimicrobial activity. Its ability to strengthen the immune system and inhibit the growth of pathogenic microorganisms has been extensively studied in microbiology and immunology (Dhama*et al.*, 2011).

Lactobacillus casei is a Gram-positive, non-spore-forming, rod-shaped bacterium that naturally inhabits the gastrointestinal tract of humans and animals and is present in a variety of fermented foods. Its probiotic properties are derived from its ability to withstand acidic environments, adhere to intestinal epithelial cells, and produce antimicrobial compounds such as lactic acid. These attributes contribute significantly to gut health and the modulation of host immunity (Yang *et al.*, 2015).

Studies have shown that *Lactobacillus casei* plays a crucial role in enhancing both mucosal and systemic immunity. It stimulates the production of immunoglobulins, activates immune cells such as macrophages and T-cells, and interacts with dendritic cells to promote immune responses. Furthermore, *Lactobacillus casei* competes with pathogenic microorganisms for adhesion sites and nutrients within the gastrointestinal tract, effectively reducing their colonization and proliferation (MarkowiakandŚliżewska, 2017).

The immunomodulatory effects of *Lactobacillus casei* are primarily mediated through its interaction with the gut-associated lymphoid tissue (GALT), where it induces the secretion of cytokines such as interleukin-10 (IL-10) and interleukin-12 (IL-12). These cytokines play

vital roles in balancing pro-inflammatory and anti-inflammatory responses, ensuring a well-regulated immune system. Additionally, *Lactobacillus casei* enhances the production of secretory immunoglobulin A (sIgA), a key component of mucosal immunity, and activates natural killer (NK) cells, macrophages, and antigen-presenting cells. These actions collectively bolster both innate and adaptive immunity (Bron*et al.*, 2012).

Staphylococcus sciuri is a coagulase-negative, Gram-positive bacterium commonly isolated from animals, environmental sources, and, less frequently, humans. While it is generally considered a commensal organism, *S. sciuri* has been increasingly recognized as an opportunistic pathogen capable of causing infections in humans and animals. It exhibits significant genetic diversity and adaptability, including the acquisition of antibiotic resistance genes, which have heightened its relevance in clinical and veterinary microbiology (Poyart*et al.*, 2001).

Infections caused by *S. sciuri* are uncommon but can be severe. They are associated with conditions such as endocarditis, septicemia, and skin and soft tissue infections in humans. In animals, including laboratory rodents, *S. sciuri* can induce systemic infections characterized by inflammation and changes in hematological parameters. The emergence of multidrug-resistant *S. sciuri* strains poses a public health challenge, emphasizing the need for alternative therapeutic approaches, including probiotics like *Lactobacillus casei* (Becker *et al.*, 2014).

Hematological parameters are critical indicators of physiological and immunological health, providing insights into the body's response to infections and other stressors. Probiotics, including *Lactobacillus casei*, have been reported to positively influence these parameters through several mechanisms. Probiotics may promote erythropoiesis, leading to improved hemoglobin levels and hematocrit, which are crucial for oxygen transport and overall metabolic function (Fernández *et al.*, 2015). By stimulating the immune system, probiotics

enhance the production and functionality of WBCs, enabling the body to effectively combat infections such as those caused by *S. sciuri* (Zhang *et al.*, 2017). Probiotics have been shown to support platelet aggregation and functionality, which are essential for maintaining hemostasis and tissue repair. Probiotics can downregulate the production of pro-inflammatory cytokines, mitigating excessive inflammation and promoting immune homeostasis (Delcenserie*et al.*, 2008).

This study aims to evaluate the hematological activities of *Lactobacillus casei* in enhancing immunity and combating *Staphylococcus sciuri* infections in wistar rats. By investigating the ability of this probiotic to modulate immune responses and improve hematological indices, the research provides insights into its potential as a sustainable alternative to antibiotics. The findings are particularly significant in the context of increasing antibiotic resistance, where probiotics offer a promising solution for managing bacterial infections naturally and effectively.

2.1. *Lactobacillus casei*

Lactobacillus casei is a gram-positive, non-spore-forming, rod-shaped bacterium belonging to the lactic acid bacteria (LAB) group (Huang *et al.*, 2018). It is widely recognized for its probiotic properties and significant contributions to human health. As a facultative anaerobe, *L. casei* thrives in a variety of environments, including the gastrointestinal tract, fermented foods, and dairy products. Its adaptability to a broad pH range and temperatures between 30°C and 40°C makes it a resilient microorganism capable of surviving the acidic conditions of the stomach, colonizing the intestines, and exerting its beneficial effects on the host.

L. casei is known for its robust metabolic flexibility, allowing it to ferment a wide array of carbohydrates and produce lactic acid as its primary metabolic byproduct. This acidification

process plays a crucial role in maintaining a healthy gut environment by creating unfavorable conditions for pathogenic microorganisms such as *Escherichia coli*, *Clostridium difficile*, and *Salmonella* (Aponte *et al.*, 2020). Its rod-shaped morphology and biofilm-forming ability facilitate adhesion to the intestinal mucosa, an essential factor for colonization and competitive exclusion of harmful bacteria (Leite*et al.*, 2015).

The probiotic potential of *Lactobacillus casei* is well-documented. It plays a vital role in restoring the balance of the gut microbiota, particularly after disturbances caused by antibiotics, infections, or dietary changes. It enhances intestinal barrier function by stimulating the production of mucin and promoting tight junction integrity, thereby preventing the translocation of pathogens and toxins into systemic circulation (Bermudez-Brito *et al.*, 2012).

The bacterium's immunomodulatory properties are particularly noteworthy. *L. casei* has been shown to stimulate the production of immunoglobulin A (IgA) and regulate the release of anti-inflammatory cytokines such as IL-10, as well as pro-inflammatory cytokines like IL-6 and TNF-α, depending on the context. These immunological effects contribute to its efficacy in reducing the severity of infections and chronic inflammatory conditions such as inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) (Hill *et al.*, 2014; Awasthi *et al.*, 2020).

In the food industry, *L. casei* is extensively utilized in the production of fermented foods such as yogurt, cheese, kefir, and fermented vegetables. It contributes to the sensory properties of these foods by enhancing flavor, texture, and shelf life. Additionally, its ability to improve lactose digestion makes it a valuable probiotic for individuals with lactose intolerance, allowing them to enjoy dairy products without adverse symptoms (Ouwehand *et al.*, 2002).

Beyond gut health, *Lactobacillus casei* has been studied for its potential in addressing systemic health issues. Research has shown its ability to reduce serum cholesterol levels by deconjugating bile acids, which are then excreted instead of reabsorbed. This mechanism has significant implications for cardiovascular health (Nagpal *et al.*, 2012).

Emerging evidence also suggests that *L. casei* plays a role in the gut-brain axis, influencing neurological and psychological health. It has been associated with reduced symptoms of depression and anxiety in animal models, likely due to its impact on the production of neurotransmitters such as gamma-aminobutyric acid (GABA) and modulation of systemic inflammation (Messaoudi*et al.*, 2011). Additionally, *L. casei* has shown promise in the management of metabolic disorders such as obesity and type 2 diabetes, partly through its role in modulating gut microbiota composition and improving insulin sensitivity (Aponte *et al.*, 2020).

Lactobacillus casei is a highly adaptable and versatile probiotic with extensive applications in health, food, and medicine. Its ability to survive in diverse environments, exert antimicrobial and immunomodulatory effects, and address systemic health issues underscores its importance in both clinical and dietary settings (Sharifi-Rad *et al.*, 2020). Continued research into its mechanisms of action and potential applications will likely expand its role in promoting human health and managing complex diseases.

2.2.*Staphylococcus scuri*

Staphylococcus scuri is a gram-positive, coagulase-negative bacterium within the *Staphylococcus* genus, known for its distinctive cell wall characteristics and the ability to grow in various environments, from skin to mucosal surfaces in humans and animals. Like other staphylococci, *S. scuri* is facultatively anaerobic, meaning it can thrive in both aerobic

and anaerobic conditions. Although *S. scuri* is a part of the normal flora in many animals, including humans, it can become pathogenic under specific conditions, particularly in hosts with weakened immune systems (KloosandSchleifer, 1975).

Despite being coagulase-negative, a key trait distinguishing it from the more virulent *Staphylococcus aureus*, *S. scuri* shares several genetic and phenotypic similarities with its more pathogenic counterpart. This includes the potential for biofilm formation, adherence to surfaces, and secretion of enzymes like proteases and lipases, which enhance its survival in hostile environments (Fadaei*et al.*, 2021).

Staphylococcus scuri is typically found in the natural microbiota of the skin, mucosal surfaces, and the gastrointestinal tracts of many mammals. It has been particularly well-documented in a variety of animal species, including cattle, poultry, and other domesticated animals. In humans, *S. scuri* can be present as part of the skin microbiome, often residing in areas such as the armpits, groin, and other moist body areas (KloosandSchleifer, 1975). This bacterium is well-adapted to survive in the skin's sebaceous secretions and can persist in the hair follicles and sweat glands, forming a long-term symbiotic relationship with its host under normal, healthy conditions.

However, when an animal or human host undergoes physical trauma, immune suppression, or stress, *S. scuri* can disrupt this balance and transition into a pathogenic state. Conditions like surgery, immunosuppressive therapy, or prolonged use of antibiotics may alter the host's normal immune response, making it easier for *S. scuri* to thrive and cause infection.

Although *S. scuri* is typically harmless in its natural habitat, it can act as an opportunistic pathogen under certain conditions. The ability of *S. scuri* to cause infections is linked to its various virulence factors. These include its ability to adhere to surfaces, produce biofilms,

and secrete enzymes that facilitate tissue invasion. In particular, its ability to form biofilms is significant, as these structures protect the bacteria from both host immune responses and antibiotics, making *S. scuri* infections more difficult to treat (Cai *et al.*, 2019).

The bacterium's pathogenic potential is especially prominent in immunocompromised hosts, where its normal role as part of the microbiome is disrupted. In such hosts, *S. scuri* can invade deeper tissues, leading to local and systemic infections. This opportunistic behavior is often observed in clinical settings, where hospital-acquired infections have been linked to *S. scuri*.

For example, *S. scuri* can be transmitted through direct contact with contaminated surfaces, wound infections, or through the blood during surgical procedures. Nosocomial infections, including those occurring in animal research facilities, are of particular concern as they can spread rapidly in environments with high levels of microbial exposure (Marples*et al.*, 2013).

2.2.1. Virulence factors of *Staphylococcus sciuri*

Staphylococcus sciuri is a coagulase-negative staphylococci (CNS) species that is commonly found in animals and the environment but has increasingly been recognized as an opportunistic pathogen in humans (Shittu *et al.*, 2004). While it is generally considered less virulent than *Staphylococcus aureus*, *S. sciuri* can still exhibit significant pathogenic potential, particularly in immunocompromised individuals. The virulence of *S. sciuri* is mediated by a variety of factors, many of which are shared with other members of the Staphylococcus genus.

One of the key virulence mechanisms of *S. sciuri* is its ability to adhere to host tissues. This is largely facilitated by surface proteins, such as fibronectin-binding proteins (FnBPs), which enable the bacteria to attach to host cells and tissues, contributing to the formation of biofilms. Biofilm formation is an important factor in chronic infections, as it protects the bacteria from

the host immune system and makes the bacteria more resistant to antibiotics (Reiter *et al.*, 2014).

Another significant virulence factor of *S. sciuri* is the production of hemolysins, such as alpha-hemolysin and beta-hemolysin. These toxins are capable of lysing red blood cells, causing tissue damage and promoting the spread of the bacteria throughout the host. Hemolysin production plays a crucial role in the bacteria's ability to cause infections, as it disrupts host tissues and facilitates bacterial invasion (Vasić*et al.*, 2016).

In addition to hemolysins, *S. sciuri* produces a range of exoenzymes, including proteases and lipases, which degrade host proteins and lipids. These enzymes allow the bacteria to break down host tissues, enabling further colonization and invasion of deeper tissues. The production of these exoenzymes contributes significantly to the pathogenicity of *S. sciuri* (Vasić*et al.*, 2016).

Furthermore, *S. sciuri* produces catalase, an enzyme that breaks down hydrogen peroxide into water and oxygen. This enzymatic activity is critical in protecting the bacteria from oxidative damage caused by reactive oxygen species (ROS) produced by the host immune system. By neutralizing hydrogen peroxide, catalase helps *S. sciuri* evade the host's defense mechanisms, thereby enhancing its survival within the host (Reiter *et al.*, 2014).

Staphylococcus sciuri is also known for its ability to resist multiple antibiotics. The bacteria can produce beta-lactamases, which break down beta-lactam antibiotics like penicillin and cephalosporins, rendering them ineffective. Some strains of *S. sciuri* have also demonstrated resistance to vancomycin, which is often used as a last-line treatment for serious staphylococcal infections (Fadaei*et al.*, 2019). This antibiotic resistance significantly complicates treatment, making infections caused by *S. sciuri* more difficult to manage.

Finally, *S. sciuri* is capable of producing various toxins, including enterotoxins, which disrupt host cell functions and stimulate inflammatory responses. These toxins contribute to tissue damage and further enhance the virulence of the bacteria. In addition, *S. sciuri* can produce capsular polysaccharides that protect the bacteria from phagocytosis, further aiding in immune evasion (Vasić*et al.*, 2016).

While *Staphylococcus sciuri* is often considered a less virulent species compared to *S. aureus*, it still possesses a variety of virulence factors that enable it to cause infections, especially in individuals with compromised immune systems. Its ability to form biofilms, produce hemolysins and exoenzymes, and evade immune responses through catalase production and capsular polysaccharides all contribute to its pathogenic potential. Moreover, its antibiotic resistance profiles complicate treatment and highlight the need for careful management of infections caused by this organism.

2.2.2. Clinical Manifestations of Staphylococcus scuri Infection

The clinical manifestations of *Staphylococcus scuri* infection in animal models, particularly wistar rats, are influenced by the site of infection, the severity of the infection, and the immune status of the host. While *Staphylococcus scuri*is generally considered a less virulent pathogen compared to other staphylococci, it can still cause a range of infections, from localized skin infections to severe, life-threatening systemic conditions, especially in immunocompromised individuals.

2.2.2.1. Skin and Soft Tissue Infections (SSTIs)

One of the most common manifestations of S. scuri infection in animal models is skin and soft tissue infections (SSTIs). These infections often occur after trauma or surgical procedures that compromise the skin barrier, allowing the bacteria to invade. In experimental settings, such as studies using wistar rat models, S. scuri can cause localized abscesses, cellulitis, and wound infections. Infected areas typically show signs of redness, swelling, and pain. Additionally, pustules or abscesses may form at the infection site. If left untreated, these infections can progress to deeper tissues, leading to more severe complications. The localized nature of these infections can make them relatively easy to identify and treat in animal models, but in some cases, they can escalate to more serious conditions if the bacteria are allowed to spread.

2.2.2.2. Systemic Infections

In immunocompromised rats, Staphylococcus scuri has the potential to cause more severe, systemic infections. These can include bacteremia, sepsis, endocarditis, and osteomyelitis. Once S. scuri enters the bloodstream, it can rapidly disseminate to various organs, leading to widespread inflammation and, in some cases, organ failure. The ability of the bacteria to evade the immune system, particularly in immunocompromised animals, allows for the development of life-threatening conditions. Bacteremia and sepsis, in particular, are critical because they involve systemic infection, which can overwhelm the body's defenses. In such cases, intensive medical intervention is required, as the infections can become fatal without prompt and effective treatment. Endocarditis, a condition in which bacteria infect the heart valves, and osteomyelitis, which involves bone infection, are also significant complications in these animals.

2.2.2.3. Urinary Tract Infections (UTIs)

Staphylococcus scuri can also lead to urinary tract infections (UTIs) in wistar rats, especially following urethral trauma or the use of catheters in experimental conditions. UTIs in these animals can present with symptoms such as dysuria (painful urination), hematuria (blood in the urine), and pyuria (pus in the urine). If not promptly treated, UTIs can progress to more serious kidney infections, including pyelonephritis, which can significantly impact the overall health of the rat. These infections, although less common than skin or systemic infections, can still have a major impact on the animals' health if left untreated.

2.3. Immune Response To *Staphylococcus scuri* Infection

The immune system plays a crucial role in managing *S. scuri* infections in wistar rats. The innate immune response is the first line of defense, with neutrophils and macrophages attempting to neutralize the bacteria through processes such as phagocytosis. These immune cells are also involved in releasing antimicrobial substances to kill the bacteria. However, *S. scuri'*s ability to form biofilms—a slimy protective layer around bacterial colonies—along with its production of immune-modulating factors, can make it difficult for the immune system to mount an effective response (Cai *et al.*, 2019). Biofilm formation helps the bacteria adhere to surfaces and evade both the immune system and antibiotics, making infections more persistent and harder to treat.

In cases where the innate immune response is insufficient, the adaptive immune system is activated. This includes the activation of T-cells and B-cells, which provide a more specific and targeted response to the infection. T-cells help in recognizing and killing infected cells, while B-cells produce antibodies to neutralize the bacteria. In immunocompromised rats, however, the adaptive immune response may be delayed or insufficient, leading to the persistence of chronic infections or the spread of infection to other parts of the body. Studies have shown that while the innate immune system is vital in controlling early stages of

infection, the adaptive immune system plays an essential role in resolving more severe or systemic infections.

The clinical manifestations of *Staphylococcus scuri* infection in wistar rats reflect a broad spectrum of possible outcomes, from localized skin infections to severe systemic conditions. The immune response, including both innate and adaptive components, is crucial in controlling the infection, although *S. scuri's* ability to evade immune defenses can make these infections particularly challenging in immunocompromised hosts.

2.4. Probiotics And Their Role In Immune Modulation

Probiotics play a pivotal role in immune modulation, demonstrating profound impacts on both innate and adaptive immunity. By definition, probiotics are live microorganisms that, when consumed in adequate amounts, confer health benefits on the host, primarily through interactions with the gut microbiota and immune system. These beneficial microorganisms are predominantly bacteria from the genera *Lactobacillus*, *Bifidobacterium*, and *Streptococcus*, as well as certain yeasts like *Saccharomyces boulardii*. According to the World Health Organization (WHO) and Food and Agriculture Organization (FAO), effective probiotics must survive the harsh conditions of the gastrointestinal tract, adhere to intestinal surfaces, and exert beneficial effects such as antimicrobial production and immune modulation (Hill *et al.*, 2014).

Probiotics are characterized by their ability to improve gut health by restoring microbial balance, producing bioactive metabolites, and influencing host immunity. Among these, *Lactobacillus casei* stands out as a widely studied species with significant health benefits. This gram-positive, rod-shaped bacterium is commonly found in fermented foods like yogurt and cheese and is known for its ability to withstand acidic stomach conditions and colonize

the intestine. Through the production of lactic acid, *L. casei* creates an environment that inhibits the growth of harmful pathogens. Its immunomodulatory properties, including enhancing the gut barrier and influencing cytokine production, make it a prime candidate for promoting health (Ouwehand *et al.*, 2002).

2.4.1. Mechanisms by Which Probiotics Modulate the Immune System

Probiotics, particularly strains like *Lactobacillus casei*, have garnered attention for their ability to modulate the immune system in beneficial ways. The mechanisms by which probiotics influence immune responses are multifaceted, affecting both the local and systemic immune functions. These mechanisms are essential for maintaining immune homeostasis and combating infections, autoimmune diseases, and even chronic inflammation. Below is an in-depth exploration of how probiotics, particularly *L. casei*, impact immune function.

2.4.1.1. Interaction with Gut Microbiota

The gut microbiota plays a pivotal role in maintaining immune function and homeostasis. The gut is home to trillions of microorganisms that interact with the host's immune system, influencing both local and systemic immunity. Dysbiosis, or an imbalance in this microbial community, has been linked to a variety of conditions, including chronic inflammation, autoimmune diseases, and increased susceptibility to infections (Galdeano*et al.*, 2007). In this context, probiotics such as *Lactobacillus casei* help restore microbial diversity by suppressing pathogenic bacteria and fostering the growth of beneficial microbial species.

One of the most important aspects of probiotics is their ability to strengthen the **Gut-Associated Lymphoid Tissue (GALT)**, which is a key player in immune surveillance and response. GALT includes structures like the Peyer’s patches and the mesenteric lymph nodes, which are essential for initiating immune responses against gut-derived pathogens. Probiotics

enhance the function of GALT by promoting the production of protective immune cells and enhancing the integrity of the intestinal mucosa. This, in turn, helps prevent the invasion of pathogens and strengthens the overall immune defense system (Galdeano*et al.*, 2007).

2.4.1.2. Cytokine Modulation

Cytokines are small signaling molecules that regulate the immune response, playing crucial roles in inflammation, immune cell activation, and resolution of infections. Probiotics like *L. casei* can significantly influence cytokine production, promoting a balanced immune response. They can upregulate anti-inflammatory cytokines, such as interleukin-10 (IL-10), and downregulate pro-inflammatory cytokines like IL-6 and tumor necrosis factor-alpha (TNF-α). This modulation helps control excessive inflammation and supports a more controlled and adaptive immune response (Dong *et al.*, 2010).

L. casei particularly affects dendritic cells, which are important antigen-presenting cells in the immune system. By interacting with dendritic cells, *L. casei* helps orchestrate a balanced Th1/Th2 immune response. Th1 cells are typically associated with responses to intracellular pathogens, while Th2 cells mediate responses to extracellular pathogens and allergens. Achieving a balance between these two responses is crucial for effectively combating infections while preventing the development of autoimmune diseases. The ability of probiotics to modulate these cytokine profiles makes them critical in managing immune responses(Dong *et al.*, 2010).

2.4.1.3. Enhanced Phagocytosis

Phagocytosis is a process in which immune cells, primarily macrophages and neutrophils, engulf and digest pathogens, dead cells, and other debris. Probiotics have been shown to enhance the phagocytic activity of these immune cells. Studies have demonstrated that *Lactobacillus casei* stimulates macrophages, improving their ability to clear pathogens from the body. This enhanced phagocytosis is vital for innate immunity, which serves as the body's first line of defense against infections (de Moreno de LeBlanc andPerdigón, 2005). By improving the function of macrophages, probiotics contribute to a more effective immune response, facilitating pathogen clearance and reducing the severity of infections.

2.4.1.4. Strengthening the Gut Barrier

A robust intestinal barrier is essential for maintaining immune function and preventing pathogens from entering the bloodstream. Probiotics like *L. casei* play a crucial role in enhancing the gut barrier by promoting the production of mucin, which is a protective glycoprotein that lines the gut epithelium (Qin *et al.*, 2022). Additionally, probiotics enhance the integrity of tight junctions between intestinal epithelial cells, reducing intestinal permeability. This strengthened gut barrier prevents the translocation of harmful bacteria and toxins from the gut into the bloodstream, reducing the risk of systemic infections and inflammation (Galdeano*et al.*, 2007). By protecting the gut barrier, probiotics support both local and systemic immune responses.

2.5. Probiotic Effects On Hematology

The impact of probiotics extends beyond gut health and has significant systemic effects, particularly on hematological parameters. These include improvements in the production and function of red and white blood cells, as well as overall immune cell activity. The modulation

of hematological parameters by probiotics, especially *Lactobacillus casei*, highlights their broader influence on immune function (Rastogi and Singh, 2022).

2.5.1. Impact on Red Blood Cells and Hemoglobin

Probiotics have been shown to enhance nutrient absorption, particularly iron, which is a critical component of hemoglobin and red blood cell production. Iron deficiency is a common cause of anemia, and probiotics like *Lactobacillus* species have been demonstrated to enhance the bioavailability of iron in the intestines. This improves iron absorption and helps increase hemoglobin levels, thereby reducing the prevalence of anemia and supporting overall red blood cell production (Ojetti*et al.*, 2014). This is particularly beneficial for individuals who may suffer from iron deficiency anemia, as probiotics can provide a natural and effective way to boost iron status.

2.5.2. Effect on White Blood Cells

White blood cells (WBCs) are critical components of the immune system that play essential roles in defending the body against infections. Probiotics, particularly *L. casei*, have been shown to positively influence WBC counts and functionality. Clinical studies have demonstrated that probiotic supplementation can increase lymphocyte, neutrophil, and natural killer (NK) cell activity, all of which are essential for the body's ability to fight off infections (Tsai *et al.*, 2012). For example, *L. casei* supplementation has been associated with increased NK cell activity, which enhances the body's ability to combat viral infections and tumor cells. Additionally, increased lymphocyte and neutrophil activity helps in pathogen clearance and improves overall immune responses.

2.5.3. Immune Cell Production and Activation

Probiotics also influence immune cell production and activation by stimulating the bone marrow, where blood cells, including immune cells, are produced. By releasing bioactive compounds that modulate hematopoiesis (the process of blood cell production), probiotics help regulate the production of T-cells, B-cells, and other immune cells that are essential for adaptive immunity (Lebeer*et al.*, 2008). The interaction between probiotics and the **gut-brain axis** can also impact immune cell activation, influencing the production of antibodies and enhancing immune responses. This ability to modulate immune cell production and activation highlights the importance of probiotics in promoting both innate and adaptive immunity.

2.6. Specific Evidence of Probiotic Influence on Immune Cell Production and Activation

Lactobacillus casei has been shown to increase the levels of interleukin-12 (IL-12), a cytokine crucial for the activation and differentiation of T-cells. This effect emphasizes the role of *L. casei* in enhancing adaptive immunity, which is critical for the body's ability to combat chronic infections and pathogens (Dong *et al.*, 2010).Numerous clinical trials have demonstrated that probiotics can significantly increase WBC counts, particularly lymphocytes and NK cells, in both healthy and immunocompromised individuals. This broad-spectrum immune enhancement helps support the body's defense against infections and improves overall immune system performance (Gill andGuarner, 2004). Studies by Perdigón*et al.* (2001) revealed that *Lactobacillus casei* enhances macrophage activity, improving their ability to clear pathogens and reduce the severity of infections. This macrophage activation is particularly important in the early stages of immune defense.

Probiotics, especially *Lactobacillus casei*, play a pivotal role in immune modulation through various mechanisms, including restoring the balance of gut microbiota, modulating cytokine

production, and enhancing both innate and adaptive immune responses. By improving gut barrier integrity, promoting phagocytosis, and influencing hematological parameters such as red and white blood cell counts, probiotics provide a comprehensive approach to boosting immune function. Their ability to enhance the immune system's ability to combat infections, improve nutrient absorption, and modulate immune cell production makes them invaluable for maintaining overall health and preventing a wide range of diseases. The growing body of evidence supporting the use of probiotics for immune modulation underscores their potential as therapeutic agents in managing immune-related conditions and enhancing overall immune resilience.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The laboratory analysis for this study was conducted at the Microbiology Laboratory, University of Benin, and the Animal House and Laboratory of the Faculty of Pharmacy, University of Benin, Edo State, Nigeria. Also ethical approval was obtained for the use of lab and animals. These facilities provided the controlled environment and equipment necessary for microbial, pathological, and immunological investigations.

3.2 Sample Collection and Preparation

Lactobacillus species were isolated from naturally fermented dairy products obtained from local vendors in Benin City, Edo State. The dairy products were transported to the laboratory under aseptic conditions and cultured on de Man, Rogosa, and Sharpe (MRS) agar to isolate *Lactobacillus* spp. A virulent strain of *Staphylococcus sciuri*, previously identified and preserved in clinical isolate banks, was obtained from the Medical and Parasitology Laboratory at the University of Benin Teaching Hospital. The isolate was sub-cultured on nutrient agar and maintained for subsequent experimental use.

3.3 Experimental Design

The experimental design was structured to evaluate the immunomodulatory and therapeutic effects of *Lactobacillus* spp. in mitigating *Staphylococcus sciuri* infections in Wistar rats. The study involved five groups, each comprising four rats:

1. **Control Group**: Received no treatment or infection, serving as the baseline.

2. **Infected Group**: Challenged to *Staphylococcus sciuri*infection without any treatment from day seven to fourteen.
3. **Probiotic Group**: Challenged with *Lactobacillus* spp. Only for fourteen days
4. **Prophylactic Group**: Pre-treated with *Lactobacillus* spp. for seven (7) days before challenged with *Staphylococcus sciuri* to assess preventive effects.
5. **Antibiotic Group**: Challenged with *Staphylococcus sciuri* and treated with a standard antibiotic (ciprofloxacin) to establish a therapeutic comparison.

Ethical approval was obtained from the Institutional Animal Care and Use Committee (IACUC), and all procedures were conducted in compliance with institutional ethical standard. All rats were housed in grouped cages under controlled environmental conditions, including a 12-hour light/dark cycle and ad libitum access to food and water.

3.4 Preparation and Sterilization of Materials

All glassware and instruments used during the study were sterilized in an autoclave at 121°C for 15 minutes. Sterilized disposable materials such as syringes, needles, and gloves were used to ensure aseptic handling. Media used in the study, including MRS agar, nutrient agar, and mannitol salt agar, were prepared according to the manufacturer's instructions and autoclaved at 121°C for 15 minutes before use.

3.5 Microbiological Analysis

3.5.1 Identification and Confirmation of Isolates

Lactobacillus spp. and *Staphylococcus sciuri* isolate was confirmed through culturing, colony morphology, Gram staining, Potassium Hydroxide testand biochemical tests, including catalase, Triple sugar iron (TSI) agar test and citrate test.

3.6 Preparation of Animal Model

3.6.1 Selection and Housing of Rats

Forty healthy Albino rats (*Rattus norvegicus*), approximately 8 weeks old and weighing between 150-200 grams, were selected for uniformity in physiological parameters. Animals were housed in polypropylene cages with sterilized bedding to reduce contamination risk. The animal room was maintained at a constant 22 ± 2°C and 45-55% humidity under a 12-hour light/dark cycle. Rats were fed a standard rodent chow diet formulated to meet their nutritional needs and were provided with filtered water. All animals were acclimatized to laboratory conditions for 14 days before starting experiments to mitigate stress-induced variability.

3.6.2 Preparation of Suspension

Following acclimatization, rats in the infected group and prophylatic groups were infected with *Staphylococcus* spp. by intraoral injection. The infection model aimed to simulate systemic infection. A single colony of *Staphylococcus* spp. was inoculated into 10ml sterile saline solution and vortex and serial dilution was carried out. The *Staphylococcus* spp. suspension (1 x 10^8 CFU/ml) was carefully administered, and signs of infection were monitored over the next 24-48 hours. Indicators such as weight loss, lethargy, and fever were recorded, providing baseline data for subsequent analysis of the treatment effects.

3.6.3. Administration of *Lactobacillus casei*

The *Lactobacillus casei* preparation was suspended in sterile phosphate-buffered saline (PBS) to achieve a concentration of 10^8 CFU/ml. The probiotic Group was challenged with *Lactobacillus* spp. The prophylaticgroup was pre-treated with *Lactobacillus* spp. for seven (7)

days before being infected with *Staphylococcus sciuri* to assess preventive effects.This pre-treatment was intended to promote gut colonization and prime the immune response against potential pathogens.

3.7 Microbial Analysis

3.7.1 Microbial Analysis of Stool Samples

Stool samples were collected from rats prior to induction with *Staphylococcus sciuri* and *Lactobacillus* spp., after induction with *Staphylococcus sciuri*, and antibiotic treatment. Approximately 1 g of stool was homogenized in 9 mL of normal saline using a vortex mixer to ensure thorough dispersion. A tenfold serial dilution was performed by transferring 1 mL of the stool homogenate into 9 mL of sterile PBS, creating dilutions from 10^{-1} to 10^{-6}.

From the 1x 10^5 dilution, 0.1 mL was plated onto Nutrient agar plates and incubated at 37°C for 24 hours. Colonies were picked from Nutrient agar platesand inoculated on Mannitol Salt Agar and incubated for 24hrs at 37°C for 24 hours and colonies were counted and identify.

8. IDENTIFICATION OF BACTERIA

3.8.1 Gram Staining

Thin smears of the bacterial isolates were made from the pure culture on clean, grease free slide. The smears were air dried and then heat fixed by passing the slide over flame. The smears were flooded with crystal violet for 60 seconds, drained and flooded with gram's iodine for another 60 seconds then rinsed with distilled water. The smears are then decolorized using 70% alcohol for 5 seconds and quickly rinsed with distilled water. The smears were then counter stained with safranin for 30 seconds, rinsed with distilled water and allow to air dried. The slides were examined under the oil immersion(X100) objective. The

gram positive cells appear blue while the gram negative cells were indicated with a pink coloration.

3.8.2. Potassium Hydroxide (KOH) test

Two drops of 3% solution of KOH were applied on a clean glass slide and a loopful of pure bacterial growth was stirred in a circular motion in the slide. The loop was occasionally raised and observed for the presence of a string of the mixture. The solution was observed to be of a viscous and mucoid consistency indicating a Gram-negative bacterium. No reaction (absence of stringing) indicates a Gram-positive bacterium (Roberts and Sandle, 2008).

3.8.3. Biochemical Test

Biochemical characteristics of each isolates carried out to identify them include catalase test, citrate utilization test, oxidase test, urease test, methyl red test, indole test, coagulase test, sugar fermentation, starch hydrolysis and Triple sugar iron fermentation.

3.8.3.1 Catalase Test

Catalase test was carried out by making a suspension of fresh culture of the test organisms using sterile distilled water on a clean glass microscope slide and few drops of hydrogen peroxide (H_2O_2) were added using a dropping pipette. Formation of bubbles indicates positive result. Lack of bubbles indicates negative result.

3.8.3.2 Citrate Utilization Test

The citrate utilization test is a part of the test used to differentiate organisms on their ability to utilize citrate as the primary energy source. A citrate test was performed to differentiate

members of Enterobacteriaceae capable of fermenting citrate in the presence of the enzyme citrate. Simon's citrate agar contained citrate as significant energy and was prepared for inoculation on Petri dishes. Well-prepared and sterilized citrate agar plates were inoculated from the pure isolated culture by streaking the surface with a sterilized loop. The plates were then incubated at 37°C for 24 hours. There were changes in colour due to bacterial growth of the organisms on the medium due to citrate metabolism, which gave a positive citrate test. The shift in pH turns the bromothymol blue indicator in the medium from green to blue (positive result). A negative test was demonstrated with no growth, no colour change, or the colour of the medium remains green.

3.8.3.3. Oxidase Test

The oxidase test was carried out to detect the presence of a cytochrome oxidase or indophenol oxidase that will catalyze electrons between electron donors in the bacteria and a redox dye known as tetramethyl-*p*-phenylene-diamine. The dye would be reduced to deep purple colour if yielded to positive reactions.

Several reagents can be used for this study but Kovacs oxidase reagent: 1% tetra-methyl-p-phenylenediamine dihydrochloride in water, was used. The filter paper was saturated with a Kovacs oxidase reagent solution, and a speck of the pure culture was smeared on it with a platinum loop. It was allowed and observed for colour development within 10 - 60 seconds. The appearance of a deep purple-blue/blue colour indicated oxidase production and the negative result was when no colour changed (Fawole and Oso, 2007)..

3.8.3.4. Indole Test

Spot indole test was carried out using a fresh culture of the test organism. Several drops of 1% p-dimethylaminocinnamaldehyde reagent were placed on a piece of filter paper. A

loopful culture of the test organism was rubbed on the reagent saturated area of the filter paper. Positive result is shown by the presence of a blue to blue-green colour change within 2-3 minutes while negative results remain colourless or appears light pink.

3.8.3.5. Triple sugar iron (TSI) agar test

An agar slant prepared of a TSI agar was used in carrying out this test in a sterile test tube at a slanted angle. The slanted medium was inoculated with TSA pure culture using a straight inoculation needle by stabbing first through the center to the bottom of the tube and streaking the agar slant's surface. After inoculations, the test tubes were covered with foil paper and left at an ambient temperature of 36°C to incubate for 24 hours. Reactions on test tubes were examined and sugar fermentations were indicated by the production of H_2S, gas and a change in colours from red (alkaline) to yellow (acid). When an alkaline/acid (red top/yellow bottom) slant reaction appeared, it only indicated dextrose (glucose) fermentation. When an acid/acid (yellow top/yellow bottom) slant reaction appeared, it showed the fermentation of dextrose, lactose and/or sucrose. The appearance of an alkaline/alkaline (red top/red bottom) slant reaction represented the absence of sugar fermentation. The blackening of the medium in the slant indicated H_2S production.Bubbles, cracks, or bottom-raised space in the slanted agar indicated gas production (formation of CO_2 and H_2).

3.9. Antibiotics Susceptibility Test

The bacterial colonies identified were utilized to assess the susceptibility and resistance of the isolates through standard Antibacterial Susceptibility Testing (AST). This analysis determined their response to commonly used antibiotics in the study area. The antibiotics tested included Amoxicillin (10 µg), Ciprofloxacin (10 µg), Gentamicin (10 µg), Tetracycline (30 µg), Chloramphenicol (30 µg), Erythromycin (10 µg), Ceftriaxone (30 µg), and

Trimethoprim-Sulfamethoxazole (30 µg). The antibiotic discs, manufactured by Oxoid, UK, were used for the disc diffusion method applied in this study. For the AST procedure, bacterial cultures grown for 18–24 hours were streaked onto Mueller-Hinton Agar (MHA) plates. The inoculum was adjusted to match a 1.5 x 10^8 CFU/mL McFarland standard, ensuring consistency in bacterial density. Using sterile forceps, antibiotic discs were carefully placed on the inoculated MHA plates. The plates were incubated at 37°C for 24 hours, after which the diameter of the inhibition zones around each disc was measured in millimeters using a ruler. The results were interpreted following the Clinical and Laboratory Standards Institute (CLSI) guidelines. The 2020 CLSI standards classified the bacterial isolates' responses to antibiotics as Resistant (R), Intermediate (I), or Sensitive (S), providing insight into their susceptibility patterns.

3.10. Hematological Analysis

Hematological parameters were assessed to determine the physiological and immune responses of the rats to infection and subsequent treatments. Blood samples were collected from the retro-orbital sinus of anesthetized rats using a capillary tube at baseline and after treatment on Day 14. The blood samples were carefully transferred into EDTA-coated tubes to prevent coagulation and ensure sample integrity for subsequent analysis.

The hematological parameters analyzed in this study included Red Blood Cell (RBC) count, White Blood Cell (WBC) count, Granulocytes (GRN), Hemoglobin (HGB), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC), and Platelet (PLT) count. These parameters were determined using an improved Neubauer hemocytometer. Each parameter was assessed as follows:

3.10.1. Red Blood Cell (RBC) Count

RBC count was measured to assess the oxygen-carrying capacity of the blood and detect conditions such as anemia or polycythemia. The number of red blood cells in a specified volume of blood was counted using the hemocytometer, and the results were expressed as cells per microliter (μL) of blood.

3.10.2. White Blood Cell (WBC) Count

White Blood Cell (WBC) count was analyzed to evaluate the immune response and detect any infections or inflammatory conditions. The total number of leukocytes in the blood sample was counted, and the results were expressed as cells per μL of blood.

3.10.3. Granulocytes (GRN)

Granulocyte count, including neutrophils, eosinophils, and basophils, was measured as a percentage of the total WBC count. This parameter provides insight into the immune system's response to infections, allergies, or other inflammatory conditions.

3.10.4. Hemoglobin (HGB)

Hemoglobin levels were measured to evaluate the oxygen-carrying capacity of the blood. A reduction in hemoglobin levels may indicate anemia, while elevated levels could suggest polycythemia or dehydration. Hemoglobin concentration was expressed in grams per deciliter (g/dL).

3.10.5. Hematocrit (HCT)

Hematocrit, also known as packed cell volume (PCV), was measured to determine the proportion of red blood cells in the blood. It provides valuable information about hydration status, anemia, or polycythemia. The results were expressed as a percentage (%).

3.10.6. Mean Corpuscular Volume (MCV)

MCV was calculated to assess the average size of the red blood cells. It is a critical parameter in identifying the type of anemia, such as microcytic or macrocytic anemia. The results were expressed in femtoliters (fL).

3.10.7. Mean Corpuscular Hemoglobin Concentration (MCHC)

MCHC was calculated to determine the average concentration of hemoglobin in the red blood cells. It helps in diagnosing and classifying different types of anemia, such as hypochromic or normochromic anemia. The results were expressed as grams per deciliter (g/dL).

3.10.8. Platelet (PLT) Count

Platelet count was determined to evaluate the blood's ability to form clots and detect any bleeding or clotting disorders. The number of platelets was expressed as cells per μL of blood.

CHAPTER FOUR

4.0. RESULTS

Table 4.1 Presents the weight and temperature variations in rats before and after exposure to *Lactobacillus casei* and St scuiri. Initial weights ranged from 147.7 ± 0.00 g to 180.35 ± 0.20 g, increasing to 169 ± 0.30 g – 214 ± 0.00 g after 21days. The probiotic-treated group recorded the highest weight gain (147.7 ± 0.00 g to 214 ± 0.00 g), while the infected group had the lowest (169.74 ± 0.40 g to 172 ± 0.30 g).

Table 4.2 Presents temperature variations in rats before and after exposure to *Lactobacillus casei* and Staphylococcus scuiriInitial temperatures varied between 37.2 ± 0.20°C and 37.9 ± 0.30°C, rising to 37.38 ± 0.30°C – 38.63 ± 0.20°C. The infected group had the highest temperature increase (36.5 ± 0.00°C to 38.63 ± 0.20°C), whereas the antibiotics group showed a slight decrease (36.0 ± 0.00°C to 37.38 ± 0.30°C).

The heterotrophic bacterial counts from stool samples before and after treatment are presented in Table 4.3. Before treatment, the highest count was in Group D (Infected) at 4.00 ± 1.41 $\times 10^6$ CFU/ml, while the lowest was in Group A (Control) at 2.17 ± 0.41 $\times 10^6$ CFU/ml. After treatment, the highest count remained in Group D (3.34 ± 0.45 $\times 10^6$ CFU/ml), while the lowest was in Group B (Probiotic Administered) at 1.34 ± 0.42 $\times 10^6$ CFU/ml.

Table 4.4: Show the morphological, biochemical, and cultural characteristics of the bacteria isolates isolated from the stool sample. The bacteria isolated from the stool samples include *Salmonella* spp. *Bacillus* spp., *E. coli*, *Streptococcus* spp., *Staphylococcus* spp. and *Lactobacillus spp*

Table 4.5presents the antibiotic susceptibility of bacterial isolates from stool samples. ***Escherichia* coli** showed resistance to Pefloxacin and Azithromycin, while ***Salmonella*** spp. was resistant to Cefazolin and Erythromycin. *Bacillus* spp. resisted Rifampicin and

Ciprofloxacin, and ***Staphylococcus*** spp**.** was only resistant to Amoxicillin. ***Streptococcus*** **spp.** was mostly susceptible but had intermediate resistance to Cefazolin. ***Shigella*** **spp.** showed resistance to Cefazolin and Erythromycin and intermediate resistance to Levofloxacin.

4.1 Displays with a bar chart which were presented in Table 4.3 which shows the difference in the Heterotrophic Bacterial Count ($\times 10^6$ CFU/ml) from stool samples before and after inoculation of ***Staphylococcus sciuri*** and *Lactobacillus casei* into the experimental animals

Table 4.1: Hematological parameters of experimental animals

Parameters	**GROUP A**	**GROUP B**	**GROUP C**	**GROUP D**	**GROUP E**
WBC ($10^3/\mu L$)	19.2± 2.00	11.1 ± 4.10	14.5± 4.70	9.2 ± 2.00	9.5 ± 2.10

LYM (%)	84.3± 3.00	68.05 ± 7.40	70.5± 5.00	68.8 ± 6.80	73.4 ± 8.7
MID (%)	25.5± 5.00	17.55 ± 2.01	19.7± 1.00	19.00 ± 2.66	14.0 ± 3.12
GRAN (%)	20.2± 2.00	14.40 ± 6.00	14.6± 2.00	12.10 ± 5.10	12.5 ± 6.00
LYM(#)	8.40 ± 0.7	7.75±1.60	5.33±0.65	6.50±1.95	7.08±0.88
MID(#)	1.65 ± 0.08	1.98±0.34	5.15±2.99	1.72±0.37	1.32±0.14
GRAN(#)	3.57±1.85	1.45±0.15	1.80±0.31	1.00±0.11	7.70±3.88
RBC (10^6/µL)	9.87± 4.00	6.63 ± 0.50	7.30± 1.00	5.89 ± 1.00	5.7 ± 0.70
HGB (g/dL)	18.4± 1.00	14.45 ± 1.37	15.9± 2.00	13.77 ± 1.35	14.2 ± 1.40
HCT (%)	53.6± 3.00	43.10 ± 2.80	48.1± 3.00	42.60 ± 3.33	44.6 ± 4.60
MCV (fL)	93.6± 3.00	65.10 ± 2.40	80.7± 4.00	73.6 ± 9.20	78.5 ± 6.5
MCH (pg)	32.4± 2.00	21.7 ± 0.74	20.1± 4.00	23.65 ± 2.37	24.9 ± 2.00
MCHC (g/dL)	39.3± 3.00	33.45 ± 1.00	39.4± 5.00	32.25 ± 1.30	31.8 ± 0.70
RDW-SD (fL)	38.5± 2.00	39.0 ± 2.00	43.7± 3.00	47.0 ± 6.30	48.1 ± 3.73
RDW-CV (%)	27.2± 1.00	17.1 ± 0.60	29.5± 3.00	18.72 ± 0.60	18.2 ± 0.64
PLT (10^3/µL)	712± 12.00	798.2 ± 397.4	562±18.00	821 ± 109.02	1143±876
MPV (fL)	8.2± 2.00	8.4 ± 0.50	11.8± 2.00	8.7 ± 0.47	10.0 ± 1.00
PDW (%)	9.7± 3.00	11.4 ± 1.60	15.2± 2.00	11.6 ± 0.87	15.5 ± 2.65
PCT (%)	0.58± 0.00	0.68 ± 0.37	0.52± 0.00	0.71 ± 0.10	1.2 ± 1.00
P-LCR (%)	7.0± 1.00	15.5 ± 1.00	21.2± 3.00	12.77 ± 2.91	21.6 ± 4.10

- Values are presented as mean ± SD
- Key:
- A= Control group
- B=Challenged group
- C=Probiotic group
- D=Prophylactic group
- E=Antibiotic group
- WBC = White blood cells
- LYM = Lymphocytes
- MID = Mid-cell absolute count
- GRAN = Granulocytes
- RBC = Red blood cells
- HGC = Hemoglobin
- HCT = Hematocrit

- MCV = Mean cell volume
- MCH = Mean corpuscular hemoglobin
- MCHC = Mean corpuscular hemoglobin concentration
- RDW-SD = Red cell distribution width-standard deviation
- RDW-CV = Red cell distribution width-coefficient of variation
- PLT = Platelet
- MPV = Mean platelet volume
- PDW = Platelet distribution
- PCT = Plateletcrit
- P-LCR = Platelet-large cell ratio
- LYMH = Lymphatic fluid
- GRANH = Granulocyte H
- MIDH = Mutated isocitrate dehydrodenase

Table 4.2: Weight of Wistar rat samples on Day 0, Day 7, Day 14 and Day 21, challenged with *Lactobacillus casei* and *Staphylococcus sciuri.*

Group	Day 0 Weight (g)	Day 7 Weight (g)	Day 14 Weight(g)	Day 21 Weight(g)
Group A (Control)	180.35± 0.20	200± 0.50	192± 0.67	200± 0.5
Group B (Probiotic Administered)	147.7± 0.00	169± 0.00	156± 0.47	214± 0.5
Group C (Prophylactic)	174.18± 0.50	172± 0.20	164± 0.20	189± 0.00
Group D (Infected)	169.74± 0.40	179± 0.66	174± 0.51	172± 0.30
Group E (antibiotics)	154.3± 0.80	169± 0.30	163± 0.86	179± 0.00

Table 4.3: Temperature of Wistar rats on Day 0, Day 7, Day 14 and Day 21, challenged with *Lactobacillus casei*, and *Staphylococcus sciuri.*

Group	Day 0 Temperature (°C)	Day 7 Temperature (°C))	Day 14 Temperature (°C)	Day 21 Temperature (°C)
Group A (Control)	36.0± 0.00	36.0± 0.00	37.2 ± 0.30	37.5 ± 0.20
Group B (Probiotic Administered)	36.8± 0.00	37.2± 0.66	37.3 ± 0.20	37.6 ± 0.30
Group C (Prophylactic)	36.2± 0.00	37.3±0.75	37.2 ± 0.20	37.6 ± 0.30
Group D (Infected)	36.5± 0.00	36.8± 0.54	37.9 ± 0.30	38.63± 0.20
Group E (antibiotics)	36.0± 0.00	38± 0.75	37.5 ± 0.30	37.38 ± 0.30

Table 4.4: Heterotrophic Bacterial Count (×10⁶ CFU/ml) from Stool Samples Before and After Inoculation

Group	Before	After
Group A (Control)	2.05 ± 0.85	2.17 ± 0.41
Group B (Probiotic Administered)	2.35 ± 0.45	1.34 ± 0.42
Group C (Prophylactic)	3.03 ± 0.35	3.49 ± 0.35
Group D (Infected)	3.34 ± 0.45	4.00 ± 1.41
Group E (Antibiotics)	3.40 ± 0.28	2.34 ± 1.03

Values represented as mean ± standard error of the mean

Table 4.5: Cultural, Morphological and Biochemical Characteristics of Bacteria isolates from stool samples

Characteristic	B1.	B2.	B3	B4.	B5	B6.	B7
Elevation	Raised	Flat	Flat	Flat	Raised	Flat	Flat
Margin	Entire	Undulate	Undulate	Entire	Entire	Entire	Entire
Color	Cream	Cream	Cream	White	Cream	White	Cream
Shape	Circular	Irregular	Irregular	Circular	Circular	Circular	Circular
Size	Medium	Large	Large	Small	Medium	Small	Medium
Gram Stain	-	+	-	+	+	+	-
Cell Type	Rod	Rod	Rod	Cocci	Cocci	Rod	Rod
Arrangement	Pair/Chains	Disperse	Disperse	Chains	Clusters	Chains	Disperse
Color (Gram Reaction)	Pink	Purple	Pink	Purple	Purple	Purple	Pink
KOH String Test	+	-	+	-	-	-	+
Catalase	+	+	+	-	+	-	-
Indole	-	-	+	-	-	-	-
Citrate	-	+	-	-	+	-	-
Oxidase	-	-	-	-	-	-	-
Glucose	+	+	+	+	+	+	+
Sucrose	-	+	-	+	+	+	-
Lactose	-	+	+	+	+	+	-
Gas Formation	+	-	+	-	-	-	-
H_2S Formation	+	-	-	-	-	-	-
\TSI (Slant/Butt) Reaction	K/AG H_2S	A/A	A/AG	A/A	A/A	A/A	K/A
Identity	*Salmonella sp.*	*Bacillus sp.*	*E. coli*	*Streptococcus sp.*	*Staphylococcus sp.*	*Lactobacillus sp.*	*Shigella sp.*

Key: (-) negative test; (+) positive test; (A) Acid; (K) Alkaline; (G) Gas production (bubbles); (H_2S) Hydrogen sulphide (black precipitate); (KOH) Potassium hydroxide test; (TSI) Triple sugar iron test

4.8: Antibiotic susceptibility patterns of bacterial isolates from stool samples

Isolates	PEF	CN	APX	Z	AM	R	CPX	AZ	LEV	E
Escherichia coli	R	S	S	S	I	S	S	R	S	S
Salmonella sp.	S	S	S	R	S	S	S	S	R	I
***Bacillus* sp.**	S	S	S	S	R	S	R	R	S	S
***Staphylococcus*sp.**	S	S	R	S	S	S	S	S	R	S
***Streptococcus* sp.**	S	S	S	S	S	S	S	i	S	S
***Shigella* sp.**	S	S	S	R	S	S	S	S	R	R

KEY: Resistance (R) = 0-10mm, Intermediate (I) = 11-16mm, Sensitive (S) = 17mm and above,

PEF	Pefloxacin
CN	Gentamycin
APX	Ampiclox
Z	Zinnacef
AM	amoxicillin
R	Rocephin
CPX	Ciprofloxacin
AZ	Azithromycin
LEV	Levofloxacin
E	Erythromycin

CHAPTER FIVE

DISCUSSION

The use of probiotics as a therapeutic and prophylactic agent has gained significant attention in recent years, particularly in the modulation of immune responses and resistance against pathogenic infections (Azad *et al*., 2018; Iqbal *et al*., 2021; Raheem *et al*., 2021). *Lactobacillus casei, a* widely studied probiotic, has been recognized for its immunomodulatory properties and its role in enhancing host defense mechanisms against various infections, including those caused by *Staphylococcus* species (Wells, 2011; Yousefi *et al*., 2019). *Staphylococcus scuiri*, a less commonly studied but potentially pathogenic member of the *Staphylococcus* genus, has been implicated in various infections in both humans and animals. The present study evaluates the haematological responses of rats exposed to *Lactobacillus casei* and *Staphylococcus scuiri,* providing insights into the probiotic's ability to enhance immunity and mitigate infection-induced alterations in blood parameters.

The haematological analysis revealed significant variations in immune-related parameters among the different treatment groups. The probiotic-treated group exhibited a notable increase in total white blood cell (WBC) count (14.5± 4.70 103/µL) compared to the infected group (9.2 ± 2.00 103/µL), indicating an enhanced immune response. This finding corroborates earlier reports that probiotics stimulate leukocyte proliferation and enhance innate immunity (Czajkowska *et al*., 2019).

Lymphocyte counts were highest in the control and probiotic groups (84.3± 3.00% and 70.5± 5.00%, respectively), supporting previous findings that probiotics enhance adaptive immunity by promoting T-lymphocyte activity (Galdeano and Perdigón, 2006). Granulocyte counts were

lower in the probiotic-treated group compared to the infected group, suggesting reduced inflammation and effective pathogen clearance (Yan and Polk, 2011).

Red blood cell (RBC) counts, hemoglobin (HGB), and hematocrit (HCT) values were significantly lower in the infected group compared to the probiotic and control groups, indicating the impact of bacterial infections on erythropoiesis. This aligns with previous reports that infections can cause hemolysis or anemia through inflammatory cytokines (Weiss and Goodnough, 2005). The improved RBC and HGB levels in the probiotic group suggest that *Lactobacillus casei* supplementation aids in hematopoiesis and reduces infection-induced anemia (Asemi *et al*., 2011).

The observed increase in body weight among probiotic-treated rats (147.7 ± 0.00 g to 214 ± 0.00 g) compared to the infected group (169.74 ± 0.40 g to 172 ± 0.30 g) by day 21 aligns with previous studies indicating that probiotic supplementation enhances gut microbiota balance, nutrient absorption, and metabolic efficiency (Ouwehand *et al*., 2016). Probiotics, including *Lactobacillus casei*, have been reported to improve feed efficiency and weight gain by modulating the gut microbiome and reducing inflammation caused by infections (Markowiak and Śliżewska, 2017). The significant weight loss in the infected group suggests that *Staphylococcus scuiri* infection impaired nutrient absorption and metabolic homeostasis, potentially due to systemic inflammation and altered gut microbiota composition (Vinderola *et al*., 2020).

Temperature variations were also significant, with the infected group showing the highest temperature increase (36.5 ± 0.00°C to 38.63 ± 0.20°C). This aligns with the findings of Yan and Polk (2020), who reported that bacterial infections often trigger systemic inflammatory responses,

leading to fever. The slight decrease in temperature in the antibiotic-treated group suggests effective bacterial suppression, consistent with the antipyretic effects of antibiotics in managing infections.

The heterotrophic bacterial counts before and after treatment revealed a significant reduction in microbial load in the probiotic-administered group ($1.34 \pm 0.42 \times 10^6$ CFU/ml), supporting previous studies that demonstrate the antimicrobial potential of *Lactobacillus casei* (Kobayashi *et al*., 2019). Probiotic administration is known to suppress pathogenic bacteria through competitive exclusion, production of bacteriocins, and modulation of immune responses (Gao *et al*., 2019).

The persistence of high microbial counts in the infected group ($3.34 \pm 0.45 \times 10^6$ CFU/ml) suggests that *Staphylococcus scuiri* colonization disrupted the normal gut flora, a common occurrence in bacterial infections (Belkaid and Hand, 2014). Furthermore, the lower microbial counts in the antibiotic-treated group suggest effective pathogen clearance, although the risk of dysbiosis remains a concern.

The antibiotic susceptibility patterns of the bacterial isolates from stool samples highlighted varying resistance profiles. Escherichia coli exhibited resistance to Pefloxacin and Azithromycin, consistent with reports of increasing fluoroquinolone and macrolide resistance among enteric bacteria (Laxminarayan *et al*., 2022). The resistance of *Salmonella* spp. to Cefazolin and Erythromycin aligns with findings by Ventola (2015), which emphasize the emergence of multidrug-resistant *Salmonella* strains. The susceptibility of *Streptococcus* spp. to most antibiotics, with intermediate resistance to Cefazolin, suggests that while certain bacterial strains remain treatable with conventional antibiotics, resistance is evolving. The findings reinforce the

necessity for alternative approaches such as probiotics to mitigate antimicrobial resistance (Hussein *et al*., 2021). The antibiotic susceptibility patterns revealed resistance to multiple antibiotics among the bacterial isolates, particularly *Escherichia coli*, *Salmonella spp.*, and *Bacillus spp.*. The probiotic-treated group demonstrated improved resistance modulation, as evident from reduced bacterial loads and enhanced immune response. These findings highlight the potential of probiotics as adjuncts to conventional antibiotics in mitigating antimicrobial resistance (AMR) (Singha *et al*., 2024).

The findings of this study provide compelling evidence for the immunomodulatory and hematological benefits of *Lactobacillus casei* in the context of bacterial infections caused by *Staphylococcus sciuri*. In our rat model, probiotic administration was associated with marked improvements in several health parameters. Notably, the probiotic-treated group exhibited enhanced weight gain, a reduction in the pathogenic microbial load, and normalization of key hematological indices such as white blood cell counts, red blood cell levels, hemoglobin concentration, and hematocrit values. These outcomes suggest that *Lactobacillus casei* not only mitigates the deleterious effects of *S. sciuri* infection but also promotes overall systemic health by modulating immune responses and reducing inflammation.

The restoration of microbial balance observed in this study is consistent with the notion that *Lactobacillus casei* exerts its effects via competitive exclusion of pathogens, secretion of antimicrobial peptides, and enhancement of mucosal immunity (Azad *et al*., 2018; Ouwehand *et al*., 2018). Moreover, the modulation of leukocyte proliferation and lymphocyte levels further underscores the probiotic's capacity to bolster both innate and adaptive immune defenses, corroborating earlier findings (Markowiak and Śliżewska, 2017; Fowler *et al*., 2018). Given the

mounting global concern over antibiotic resistance (Laxminarayan *et al*., 2022), these results underscore the potential of probiotic interventions as adjuncts or alternatives to conventional antimicrobial therapies.

While these preclinical findings are promising, further research is warranted to elucidate the precise molecular and cellular mechanisms underlying these beneficial effects. Determining the optimal dosing regimens, administration duration, and formulation of *Lactobacillus casei* is critical to maximize its therapeutic potential. Additionally, the translation of these findings into clinical practice will require comprehensive long-term studies in human populations.

5.1. Recommendations

1. Further studies should explore the molecular mechanisms underlying the immunomodulatory effects of *Lactobacillus casei.*
2. Long-term clinical trials should be conducted to evaluate the sustained impact of probiotic supplementation on bacterial infections and immune function.
3. Optimal probiotic dosing and administration protocols should be investigated to enhance therapeutic outcomes.
4. Combination therapies involving probiotics and conventional antimicrobials should be explored to mitigate antimicrobial resistance while maximizing treatment efficacy.
5. Public awareness programs should be implemented to promote the benefits of probiotics in managing infections and improving overall health.
6. Regulatory guidelines should be established for probiotic use in therapeutic interventions to ensure safety and efficacy.

5.2. Conclusion

This study provides substantial evidence supporting the immunomodulatory and hematological benefits of *Lactobacillus casei* in combating *Staphylococcus scuiri* infection. The probiotic's ability to restore microbial balance, enhance hematological parameters, and regulate immune responses underscores its potential as a complementary therapeutic strategy. These findings reinforce the growing recognition of probiotics as valuable alternatives to conventional antimicrobial treatments, particularly in the face of increasing antibiotic resistance.

Future research should explore the molecular mechanisms underlying these effects and evaluate the long-term impact of probiotic supplementation in managing bacterial infections. Additionally, further investigations into optimal probiotic dosing and combination therapies with conventional treatments will be critical in maximizing therapeutic outcomes for bacterial infections and immune-related disorders.

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