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Experimental Evaluation of Immunological Response for Flagellin Extractions from Various Pathogenic Bacteria in Rat Models

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Abstract - **Flagellin, the bacterial flagella structural protein, is one of the important pathogen-associated molecular patterns (PAMP) detected by Toll-like receptor 5 (TLR5), which stimulates innate immunity. The aim of this study was to examine the immunological and histopathological impacts of flagellin purified from nine pathogenic bacterial species on rat models. The successful purification of flagellin was confirmed by SDS-PAGE analysis with molecular weights between 35–45 kDa. Enzyme-linked immunosorbent assay (ELISA) revealed high strain-dependent variation in TLR5 levels, with *Escherichia coli*, *Pseudomonas fluorescens* , and *Pseudocitrobacter faecalis* evoking peak responses at day 7 postinjection, suggesting vigorous immune activation. While, *Serratia marcescens* and *Salmonella enterica*, produced lower TLR5 levels that can be suggestive of immune tolerance or receptor down regulation. Histopathology of mesenteric lymph nodes demonstrated variable inflammation patterns, including necrotizing lymphadenitis, granulomatous inflammation, and reactive hyperplasia, in accordance with the immunogenicity of each flagellin variant. Worth noting, environmental isolates such as *Pseudomonas rhizosphaerae* induced milder tissue reactions, reflecting lower pathogenicity. These results indicate the effect of flagellin origin on tissue pathology and host immunity dynamics. The research sets the therapeutic potential of selected flagellins as drugs for immunotherapy, and furthering our understanding of host-pathogen interaction. Further structural and functional investigations are required to identify the mechanisms behind these differential effects.**

Conclusion: The integration of TRL5 immune response profiles and histopathological findings provides valuable information regarding the immunogenicity and tissue-specific pathogenicity of the nine bacterial strains that were studied. The ELISA analysis revealed that *Escherichia coli, Pseudomonas fluorescens, and Pseudocitrobacter faecalis* elicited the most intense TRL5 response, with levels increasing significantly by day 7 (4.98, 4.77, and 4.82 ng/mL, respectively). These pathogens also induced histopathological manifestations of acute immune activation: *E. coli* and *P. fluorescens* evoked necrotizing inflammation with lymphoid hyperplasia and vascular damage, while *P. faecalis* compared lymphoid depletion to focal hyperplasia, suggesting an ineffective but dysregulated immune activation. In contrast, *Salmonella enterica* and *Serratia marcescens* were manifested decreaseing TRL5 levels over time (3.56 and 3.74 ng/mL on day 7), as they had histopathological profiles marked by lymphoid depletion and extensive necrosis. This inhibition/inverse relationship with tissue damage indicates potential mechanisms of immune evasion, such as toxin-induced lymphocyte apoptosis or blocking of TLR5 signaling pathways. Strains like *Burkholderia metallica* and *Bacillus cereus* elicited modest TRL5 increases (4.32 and 4.26 ng/mL) reflecting their pyogranulomatous inflammation and toxin-mediated stromal remodeling, respectively. The control group's stable TRL5 levels (3.75–3.86 ng/mL) and absence of histopathological changes highlight the specificity of the reaction. Curiously, *Bacillus rhizoplanae* and *Pseudomonas rhizosphaerae* caused compartmentalized inflammation and vascular enlargement with minimal noticeable lymphoid depletion, mirroring their intermediate TRL5 levels (4.54 and 4.59 ng/mL).

Keywords *- Falgellin, Toll like receptors, Histopathology, ELISA, Immunology .*

INTRODUCTION

The innate immune system creates an efficient and quick first line of defense against microbial pathogens by employing pattern recognition receptors (PRRs) to recognize conserved microbial molecules that are referred to as pathogen-associated molecular patterns (PAMPs) (1). Toll-like

receptor 5 (TLR5) is one of the PRRs that recognizes flagellin, which is the primary bacterial flagella protein. Upon detection, TLR5 activates a signaling cascade that ultimately activates nuclear factor-kappa B (NF-κB), producing pro-inflammatory cytokines and activating immune defense (2). This makes flagellin not only a crucial mediator of microbial detection but also an exciting target for therapeutic applications like vaccine adjuvants and immune modulators (3).

While flagellin is generally conserved in motile bacteria, variations in sequence and structure among bacteria can have a profound effect on its capacity to bind TLR5 and induce immune activity (4,5). Certain flagellins are more potent at stimulating TLR5, whereas others elicit weaker or more controlled responses. This has been explained due to variation within the D1 domain—the site of recognition for TLR5—and other structural motifs that influence the immunogenicity of the molecule (6). Therefore, comparison of immunological and pathological responses to flagellin of different bacterial species gives an indication of the extent of immune reactivity and related inflammatory risks of such proteins (7,8). Here, we investigate the immunological and histopathological activity of flagellin from nine different species of bacteria, environmental and clinically significant pathogens (9).

They include *Serratia marcescens*, *Pseudomonas rhizosphaerae*, *Escherichia coli*, *Bacillus cereus*, *Bacillus rhizoplanae*, *Pseudocitrobacter faecalis*, *Burkholderia metallica*, *Pseudomonas fluorescens*, and *Salmonella enterica*. These bacteria were selected because of their reported motility, pathogenicity, and impact on human and environmental health (9). The diversity within these bacteria provides a broad context for establishing how flagellin structural variation is translated into differential immune responses (10). In pursuit of studying such effects, rat models were utilized since they are best suited to study systemic immune effects and tissue level effects after antigen administration (11). Following intraperitoneal injection of purified flagellin, the immunologic effect was evaluated by measuring levels of expression of TLR5 and pro-inflammatory cytokines interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and interleukin-1 beta (IL-1β) (12). Such markers serve as good gauges of the extent of innate immune stimulation via each variant of flagellin (13).

Notably, the histopathological impact of these flagellin injections was assessed with special attention to the mesenteric lymph nodes (MLNs). MLNs are optimally positioned to drain antigens from the gut and are a critical location for the induction of mucosal immune responses. They contain a high concentration of lymphocytes, macrophages, and dendritic cells—highly antigen-sensitive and hence optimally placed to assess immune-mediated tissue alterations (14). Here, MLNs were removed and subjected to histological analysis to compare the extent of pathological alterations induced by different bacterial flagellins. Histopathology was assessed by looking for significant signs of inflammation and immune activation, which are: Follicular hyperplasia, Cortical and paracortical enlargement, Sinus histiocytosis, Lymphoid depletion or necrosis and Vascular congestion and cellular infiltration (8).

By comparing these histological markers between groups of experiments, we aim to assess correlations between flagellin source, immune activation by TLR5, and level of tissue pathology in mesenteric lymph nodes (14). This provides us with a two-level picture functional (immune activity) and structural (tissue integrity) of the biological impact of flagellin exposure (15). Most past studies have used model organisms like *Salmonella enterica* and *E. coli*, so there is a gap in the knowledge of how flagellins from environmental or less-studied clinical isolates influence host immune responses. Taken together, the strains like *Pseudocitrobacter faecalis, Burkholderia metallica*, and *Bacillus rhizoplanae* provides a more comprehensive perspective on flagellin diversity and its functional implications.

This study thus offers a comparative platform to determine the pathogenic and immunostimulatory capability of flagellins from different bacteria. Not only does it inform TLR5 biology and host pathogen interactions, but it is also relevant to the design of flagellin-based immunotherapies, where selection of bacterial source is considerations to balance efficacy with safety. In addition, the identification of flagellins with minimal histopathological alterations without sacrificing immune stimulation may lead to the development of next-generation immune modulators or vaccine adjuvants (6). Overall, the study will solve the clue between molecular immunology and tissue pathology though an investigation of the effect of structurally distinct flagellins on systemic immune responses and mesenteric lymph node organization. The results will expand the knowledge regarding bacterial immune evasion, inflammatory capacity, and the safe use of microbial products in immunotherapy.

MATERIALS AND METHOD

Flagellin Extraction

Nine bacterial strains were isolated form different niches at this study which are: *Serratia marcescens, Pseudomonas rhizosphaerae, Escherichia coli, Bacillus cereus, Bacillus rhizoplanae, Pseudocitrobacter faecalis, Burkholderia metallica, Pseudomonas fluorescens, and Salmonella enterica.* The strains were all cultured in LB broth aerobically at 37°C with shaking (200 rpm) for 18–24 hours to the late log phase. Flagellin was isolated from the bacterial cultures by a mechanical shearing procedure. Briefly, bacterial suspensions were centrifuged at 5000 rpm for 10 minutes at 4°C to pellet the cells. The pellets were

resuspended in phosphate-buffered saline (PBS) and were blended to shear the flagella directly of bacterial body (16). The suspensions were centrifuged at 10,000 rpm for 20 minutes to remove cellular debris, and the supernatants containing flagellin were collected. Then, the extracted flagellin that purified by precipitation with ammonium sulfate (60% saturation) and dialyzed in PBS to remove residual salts (16).

SDS-PAGE Analysis of Flagellin

Molecular weight and flagellin protein purity isolated were determined by SDS-PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis). Protein samples were heat-denatured in Laemmli buffer containing β-mercaptoethanol and loaded on a 12% polyacrylamide gel. Electrophoresis was performed at 120 V for 90 minutes. Then, gel was stained with Coomassie Brilliant Blue R-250, and molecular weights were estimated using a standard protein ladder (7,17). Flagellin generally appeared as a distinct band at approximately 35-45 kDa (18).

Animal Experiment and Flagellin Injection

Thirty of Adult healthy male Wistar rats (180–220 g) were used in the study for nine groups as well as control group, each group included three animals. All animal procedures were sanctioned by the Institutional Animal Care and Use Committee (IACUC) and performed according to ethical standards (Date : 1/10 /2024 & Ref. UM.VET.2024.117). Rats were randomly distributed into experimental groups (n = 3 per bacterial flagellin type and one PBS control group). Every rat was administered an intraperitoneal by injection of purified flagellin (25 µg in 0.5 mL PBS). Administration was repeated on day 3 and day 7 to immunostimulate the immune system (19). Control animals were administered the same volume of sterile PBS lacking flagellin (20).

Serum Collection and TLR5 Quantification

Blood samples were collected from the retro-orbital plexus under light anesthesia on day 3 and day 7 by following the respective injections. Then, blood was allowed to clot at room temperature, and serum was separated by centrifugation at 3000 rpm for 15 minutes and stored at −20°C until analysis. Serum TLR5 concentrations were ascertained by a commercial ELISA kit (specific for rat TLR5) following the manufacturer's protocol. Absorbance at 450 nm was quantified using a microplate reader, and concentrations were calculated from a standard curve constructed from known concentrations of recombinant TLR5(21).

Mesenteric Lymph Node Collection and Histopathological Analysis

An end of experiment after fourteen days, rats were euthanized under deep anesthetic conditions. Mesenteric lymph nodes (MLNs) were carefully dissected and excised from intestinal mesentery. The tissues were fixed promptly in 10% neutral-buffered formalin for 48 hours (22). Following fixation, the tissues were graded alcohol-processed, xylene-cleared, and paraffin-embedded. Tissue sections of 4–5 µm thickness were cut and stained with hematoxylin and eosin (H&E) to examine histopathologically under a light microscope (23).

RESULTS AND DISCUSSION

The results were carried out to evaluate the separation of flagellin proteins from nine bacteria strains via SDS-PAGE: *Serratia marcescens*, *Pseudomonas rhizosphaerae, Escherichia coli*, *Bacillus cereus*, *Bacillus rhizoplanae, Pseudocitrobacter faecalis*, *Burkholderia metallica*, *Pseudomonas fluorescens*, and *Salmonella enterica*. The electrophoretic patterns exhibited distinct bands for the flagellin proteins, ranging generally from 35 to 45 kDa. *Serratia marcescens* presented a strong band at approximately 42 kDa, while *Pseudomonas rhizosphaerae* exhibited a strong band near 40 kDa. *Escherichia coli* also exhibited a clear band at 38 kDa, and *Bacillus cereus* demonstrated a strong band at approximately 44 kDa. While, *Bacillus rhizoplanae* that exhibited a fragment at 43 kDa but was slightly broader. In contrast, *Pseudocitrobacter faecalis* molecular weight at 39 kDa, reflecting lower concentration of flagellin. *Burkholderia metallica* presented a distinct band at 41 kDa with few faint additional bands. *Pseudomonas fluorescens* presented a well-defined band at 40 kDa, indicating high purity, and *Salmonella enterica* presented a typical band at 37 kDa. The results confirm the effective isolation of flagellin proteins from all the tested strains, with the slight variation in molecular weight and band intensity being species-specific figure (1).

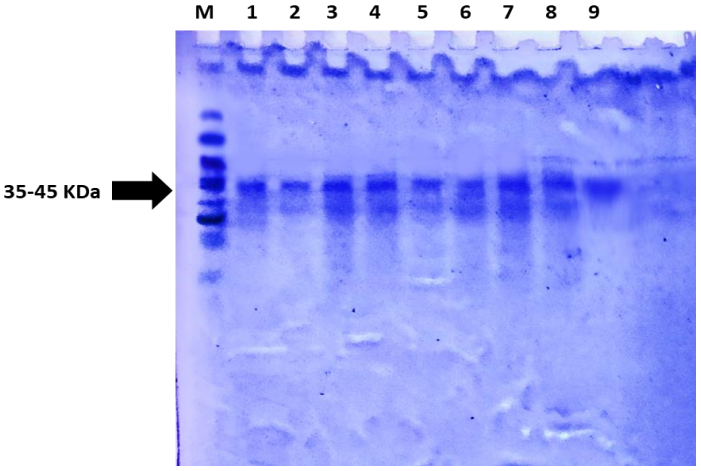

Figure 1.SDS-PAGE profile of flagellin proteins from nine bacterial isolates. Lane M: Protein molecular weight marker;

Lanes 1–9: bacterial flagellin samples. Lane 1: *Serratia marcescens*, Lane 2: *Pseudomonas rhizosphaerae*, Lane 3: *Escherichia coli*, Lane 4: *Bacillus cereus*, Lane 5: *Bacillus rhizoplanae*, Lane 6: *Pseudocitrobacter faecalis*, Lane 7: *Burkholderia metallica*, Lane 8: *Pseudomonas fluorescens*, Lane 9: *Salmonella enterica.*

Enzyme linkage immunoassay (ELISA) to quantify Toll like receptor 5 (TLR5) immune response (in ng/mL) in serum samples taken at 7 and 14 days post-immunization with nine different bacterial strains indicated that a majority of strains had a concentration of TLR5 over time indicating chronic immune response.A standard curve was generated by using standard TLR5 concentrations (0, 1.5, 3, 6, 12, and 24 ng/mL) based on determine the final concentrations of TLR5 (table1) by bloating serum samples value figure (2).On day 3, the contents of TLR5 ranged from 3.81 ng/mL for immunized *Serratia marcescens* rats to 4.59 ng/mL in immunized *Pseudocitrobacter faecalis* rats. The level of control was a background reading of 3.86 ng/mL, and it signified the absence of specific immunostimulation.

By compared to day 14, TLR5 levels higher than day 7 were obtained for some of the bacterial strains. The maximum elevation was seen with rats immunized with *Escherichia coli K12*, for which TLR5 showed an increase from 4.18 ng/mL to 4.98 ng/mL. An increase was from 3.85 ng/mL to 4.77 ng/mL by *Pseudomonas fluorescens*, and an increase from 4.59 ng/mL to 4.82 ng/mL was seen with *Pseudocitrobacter faecalis*. there was Slight raised also recorded with *Bacillus*

rhizoplanae (4.13 to 4.54 ng/mL), *Bacillus cereus* (3.81 to 4.26 ng/mL), and *Burkholderia metallica* (4.12 to 4.32 ng/mL). Immune responses in rats immunized with *Serratia marcescens* and *Salmonella enterica*, however, showed slight declines, from 4.03 to 3.56 ng/mL and 4.43 to 3.74 ng/mL, respectively. The control was constant at 3.86 ng/mL on day 3 and 3.75 ng/mL on day7 figure (4).

Generally, the results reveal what most of the bacterial strains, particularly *Escherichia coli*, Pseudomonas *fluorescens*, and *Pseudocitrobacter faecalis*, evoked strong TLR5 immune reactions after some time, indicating that they are likely to be strong immunogenic candidates.

Table 1. Different concentrations of TLR5 and their optical density, in order to blotting unknown results to get final concentrations

Concentrations ng/ml	O.D=450
0	0.215
1.5	0.495
3	0.717
6	1.409
12	1.843
24	2.389

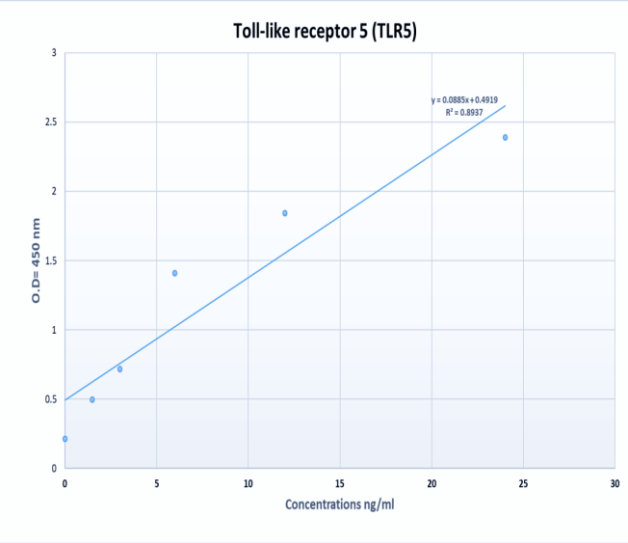

Figure 2. Standard curve for TLR5 ELISA quantitation. Graph of known TLR5 protein concentrations (0, 1.5, 3, 6, 12, and 24 ng/mL) against their respective optical density (OD) readings at 450 nm was drawn. The curve was used to interpolate test serum sample TLR5 concentrations.

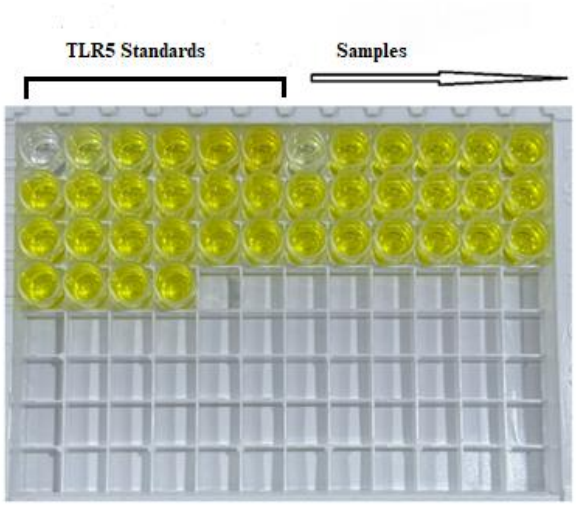

Figure 3. Microplate of ELISA results

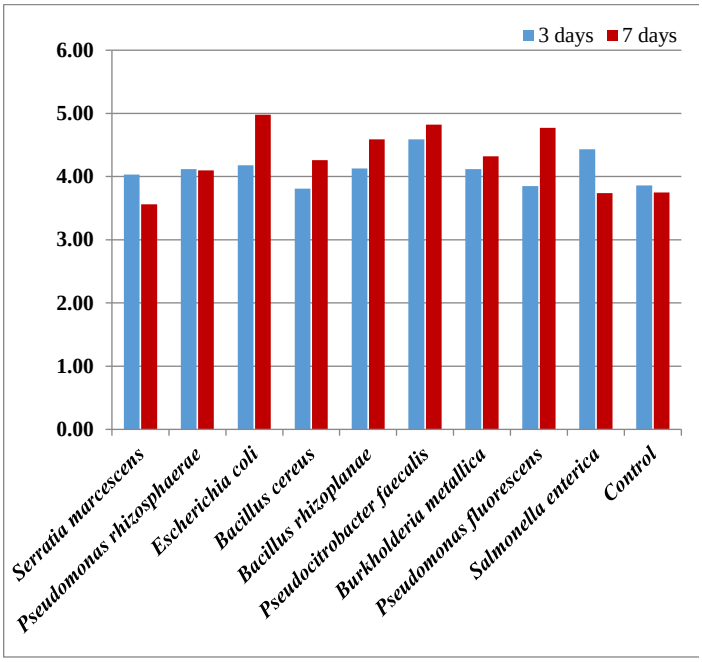

Figure 4. Histogram of TLR5 levels (ng/mL) in serum at days 3 and 7 after immunization with nine bacterial species, compared to control. Bars represent the mean ELISA-determined TLR5 level for each of the nine bacterial species on both days. *Escherichia coli, Pseudomonas fluorescens*, and *Pseudocitrobacter faecalis* showed the largest increase by day 7. Control had low, stable TLR5 levels without a notable trend over time.

Histopathological examination of the mesenteric lymph nodes experimental infected with nine strains of bacteria depicted typical tissue damage patterns, which are: inflammation, and immune dysregulation, which focusing species-specific pathogenic mechanisms. *Serratia marcescens* and *Salmonella enterica* induced systemic necrotizing lymphadenitis, which was marked by medullary and cortical necrosis, eosinophilic debris deposition, and vascular thrombosis. Whereas Serratia was marked by thrombus formation and lymphoid follicular hyperplasia, Salmonella exhibited cortical lymphoid depletion and medullary inflammatory cell infiltration, consistent with its enteric pathogenicity figure (4). *Burkholderia metallica* elicited pyogranulomatous inflammation, characterized by neutrophilic and macrophage aggregates, follicular germinal center apoptosis, and necrotic foci, distinguishing it from *Pseudomonas fluorescens*, which elicited lymphoid hyperplasia in addition to vascular congestion and thrombus formation, consistent with its dual immunostimulant and endothelial insult effects.

Lymphoid reactions were very varied: *Bacillus cereus* and *Pseudocitrobacter faecalis* induced major lymphoid depletion, the former leading to paracortical loss of lymphocytes with reorganization of the stroma, suggestive of cytotoxicity induced by the toxin. But Pseudocitrobacter paradoxically added lymphoid atrophy to local hyperplasia, suggestive of immune dysregulation. *Bacillus rhizoplanae* and *Pseudomonas rhizosphaerae* induced compartmentalized inflammation, with *B. rhizoplanae* inducing subcapsular follicular hyperplasia with vascular dilatation, whereas *P. rhizosphaerae* sustained chronic lymphoid activation in the absence of acute suppuration. Escherichia coli caused acute necrotizing lesions with eosinophilic debris and focal inflammatory aggregates, which contrasted with the granulocyte-predominant responses of Salmonella and Burkholderia.

The present study attempted to investigate immunological and histopathological host reactions induced by flagellin isolated from nine different pathogenic bacterial species in rats as models (24). The investigation discovers widespread divergence in both activation of innate immunity (as measured by TLR5 expression) and in pathology of mesenteric lymph node, suggesting that structural difference of flagellin among bacterial species predominantly dictates host reactions (25). SDS-PAGE analyses revealed effective extraction and purification of flagellin from nine strains with all nine strains yielding protein fragments in the expected size range of 35–45 kDa (26). Variations in band intensity and molecular weight are in agreement with previously documented bacterial flagellin structural diversity, especially in the D1 and D0 domains essential for TLR5 recognition (27). ELISA analysis for TLR5 indicated that *Pseudomonas fluorescens*, *Escherichia coli*, and *Pseudocitrobacter faecalis* all stimulated most of the TLR5 responses by 7 days, revealing that the immune stimulation was still present (or potentially even greater) at that time. This data confirms prior observations that the previously identified E. coli flagellin is a potent TLR5 agonist. As opposed to this, *Serratia marcescens* and *Salmonella enterica* flagellins down-regulated TLR5 levels by day 7 that may indicate immune tolerance mechanisms or differences in TLR5 affinity. Such dynamics demonstrate how even conserved PAMPs like flagellin are functionally divergent depending on bacterial source (28). Histopathological analysis further highlighted such differences, with certain strains eliciting severe lymph node pathology (29). *Serratia marcescens* and *Salmonella enterica* both caused necrotizing lymphadenitis, indicating a strong cytopathic or proinflammatory response that would be expected to be related to both their flagellin and other co-purified virulence factors. Of interest, *Burkholderia metallica* and *Bacillus cereus* also caused extensive tissue damage, including granulomatous inflammation and stromal reorganization, respectively (30). These findings suggest that the flagellin of these bacteria may have immune-modulatory motifs distinct from TLR5 activation, possibly modulation of inflammasome pathways or interaction with other receptors such as

NLRC4 (31). In contrast, *Pseudomonas rhizosphaerae* and *Bacillus rhizoplanae* were associated with more compartmentalized and chronic inflammation without widespread necrosis (32). This could suggest more controlled immune response or diminished intrinsic flagellin toxicity, rendering them potential safer immunomodulatory candidates (33). *Pseudocitrobacter faecalis* exhibited a pattern of atrophy and hyperplasia, suggesting immune dysregulation rather than a uniform proinflammatory effect possibly as a result of host-specific immune response fluctuation or unique structural characteristics of its flagellin (34). Together, the data validate the hypothesis that flagellin from different bacterial species is able to cause qualitatively and quantitatively different immunological and histological responses. The satisfactory correlation between elevated TLR5 levels and extreme lymph node pathology in some strains (e.g., *E. coli*, *Pseudomonas fluorescens*) provides the central role of TLR5-mediated signaling in triggering early immune activation. But the difference in histopathological patterns between hyperplasia and necrosis suggests that additional downstream pathways and sensitivities of host tissue further decide the ultimate outcome. These findings have important implications for the development of flagellin-based immunotherapeutics. Overall, the discovery of flagellin variants with high immunostimulatory activity and minimal tissue pathology could render vaccines and adjuvants less toxic. Possible sources for this are environmental isolates such as *Pseudomonas rhizosphaerae* and *Bacillus rhizoplanae*. Our study further expands bacterial flagellin diversity beyond the most common clinical strains and fills a gap in the scientific literature regarding environmental and less commonly examined pathogenic species.

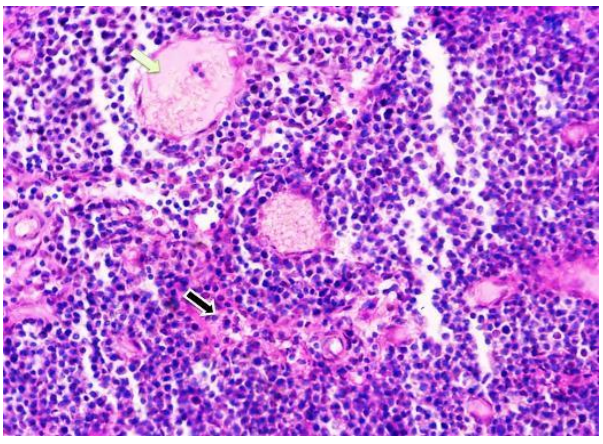

Figure 5. Microscopic section of mesenteric L. node revealed, hemorrhage (black arrow) congestion and thrombus of blood vessels (yellow arrow) as well as infiltration inflammatory cells. (H&E stain, 40x magnification).for *Serratia marcescens*.

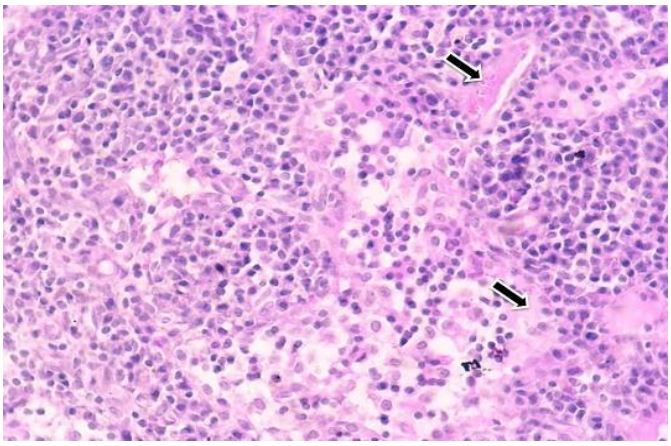

Figure 6. Microscopic section of mesenteric L. node revealed, necrosis and inflammatory cells hyperplasia mainly lymphocytes and macrophages as well as congestion of blood vessels with esinophylic debris deposition (black arrow) (H&E stain, 40x magnification). for *Salmonella enterica*.

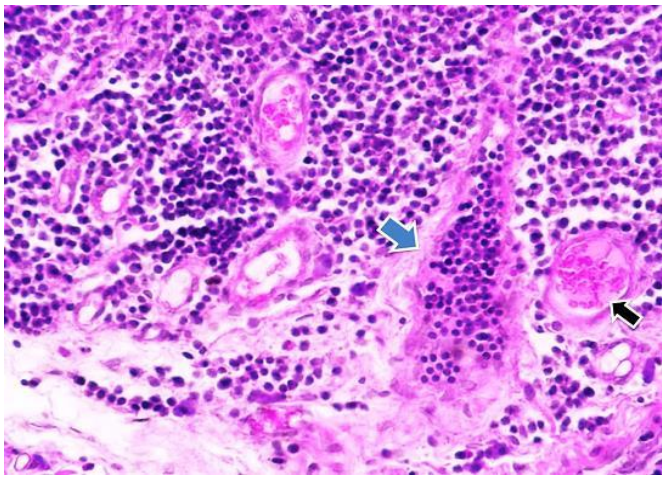

Figure 7. Microscopic section of mesenteric L. node revealed, lymphoid and inflammatory cells hyperplasia in the parenchymal tissue as well as inside blood vessels (Blue arrow).with marked blood vessels congestion and thrombus formation(black arrow) .for *Bulkholderia metallica* .

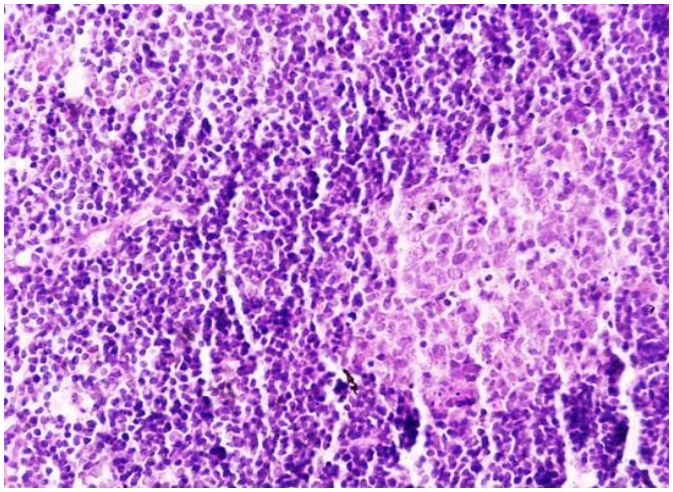

Figure 8. Microscopic section of mesenteric L. node revealed, lymphoid, pyogranulomatous lymphadenitis marked by abundant neutrophils and macrophages. As well as necrosis and apoptosis in the follicular germinal center. (H&E stain, 40x magnification). for *Pseudomonas fluorescens*.

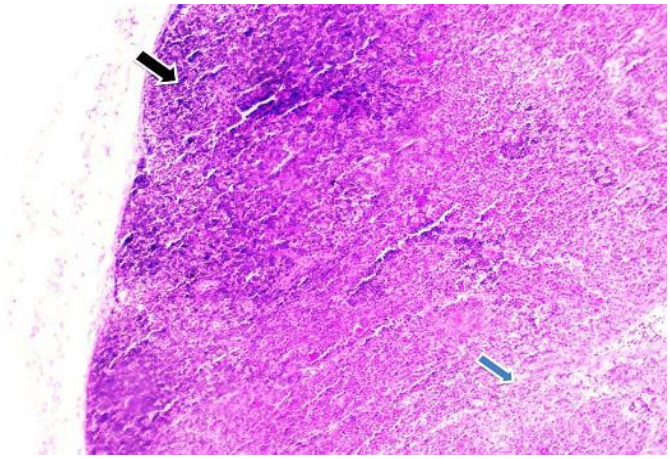

Figure 9. Microscopic section of mesenteric L. node revealed, the stromal cells may become more prominent, as well as nnecrotic area (blue arrow). (H&E stain, 40x magnification). for *Bacillus cereus.*

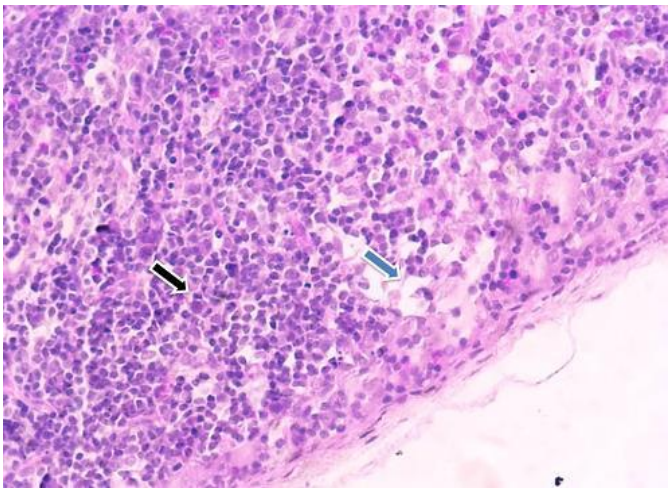

Figure 10. Microscopic section of mesenteric L. node revealed, area of necrosis and lymphoid depletion (blue arrow) with hyperplasia of lymphoid cells (H&E stain, 40x magnification). for *Pseudocitrobacter faecalis*

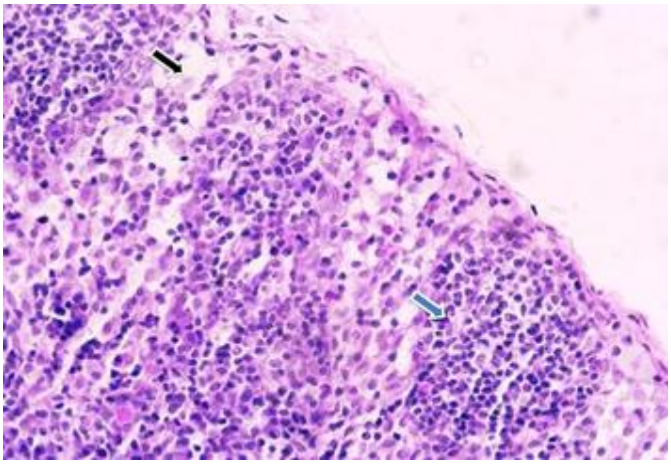

Figure 11. Microscopic section of mesenteric L. node revealed, lymphadenitis with necrosis area in the medulla (black arrow) as well as hyperplasia of lymphoid follicles (blue arrow). (H&E stain, 40x magnification). for *Pseudomonas rhizophaerae*

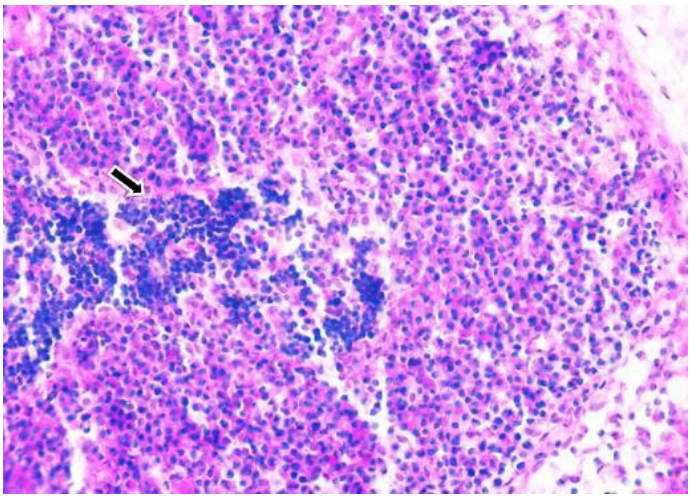

Figure 12. Microscopic section of mesenteric L. node revealed, lymphadenitis with focal aggregation of inflammatory cells (black arrow) as well as hyperplasia of lymphoid cells (blue arrow. (H&E stain, 40x magnification). for *Escherichia coli* .

CONCLUSION:

The integration of TRL5 immune response profiles and histopathological findings provides valuable information regarding the immunogenicity and tissue-specific pathogenicity of the nine bacterial strains that were studied. The ELISA analysis revealed that *Escherichia coli, Pseudomonas fluorescens, and Pseudocitrobacter faecalis* elicited the most intense TRL5 response, with levels increasing significantly by day 7 (4.98, 4.77, and 4.82 ng/mL, respectively). These pathogens also induced histopathological manifestations of acute immune activation: *E. coli* and *P. fluorescens* evoked necrotizing inflammation with lymphoid hyperplasia and vascular damage, while *P. faecalis* compared lymphoid depletion to focal hyperplasia, suggesting an ineffective but dysregulated immune activation.

In contrast, *Salmonella enterica* and *Serratia marcescens* were manifested decreaseing TRL5 levels over time (3.56 and 3.74 ng/mL on day 7), as they had histopathological profiles marked by lymphoid depletion and extensive necrosis. This inhibition/inverse relationship with tissue damage indicates potential mechanisms of immune evasion, such as toxin-induced lymphocyte apoptosis or blocking of TLR5 signaling pathways. Strains like *Burkholderia metallica* and *Bacillus cereus* elicited modest TRL5 increases (4.32 and 4.26 ng/mL) reflecting their pyogranulomatous inflammation and toxin-mediated stromal remodeling, respectively.

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