Republic of Iraq

Ministry of Higher Education

and Scientific Research

University of Babylon \College of Science

Department of Biology

Effect of Bacterial Infection on IL-17; Dectin-1; sIgA and Gene Polymorphism of TLR4 with Appendicitis Patients

A thesis

Submitted to the Council of the College of Science- University of Babylon in a Partial Fulfillment of the Requirements for the Master Degree in Biology

By

Zahraa Abdulhussien Abdul Wahid Abbas

B.Sc. Biology-Microbiology/ College of Science / University of Babylon -2018

Supervisor by:

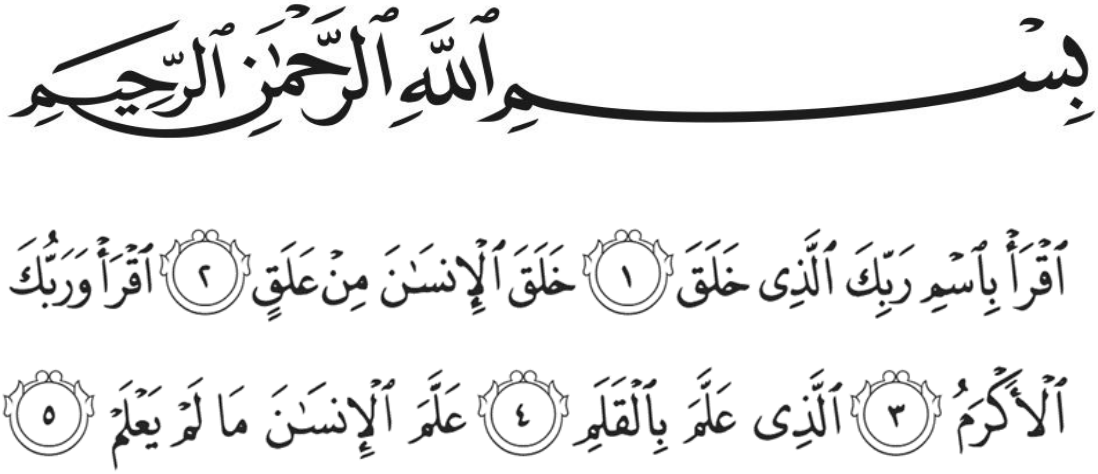
Prof.

Dr. Frial Gemeel Abd Atiea

Prof.

Dr. Ibtihal Muiz Abdul Mahdi Al-Hussaini

2025 A.D **1447 A.H**



صَدَقَ اللَّهُ العَلِيُّ العَظِيمْ

سورة العلق: الايات (١ – ٥)

Supervisor Certification

We certify that the preparation of this thesis entitled **(Effect of Bacterial Infection on IL-17; Dectin-1; sIgA and Gene Polymorphism of TLR4 with Appendicitis Patients)** was made by **Zahraa Abdulhussien Abdul Wahid** under our supervision at the Department of Biology, College of Science / University of Babylon, as a partial fulfillment of the requirements for the degree of Master of Science in Biology.

Supervisor:

Signature

Name: Dr. Frial Gemeel Abd Atiea

Title: Professor

Address: Department of Biology

University of Babylon

College of Science

Date: / / 2025

Signature

Name: Dr.Ibtihal Muiz Mahdi Al- Hussaini

Title: Professor

Address: Department of Biology

University of Babylon

College of Science

Date: / / 2025

In view of the available recommendation, I forward this thesis for debate by the examining committee.

Signature:

Name: Prof.Dr. Basheer Abdulhamza Mohammed Alalwani

Address: Head of Biology Department, College of Science,

University of Babylon

Date: / / 2025

Dedication

To the two lights of my life,
*To **my mother**, whose prayers wrapped me in peace, and to **my father**, whose strength supported every step — your love stays forever in my heart.*

*To my dear **sisters**,*
Your kindness, laughter, and support made every tough day easier and every moment brighter.

To the family I found through love,
*to my **mother-in-law**, **father-in-law**, and **sisters-in-law** — thank You for your unwavering support and encouragement that helped me keep going and complete my master's journey.*

To the soul who walks beside me,
*To **my husband** — your patience, support, and belief in me turned every stumbling block into a stepping stone.*

To the piece of my heart that walks outside my body,
*To my son **Yousif**, who gave every effort a deeper meaning — this is all for you.*

*To my dear **friends**,*
Thank you for being my light in the dark, my calm in the storm, and my strength when I felt weak.

With all my love and gratitude,
***Zahraa** 2025*

Acknowledgements

First and foremost, I am deeply grateful to **Allah,** whose infinite wisdom and mercy granted me the strength, patience, clarity of thought, and well-being throughout the journey of completing this work.

I would like to express my sincere gratitude to the **Department of Biology / College of Science / University of Babylon** for their continuous support and for providing the facilities necessary to accomplish this study.

My deepest appreciation and respect go to my supervisors, **Prof. Dr. Frial Gemeel Abd Atiea** and **Prof. Dr. Ibtihal Muiz Al-Hussaini**, for their invaluable guidance, unwavering support, and insightful advice throughout every stage of this research.

I also extend my heartfelt thanks to **Dr. Hayder Muneer Tajeddin**, Consultant in General Surgery, and **Dr. Nora Abdulhussien Abdul Wahid**, General Surgeon, both at Al-Hilla Teaching Hospital, for their significant support and guidance during the course of this study.

Additionally, I am grateful to all the staff members of the **Operating Room and Laboratory Division at Al-Hilla Teaching Hospital** for their kind cooperation and assistance.

I express my sincere appreciation to all the patients and healthy volunteers who agreed to participate in this study, as their contribution was essential to the success of this work.

Finally, I would like to thank everyone who provided help, directly or indirectly, throughout this research journey.

Zahraa 2025

Summary

Appendicitis is one of the most common surgical emergencies, especially in adolescents and young adults. It involves inflammation of the vermiform appendix, often due to obstruction of the lumen, which leads to microbial infection and immune activation. The current study aimed to evaluate the role of bacterial and fungal infections in appendicitis and their relationship with specific immunological markers including Toll-like receptor 4 (TLR4), Interleukin-17 (IL-17), Dectin-1, and Secretory Immunoglobulin A (sIgA) in both blood and appendix tissue.

This case-control study was conducted at Al-Hilla Teaching Hospital from September 2024 to December 2024. The study population comprised 100 patients diagnosed with appendicitis, whose ages ranged from 14 to 65 years, in addition to 50 apparently healthy controls (AHC). Blood and appendix tissue specimens were obtained from the patients, while only blood samples were collected from the control group. The highest proportion of patients was observed within the 14–24 years age group (48%). Females accounted for a slightly higher percentage (54%) compared with males (46%). Furthermore, rural residents represented the majority of the patient group (66%).

Specimens collected from appendectomy patients were cultured to identify bacterial and fungal pathogens. Bacterial isolates were obtained from appendix swabs of 100 patients diagnosed with appendicitis. After surgical removal, specimens were collected using gel transport swabs and cultured on Blood agar, MacConkey agar, UTI Chromogenic agar, and EMB

agar. All specimens showed bacterial growth after 24–48 hours of incubation. *Escherichia coli* was the most frequently isolated organism (88%), followed by *Klebsiella pnemoniae* (12%). All isolates were Gram-negative and confirmed by Gram stain and the VITEK 2 Compact System.

Fungal culture was performed using Sabouraud Dextrose Agar and Candida Chromogenic Agar, incubated at both 25°C and 37°C for 48 to 72 hours. No fungal growth was detected in any of the specimens. These results suggest that fungi are unlikely to be involved in the pathogenesis of appendicitis in the studied cases.

The levels of TLR4, IL-17, Dectin-1, and sIgA were measured in both serum and tissue using Enzyme-Linked Immunosorbent Assay (ELISA). Mean serum TLR4 levels were 18.72 ng/ml in patients and 18.67 ng/ml in controls, with no statistically significant difference (P = 0.083). However, in patients, serum levels were significantly higher than tissue levels (P = 0.000). IL-17 levels were significantly elevated in serum in patients compared to controls (257.22 ± 132.93 ng/L vs. 109.71 ± 62.60 ng/L) and tissue (501.98 ± 183.24 ng/L)compared to serum among patients (P = 0.000 for both).

Dectin-1 levels showed no significant difference between patients and controls; however, within patients, tissue levels of Dectin-1 were slightly higher than serum levels. sIgA was significantly higher in appendix tissue than in serum within the patient group (3.10 ± 1.30 µg/ml vs. 1.60 ± 0.74 µg/ml), while serum levels were lower in patients than in controls but without statistical significance.

Tetra-ARMS PCR was used to analyze TLR4 rs4986790 A/G polymorphism. The G allele was significantly associated with susceptibility to appendicitis (OR = 2.025; P = 0.028). The GG genotype occurred more frequently in males (30.43%) than in females (7.41%), with an odds ratio of 5.47 (P = 0.0491). The G allele was also more frequent in males (45.65%) compared to females (25.93%) with an odds ratio of 2.40 (P = 0.0413).

When analyzing the immune parameters based on genotypes, no significant differences were observed in TLR4, IL-17, or Dectin-1 levels among AA, AG, and GG groups (P > 0.05). However, sIgA levels differed significantly, with the highest levels in the GG group (1.76 µg/ml), followed by AG (1.51 µg/ml) and AA (0.88 µg/ml) (P = 0.007). These results suggest that the TLR4 polymorphism may influence mucosal immune responses by modulating sIgA production.

In conclusion, this study highlights the major role of Gram-negative bacteria, particularly *Escherichia coli*, in the pathogenesis of appendicitis. IL-17 and sIgA were significantly elevated, especially in appendix tissue, indicating their involvement in local immune defense. Although fungi were not detected, Dectin-1 levels may still reflect immune modulation. The TLR4 rs4986790 G allele may be linked to increased disease risk and influence sIgA levels. These findings may support future approaches in understanding immune responses in appendicitis.

List of Contents

List of Tables

List of Figures

List of Abbreviations

Abbreviation	Terms
AA	Acute Appendicitis
AHC	Apparently Healthy Controls
ANOVA	Analysis of Variance
ARMS-PCR	Amplification Refractory Mutation System Polymerase Chain Reaction
Bp / bp	Base Pair
CA	Chronic Appendicitis
CD	Crohn's Disease
CD4+	Cluster of Differentiation 4 Positive (T Helper Cells)
CFU	Colony Forming Unit
CI	Confidence Interval
CLR	C-type Lectin Receptor
CRD	Carbohydrate Recognition Domain
CRP	C-Reactive Protein
CT	Computed Tomography
DC	Dendritic Cell
DNA	Deoxyribonucleic Acid
DW	Distilled Water
ED	Emergency Department
EDTA	Ethylenediaminetetraacetic Acid (anticoagulant)
ELISA	Enzyme-Linked Immunosorbent Assay
EMB	Eosin Methylene Blue
ESR	Erythrocyte Sedimentation Rate
GWAS	Genome-Wide Association Study
HRP	Horseradish Peroxidase
IBD	Inflammatory Bowel Disease

IgA	Immunoglobulin A
IL-17	Interleukin-17
LPS	Lipopolysaccharide
M	Marker (used in gel electrophoresis)
MALT	Mucosa-Associated Lymphoid Tissue
MAPK	Mitogen-Activated Protein Kinase
M Cells	Microfold Cells
NF-κB	Nuclear Factor kappa-light-chain-enhancer of activated B cells
NK	Natural Killer (Cells)
NS	Not Significant
OD	Optical Density
OR	Odds Ratio
PAMPs	Pathogen-Associated Molecular Patterns
PCR	Polymerase Chain Reaction
PRR	Pattern Recognition Receptor
ROS	Reactive Oxygen Species
S	Significant
SC	Secretory Component
SD	Standard Deviation
sIgA	Secretory Immunoglobulin A
SNP	Single Nucleotide Polymorphism
SPSS	Statistical Package for the Social Sciences
T-ARMS-PCR	Tetra-Primer Amplification Refractory Mutation System PCR
Th1	T-helper 1 cells
Th17	T helper 17 Cells
TLR	Toll-Like Receptor
TLR4 / TLR-4	Toll-Like Receptor 4
Tris.HCl	Tris(hydroxymethyl)aminomethane Hydrochloride
TBE	Tris-Borate-EDTA (Buffer)
UC	Ulcerative Colitis
US	Ultrasound
UV	Ultraviolet
UTI	Urinary Tract Infection
W1 buffer	Wash buffer 1
WBC	White Blood Cell
YLDs	Years Lived with Disability

CHAPTER ONE
INTRODUCTION

1-1 Introduction:

Appendicitis, defined as inflammation of the vermiform appendix, is the most common cause of emergency abdominal surgery worldwide (Moris *et al*., 2021).

Numerous studies have sought to examine the etiology of acute appendicitis; however, a consensus on its cause remains elusive. The traditional explanation for the pathophysiology of acute appendicitis has been direct luminal obstruction, which is frequently linked to parasites, lymphoid hyperplasia, fecoliths, and, in rare cases, cecal or appendiceal cancer (Lee *et al*., 2022).

Numerous bacteria colonize the appendix, but the most frequent pathogens are *Escherichia coli* and bacteria from the *Bacteroides fragilis* group. Bacterial colonization and overgrowth in the appendix are encouraged when luminal obstruction takes place. Appendix infection and inflammation may result from the stagnant contents and elevated bacterial load causing an inflammatory response. Cytokines, chemokines, and other pro-inflammatory mediators are released as part of the inflammatory process in acute appendicitis. Immune cells like neutrophils and macrophages are drawn to the site of inflammation by these signaling molecules (Petruzziello *et al*., 2023).

Although appendicitis is typically associated with bacterial infections, recent studies have suggested a possible involvement of fungal pathogens, especially in immunocompromised individuals, with *Candida albicans* occasionally identified in appendiceal specimens in such cases (Constantin *et al*., 2023).

The vermiform appendix is increasingly recognized not as a vestigial structure but as an immunologically active organ that plays a role in maintaining gut homeostasis. It serves as a reservoir for commensal microbiota and contributes to the development of mucosal immunity through the production of secretory immunoglobulin A (sIgA) and the activation of gut-associated lymphoid tissue (GALT) (Vitetta *et al*., 2019).

Toll-like receptor 4 (TLR4) plays a central role in the recognition of Gram-negative bacteria through lipopolysaccharide (LPS) sensing. Upon activation, TLR4 triggers downstream inflammatory pathways, including the Nuclear Factor kappa B (NF-κB) signaling cascade and cytokine production (Zamyatina & Heine, 2020).

Elevated expression of TLR4 has been associated with the severity of inflammation in appendicitis patients (Su *et al*., 2025). A recent study reported significantly elevated TLR4 gene expression in patients with appendicitis compared to healthy controls, suggesting its potential involvement in the pathogenesis of the disease (Mahdi & AlSaimary, 2024a).

IL-17, a pro-inflammatory cytokine generated by Th17 cells, is essential to immune responses. In appendicitis, IL-17 recruits neutrophils and increases the production of other cytokines and chemokines, escalating inflammation. High IL-17 levels in appendicitis indicate its role in its development (Reismann, 2022).

C-type lectin receptor Dectin-1 is expressed on myeloid cells such as macrophages, dendritic cells, and neutrophils. It detects β-glucans in fungal and bacterial cell walls. Phagocytosis, ROS generation, and

cytokine release occur when Dectin-1 is activated. Moreover, Dectin-1 signaling supports adaptive immunity by transforming naïve CD4+ T cells into Th17 cells, which regulate inflammation and immunity (Kalia *et al*., 2021).

Secretory IgA (sIgA) plays a critical role in maintaining intestinal homeostasis by neutralizing pathogens and shaping the gut microbiota. Deficient or dysregulated sIgA responses may compromise mucosal defense and contribute to inflammatory conditions such as appendicitis (Li *et al*., 2020)

1.2. Aims of Study:

The study aimed to investigate the relationships among bacteria, fungi, and some immunological parameters in patients with appendicitis through the following objectives:

1. Collect the blood and appendix tissues after appendectomy.

2. Isolation and identification of bacteria and fungi from tissue by using different methods and confirmed with the VITEK 2 system.

3. Evaluate IL-17, dectin-1, secretory IgA, and TLR4 concentrations by ELISA.

4. Polymorphism of TLR4.

CHAPTER TWO
LITERATURE REVIEW

2. Literature Review:

2.1 Appendicitis

Appendicitis is the most common general surgical emergency in children and young adults and is defined as inflammation of the vermiform appendix. Despite its prevalence, diagnosis remains challenging, even for experienced surgeons, due to its widely varying clinical presentations (Dixon & Singh, 2020). Acute appendicitis is among the leading causes of admission to Emergency Departments (ED), affecting nearly 70% of individuals under 30 years old and about 10% of those over 60 (Petruzziello *et al.*, 2023).

The appendix is a blind-ended organ, and it is widely accepted that appendicitis results from obstruction of the appendiceal lumen. This obstruction leads to a localized infection that cannot drain properly. Consequently, swelling induced by the blockage can cause local ischemia, tissue necrosis, bacterial invasion, and may ultimately result in localized abscess formation or widespread peritonitis, potentially leading to perforation (Dixon & Singh, 2020).

The appendix normally harbors both aerobic and anaerobic bacteria, including *Escherichia coli* and *Bacteroides* species. Obstruction of the appendiceal lumen facilitates bacterial overgrowth, which triggers acute inflammation and abscess development. Studies using next-generation sequencing techniques have demonstrated that patients with complicated, perforated appendicitis carry a significantly higher bacterial phyla load compared to those with uncomplicated appendicitis (Bhangu *et al.*, 2015).

Delayed diagnosis and treatment of acute appendicitis can result in serious complications such as abscess formation, peritonitis, sepsis, ileus, and even mortality (Podany *et al.*, 2017; Hong *et al.*, 2020).

2.2 Epidemiology of appendicitis

The global incidence of appendicitis ranges between 100 and 300 cases per 100,000 individuals annually, with higher rates generally reported in developed countries (Ferris *et al.*, 2017). The condition primarily affects adolescents and young adults, with most cases occurring between the ages of 10 and 30 years (Ohmann *et al.*, 2002; De Costa, 2022).

Geographic and environmental factors significantly influence the epidemiological patterns of appendicitis. Studies have shown marked regional differences in incidence, which may be attributed to variations in hygiene standards, dietary habits, microbial exposure, and healthcare accessibility (De Costa, 2022). Seasonal fluctuations have also been documented, with a noticeable increase in appendicitis cases during summer months, suggesting the involvement of environmental triggers (Danwang *et al.*, 2020).

According to the Global Burden of Disease Study 2019, the age-standardized prevalence, incidence, and years lived with disability (YLDs) due to appendicitis increased steadily between 1990 and 2019. The highest rates were reported among adolescents, with no significant differences observed between males and females (Guan *et al.*, 2023).

National data from Iraq largely mirror these global trends. Several hospital-based studies have reported a predominance of cases among males under the age of 30. For example, demographic analyses conducted in

Basrah and Baghdad identified peak incidence rates among males aged 20–30 years (Shaker *et al.*, 2024; Saleem & Alkawaz, 2021). Similar patterns were reported in Hilla and Sulaymaniyah, further confirming the consistency of age and gender distribution across different Iraqi regions (Al-Janabi *et al.*, 2019; Hama *et al.*, 2021).

In terms of geographic distribution, recent observational studies in Iraq have reported a higher incidence of appendicitis cases among individuals residing in urban areas compared to those from rural or peripheral regions. An investigation in Basrah revealed that 56.8% of patients were from urban districts (Alshawi, 2024), while data from Baghdad demonstrated a similar trend, with a greater proportion of cases originating from city centers (Wahhab *et al.*, 2024).

2.3 Pathophysiology of Acute Appendicitis

Although the exact etiology of acute appendicitis (AA) remains unclear, several contributing factors have been identified, including luminal obstruction, ischemia, bacterial overgrowth, inflammatory and infectious processes, traumatic injury, as well as dietary, genetic, and hygienic influences (Snyder *et al*., 2018; Petruzziello *et al*., 2023). Luminal obstruction typically results from lymphoid hyperplasia or fecalith formation, both of which impede the normal drainage of the appendiceal contents and lead to progressive distension of the lumen (Park *et al*., 2017; Di Saverio *et al*., 2020)

Lymphoid hyperplasia of the mucosa or submucosa is considered the most common mechanism causing obstruction and may be triggered by infections (bacterial, viral, fungal, parasitic) or inflammatory conditions such as inflammatory bowel disease. Less common causes include

parasites (more prevalent in developing countries), fibrous bands, foreign bodies, carcinoid tumors, and cecal carcinoma. Symptom onset tends to be gradual with lymphoid hyperplasia, whereas fecalith-related obstruction is usually associated with a more abrupt clinical course (D'souza & Nugent, 2016).

The pathological cascade of AA begins with inflammation of the appendiceal wall, which may progress to localized ischemia, perforation, and the development of either a confined abscess or diffuse peritonitis (Figure 2-1) (Moris *et al*., 2021).

At the molecular level, the condition is driven by a complex interplay of inflammatory mediators—including proinflammatory cytokines, chemokines, and adhesion molecules—that orchestrate the recruitment and activation of immune cells such as neutrophils and macrophages. These mediators not only underlie the clinical manifestations of appendicitis but also play a critical role in its complications, including perforation and abscess formation (Bhangu *et al*., 2015; Elwan *et al*., 2023).

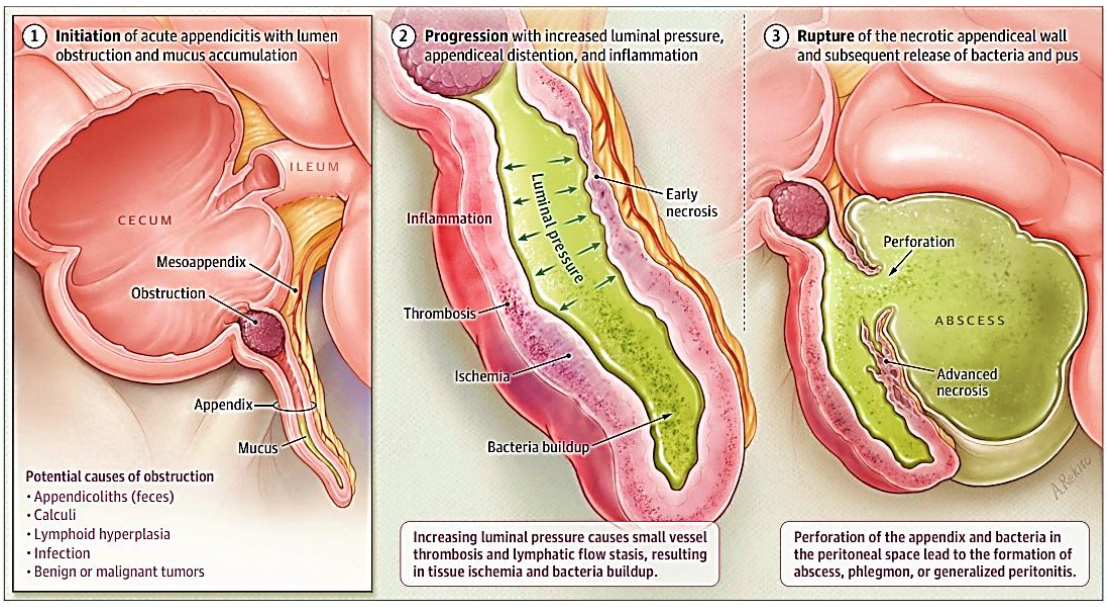


Figure (2-1) Pathophysiology of Acute Appendicitis (Moris *et al*., 2021).

2.4 Diagnosis of appendicitis

Although acute appendicitis (AA) is one of the most common causes of surgical emergency visits, it continues to pose a diagnostic challenge. Traditionally, diagnosis has been based on patient history, clinical evaluation, and laboratory investigations. However, the diagnostic accuracy of clinical assessment alone—without preoperative imaging—remains suboptimal, especially in mixed-gender populations (Sippola *et al.*, 2021).

Laboratory tests can improve diagnostic confidence. One useful marker is the neutrophil count, a component of the white blood cell (WBC) profile, which is often elevated in patients with acute appendicitis compared to those with a normal appendix (Peksöz & Bayar, 2021). C-reactive protein (CRP) is another important inflammatory biomarker that has shown higher specificity and diagnostic value than WBC or erythrocyte sedimentation rate (ESR) (Ramrao *et al*., 2020). A normal CRP level does not rule out appendicitis and should prompt further evaluation through ultrasonography or diagnostic laparoscopy. Combining CRP with clinical history and physical examination significantly improves diagnostic accuracy (Lateef *et al*., 2009; Ramrao *et al*., 2020).

Imaging studies are essential in confirming the diagnosis. The most frequently used preoperative imaging modalities are ultrasound (US) and computed tomography (CT). If imaging confirms appendicitis, surgical intervention is usually warranted. In cases where ultrasound findings are inconclusive, CT is commonly used as a second-line investigation. If CT results are also non-diagnostic, the patient may undergo diagnostic laparoscopy or be observed clinically (D'Souza *et al*., 2021).

In addition to imaging and laboratory tests, clinical scoring systems are used to stratify patients based on their risk of appendicitis. The most

widely utilized is the Alvarado score, which incorporates symptoms, clinical signs, and laboratory findings. While the score demonstrates good sensitivity, especially in male patients, its specificity is relatively low. Therefore, it is often used as a supportive tool rather than a definitive diagnostic method (Ohle *et al.*, 2011; Bhangu *et al*., 2020).

2.5 Classification of appendicitis

Appendicitis can be categorized based on severity as either complicated or uncomplicated. Uncomplicated appendicitis is characterized by acute appendicitis devoid of clinical or radiographic indicators of perforation, such as inflammatory mass, phlegmon, or abscess. An appendiceal rupture followed by the formation of an abscess or phlegmon is a sign of complicated appendicitis (Krzyzak & Mulrooney, 2020).

Appendicitis may also be classified as acute or chronic based on clinical presentation. Acute appendicitis (AA) is the most frequent form and a leading cause of emergency abdominal surgery. Most cases, particularly those involving the development of appendicoliths (fecoliths), require immediate surgical removal of the inflamed appendix (Moris *et al*., 2021).

The rare medical condition known as chronic appendicitis (CA), on the other hand, is characterized by less severe and ongoing abdominal pain as well as a clinical picture that lasts for months or even years rather than just one or two days. However, using it as a preliminary diagnosis is not always feasible. (Kanat *et al*., 2021). Although the exact cause of chronic appendicitis is unknown, it is thought to be caused by temporary or partial

obstruction of the appendix and is not a surgical emergency (Lee *et al*., 2021).

From a histopathological perspective, appendicitis can be categorized as either gangrenous or non-gangrenous based on the presence of necrosis in the appendiceal tissue. Gangrenous appendicitis is characterized by areas of necrosis and severe transmural inflammation, indicating extensive tissue damage and disease progression. In contrast, non-gangrenous appendicitis lacks necrosis and typically presents with localized inflammation confined to specific layers of the appendiceal wall. (Peeters et al., 2020; Peeters et al., 2023).

2.6 Appendicitis risk factors

2.6.1 Age and Sex

Appendicitis is most frequently diagnosed between the ages of 10 and 20 years, with a reported male-to-female ratio of approximately 1.4:1. In the United States, the lifetime risk of developing appendicitis is estimated at 8.6% for men and 6.7% for women (Krzyzak & Mulrooney, 2020). In another study, the incidence of acute appendicitis was reported as 16.33% in men and 16.34% in women, predominantly affecting individuals in their second and third decades of life (Fikri *et al.,* 2023).

2.6.2 Lifestyle Factors

Lifestyle factors, particularly dietary habits, have been associated with the risk of developing appendicitis. Diets low in fiber and high in sugars, along with insufficient intake of fruits and vegetables, have been linked to an increased incidence of acute appendicitis (Peeters *et al.,* 2023). Adequate dietary fiber intake is recognized as important for maintaining gastrointestinal health and reducing the risk of various conditions,

including appendicitis (Siraj *et al*., 2020). In addition, smoking has been reported as a contributing risk factor for appendicitis (Peeters *et al*., 2023).

2.6.3 Genetic Factors

A family history of appendicitis is associated with a higher risk of developing the condition. Individuals with a positive family history are approximately three times more likely to develop appendicitis compared to those without such a history (Gaitanidis *et al*., 2021). Although no single gene has been definitively linked to appendicitis, multiple candidate gene studies and genome-wide association studies (GWAS) have supported a genetic contribution to its pathogenesis (Ricaño-Ponce *et al*., 2022). It has been reported that 6–10% of patients undergoing appendectomy have at least one family member with a history of appendicitis. Furthermore, patients with abdominal pain and a positive family history of appendicitis exhibit a 1.4 to 3-fold increased risk of developing the condition (Tartar *et al*., 2022).

2.6.4 Seasonal Variation

Seasonal variation in the incidence of appendicitis has been observed in several countries, with higher rates typically reported during the summer months. Factors such as dehydration-related constipation and potential interactions between elevated temperatures and genetic predispositions have been suggested as possible explanations for this pattern (Simmering *et al.*, 2022).

2.6.5 Socioeconomic Factors

Socioeconomic status has also been examined in relation to appendicitis incidence. Regions with higher socioeconomic status and educational attainment tend to have lower rates of acute appendicitis, while

the incidence of perforated appendicitis appears to be less influenced by these socioeconomic factors (Golz et al., 2020).

2.7 Gut Microbiota

The gut microbiota is a dynamic and diverse community of microorganisms inhabiting the human gastrointestinal tract. While bacteria constitute the majority of this community in terms of both biomass and diversity, smaller populations of archaea, eukaryotes (such as fungi and parasites) are also present and contribute to the overall ecosystem. The collective genetic and functional attributes of these microbial species are referred to as the gut microbiome (Ursell et al., 2012; Al Bander et al., 2020; Golz et al., 2020)

These microbes maintain a symbiotic relationship with the human host, performing several vital functions such as regulating host metabolism, synthesising essential vitamins, and providing protection against pathogenic organisms (LeBlanc *et al*., 2013; Kamada *et al*., 2013). In exchange, the host offers a nutrient-rich environment that supports microbial survival. The gut microbiota also plays a critical role in digestion by facilitating the fermentation and anaerobic degradation of otherwise indigestible substrates, thereby enhancing caloric extraction and contributing to gut homeostasis (Sekirov *et al*., 2010).

Beyond its well-established roles in metabolism and immunity, emerging evidence suggests that alterations in gut microbiota composition—referred to as dysbiosis—may contribute to the pathogenesis of various gastrointestinal diseases, including acute appendicitis (Lee *et al*., 2022).

2.7.1 The role of the gut microbiome in the pathogenesis of appendicitis

The gut microbiota has been implicated in the development of various diseases, including inflammatory bowel disease, obesity, and mental health disorders. Alterations in both the composition and function of the gut microbiome have also been associated with the onset of appendicitis (Lozupone *et al*., 2012; Zhao *et al*., 2020).

Several theories have been proposed regarding the contribution of the gut microbiota to appendicitis, which is an inflammatory condition of the appendix. However, the precise mechanisms remain unclear (Elwan *et al*., 2023).

One theory suggests that appendicitis may arise from an inflammatory response triggered by an imbalance or dysbiosis in the gut microbiota (Lee *et al*., 2022). Studies have demonstrated that changes in microbial diversity, such as an increase in pathogenic bacteria and a decrease in beneficial species, may elevate the risk of appendicitis (Lee *et al*., 2022; Lobionda *et al*., 2019).

Another hypothesis proposes that the gut microbiota influences appendicitis development through modulation of the immune system. Dysbiosis can provoke abnormal immune responses in the appendix, resulting in inflammation and disease. The gut microbiota plays a critical role in training and regulating the host immune system (Kooij *et al*., 2016). In addition, research suggests that the gut microbiota may contribute to the formation of appendiceal biofilms, which microbial communities are adhering to the appendix lining. These biofilms have been implicated in the pathophysiology of various inflammatory diseases, including appendicitis (Bollinger *et al*., 2007; Elwan *et al*., 2023).

2.7.2 Role of Bacteria in the Pathogenesis of Appendicitis

Bacteria have long been implicated in the pathogenesis of appendicitis, traditionally believed to result from obstruction of the appendix's lumen by feces, stool, or lymphoid hyperplasia, which subsequently leads to inflammation and bacterial overgrowth. While obstruction remains a key factor, recent studies have highlighted the potential role of bacterial infection and dysbiosis in the development of appendicitis. Enteric bacteria are the most prevalent organisms associated with appendicitis, with *Escherichia coli* and *streptococci* identified as the most frequent aerobic isolates (Jung *et al*., 2022).

Among gut bacteria, certain opportunistic pathogens may contribute significantly to disease progression. *Prevotella*, a common gut microbe linked to systemic infections, has been suggested to influence the pathophysiology of acute appendicitis by impairing gut barrier function. Its overrepresentation in the appendix may trigger an inflammatory immune response through the release of pro-inflammatory mediators (Bi *et al*., 2022).

In addition, alterations in the appendiceal microbiome have been observed in patients with acute appendicitis. Oh *et al*. (2020) reported elevated levels of *Campylobacter jejuni* in affected individuals, suggesting a potential pathogenic role for this bacterium. Although the precise mechanisms underlying bacterial involvement in appendicitis remain incompletely understood, several hypotheses have been proposed. An imbalance favoring pathogenic bacteria may provoke appendiceal inflammation (Lee *et al*., 2022).

Notably, patients with appendicitis exhibit reduced levels of beneficial *Bifidobacterium* and *Lactobacillus*, which are known for their

anti-inflammatory properties, and increased Enterobacteriaceae, which produce pro-inflammatory molecules (Lobionda *et al*., 2019). Moreover, certain bacteria within the gut microbiota have been shown to stimulate immune cell and cytokine production, thereby exacerbating inflammation and tissue damage in the appendix (Li *et al*., 2022).

Another contributory factor may be the formation of bacterial biofilms within the appendix. These structured microbial communities can sustain inflammation and are implicated in the pathogenesis of appendicitis (Elwan *et al*., 2023).

2.7.3 Role of Fungi in the Pathogenesis of Appendicitis

While bacterial infections are the primary cause of appendiceal inflammation, fungal infections may also occur, especially in immunocompromised patients. (Carlini *et al*., 2016) Fungi can colonize the appendiceal mucosa or surrounding vessels, leading to inflammation that clinically resembles typical acute appendicitis (Constantin *et al*., 2023).

Among fungal pathogens, *Candida* species are of particular relevance, as they are a normal component of the gastrointestinal flora. However, in states of immunosuppression, *Candida spp.* can become opportunistic pathogens capable of invading the gastrointestinal tract, including the appendix (Khoury *et al*., 2010; Larbcharoensub *et al*., 2013). Such invasion typically results in ulceration, pseudomembrane formation, inflammatory masses, and in severe cases, systemic fungal infections via hematogenous spread (Larbcharoensub *et al*., 2013).

In addition to *Candida,* other fungal pathogens such as mucormycosis, histoplasmosis, and aspergillosis have been implicated in

case reports of fungal appendicitis (Constantin *et al*., 2023). One study estimated the incidence of fungal appendicitis at approximately 1.15% of appendicitis cases, though available data are largely limited to individual case reports and small series, particularly involving immunocompromised individuals (Carlini *et al*., 2016; Jung *et al*., 2022).

It is hypothesized that fungal invasion of the appendiceal wall triggers an inflammatory response that may progress to complications such as perforation and peritonitis if not promptly managed (Choy *et al*., 2019).

2.8 Gastrointestinal mucosal immunity

The mucosal immune system is a tightly regulated mechanism that protects mucosal surfaces from invading pathogens while preventing inappropriate immune responses to harmless antigens such as food or commensal microbes. Antigen entry into the mucosal immune system is facilitated through specialized immune processes that initiate responses at inductive sites located within mucosa-associated lymphoid tissue (MALT) (Velikova *et al.*, 2024).

In humans, the gut-associated lymphoid tissue (GALT), a component of MALT, comprises three primary structures: Peyer's patches, which are rich in lymphoid follicles located in the ileum; the appendix; and numerous isolated lymphoid follicles distributed throughout the intestinal tract. These structures are essential for antigen sampling and the activation of lymphocytes that participate in mucosal immune responses (Mörbe *et al.*, 2021).

The GALT includes both aggregated and non-aggregated lymphoid components. The aggregated structures comprise the appendix, intestinal

Peyer's patches, and organized lymphoid nodules. In contrast, non-aggregated components consist of intraepithelial lymphocytes and lymphoid cells within the lamina propria, all of which contribute to the immune defense of the gastrointestinal mucosa (Velikova *et al*., 2024).

2.9 Immunological Role in Appendicitis

2.9.1 The immunological functions of the Appendix

The vermiform appendix is not a vestigial organ; rather, it plays a significant role in the immune system as a unique component of the gut-associated lymphoid tissue (GALT), distinct from lymphoid tissues found in other intestinal regions. Its primary function involves interaction with and regulation of gut microbiota. The appendix contributes to GALT formation and facilitates recovery following diarrheal illnesses by promoting the recolonization of the colon with commensal bacteria (Kooij *et al*., 2016).

Structurally, the appendix contains abundant lymphoid tissue that supports local protective immune mechanisms. This immune function is mediated by a complex network of immune cells. Antibody production within the appendix has been well documented, with IgA contributing to mucosal immunity and IgG supporting systemic immune responses (Hanson & Lanning, 2008; Vitetta *et al*., 2019).

Anatomically, although the appendix is attached to the cecum and resembles the colon in structure, it differs in cellular composition and immunological function. Notably, the submucosa and lamina propria of the appendix wall contain a higher density of lymphoid follicles compared to the sparse lymphoid elements in the colon's lamina propria (Figure 2-2) (Schumpelick *et al*., 2000; Arjomand Fard *et al*., 2023).

Microfold (M) cells play a critical role in recognizing pathobionts and initiating immune responses. These cells are present in mucosa-associated lymphoid tissues, including Peyer's patches and the lymphoid follicles of the appendix. M cells are capable of sampling antigens from the lumen and delivering them to dendritic cells and macrophages to initiate adaptive immune responses (Corr *et al*., 2008; Vitetta *et al*., 2019).

Extending from the cecum, the appendix is enriched with immune cells and functions as an important site for GALT development, resembling Peyer's patches. It has been proposed that the appendix acts as a reservoir for commensal bacteria. This, combined with its immune cell enrichment, enables the appendix to play a crucial role in modulating intestinal immune responses under both homeostatic and inflammatory conditions (Bollinger *et al*., 2007; The *et al*., 2024).

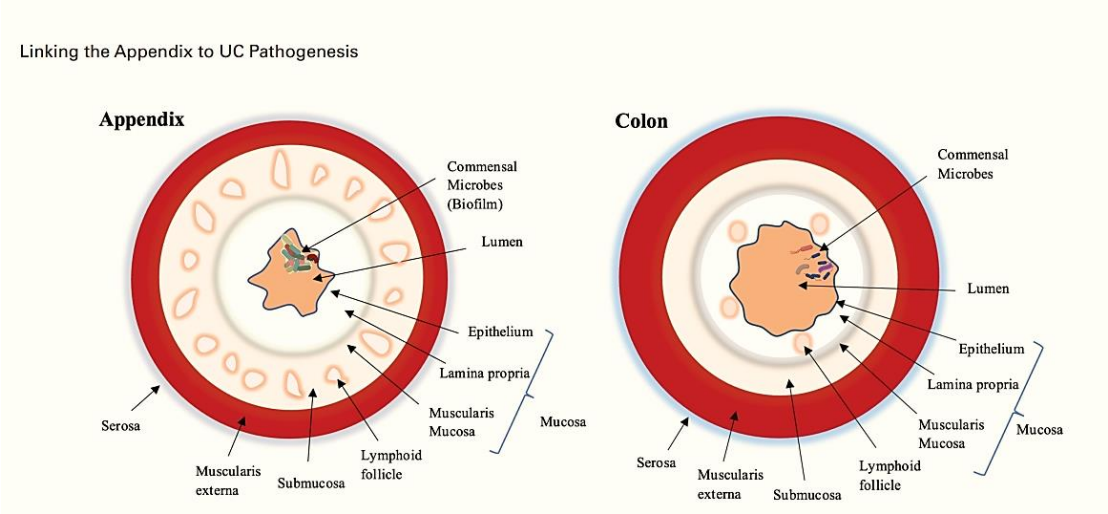


Figure (2-2) A diagram showing the layers of the colon and appendix (Arjomand Fard *et al*., 2023)

2.9.2 Immune Response in Appendicitis

Inflammation during appendicitis involves the activation of various immune cells and their complex interactions, orchestrated by numerous pro-inflammatory and anti-inflammatory cytokines, primarily released by macrophages and T lymphocytes (Stankovic *et al*., 2019; Mahdi & AlSaimary, 2024a).

The appendix is predominantly colonized by *Escherichia coli* and *Bacteroides fragilis*. Luminal obstruction can lead to bacterial overgrowth and the accumulation of stagnant contents, ultimately triggering an inflammatory response. This process is characterized by the release of cytokines, chemokines, and other pro-inflammatory mediators. These signaling molecules recruit immune cells such as neutrophils and macrophages to the site of inflammation (Petruzziello *et al*., 2023; Reismann, 2022).

Moreover, previous studies have demonstrated that the frequencies of CD4+ and CD8+ T cells differ between simple and complex appendicitis, highlighting the involvement of adaptive immune responses during appendiceal inflammation (Gorter *et al*., 2017; The *et al*., 2024).

2.10 Toll like receptors (TLRs)

Toll-like receptors (TLRs) are a family of pattern recognition receptors (PRRs) that play a critical role in the development and maintenance of the immune system. These receptors recognize both endogenous ligands associated with cellular damage and a broad spectrum of pathogens. Activation of TLR signaling pathways leads to the production of pro-inflammatory cytokines and other mediators of the immune response (Aluri *et al*., 2021).

TLRs are expressed on all immune cell types, including innate immune cells such as dendritic cells (DCs), macrophages, and natural killer (NK) cells, as well as adaptive immune cells like T and B lymphocytes. In addition, non-immune cells—particularly epithelial and endothelial cells—also express TLRs (Delneste *et al*., 2007; Kim *et al*., 2023). Among these, DCs and macrophages serve as the primary sources of TLR expression, although expression levels may vary across different cell subsets (Nouri *et al*., 2021).

In humans, ten distinct TLRs have been identified, each exhibiting unique ligand specificity. Based on their cellular localization, TLRs are categorized into two main groups: those located on the cell surface (TLR1, TLR2, TLR4, TLR5, TLR6, and TLR10) and those residing within endosomal compartments (TLR3, TLR7, TLR8, and TLR9). Surface TLRs primarily recognize structural components of bacterial cell walls, whereas endosomal TLRs detect both microbial and endogenous nucleic acids (Wicherska-Pawłowska *et al*., 2021).

2.10.1 Toll like receptor 4

Toll-like receptor 4 (TLR4) is a transmembrane protein composed of three domains: an extracellular domain, a transmembrane domain, and a cytoplasmic domain. The extracellular domain contains leucine-rich repeat (LRR) motifs, each comprising 20–29 residues, which serve as binding sites for ligands. Lipopolysaccharide (LPS), derived from Gram-negative bacteria, is the primary ligand for TLR4 (Kim *et al*., 2007; Kim *et al*., 2023).

Upon interaction with LPS, TLR4 initiates intracellular signaling cascades, including the nuclear factor kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) pathways. Activation of these pathways

leads to the production of pro-inflammatory cytokines—such as TNF-α, IL-1β, IL-6, and IL-12—and type I interferon, which are crucial for amplifying the inflammatory response and promoting pathogen clearance (Figure 2-3) (Lu *et al.*, 2008; Swanson *et al.*, 2020).

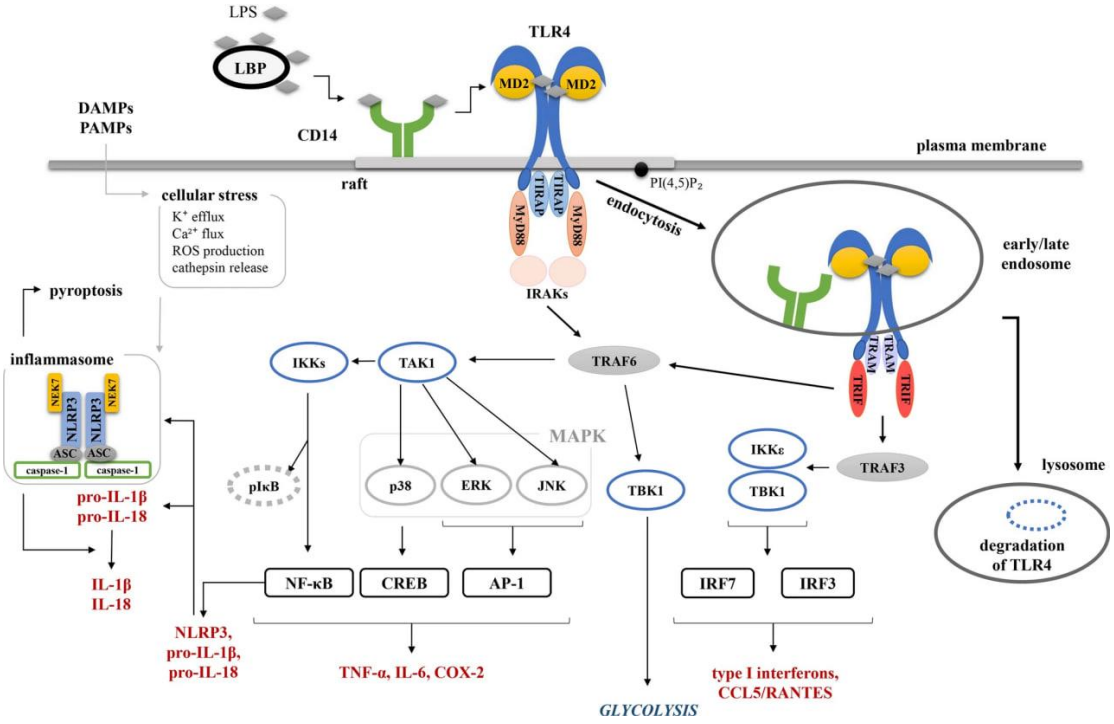


Figure (2-3) Pro-inflammatory signaling pathways of TLR4 (Ciesielska *et al.*, 2021).

2.10.2 Toll like receptor 4 and appendicitis

Stimulation of Toll-like receptors (TLRs) on myeloid cells of the innate immune system initiates signaling pathways that instruct lymphocytes to generate robust immune responses aimed at eradicating infections and maintaining homeostasis. Conversely, inappropriate TLR activation can contribute to the development of cancer, autoimmune diseases, and both acute and chronic inflammatory conditions (Wang *et al.*, 2024).

LPS-induced TLR4-mediated signaling, in conjunction with caspase-4/11 activation, promotes inflammasome assembly and the development of inflammation. This mechanism enables the host immune system to mount an effective defense against infectious challenges (Zamyatina & Heine, 2020). Recent studies suggest that bacterial lipopolysaccharide (LPS) may contribute to the pathogenesis of appendicitis by inducing a systemic anti-inflammatory response (Péterfi *et al*., 2006; Mahdi *et al*., 2024b).

Furthermore, prior research has shown that plasma levels of Toll-like receptors TLR1 and TLR4 are significantly elevated in patients with appendicitis compared to healthy controls, indicating that these receptors may play a key role in driving the inflammatory response and the progression of the disease (Mahdi *et al*., 2024).

2.11 Interleukin 17 (IL-17)

The IL-17 cytokine family comprises six proteins (IL-17A to IL-17F) and five receptors (IL-17RA to IL-17RE), which are structurally distinct from all other known cytokine receptors (De Morales *et al*., 2020). Interleukin 17 (IL-17) is a pivotal cytokine involved in host defense against mucosal infections and serves as a key pathological mediator and therapeutic target in various autoimmune, inflammatory, and malignant diseases (Huangfu *et al*., 2023).

Members of the IL-17 cytokine family perform diverse biological functions, including driving inflammatory pathology in the context of infection and autoimmunity, while also promoting protective immunity against numerous pathogens. In response to IL-1β and IL-23, IL-17A and IL-17F are primarily produced by CD4+ and CD8+ T cells, γδ T cells, and other innate immune cell populations. These cytokines contribute to host

defense by enhancing epithelial barrier function, promoting neutrophil recruitment, and stimulating the production of antimicrobial peptides.

IL-17-mediated inflammation is typically regulated by regulatory T cells and anti-inflammatory cytokines such as IL-10, TGF-β, and IL-35. However, when dysregulated, as observed in chronic infections or autoimmune diseases, IL-17 responses can exacerbate immunopathology (Mills, 2023).

2.11.1 Interleukin 17 and appendicitis

Studies in the literature demonstrate a significant increase in inflammatory markers associated with Th1 and Th17 cell responses in appendicitis. Notably, IL-17 plays a critical role in recruiting neutrophils to the site of infection and stimulating the production of cytokines and chemokines. The involvement of IL-17 in initiating immune responses during appendiceal inflammation has been extensively documented (Reismann, 2022).

Escherichia coli, the most commonly isolated bacterium in acute complicated appendicitis, has been shown to induce IL-17 expression during infection. Moreover, gangrenous appendicitis has been linked to the Th17-associated IL-23/IL-17 signaling pathway, characterized by elevated levels of IL-27, IL-17A, and IL-23A (Reismann, 2022).

Furthermore, studies report that children with complex appendicitis produce higher levels of cytokines, particularly IL-17A from CD4+ T cells, compared to those with simple appendicitis (The *et al*., 2024).

2.12 Dectin-1

Innate immune cells are equipped with various pattern recognition receptors (PRRs) to initiate innate immune responses. Upon recognition of

antigens, PRRs activate these cells, triggering a range of reactions such as phagocytosis and secretion of inflammatory mediators. Among the diverse PRRs, C-type lectin receptors (CLRs) are essential for pathogen recognition. Dectin-1, a member of the CLR family, is a type II transmembrane lectin highly expressed in cells of the myeloid lineage, including neutrophils, dendritic cells (DCs), and macrophages (Mata-Martínez *et al*., 2022; Zahra'a *et al*., 2023).

Dectin-1 recognizes various ligands of both endogenous and microbial origin. This recognition initiates multiple responses, including pro-inflammatory processes such as phagocytosis, reactive oxygen species (ROS) production, and cytokine release. Additionally, ROS generation is influenced by mitochondrial metabolism upon Dectin-1 ligand binding. These Dectin-1-mediated signaling pathways present promising targets for therapeutic intervention. Importantly, Dectin-1 facilitates immune responses against a broad spectrum of pathogens, including bacteria, parasites, and fungi (Mata-Martínez *et al*., 2022).

Structurally, Dectin-1 contains a single carbohydrate recognition domain (CRD) that specifically binds β-(1→3)/(1→6)-glucans, polysaccharides present in the cell walls of certain bacteria, fungi, and other pathogens (Brown, 2006; Kalia *et al*., 2021).

Activation of Dectin-1 triggers numerous downstream cellular events, including the production of cytokines such as TNF-α, IL-1β, IL-2, IL-8, IL-10, IL-12, and CXCL2, induction of phagocytosis, and generation of ROS. Beyond these innate immune functions, Dectin-1 signaling also modulates adaptive immunity by promoting CD8+ T cell activation and the differentiation of naïve CD4+ T cells into T helper (Th)1 or Th17 subsets. Thus, as depicted in Figure (2-4), Dectin-1 signaling orchestrates a

coordinated immune response involving both innate and adaptive immunity (Mata-Martínez *et al*., 2022).

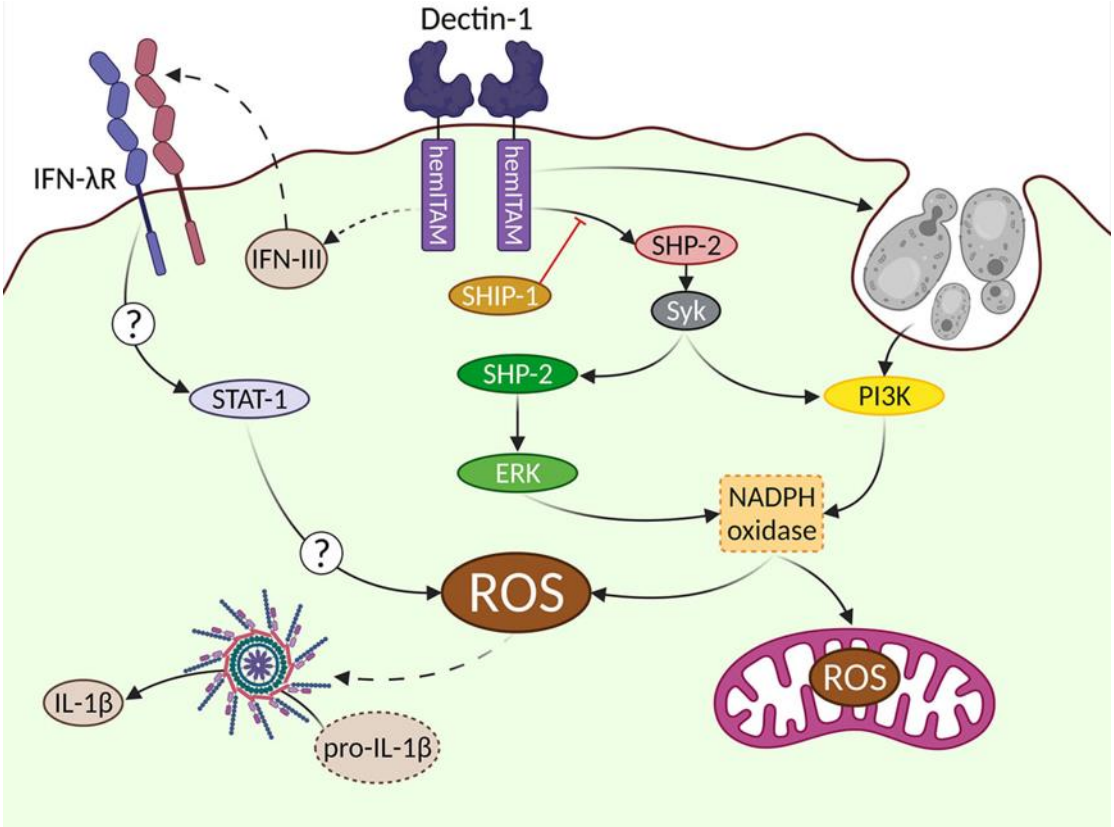


Figure (2-4) Main signaling pathways downstream Dectin-1(Mata-Martínez *et al*., 2022).

2.13 Secretory immunoglobulin A

Secretory immunoglobulin A (SIgA) is the predominant antibody found in mucosal secretions, especially within the gastrointestinal tract, where it plays a vital role in maintaining mucosal homeostasis and providing defense against pathogens (Li *et al*., 2020; Riedel *et al*., 2019).

Structurally, mucosal SIgA primarily exists as a polymer composed of two IgA monomers linked by a joining (J) chain and associated with a secretory component (SC). This secretory component facilitates SIgA

transport across mucosal surfaces and enhances its stability in the harsh mucosal environment (Woof & Russell, 2011). The glycan-rich structure of SIgA enables broad interactions with microbial antigens, mediating immune exclusion by preventing pathogen adhesion and invasion, neutralizing toxins, and entrapping antigens within the mucus layer (Mantis *et al*., 2011; Pietrzak *et al*., 2020).

Beyond serving as a protective barrier, SIgA plays a regulatory role in shaping the gut microbiota composition and modulating immune responses to maintain tolerance toward commensal microorganisms and dietary antigens, thereby preventing chronic inflammation (Sutherland & Fagarasan, 2012; Li *et al*., 2020).

Furthermore, SIgA promotes antigen sampling by facilitating the delivery of luminal antigens to dendritic cells within the gut-associated lymphoid tissue (GALT), contributing to mucosal immune education and tolerance (Mantis *et al*., 2011).

2.13.1 Secretory immunoglobulin A and appendicitis

The human appendix is considered a peripheral lymphoid organ due to its rich concentration of lymphoid follicles, which support mucosal immunity by producing immunoglobulin A (IgA). Studies have shown that individuals who undergo appendectomy have significantly lower IgA levels in both serum and colonic secretions. These findings suggest that the appendix may play an important role in regulating both systemic and mucosal immune responses (Andreu-Ballester et al., 2007; Liu et al., 2017).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Materials

3.1.1 Instruments and apparatuses

The equipment and apparatus utilized in this study are detailed in table 3-1.

Table (3-1) Equipment and Instruments.

NO.	Equipment and Instruments	Company	Country Origin
1	Autoclave	Tripod	UK
2	Burner	Amal	Turkey
3	Centrifuge	Memmert	Germany
4	Digital Camera	Samsung	China
5	Disposable (Gel tube, Surgical blade, Syringe)	Citro	China
6	Electrophoresis	Clarivate	UK
7	ELISA System	Biotech	USA
8	Eppendorf Tubes	Eppendorf	Germany
9	Exispin Vortex Centrifuge	Bioneer	Korea
10	Filter Paper	Hettich	UK
11	Gel Electrophoresis	Bioneer	Korea
12	Glassware	Hettich	UK
13	High Speed Cold Centrifuge	Eppendorf	Germany
14	Hood	Bio LAB	Korea
15	Incubator	Memmert	Germany
16	Light Microscope	Olympus	Japan
17	Microwave	Argose	Germany
18	Microcentrifuge Tubes	Biobasic	Canada
19	Micropipettes (5-50, 0.5-10, 100-1000 µl)	CYAN	Belgium
20	Oven	Olympus	Japan
21	Pasteur Pipettes	Afco	Jordan

22	Petri Dish	Sterilin	England
23	Plain Tubes	Citro	China
24	Power Supply	Biorad	USA
25	Refrigerator	Concord	Lebanon
26	Sensitive Balance	Sartorius	Germany
27	Slide	Sail Brand	China
28	Thermocycler PCR	BioRad	USA
29	Thermostatic Incubator	Zxinstrument	China
30	Tips (Different Sizes)	Jippo	Japan
31	UV Transilluminator	ATTA	Korea
32	Vitek 2 System	Biomerieux	France
33	Vortex	CYAN	Belgium
34	Water Bath	Memmert	Germany
35	Water Distillatory	GFL	Germany

3.1.2 Chemical materials

The chemical ingredients, reagents, stains, and solutions utilized in this work are presented in Table 3-2.

Table (3-2) Chemical materials

NO.	Chemical	Company and Origin
1	Absolute Ethanol	Scharlau (Spain)
2	Agarose	BioBasic (Canada)
3	DNA Marker Ladder 100bp	INtRON (Korea)
4	Ethidium Bromide 10mg/ml	BioBasic (Canada)
5	Glycerol (C3H8O3)	Merck (England)
6	Gram Stain Set	BDH (England)
7	Nuclease Free Water	Bioneer (Korea)
8	TBE Buffer 10X	BioBasic (Canada)

3.1.3 Culture media

Culture media were used in the present study were illustrated in Table (3-3).

Table (3-3) Culture media

NO.	Type of Media	Manufacturing Company	Origin
1	Blood Agar Base	Himedia	India
2	Brain Heart Infusion Broth	Himedia	India
3	Candida Chromogenic Agar	Condalab	Spain
4	Eosin Methylene Blue Agar	Himedia	India
5	MacConkey Agar	Condalab	Spain
6	Nutrient Agar	Himedia	India
7	Sabouraud Dextrose Agar	Condalab	Spain
8	Urinary Tract Infections Chromogenic Agar (UTIC)	Condalab	Spain

3.1.4: Commercial kits

The commercial kits utilized in the current study are presented in Table 3-4.

Table (3-4) Commercial kits

No.	Kit / Chemical	Company	Country
1	gSYAN DNA Extraction Kit	Geneaid	Taiwan
2	GoTaq® G2 Green Master Mix Kit	Promega	Korea
3	Gram Stain	BDH	UK
4	Human Dectin-1 Kit	BT LAB	China
5	Human Interleukin 17 (IL-17) Kit	BT LAB	China
6	Human Secretory Immunoglobulin A Kit	BT LAB	China
7	Human Toll-like Receptor 4 (TLR4) Kit	BT LAB	China
8	Vitek 2 System Kit	Biomerieux	France

3.1.5 Molecular kits and reagents for genetic detection

3.1.5.1 Genetic Detection Kits and Reagents kits

Kits and reagents are used in this study in Table (3-5).

Table (3-5) The kits used in this study with their companies and countries of origin.

No.	Kit	Company	Country
1	**gSYAN DNA Extraction Kit**	**Geneaid**	**Taiwan**
	GST buffer		
	GSB buffer		
	W1 buffer		
	Wash buffer		

	Elution buffer		
	GD column		
	Collection tube 2ml		
	Proteinase K 10mg/ml		
2	**GoTaq® G2 Green Master Mix kit**	**Promega**	**Korea**
	Taq DNA polymerase		
	dNTPs (dATP, dCTP, dGTP, dTTP)		
	Tris.HCl pH 9.0		
	KCl		
	$MgCl_2$		
	Loading dye		

3.1.5.2 DNA Primers

The gene polymorphism Tetra-ARMS-PCR Primers were designed in study using NCBI-SNP data base and ARMS-PCR Primers design server (https://snp.biotech.edu.lk/arms.php). These primers were provided from (ScientificReseracher. Co. Ltd. Iraq), as shown in the following table.

Table (3-6) The Tetra-ARMS-PCR Primers for TLR4-rs4986790 A/G gene polymorphisms with their sequence and amplicon size

T-ARMS-PCR Primer	Sequence (5'-3')	Product size
Forward inner primer (A allele):	GCATACTTAGACTACTACCTCGAGGA	163
Reverse inner primer (G allele)	TGTCAAACAATTAAATAAGTCAATAAGAC	209
Forward outer primer	GTTTAAATGTAATGAAAACTTGTATTCAA	317
Reverse outer primer	CTGTCCAAATTTACAGTTAACTAATTCT	

3.1.6 Enzyme –linked immunosorbent assay (ELISA) kits

3.1.6.1 Human Toll-like Receptor 4, TLR4 ELISA Kit

The levels of TLR4 in serum and tissue were measured using a human Enzyme-Linked Immunosorbent Assay (ELISA) kit. The components of the kit and specimens were equilibrated at a temperature of 2-8°C prior to use, and their items are detailed in Table 3-7.

Table (3-7) Contents of human TLR4 ELISA kit

Components	Quantity (96T)	Quantity (48T)
Biotinylated Human TLR4 antibody	1 ml × 1	1 ml × 1
Plate sealer	2 pcs	2 pcs

Pre-coated ELISA plate	12 × 8-well strips × 1	12 × 4-well strips × 1
Standard diluent	3 ml × 1	3 ml × 1
Standard solution (16 ng/ml)	0.5 ml × 1	0.5 ml × 1
Stop solution	6 ml × 1	3 ml × 1
Streptavidin-HRP	6 ml × 1	3 ml × 1
Substrate solution A	6 ml × 1	3 ml × 1
Substrate solution B	6 ml × 1	3 ml × 1
User instruction	1	1
Wash buffer concentrate (25×)	20 ml × 1	20 ml × 1

3.1.6.2 Human interleukin 17, IL-17 ELISA Kit.

Serum and tissue levels of IL-17 were quantified using a human Enzyme-Linked Immunosorbent Assay (ELISA) kit. The kit components and specimens were equilibrated at a temperature of 2-8°C before usage, with their details provided in Table 3.8.

Table (3-8) Contents of human IL-17 ELISA kit

Components	**Quantity (96T)**	**Quantity (48T)**
Biotinylated Human IL-17A antibody	1 ml × 1	1 ml × 1
Plate sealer	2 pcs	2 pcs
Pre-coated ELISA plate	12 × 8-well strips × 1	12 × 4-well strips × 1
Standard diluent	3 ml × 1	3 ml × 1
Standard solution (640 ng/L)	0.5 ml × 1	0.5 ml × 1
Stop solution	6 ml × 1	3 ml × 1
Streptavidin-HRP	6 ml × 1	3 ml × 1
Substrate solution A	6 ml × 1	3 ml × 1

Substrate solution B	6 ml × 1	3 ml × 1
User instruction	1	1
Wash buffer concentrate (25×)	20 ml × 1	20 ml × 1

3.1.6.3 Human Dectin-1 ELISA Kit

The concentrations of dectin-1 in serum and tissue were quantified utilizing a human Enzyme-Linked Immunosorbent Assay (ELISA) kit. The kit components and specimens were equilibrated at a temperature of 2-8°C before usage, with their details provided in Table 3-9.

Table (3-9) Contents of human Dectin-1 ELISA kit

Components	**Quantity (96T)**	**Quantity (48T)**
Biotinylated Human Dectin-1 Antibody	1 ml × 1	1 ml × 1
Plate Sealer	2 pcs	2 pcs
Pre-coated ELISA Plate	12 × 8-well strips × 1	12 × 4-well strips × 1
Standard Diluent	3 ml × 1	3 ml × 1
Standard Solution (4800 ng/L)	0.5 ml × 1	0.5 ml × 1
Stop Solution	6 ml × 1	3 ml × 1
Streptavidin-HRP	6 ml × 1	3 ml × 1
Substrate Solution A	6 ml × 1	3 ml × 1
Substrate Solution B	6 ml × 1	3 ml × 1
User Instruction	1	1
Wash Buffer Concentrate (25×)	20 ml × 1	20 ml × 1
Zipper bag	1 pc	1 pc

3.1.6.4 Human secretory immunoglobulin A, sIgA ELISA Kit.

Serum and tissue sIgA levels were quantified utilizing a human Enzyme-Linked Immunosorbent Assay (ELISA) kit. The kit components and specimens were equilibrated at a temperature of 2-8°C before use, with details provided in Table 3-10.

Table (3-10) Contents of human sIgA ELISA kit

Components	Quantity (96T)	Quantity (48T)
Biotinylated Human sIgA Antibody	1 ml × 1	1 ml × 1
Plate Sealer	2 pcs	2 pcs
Pre-coated ELISA Plate	12 × 8-well strips × 1	12 × 4-well strips × 1
Standard Diluent	3 ml × 1	3 ml × 1
Standard Solution (8 µg/ml)	0.5 ml × 1	0.5 ml × 1
Stop Solution	6 ml × 1	3 ml × 1
Streptavidin-HRP	6 ml × 1	3 ml × 1
Substrate Solution A	6 ml × 1	3 ml × 1
Substrate Solution B	6 ml × 1	3 ml × 1
User Instruction	1	1
Wash Buffer Concentrate (25×)	20 ml × 1	20 ml × 1

Zipper bag	1 pc	1 pc

3.1.7: Culture Media Used for Microbial Isolation

Various culture media were utilized for the isolation and identification of bacterial and fungal pathogens from appendiceal tissue samples. Each medium was selected based on its specificity for certain microbial groups and its diagnostic properties.

3.1.7.1 Blood agar medium

Blood agar is an enriched medium commonly used for the cultivation of fastidious organisms. It also facilitates the differentiation of pathogenic bacteria based on hemolytic activity (MacFaddin, 2000).

3.1.7.2 MacConkey agar medium

A selective and differential medium designed to support the growth of Gram-negative bacteria while differentiating lactose fermenters from non-fermenters (MacFaddin, 2000).

3.1.7.3 Nutrient Agar Media

Used to activate the isolates and as short-term storage (MacFaddin, 2000).

3.1.7.4 Brain heart infusion broth

It serves as long-term storage with the incorporation of 15% glycerol **(MacFaddin, 2000).**

3.1.7.5 UTI chromogenic agar medium

Chromogenic differential media for the identification, differentiation, and confirmation of enteric bacteria from materials such as urine (Abdullah *et al*., 2009).

3.1.7.6 Eosin methylene blue agar

EMB agar was used to differentiate *Escherichia coli* from other members of the Enterobacteriaceae family based on characteristic colony morphology and color (Forbes *et al*., 2007).

3.1.7.7 Candida Chromogenic Agar

This medium enhances the isolation and presumptive identification of *Candida spp.* based on colony coloration (Ghelardi *et al*., 2008).

3.1.7.8 Sabouraud Dextrose Agar

Its low pH renders it more conducive to the separation of fungus rather than bacteria, while simultaneously facilitating identification by promoting the formation of distinctive spores and pigments in fungi (O'Day *et al*. 1979; Das *et al*. 2010).

3.2 Methods

3.2.1 Design of study

The specimens were processed according to the study design illustrated in Figure 3-1.

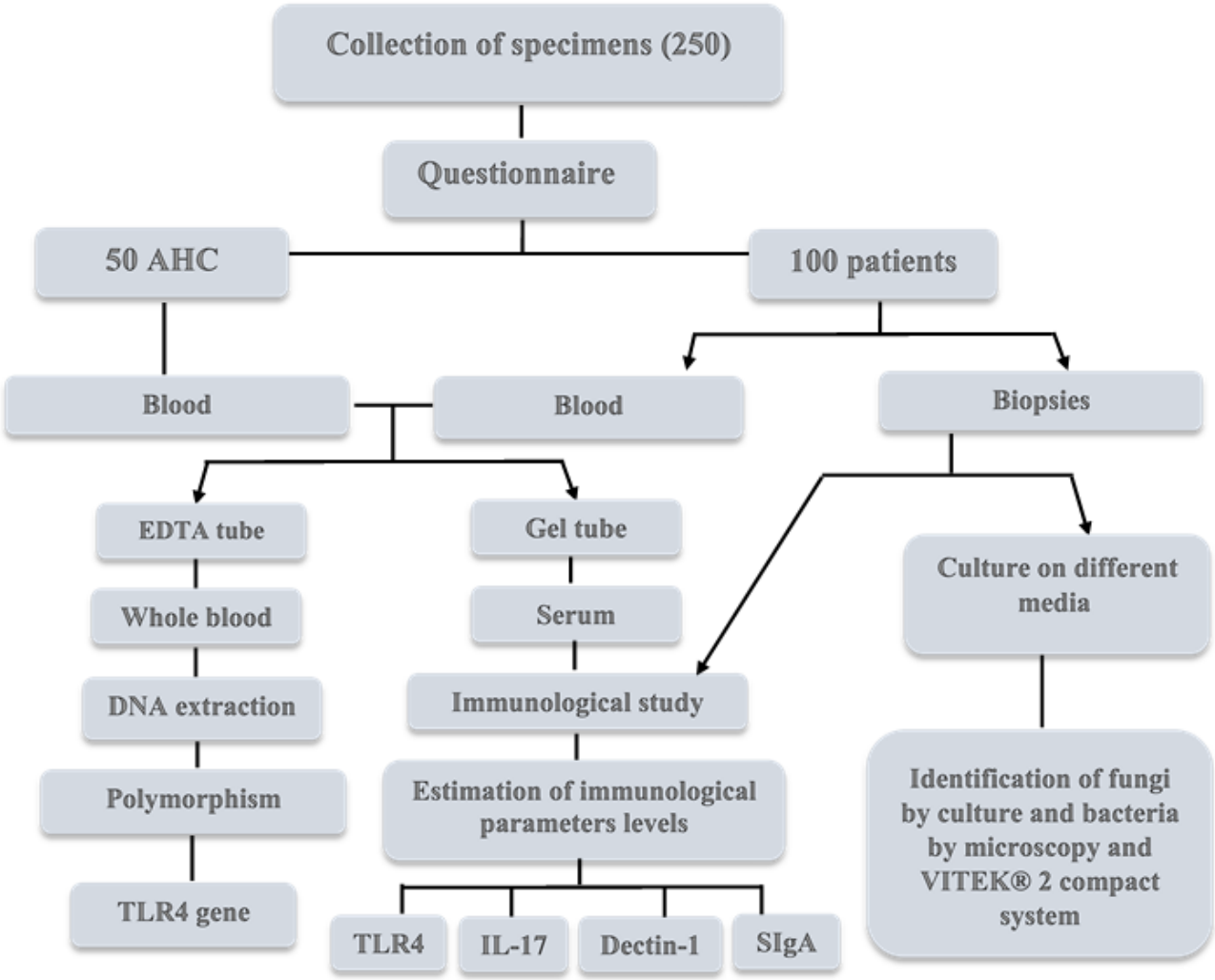


Figure (3-1) Study Design

3.2.2 Study of population

A total of 250 specimens were divided into**:**

Patients: 100 blood specimens and 100 appendix tissue specimens were obtained from individuals aged 14 to 65 years, both males and females, who underwent appendectomy at Al-Hilla Teaching Hospital, Babylon Province, Iraq, between September and December 2024.

Control: Blood specimens from 50 apparently healthy individuals of similar age groups were collected and used as the control group for this study.

3.2.3 Specimens collection

3.2.3.1 Blood collection

Five milliliters of venous blood were collected from a total of 150 individuals, including 100 patients diagnosed with appendicitis and 50 healthy controls. One milliliter of blood from each individual was transferred into EDTA tubes for genetic analysis, while the remaining four milliliters were placed into disposable tubes containing separating gel. The EDTA tubes were stored at −20ºC until further analysis. Blood in the gel tubes was allowed to clot at room temperature for 30 minutes, then centrifuged at 2000 × g for approximately 15 minutes. The serum was collected and stored at −20ºC until use (Mei, 2014).

3.2.3.2 Tissue collection

A total of 100 appendix tissue specimens were collected following appendectomy surgery with the assistance of surgical doctors. Secretory materials were extracted from the appendix based on the method described by Shnawa and Abd (2005), with some modifications. The appendix was placed in a petri dish containing normal saline, then opened lengthwise using scissors and washed with saline. The mucous layer was gently scraped and suspended in 5 ml of normal saline, followed by centrifugation at 3500 rpm for 30 minutes. The resulting supernatant was used to estimate immunological parameters.

Additionally, a specimen was directly obtained from the inner mucosal surface of the opened appendix using a gel transport swab. This swab was cultured on appropriate media for bacterial and fungal isolation.

3.2.4 Sterilization Methods

Most culture medium and heat-resistant solutions were sterilized using an autoclave at 121°C and a pressure of 15 lbs/in² for 15 minutes. Glassware was sterilized in an electric oven at 180°C for 2 hours.

3.2.5 Preparation of Culture Media

All culture media were prepared according to the company specifications and utilized as per (MacFaddin 2000; Forbes *et al*.2007).

3.2.5.1 Blood Agar Base Media

Blood agar base medium was prepared according to the manufacturer's instructions and sterilized using an autoclave. After sterilization, the medium was cooled to 45°C, then supplemented with 5% blood solution. The mixture was gently stirred, poured into sterile petri dishes, and allowed to solidify.

3.2.5.2 Brain Heart Infusion Broth Media

Brain Heart Infusion broth was prepared following the manufacturer's guidelines (Himedia, India) by dissolving 37 g of powder in 1 liter of distilled or deionized water. The solution was thoroughly mixed, heated to boiling with frequent stirring until completely dissolved, and then dispensed into final containers.

3.2.5.3 Nutrient Agar Media

Nutrient agar was prepared according to the manufacturer's instructions (Himedia, India). Specifically, 28.0 grams of the medium were suspended in 1000 ml of purified or distilled water. The mixture was heated to boiling with continuous stirring until fully dissolved, then sterilized by autoclaving at 121°C under 15 lbs/in² pressure for 15 minutes.

3.2.5.4 Macconkey Agar Media

MacConkey agar powder (51.5 g) was suspended in 1 liter of distilled or deionized water, mixed thoroughly, and heated to boiling for 1 minute with regular stirring until fully dissolved (MacFaddin, 2000).

3.2.5.5 Eosin-Methylene Blue Agar Media

This medium was prepared based on the manufacturer company by dissolving 40 gm of its powder into 1000 ml DW. After complete mixing and dissolving, it was autoclaved at 121 ºC for 15 min and pressure 15 pounds per square (MacFaddin, 2000).

3.2.5.6 UTI Chromogenic Agar Media

It was prepared according to the manufacturing company (Condalab, Spain). Suspend 47.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121 ºC for 15 minutes. Cool to 45-50 ºC, mix well and dispense into plates.

3.2.5.7 Candida Chromogenic Agar

This medium was prepared according to the manufacturer's instructions (Condalab, Spain). Specifically, 36.9 grams of the powder were suspended in 1 liter of distilled water. The mixture was heated with

constant stirring until fully dissolved, boiled for 1 minute, then poured into sterile petri dishes and allowed to solidify.

3.2.5.8 Sabouraud Dextrose Agar

A total of 65 g of the medium was suspended in 1 liter of distilled water. The mixture was thoroughly mixed and dissolved by heating with frequent agitation, then boiled for one minute until completely dissolved. The medium was subsequently distributed into appropriate containers and sterilized in an autoclave at 121 ºC for 15 minutes.

3.2.6 Isolation and Identification of the Bacterial Isolates

3.2.6.1 Culture Identification

The swab collected from the mucosal surface of the appendix tissue was cultured using appropriate media (Blood Agar, MacConkey Agar, Eosin-Methylene Blue Agar, UTI Chromogenic Agar, Sabouraud Dextrose Agar, and Candida Chromogenic Agar) for the identification and differentiation of bacterial and fungal species based on their characteristics in these media.

3.2.6.2 Microscopic identification

A single colony was selected from the positive culture, and identification was based on morphological characteristics such as colony size, shape, color, pigment, translucency, edge, elevation, and texture. A small portion of the colony was smeared on slides and stained using Gram stain to determine Gram-positive or Gram-negative status and to observe bacterial morphology. Subsequently, bacterial isolates were definitively identified using the VITEK 2 system (MacFaddin, 2000).

3.2.6.3 Vitek 2 System

The VITEK 2 System (bioMérieux, France) was used to validate the biochemical identification of bacterial isolates, following the manufacturer's protocol. This automated system is designed to enhance the accuracy and efficiency of microbial identification through a fully integrated process that includes inoculum preparation, card loading, incubation, and data interpretation.

A loopful of a single colony (24 hours old) was suspended in 3 ml of normal saline in a sterile test tube. The suspension was then standardized to a McFarland 0.5 turbidity (equivalent to 1.5×10^8 cells/ml) using a densitometer. The standardized suspension was loaded into the identification card, which was then associated with a sample ID via barcode entry into the system software. The card was inserted into the filler module, and then transferred to the reader-incubator module. The instrument automatically controlled all subsequent steps, including incubation temperature, optical readings, and real-time data transfer for analysis. This process reduced the need for manual testing and improved both diagnostic precision and operator safety.

1. Standardization

Following primary isolation, the inoculum preparation, standardization, and dilution processes were streamlined. The standardized inoculum was introduced into the cassette, and the specimen identification number was entered into the computer software using a barcode.

2. Traceability

The barcode on the VITEK 2 card was scanned during manufacturing to link the card with the corresponding specimen ID. These manufacturer-provided barcodes connect the card to the patient's information through a straightforward scanning process.

3. Load and Go

The cassette was inserted into the filler module. Once the cards were filled, the cassette was transferred to the reader/incubator module. All subsequent procedures were managed automatically by the device.

3.2.7 Preservation of Bacterial Isolates

1- Short-term Preservation

Tubes with slant nutritional agar were inoculated via streaking, incubated at 37 °C for 24 hours, and thereafter stored at 4 °C until required. Isolates were cultivated monthly on nutrient agar and subsequently on slant nutrient agar (MacFaddin, 2000).

2- Long-term Preservation

A medium for the long-term preservation of isolates was created by combining 15 mL of glycerol with 85 mL of brain-heart infusion broth, which was subsequently distributed into many sterile, heat-resistant tubes for sterilization by incubation. Subsequent to cooling to ambient temperature, the tubes were injected with colonies cultivated on nutrient agar and incubated at 37 °C for 24 hours, followed by storage at -20 °C, with the understanding that the isolates may last for about 6 to 8 months. (McFadden, 2000).

3.2.8 Immunity Study by ELISA Test

3.2.8.1 Estimation of human TLR4 Concentration

1-Assay Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human TLR4 antibody. TLR4 present in the specimen is added and binds to antibodies coated on the wells. And then biotinylated Human TLR4 Antibody is added and binds to TLR4 in the specimen. Then Streptavidin HRP is added and binds to the Biotinylated TLR4 antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human TLR4. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

2-Reagent Preparation

- All reagents were brought to room temperature before use.
- **Standard** The 120 µL of the standard (16 ng/mL) was reconstituted with 120 µL of standard diluent to generate an 8 ng/mL standard stock solution. The standard was allowed to sit for 15 minutes with gentle agitation before making dilutions. Duplicate standard points were prepared by serially diluting the standard stock solution (8 ng/mL) 1:2 with standard diluent, producing 4 ng/mL, 2 ng/mL, 1 ng/mL, and 0.5 ng/mL solutions.
- **Wash Buffer** Wash Buffer Concentrate 25x was Dilute 20 ml of it into deionized or distilled water to yield 500 ml of 1x Wash Buffer, then mixed gently until the crystals have completely dissolved.

3-Assay Procedure

1. All reagents, standard solutions, and specimens were prepared according to instructions. Equilibrated all reagents to room temperature prior to use. The experiment was conducted at room temperature.
2. Ascertain the quantity of strips necessary for the test. Inserted the strips into the frames for utilization. Preserved the unused strips at 2–8°C.
3. A volume of 50 µl of standard solution was added to the standard well without adding biotinylated antibody, as the standard solution contained biotinylated antibody.

4. A total of 40 µL of specimen was added to the specimen wells, followed by 10 µL of Human TLR4 antibody. Then, 50 µL of streptavidin-HRP was added to both the specimen and standard wells, excluding the blank control well. The solution was thoroughly mixed, and a sealant was applied to the plate. The plate was incubated at 37°C for 60 minutes.

5. The sealer was removed, and the plate was rinsed five times with wash buffer. Each wash included incubation with 300 µL of wash buffer for 30 seconds to 1 minute. For automated washing, each well was aspirated or decanted and washed five times with wash buffer. After washing, the plate was blotted onto paper towels or other absorbent material.
6. A volume of 50 µL of substrate solution A was added to each well, followed by the addition of 50 µL of substrate solution B. The plate was then sealed with a fresh cover and incubated for 10 minutes at 37°C in the dark. Subsequently, 50 µL of Stop Solution was added to each well, resulting in an immediate color change from blue to yellow.

8. The optical density (OD) of each well was promptly measured using a microplate reader calibrated to 450 nm, within 10 minutes after the addition of the Stop Solution.

3.2.8.2 Estimation of human IL-17 Concentration

1- Assay Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human IL17A antibody. IL17A present in the specimen is added and binds to antibodies coated on the wells. And then Biotinylated Human IL17A Antibody is added and binds to IL17A in the specimen. Then Streptavidin-HRP is added and binds to the Biotinylated IL17A antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human IL17A. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

2- Reagent Preparation

- Prior to use, all reagents were allowed to come to room temperature.
- **Standard** A volume of 120 µL of the standard (640 ng/L) was reconstituted with 120 µL of standard diluent to obtain a 320 ng/L standard stock solution. The solution was allowed to sit for 15 minutes with gentle agitation prior to dilution. Duplicate standard points were prepared by serial 1:2 dilution of the 320 ng/L stock solution with standard diluent, yielding concentrations of 160 ng/L, 80 ng/L, 40 ng/L, and 20 ng/L. Any remaining solution was stored at –20°C and used within one month.

- **Wash Buffer** Twenty milliliters (20 mL) of Wash Buffer Concentrate (25×) were diluted with deionized or distilled water to a final volume of 500 mL, resulting in 1× Wash Buffer. If crystals had formed in the concentrate, the solution was gently mixed until fully dissolved.

3- Assay Procedure

1. All reagents, standard solutions, and specimens were prepared as specified. Brought all reagents to room temperature before use. The test was performed at room temperature.
2. The required number of strips for the experiment was determined. The strips were inserted into the frames for use. Unused strips were stored at 2–8°C.
3. Fifty microliters (50 µL) of standard solution was added to the standard well. The antibody was not added to the standard well since the standard solution contained a biotinylated antibody.
4. Forty microliters (40 µL) of specimen was added to the specimen wells, followed by the addition of 10 µL of Human anti-IL17A antibody. Then, 50 µL of streptavidin-HRP was added to both specimen wells and standard wells (excluding the blank control wells). The contents were mixed thoroughly. The plate was covered with a sealant and incubated for 60 minutes at 37°C.
5. The sealant was removed, and the plate was washed five times with wash buffer. Each wash included soaking the wells with 300 µL of wash buffer for 30 seconds to 1 minute. For automated washing, each well was aspirated or decanted and washed five times with wash buffer. The plate was then blotted onto paper towels or other absorbent materials.
6. Next, substrate solution A (50 µL) was added to each well, followed by 50 µL of substrate solution B. The plate was resealed and incubated in the

dark at 37°C for 10 minutes.
7. A volume of 50 µL of Stop Solution was added to each well, resulting in an instantaneous color shift from blue to yellow.

8. Finally, the optical density (OD) at 450 nm was measured promptly within 10 minutes using a calibrated microplate reader.

3.2.8.3 Estimation of human Dectin-1 Concentration

1- Assay Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human DECTIN-1 antibody. DECTIN-1 present in the specimen is added and binds to antibodies coated on the wells. And then Biotinylated Human DECTIN-1 Antibody is added and binds to DECTIN-1 in the specimen. Then Streptavidin-HRP is added and binds to the Biotinylated DECTIN-1 antibody. After incubation unbound Streptavidin HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human DECTIN-1A.The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450nm.

2- Reagent Preparation

- All reagents were brought to room temperature before use.
- **Standard** Reconstitution of the standard was performed by combining 120 µl of the 4800 ng/L standard with an equal volume (120 µl) of standard diluent, resulting in a 2400 ng/L stock solution. The mixture was incubated for 15 minutes with gentle agitation before further dilution. Serial 1:2 dilutions of the stock solution were prepared in duplicate to

obtain concentrations of 1200 ng/L, 600 ng/L, 300 ng/L, and 150 ng/L. The standard diluent was used as the zero calibrator (0 ng/L). Any leftover solution was stored frozen at –20°C and used within one month.

- **Wash Buffer** Preparation of the wash buffer involved diluting 20 mL of 25× Wash Buffer Concentrate with deionized or distilled water to reach a final volume of 500 mL of 1× Wash Buffer. The solution was mixed gently until all crystals were fully dissolved.

3- Assay Procedure

1. All reagents, standard solutions, and specimens were prepared as instructed. They were brought to room temperature before use. The assay was conducted at room temperature.

2. The number of strips required for the assay was determined, and the strips were inserted into the frames for use. Unused strips were stored at 2–8°C.

3. Fifty microliters (50 µL) of standard solution was added to the standard well without adding biotinylated antibody, as the standard solution contained biotinylated antibody.

4. A 40µl of the specimens was added to the specimen wells, followed by 10 µL of anti-DECTIN-1 antibody. Then, 50 µL of streptavidin-HRP was added to both specimen and standard wells (excluding the blank control wells). The contents were mixed thoroughly. The plate was covered with a sealer and incubated for 60 minutes at 37°C.

5. The sealer was removed, and the plate was washed five times with wash buffer. Each wash included soaking the wells with 300 µL of wash buffer for 30 seconds to 1 minute. For automated washing, each well was

aspirated or decanted and washed five times. The plate was then blotted onto paper towels or other absorbent material.

6. Fifty microliters (50 µL) of substrate solution A was added to each well, followed by 50 µL of substrate solution B. The plate was covered with a fresh sealer and incubated in the dark at 37°C for 10 minutes.

7. A volume of 50 µL of Stop Solution was added to each well, resulting in an immediate color change from blue to yellow.

8. The optical density (OD) of each well was promptly measured using a microplate reader set to 450 nm within 10 minutes after adding the Stop Solution.

3.2.8.4 Estimation of human sIgA Concentration

1- Assay Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human sIgA antibody. sIgA present in the specimen is added and binds to antibodies coated on the wells. And then biotinylated Human sIgA Antibody is added and binds to sIgA in the specimen. Then Streptavidin-HRP is added and binds to the Biotinylated SIgA antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human sIgA. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

2- Reagent Preparation

- All reagents were brought to room temperature before use .

- **Standard** A diluent was used to generate a 4 μg/ml standard stock solution. The standard sat for 15 minutes with gentle agitation before making the necessary dilutions. Duplicate standard points were prepared by serially diluting the stock solution (4 μg/ml) 1:2 with standard diluent, resulting in 2 μg/ml, 1 μg/ml, 0.5 μg/ml, and 0.25 μg/ml solutions. The standard diluent served as the zero standard (0 μg/ml). Any remaining solution was frozen at -20°C and was to be used within one month.
- **Wash Buffer** Twenty milliliters of Wash Buffer Concentrate 25x was diluted into deionized or distilled water to yield 500 milliliters of 1x Wash Buffer. If crystals had formed in the concentrate, they were mixed gently until completely dissolved.

3- Assay Procedure

1. All reagents, standard solutions, and specimens were prepared as instructed. All reagents were brought to room temperature prior to use. The assay was performed at room temperature.

2. The number of strips required for the assay was determined. The strips were inserted into the frames for use. Unused strips were stored at 2–8°C.

3. Fifty microliters (50 μL) of standard solution was added to the standard well. Biotinylated antibody was not added to the standard well since the standard solution contained it.

4. A volume of 40 μL of specimen was added to the specimen wells, followed by 10 μL of Human Anti-sIgA antibody. Then, 50 μL of streptavidin-HRP was added to both specimen and standard wells (excluding blank control wells). The contents were mixed thoroughly.

The plate was covered with a sealer and incubated for 60 minutes at 37°C.

5. The sealer was removed, and the plate was washed five times with wash buffer. Each wash involved soaking the wells with 300 µL of wash buffer for 30 seconds to 1 minute. For automated washing, each well was aspirated or decanted and washed five times. The plate was blotted onto paper towels or other absorbent material.

6. Fifty microliters (50 µL) of substrate solution A was added to each well, followed by 50 µL of substrate solution B. The plate was covered with a fresh sealer and incubated in the dark at 37°C for 10 minutes.

7. A 50 µL of Stop Solution was added to each well, resulting in an immediate color change from blue to yellow.

8. The optical density (OD) of each well was immediately determined using a microplate reader set to 450 nm within 10 minutes after the addition of the Stop Solution.

3.2.8.5 Calculating of Results of ELISA Test

The standard curve was generated by plotting the average optical density (OD) for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis, followed by the construction of a best-fit curve through the data points on the graph. These calculations are optimally executed using computer-based curve-fitting software, and the best-fit line can be obtained through regression analysis.

Figure (3-2) show the standard curve in TLR4, IL-17, Dectin-1, and sIgA.

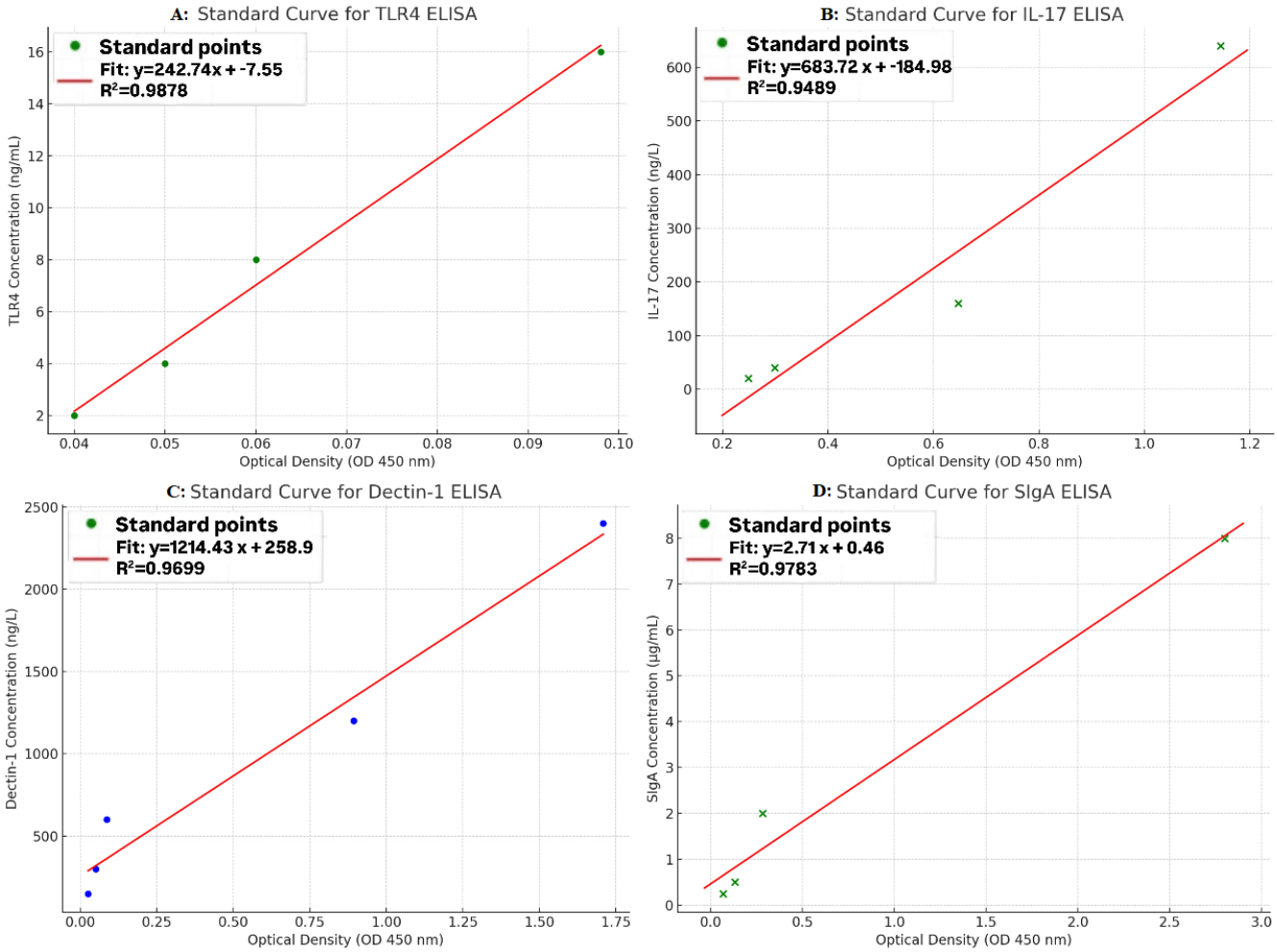


Figure (3-2) The standard curve of immunological parameter

A: TLR4 B: IL-17 C: Dectin-1 D: SIgA

3.2.9 Molecular Examination

3.2.9.1 Genomic DNA Extraction

Genomic DNA from blood specimens were extracted by using gSYAN DNA kit extraction kit (Frozen Blood) Geneaid. USA, and done according to company instructions as following steps:

1. A 200µl of frozen blood was transferred to sterile 1.5ml microcentrifuge tube, and then added 30µl of proteinase K and mixed by vortex. And incubated at 60℃ for 5 minutes.

2. After that, 200µl of lysis buffer GSB was added to each tube and mixed by vortex vigorously, and then all tubes were incubated at 70℃ for 10 minutes, and inverted every 3 minutes through incubation periods.

3. A 200µl absolute ethanol were added to lysate and immediately mixed by shaking vigorously.

4. DNA filter column was placed in a 2 ml collection tube and transferred all of the mixture (including any precipitate) to column. Then centrifuged at 10000rpm for 5 minutes. And the 2 ml collection tube containing the flow through were discarded and placed the column in a new 2 ml collection tube.

5. A volume of 400µl W1 buffer were added to the DNA filter column, then centrifuge at 10000rpm for 30 seconds. The flow through was discarded and placed the column back in the 2 ml collection tube.

6. A 600µl Wash Buffer (ethanol) was added to each column. Then centrifuged at 10000rpm for 30 seconds. The flow through was discarded and placed the column back in the 2 ml collection tube.

7. All the tubes were centrifuged again for 3 minutes at 10000 rpm to dry the column matrix.

8. The dried DNA filter column was transferred to a clean 1.5 ml microcentrifuge tube and 50 µl of pre.heated elution buffer were added to the center of the column matrix.

9. The tubes were let stand for at least 5 minutes to ensure the elution buffer was absorbed by the matrix. Then centrifuged at 10000 rpm for 30 seconds to elute the purified DNA.

3.2.9.2 Genomic DNA estimation

The extracted blood genomic DNA was checked by using Nanodrop spectrophotometer (THERMO. USA), which measured DNA concentration (ng/µL) and check the DNA purity by reading the absorbance at (260 /280 nm) as following steps:

1. After opening up the Nanodrop software, chosen the appropriate application (Nucleic acid, DNA).
2. A dry wipe was taken and cleaned the measurement pedestals several times. Then carefully pipette 2µl of free nuclease water onto the surface of the lower measurement pedestals for blank the system.
3. The sampling arm was lowered and clicking OK to initialized the Nanodrop, then cleaning off the pedestals and 1µl of blood genomic DNA was added to measurement.

3.2.9.3 Tetra- ARMS-PCR Method

T-ARMS-PCR method was performed for detection and genotyping of TLR4- rs4986790 gene polymorphism in patients and healthy control specimens. This method was carried out according to following steps:

3.2.9.3.1 T-ARMS-PCR master mix preparation

T-ARMS-PCR master mix was prepared by using (GoTaq® G2 Green Master Mix kit) and this master mix done two reactions for each specimens according to company instructions as following tables (3-11):

Table (3-11) Standard T-ARMS-PCR reaction Mix

T-ARMS-PCR Master mix	Volume
DNA template	5µl
Forward inner primer (wild allele) (10pmol)	1µl
Reverse inner primer (mutant allele) (10pmol)	1µl
Forward outer primer (10pmol)	1µl
Reverse outer primer (10pmol)	1µl
G2 Green Master Mix	12.5µl
Nuclease free water	3.5µl
Total volume	25µl

After that, these PCR master mix component that mentioned in table above were transferred into Exispin vortex centrifuge at 3000rpm for 3 minutes. Then placed in PCR Thermocycler (BioRad. USA).

3.2.9.3.2 PCR Thermocycler Conditions

The PCR Thermocycler conditions for the TLR4-rs4986790 gene were applied according to the protocol shown in Table (3-12).

Table (3-12): PCR Thermocycler Conditions

PCR step	Temp.	Time	repeat
Initial denaturation	95°C	5min.	1
Denaturation	95°C	30 sec.	35cycle
Annealing	62°C	30 sec.	

Extension	72°C	30 sec.	
Final extension	72°C	5min	1
Hold	4°C	Forever	-

3.2.9.3.3 T-ARMS-PCR product analysis

The T-ARMS-PCR products were analyzed by agarose gel electrophoresis following steps (Sambrook, and Russell, 2001):

1. Agarose gel (2%) was prepared in using 1X TBE and dissolving in water bath at 100 °C for 15 minutes, after that, left to cool 50°C.
2. Then 3µL of ethidium bromide stain were added into Agarose gel solution.
3. Agarose gel solution was poured in tray after fixed the comb in proper position after that, left to solidify for 15 minutes at room temperature, then the comb was removed gently from the tray.
4. The gel tray was fixed in electrophoresis chamber and fill by 1X TBE buffer.
5. A 10µl PCR product were added in to each comb well and 3µl of (100bp Ladder) in First well.
6. The electric current was performed at 100 volt and 80 mA for 1hour.
7. The T-ARMS-PCR products were visualized by using UV transilluminator.

3.2.10 Ethical approval

1. The study was conducted, and the cases were collected following the patients' consent (verbal agreement).

2. Approval of the Babylon Science College Ethical Committee.

3. Prior to commencing the study, permission was granted by the Babylon Health Presidency, and the Ethical Committee of the Babil Health Directorate accepted the research plan on September 1, 2024 (M240903).

3.2.11 Statistical Analysis

All statistical analyses were performed using SPSS version 25. Data were presented as mean ± standard deviation (SD). ELISA concentrations for TLR4, IL-17, Dectin-1, and sIgA were calculated using Microsoft Excel 2010 (trend function). The independent t-test was used to determine the significance of differences between appendicitis patients and healthy controls, as well as between serum and tissue specimens. Pearson's correlation test was applied to assess the relationship between serum and tissue levels of the studied markers. One-way ANOVA was used to evaluate the association between TLR4 rs4986790 genotypes and immunological marker levels. Odds ratios (OR) with 95% confidence intervals (CI) were calculated to estimate the risk associated with genotypes. A p-value less than 0.05 was considered statistically significant.

CHAPTER FOUR
RESULTS
AND
DISCUSSION

4.1 Demographic Characteristics of the Participants

The current study included 100 patients diagnosed with appendicitis and 50 apparently healthy individuals as a control group (AHC). To analyze the distribution of appendicitis cases, three demographic variables were considered: age, sex, and place of residence.
The age group 14–24 years represented the highest percentage of cases (48%), followed by the age group 25–35 years (30%), while the groups 36–46 years and 47–57 years accounted for 10% and 4% respectively, and the lowest percentage was observed in the age group above 58 years (8%).
In terms of sex distribution, 54% of patients were female and 46% were male.
Regarding geographic distribution, patients were categorized as residing either in central (urban area) or peripheral (rural) areas. In this study, 34% of patients were from urban area, while 66% were from rural areas, as demonstrated in Table 4-1.

Table (4-1): Distributions of the Participants

Groups	NO. (%)
Age (Years)	
14-24	48 (48%)
25-35	30 (30%)
36-46	10 (10%)

47-57	**4 (4%)**
>58	**8 (8%)**
Total	**100 (100%)**
Sex groups	
Male	**46 (46%)**
Female	**54 (54%)**
Place of residence	
Rural area	**66 (66%)**
urban area	**34 (34%)**

These results are consistent with the findings of Saleem and Alkawaz (2021) in Baghdad, who reported the highest frequency of appendicitis among patients aged 11–20 years (54.3%), which is comparable to the present study where the 14–24 years group represented the highest percentage (48%). However, they differ from the findings of Al Sehlany et al. (2022) in Hilla, Iraq, who reported the highest incidence (39.13%) in the 20–30-year age group.

Regarding sex, the current study found that 54% of patients were female, aligning with the findings of Saleem and Alkawaz (2021), who

reported a higher prevalence among females (54.3%) compared to males (45.7%), and with Al Sehlany *et al*. (2022), who also observed a female predominance (53.3%). In contrast, Majrashi *et al*. (2018) reported a significantly higher proportion of male patients (69%) compared to females (30.9%).

With respect to geographic distribution, the present study revealed that 66% of patients resided in rural areas, while only 34% were from urban area. This finding contrasts with a Basra-based study by Alshawi (2024), which reported a higher incidence of appendicitis in urban areas (56.8%) compared to rural areas (43.2%). The discrepancy may be attributed to differences in healthcare accessibility and the likelihood of early medical intervention in urban settings, as well as differences in lifestyle between rural and urban populations.

4.2 Isolation and identification of bacteria and fungi

4.2.1 Bacterial studies

In this study, bacterial isolates were obtained from appendiceal swab specimens from 100 patients who had acute appendicitis. Following appendectomy, specimens were obtained using gel transport swabs and cultivated on various media such as Blood Agar, MacConkey Agar, UTI Chromogenic Agar, and Eosin Methylene Blue Agar. All the cultures showed bacterial growth after 24 to 48 hours of incubation, with no cases of "no growth" observed.

The morphological and cultural properties were evaluated by examining colony size, shape, color, edge, and transparency on these various media as figure 4-1 and figure 4-2 demonstrated.

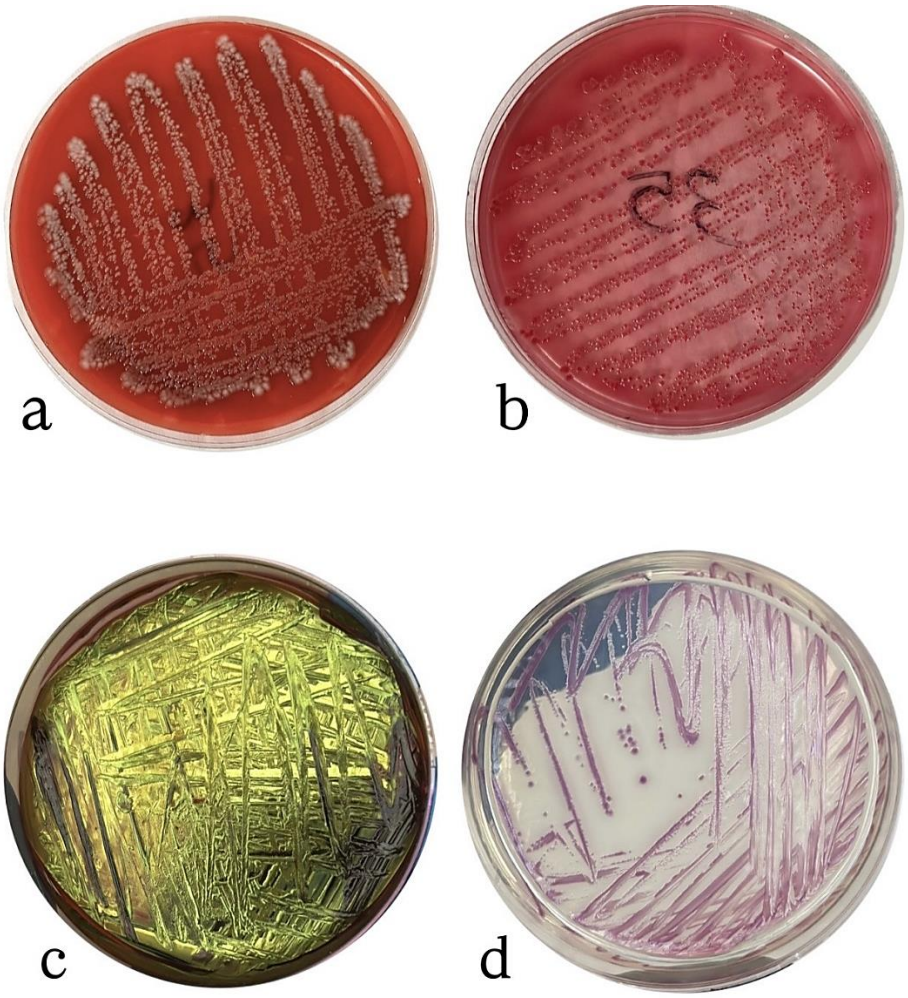


Figure (4-1) Morphological properties of *Escherichia coli* colonies on different media.

***a- Escherichia coli* on Blood agar** **b- *Escherichia coli* on MacConkey Agar**

c- *Escherichia coli* on EMB Agar **d- *Escherichia coli* on UTI chromogenic Agar**

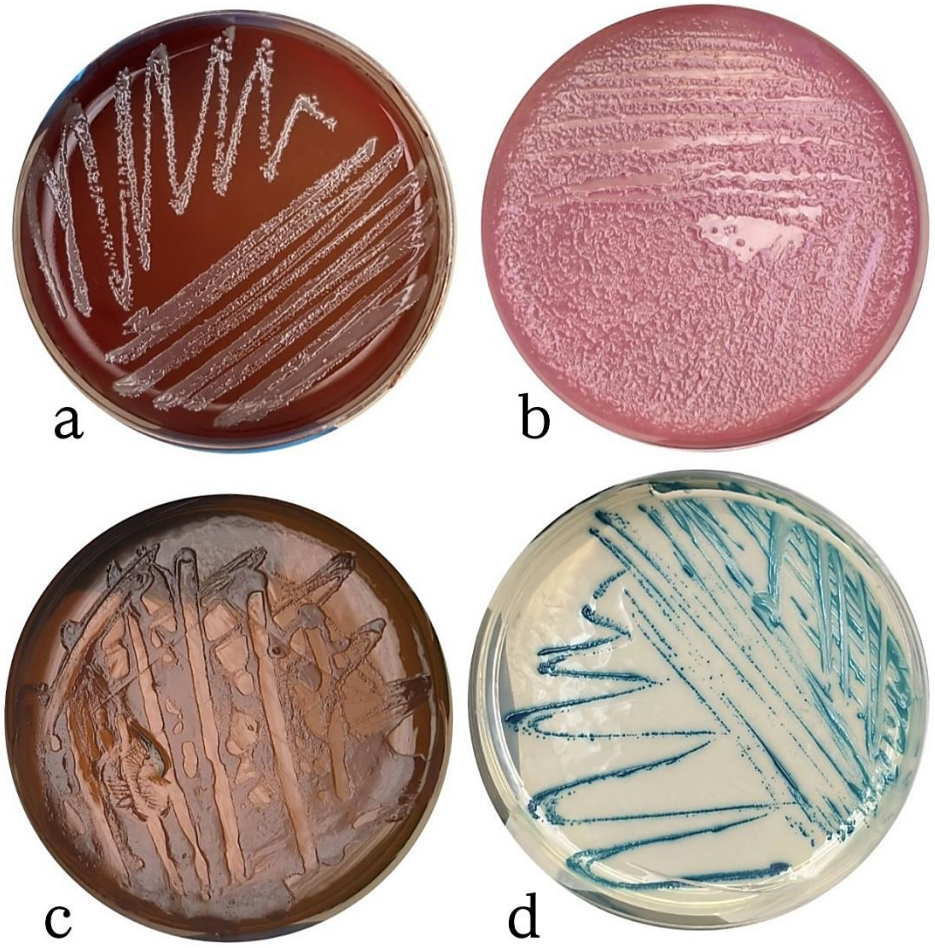


Figure (4-2) Morphological properties of *klebsiella pnemoniae* on different media.

***a- Klebsiella pnemoniae* on Blood agar b- *Klebsiella pnemoniae* on MacConkey Agar**

c- *Klebsiella pnemoniae* on EMB Agar d- *Klebsiella pnemoniae* on UTI chromogenic Agar

Each bacterial isolate was also Gram stained, and the reaction was performed and observed using an oil immersion lens. The results revealed that all isolates were Gram-negative bacteria. These findings align with a study that revealed 73.6% of the isolated bacteria in the appendix lumen were Gram-

negative, while 23.4% were a mixed population of Gram-positive and Gram-negative microorganisms. No culture included Gram-positive bacteria exclusively (Zachos *et al*., 2023).

The VITEK 2 System, which offers automated identification based on biochemical profiling, was used to further identify the isolates.

In the present study, *Escherichia coli* was identified as the predominant bacterial species 88 (88%) isolated from appendicitis patients, followed by *Klebsiella pneumonia 12* (12%). (Figure 4-3).

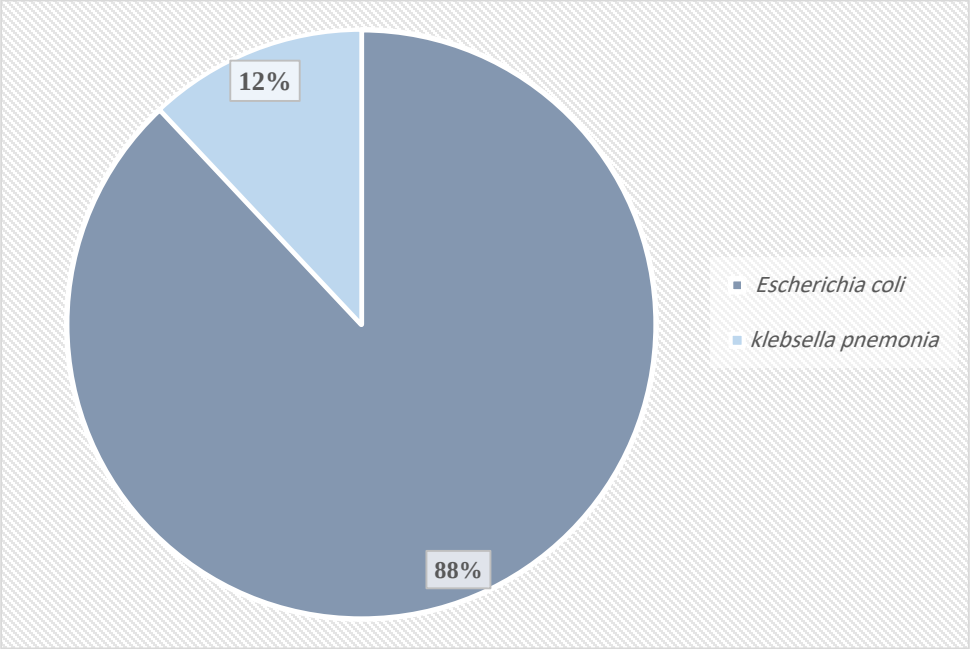


Figure (4-3): Distribution of bacterial isolates identified in appendicitis patients.

Several studies have investigated the bacterial profile associated with acute appendicitis. The study conducted by Abdurrazzaaq *et al*. (2018) investigated bacterial isolates in acute appendicitis at the University College

Hospital in Ibadan, Nigeria. The most commonly identified bacteria were *Escherichia coli* (50%), *Klebsiella* spp. (15%), and *Proteus* spp. (8.3%).

In Kirkuk province, Iraq, researchers conducted a study to determine how some antibiotics affected bacteria isolated from appendices. According to Bazzaz *et al*. (2018), the culture results showed that 94% of the specimens had a single isolate, 2% had negative growth, and 4% had mixed growth. The gram-positive bacteria isolates accounted for 11.77%, which is less than the gram-negative isolates at 88.23%. There were a total of 51 bacteria isolates, with *Escherichia coli* being the most common at 34 (66.66%), followed by *Enterococcus faecalis* and *Klebsiella pneumoniae* both at 3 (5.89%), *Citrobacter youngae*, *Ralstonia terrigena*, and *Pseudomonas aeruginosa* each at 2 (3.92%), and other bacteria, each at 1 (1.96% or 1.92%).

A study was conducted in Babylon province, Iraq, to identify Bacterial Infections in Patients with Appendicitis. According to (Al-Janabi *et al*.2019), the investigation discovered several bacterial strains, and found that there is a relation between bacterial infection with appendicitis and *Escherichia coli* was predominant with 50.9% followed by *Streptococcus pneumoniae* (21.5%), *Pseudomonas aeruginosa* (13.7%), *and Klebsiella pneumoniae* (9.8%), while *Staphylococcus aureus* has shown a lowest isolation rate.

A study in Sulaymaniyah city, Iraq, by Hama *et al*. (2021) found that the most common bacteria in patients with appendicitis was *Escherichia coli* (27.27%), followed by *Staphylococcus aureus* and anaerobic *Bacteroides fragilis* (7.8%), *Klebsiella pneumoniae* (4.2%), and other bacteria.

A study by Cimpean *et al*. (2022) looked into the bacteriological profiles of patients with acute appendicitis that was both complicated and uncomplicated. With an isolation rate of 37.9% in cases of uncomplicated appendicitis and 48.6% in cases of complicated appendicitis, *Escherichia coli* was the most commonly isolated organism in both groups. Members of the *Streptococcus anginosus* group and *Bacteroides* species were among the other frequently found bacteria.

Madhkoor *et al*. (2024) conducted a recent study in Basra Province. The research sought to discover the pathogenic microorganisms associated with appendicitis. Of the specimens, 80 (88.9%) yielded positive cultures, whereas 10 (11.1%) resulted in negative cultures. The analysis identified 15 distinct bacterial isolates, with *Escherichia coli* being the predominant species at 80 (44.9%), while other species were present in lesser proportions.

4.2.2 Fungal studies

In the current study, the appendiceal specimens were cultured on Sabouraud Dextrose Agar and Candida Chromogenic Agar to check for fungi and incubated for 48 to 72 hours at 25°C and 37°C to allow for potential fungal growth. None of the specimens showed any discernible fungal growth. This finding aligns with previous literature indicating that fungi constitute only a minor component of the normal human gut microbiota compared to bacteria (Hallen-Adams & Suhr, 2017).

In Ukraine, research was carried out to find microbial agents linked to children's acute appendicitis. Two (2.7%) patients had *Candida* fungus, according to Zahrychuk *et al*. (2023). These cases of gangrene and gangrenous-perforated appendicitis suggest that fungal infections could exist in severe forms of the disease.

In a study by Larbcharoensub *et al*. (2013), researchers looked at 262 appendectomy cases and discovered that about 1.15% were caused by fungal infections, specifically types of *Candida* and *Aspergillus*. It was noted that all the patients had weakened immune systems, and tests revealed pus-filled inflammation in the appendix wall along with visible fungal growth. These results indicate that fungal infections in the appendix, although rare, are possible, especially in immunocompromised patients.

In 2019 Choy *et al*. documented a rare case of fungal appendicitis in a woman without an immunosuppressive condition. Though not known to have any immunosuppressive properties, the appendiceal tissue included *Candida* fungus. After the patient had an appendectomy, antifungals proved to be efficient in treating the infection, so highlighting the need of considering fungal infections even in immunocompetent individuals.

4.3 Immunological study

4.3.1 Estimation of TLR-4 in serum and tissue in patients and AHC

The TLR 4 concentrations in the serum of patients and the apparently healthy control group were determined by enzyme-linked immunosorbent

assay, yielding a mean value of 18.72764 ng/ml and a standard deviation of 0.028774 ng/ml, while the control group had a mean of 18.67014 ng/ml and a standard deviation of 0.156426 ng/ml. Although the mean value was slightly higher in patients compared to controls, this difference was not statistically significant ($p = 0.083$), as shown in Table 4-2.

Table (4-2) The concentration of TLR4 in serum of patients and AHC.

TLR4 serum	M±SD ng\ml	P value
Patient	18.72764 ±0.028774	0.083 ^{NS}
AHC	18.67014±0.156426	

SD: standard deviation; **NS**: not significant at P > 0.05

The results align with a recent study in 2024 in Basra province, Iraq, to identify the immunohistochemical expression of Toll-like receptors TLR1, TLR4, and TLR5 in patients with acute appendicitis. The study revealed a significant elevation in the plasma concentrations of Toll-like receptors TLR1 and TLR4 in appendicitis patients compared to the AHC group (Mahdi *et al*., 2024b).

Another study by Arlt *et al*. (2015) looked at TLRs in the tissues of inflamed appendices compared to normal appendices and found that TLR4 levels were much higher in patients with acute appendicitis than in those with

non-inflamed appendices (P = 0.01), with TLR4 amounts increasing from 2486 ± 381 to 4694 ± 963 copies/20 ng mRNA.

Molteni *et al*. (2016) concurred that toll-like receptors are highly conserved entities that identify conserved pathogen-associated molecular patterns (PAMPs), hence serving as the initial defense against infections. TLR4 has been acknowledged as the receptor that detects gram-negative lipopolysaccharide (LPS). TLR4-mediated inflammation, initiated by exogenous or endogenous ligands, is implicated in various acute and chronic illnesses.

The current study appeared to demonstrate a significant increase (P= 0.000) in TLR 4 concentrations in serum (18.727642 ±0.0287743) ng\ml compared to tissue (18.635642±0.0578208) ng\ml; no previous studies had provided an explanation. As table 4-3 demonstrates.

Table (4-3): The concentrations of TLR4 in serum and tissue of patient.

TLR4	M±SD ng\ml	P value
Serum	18.727642 ±0.0287743	0.000 S
Tissue	18.635642±0.0578208	

SD: standard deviation; **S:** Significant at $P \leq 0.05$

The results of the current study found no significantly increased TLR-4 in the serum and tissue of males compared to females; this could be due to

dietary differences, such as a higher consumption of fast food among males. As in table 4-4.

Table (4-4): The concentrations of TLR4 in serum and tissue specimens of patients according to sex

Parameters	TLR4 M±SD ng\ml		P value
	Male	Female	
Serum	18.7298±0.02904	18.7255±0.02964	0.728 NS
Tissue	18.6278±0.05673	18.6435±0.06031	0.518 NS

SD: standard deviation; **NS**: not significant at P > 0.05

4.3.2 Correlation between TLR-4 serum and tissue in patients

Table 4-5 shows a correlation coefficient of 0.464 for both serum and tissue TLR-4 levels, with two-tailed p-values of 0.022. This correlation coefficient suggests a moderate positive relationship between TLR-4 levels in serum and tissue. Moreover, since the p-values are less than the commonly used significance level of 0.05, the correlations are considered statistically significant.

Table (4-5): Correlation of TLR4 in serum and tissue of patient.

TLR4		Serum	Tissue
Serum	Pearson Correlation	**1**	**0.464**
	Sig. (2-tailed)		**0.022** S
Tissue	Pearson Correlation	**0.464**	**1**
	Sig. (2-tailed)	**0.022** S	

S: Significant at $P \leq 0.05$

An old study in 2004 examined the correlation between certain genes associated with innate immunity, including changes in TLR4, and the severity of appendicitis; however, they did not directly quantify TLR4 protein levels in blood or tissue specimens (Rivera-Chavez *et al*. 2004). The current findings provide new visions by quantitatively evaluating TLR4 levels in blood and appendiceal tissue, highlighting the possible immunological role of TLR4 in local and systemic responses during appendicitis.

4.3.3 Estimation of IL-17 in serum and tissue in patients and AHC

Table (4-6) displays the mean and standard deviation (M±SD) for serum IL-17 concentrations in both patients and the AHC group. Appendicitis patients had noticeably higher serum IL-17 concentrations 257.23±132.93

ng/L, whereas the AHC group had a lower average of 109.72±62.60 ng/L. The p-value of 0.000, marked with an asterisk, indicates a statistically significant difference between the two groups (P<0.05).

Table (4-6): The concentration of IL-17 in serum of patient and AHC.

IL-17 serum	M±SD ng\L	P value
Patient	257.2286±132.93146	0.000 S
AHC	109.7160± 62.60039	

SD: standard deviation; **S:** Significant at $P \leq 0.05$

This increase is consistent with earlier studies that found appendicitis and bacterial infections can affect IL-17 levels. A study by. Al-Khawaja & Al-Shabaa, (2021) reported significantly elevated serum IL-17 levels in patients with acute appendicitis (210.1 pg/mL) compared to apparently healthy controls (52.43 pg/mL), with a statistically significant difference (P = 0.004). This study suggested that IL-17 could serve as a diagnostic biomarker for acute appendicitis.

A study by Rubér *et al*. (2010) reported that patients with gangrenous appendicitis exhibited significantly elevated levels of proinflammatory markers in serum, including IL-17, when compared to patients with phlegmonous appendicitis and nonspecific abdominal pain (P ≤ 0.04).

The mean IL-17 level in the serum of patients with appendicitis was 257.2286 ng/L, while tissue specimens exhibited a markedly elevated level of 501.99 ng/L, showing a significant difference (P = 0.000) as shown in Table 4-7.

Table (4-7): The concentrations of IL-17 in serum and tissue of patient

IL-17	M±SD ng\L	P value
Serum	257.2286±132.93146	0.000 S
Tissue	501.9877±183.24266	

SD: standard deviation; **S:** Significant at $P \leq 0.05$

A Recent study by The *et al*. (2024) found that IL-17A-producing CD4+ T cells were significantly increased in the appendix tissue of children with complex appendicitis compared to those with simple appendicitis. Increased local inflammation and microbial dysbiosis, specifically an increase in Proteobacteria, were linked to this rise in IL-17A levels. According to their findings, the severity of appendicitis may be influenced by a robust local Th17 immune response.

A study conducted by Elliver *et al*. (2024) aimed to assess the relationship between serum levels of various Th1-associated cytokines, including IL-17A, and the risk of complicated appendicitis in children. The findings indicated that serum IL-17A concentrations did not significantly differ between children with uncomplicated and complicated appendicitis,

suggesting that the systemic inflammatory response in complicated appendicitis is intricate and not exclusively Th1-dependent.

The results of the current study found no significant difference in IL-17 concentrations between males and females in either serum (P = 0.367) or tissue (P = 0.871). The mean serum IL-17 level was slightly higher in females (288.34 ± 158.58 ng/L) compared to males (226.12 ± 102.50 ng/L), while the tissue level was marginally higher in males (445.83 ± 260.76 ng/L) than in females (429.41 ± 209.17 ng/L). However, the lack of statistical significance indicates that sex does not influence IL-17 expression in this study population, as Table 4-8 demonstrated.

Table (4-8): The concentrations of IL-17 in serum and tissue specimens of patients according to sex

Parameters	IL-17 M±SD ng\L		P value
	Male	Female	
Serum	226.1151±102.49719	288.3420±158.57991	0.367 NS
Tissue	445.8268±260.7627	429.4050±209.17499	0.871 NS

SD: standard deviation; **NS**: not significant at P > 0.05

Currently, there is no evidence of significant sex-based differences in IL-17 levels in serum or tissue, which is consistent with the findings of the present study.

4.3.4 Correlation between IL-17 in serum and tissue in patients

Pearson correlation analysis revealed no significant association between IL-17 levels in serum and tissue among patients (r = 0.025, p = 0.911), suggesting that IL-17 concentrations in these two compartments are not strongly related, as in table 4-9.

Table (4-9): Correlation of IL-17 in serum and tissue of patient

	IL-17	Serum	Tissue
Serum	Pearson Correlation	1	0.025
	Sig. (2-tailed)		0.911 NS
Tissue	Pearson Correlation	0.025	1
	Sig. (2-tailed)	0.911 NS	

NS: not significant at P > 0.05

A study by The *et al*. (2024) reported increased IL-17A expression in appendiceal tissue of children with complex appendicitis; however, no comparison with serum levels was conducted, leaving the relationship between local and systemic IL-17 responses unclear.

In a study on inflammatory bowel disease (IBD), Fujino *et al*. (2003) reported elevated IL-17 levels in both serum and mucosal tissue of patients with active disease. While this finding highlights IL-17's potential role in IBD pathogenesis, the study did not confirm a direct correlation between systemic and mucosal IL-17 levels, indicating that local and systemic immune responses may function independently.

4.3.5 Estimation of Dectin-1 in serum and tissue in patients and AHC

The mean serum level of Dectin-1 among appendicitis patients was 601.21 ± 332.11 ng/L, while it was 588.16 ± 238.59 ng/L in the AHC group. Statistical analysis showed no significant difference between the two groups (P = 0.902).

Table (4-10): The concentrations of Dectin-1 in serum of patients and AHC

Dectin-1 serum	M±SD ng\L	P value
Patients	601.2088±332.10803	0.902 NS
AHC	588.1647±238.58534	

SD: standard deviation; **NS**: not significant at P > 0.05.

Despite the slight increase in serum Dectin-1 levels observed in appendicitis patients, the absence of statistical significance suggests that acute appendicitis does not markedly influence systemic Dectin-1 concentrations in this specimens. This finding implies that circulating Dectin-1 may not accurately

reflect localized immune responses within the appendix, or that serum measurements lack the sensitivity to detect subtle inflammation-associated changes in appendicitis.

In the study by Al Madhoun *et al*. (2022), Dectin-1 levels were elevated in adipose tissue of individuals with obesity, suggesting its association with chronic metabolic inflammation. This indicates that Dectin-1 could be a marker of ongoing inflammation in adipose tissue. However, their study did not examine serum Dectin-1 levels, so it cannot be directly compared to this findings on serum levels in appendicitis patients.

The mean serum Dectin-1 level was 601.21 ± 332.11 ng/L, while the mean tissue level was 693.45 ± 300.75 ng/L. However, statistical analysis revealed no significant difference between the two groups, with a P-value of 0.562, as table 4-11 illustrates.

Table (4-11): The concentrations of Dectin-1 in serum and tissue of patients

Dectin-1	M±SD ng\L	P value
Serum	**601.21 ± 332.11**	**0.562** ^{NS}
Tissue	**693.45 ± 300.75**	

SD: standard deviation; **NS**: not significant at P > 0.05.

The current findings indicate that tissue concentrations were greater than those in serum; this may be attributed to Dectin-1, which serves to protect the

host from invading fungi while also potentially contributing to increased inflammation (Iliev, 2015).

A study by Wang *et al*. (2022) demonstrated that Dectin-1 plays a critical role in regulating the bacterial microbiota in the gut, which is essential for maintaining immune homeostasis, although their research focused on the gut, it highlights how Dectin-1 can influence inflammatory responses and immune modulation in specific tissues.

The higher levels of Dectin-1 observed in tissue specimens may reflect a localized immune response, in which Dectin-1 participates in both pathogen defense and inflammation modulation, particularly in inflammatory conditions such as appendicitis (Rosas et al., 2008).

De Vries *et al*. (2009) demonstrated that Dectin-1 expression was significantly increased in inflamed colonic tissues of patients with inflammatory bowel disease (IBD), compared to non-inflamed regions of the same individuals. This upregulation was particularly observed on macrophages, neutrophils, and other immune cells involved in the inflammatory response. Their findings reinforce the concept that Dectin-1 is locally upregulated in inflamed tissues, supporting the current observation of elevated Dectin-1 levels in appendix tissue versus serum.

In terms of sex comparisons, the average serum Dectin-1 levels were elevated in females (649.18 ± 413.21 ng/L) compared to males

(553.24 ± 245.65 ng/L); however, this disparity was not statistically significant (P = 0.581). Tissue concentrations of Dectin-1 were elevated in females (744.66 ± 340.84 ng/L) relative to males (642.25 ± 264.84 ng/L), with no statistically significant difference (P = 0.487), indicating that sex did not markedly affect Dectin-1 levels in blood or tissue specimens among appendicitis patients, as emphasized in Table 4-12.

Table (4-12) The concentrations of Dectin-1 in serum and tissue specimens of patients according to sex

Parameters	Dectin-1 M±SD ng\L		P value
	Male	Female	
Serum	553.2402±245.65033	649.1778±413.21169	0.581 NS
Tissue	642.2456±264.84015	744.6600±340.84479	0.487 NS

SD: standard deviation; **NS**: not significant at P > 0.05.

To the best of current knowledge, no studies have compared Dectin-1 expression based on sex in serum or appendix tissue from appendicitis patients. Al Madhoun et al. (2022) similarly examined Dectin-1 in adipose tissue and found no sex-related differences in protein levels, suggesting that its upregulation during tissue inflammation is sex-independent. Although their focus was metabolic inflammation, their results align with the present finding of equivalent Dectin-1 levels in male and female appendix tissue and serum.

4.3.6 Correlation between Dectin-1 in serum and tissue in patients

A weak positive correlation was observed between Dectin-1 levels in serum and tissue (r = 0.250, p = 0.410). However, this correlation was not statistically significant, indicating no meaningful association between serum and tissue concentrations of Dectin-1 in appendicitis patients, as table 4-13 shows.

Table (4-13) Correlation of dectin-1 in serum and tissue of patient.

Dectin-1		Serum	Tissue
Serum	Pearson Correlation	1	0.250
	Sig. (2-tailed)		0.410 NS
Tissue	Pearson Correlation	0.250	1
	Sig. (2-tailed)	0.410 NS	

NS: not significant at P > 0.05.

No studies have yet directly investigated the correlation between Dectin-1 levels in serum and appendix tissue in cases of acute appendicitis. This investigation discusses that gap for the first time. A weak positive correlation was noted; however, it lacked statistical significance, suggesting no clear relationship between systemic and local Dectin-1 expression in this condition.

4.3.7 Estimation of sIgA in serum and tissue in patients and AHC

Table 4-14 reveals that a p-value of 0.285 shows no significant difference in serum Secretory IgA levels between appendicitis patients (1.6076 ± 0.7493 ng/L) and the AHC group (2.0255 ± 1.7382 ng/L). This suggests that Secretory IgA levels are not significantly affected by appendicitis in this study.

Table (4-14): The concentrations of sIgA in serum of patient and AHC

Secretory IgA serum	M±SD µg\ml	P value
Patient	1.607612±0.749275	0.285 NS
Control	2.025496±1.738187	

SD: standard deviation; **NS:** not significant at P > 0.05.

This finding aligns with several previous studies that examined the relationship between sIgA and different groups. For example, Carvalho *et al*. (2022) found no significant differences in IgA serum levels between patients and controls, which is consistent with our results, suggesting no clear association between sIgA in serum and acute appendicitis.

The mean concentration of secretory IgA (sIgA) in patient serum was 1.607612 µg\ml, whereas in appendiceal tissue it was 3.103411 µg\ml, indicating a statistically significant difference (P = 0.000) as illustrated in Table 4-15.

Table (4-15): The concentrations of sIgA in serum and tissue of patient.

Secretory IgA	M±SD µg\ml	P value
Serum	1.607612±0.749275	0.000 S
Tissue	3.103411±1.305151	

SD: standard deviation; **S:** Significant at $P \leq 0.05$

The current study showed a significant decrease in sIgA concentrations in the serum of appendicitis patients compared to apparently healthy controls, while sIgA concentrations were significantly higher in tissue specimens compared to serum in the same patients.

This finding aligns with Mantis *et al*. (2011), who noted that SIgA can directly neutralize bacterial virulence factors and influence intestinal microbiota composition through both Fab-dependent and -independent mechanisms.

The increased SIgA levels in appendix tissue after appendectomy may be due to the high density of mucin and sIgA produced by B cells in the appendix mucosa, which creates a pro-microbiotic environment, reinforcing the appendix's role as a "safe house" for beneficial microbes (Kooij *et al*., 2016). These results are consistent with those of Andreu-Ballester *et al*. (2007), who found that sIgA levels in both serum and colon decreased significantly after appendectomy in individuals of various ages.

This decrease is attributed to the exposure of antigens to Peyer's patches and follicles, leading to lymphocyte proliferation and the generation of memory

cells that migrate back to the lamina propria, where they persist (Andreu-Ballester *et al*., 2007; Liu *et al*., 2017).

The results of the current study found no significant difference in secretory IgA (sIgA) concentrations between males and females in either serum (P = 0.718) or tissue (P = 0.644), with the mean serum sIgA level being slightly higher in females (1.6646 ± 0.701 µg\ml) compared to males (1.5506 ± 0.822 µg\ml), while the tissue level was marginally higher in males (3.2305 ± 1.1536 µg\ml) than in females (2.9764 ± 1.4818 µg\ml; however, the lack of statistical significance indicates that sex does not influence sIgA expression in this study population, as shown in Table 4-16.

Table (4-16) The concentrations of sIgA in serum and tissue specimens of patients according to sex.

Parameters	sIgA M±SD µg\ml		P value
	Male	Female	
Serum	1.55057±0.82183	1.66464±0.70097	0.718 NS
Tissue	3.2305±1.15363	2.9764±1.48176	0.644 NS

SD: standard deviation; **NS**: not significant at P > 0.05.

Although the differences in SIgA levels between males and females were not statistically significant in the present study, the observed trends suggest potential sex-related variations in immune responses. Serum SIgA levels were

higher in females, aligning with the findings of Klein and Flanagan (2016), who referenced data from Nigerian children aged 5–12 years, where males had higher serum IgA levels than females. While this finding reflects a specific population and age group, it supports the idea that immunoglobulin A expression may vary by sex. Additionally, Hodges-Simeon *et al*. (2019) reported that testosterone is positively associated with mucosal sIgA, whereas estradiol has a negative effect. This hormonal influence may explain the elevated tissue sIgA in males observed in this study, despite the lack of statistical significance.

4.3.8 Correlation of sIgA in serum and tissue of patient.

Table 4-17 presents the correlation analysis between serum and tissue levels of Secretory IgA (sIgA). The Pearson correlation coefficient between the two compartments is 0.320, with a two-tailed p-value of 0.128. As expected, the correlation coefficients for serum and tissue with themselves are both 1. The correlation value of 0.320 indicates a weak positive association between serum and tissue sIgA levels. However, since the p-value is greater than 0.05, the result is not statistically significant.

In practical terms, this weak and non-significant association implies that there is no consistent linear relationship between the concentrations of sIgA in the blood and those in appendix tissue. This suggests that systemic and mucosal sIgA responses in appendicitis may be regulated independently, as further supported by the differing trends observed in their mean values between sexes.

Table (4-17) Correlation of sIgA in serum and tissue of patient.

Secretory IgA		Serum	Tissue
Serum	Pearson Correlation	1	0.320
	Sig. (2-tailed)		0.128 NS
Tissue	Pearson Correlation	0.320	1
	Sig. (2-tailed)	0.128 NS	

NS: not significant at P > 0.05.

Sheikh-Mohamed *et al*. (2022) directly compared SARS-CoV-2–specific IgA responses in serum versus mucosal secretions (saliva) following mRNA vaccination. They found only a modest correlation between systemic and mucosal IgA at peak response (Pearson r ≈ 0.30–0.40), and noted that strong local (mucosal) IgA induction often occurred independently of serum levels. This finding is consistent with the present study's observation of a weak, non-significant correlation between serum and tissue sIgA (r = 0.320, p = 0.128) in appendicitis patients, suggesting that local tissue factors such as antigen exposure at the mucosal site are more influential in driving sIgA production than systemic signals. Therefore, serum sIgA may not reliably reflect mucosal/tissue immunity in localized inflammatory conditions.

Lin *et al.* (2018) demonstrated that fecal IgA levels in IBD patients rise markedly, while serum IgA remains within the normal range, indicating that mucosal stimuli drive local SIgA production independently of systemic levels. This finding parallels the present study's observation of a weak, non-significant correlation between serum and tissue sIgA levels in appendicitis patients.

4.4 Molecular study

4.4.1 DNA Extraction and determination of purity

DNA extraction Genomic DNA from blood specimens were extracted by using Geneaid DNA kit (Frozen Blood), and checked using Nano drop spectrophotometer at (260/280 nm), it was ranging between 1.8- 2.2, with purity average equal 2, and DNA concentration mean was 20 ng/µl.

4.4.2 Detection of TLR4 (rs4986790) A/G Polymorphism

The distribution of TLR4 rs4986790 A > G Polymorphism was detected by ARMS-PCR technique. At this locus there are three genotypes; GG, GA and AA. (AA) wild type homozygote was showed only A allele at 163bp. (GG) mutant type homozygote was showed only G allele at 209bp T-ARMS-PCR product, whereas the (AG) heterozygote were showed as both A and G allele at 163bp and 209bp respectively, Figure (4-4) The genotype distribution had no deviation from Hardy-Weinberg equilibrium in all study groups.

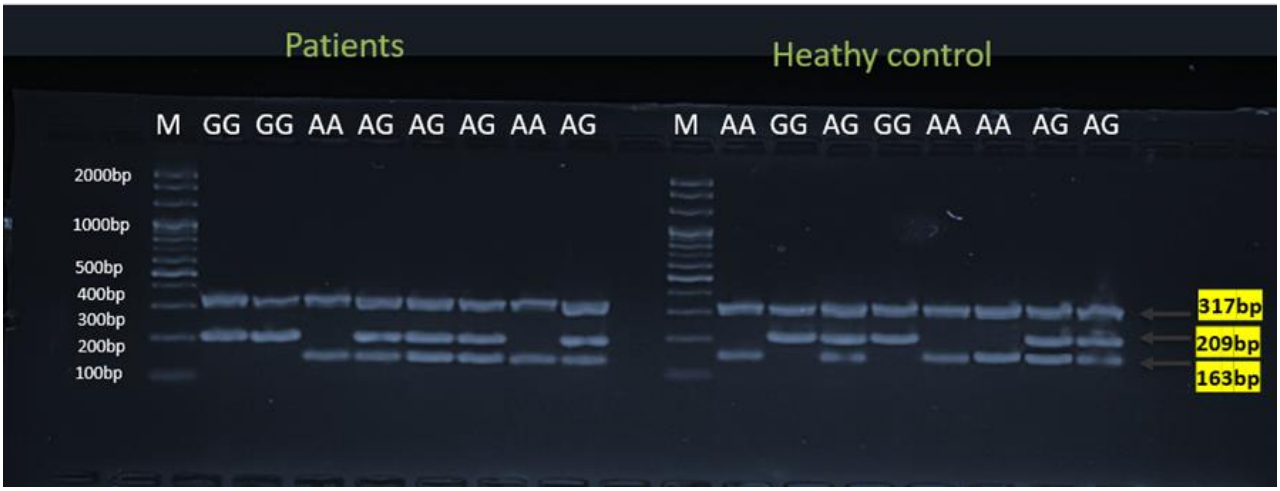


Figure (4-4): Agarose gel electrophoresis image that showed the T-ARMS-PCR product analysis for TLR4 rs4986790 A > G gene polymorphism FROM patients and apparently healthy control specimens. Where M: marker (2000-100bp). The lane (AA) wild type homozygote was showed only A allele at 163bp T-ARMS-PCR product. The lane (GG) mutant type homozygote was showed only G allele at 209bp T-ARMS-PCR product, whereas the (AG) heterozygote were showed as both A and G allele at 163bp and 209bp T-ARMS-PCR product. The outer internal control was observed at 317bp T-ARMS-PCR product.

4.4.3 Genotypic and Alleles Analysis for studied gene in patients and apparently healthy control.

The comparison of genotypes and allele frequencies concerning TLR4 rs4986790 A > G SNP between patients with appendectomy and apparently healthy control is shown in Table (4-18). Risk analysis revealed that the homozygous GG genotype was significant risk factor (OR=1.98), which means that patients with heterozygous AG genotype are approximately two time more liable to develop disease in comparison with other genotypes. Furthermore, a statistically significant association was found between the G

allele and the risk of appendicitis (OR = 2.025; 95% CI = 1.075–3.814; p = 0.028), suggesting that carriers of the G allele have a higher susceptibility to the condition.

Table (4-18) Distributions of Genotypes and Allele Frequency for TLR4 rs4986790 A > G in appendicitis patients and control

Genotype	Patients	AHC	P value	Odd ratio	CL 95%
AA	24 (48%)	34 (68%)	Reference		
AG	17 (34%)	11 (22%)	0.1841 NS	1.83	(0.75-4.4427)
GG	9 (18%)	5 (10%)	0.2550 NS	1.98	(0.611-6.37)
Total	50	50			
Alleles frequency					
A	65(0.65)	79(0.79)	0.028 S	Reference	
G	35(0.35)	21(0.21)		2.025	(1.075 -3.814)

S: Significant at P ≤ 0.05; **NS**: not significant at P > 0.05.

The findings of the current study are consistent with those of Rivera-Chavez *et al*. (2004), who reported that genetic variations in innate immunity genes, including TLR4, influence the severity of acute appendicitis. Their study showed that certain TLR4 polymorphisms were associated with more

severe outcomes, such as perforated or gangrenous appendicitis, likely due to impaired innate immune responses and delayed bacterial clearance.

Similarly, the present study identified the G allele of the TLR4 rs4986790 (Asp299Gly) polymorphism as a significant risk factor for appendicitis, with an odds ratio of 2.025, indicating a strong association with disease susceptibility. This polymorphism leads to a non-synonymous amino acid substitution in the third exon of the TLR4 gene, replacing aspartic acid with glycine at position 299, which is located in the extracellular domain. This substitution is known to reduce TLR4's ability to recognize lipopolysaccharides (LPS) from Gram-negative bacteria like *E.coli*, a common pathogen in appendicitis (Ohto *et al*., 2012; de Oliveira and Silva, 2012).

Silva *et al*. (2022) revealed a notable correlation between the G allele of rs4986790 and susceptibility to infectious diseases, however their research did not directly address appendicitis. Nonetheless, their findings correspond the idea that this mutation impairs TLR4 signaling and impairs the host's capacity to respond effectively to bacterial infections.

The TLR4 gene, located on chromosome 9q33.1, comprises three exons and is essential for non-specific immunity by identifying a diverse range of microbial ligands (Vaure and Liu, 2014). The rs4986790 A > G polymorphism (Asp299Gly) is among the most extensively examined variants and has been associated with the development of various diseases. Research indicates a correlation with increased susceptibility to pneumonia, inflammatory bowel

disease, Helicobacter pylori infection, and several types of cancer, as documented by Cai *et al*. (2015), Lin *et al*. (2019), and He and Jiang (2022). On the other hand, certain studies indicated a lack of association with conditions like asthma, coronary artery disease, and preeclampsia (Zhang et al., 2012; Jin et al., 2016; Guo et al., 2024).

There is also increasing evidence indicates that TLR4 polymorphism correlates with the onset of ulcerative colitis (UC) and Crohn's disease (CD), as the allele frequencies of the TLR4 Asp299Gly polymorphism were found to be significantly elevated in patients with UC and CD (Franchimont *et al*., 2004).

The serum levels of TLR4 rs4986790 (A/G) genotypes have been evaluated in appendicitis patients and compared to those in apparently healthy controls (AHC).The AA genotype showed identical mean serum TLR4 concentrations in both patients and controls (18.7219 ± 0.0264 ng/ml vs. 18.700 ± 0.04128 ng/ml), with no statistically significant difference (P = 0.155). For the AG genotype, the mean concentration in patients (18.7450 ± 0.03305 ng/ml) was slightly higher than in controls (18.7054 ± 0.02001 ng/ml), and this difference was statistically significant (P = 0.055). In contrast, the GG genotype showed a non-significant difference between patients (18.7179 ± 0.02497 ng/ml) and controls (18.3330 ± 0.53344 ng/ml) with a P-value of 0.075 as table 4-19 demonstrated.

Table (4-19) Concentration of TLR4 in serum according to SNPs in appendicitis patients and AHC Group Statistics

TLR4-rs4986790 A/G	TLR4 concentration ng\ml M±SD		P – value
	Serum patients	AHC	
AA	18.7219 ± 0.0264	18.700 ± 0.04128	0.155 NS
AG	18.7450 ± 0.03305	18.705 ± 0.02001	0.055 S
GG	18.7179 ± 0.02497	18.3330±0.53344	0.075 NS

SD: standard Deviation; **NS**: not significant at P > 0.05; **S:** Significant at P ≤ 0.05

The analysis of serum TLR4 concentrations among different rs4986790 genotypes revealed no statistically significant differences between appendicitis patients and healthy controls. The AA genotype appeared to have no effect on TLR4 expression, as concentrations were nearly identical across groups. The AG genotype showed a slightly higher concentration in patients, with a borderline significance, suggesting a potential but limited influence. In contrast, although the GG genotype showed a noticeable reduction in controls, this difference did not reach statistical significance. Overall, these findings indicate that the rs4986790 polymorphism may not play a major role in modulating serum TLR4 levels in appendicitis.

Mean ± standard deviation (M±SD) of TLR4 protein concentrations (ng/ml) in both the blood and appendix tissue of individuals with various TLR4 rs49867 (A/G) genotypes (AA, AG, GG) shown in Table 4-20, all genotype groups had statistically significant variations in serum and tissue levels ($P < 0.05$).

Table (4-20) Concentration of TLR4 in serum and tissue according to SNPs in appendicitis patients

TLR4-rs4986790 A/G	M±SD concentration ng\ml		P – value
	Serum patients	Tissue patient	
AA	18.721 ± 0.0264	18.626 ± 0.0520	0.000 S
AG	18.745 ± 0.0330	18.645 ± 0.828	0.012 S
GG	18.717 ± 0.0249	18.642 ± 0.0368	0.002 S

SD: standard Deviation; **S:** Significant at $P \leq 0.05$

Table 4-21 demonstrates the distribution of TLR4 rs4986790 genotypes (AA, AG, and GG) and allele frequencies (A and G) in male and female patients. The AA genotype serves as the reference group. The frequency of the AG genotype is comparable between males and females ($P = 0.6237$). The GG genotype exhibits a statistically significant disparity, occurring more frequently in males (30.43%) than in females (7.41%), with an odds ratio of 5.47 ($P = 0.0491$), suggesting that males are more predisposed to possess the GG genotype.

The G allele exhibits a markedly higher frequency in males (45.65%) compared to females (25.93%), with an odds ratio of 2.40 (P = 0.0413). This indicates that the G allele may correlate with sex disparities in this group.

Table (4-21) Distributions of Genotypes and Allele Frequency of TLR4-rs4986790 A/G genotypes and in appendicitis patients male and female

Alleles	Number / percentage		Odd ratio	P value
	Male	Female		
AA	9 (39.13%)	15 (55.56%)	Reference	
AG	7 (30.43%)	10 (37.04%)	0.7438(0.2279 - 2.4272)	0.6237 NS
GG	7 (30.43%)	2 (7.41%)	5.4688(1.0069-29.7015)	0.0491 S
Total	23	27		
Alleles frequency				
A allele	25 (54.35%)	40 (74.07%)	Reference	
G allele	21 (45.65)	14 (25.93%)	2.400(1.0352 - 5.5640)	0.0413 S

S: Significant at $P \leq 0.05$; **NS**: not significant at P > 0.05.

The current study indicated that the G allele increased in males; this might be due to changing bad living habits, such as smoking, high-salt and high-fat diets, lack of exercise, etc.

4.4.5 The association between ARMS-PCR finding and Immunological parameters (TLR4, IL-17, Dectin-1, SIgA) level in appendicitis patients

The association between ARMS-PCR finding and Immunological parameters (TLR4, IL-17, Dectin-1, and SIgA) levels in patients with appendicitis is shown in Table (4-22), the mean levels of TLR4 were 18.72 ± 0.026, 18.74 ± 0.033, and 18.71 ± 0.024 in patients with AA, AG, and GG genotypes respectively. No significant differences were observed among the genotypes (P = 0.169).

Similarly, the mean levels of IL-17 were 288.17 ± 155.36, 210.63 ± 79.37, and 252.37 ± 161.71 in AA, AG, and GG genotypes respectively, with no statistically significant difference between groups (P = 0.623). For Dectin-1, the mean levels were 422.84 ± 143.61, 522.42 ± 262.90, and 675.84 ± 242.20 in AA, AG, and GG genotypes respectively; again, the difference was not statistically significant (P = 0.233).

In contrast, SIgA levels showed a significant difference among the genotypes. The mean values were 0.886 ± 0.331, 1.5124 ± 0.4521, and 1.7600 ± 0.4447 in AA, AG, and GG genotypes respectively, with the highest level observed in the GG genotype group (P = 0.007)

Table (4-22): The association between ARMS-PCR finding and Immunological parameters (TLR4, IL-17, Dectin-1, SIgA) level in appendicitis patients

Immunological parameters	ARMS-PCR finding			
	AA genotype n = 24	AG genotype n = 17	GG genotype n = 9	P-value
TLR4 (ng/ml)				
Mean± SD	18.72 ± 0.026	18.74 ± 0.033	18.71 ± 0.024	**0.169** NS
IL-17 (ng/L)				
Mean± SD	288.17 ± 155.36	210.63 ± 79.372	252.37 ± 161.71	**0.623** NS
Dectin-1 (ng/L)				
Mean± SD	422.84 ± 143.61	522.42 ± 262.90	675.84 ± 242.20	**0.233** NS
SIgA (µg/ml)				
Mean± SD	0.886 ± 0.331	1.5124 ± 0.4521	1.7600 ± 0.4447	**0.007** S

SD: standard deviation; **S**: Significant at $P \leq 0.05$; **NS**: not significant at $P > 0.05$.

In this study, no significant differences were observed in TLR4 protein levels (ng/ml) across the genotypes of rs4986790 (P = 0.169), suggesting that the mutation may not significantly affect the overall expression of TLR4 in serum under normal conditions. This is consistent with previous studies indicating that the Asp299Gly mutation in TLR4 mainly impacts the functional responsiveness to microbial stimuli rather than altering protein expression levels (Arbour *et al*., 2000).

When examining IL-17 levels in relation to the rs4986790 polymorphism, a decrease was observed in individuals with AG and GG genotypes compared to the AA genotype, although the differences were not statistically significant. This pattern may indicate a possible functional impact of the TLR4 rs4986790 variation on mucosal immune responses, specifically regarding IL-17 generation. IL-17, a pro-inflammatory cytokine generated by Th17 cells, is tightly linked to TLR4-mediated immune activation. The reduced levels observed among AG and GG carriers may indicate impaired TLR4 signaling pathways.

This discovery aligns with data by Flores-Gonzalez *et al*. (2024), which indicated that people with the GG genotype demonstrated reduced TLR4 activation, leading to decreased production of pro-inflammatory cytokines including IL-6, TNF-α, and FasL. The rs4986790 mutation, especially in the GG genotype, induces structural alterations in the TLR4 protein, diminishes its interaction with downstream signaling molecules, and fosters a hyporesponsive immunological response. These molecular modifications might clarify the decreased IL-17 response observed in the current study.

Dectin-1 levels did not show any statistically significant differences across the rs4986790 genotypes. The average concentrations among those with AA, AG, and GG genotypes revealed no appreciable variations ($p = 0.233$). This result implies that, in regard to appendicitis, the TLR4 rs4986790 polymorphism may not be dramatically influencing Dectin-1.

The significant differences in SIgA levels considered across the TLR4 rs4986790 genotypes in the present study might be linked to changes in immunological signaling caused by this polymorphism. By preventing the attachment of pathogens to epithelial surfaces, secretory IgA (SIgA) is fundamental in mucosal immunity. Crucially pattern recognition receptor TLR4 helps to control mucosal immune responses, including SIgA production regulation.

According to Wells *et al*. (2011), TLR4 activation influences immunoglobulin production, including SIgA, through interactions with dendritic cells and T cells in the mucosal immune environment. Furthermore, Arbour *et al*. (2000) demonstrated that the rs4986790 polymorphism reduces TLR4 sensitivity to bacterial components, particularly lipopolysaccharides (LPS), potentially leading to altered mucosal immune responses. This reduced responsiveness may impact the production of mucosal antibodies like SIgA. Together, these findings support the notion that variations in TLR4, especially rs4986790, may influence SIgA levels, contributing to the immunological variability observed between genotypes.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

1. Appendicitis was most common among individuals aged 14–24 years, with a slightly higher occurrence in females than males, and it was more frequent in rural areas compared to urban areas.
2. *Escherichia coli* was the most commonly isolated bacterium from appendix tissue specimens.
3. No fungal growth was detected in appendicitis specimens, indicating that fungi are unlikely to play a role in the pathogenesis of the disease in the studied cases.
4. The appendicitis induced Toll like receptors 4 and increased, IL-17 and Dectin-1 in serum compared the control.
5. The concentrations of immunological parameters differed between serum and tissue specimens from the same patients.
6. The levels of IL-17, Dectin-1, and sIgA in patients were higher in tissue specimens than in serum.
7. Analysis of TLR4 rs4986790 A/G polymorphism using tetra-ARMS PCR revealed that the A allele may be protective, while the G allele was associated with increased risk of appendicitis.
8. Higher concentrations of TLR4 were detected in individuals with the AG genotype compared to other genotypes.
9. TLR4 polymorphism may influence mucosal immune responses through modulation of sIgA production.

Recommendations

1. Further investigation of the virulence factors of bacterial isolates from appendix tissues is recommended.
2. Study the IL-17 gene polymorphism in relation to appendicitis using tissue-based analysis.
3. Evaluate additional cytokines and immune markers in appendix tissue to better understand the local immune response.

REFERENCES

Abdullah, A. M., Abdullah, R. M., and Salman, S. L. (2009). Use of chromogenic agar in detection of urinary tract pathogens and antimicrobial susceptibility. *Journal of the Faculty of Medicine Baghdad*, *51*(1), 39-42. https://doi.org/10.32007/jfacmedbagdad.5111168

Abdurrazzaaq, A., Afuwape, O., Ademola, A., & Fasina, O. (2018). Bacterial pattern in acute appendicitis. *Annals of African Surgery, 15*(1). DOI: 10.4314/aas.v15i1.3

Al Bander, Z., Nitert, M. D., Mousa, A., & Naderpoor, N. (2020). The Gut Microbiota and Inflammation: An Overview. *International Journal of Environmental Research and Public Health*, *17*(20), 7618. https://doi.org/10.3390/ijerph17207618

Al Madhoun, A., Kochumon, S., Al-Rashed, F., Sindhu, S., Thomas, R., Miranda, L., Al-Mulla, F., & Ahmad, R. (2022). Dectin-1 as a Potential Inflammatory Biomarker for Metabolic Inflammation in Adipose Tissue of Individuals with Obesity. *Cells*, *11*(18), 2879. https://doi.org/10.3390/cells11182879

Al-Janabi, M. S., Al-Janabi, S. A., & Al-Mahdawi, A. M. (2019). Bacterial infections in patients with appendicitis in Hilla City, Iraq. *Medical Journal of Babylon*, 16(1), 55–57. https://doi.org/10.4103/MJBL.MJBL_4_19

AL-Khawaja, N. Q. A., & Al-Shabaa, K. F. H. (2021). Study Some Immunological Factor (IL-17, INF-γ, IL-8, and CA-12) as Predicator in

The Diagnosis of Appendicitis. *Al-Kufa University Journal for Biology*, *13*(3), 12-25. https://doi.org/10.36320/ajb/v17.i1

Alshawi, M. H. S. (2024). Appendicitis in Basrah City: An Observational Study. *J Gastro Hepato*, *10*(13), 1-4.

Al Sehlany, R. G., Al-Gazally, M. E., & Alshalah, M. A. (2022). Accuracy of C-reactive protein and procalcitonin in differentiating between complicated and non-complicated acute appendicitis patients in Babylon province. *Medical Journal of Babylon*, *19*(4), 709-713. https://doi.org/10.4103/MJBL.MJBL_230_22

Aluri, J., Cooper, M. A., & Schuettpelz, L. G. (2021). Toll-Like Receptor Signaling in the Establishment and Function of the Immune System. *Cells*, *10*(6), 1374. https://doi.org/10.3390/cells10061374

Andreu-Ballester, J. C., Pérez-Griera, J., Ballester, F., Colomer-Rubio, E., Ortiz-Tarín, I., & Otero, C. P. (2007). Secretory immunoglobulin A (sIgA) deficiency in serum of patients with GALTectomy (appendectomy and tonsillectomy). *Clinical Immunology*, *123*(3), 289-297. https://doi.org/10.1016/j.clim.2007.02.004

Arbour, N. C., Lorenz, E., Schutte, B. C., Zabner, J., Kline, J. N., Jones, M., ... & Schwartz, D. A. (2000). TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. *Nature genetics*, *25*(2), 187-191. https://doi.org/10.1038/76048

Arjomand Fard, N., Armstrong, H., Perry, T., & Wine, E. (2023). Appendix and Ulcerative Colitis: a Key to Explaining the Pathogenesis and Directing Novel Therapies?. *Inflammatory Bowel Diseases*, *29*(1), 151-160. https://doi.org/10.1093/ibd/izac106

Arlt, A., Bharti, R., Ilves, I., Häsler, R., Miettinen, P., Paajanen, H., ... & Herzig, K. H. (2015). Characteristic changes in microbial community composition and expression of innate immune genes in acute appendicitis. *Innate immunity*, *21*(1), 30-41. https://doi.org/10.1177/1753425913515033

Bazzaz, A.A., Lor, D.A.K. and Mahdi, N.B. (2018). Impact of Some Antibiotics on Bacteria Isolated from Appendices in Kirkuk Province, Iraq. *Advances in Bioscience and Biotechnology*, *9*, 1-10. https://doi.org/10.4236/abb.2018.91001

Bhangu, A., Søreide, K., Di Saverio, S., Assarsson, J. H., & Drake, F. T. (2015). Acute appendicitis: Modern understanding of pathogenesis, diagnosis, and management. *The Lancet*, *386*(10000), 1278–1287. https://doi.org/10.1016/S0140-6736(15)00275-5

Bi, Y., Yang, Q., Li, J., Zhao, X., Yan, B., Li, X., & Cui, H. (2022). The gut microbiota and inflammatory factors in pediatric appendicitis. Disease *Markers*, *2022*(1), 1059445. https://doi.org/10.1155/2022/1059445

Bollinger, R. R., Barbas, A. S., Bush, E. L., Lin, S. S., & Parker, W. (2007). Biofilms in the large bowel suggest an apparent function of the human vermiform appendix. *Journal of theoretical biology*, *249*(4), 826-831. https://doi.org/10.1016/j.jtbi.2007.08.032

Brown, G. D. (2006). Dectin-1: a signalling non-TLR pattern-recognition receptor. *Nature Reviews Immunology*, *6*(1), 33-43. https://doi.org/10.1038/nri1745

Cai, X., Fu, Y., & Chen, Q. (2015). Association between TLR4 A299G polymorphism and pneumonia risk: A meta-analysis. *Medical Science Monitor*, *21*, 625–631. https://doi.org/10.12659/MSM.892557

Carlini, V., Calcaterra, V., Decembrino, N., Rubert, L., Pasqua, N., Novario, M., ... & Pelizzo, G. (2016). Fungal appendicitis in immunocompromised children. Indications and contraindications for laparoscopic appendectomy: a report on 2 successful cases. *Journal of Pediatric Hematology/Oncology*, *38*(7), 581-584

Carvalho, N., Carolino, E., Carvalho, I., Lisboa, V., Coelho, H., Trindade, M., ... & Costa, P. (2022). Immunoglobulin serum concentrations do not correlate with acute appendicitis. *Portuguese Journal of Surgery*, (53), 37-45. https://doi.org/10.34635/rpc.970

.

Choy, K. T., Chiam, H. C., & Zardawi, I. (2019). Fungal appendicitis in a non-immunocompromised woman. *BMJ case reports, 12*(7), e229509. https://doi.org/10.1136/bcr-2019-229509

Ciesielska, A., Matyjek, M., & Kwiatkowska, K. (2021). TLR4 and CD14 trafficking and its influence on LPS-induced pro-inflammatory signaling. *Cellular and molecular life sciences, 78*, 1233-1261. https://doi.org/10.1007/s00018-020-03656-y

Cimpean, S., Barranquero, A. G., Surdeanu, I., Cadiere, B., & Cadiere, G. B. (2022). Implications of bacteriological study in complicated and uncomplicated acute appendicitis. *Annals of Coloproctology*. https://doi.org/10.3393/ac.2022.00157.0022

Constantin, M., Petrescu, L., Mătanie, C., Vrancianu, C. O., Niculescu, A. G., Andronic, O., & Bolocan, A. (2023). The Vermiform appendix and its pathologies. *Cancers, 15(*15), 3872. https://doi.org/10.3390/cancerkls15153872

Corr, S. C., Gahan, C. C., & Hill, C. (2008). M-cells: origin, morphology and role in mucosal immunity and microbial pathogenesis. FEMS Immunology & Medical Microbiology, 52(1), 2-12. https://doi.org/10.1111/j.1574-695X.2007.00359.x

D'Souza, N., Hicks, G., Beable, R., Higginson, A., & Rud, B. (2021). Magnetic resonance imaging (MRI) for diagnosis of acute appendicitis.

Cochrane Database of Systematic Reviews, (12). https://doi.org/10.1002/14651858.CD012028.pub2

D'souza, N., & Nugent, K. (2016). Appendicitis. *American family physician*, *93*(2), 142-143.

Danwang, C., Bigna, J. J., Tochie, J. N., Mbonda, A., Mbanga, C. M., Nzalie, R. N. T., ... & Essomba, A. (2020). Global incidence of surgical site infection after appendectomy: a systematic review and meta-analysis. *BMJ open*, *10*(2), e034266. doi: 10.1136/bmjopen-2019-034266

Das, S., Sharma, S., Kar, S., Sahu, S. K., Samal, B., & Mallick, A. (2010). Is inclusion of Sabouraud dextrose agar essential for the laboratory diagnosis of fungal keratitis*?*. *Indian journal of ophthalmology*, *58*(4), 281-285. DOI: 10.4103/0301-4738.64122

De Costa, A. (2022). The appendix-mucosal immunity and tolerance in the gut: consequences for the syndromes of appendicitis and its epidemiology. ANZ journal of surgery, 92(4), 653-660. https://doi.org/10.1111/ans.17522

de Morales, J. M. G. R., Puig, L., Daudén, E., Cañete, J. D., Pablos, J. L., Martín, A. O., ... & González-Gay, M. Á. (2020). Critical role of interleukin (IL)-17 in inflammatory and immune disorders: An updated review of the evidence focusing in controversies. *Autoimmunity reviews*, *19*(1), 102429. https://doi.org/10.1016/j.autrev.2019.102429

De Oliveira, J. G., & Silva, A. E. (2012). Polymorphisms of the TLR2 and TLR4 genes are associated with risk of gastric cancer in a Brazilian population. *World Journal of Gastroenterology*, *18*(11), 1235–1242. https://doi.org/10.3748/wjg.v18.i11.1235

de Vries, H. S., Plantinga, T. S., van Krieken, J. H., Stienstra, R., van Bodegraven, A. A., Festen, E. A., ... & de Jong, D. J. (2009). Genetic association analysis of the functional c. 714T> G polymorphism and mucosal expression of dectin-1 in inflammatory bowel disease. *PLoS One*, *4*(11), e7818. https://doi.org/10.1371/journal.pone.0007818

Delneste, Y., Beauvillain, C., & Jeannin, P. (2007). Innate immunity: structure and function of TLRs. *Medecine Sciences: M/S, 23(*1), 67-73. https://doi.org/10.1051/medsci/200723167

Di Saverio, S., Podda, M., De Simone, B., Ceresoli, M., Augustin, G., Gori, A., Boermeester, M., Sartelli, M., Coccolini, F., Tarasconi, A., et al. (2020). Diagnosis and treatment of acute appendicitis: 2020 update of the WSES Jerusalem guidelines. *World Journal of Emergency Surgery*, *15(*27). https://doi.org/10.1186/s13017-020-00306-3

Dixon, F., & Singh, A. (2020). Acute appendicitis. *Surgery (Oxford)*, *38(*6), 310-317. DOI: 10.1016/j.mpsur.2020.03.015

Elliver, M., Salö, M., Roth, B., Ohlsson, B., Hagander, L., & Gudjonsdottir, J. (2024). Associations between Th1-related cytokines

and complicated pediatric appendicitis. *Scientific Reports, 14(*1), 4613. https://doi.org/10.1038/s41598-024-53756-z

Elwan, T. H., Abdennour, A. E. K., Akl, U., Elshennawy, A. T. M., & Shatla, I. M. (2023). Unveiling the role of inflammatory mediators and gut microbiome in appendicitis: types and applications in clinical scoring. *Advanced Gut & Microbiome Research, 2023*(1), 1080495. https://doi.org/10.1155/2023/1080495

Felber, J., Gross, B., Rahrisch, A., Waltersbacher, E., Trips, E., Schröttner, P., & Schultz, J. (2023). Bacterial pathogens in pediatric appendicitis: a comprehensive retrospective study. *Frontiers in Cellular and Infection Microbiology, 13*, 1027769. https://doi.org/10.3389/fcimb.2023.1027769

Ferris, M., Quan, S., Kaplan, B. S., Molodecky, N., Ball, C. G., Chernoff, G. W., ... & Kaplan, G. G. (2017). The global incidence of appendicitis: a systematic review of population-based studies. *Annals of surgery*, *266*(2), 237-241. https://doi.org/10.1097/SLA.0000000000002188

Fikri, E., Alnaz, A. R. M., & Alnaz, F. M. (2023). Endothelial dysfunction in appendicitis. *In Endothelial Dysfunction-A Novel Paradigm.* IntechOpen. DOI: 10.5772/intechopen.107480

Flores-Gonzalez, J., Chavez-Galan, L., Falfán-Valencia, R., Roldán, I. B., Fricke-Galindo, I., Veronica-Aguilar, A., ... & Pérez-Rubio, G.

(2024). Variant rs4986790 of toll-like receptor 4 affects the signaling and induces cell dysfunction in patients with severe COVID-19. *International Journal of Infectious Diseases*, *138*, 102-109. https://doi.org/10.1016/j.ijid.2023.11.032

Forbes, B. A., Sahm, D. F., & Weissfeld, A. S. (2007). *Diagnostic microbiology* (pp. 288-302). St Louis: Mosby.

Franchimont, D., Vermeire, S., El Housni, H., Pierik, M., Van Steen, K., Gustot, T., ... & Rutgeerts, P. (2004). Deficient host–bacteria interactions in inflammatory bowel disease? The Toll-like receptor (TLR)-4 Asp299Gly polymorphism is associated with Crohn's disease and ulcerative colitis. *Gut*, *53*(7), 987–992. https://doi.org/10.1136/gut.2003.030205

Fujino, S., Andoh, A., Bamba, S., Ogawa, A., Hata, K., Araki, Y., ... & Fujiyama, Y. (2003). Increased expression of interleukin 17 in inflammatory bowel disease. *Gut*, *52*(1), 65-70. https://doi.org/10.1136/gut.52.1.65

Gaitanidis, A., Kaafarani, H. M., Christensen, M. A., Breen, K., Mendoza, A., Fagenholz, P. J., ... & Farhat, M. R. (2021). Association between NEDD4L variation and the genetic risk of acute appendicitis: a multi-institutional genome-wide association study. *JAMA surgery*, *156*(10), 917-923. doi:10.1001/jamasurg.2021.3303

Ghelardi, E. M. I. L. I. A., Pichierri, G., Castagna, B., Barnini, S., Tavanti, A. R. I. A. N. N. A., & Campa, M. A. R. I. O. (2008). Efficacy of Chromogenic Candida Agar for isolation and presumptive identification of pathogenic yeast species. *Clinical microbiology and infection*, *14*(2), 141–147. https://doi.org/10.1111/j.1469-0691.2007.01872.x

Golz, R. A., Flum, D. R., Sanchez, S. E., Liu, X., Donovan, C., & Drake, F. T. (2020). Geographic association between incidence of acute appendicitis and socioeconomic status. *JAMA surgery*, *155(*4), 330-338. doi:10.1001/jamasurg.2019.6030

Gorter, R. R., Wassenaar, E. C., de Boer, O. J., Bakx, R., Roelofs, J. J., Bunders, M. J., ... & Heij, H. A. (2017). Composition of the cellular infiltrate in patients with simple and complex appendicitis. *Journal of Surgical Research*, *214*, 190-196. https://doi.org/10.1016/j.jss.2017.02.062

Guan, L., Liu, Z., Pan, G., Zhang, B., Wu, Y., Gan, T., & Ouyang, G. (2023). The global, regional, and national burden of appendicitis in 204 countries and territories, 1990–2019: a systematic analysis from the Global Burden of Disease Study 2019. *BMC gastroenterology*, *23*(1), 44. https://doi.org/10.1186/s12876-023-02678-7

Guo, N., Tian, H., Song, T., & Peng, Y. (2024). Association of TLR4 gene rs4986790 and rs4986791 polymorphisms with asthma susceptibility: Meta-analysis and trial sequential analysis. *Annals of*

Saudi Medicine, *44*(3), 183–194. https://doi.org/10.5144/0256-4947.2024.183

Hallen-Adams, H. E., & Suhr, M. J. (2017). Fungi in the healthy human gastrointestinal tract. *Virulence*, *8*(3), 352–358. https://doi.org/10.1080/21505594.2016.1247140

Hama, N., Arif, H., & Ameen, S. (2021). Antibiotic susceptibility of isolated bacteria from cases of appendicitis in Sulaymaniyah city, Iraq. *Journal of Zankoy Sulaimani-Part A*, *23*(1), 7-16. DOI: 10.17656/jzs.10836

Hanson, N. B., & Lanning, D. K. (2008). Microbial induction of B and T cell areas in rabbit appendix. Developmental & Comparative *Immunology*, *32*(8), 980-991. https://doi.org/10.1016/j.dci.2008.01.013

He, W., & Jiang, M. (2022). TLR4 rs4986790 polymorphism confers risk to Helicobacter pylori infection in Zhejiang, China and its enlightenment to nursing care. *Journal of Clinical Laboratory Analysis*, *36*(6), e24453. https://doi.org/10.1002/jcla.24453

Hodges-Simeon, C. R., Asif, S., Gurven, M., Blackwell, A. D., & Gaulin, S. J. (2019). Testosterone is positively and estradiol negatively associated with mucosal immunity in Amazonian adolescents. *American Journal of Human Biology*, *31*(5), e23284. https://doi.org/10.1002/ajhb.23284

Hong Duc, P., Minh Xuan, N., Huu Thuyet, N., & Quang Huy, H. (2020). Intestinal obstruction due to acute appendicitis. *Case reports in gastroenterology*, *14*(2), 346-353. https://doi.org/10.1159/000508435

Huangfu, L., Li, R., Huang, Y., & Wang, S. (2023). The IL-17 family in diseases: from bench to bedside. *Signal Transduction and Targeted Therapy, 8(*1), 402. https://doi.org/10.1038/s41392-023-01620-3

Iliev, I. D. (2015). Dectin-1 exerts dual control in the gut. *Cell host & microbe*, *18*(2), 139-141. http://dx.doi.org/10.1016/j.chom.2015.07.010

Jin, S. H., Guan, X. Y., Liang, W. H., Bai, G. H., & Liu, J. G. (2016). TLR4 polymorphism and periodontitis susceptibility: *A meta-analysis. Medicine*, *95*(36), e4845. https://doi.org/10.1097/MD.0000000000004845

Jung, K. U., Yoon, K. W., Do, I. G., & Lee, D. (2022). Aspergillus appendicitis complicating chemotherapy of leukemia: A case report and review of the literature. *International Journal of Surgery Case Reports*, *100*, 107738. https://doi.org/10.1016/j.ijscr.2022.107738

Kalia, N., Singh, J., & Kaur, M. (2021). The role of dectin-1 in health and disease. Immunobiology, 226(2), 152071. https://doi.org/10.1016/j.imbio.2021.152071

Kamada, N., Seo, S.U., Chen, G.Y., & Núñez, G. (2013). Role of the gut microbiota in immunity and inflammatory disease. *Nature Reviews Immunology*, **13**, 321–335. https://doi.org/10.1038/nri3430.

Kanat, B. H., Solmaz, Ö. A., Bozdağ, P., Doğan, S., Kutluer, N., Kurt, F., & Öndes, B. (2021). Chronic appendicitis: the process from pre-diagnosis to pathology. *Eur Rev Med Pharmacol Sci*, *25*(24), 7898-7902. DOI: 10.26355/eurrev_202112_27639

Khoury, W., Szold, O., Soffer, D., Kariv, Y., Wasserlauf, R., Klausner, J. M., ... & Weinbroum, A. A. (2010). Prophylactic fluconazole does not improve outcome in patients with purulent and fecal peritonitis due to lower gastrointestinal perforation. *The American Surgeon*, *76*(2), 197-202.

Kim, H. J., Kim, H., Lee, J. H., & Hwangbo, C. (2023). Toll-like receptor 4 (TLR4): new insight immune and aging. *Immunity & Ageing*, *20*(1), 67. https://doi.org/10.1186/s12979-023-00383-3

Kim, H. M., Park, B. S., Kim, J. I., Kim, S. E., Lee, J., Oh, S. C., ... & Lee, J. O. (2007). Crystal structure of the TLR4-MD-2 complex with bound endotoxin antagonist Eritoran. *Cell*, *130*(5), 906-917. https://doi.org/10.1016/j.cell.2007.08.002

Klein, S. L., & Flanagan, K. L. (2016). Sex differences in immune responses. *Nature Reviews Immunology*, *16*(10), 626-638. https://doi.org/10.1038/nri.2016.90

Kooij, I. A., Sahami, S., Meijer, S. L., Buskens, C. J., & Te Velde, A. A. (2016). The immunology of the vermiform appendix: A review of the

literature. *Clinical & Experimental Immunology*, *186*(1), 1-9. https://doi.org/10.1111/cei.12821

Krzyzak, M., & Mulrooney, S. M. (2020). Acute appendicitis review: background, epidemiology, diagnosis, and treatment. *Cureus*, *12*(6). https://doi.org/10.7759/cureus.8562

Larbcharoensub, N., Boonsakan, P., Kanoksil, W., Wattanatranon, D., Phongkitkarun, S., Molagool, S., & Watchararanan, S. P. (2013). Fungal appendicitis: A case series and review of the literature. *Southeast Asian Journal of Tropical Medicine and Public Health*, *44*(4), 681–689. https://pubmed.ncbi.nlm.nih.gov/24050103

Lateef, A., Arshad, A., Misbah, J., & Hamayun, M. (2009). Role of Leucocyte Count in the Diagnosis of Acute Appencicitis. *Gomal Journal of Medical Sciences*, *7*(2). Retrieved from https://gjms.com.pk/index.php/journal/article/view/317

LeBlanc, J.G., Milani, C., de Giori, G.S., Sesma, F., van Sinderen, D., & Ventura, M. (2013). Bacteria as vitamin suppliers to their host: a gut microbiota perspective. *Current Opinion in Biotechnology*, *24*(2), 160–168. https://doi.org/10.1016/j.copbio.2012.08.005

Lee, C. K., Pelenyi, S. S., Fleites, O., Velez, V., Alaimo, K. L., Ramcharan, D. N., & Tiesenga, F. (2021). Chronic appendicitis, the lesser-known form of appendiceal inflammation: a case report. *Cureus*, *13*(11). DOI:10.7759/cureus.19718

Lee, M. S., Sulit, A., Frizelle, F., & Purcell, R. (2022). The microbiome in adult acute appendicitis. *Gut Microbiome*, *3*, e8. doi:10.1017/gmb.2022.7

Li, X., Zhang, S., Guo, G., Han, J., & Yu, J. (2022). Gut microbiome in modulating immune checkpoint inhibitors. *EBioMedicine*, *82*. https://doi.org/10.1016/j.ebiom.2022.104163

Li, Y., Jin, L., & Chen, T. (2020). The effects of secretory IgA in the mucosal immune system. *BioMed research international*, *2020*(1), 2032057. https://doi.org/10.1155/2020/2032057

Lin, R., Chen, H., Shu, W., Sun, M., Fang, L., Shi, Y., ... & Liu, Z. (2018). Clinical significance of soluble immunoglobulins A and G and their coated bacteria in feces of patients with inflammatory bowel disease. *Journal of translational medicine*, *16*, 1-15. https://doi.org/10.1186/s12967-018-1723-0

Lin, Z., Huang, S., Sun, J., Xie, B., & Zhong, Y. (2019). Associations between TLR4 polymorphisms and open angle glaucoma: A meta-analysis. *BioMed Research International*, *2019*, Article ID 6707650. https://doi.org/10.1155/2019/6707650

Liu, S., Pei, F., Wang, X., Li, D., Zhao, L., Song, Y., Chen, Z., & Liu, B. (2017). The immune impact of mimic endoscopic retrograde appendicitis therapy and appendectomy on rabbits of acute

appendicitis. *Oncotarget*, *8*(39), 66528–66539. https://doi.org/10.18632/oncotarget.16236

Lobionda, S., Sittipo, P., Kwon, H. Y., & Lee, Y. K. (2019). The Role of Gut Microbiota in Intestinal Inflammation with Respect to Diet and Extrinsic Stressors. *Microorganisms*, *7*(8), 271. https://doi.org/10.3390/microorganisms7080271

Lozupone, C. A., Stombaugh, J. I., Gordon, J. I., Jansson, J. K., & Knight, R. (2012). Diversity, stability and resilience of the human gut microbiota. *Nature*, *489*(7415), 220-230. https://doi.org/10.1038/nature11550

Lu, Y. C., Yeh, W. C., & Ohashi, P. S. (2008). LPS/TLR4 signal transduction pathway. *Cytokine*, *42*(2), 145-151. https://doi.org/10.1016/j.cyto.2008.01.006

MacFaddin, J. F. (2000). Biochemical tests for identification of medical bacteria, williams and wilkins. *Philadelphia, PA*, *113*(7).

Madhkoor, H. A., Almazini, M. A., & Al Mallak, M. K. (2024). Determination genes of Beta-lactamase in E. coli and histological state for appendicitis patients in Basra province. *Al-Kunooze Scientific Journal*, *8*(1), 1-20.

Mahdi, D. S., & AlSaimary, I. E. (2024a). Molecular detection of Toll-like receptors 1, 4, and 5 genes among patients with appendicitis.

Anaesthesia, Pain & Intensive Care, 28(6), 1050-1060. https://doi.org/10.35975/apic.v28i6.2622

Mahdi, D. S., Alsaimary, I. E., & Hoka, S. I. I. (2024b). Toll-like (Tlrs 1, 4, 5) Immunodetection of Some Toll-like Receptors as an Immune Response to Patients with Acute Appendicitis and Its Effact on Clinical Features in Basra/Iraq. *Journal of Medical Genetics and Clinical Biology*, *1*(9), 23-36. https://doi.org/10.61796/jmgcb.v1i9.919

Majrashi, S. A., Almalki, M., & Bardisi, A. (2018). Retrospective study to explore a new predictor for the early diagnosis of perforated acute appendicitis. *J Clin Case Stu, 3*(5), 11-13. http://dx.doi.org/10.16966/2471-4925.179

Mantis, N. J., Rol, N., & Corthésy, B. J. (2011). Secretory IgA's complex roles in immunity and mucosal homeostasis in the gut. *Mucosal Immunology*, *4(*6), 603-611. https://doi.org/10.1038/mi.2011.41

Mata-Martínez, P., Bergón-Gutiérrez, M., & Del Fresno, C. (2022). Dectin-1 signaling update: new perspectives for trained immunity. *Frontiers in immunology*, *13*, 812148. https://doi.org/10.3389/fimmu.2022.812148

Mei, J. (2014). Dried blood spot sample collection, storage, and transportation. Dried blood spots: Applications and techniques, 21-31

Mills, K. H. (2023). IL-17 and IL-17-producing cells in protection versus pathology. Nature Reviews Immunology, 23(1), 38-54. https://doi.org/10.1038/s41577-022-00746-9

Molteni, M., Gemma, S., & Rossetti, C. (2016). The role of toll-like receptor 4 in infectious and noninfectious inflammation. *Mediators of inflammation*, *2016(*1), 6978936. https://doi.org/10.1155/2016/6978936

Moris, D., Paulson, E. K., & Pappas, T. N. (2021). Diagnosis and management of acute appendicitis in adults: a review. Jama, 326(22), 2299-2311. https://doi.org/10.1001/jama.2021.20502

Mörbe, U. M., Jørgensen, P. B., Fenton, T. M., von Burg, N., Riis, L. B., Spencer, J., & Agace, W. W. (2021). Human gut-associated lymphoid tissues (GALT); diversity, structure, and function. *Mucosal immunology*, *14(*4), 793-802. https://doi.org/10.1038/s41385-021-00389-4

Nouri, Y., Weinkove, R., & Perret, R. (2021). T-cell intrinsic Toll-like receptor signaling: implications for cancer immunotherapy and CAR T-cells. *Journal for immunotherapy of cancer*, *9*(11), e003065. https://doi.org/10.1136/jitc-2021-003065

O'Day, D. M., Akrabawi, P. L., Head, W. S., & Ratner, H. B. (1979). Laboratory isolation techniques in human and experimental fungal infections. *American journal of ophthalmology*, *87*(5), 688-693. https://doi.org/10.1016/0002-9394(79)90305-2

Ohmann, C., Franke, C., Kraemer, M., & Yang, Q. (2002). Status report on epidemiology of acute appendicitis. Der Chirurg; Zeitschrift fur alle Gebiete der operativen Medizen, 73(8), 769-776. https://doi.org/10.1007/s00104-002-0512-7

Ohle, R., O'Reilly, F., O'Brien, K. K., Fahey, T., & Dimitrov, B. D. (2011). The Alvarado score for predicting acute appendicitis: a systematic review. *BMC medicine, 9*, 1-13.

Oh, S. J., Pimentel, M., Leite, G. G., Celly, S., Villanueva-Millan, M. J., Lacsina, I., ... & Pimentel, M. (2020). Acute appendicitis is associated with appendiceal microbiome changes including elevated Campylobacter jejuni levels. *BMJ open gastroenterology*, *7*(1).

Ohto, U., Yamakawa, N., Akashi-Takamura, S., Miyake, K., & Shimizu, T. (2012). Structural analyses of human Toll-like receptor 4 polymorphisms D299G and T399I. *Journal of Biological Chemistry, 287(48), 40611–40617*. https://doi.org/10.1074/jbc.M112.404608

Park, H. C., Kim, M. J., & Lee, B. H. (2017). Randomized clinical trial of antibiotic therapy for uncomplicated appendicitis. British Journal of Surgery, 104(12), 1785–1790. https://doi.org/10.1002/bjs.10660

Peeters, T., Houben, B., Cools, P., Thys, Y., D'Onofrio, V., Martens, S., ... & Gyssens, I. C. (2023). An observational study on lifestyle and environmental risk factors in patients with acute appendicitis. *Heliyon*, *9*(4). https://doi.org/10.1016/j.heliyon.2023.e15131

Peeters, T., Martens, S., D'Onofrio, V., Stappers, M. H., van der Hilst, J. C., Houben, B., ... & Gyssens, I. C. (2020). An observational study of innate immune responses in patients with acute appendicitis. *Scientific RepoRtS, 10(*1), 17352. https://doi.org/10.1038/s41598-020-73798-3

Peksöz, R., & Bayar, B. (2021). The role of complete blood count parameters in diagnosing acute appendicitis and measuring the severity of inflammation. *Ulusal Travma Ve Acil Cerrahi Dergisi-Turkish Journal Of Trauma & Emergency Surgery, 27*(6). DOI: 10.14744/tjtes.2020.69195

Petruzziello, C., Saviano, A., & Ojetti, V. (2023). Probiotics, the immune response and acute appendicitis: a review. *Vaccines, 11*(7), 1170. https://doi.org/10.3390/vaccines11071170

Pietrzak, B., Tomela, K., Olejnik-Schmidt, A., Mackiewicz, A., & Schmidt, M. (2020). Secretory IgA in Intestinal Mucosal Secretions as an Adaptive Barrier against Microbial Cells. *International Journal of Molecular Sciences, 21*(23), 9254. https://doi.org/10.3390/ijms21239254

Podany, A. B., Tsai, A. Y., & Dillon, P. W. (2017). Acute appendicitis in pediatric patients: an updated narrative review. *J Clin Gastroenterol Treat, 3*(1), 1-9. https://doi.org/10.23937/2469-584X/1510042

PÉTerfi, Z., Kovács, K., Antal, A., & KocsiDEs, B. (2006). *Anti-lipopolysaccharide antibodies in acute appendicitis detected by ELISA.*

Apmis, *114*(4), 265-269. https://doi.org/10.1111/j.1600-0463.2006.apm_346.x

Ramrao, L. Y., Gajbhiye, V., Vaidya, V. P., Akther, M. J., & Padmawar, M. (2020). Role of c reactive protein in acute appendicitis: A cross-sectional study. *International Journal of Current Research and Review*, *12*(20), 66-69. http://dx.doi.org/10.31782/IJCRR.2020.12208

Reismann, M. (2022). A concise pathophysiological model of acute appendicitis against the background of the COVID-19 pandemic. *Frontiers in Pediatrics*, *10*, 908524. https://doi.org/10.3389/fped.2022.908524

Ricaño-Ponce, I., Peeters, T., Matzaraki, V., Houben, B., Achten, R., Cools, P., ... & Kumar, V. (2022). Impact of Human Genetic Variation on C-Reactive Protein Concentrations and Acute Appendicitis. *Frontiers in Immunology*, *13*, 862742. https://doi.org/10.3389/fimmu.2022.862742

Riedel, S., Hobden, J. A., Miller, S., Morse, S. A., Mietzner, T. A., Detrick, B., Mitchell, T. G., Sakanari, J. A., Hotez, P., & Mejia, R. (2019). *Jawetz, Melnick, & Adelberg's Medical Microbiology* (28th ed., p. 138). McGraw-Hill Education.

Rivera-Chavez, F. A., Peters-Hybki, D. L., Barber, R. C., Lindberg, G. M., Jialal, I., Munford, R. S., & O'Keefe, G. E. (2004). Innate immunity genes influence the severity of acute appendicitis. *Annals of surgery*, *240*(2), 269-277. DOI: 10.1097/01.sla.0000133184.10676.26

Rosas, M., Liddiard, K., Kimberg, M., Faro-Trindade, I., McDonald, J. U., Williams, D. L., ... & Brown, G. D. (2008). The induction of inflammation by Dectin-1 in vivo is dependent on myeloid cell programming and the progression of phagocytosis. *Journal of Immunology*, *181*(5), 3549–3557. https://doi.org/10.4049/jimmunol.181.5.3549

Rubér, M., Andersson, M., Petersson, B. F., Olaison, G., Andersson, R. E., & Ekerfelt, C. (2010). Systemic Th17-like cytokine pattern in gangrenous appendicitis but not in phlegmonous appendicitis. *Surgery*, *147*(3), 366-372. https://doi.org/10.1016/j.surg.2009.09.039

Saleem, Z. G., & Alkawaz, H. S. (2021). Evaluation of the Ohmann Scoring System in the Diagnosis of Acute Appendicitis in Al-Yarmouk Teaching Hospital. *Indian Journal of Forensic Medicine & Toxicology*, *15*(3), 4588-4593. DOI**:** 10.37506/ijfmt.v15i3.16014

Sambrook, J.F. and Russell, D.W.(2001). Cold Spring Harbor Laboratory Press, , 2100 pp., soft cover.

Schumpelick, V., Dreuw, B., Ophoff, K., & Prescher, A. (2000). Appendix and cecum: *embryology, anatomy, and surgical applications. Surgical Clinics*, *80*(1), 295-318. https://doi.org/10.1016/S0039-6109(05)70407-2

Sekirov, I., Russell, S. L., Antunes, L. C. M., & Finlay, B. B. (2010). Gut microbiota in health and disease. *Physiological reviews*. https://doi.org/10.1152/physrev.00045.2009

Shaker, Z. N., Mahdi, D. S., & Alsaimary, I. E. (2024). Demographical Study of Appendicitis Patients in Basra/Iraq. *European Journal of Medical and Health Sciences*, *6*(4), 1-5. 10.24018/ejmed.2024.6.4.2136

Sheikh-Mohamed, S., Isho, B., Chao, G. Y., Zuo, M., Cohen, C., Lustig, Y., ... & Gommerman, J. L. (2022). Systemic and mucosal IgA responses are variably induced in response to SARS-CoV-2 mRNA vaccination and are associated with protection against subsequent infection. *Mucosal immunology*, *15(*5), 799 808.https://doi.org/10.1038/s41385-022-00511-0

Shnawa, I. M. S., & Abd, F. G. (2005). The role of carbohydrate binding complement components: The lectins in plotting the immunophyletic tree of vertebrates. Al-Qadisiya.

Silva, M. J. A., Santana, D. S., de Oliveira, L. G., Monteiro, E. O. L., & Lima, L. N. G. C. (2022). The relationship between 896A/G (rs4986790) polymorphism of TLR4 and infectious diseases: A meta-analysis. *Frontiers in Genetics*, *13*, 1045725. https://doi.org/10.3389/fgene.2022.1045725

Simmering, J. E., Polgreen, L. A., Talan, D. A., Cavanaugh, J. E., & Polgreen, P. M. (2022). Association of appendicitis incidence with

warmer weather independent of season. *JAMA network open*, *5*(10), e2234269-e2234269. doi:10.1001/jamanetworkopen.2022.34269

Sippola, S., Haijanen, J., Grönroos, J., Rautio, T., Nordström, P., Rantanen, T., ... & Salminen, P. (2021). Effect of oral moxifloxacin vs intravenous ertapenem plus oral levofloxacin for treatment of uncomplicated acute appendicitis: the APPAC II randomized clinical trial. *Jama*, *325*(4), 353-362. doi:10.1001/jama.2020.23525

Siraj, F., Qureshi, M. A., Ahmad, M., Siddiqi, O., & Hassan, B. (2020). Relationship between fiber in diet and acute appendicitis. *In Medical Forum Monthly* (Vol. 31, No. 9**).**

Snyder, M. J., Guthrie, M., & Cagle, S. (2018). Acute Appendicitis: Efficient Diagnosis and Management. *American family physician*, *98*(1), 25–33.

Stankovic, N., Surbatovic, M., Stanojevic, I., Simić, R., Djuricic, S., Milickovic, M., ... & Vojvodic, D. (2019). Possible cytokine biomarkers in pediatric acute appendicitis. *Italian journal of pediatrics*, *45*, 1-9. https://doi.org/10.1186/s13052-019-0726-7

Sutherland, D. B., & Fagarasan, S. (2012). IgA synthesis: a form of functional immune adaptation extending beyond gut. Current opinion in immunology, 24(3), 261-268. https://doi.org/10.1016/j.coi.2012.03.005

Su, W., Yang, T., Hu, X. J., Song, J., He, J. J., Huang, D., Zhang, B., Zhao, X. J., & Tang, F. (2025). Relationship between peripheral blood

endotoxin, toll-like receptor 4 expression, and postoperative infection following surgery for acute appendicitis. *World journal of gastrointestinal surgery*, *17*(4), 104443. https://doi.org/10.4240/wjgs.v17.i4.104443

Swanson, L., Katkar, G. D., Tam, J., Pranadinata, R. F., Chareddy, Y., Coates, J., ... & Ghosh, P. (2020). TLR4 signaling and macrophage inflammatory responses are dampened by GIV/Girdin. *Proceedings of the National Academy of Sciences*, *117(*43), 26895-26906. https://doi.org/10.1073/pnas.2011667117

Tartar, T., Akdeniz, I., Onalan, E., & others. (2022). Investigation of VGLL3 and sub-target genes in the aetiology of acute appendicitis. *Research Square*. https://doi.org/10.21203/rs.3.rs-2392719/v1

The, S. M. L., Schreurs, R. R. C. E., Drewniak, A., Bakx, R., de Meij, T. G. J., Budding, A. E., Poort, L., Cense, H. A., Heij, H. A., van Heurn, L. W. E., Gorter, R. R., & Bunders, M. J. (2024). Enhanced Th17 responses in the appendix of children with complex compared to simple appendicitis are associated with microbial dysbiosis. *Frontiers in immunology*, *14*, 1258363. https://doi.org/10.3389/fimmu.2023.1258363.

Ursell, L. K., Metcalf, J. L., Parfrey, L. W., & Knight, R. (2012). Defining the human microbiome. *Nutrition reviews*, *70*(suppl_1), S38-S44. doi:10.1111/j.1753-4887.2012.00493.x.

Vaure, C., & Liu, Y. (2014). A comparative review of toll-like receptor 4 expression and functionality in different animal species. *Frontiers in Immunology*, *5*, 316. https://doi.org/10.3389/fimmu.2014.00316

Velikova, T., Kaouri, I. E., Bakopoulou, K., Gulinac, M., Naydenova, K., Dimitrov, M., ... & Lazova, S. (2024). Mucosal Immunity and Trained Innate Immunity of the Gut. *Gastroenterology Insights*, *15*(3), 661-675.

Vitetta, L., Chen, J., & Clarke, S. (2019). The vermiform appendix: an immunological organ sustaining a microbiome inoculum. *Clinical Science*, *133*(1), 1-8. https://doi.org/10.1042/CS20180956

Wahhab, R. A. S. A., & Mohammed, L. K. (2024). Incidental gynecological conditions in patients presented with acute appendicitis. *Medical Journal of Babylon*, *21*(2), 259–262. https://doi.org/10.4103/MJBL.MJBL_153_23

Wang, K., Huang, H., Zhan, Q., Ding, H., & Li, Y. (2024). Toll-like receptors in health and disease. *MedComm*, *5(*5), e549. https://doi.org/10.1002/mco2.549

Wang, Y., Spatz, M., Da Costa, G., Michaudel, C., Lapiere, A., Danne, C., & Richard, M. L. (2022). Deletion of both Dectin-1 and Dectin-2 affects the bacterial but not fungal gut microbiota and susceptibility to colitis in mice. *Microbiome*, *10*(1), 91. https://doi.org/10.1186/s40168-022-01273-4

Wells, J. M., Rossi, O., Meijerink, M., & van Baarlen, P. (2011). Epithelial crosstalk at the microbiota–mucosal interface. *Proceedings of the national academy of sciences*, *108*(supplement_1), 4607-4614. J.M. https://doi.org/10.1073/pnas.1000092107

Wicherska-Pawłowska, K., Wróbel, T., & Rybka, J. (2021). Toll-Like Receptors (TLRs), NOD-Like Receptors (NLRs), and RIG-I-Like Receptors (RLRs) in Innate Immunity. TLRs, NLRs, and RLRs Ligands as Immunotherapeutic Agents for Hematopoietic Diseases. *International Journal of Molecular Sciences*, *22*(24), 13397. https://doi.org/10.3390/ijms222413397

Woof, J. M., & Russell, M. W. (2011). Structure and function relationships in IgA. *Mucosal immunology*, *4*(6), 590-597. https://doi.org/10.1038/mi.2011.39

Zachos, K., Kolonitsiou, F., Panagidis, A., Gkentzi, D., Fouzas, S., Alexopoulos, V., & Sinopidis, X. (2023). Association of the bacteria of the vermiform appendix and the peritoneal cavity with complicated acute appendicitis in children. Diagnostics, 13(11), 1839.

Zahra'a, A. A., Idan, E. M., & Ardalan, N. M. (2023). Role of IL-37 and Dectin-1 during Toxoplasmosis. Baghdad Science Journal, 20(3), 0746-0746.DOI: 10.21123/bsj.2022.7618

Zahrychuk, O. M., Mykhailyshyn, H. I., Volch, I. R., Klumnyuk, S. I., & Romanyuk, L. B. (2023). Species characteristics of causative

agents of acute appendicitis in children and determination of their susceptibility to antibiotics. Mikrobiolohichnyi Zhurnal, 85(3), 22-31.

Zamyatina, A., & Heine, H. (2020). Lipopolysaccharide Recognition in the Crossroads of TLR4 and Caspase-4/11 Mediated Inflammatory Pathways. Frontiers in immunology, 11, 585146. https://doi.org/10.3389/fimmu.2020.585146

Zhang, K., Zhang, L., Zhou, B., Wang, Y., Song, Y., Rao, L., & Zhang, L. (2012). Lack of association between TLR4 Asp299Gly polymorphism and atherosclerosis: Evidence from meta-analysis. Thrombosis Research, 130(4), e203–e208. https://doi.org/10.1016/j.thromres.2012.07.008

Zhao, L., Fang, X. D., Jia, W., & Bian, Z. X. (2020). Managing chronic diarrhea from a gut microbiota-bile acid perspective. Clinical and translational gastroenterology, 11(8), e00208.

APPENDIX

bioMérieux Customer:

Microbiology Chart Report

Printed October 27, 2024 1:41:36 PM AST

Patient Name:
Location:
Lab ID: 24102613

(13) ميس

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Escherichia coli
BP Infection Site:

Source:

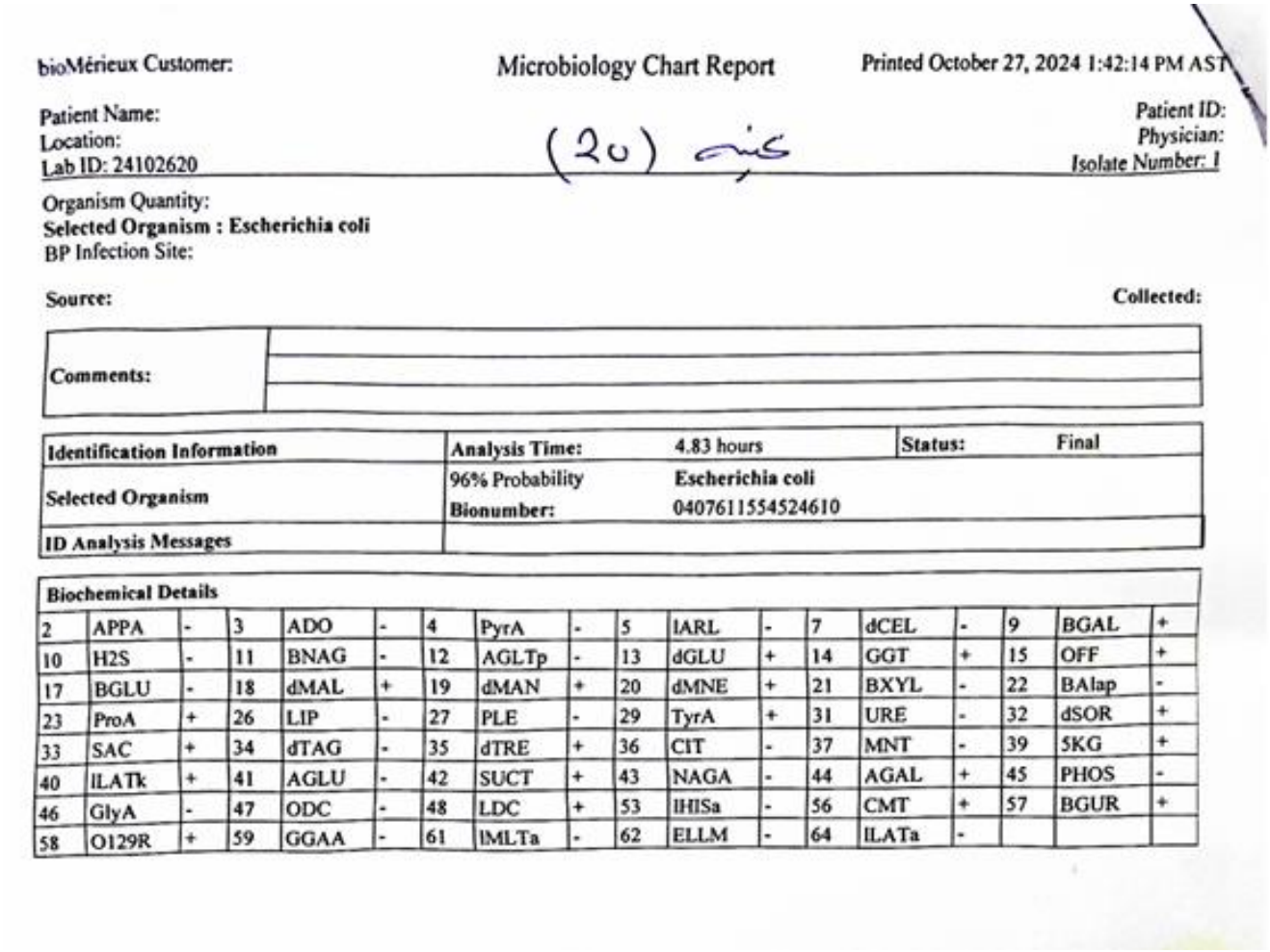
Collected:

Comments:	

Identification Information	**Analysis Time:**	3.87 hours	**Status:** Final
Selected Organism	99% Probability	**Escherichia coli**	
	Bionumber:	0005610540466611	
ID Analysis Messages			

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	-	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	+
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	lLATk	-	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	+
46	GlyA	-	47	ODC	+	48	LDC	+	53	lHISa	-	56	CMT	+	57	BGUR	+
58	O129R	+	59	GGAA	-	61	lMLTa	-	62	ELLM	+	64	lLATa	-			



bioMérieux Customer:

Microbiology Chart Report

Printed October 27, 2024 1:42:14 PM AST

Patient Name:
Location:
Lab ID: 24102620

(20) ais

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Escherichia coli
BP Infection Site:

Source:

Collected:

Comments:	

Identification Information	**Analysis Time:**	4.83 hours	**Status:**	Final
Selected Organism	96% Probability	**Escherichia coli**		
	Bionumber:	0407611554524610		
ID Analysis Messages				

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	-	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	+
40	lLATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	+	53	lHISa	-	56	CMT	+	57	BGUR	+
58	O129R	+	59	GGAA	-	61	lMLTa	-	62	ELLM	-	64	lLATa	-			

bioMérieux Customer:

Microbiology Chart Report

Printed November 7, 2024 10:13:55 AM AST

Patient Name: jamil, zahra
Location:
Lab ID: 20241161 3

Patient ID: 20241161 3
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Klebsiella pneumoniae ssp pneumoniae

Source:

Collected:

Comments:	

Identification Information	**Analysis Time:**	3.85 hours	**Status:**	Final
Selected Organism	99% Probability	**Klebsiella pneumoniae ssp pneumoniae**		
	Bionumber:	6607734753565010		
ID Analysis Messages				

Biochemical Details																
2	APPA	-	3	ADO	+	4	PyrA	+	5	lARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	+	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	+	29	TyrA	+	31	URE	+	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	+	39	5KG	-
40	lLATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	+
46	GlyA	+	47	ODC	-	48	LDC	+	53	lHISa	-	56	CMT	-	57	BGUR	-
58	O129R	+	59	GGAA	-	61	lMLTa	-	62	ELLM	-	64	lLATa	-			

الخلاصة

تُعدّ الزائدة الدودية من أكثر حالات الطوارىء الجراحية ، خصوصًا لدى المراهقين والشباب. تنشأ الإصابة نتيجة التهاب الزائدة الدودية، غالبًا بسبب انسداد في تجويفها نتيجة تحفيز عدوى بكتيرية وتنشيط الاستجابة المناعية. هدفت الدراسة الحالية إلى تقييم دور العدوى البكتيرية والفطرية في التهاب الزائدة الدودية، ودراسة علاقتها بعدد من المؤشرات المناعية المهمة، وهي مستقبلات-Toll like receptor 4 (TLR4)، الإنترلوكين-17(IL-17) ، ديكتين-1(Dectin-1) ، والأجسام المضادة المناعية الإفرازية (SIgA) في كل من الدم وأنسجة الزائدة.

أُجريت هذه الدراسة في مستشفى الحلة التعليمي خلال الفترة من أيلول 2024 إلى كانون الأول 2024، وشملت الدراسة (100) مريض مشخصين بالتهاب الزائدة الدودية، وتراوحت أعمارهم بين (14–65) سنة، إضافة إلى (50) شخصاً من الأصحاء ظاهرياً كمجموعة سيطرة (AHC). تم الحصول على عينات دم وأنسجة الزائدة من المرضى، في حين جُمعت عينات دم فقط من مجموعة السيطرة. وقد تبيّن أن أعلى نسبة من المرضى كانت ضمن الفئة العمرية (14–24) سنة وبنسبة (48%). كما سُجِّلت نسبة أعلى قليلاً لدى الإناث (54%) مقارنة بالذكور (46%). في حين شكّل سكان المناطق الريفية الغالبية العظمى من المرضى بنسبة (66%).

تمت زراعة عينات الزائدة الدودية على أوساط غذائية مختلفة للكشف عن وجود البكتيريا أو الفطريات. وقد أظهرت جميع العينات نموًا بكتيريًا، وكانت جميع العزلات من البكتيريا سالبة الصبغة بصبغة كرام، حيث شكّلت بكتيريا الإشريكية القولونية النسبة الأعلى (88%)، تلتها الكلبسيلا الرئوية بنسبة (12%). تم تحديد نوع البكتيريا باستخدام الطرق التشخيصية المعتمدة، والتي شملت الزرع على الأوساط المناسبة والفحص المجهري، وتم تأكيد التشخيص باستخدام جهاز الفايتك VITEK 2 Compact System. في المقابل، لم يُلاحظ أي نمو فطري في العينات، مما يشير إلى عدم وجود دور للفطريات في مسببات التهاب الزائدة الدودية في هذه الحالات.

تم قياس مستويات المؤشرات المناعية TLR4 ، IL-17، Dectin-1، و SIgA في كل من المصل وأنسجة الزائدة باستخدام تقنية المقايسة الامتصاصية المناعية للانزيم المرتبط (ELISA).أظهرت النتائج أن متوسط مستوى TLR4 في المصل لدى المرضى كان 18.72 نانوغرام/مل، مقابل 18.67 نانوغرام/مل في مجموعة السيطرة، دون اختلاف معنوي إحصائي

.(P = 0.083)لكن داخل مجموعة المرضى، كانت مستويات TLR4 في المصل أعلى بكثير مقارنة بمستوياتها في الأنسجة .(P = 0.000) أما IL-17 ، فكانت مستوياته مرتفعة بشكل واضح في مصل المرضى مقارنة بمجموعة السيطرة ، (257.22 ± 132.93 نانوغرام/لتر مقابل 109.71 ± 62.60 نانوغرام/لتر) كما كانت مستويات IL-17 في أنسجة الزائدة الدودية لدى المرضى أعلى بكثير منها في المصل، مع دلالة إحصائية قوية (P = 0.000).

بالنسبة لـDectin-1 ، لم تُلاحظ فروق معنوية بين المرضى ومجموعة السيطرة ، رغم وجود ارتفاع طفيف في مستوياته داخل المرضى في الأنسجة مقارنة بالمصل. أماSIgA ، فكان مرتفعًا بشكل كبير في أنسجة الزائدة مقارنة بالمصل داخل المرضى 3.10 ± 1.30 ميكروغرام/مل مقابل 1.60 ± 0.74 ميكروغرام/مل، في حين لم يكن هناك فرق معنوي في مستويات المصل بين المرضى ومجموعة السيطرة.

أُجري تحليل تعدد الأشكال الجيني (polymorphism) في جين TLR4 عند الموقع rs4986790 A/G باستخدام تقنية Tetra-ARMS PCR ، وأظهرت النتائج ارتباطًا معنويًا للأليل G مع زيادة قابلية الإصابة بالتهاب الزائدة الدودية) نسبة الأرجحية OR = 2.025 ؛ P = .(0.028لوحظ أن النمط الوراثي GG كان أكثر انتشارًا بين الذكور (30.43%) مقارنة بالإناث (7.41%)، مع نسبة أرجحية مرتفعة بلغت 5.47 .(P = 0.0491) كما كان الأليل G أكثر تكرارًا بين الذكور (45.65%) مقارنة بالإناث (25.93%)، بنسبة أرجحية 2.40 (P = 0.0413).

عند تحليل العلاقة بين الأنماط الوراثية ومستويات المؤشرات المناعية، لم تظهر فروق ذات دلالة إحصائية في مستويات TLR4 ، IL-17 أو Dectin-1 بين مجموعاتAA ، AG و GG (P > 0.05).ومع ذلك، لوحظت فروقات معنوية في مستوياتSIgA، حيث سجلت أعلى المستويات في مجموعة GG (1.76 مايكروغرام/مل) ،تلتها مجموعة AG (1.51 مايكروغرام/مل) وAA (0.88 مايكروغرام/مل)(P= 0.007). تشير هذه النتائج إلى أن تعدد الأشكال الجيني لـ TLR4 قد يؤثر في الاستجابات المناعية المخاطية من خلال تنظيم إنتاج SIgA .

تؤكد هذه الدراسة الدور الأساسي للبكتيريا سالبة الغرام، وخصوصًا الاشريشيا القولونية، في تطور التهاب الزائدة الدودية. كما توضح النتائج ارتفاعًا معنويًا في IL-17 وSIgA، خاصة في أنسجة الزائدة، مما يدل على دورهما في الدفاع المناعي الموضعي. رغم عدم الكشف عن الفطريات،

قد يعكس مستوى Dectin-1 تأثيرًا مناعيًا معينًا. بالإضافة إلى ذلك، يرتبط الأليل G في جين TLR4بزيادة خطر الإصابة، وله تأثير محتمل على مستويات SIgA . تسهم هذه النتائج في تعميق الفهم حول الاستجابات المناعية في التهاب الزائدة الدودية وقد تفتح آفاقًا جديدة لتطوير استراتيجيات علاجية مستهدفة في المستقبل.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بابل / كلية العلوم
قسم علوم الحياة

تأثير الإصابة البكتيرية على الإنترلوكين-١٧؛ ديكتين-١؛ الكلوبيولين المناعي الافرازي A وتعدد الأنماط الجينية للمستقبل الشبيه بالتول-٤ لدى مرضى التهاب الزائدة الدودية

رسالة مقدمة الى

مجلس كلية العلوم /جامعة بابل

وهي جزء من متطلبات نيل درجة الماجستير في العلوم/ علوم الحياة

من قبل الطالبة

زهراء عبد الحسين عبد الواحد عباس

(بكالوريوس علوم / علوم الحياة- 2018)

بإشراف

أ.د. فريال جميل عبد عطيه

أ.د. ابتهال معز مهدي الحسيني

١٤٤٧هـ

٢٠٢٥ م