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

التغايرات الوراثية للانترليوكينات 17 و1- بيتا في مرضى حمى التيفوئيد

اطروحة مقدمة الى

مجلس كلية العلوم / جامعة بابل كجزء من متطلبات نيل درجة دكتوراه فلسفة في العلوم / علوم الحياة

من قبل

زينب فريد حسن موسى

ماجستير علوم حياة / 2011

بإشراف

ا. د. شيماء جاسم السلطاني

2022 م 1444هـ

Ministry of Higher Education and Scientific Research

University of Babylon

College of Science

Department of Biology

Genetic Polymorphisms of IL-17A and IL-1beta in Patients with Typhoid Fever

A Thesis

Submitted to the Council of the College of Science, University of Babylon as a Partial Fulfillment of the Requirements for the Degree of Doctorate of Philosophy in Science / Biology

By :

Zaineb Fareed Hassan Musaa AL Eatia

M.Sc. in Biology / 2011

***Supervised by* :**

Prof. Dr.

Shaimaa Jassim Al Sultany

2022 A.D **1444 A.H**

بسم الله الرحمن الرحيم

﴿ وَلَقَدْ آتَيْنَا دَاوُودَ وَسُلَيْمَانَ عِلْمًا وَقَالَا الْحَمْدُ لِلَّهِ الَّذِي فَضَّلَنَا عَلَى كَثِيرٍ مِنْ عِبَادِهِ الْمُؤْمِنِينَ ﴾

صدق الله العلي العظيم

سورة النمل / الآية

١٥

Dedication

To the master of mankind, the messenger of mercy our Prophet Muhammad (May the peace and blessings of God be upon him) and to him are the good and pure...

To my country with everything…

To my father who... exerted himself to build us... and exhausted his strength... to be strong Dad, may God have mercy on him.

To my lovely mother who... stayed up for our sleep... I made it clear so that we could live...

To my dear husband who... sits on the throne of my heart without any competition…

To those who are they the pleasures of my liver... and the adornment of life … Mustafa and Sara

Zaineb

الخلاصة

صممت الدراسة الحالية لتحديد أهمية انترلوكين 17A وانترلوكين 1- بيتا المناعية وتعدد الأشكال الوراثي في المرضى العراقيين المصابين بعدوى حمى التيفوئيد في محافظة بابل. تم جمع إجمالي 263 عينة سريرية من دم مرضى حمى التيفوئيد الذين حضروا إلى مستشفى الحلة الجراحي التعليمي والمختبرات الخاصة في الحلة / محافظة بابل ، خلال الفترة من فبراير إلى أغسطس 2021. فقط 50 (19٪) من 263 شخصًا ثبتت إصابتهم بعدوى بكتريا التيفوئيد باستخدام مزرعة الدم والاختبارات البيوكيميائية ونظام VITEK-2 المضغوط.

وفقًا للنتائج ، تم تقسيم العينات السريرية إلى 50 (19٪) مزرعة دم إيجابية لـ *S.typhi* و 213 (81٪) مزرعة دم سلبية لـ *Salmonella.typhi* أسفر اختبار ويدال عن نتائج إيجابية بنسبة 100٪ بالنسبة لـ *S typhi*.

كان متوسط عمر المرضى (36.66 ± 12.32) سنة ، بمدى من (15-65) سنة ، و (50) فردًا كمجموعة سيطرة تم تضمينهم في هذه الدراسة. أظهرت الدراسة التوزيع النسبي لمجموعتي الدراسة على أساس الجنس حيث كان هناك 32 (64٪) من الحالات للذكور و 18 (36٪) من الحالات للإناث للإصابة بحمى التيفوئيد.

أثبتت الدراسة أن العزلات كانت ذات مقاومة عالية للبنسلينات: تيكارسيلين ، تيكارسيلين / كلافيولونك اسد، بيبرسيلين كانت 100٪ وبيبرسيلين / تازوبكتام كانت 28٪؛ بالنسبة للمونوبكتام: كانت للأزتريونام 76٪؛ للأمينوغليكوزيدات: أميكاسين كانت 80٪، جنتاميسين كانت 100٪ وتوبراميسين كانت 92٪؛ بالنسبة للكاربنيمز: ميروبنيم كانت 24٪ وابيمنيم كانت 8٪؛ بالنسبة لـ (الكينولونات والفلوروكينولونات): سيبروفلوكساسين كانت 100٪؛ بالنسبة للسيفالوسبورينات: السيفيبيم والسيفتازيديم كانت 100٪ والسلفوناميدات: ترايميثوبريم / سلفميثوكزول كانت 36٪. أظهرت نتائج العزلات مقاومة متعددة للمضادات لأن عزلات *S.typhi* كانت مقاومة لأكثر من ثلاث مجموعات من المضادات الحيوية.

كان متوسط تركيز IL- 17A في مصل الدم 27.06 ± 8.76 بيكوغرام / مل بينما كان التركيز في مجموعة السيطرة 13.41 ± 5.27 بيكوغرام / مل وكانت هناك اختلافات معنوية ($P \leq 0.000$) مقارنة بمجموعة السيطرة. كان متوسط تركيز IL-1β في مصل الدم 15.38 ± 6.45 بيكوغرام /

مل بينما كان التركيز في مجموعة السيطرة 7.23 ± 3.85 بيكوغرام / مل وكانت هناك اختلافات معنوية ($P \leq 0.000$) مقارنة بمجموعة السيطرة.

كان أليل IL-17A (C) أقل شيوعًا بين المرضى ($n = 28$) من أليل التحكم ، وكان أليل (T) اقل تكرارا بين المرضى منه بين مجموعة السيطرة ($n = 38$). أظهرت الأنماط الجينية وتواتر الأليل لـ rs1974226 أن الأفراد الذين يحملون النمط الجيني TT تم تمثيلهم بشكل كبير بين المرضى الذين يعانون من حمى التيفوئيد: 14 (28٪) ، $P < 0.0061$، مقارنة مع الأشخاص الأصحاء ، 6 (12٪) ، وكان لديهم خطر متزايد للإصابة. الإصابة بعدوى حمى التيفوئيد.

وفقًا للنتائج ، تم ملاحظة وجود الأفراد الذين يحملون النمط الجيني GA بشكل كبير بين المرضى الذين يعانون من حمى التيفوئيد: 33 (66٪) ، قيمة $P = 0.0030$ ، مقارنة مع الأشخاص الأصحاء ، 18 (36٪) ، وكان لديهم خطر متزايد للإصابة بعدوى حمى التيفوئيد. كان أليل IL-1β (G) أقل تكرارا بين المرضى (51٪ ، $n = 51$) من مجموعة السيطرة ، وكان الأليل (A) أكثر تكرارا بين المرضى منه بين مجموعة السيطرة (49٪ ، $n = 49$) .

تم اكتشاف تعدد أشكال الحمض النووي (SNP) في هذه الدراسة في العينات التي تم فحصها ، حيث تم استبدال السايتوزين بالثيامين في الموضع 226 ، وهو C226T للجين IL-17 ، وتم اكتشاف (SNP) في العينات التي تم فحصها لجين انترليوكين IL-1β ، حيث تم استبدال الكوانين بالأدينين في الموضع 122 ، أي G122A.

Summary

The present study is designed to determine the importance of interleukin-17A and interleukin-1β immunological and genetic polymorphisms among patients with typhoid fever infection in Babylon province. A total of 263 clinical samples were collected from the blood of patients with typhoid fever who attended Al-Hillah Surgical Teaching Hospital and private laboratories in AL-Hillah / Babylon province, during the period from February to August 2021. Only 50 (19%) of 263 patients tested positive for *Salmonella typhi* infection using blood culture, biochemical tests, and the VITEK-2 compact system.

According to the findings, clinical samples were divided into 50 (19%) positive blood cultures for *Salmonella.typhi* and 213 (81%) negative cultures for *S typhi*.

The mean age of the patients was 36.66 ± 12.32 years old, with a range of 15–65 years old, and 50 control individuals were included in this study. The study shows the percentage distribution of the two study groups based on gender that there were 32 (64%) of cases for males and 18 (36%) of cases for females' susceptibility to typhoid fever infection.

The study proved that the isolates had a high resistance rate for penicillins: Ticarcillin, Ticarcillin/Clavulanic Acid, Piperacillin were 100% and Piperacillin/Tazobactam were 28%; for Monobactam: Aztreonam was 76%; for Aminoglycosides: Amikacin was 80%, Gentamicin was 100% and Tobramycin 92%; for Carbapenems: Meropenem was 24% and Imipenem was 8% ; for (Quinolones and fluoroquinolones): Ciprofloxacin was 100%; for Cephalosporins: Cefepime and Ceftazidime were 100% and for

Sulfonamides: Trimethoprim/Sulphamethoxazole was 36%. The isolates' results revealed multidrug resistance because the *S typhi* isolates were resistant to more than three groups of antibiotics.

The mean of IL-17A concentration in the serum of patients was 27.06±8.76 pg/ml while the control was 13.41±5.27 pg/ml. There were significant differences ($P \leq 0.000$) compared with control group. The mean of IL-1β concentration in the serum of patients was 15.38±6.45 pg/ml while the control was 7.23±3.85 pg/ml. There were significant differences ($P \leq 0.000$) compared with control group.

The genotype A (C) allele was less frequent among patients (n =28) than control, and the (T) allele was less frequent among patients than control (n =38). Genotypes and allele frequency for rs1974226 showed individuals with genotype TT were significantly represented among the patients with typhoid fever: 14 (28%), $P < 0.0061$, as compared with healthy control subjects, 6 (12%), and had an increased risk of developing typhoid fever infection. According to the results, individuals with genotype GA were significantly represented among the patients with typhoid fever: 33 (66%), $P< 0.0067$, as compared with healthy control subjects, 18 (36%), and had an increased risk of developing typhoid fever infection. The IL-1β (G) allele was less frequent among patients (51%, n =51) than control, and the (A) allele was more frequent among patients than control (49%, n =49). A highly interesting nucleic acid polymorphism (SNP) was detected in this study in the investigated samples, in which cytosine was replaced with thymine at position 226, namely C226T for the IL-17 gene, and (SNP) was detected in the investigated samples, in which guanine was replaced with adenine at position 122, namely G122A for the IL-1β gene.

Certification

I certify that the preparation of this thesis **((Genetic Polymorphisms of IL-17A and IL-1 beta genes in Patients with Typhoid Fever))** was made by **(Zaineb Fareed Hassan Musa)** under my supervision at University of Babylon ,College of science, Department of Biology, as a partial fulfillment of the requirements for the Degree of Doctorate of Philosophy in Science of Biology – Microbiology**.**

Signature

Name: Dr. Shaimaa Jassim Al-Sultany

Address :Department of Biology

College of science

University of Babylon

Date: / / 2022

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

Signature:

Name : **Assist. Prof. Dr. Adi Jassim Abd Al.-Rezzaq**

Address: Head of Department of Biology , College of Science

University of Babylon

Date : / / 2022

Acknowledgements

I would like to express my thanks to "Allah" the Most Gracious and to Most Merciful, and to His prophet "Mohammad" God's Blessings and Peace Be Upon Mohammed and His Family for enabling me to complete this study.

Thank you, God, for providing us the strength and patience to carry out this task, which we hope will be beneficial and objective. I'd like to convey my heartfelt appreciation to my Supervisor, Prof. Shaimaa Jassim Al Sultany (Ph.D.), for her wise counsel and scientific direction during this work.

Special thanks are for the Head of Department of Biology , College of Science Assist. Prof. Adi Jassim Abd Al.-Rezzaq (Ph.D.) and Wassn Modhar (Ph.D.) during period of research.

My sincere thanks go to all the people and patients who helped me in one way or another in the completion of my work.

I wish to express my thanks and , my deep gratitude to my best friends for their scientific advices and support during the period of the research.

Zaineb

Decision of Examination Committee

We, the examination committee, certify that we have read the thesis entitled (**Genetic Polymorphisms of IL-17A and IL-1beta in Patients with Typhoid Fever**) and have examined the student (**Zaineb Fareed Hassan Musaa**) in its contents , and that in our opinion it is accepted as a thesis for Degree of Doctorate of Philosophy in Science of Biology – Microbiology with excellent estimation.

Signature

Professor Dr. Samah Ahmad Kadhum

College of pharmacy ,University of Babylon

(Chairman)

Signature

Assist prof Dr.Yazi Abdullah Jassim Nayif

College of Science, University of Babylon

(Member)

Signature

Assist prof Dr. Eman Mobder

AL-Mustaqbal, University College

Department medical laboratories

(Member)

Signature

Assist .Prof .Dr. Noor Salman Kadhim

College of Science, University of Babylon

(Member and Supervisor)

Contents

List of Tables

List of Figures

List of Abbreviations

Symbols	Meaning
ALS	Antibody-in-lymphocyte-supernatant
AST-N222	Anti-sensitivity test number 222
BLAST	Basic local alignment search tool
BHI	Brain heart infusion
CD4	cluster of differentiation 4
CFTR	Cystic fibrosis trans-membrane conductance regulator
DAMPs	Damage associated molecular patterns
DNA	Deoxy nucleotide triphosphate
GnomAD	Genome Aggregation Database
GTPase	Guanine trinucleotide phosphatases
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HLA-B27	
IpaB	Invasion plasmid antigen B
Ipaf	
LPS	Lipopolysaccharide
M cells	Membrane cells
MDR	Multi drug resistance
NAIP	Neuronal apoptosis inhibitory protein
NCBI	National Center for Biotechnology Information
NLR	Nod-like receptor
NOD	Nucleotide binding

RDTs	Rapid diagnostic tests
SNPs	Single nucleotide polymorphisms
SS agar	*Salmonella Shigella* agar
SSCP	Single strand conformation polymorphism
SPI	*Salmonella* Pathogenicity Island
TBE	Tris borate-EDTA
TCVs	Typhoid conjugate vaccines
TLR_4	Toll like receptor $_4$
TNFα	Tumour necrosis factor α
TOPMED	Trans-Omics for Precision Medicine
TTSS	Type three secretion system
PAMPs	pathogen-associated molecular patterns
PCR	Polymerase chine reaction
PRR_S	Pattern recognition receptors
UTRs	Un translated regions
XDR	Extremely drug-resistant
XLD agar	Xylose lysine deoxycholate agar

List of Abbreviations

Symbols	Meaning
ALS	Antibody-in-lymphocyte-supernatant
AST-N222	Anti-sensitivity test number 222
BLAST	Basic local alignment search tool
BHI	Brain heart infusion
CD4	cluster of differentiation 4
CFTR	Cystic fibrosis trans-membrane conductance regulator
DAMPs	Damage associated molecular patterns
DNA	Deoxy nucleotide triphosphate
GnomAD	Genome Aggregation Database
GTPase	Guanine trinucleotide phosphatases
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HLA-B27	
IpaB	Invasion plasmid antigen B
Ipaf	
LPS	Lipopolysaccharide
M cells	Membrane cells
MDR	Multi drug resistance
NAIP	Neuronal apoptosis inhibitory protein
NCBI	National Center for Biotechnology Information
NLR	Nod-like receptor
NOD	Nucleotide binding
RDTs	Rapid diagnostic tests

SNPs	Single nucleotide polymorphisms
SS agar	*Salmonella Shigella* agar
SSCP	Single strand conformation polymorphism
SPI	*Salmonella* Pathogenicity Island
TBE	Tris borate-EDTA
TCVs	Typhoid conjugate vaccines
TLR_4	Toll like receptor $_4$
TNFα	Tumour necrosis factor α
TOPMED	Trans-Omics for Precision Medicine
TTSS	Type three secretion system
PAMPs	pathogen-associated molecular patterns
PCR	Polymerase chine reaction
PRR_S	Pattern recognition receptors
UTRs	Un translated regions
XDR	Extremely drug-resistant
XLD agar	Xylose lysine deoxycholate agar

1.1: Introduction

Typhoid fever is a major public health issue in many poor and middle-income nations, which includes gastroenteritis, enteric fever, and septicemia. The existence of antibiotic medicine and improved water quality and sanitation constitute ultimate solutions to this problem, and as recommended by WHO, vaccination in high-risk areas is a potential control strategy (Antillón *et al*., 2017). Typhoid fever is a potentially fatal infection of the digestive system and circulation caused by pathogenic bacteria. *Salmonella enterica* serotype *typhi* is a gram-negative, non-capsulated, non-sporulation, rod-shaped, facultative anaerobe of the enterobacteriaceae family that has flagella, somatic and outer coat antigens that only live in humans because it is an infectious illness that is spread orally through contact person-to-person or spread through contaminated food or impure water (Pouzol *et al*., 2019 ; Phetsavanh *et al*., 2020). This bacteria may infect food products of animal origin like poultry and dairy products (Alzwghaibi *et al*., 2018).

Typhoid fever is not a specific clinical condition because the presenting signs and symptoms are numerous and similar to those of other common febrile diseases, such as malaria and dengue fever, and can only be identified with certainty by isolating the pathogen from clinical specimens in humans. Clinical studies have revealed that this disease stimulates both the digestive mucosal and humeral reactions, both of which play an important role in pathogen control (Alikhan *et al* ., 2018). When *S typhi* enters the body by the fecal-oral route, immunological recognition occurs during the invasion of intestinal epithelial cells, which can recognize harmful bacteria and start an inflammatory response through the recruitment of multiple phagocytic cells. When TLR_4 recognizes bacterial LPS, these cells produce cytokines and

chemokines, which serve as the initial signal for phagocytic cell recruitment such as interleukin-17A and interleukin-1β (Kaiser and Hardt, 2011). Researchers have accumulated a lot of information on the function of cytokines in protective immunity against intracellular bacterial pathogens in recent years. IL-17A & IL-1β are a predominance of pro-inflammatory cytokines in typhoid patients, and their levels are higher when compared to patients with other diseases (Alvi *et al*., 2014).

Interleukin-17A is a proinflammatory cytokine with many biological functions, including up-regulating proinflammatory gene expression. IL-17 attracts neutrophils and induces neutrophil-attracting chemokines, such as IL-1 and TNF-, to the site of inflammation, thereby amplifying inflammatory responses has been found to play a significant role in mucosal immunity and protection against intracellular microorganisms (Khader *et al*., 2009). IL-17A relatives would be included in cytokines and are involved in facilitating nearby tissue aggravation by the arrival of proinflammatory and neutrophil-mobilizing cytokines (Kawaguchi *et al*., 2006).

Interleukin-1β is a member of the interleukin-1 family, a key mediator of the inflammatory response that is thought to have a variety of physiological and pathological activities and to play an essential role in the host-response and resistance to pathogenic bacteria in healthy and sick humans. Bacterial resistance to antimicrobial agents is a severe problem across the world. In the last two decades, there were development and spread of multidrug resistance against ordinary anti-typhoid drugs among typhoid fever patients. *S typhi* has evolved resistance to many medicines, leading to the formation of multi-drug resistant *S typhi* strains; these strains have demonstrated resistance to first-line treatments especially in countries with poor sanitation

and low income (Klemm *et al*., 2018). According to a distributing investigation in the Middle East and Central Asia, Iraq has the highest prevalence of MDR after Pakistan, which was the first to be infected with multidrug-resistant *S typhi* (Rahman *et al*., 2014). Gene polymorphisms in IL-17 and IL-1β cytokines alter the activity of interleukins and may alter cytokine function, therefore lead to disorder in the cytokines expression (Ishigame *et al*., 2009).

1.2: Aim of Study

The main objective of this study is to find the immunological importance of interleukin-17A and interleukin-1β immunological and genetic polymorphisms in Iraqi patients with typhoid fever infection. These objectives were met by completing the following:

1-Isolation and identification of *S typhi* from patients who are suffering from typhoid fever by using Widal test, blood culture, biochemical examination and Vitek compact system.

2-Studying of *S typhi* for the antibiotic sensitivity test.

3-Estimate concentration of human IL-17A and IL-1β in the serum of patients by ELISA.

4-Gene polymorphisms of IL-17A and IL-1β in the whole blood of patients with typhoid fever.

2: Review of Literatures

2.1: Typhoid Fever Definition

Typhoid fever seems to be a multi-systemic illness that is a serious and dangerous and considered a significant medical issue on the planet and in several non-industrial nations. This disease has been a public health problem, It affects about 10.9 million people (Stanaway *et al*., 2019).

Typhoid fever is an acute infectious disease caused by the bacterium *Salmonella enterica* serovar *typhi*, whose name comes from the oldest Greek word typhos, which means' to smoke'. *Salmonella* is named after the scientist Salmon who isolated Bacillus from pigs (Shen *et al*., 2007).

The bacteria usually enters the body through the mouth by the ingestion of contaminated food or water, penetrates the intestinal wall, and multiplies in lymphoid tissue; it then enters the bloodstream and causes bacteremia (Pegues *et al*., 2002).

Typhoid fever has become less common as a result of extensive research and public health interventions. The disease course ranges from early gastrointestinal distress to nonspecific systemic illness but ultimately may lead to multiple complications. *Salmonella* is said to spread by the four: flies, fomites, fingers, and feces. Fever characteristically comes in a step-wise pattern when it rises and falls, alternatively followed by headache and abdominal pain (Parry *et al*., 2002).

2.2: Typhoid Fever Epidemiology

The epidemiology of typhoid fever began with Austin's standard research. Data shows the annual load of disease to be 21 million cases throughout the world, with over 200 000 deaths due to typhoid fever (Crump *et al*., 2004). Before this, it was observed to be 16 million with over 600,000 deaths (Merican, 1997). This clearly suggests an increase in morbidity accompanied by a decrease in death. Asia has the greatest typhoid fever fatality rate, accounting for 93 percent of all infected cases worldwide. Asia also has the greatest regional frequency rate of 274 instances per 100,000 people, which is five times higher than Latin America, which has the second highest rate. Southeast Asia has an incidence of 110 cases per 100,000 population, which is the third-highest occurrence rate for any region (Crump *et al*., 2004).

An estimated 26 million cases of typhoid fever occur worldwide each year, causing 215,000 deaths (Chiodini, 2017). The fecal-oral pathway is used to spread infection from person to person. Humans are the only known reservoirs when contaminated food (Sharma *et al*., 2009) and water (Kelly-Hope *et al*., 2007) have been recognized as the major associated risk factors for typhoid prevalence. According to a study in 2003, ground water infections caused 250,000 deaths every year in Pakistan, among which typhoid fever is the leading cause (Crump, 2004). Close contact with typhoid cases or carriers (Tran *et al*., 2005), larger household size, education level, proximity to water bodies (Sur *et al*., 2007), flooding (Vollaard *et al*., 2004), personal hygiene (Kelly-Hope *et al*., 2007), poor lifestyle (Kothari *et al*., 2008), and travel to endemic areas (Whitaker *et al*., 2009). Climatic related factors such as rainfall, vapor pressure, and temperature have a major effect

on the burden and distribution of typhoid infections in human populations (Wang *et al*., 2012).

2.3: Features of *Salmonella typhi*

Salmonella typhi, a member of the Enterobacteriaceae family, is the primary cause of typhoid fever. Salmonella is a genus with two species, Salmonella enterica serovar and enteritidis, which were identified through extensive multiplex quantitative polymerase chain reaction analysis (Heymans *et al*., 2018). Members of this genus, known as chemoorganotrophic organisms, can metabolize nutrients via both respiratory and fermentative pathways (Popoff and Le Minor, 2005). Many of the virulence factors required for *Salmonella* infection are encoded by Salmonella pathogenicity islands (SPIs), which differ between serovars (Brenner *et al*., 2000).

Colonies on blood agar are 2–3 mm in diameter. It appears colorless on MacConkey and eosin methylene blue agar. *S typhi* is an exception to triple sugar iron because it does not form gas and produces only a small amount of H_2S. *S typhi* is resistant to certain chemicals that inhibit other enteric bacteria, such as brilliant green and sodium deoxycholate. These compounds are useful for inclusion in media to isolate *S typhi* from feces (Brooks *et al*., 2004).

Serological tests and agglutination with polyvalent O and H antisera can be used to identify members of the genus (Lee *et al*., 2015). Some H antigens may not develop well on some solid agar (Levinson, 2004).

The flagellar protein groups are known as H flagellar determinant antigens. They are both heat and alcohol-labile. Heating at $60C^{O}$ or higher

causes the flagella to detach from the bacteria, and heating at $100C^0$ for 30 minutes works well. Suspension of bacilli liberated from detached flagella by centrifugation and washing at 100 C^0 for 2 hours. The findings suggest that Vi may be important in the bacterium's survival inside the macrophage but not in the macrophage's or the intestinal wall's cellular invasion. Organisms can become immobile after losing their H antigens. The loss of O antigen results in a shift in colony morphology from smooth to rough (Brooks *et al*., 2007).

Salmonella comprises a type III secretion system (TTSS), which is one of the virulence mechanisms that permits toxins to be delivered to the host's cells. *Burkholderia*, Escherichia, and Shigella are among the other enterobacteriaceae possessing TTSS genes (Winstanley and Hart, 2001).

Many of these gene products are encoded on Salmonella pathogenicity islands such as SPI-15 and SPI-18, which are exclusive to it (Dougan and Baker, 2014). They also carry typhoid-toxin, an exotoxin that is considered to have a role in the pathogenesis of enteric fever (Gala'n, 2016).

Certain Salmonella strains possess a plasmid containing virulence genes with a high or low copy number. The size of Salmonella virulence plasmids varies (50–90 kb), but they all contain a 7.8 kb region known as salmonella pathogenicity virulence, which is necessary for bacterial growth in the reticuloendothelial system (AL-Quraishi, 2018).

The Kauffman and White classification is a phylogenetic classification scheme for *Salmonella* subspecies that divides *Salmonella* into serotypes based on three key antigenic determinants: somatic (O), capsular (Vi), and flagella (H) (Brenner *et al*., 2000). The somatic (O) antigen is found on the

outside bacterial cell membrane, is heat stable, and produces the oligosaccharide component of the bacterial cells' lipopolysaccharide. For example, a particular Salmonella serotype may express many O antigens (Popoff *et al*., 2005).

Salmonella spp. has two distinct genes that encode for flagellar proteins. These bacteria might be diphasic (phase I and II), which implies they can only express one protein at a time. Some serotypes may express phase I H antigens, which are important for immunological identification, whereas phase II antigens are non-specific antigens that can be found in different serotypes (McQuiston *et al*., 2008).

Many virulence factors play a variety of roles in the pathogenesis of *Salmonella* infections included flagella, capsules, plasmids, adhesion systems, and type3 secretion systems encoded on the Salmonella pathogenicity island SPI-1 and SPI-2 and other SPIs (Sabbagh et al., 2010). These factors, alone or in combination, allow Salmonella to colonize its host by attaching, invading, surviving, and by passing host defense mechanisms such as gastric acidity, gastrointestinal proteases, and defensins (Yue and Schifferli, 2014).

Fimbriae plays an important role in the pathogenesis of Salmonella, and recently it has been shown to represent a source of diversity among Salmonella serovars (Dufresne and Daigle, 2017). Fimbriae represent the most common adhesion systems, which are differentially expressed and are found in specific patterns among each serovar (Townsend et al., 2001). They mediate the adhesion of Salmonella to the hosts' cells and food. It's been implicated in a variety of other roles, namely biofilm formation,

hemagglutination, cellular invasion, and macrophage interactions (Sabbagh et al., 2010).

2.4: The Pathogenesis of *Salmonella typhi*

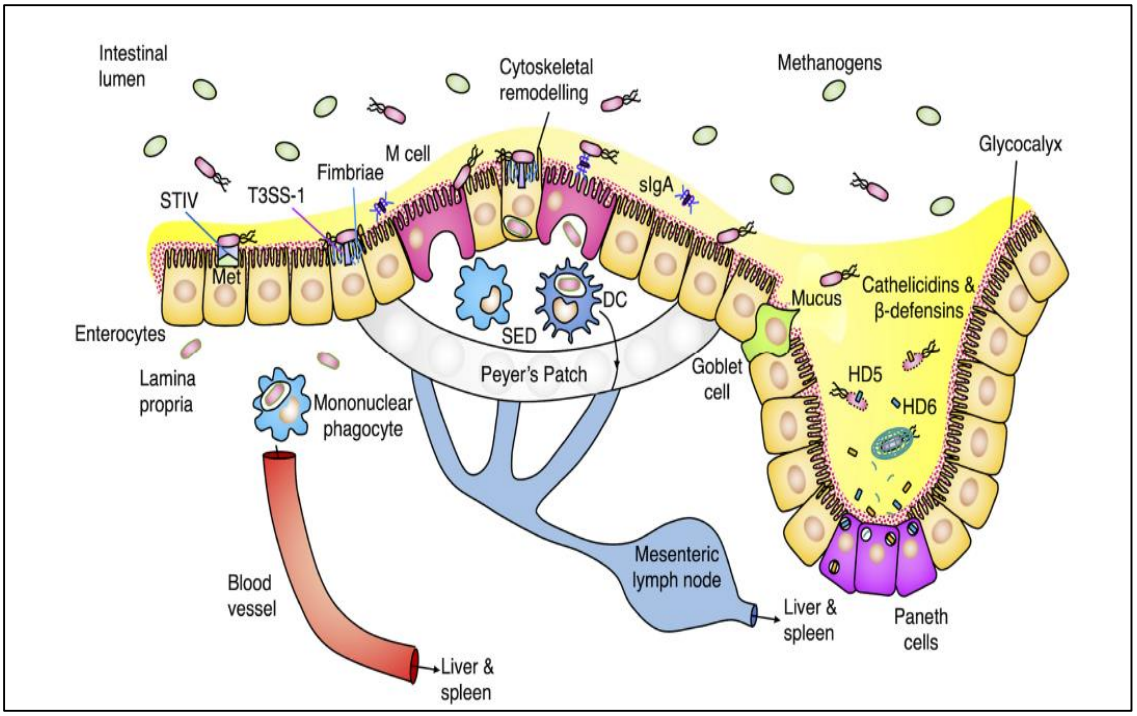
Salmonella typhi is one species of gram-negative enterobacteriaceae that is related to human disease (Alikhan *et al*., 2018). It is a multi–organ pathogen that inhibits lymphatic tissues in the small intestine, liver, spleen, and bloodstream of infected humans (Parry, 2006).

The pathogenesis of typhoid fever depends upon a number of factors, including infectious species, virulence, the host's immunity, and infectious dose. The greater the infectious dosage, the shorter the incubation time and the greater the attack rate. Typhoid fever is more severe in weakness and immunocompromised people, such as HIV patients, those on glucocorticoid medication, and those with impaired phagocyte function, such as malaria patients. Except for a few resistant strains, *Salmonella* is an acid-sensitive bacterium that is normally killed in the stomach by gastric acid unless a sufficient amount is consumed (Lianou *et al*., 2017).

Because the gastrointestinal system is the principal location of *Salmonella* invasion, when it is swallowed in contaminated food or drink, it passes past the stomach and invades the gut epithelium. Before invasion to occur, the bacteria may need to stimulate active attachment, which most likely requires undiscovered adhesion molecules on the bacterium interacting with receptors on the host cell, which includes 12 fimbriae operons, including typhi colonization factor, which is specific to *S typhi* (Townsend *et al*., 2001). The wide family of fimbriae may be the outcome of host immune response selection (Nicholson and Baumler, 2001).

Typhoid toxin, Vi antigen (polysaccharide capsule), lipopolysaccharide O antigen, and flagellar H antigen dictate its pathogenicity. The major function of the Vi antigen is to serve as an anti-phagocytic agent, inhibiting macrophages from acting, limiting complement deposition, lowering immunological activation, aiding with phagocytosis evasion, and suppressing serum bactericidal activity are all immune modulatory features of the Vi capsule that are hypothesized to contribute to disease etiology (Dougan and Baker, 2014; Hart *et al*., 2016).

The flagellar H antigen promotes bacterial motility and adhesion to the mucosa of the gut wall. Flagella aid in invasion of the gut wall, and the type III secretion system is capable of transporting bacterial protein into enterocytes and M cells, which are specialized epithelial cells that function as antigen-presenting cells in the gut mucosa, or via direct mucosal penetration. Bacteria linked to M cells are absorbed and extruded into the luminal space by bacteria in pinched off cytoplasm. M cells are destroyed during this process, and the basal lamina is exposed. It allows microorganisms free access for invasion, which worsens the illness. The transferred proteins activate the host cell GTPases, which triggers the actin rearrangement so that bacterial protein uptake happened in the phagosome where the bacteria can grow. *Salmonella* also produces a molecule that stimulates the epithelial release of chemo attractant eicosanoid, which sequesters neutrophils into the lumen and potentiates mucosal damage (Vande-Vosse *et al*., 2005).



S typhi invasion is impeded by mucus and the glycocalyx acting as physical barriers, as well as the action of defensins, cathelicidins and IgA in the intestinal mucosa. Those bacteria that successfully adhere to the epithelium using fimbriae cross by T3SS-1- or STIV mediated invasion of intestinal epithelial cells. Bacteria in the lamina propria and subepithelial dome (SED) are then phagocytosed and systemically disseminated to the liver and spleen via the blood and lymph.

Figure (2-1): *S typhi* invasion intestinal epithelium (Barton *et al*., 2021)

Diarrhea is often non-bloody and loose, with the possibility of large-volume watery stools, bloody stools, and dysentery symptoms. While infection may occur in almost every organ system, problems outside of the stomach intestinal tract are infrequent. Septicemia is caused by the widespread spread of bacteria, which results in multi-organ failure (Kumwenda and Iroh, 2019).

Hepatic and splenic abscesses are caused by intra-abdominal infections. Lung abscesses and bronchi pleural fistula development are two other pulmonary consequences, albeit the majority of instances occur in people

with lung cancer, glucocorticoid usage, and other structural lung problems (Esmailpour *et al*., 2006).

Headaches are frequently described in conjunction with other neurologic symptoms such as sleep disturbances, acute psychosis, myelitis, meningitis, muscular stiffness, and focal neurologic abnormalities. Myocarditis and nephritis are side effects of toxic events (Majid *et al*., 2019). Infections of the long bones, particularly the femur and tibia, are more prevalent in children with sickle cell anemia and previous bone disease (Rohilla *et al*., 2019).

Patients with HLA-B27 antigens are more likely to develop reactive arthritis. Complications are enhanced in debilitated patients with chronic illnesses such as cancer, tuberculosis, and HIV by a prolonged duration of disease prior to admission, a protracted period of hospitalization, antibiotic treatment, and immunological compromise. Patients who do not receive proper typhoid treatment continue to excrete germs and are termed chronic carriers. The bacteria in the chronic stage of typhoid invade the gallbladder and have been related to gallbladder cancer if not managed (Gunn *et al*., 2014; Koshiol *et al.,* 2016).

Bacilli disperse and multiply in numerous organs, but they are most varied in organs that have critical phagocytic movement, in particular the liver, spleen, and bone marrow. Maintaining enough hydration during diarrhea, as well as proper ventilation and oxygenation for pulmonary issues, should be offered as supportive treatment for metastatic complications, along with analgesics and antipyretics. Corticosteroids have been proposed as a treatment for severe instances of encephalitis (Mellon *et al*., 2017).

2.5: Contamination and Transmission by *Salmonella typhi*

Typhoid fever is a serious food and waterborne infection spread by shellfish and dairy products, and less frequently by direct contact with urine, feces, or other bodily fluids. Typhoid fever is no longer common in countries with adequate sewage and drainage infrastructure, as well as safe drinking water (House *et al*., 2001). Humans are the only natural hosts and reservoirs of *S typhi* infection. It is spread through the consumption of feces-contaminated food or water. Typhoid fever is primarily found in South Asia and Africa. Children under the age of 14 bear the greatest burden. In 2017, it was estimated that enteric fever caused 9.8 million infection and 136,004 deaths, person-to-person transmission can take place primarily through fecal-oral or oral-oral routes (Stanaway *et al*., 2019).

Water and food are important vehicles for the microbe's spread. Ice cream has been identified as a significant risk factor for the spread of typhoid fever. Shellfish from contaminated water, as well as raw fruits and vegetables fertilized with sewage also considered as risk factors. The most common incidence occurs when water supplies serving large populations become contaminated with feces. The size of the inoculum and the type of vehicle in which the organisms are ingested have a significant impact on both the attack rate and the incubation period. In one study on the distribution of *Salmonella* isolates in China using whole-genome sequencing, 57% of samples were positive (Gu *et al*., 2020).

Transmission can occur through short-cycle or long-cycle consumption of contaminated food or water. Short-cycle transmission is defined as the contamination of food and water in the immediate environment as a result of

inadequate hygiene and in adequate sanitation, caused by either acute or chronic carrier. Long-cycle transmission is defined as contamination of the broader environment, such as sewage contamination of water supplies or insufficient treatment of water. Chronic carriers may be responsible for disease transmission at a low level, complicating disease through sanitation and vaccination programs (Baker *et al*., 2011), safe drinking water and avoiding overcrowding all contribute significantly to a decrease in the number of cases (Darton *et al*., 2017).

Since the invention of *S typhi* vaccination, the burden of typhoid fever has been reduced when vaccination prophylaxis is used. Those traveling to areas where there is a risk of exposure should get the vaccine. There are two types of licensed, unconjugated vaccines in the United States (Jackson *et al*., 2015).

The intramuscular Vi capsular polysaccharide vaccine is recommended for people over the age of two years old. It should be given at least two weeks before traveling, and a booster should be given every two years. A live attenuated oral vaccine (Serotype Typhi Ty21a strain) boosts immunity by stimulating the production of endogenous antibodies. It is recommended for people over the age of six who are traveling to endemic areas or who are in close contact with chronic carriers or infected patients. Both vaccines have 50 to 80 percent efficacy rates, and travelers must take precautions in addition to the vaccine. In 2017, the World Health Organization's Strategic Advisory Group of Immunization Experts recommended the use of typhoid conjugate vaccines (TCVs) in typhoid-endemic countries for the first time (Burki, 2018). Intramuscular, single-dose TCVs for those 6 months and older are now registered in Nepal, India, Nigeria, and Cambodia, and are

being studied further for use in endemic areas and during outbreaks (Appiah *et al*., 2020).

TCV was found to be safe in children aged 6 to 10 months during an outbreak of extensively drug-resistant typhoid in Pakistan in 2018 (Qamar *et al*., 2020). The World Health Organization is currently proposing two immunizations for the prevention of typhoid fever. Vaccination in high-risk areas is a possible short-to-medium-term control strategy recommended by the World Health Organization (WHO, 2018). Surgery could be an option when gallstones are present in conjunction with a carrier state. Cholecystectomy can be curative (Lee *et al* ., 2020).

2.6: Diagnosis of *Salmonella typhi*

Clinical care should be provided to typhoid patients. Patients presenting with febrile illness for more than three days and gastrointestinal manifestations such as pain and diarrhea who live in areas with poor sanitation, contaminated drinking water, or a history of travel from endemic areas are highly suspicious. In the first week, diagnosis is challenging, although a range of laboratory testing can aid (Bhutta, 2006).

Blood culture is still the most common method of confirming a typhoid fever diagnosis because it is not expensive or technically difficult, it is widely available and is the most commonly performed test. When large volumes of blood are collected, the efficacy of the blood culture is increased. Blood cultures performed during secondary bacteremia are more reliable, though 30% to 50% of cultures may be falsely negative depending on the technique and time series used (Mogasale *et al*., 2016).

In the bacteremic phase of the disease, stool culture is less effective. In the second and third weeks, stool culture is diagnostic. The amount of stool samples obtained and the period of sickness influence the sensitivity of stool culture. Chronic carriers pass pathogens in feces for an extended period of time (Näsström *et al*., 2018).

The gold standard for typhoid diagnosis is bone marrow culture. Because of the greater number of microorganisms present in bone marrow, aspirated bone marrow samples are more sensitive than blood cultures. however, the test is expensive, so it is not routinely used for the diagnosis and treatment of typhoid (Mawazo *et al*., 2019).

The Widal test is a serological test that detects antibodies against O (surface) and H (flagellar) antigens. An antibody titer of greater than 1:160 for anti-H antigen and greater than 1:80 for anti-O antigen is considered the cut-off level for predicting recent typhoid fever infection in an endemic area. O antibodies normally present 6–8 days after illness start, while H antibodies occur 10–12 days later. This test is unreliable because to its frequent false-negative and false-positive outcomes, poor concordance with blood culture, and poor performance. Because sensitivities can be low due to low bacterial concentrations during bacteremia, Polymerase Chain Reaction can provide DNA-based gene identification of several serotypes, such as the H antigen gene and O antigen gene (Goay *et al*., 2016).

There is still a pressing need to improve current enteric fever diagnostics and create a new generation of tests that are easy to use, inexpensive, sensitive, and specific (Andrews and Ryan, 2015). Rapid diagnostic tests (RDTs) for typhoid fever could theoretically be combined with clinical

algorithms to differentiate febrile patients and guide management, especially in areas with limited laboratory resources (Mogasale *et al*., 2014).

Several rapid diagnostic tests for enteric fever diagnosis have been developed, the most common of which are the Typhidot/Typhidot-M test and the tubex test. The current generation of typhoid RDTs has only moderate sensitivity and specificity as determined by meta-analyses, and there is insufficient evidence to support their sole use for the diagnosis and management of enteric fever (Wijedoru *et al*., 2017).

Other diagnostics under development include antibody-in-lymphocyte-supernatant (ALS), which has demonstrated high sensitivity and specificity in endemic settings (Farhana *et al*., 2013; Islam *et al*., 2016; Darton *et al*., 2017). The sensitivity of PCR-based assays can be increased by including a pre-enrichment step (Zhou and Pollard, 2010), which was able to differentiate typhoid from paratyphoid fever and enteric fever febrile, typhoid-negative controls, and chronic carriers (Näsström *et al*., 2018). Transcriptional data from people with acute typhoid fever could also be used to identify signatures that reliably identify cases of enteric fever (Thompson *et al*., 2009; Blohmke *et al*., 2016).

2.7: Antibiotic Resistance

Multidrug resistant strains have hindered treatment in many endemic locations, particularly in India and Southeast Asia. The mode of therapy is determined by the severity of the disease, its length and spread. The initial pharmacological therapy of choice is determined by the strains' susceptibility. Fluoroquinolones are the most effective medicine of choice in most cases. Fluoroquinolones can be administered on clinical suspicion prior

to the results of the diagnostic culture test in severe cases that demand immediate treatment (Iwamoto *et al*., 2017).

The most effective fluoroquinolone is ciprofloxacin, 500 mg orally twice a day for 5-7 days. Alternative treatments for adults who are completely susceptible include amoxicillin 750 mg orally four times daily for approximately two weeks, trimethoprim-sulfamethoxazole 160 mg twice daily for two weeks, and chloramphenicol 500 mg four times daily for 2-3 weeks outside of the United States. Over the years, antimicrobial resistance has posed a severe public health problem since antibiotics are no longer effective against bacterial agents, resulting in treatment failures, high mortality rates, and increased hospitalization time, among other things (Founou *et al*., 2016).

In endemic locations, multidrug-resistant (MDR) and extensive drug-resistant (XDR) strains have emerged. Bacterial intracellular protects them from extracellular antibiotics (Wen *et al*., 2017). In MDR instances, third-generation cephalosporins and azithromycin are the preferred treatments, with ciprofloxacin as a backup option. The failure rate of this therapy is around 5% to 10%, with recurrence rates ranging from 3% to 6%. With a fecal carriage rate of less than 3%, these drugs eliminate fever in a week. The combination of azithromycin and cefixime minimizes the incidence of failure and length of hospitalization (Tiwaskar., 2019). A study found that the MDR *Salmonella* is increasing in Africa, which may complicate the treatment of human salmonellosis (Smith *et al*., 2016).

Multi drug resistance for *Salmonella* has been found in Japanese quails, raising concerns about public health hazards from direct ingestion of these

birds or contact with carriers (Omoshaba *et al*., 2017). The non typhoidal salmonellosis isolates from chicken had the highest degree of resistance to sulfonamides, nalidixic acid, and tetracycline. Those of human origin were also resistant to sulfonamide, tetracycline, and ampicillin (Voss-Rech *et al*., 2017).

The rising frequency of MDR for *Salmonella,* as well as resistance to therapeutically significant antimicrobials such as fluoroquinolones, has emerged as a global issue (Brands *et al*., 2005). In a similar study conducted in Kuwait and the United Arab Emirates by Rotimi *et al*. (2008), the increasing trend of MDR among *Salmonella* isolates was further demonstrated, and the rate of resistance to third-generation cephalosporins such as ceftriaxone and cefotaxime was reported to have increased five-fold. *Salmonella* ceftriaxone resistance is a critical public health concern since it is routinely used to treat severe *Salmonella* infections, particularly in children (Iwamoto *et al*., 2017).

The antibiotic drugs amoxicillin/Clavulanic acid, ampicillin, cefoxitin, chloramphenicol, streptomycin, sulfonamides, and tetracycline had the highest percentage of resistant by *Salmonella* isolates, and the percentage of resistant isolates to these drugs has increased since 1997 (FDA, 2010).

2.8: Proinflammatory Cytokine Interleukin 17 (IL-17)

IL-17 is a specific pro-inflammatory factor mainly secreted by activated memory $CD4^+$ T lymphocytes (Th17 cells) that can promote the body's local production of cytokines such as IL-8 and IL-6, lead to rapid growth of monocytes and neutrophils, enhance local inflammation and induce pro-inflammatory cytokines. TNF-α and IL-6 from activated macrophages and

dendritic cells promote Th17 cell differentiation, whereas IL-12 and interferon- stimulate Th1 cell development. There are six types of IL-17 (IL-17A to IL-17F) and there are five receptors for these cytokines (IL-17RA to IL-17RE) (Gaffen, 2009).

Interleukin 17 (IL-17) is an inflammatory cytokine and it plays a protective role against infection (Amatya *et al*., 2017). IL-17A is produced by Th17 $CD4^+$ T cells and other leukocytes such as natural killer cells, lymphoid tissue inducer-like cells, and neutrophils (McGeachy *et al*., 2019).

Among IL-17 family members, the IL-17A isoforms have the highest sequence homology with IL-17F and are located adjacent to each other on human chromosome 6 (Zhang *et al*., 2013).

2.9: Role of Interleukin-17 in Typhoid Fever

IL-17A and associated Th17 cytokines promote neutrophil recruitment via induction of chemokines and granulopoietic factors (Khader *et al*., 2009). IL-17 has been shown to regulate Th1 responses by altering IL-12 production by dendritic cells. IL-17 has been increasingly implicated in host responses against intracellular pathogens (Khader and Gopal, 2010). *Salmonella* infection activates Th17 cells and is associated with IL-17A production by classical Th17 cells, T cells, and other $CD4^+$T lymphocytes (Schulz *et al*., 2008).

The receptor for IL-17A is ubiquitously expressed in different tissues, leading to pleiotropic effects of the cytokine (Alber *et al.*, 2007; Ouyang *et al*., 2008). IL-17-mediated recruitment of polymorphic nucleotide is required for protective immunity after infection with several pathogens such as *Escherichia coli, Candida albicans,* and *Mycoplasma pneumonia* (Huang *et*

al., 2004; Shibata *et al*., 2007). Pathophysiological processes and the development of organ-dependent autoimmunity, such as experimental autoimmune encephalomyelitis and inflammatory bowel disease, have been revealed to be mediated by the IL-23/IL-17A pathway (Yen *et al.*, 2006).

Interleukin-17A is involved in protection against extracellular and intracellular bacterial pathogens, as well as fungi and viruses (Raffatellu *et al.*, 2008). IL-17 has been increasingly implicated in host responses against intracellular pathogens (Khader and Gopal, 2010). The importance of IL-17 in mucosal immune responses to intracellular enteric pathogens has been demonstrated in animal models (Sellge *et al.*, 2010). It was shown that depletion of Th17 cells during simian immunodeficiency virus infection results in increased dissemination of *Salmonella typhimurium* from the gut (Raffatellu *et al.*, 2008).

It was of great importance to initiate studies to evaluate whether IL-17A might play a role in protection from *S typhi* because the gastrointestinal mucosa is the first point of contact for it. Mucosal immune responses are likely to play an important role in protection as shown in figure (2-2). Thymus output may especially be important during *Salmonella* infection, where $CD4^+$ T cells have been shown to be essential for resistance against both acute and persistent infection, It is known that control of both acute and persistent infection relies on the presence of $CD4^+$ T cells (Kurtz *et al*., 2020).

Interleukin-17A deficient mice demonstrate a modest increase in bacterial dissemination following oral *Salmonella* infection, suggesting a protective

role for IL-17 in the maintenance of the mucosal barrier during early infection.

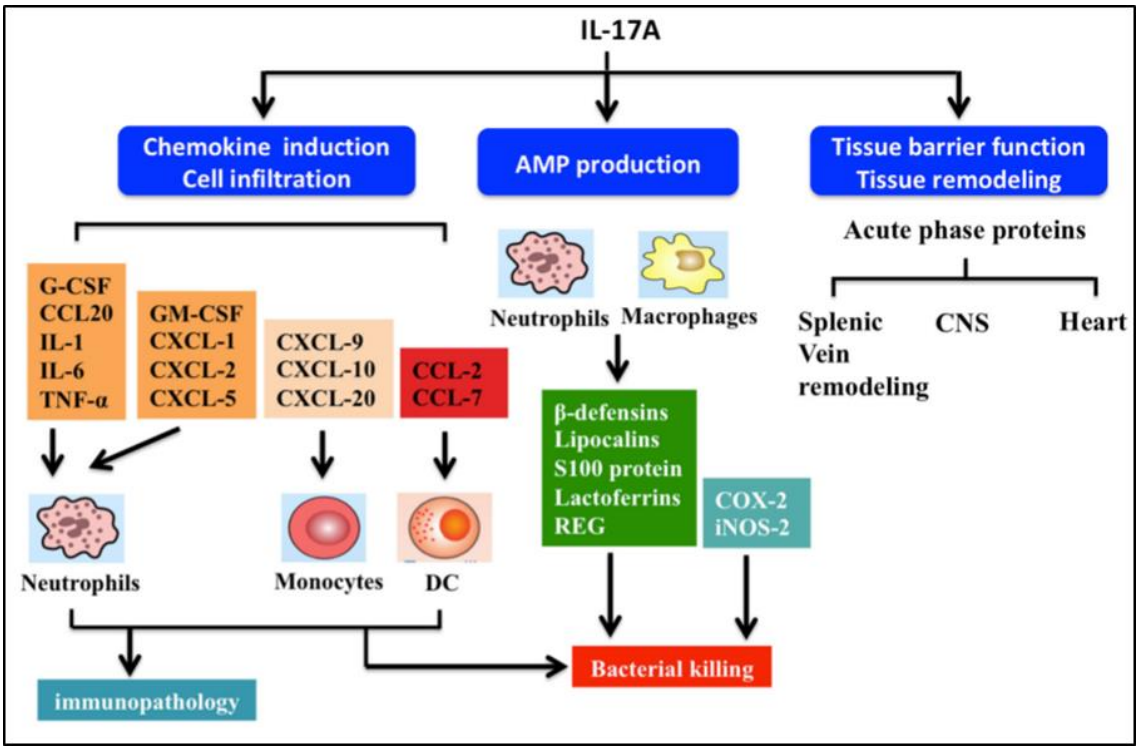


Figure (2-2): Function of Interleukin-17A (Ge *et al*., 2020).

IL-17 induces the expression and secretion of antimicrobial peptides including lipocalin-2 and β defensins (Ishigame *et al.*, 2009). Some of these antimicrobial peptides may control dissemination from the mucosa (Zheng *et al.*, 2008). While lipocalin-2 suppresses the growth of resistant pathogens (Raffatellu *et al.*, 2009). Infection with *S.enteriditis* leads to the appearance of antigen-specific IL-17A cells, and IL-17A knockout animals exhibit a higher bacterial load in the liver and spleen than wild-type mice (Schulz *et al*., 2008). Decreased levels of IL-17A are correlated with an increased risk of bacteremia (Ye *et al.*, 2001).

2.10: Proinflammatory Cytokine Interleukin-1β (IL-1β)

Interleukin-1beta protects against infections by inducing a number of reactions, including the fast recruitment of neutrophils to inflammatory areas, activation of endothelium adhesion molecules, generation of cytokines and chemokines, and development of the febrile response. IL-1β is both pro-inflammatory and pyrogenic cytokines released by numerous cells, particularly innate immune cells such as monocytes, macrophages and dendritic cells (Dinarello , 2006).

IL-1beta is synthesized as a 269 amino acids precursor protein and then processed to the C-terminal 153 amino acids as mature IL-1beta by caspase-1, also known as the IL-1beta converting enzyme activated in inflammasomes (Poyet *et al*., 2002). Chymases, granzyme A, and cathepsin G are neutrophil proteases that cleave the IL-1beta precursor into a secreted, physiologically active form (Robertson *et al.*, 2006).

Inflammasomes are multi-protein complexes that facilitate caspase-1 activation. Active caspase-1 cleaves the preforms of the cytokines IL-1β and IL-18 and the pore-forming protein gasdermin D, leading to cytokine maturation and their release from the cell through the pores. *Salmonella* infection has been shown to have a redundant function in macrophage NAIP/NLRC4 and NLRP3 inflammasome activation (Qu *et al*., 2016; Bierschenk *et al*., 2019).

The NAIP/NLRC4 inflammasome is well characterized in terms of its molecular activation mechanism (Sharma and Kanneganti, 2016). Knockout of NLRC4 leads to loss of inflammasome activation at early time points during infection (Man *et al*., 2014). Inflammasomes arise when bacteria

detect the bacterial protein ligands flagellin and the needle and/or rod of type III secretion systems (Miao *et al*., 2006; Zhao *et al*., 2011; Yang *et al.*, 2013).

IL-1beta is produced in response to a number of PAMPs and DAMPs, which can activate a variety, or in some cases, several, PRRs, resulting in the formation of inflammasomes. Extracellular ATP acting via the receptor induces caspase-1-dependent release of IL-1β (Le Feuvre *et al.*, 2002) and is dependent upon the formation of the NLRP3 inflammasome (Mariathasan *et al*., 2006).

A consequence of caspase-1 activation in macrophages following infection by NLRC4-activating pathogenic bacteria is a rapid, and caspase-1-dependent cell death called pyroptosis. This is a pro-inflammatory form of cell death that causes an infected macrophage to kill itself, and at the same time release pro-inflammatory cytokines such as IL-1β (Bergsbaken *et al*., 2009). Pyroptosis of macrophages following infection with *Salmonella* occurs after the caspase-1-dependent formation of pores causes the dissipation of ionic gradients and the osmotic lysis of the cell in the plasma membrane (Bergsbaken *et al*., 2009).

2.11: Role of Interleukin-1beta in Typhoid Fever

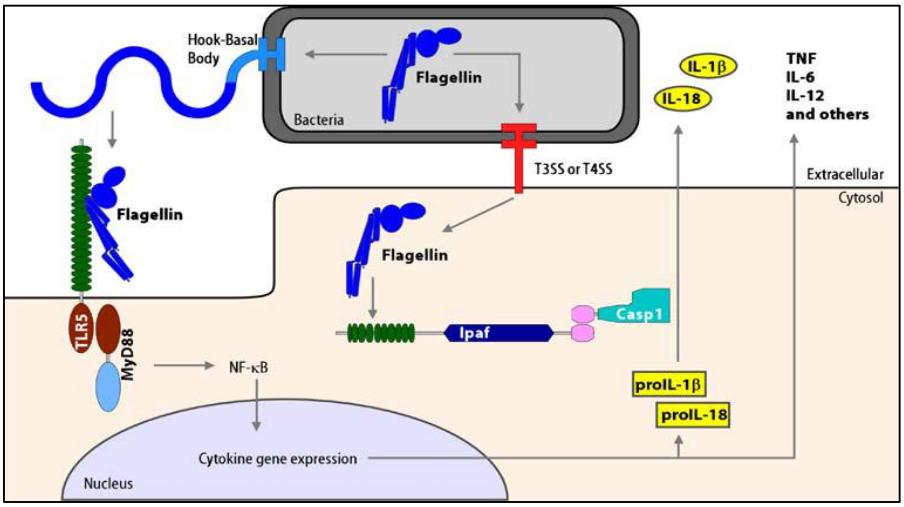
The ability of the host to detect the presence of an infectious agent as well as the ability to kill the invading pathogen is required for an immune response to microbial infections. The presence of infection is detected through pathogen recognition molecules that sense unique microbial components called pathogen-associated molecular patterns (Akira *et al*., 2006). The recognition of bacterial PAMPs is mediated by several host

molecules, including Toll-like receptors that are present on the cell surface and endosomal compartments and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) that sense the presence of PAMPs in the cytosol (Janeway and Medzhitov, 2002). IL-1beta processing induced by specific bacterial infection (Sutterwala *et al*., 2006).

Proteins such as cryopyrin and Ipaf play a crucial role in processing mature IL-1beta. Ipaf referred to a receptor that activates caspase-1 by recognizing flagellin generated by certain bacteria, such as *Salmonella*. The inflammasome is activated by lipopolysaccharides identification via a toll-like receptor and type I interferon (Rathinam and Fitzgerald, 2016).

Caspase-1activation during intracellular bacterial infection involves active contribution from the bacteria. activation occurs following bacterial type III secretion dependent introduction into the cytosol *Salmonella* enterica flagellin (Franchi *et al*., 2006). Caspase-1 activation in the infection with Salmonella spp. occur after exposure to bacterial DNA or flagellin (Muruve *et al*., 2008). Infection of macrophages with *E. coli* LPS or with *Chalmydia muridarum* amplified the secretion of IL-1β approximately five to ten fold (Prantner *et al*., 2009). After lipopolysaccharides stimulation monocytes, IL-1beta mRNA levels rise fast and then begin to fall over the next 4 hours due to mRNA half-life activity. When compared to microbial stimulants, IL-1beta mRNA levels are prolonged for more one day. Active caspase-1 can break the IL-1beta precursor in either specialized secretory lysosomes or the cytoplasm. There appears to be more than one mechanism for digested IL-1beta to escape the cell. These include exocytosis of the secretory lysosomes (Andrei *et al*., 2004) shedding of plasma membrane micro vesicles, direct release via transporters or multi vesicular bodies containing exosomes (Qu *et*

al., 2007) or a process termed pyroptosis (Bergsbaken *et al.*, 2009). The release of processed IL-1β takes place before there is a significant release of lactate dehydrogenase (Brough and Rothwell, 2007) as shown in figure (2-3). Pyroptosis is a process of cell death used by some bacteria in conjunction with Ipaf (Suzuki *et al.*, 2007).



TLR_5 and Ipaf respond to extracellular and cytosolic flagellin. TLR5 detects extracellular flagellin monomers and activates transcriptional responses through MyD88 that result in proinflammatory cytokine expression. Ipaf responds to cytoslic flagellin by activating caspase-1. IL-1β and IL-18 are two cytokines that require dual stimuli to induce their secretion. TLR5 or other TLRs stimulate the expression of proIL-1β and proIL-18, which are retained in the cytosol. Ipaf or other NLRs activate caspase-1, which proteolytically processes proIL-1β and proIL-18, promoting their secretion. The hypothesized mechanism by which flagellin accesses the cytosol during bacterial infection is illustrated. Bacterial protein transport systems (T3SS in *Salmonella* and T4SS in Legionella) are predicted to transport flagellin monomers directly from the bacterial cytosol to the mammalian cell cytosol.

Figure (2-3): Flagellin activation process by IL-1β (Miao *et al.*, 2007)

2.12: Polymorphism in Genetics

Polymorphisms are changes in human DNA sequences that may be associated with any disease characteristics. Polymorphisms are common in the human genome and can be found in almost every gene (Akalin and Murphy, 2001).

Each individual gene has two copies, one of which is located on one of the twenty-three father chromosomes, and each copy of the gene is what geneticists call an allele, which represents a person's genotype. The polymorphic gene that has two similar alleles is known as homozygous, whereas the polymorphic gene that has two different alleles is known as heterozygous (Kozak *et al*., 2020).

Most biallelic point mutations are present within a population at a frequency higher than 1%. SNPs are also thought to be the primary source of variation in humans, particularly when they alter gene expression or function based on their placement in the DNA sequence. In complicated disorders such as infectious and autoimmune diseases, as well as cancer, a substantial number of SNPs in cytokine loci have been discovered and examined (Pacheco and Moraes, 2009).

The SNP or mutation may be related to disease susceptibility, disease pathogenesis, or the efficacy of specific drugs. It is important to detect SNPs or mutations clinically (Matsuda, 2017).

Single nucleotide polymorphisms are located in different regions of genes, such as promoters, exons, introns, and 5′- and 3′-UTRs. The promoter region SNPs affect gene expression by altering promoter activity, transcription-factor binding, DNA methylation, and histone modifications (Schirmer *et al*., 2016).

2.13: Effect of SNPs on (IL-17 and IL-1β) Genes among patients with typhoid fever

Single nucleotide polymorphisms are one of the most common types of genetic variations in the human genome. SNPs in genes that control DNA

and metabolism are associated with a higher risk of cancer (Devilee and Rookus, 2010; Ulaganathan *et al.*, 2015). Elevated production of inflammatory cytokines can be caused by genetic variation such as single nucleotide polymorphisms in promoter or regulatory elements of cytokine genes. Furthermore, SNPs, in particular pro-inflammatory cytokine alleles, led to increased susceptibility to a variety of illnesses (Keshavarz *et al*., 2019).

Interleukin-17 deficiency or variation, a cytokine primarily secreted by Th17 cells, has been linked to increased susceptibility to infection by a wide range of pathogens (Dhaouadi *et al*., 2018). The rs2275913, which is found in the promoter of the IL-17A gene, has been linked to a variety of disorders. The presence of the A allele at rs2275913 has been linked to an increase in IL-17A secretion (Ohka *et al*., 2018).

A meta-analysis study showed that the IL-17A rs2275913 polymorphism is remarkably linked to the risk of many types of cancer (Dai *et al*., 2016; Rolandelli *et al.*, 2017). Recent research found that the IL-17A gene is linked to viral infection and that might result in IL-17 variants (Pinto *et al*., 2017). Polymorphisms in the IL-1beta gene have been linked to the development of gastric cancer. Those found in the IL-1beta gene are linked to the inhibitory impact of cytosine on stomach acid output. This inhibition promotes colonization and infection by pathogens such as *Helicobacter pylori*, as well as the emergence of pre-neoplastic states that can lead to cancer formation (Arango *et al*., 2010). At present, IL-17A and IL-17F gene polymorphisms have been found to be associated with a series of diseases. Wang *et al*. (2014) demonstrated that IL-17 G197A (rs2275913) A allele and rs3748067 C allele were associated with an increased gastric cancer risk

in contrast with normal controls, which suggests that some IL-17 gene polymorphisms significantly increase gastric cancer risk (Wang *et al*., 2014).

Another study on cervical cancer patients revealed that IL17A G197A gene polymorphism was associated with the susceptibility of the cancer in Chinese women (Kawaguchi *et al*., 2006). Other researchers have recently reported that IL-17A rs2275913 polymorphisms were associated with the susceptibility to rheumatoid arthritis and ulcerative colitis (Arisawa *et al*., 2008; Nordang *et al*., 2009).

One of the usual functional SNPs was discovered in the IL-1beta promoter, T to C transition (rs16944). It is found in an IL-1beta TATA-box motif and influences IL-1beta transcription activity by binding various transcription factors (Zhang *et al*., 2014). When the rs16944 polymorphism in IL-1beta contributes to an elevated risk of gastric cancer with a proinflammatory phenotype in Caucasian carriers, it has been established that an increase in IL-1beta cytokine promotes lung damage after influenza A infection (El-Omar, 2000). Camargo *et al*. (2016) discovered that IL-1beta levels in the gastric mucosa are higher than usual in *H. pylori* patients and bearers of the IL-1B511TT or IL-1RNA2-A2 genotypes. Rogo and colleagues recently documented the impact of specified SNPs in IL-1 and IL-17 on influenza virus in the Iranian population, which demonstrated a greater chance of acquiring a severe illness (Rogo *et al*., 2016).

3: Materials and Methods

3.1: Materials

3.1.1: Laboratory Instruments

The laboratory tools and apparatuses utilized in this investigation are listed in table (3-1).

Table (3-1): Instruments and Apparatuses

Instrument	Company	Origin
Autoclave	Tripod	UK
Bench centrifuge	Memmert	Germany
Burner	Amal	Turkey
Cooling centrifuge	Hitachi	Germany
Deep freezer	Al-Mateen	China
Different size tips	Bio basic	China
Different size tube	Bio basic	China
Digital camera	Sony	Japan
Distillatory	GFL	Germany
DNA extraction tubes	Eppendorf	Germany
ELISA reader	Biotech	USA
Flask	Chemical-Lab	China
Hood	Labogene	Denmark
Horizontal gel electrophoresis	Bio-Rad	Italy
Hot plate	Heiddph	Germany
Incubator	Memmert	Germany
Light microscope	Olympus	Japan
Nanodrop	Bio-Rad	Italy

Oven	Memmert	Germany
PCR system	Clever	Germany
PCR tubes	Eppendorf	Germany
Petri dishes	Sterilin	England
Plain tubes	DMD-DISPO	Syria
Polyetheleyne tubes	DMD-DISPO	Syria
Refrigerator	Concord	Italy
Sensitive electron balance	Sauter	Switzerland
Thermocycler	Clever, BIO -RAD	UK, USA
Vitek-2	BioMérieux	France
Vortex mixer	Griffin	Germmy
Water bath	Memmert	Germany

3.1.2: Chemical Materials

The chemical materials, stains, reagents, and solution that used are listed in table (3-2).

Table (3-2): Chemical Materials

Chemicals	**Company / Origin**
Acrylamide	Fluka / Germany
Agarose	Pronadisa / Spain
Barritt's reagent	Himedia / India
Bis acrylamide	Scr / China
DNA ladder marker	Promega / USA
EDTA	Thomas baker / India
Ethanol (70% and 99%)	Fluka chemika / Switerland
Ethidium bromide	promega / USA
Gram stain kit	Sigma / Germany
Hydrogen peroxide	Fluka / England

Kovac's reagent	Himedia / India
McFarland’s standard solution	Biomerieux / France
Oxidase reagent	Himedia / India
Tris-Borate-EDTA (TBE)	Bio Basic / Canada
Tris-base	Bio Basic / Canada

3.1.3: Culture Media

Culture media that were used are listed in table (3-3).

Table (3-3): Culture media

Culture Media	Company / Origin
Brain Heart Infusion Broth, Simmon Citrate Agar, MacConkey Agar , Blood agar, *Salmonella Shigella* agar , XLD agar	Conda / Spain
Triple sugar iron	Himedia / India
peptone water, MR-VP broth	Himedia / India

3.1.4: Kits

Kits were used in this study are listed in table (3-4).

Table (3-4): kits

Type of Kit	Company / Origin
DNA extraction Kit	Favorgen / Taiwan
Green master mix	Promega / USA
ELISA Kit forHuman IL-17A and IL-1β	Bioassay / China

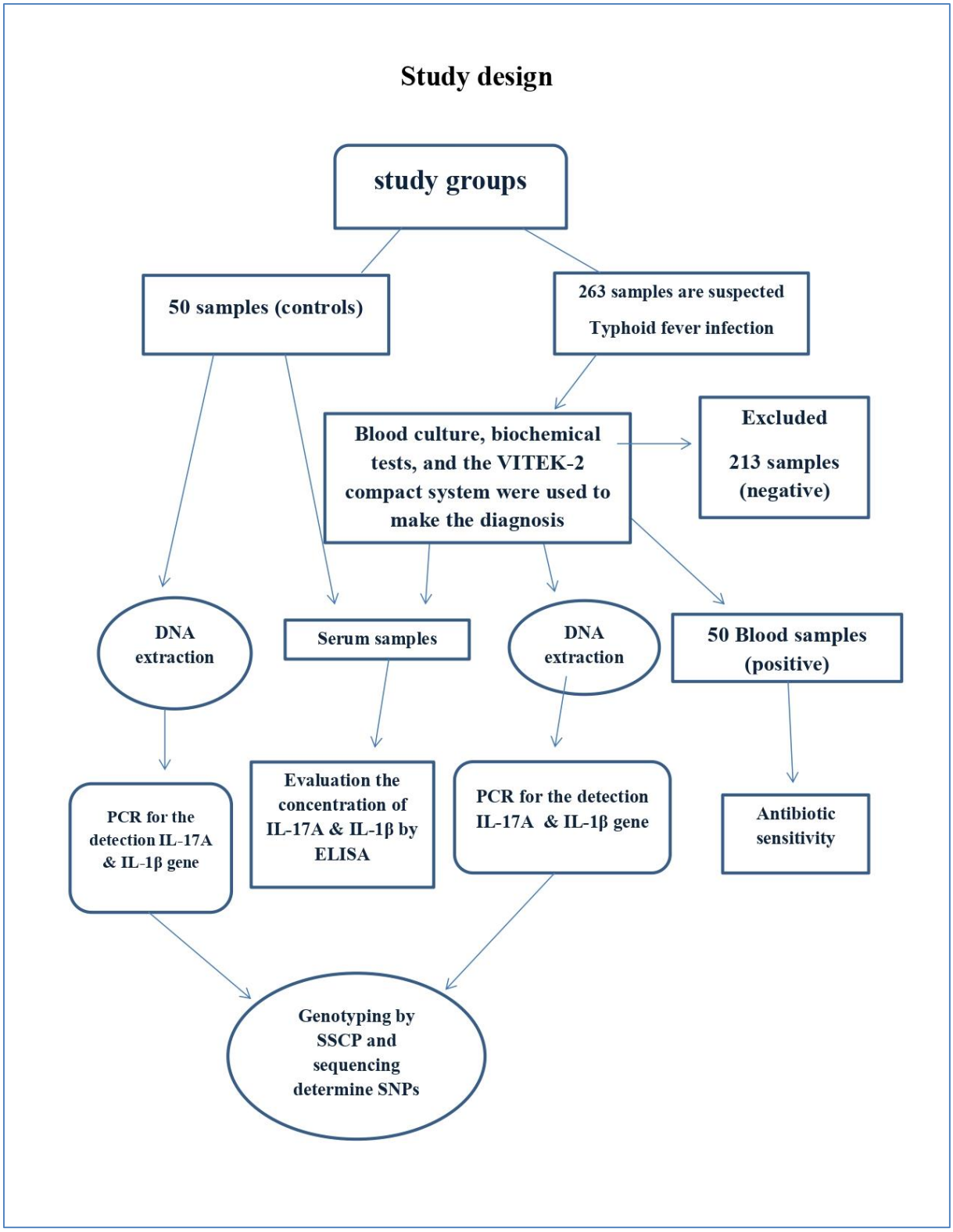
Table (3-5): Primers of IL-17A and IL-1β genes

Gene type	Primer name	Primer sequence 5′ to 3′	Melting temp	Product size
IL-17A	ZF25	F: 5′-GACCTTGGGGGCGGAAA-3′	62.49 Cº	301 bp
		R : 5′-CCATAGTCAGAACCCAGCGTTT-3′	60.62 Cº	
	ZF26	F : 5′-GGGGAAAATGAAACCCTCCCC-3′	60.90 Cº	328 bp
		R : 5′-GGGGCGAAAATGGTTACGATG-3′	59.94 Cº	
IL-1β	ZF27	F : 5′-TGTGCCTCGAAGAGGTTTGG-3′	60.25 Cº	368 bp
		R : 5′-GTGTCTTCCACTTTGTCCCAC-3′	59.05 Cº	
	ZF29	F : 5′-GGTGCTCCCTGTTGGATCTT-3′	59.67 Cº	369 bp
		R : 5′-TTACAGGTCAGTGGAGACAC-3′	59.40 Cº	

***All primers design in this study by program primer BLAST**

F= forward, R= reverse, temp = temperature, bp= base pair

3.2.1: Study design



The methods used in this study

3.3 :Methods

3.3.1: Patients and Clinical Samples

A total of 263 samples were collected from the blood of patients with typhoid fever who attended Al-Hillah Surgical Teaching Hospital and private laboratories in AL-Hillah/Babylon province, during the period from February to August 2021. Ten ml of blood was drawn from patients suffering from typhoid fever symptoms aged 15–65 years. 5 ml of blood was injected into bottles containing 20 ml of brain heart infusion broth and incubated for 3 days at 37ºC. If positive, each sample was inoculated using the direct method of inoculation on MacConkey, Blood, XLD, and SS agar and incubated for 24 hours at 37ºC. These samples were compared with 50 samples of apparently healthy individuals who appeared to be disease-free.

3.3.2: Moral Approval

The appropriate ethical permission from the ethics committees of Babylon Health Office, the hospital's ethical committee, as well as patients and their supporters, must be acquired. Furthermore, prior to the collection of samples, all participants engaged in this work are informed, and the agreement necessary for conducting the tests and publishing this work is acquired from each one.

3.4 : Culture Media Preparation

3.4.1: Blood Agar Medium

Dissolving 40 grams of blood agar base in 1000 distal water yielded blood agar medium. It was autoclaved for 15 minutes at 121C^{o} before being cooled to 50C^{o}. Human blood was added at a rate of 5%. This medium was used to

cultivate bacterial isolates and to test bacteria's capacity to hemolyze blood cells (McFadden, 2000).

3.4.2: MacConkey Agar

This medium is used to separate and distinguish bacteria that ferment and bacteria that do not ferment lactose. It was made by weighing 51.5 grams of medium, dissolving it in one liter of distilled water, heating to boiling with agitation to dissolve the medium. It was autoclaved for 15 minutes at 121 C^o cooling to 55C^o, and pouring it onto petri dishes (McFadden, 2000).

3.4.3: *Salmonella–Shigella* Agar

This medium was used to isolate and identify *Salmonella* and *Shigella*. It is made by dissolving 63 grams of medium in one liter of distilled water, heating it to boiling with frequent agitation to completely dissolve the medium, cooling it to 55C^o in a water bath, and pouring it into petri dishes (McFadden, 2000).

3.4.4: Xylose-Lysine Deoxycholate Agar

This medium was made by dissolving 56.68 grams of powder in 1000 ml distilled water and thoroughly mixing it with a magnetic-stirring apparatus, then heating it with frequent agitation with a magnetic-stirring apparatus until the medium just boiled to dissolve completely, then pouring it into plates, and leaving it to solidify (Wallace *et al*., 2011).

3.4.5: Brain Heart Infusion Broth

This medium is used to activate bacteria; it is made by dissolving 37 grams of media in one liter of distilled water and sterilizing it by autoclaved for 15 minutes at 121C^{o} (MacFaddin, 2000).

3.4.6: Peptone Water medium

This medium is used to test bacteria's capacity to synthesize an indole ring from tryptophan. It is made by dissolving 15 grams of medium in one liter of distilled water, then filling sterile test tubes and sterilizing them in an autoclave for 15 minutes at 121C^{o} (McFadden, 2000).

3.4.7: Triple sugar Iron Agar

This medium is made by dissolving 55 grams of medium in one liter of distilled water and sterilizing it by autoclave for 15 minutes at 121C^{o}. This Agar test employs a medium for the identification of Enterobacteriaceae, based on double sugar fermentation and hydrogen sulphide production. The medium is then distributed in five ml amounts as a slant in sterile tubes and placed in a slant position (McFadden, 2000).

3.4.8: Methyl Red Voges-Proskauer Broth

This medium can be made by dissolving 17 grams in one liter of distilled water, heating if required to thoroughly dissolve the medium, then distributing in test tubes in ten ml portions and sterilizing by autoclave for 15 minutes at 121C^{o}. This test is used to determine which fermentation pathway is used to utilize glucose (McFadden, 2000).

3.4.9: Simmon's citrate medium

Simmon's citrate medium was used for determining the ability of bacteria to utilize citrate as the sole carbon source. Prepared by dissolved 23 grams in 1000 ml of distilled water. The medium is boiled for a few seconds until the ingredients are completely dissolved. Distribute in tubes or bottles and sterilize by autoclaving at 121ºC for 15 minutes. Allow the tubes to cool on a slope (McFadden, 2000).

3.4.10: Gram's Staining

Bacteria were identified using a Gram stain kit. It is made up of the following ingredients: crystal violet, iodine, alcohol, and safranin stain. Take distal water by loop full, and a single colony from culture was taken by loop and spread on a clean slide, then waited for it to dry and be fixed by heat, staining with gram stains, and examining the bacterial cell under a microscope (Jawetz *et al.*, 2007).

3.5.1: Blood Samples

Two ml of blood were drawn and stored at -20ºC in an ethylinditetracitic acid (EDTA) tube for DNA extraction for molecular analysis.

3.5.2: Serum Samples

Three ml of blood kept in the jelly tube without anticoagulant. The latter was undergone centrifugation at 2500 rpm for 15 minutes then the serum was collected and preserved at -20ºC until being used to avoid loss of bioactive interleukins of human.

3.6: Preparation of Solutions

3.6.1: Normal Saline Solution

It was prepared by dissolving 0.85 g of NaCl in 90 ml distilled water and further completed to 100 ml with D.W. Then autoclaved at 121C^{o} for 15 min and stored at 4 C^{o} until be used. Normal saline was utilized in all tests and investigations that required it (McFadden, 2000).

3.6.2: McFarland's Turbidity Standard

McFarland's standard was made by adding (0.05) ml of (1.175%) barium chloride to (1%) sulfuric acid (9.95) ml in order to obtain a barium sulfate precipitate. The solution was used to visually compare the turbidity of a suspension of bacteria with the turbidity of the (0.5) McFarland's standard. Then checked by spectrophotometer (Murray *et al*., 2003).

3.6.3: Phosphate Buffer Saline PH 7.2 (PBS)

It was prepared by dissolving one pill of PBS in 100 ml distil water then autoclaved at 121^{o}C for 15 min and stored at 4^{o}C until be used.

3.6.4: Tris-Borate-EDTA (TBE) Buffer

Tris-Borate-EDTA Buffer was used at 1X(1:10 dilution of the concentration stock). The stock solution was diluted by D.W. and stored at room temperature. Also (TBE) buffer is used and prepared by Promega company/USA.

3.6.5: Tris-EDTA Buffer (TE)

This buffer was prepared by adding 0.05 M of Tris-OH and 0.001M of EDTA to 800 ml D.W.; the pH was adjusted to 8 and completed to one litter by D.W., then autoclaved at 121^{o}C for 15 min and stored at 4^{o}C until be used. TE buffer is used and prepared from Promega company/USA.

3.6.2: Reagents

3.6.2.1: Catalase Reagent

The reagent was created by combining 3 ml of H_2O_2 with 100 ml of distilled water and keeping it in a dark jar. The reagent was used to test bacteria's ability to produce the catalase enzyme (Forbes *et al*., 2007).

3.6.2.2: Methyl red reagent

The reagent was made by dissolving 0.1 gram of methyl red in 300 ml of (99 %) ethanol and then adding distilled water to make the volume (500) ml. This reagent was used to determine the total amount of glucose hydrolysis (Forbes *et al*., 2007).

3.7: Laboratory Diagnosis

3.7.1: Serology Test

The Widal test was used to identify typhoid antibodies in blood samples. The presence of typhoid antibodies in fresh blood samples was determined using the O and H antigens as follows:

1- Five milliliter of blood was centrifuged for five minutes to separate the serum.

2- On a clean glass slide, a single drop of each O and H antigen was applied individually.

3- One drop of serum was extracted from the blood and thoroughly mixed with each antigen. Then waited two minutes. Agglutination was reported as a positive outcome if it was seen

3.7.2: Isolation and Identification of *S typhi*

Blood cultures were evaluated on a regular basis for the following signs of microbial development: turbidity, gas production, color change, and other signs of microbial growth. Before reporting negative results, cultures should be cultured for at least seven days. Blood agar and MacConkey agar were used to subculture probable positive bottles. Pale MacConkey agar colonies were subcultured on Xylose Lysine Deoxycholate agar and *Salmonella Shigella* agar and incubated at 37C^{o} for 24–48 hours. If a colony was visible, it was recognized based on its morphology and then inspected under a light microscope after being stained with Gram's stain.

3.7.3: Biochemical Test

3.7.3.1: Oxidase Test

The test was used to identify the presence of an oxidase enzyme in bacteria, which catalyzes electron transport among the bacteria's electron donors, and a redox dye, which had been reduced to a purple color. A few bacteria colonies were dispersed on the filter paper saturated with oxidase reagent by altering the color to purple for ten seconds, yielding a good result (Forbes *et al.,* 2007).

3.5.3.2: Catalase Test

Catalase is an enzyme that catalyzes the oxygen release from hydrogen peroxide. Using a sterile wooden stick, a small amount of bacterial growth was transferred to the surface of a clean, dry glass slide, and one drop of 3 percent H_2O_2 was added to it. The production of gas bubbles suggested a successful outcome (Forbes *et al.*, 2007).

3.7.3.3: Indole Test

Fresh colony was cultured on peptone water then incubated at 37C^{o} for (24-48) hrs. Drops from Kovacs reagent were added. The presence of pink ring after adding of reagent considered as positive result of the test (Forbes *et al.,* 2007).

3.7.3.4: Methyl Red Test

Five drops of methyl red reagent were applied to a broth of bacteria after incubated at 37^{o}C for 24 hours. The next step was to study to read results. The color change to red (Forbes *et al* ., 2007).

3.7.3.5: Voges-Proskauer Test

A young colony was grown on methyl red-Voges-Proskauer broth and incubated at 37^{o}C for 48 hours then added Britt's reagent. A favorable reaction was confirmed by the appearance of a pink tint within 20 minutes (Forbes *et al*., 2007).

3.7.3.6: Citrate Test

Young colony was cultured on Simmons citrate slant, then incubated at 37^{o}C for 48-72 hours, The color changed from green to blue that considered a positive result (Forbes *et al*., 2007).

3.7.4: VITEK-2 Compact System

The VITEK-2 compact system was used to confirm the manual biochemical test findings, and it was recently employed to identify bacteria (Pincus, 2010). It was provided with the essential identification data base for all standard identification tests, allowing for greater efficiency in

microbiological diagnosis by minimizing the time and need for any additional tests that are safe for the system's user. This system was created in compliance with the specifications provided by the manufacturer (BioMérieux-France).

A personal device, a reader incubator, and a variety of internal components such as a card cassette, a card filler mechanism, a cassette loading processing mechanism, a card sealer, a bar code reader, a cassette carousel, and an incubator comprise this system. Transmittance optics, waste processing, instrument control electronics, and software are also included in the system. In a plane test tube, three milliliters of normal saline were poured and infected with a lope full of isolated colonies before being introduced into a dens check machine to standardize the colony to McFarland's standard solution ($1.5x10^8$ cells/ml).

The standardized inoculums were inserted into the cassette, and a barcode-encoded sample identification number was entered into the computer software. The VITEK-2 card type is then read from the barcode placed on the card during manufacturing, and the card is thus linked to the sample ID number. The tape was then put into the filler module.

The cassette was transferred to the reader/incubator module when it was full. All the following activities, such as adjusting the incubation temperature, optically reading the cards, and continually monitoring and sending test findings to the computer for analysis, were handled by the equipment.

3.7.5: Antibiotics by VITEK-2 Compact System

The automated VITEK-2 compact system was used for antibiogram testing, which was based on MIC method determination employing AST-N222 cards: Piperacillin, Piperacillin/Tazobactam, Piperacillin/Clavulanic acid, Cefepime, Aztreonam, Imipenem, Meropenem, Gentamicin, Tobramycin, Ciprofloxacin, Ticarcillin, Amikacin, Ceftazidime and Trimethoprim/Sulphamethoxazole. The AST-N222 cards were infected in the same way as specified in the VITEK-2 compact system, which analyzes each organism's growth pattern in the presence of the antibiotic in relation to growth control well. Several factors are employed to give acceptable input for MIC estimations based on the observed growth characteristics. To identify a category interpretation, the MIC result must be connected to an organism identity.

3.8: Immunological Study

3.8.1: IL-17A Level in Serum by ELISA

3.8.1.1: Principal and Methods

The enzyme-linked immunosorbent assay is a medical diagnostic test that employs antibodies and antigens and color change. The micro ELISA plate has been pre-coated with a human IL-17A antibody. Standards or samples are mixed with the particular antibody in the micro ELISA plate wells. Then, in each microplate well, a biotinylated detection antibody specific for human IL-17 and an Avidin-Horseradish Peroxidase conjugate are added and incubated. When stop solution is added to the enzyme-substrate reaction, the color turns yellow. A spectrophotometer with a wavelength of 450 nm is used to measure the optical density.

3.8.1.2: Assay Procedure

1- All reagents, stander solutions, and samples were prepared as instructed, and all reagents were brought to room temperature before use. The assay is performed at room temperature.

2- A 50µl of the standard was added to the standard well.

3- A 40µl of the sample was added to sample wells and then was added 10µl anti- IL-17A antibody to sample wells, then was added 50µl streptavidin-HRP to sample wells and standard wells (not blank control well). Mixed well, and cover the plate with a sealer and incubated for 60 minutes at 37^{o}C.

4- Removed the sealer and washed the plate five times with wash buffer. Soaked wells with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash.

5- A 50µl substrate solution A was added to each well and then was added 50µl substrate solution B to each well. Incubation plate covered with a new sealer for 10 minutes at 37^{o}C in the dark.

6- A 50µl of stop solution was added to each well, the blue color will change into yellow immediately.

7- The optical density (OD value) of each well determined immediately by using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

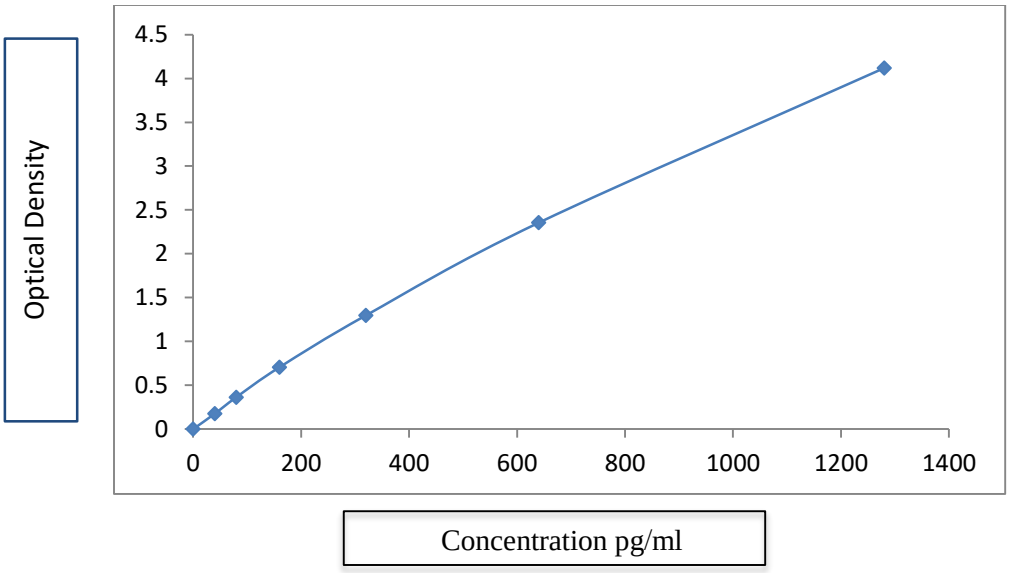


Figure (3-3): Standard curve for IL-17A concentration

3.8.2: IL-1β Level in Serum by ELISA

3.8.2.1: Principal and Methods

The enzyme-linked immunosorbent assay is a medical diagnostic test that employs antibodies and antigens and color change. The microplate has been pre-coated with a human IL-1beta antibody. Standards or samples are mixed with the particular antibody in the micro ELISA plate wells. Then, in each microplate well, a biotinylated detection antibody specific for human IL-1 and an Avidin-Horseradish Peroxidase conjugate are added and incubated. When stop solution is added to the enzyme-substrate reaction, the color turns yellow. A spectrophotometer with a wavelength of 450 nm is used to measure the optical density.

3.8.2.2: Assay Procedure

1- All reagents, stander solutions, and samples were prepared as instructed, and all reagents were brought to room temperature before use. The assay is performed at room temperature.

2- A 50µl of the standard was added to the standard well.

3- A 40µl of the sample was added to sample wells and then was added 10µl anti- IL-1beta antibody to sample wells, then was added 50µl streptavidin-HRP to sample wells and standard wells (not blank control well). Mixed well, and cover the plate with a sealer and incubated for 60 minutes at 37ºC.

4- Removed the sealer and washed the plate five times with wash buffer. Soaked wells with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash.

5- A 50µl substrate solution A was added to each well and then was added 50µl substrate solution B to each well. Incubation plate covered with a new sealer for 10 minutes at 37ºC in the dark.

6- A 50µl of stop solution was added to each well, the blue color will change into yellow immediately.

7- The optical density (OD value) of each well determined immediately by using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

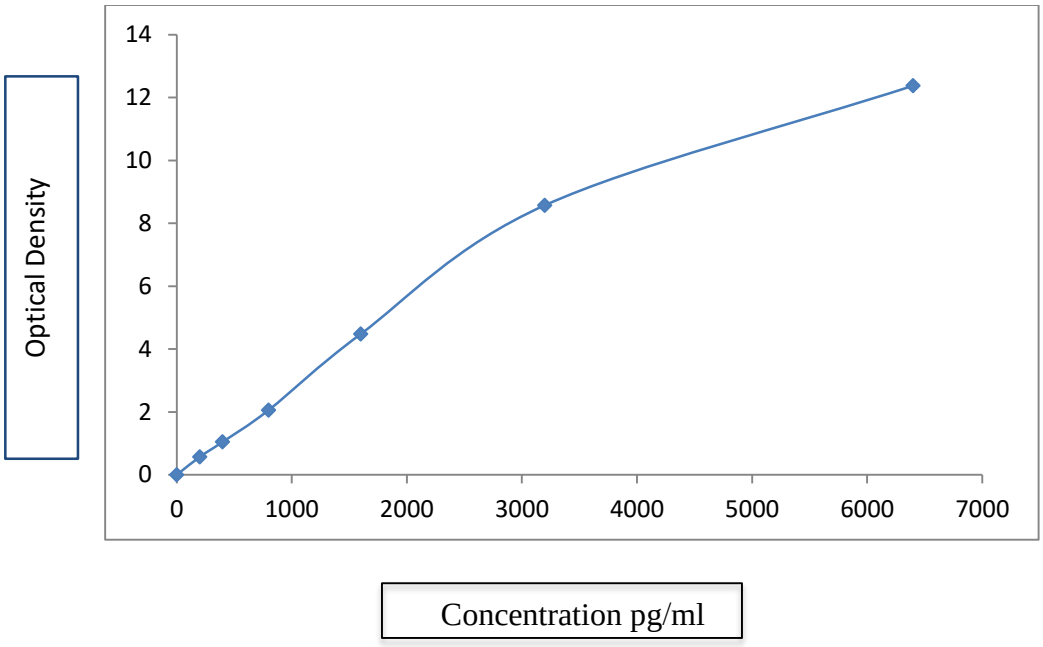


Figure (3-3): Standard curve for IL-1beta concentration

3.9: Molecular Study

3.9.1: DNA Extraction

Genomic DNA from blood samples was extracted using the Genomic DNA extraction kit procedure for frozen blood (Favorgen/Taiwan) and the following stages were followed:

1- A volume of 200 µl of frozen blood were put into a sterile 1.5 ml micro centrifuge tube, and then 20 µl (10 mg/ml) of proteinase K were added and mixed well then incubated for 15 minutes at 60°C.

2-Followed by adding 200 µl of GSB buffer to each tube and forcefully mixed by vortex, and all tubes were incubated at 60ºC for 15 minutes, inverting every three minutes throughout the incubation period.

3- A volume 200 µl of absolute ethanol (96-100%) were added to the lysate sample and mixed by shaking vigorously for 10 seconds.

4- A GSB Mini Column was inserted into a 2 ml collection tube, and the entire mixture (including any precipitate) was transferred to the column. The mixture was then centrifuged at 10,000 xg for 5 minutes.

5- A 400 µl W1 buffer were added to the GSB Mini Column and centrifuged at 10,000 xg for 30 seconds before discarding the flow through and placing the column back in the 2 ml collection tube.

6-Each column received a washing buffer. The column was then centrifuged at 10,000 xg for 30 seconds, the flow through was discarded, and the column was put back in the 2 ml collecting tube.

7-After centrifuging all of the tubes for 3 minutes at 10,000 xg to dry the DNA, it was transferred to a clean 1.5 ml micro centrifuge tube and 100 µl of warmed elution buffer was added to the middle of the column matrix.

9-The tubes were left for at least 5 minutes to allow the elution buffer to be absorbed by the matrix. The purified DNA was then centrifuged at 10,000 xg for 30 seconds to elute it.

10- The DNA was stored at -4^{o}C until used it.

3.9.2: DNA Concentration

The collected blood genomic DNA was tested using a nanodrop spectrophotometer, which evaluated DNA content (ng/L) and DNA purity by reading absorbance at (260/280 nm) as shown below.

3.9.3: PCR Amplification

3.9.3.1:Principle of PCR

Polymerase chain reaction is a technique commonly used in molecular biology. The basic PCR idea is straightforward. It is a chain reaction, as the name implies: one DNA molecule is utilized to generate two copies, then four, then eight, and so on. Specific proteins are responsible for this constant doubling. Polymerases are enzymes that can link together individual DNA building pieces to generate lengthy molecular strands.

3.9.3.2: Preparing the Primers

Primers were made according to the manufacturer's instructions to a supply of primers. Each primer was treated with lyophilized primer dissolved in nuclease-free water to create a master stock that would be utilized to create a working stock.

3.9.3.3: Agarose Gel Electrophoresis

1-To make the gel (1%), dissolve 0.3 grams of agarose in 30 ml of 0.5XTBE buffer and heat in a microwave oven for 45 seconds.

2-Agarose was homogenized and chilled to 55C^{o}. The gel was then combined with 2µl of ethedium bromide by swirling.

3-The gel was then put into the gel attempt and put comb to form wells then allowed to polymerize for 30 minutes. The polymerized gel was then transferred to the electrophoresis apparatus after removed comb and immersed in 0.5 TBE running buffer. 10µl of extracted DNA were combined with 2µl of loading dye and mechanically pipetted onto the gel wells.

4-The electrophoresis was performed by setting the apparatus to 100 volts for one hour, imaging the gel, and analyzing the image to determine the extracted DNA molecular weight.

3.9.3.4: Optimization of IL-17A and IL-1β Genes PCR Product

Different annealing temperatures were used to optimize primer pairs which produced (301 bp) gene of first region and (328) second region of the IL-17A, while the optimize primer pairs of the IL-1β gene which produced (368 bp) first region and (369 bp) second region. A gradient of (55-66^{o}C) annealing temperature were used to optimize the PCR amplification.

3.9.3.5: PCR Amplification of IL-17A and IL-1β genes

Table (3-6): Mixture optimized reaction of PCR

Composition		Volume
1	Master mix	8 µl
2	10µ M Forward Primer	1 µl
3	10µ M Reverse Primer	1 µl
4	DNA sample	2 µl
5	Nuclease-free water	7.5 µ
6	$Mgcl_2$	0.5 µl
Total		20 µl

Table (3-10): Conditions of PCR thermal cycling

Stage	Step	Temperature	Time	No. of Cycles
1	Initial denaturation	94 C^0	5 min	1
2	Denaturation	94 C^0	30 sec	35
	Primer annealing	60 C^0	30 sec	
	Extension	72 C^0	30 sec	
3	Final extension	72 C^0	5 min	1

3.10: Single Strand Conformation Polymorphism

Mutation scanning approaches, such as PCR-coupled single-strand conformation polymorphism, provide substantial benefits over many other nucleic acid techniques for analyzing allelic and mutational sequence variation. The current protocol describes the SSCP method of analysis, including all steps from small-scale genomic DNA isolation and PCR implication of target sequences to gel-based amplicon separation and SSCP scanning for mutations. The sequencing analysis of polymorphic bands recovered from gels that follow is also addressed. The SSCP procedure may identify point mutations in amplicons as small as 450-500 bp and typically takes one day to complete. This user-friendly, low-cost, possibly high-throughput disease detection method has the potential to be applied to any gene from any creature (Gasser *et al*., 2006).

3.10.1: Procedure of SSCP

1-The glass plates were carefully washed with warm tap water before being rinsed with tap water, deionized water, and finally ethanol. They were either wiped dry or air dried.

2-First, the long plate was placed on a clean surface, followed by the left and right spacers positioned along the long plate's sides. The short plate was placed on top of the spacers, flush with the bottom border of the long plate.

3-After loosening the single screw on each sandwich clamp, each clamp was positioned on the proper side of the gel sandwich, with the locating arrows facing up and toward the glass sandwich.

4-The gel sandwich was tightly gripped and inserted into the left and right clamps. The screws were tightened sufficiently to keep the plates in place. The plates and spacers were checked for evenness at the bottom. If this was not the case, the plates and spacers were repositioned in order to achieve a proper seal. Failure to do so might have resulted in gel leakage during casting and buffer leakage during the run.

5-The gray sponge was inserted into the casting slot in the front. With the short glass plate facing front, the sandwich assembly was put on the sponge. To secure the sandwich in position, the sandwich was forced down and the camshaft handles were held down as well.

6-A gel solution was prepared with the necessary polyacrylamide percentage volume of reagents used to cast polyacrylamide gel (12%) containing H_2O (4ml), TBE (2ml) and 30% Acrylamide (4 ml). The sandwich was dipped in the gel solution.

7-To produce the sample wells, a comb was put into the top of the sandwich and allowed to polymerize at room temperature for at least 1 hour. After polymerization, the comb was removed by gently drawing it straight up. The gel sandwich was removed from the casting stand and secured to the core,

with the short glass plate facing the core. The core was flipped over and the second gel sandwich was connected.

8-Using a syringe and needle, the upper and lower chambers were filled with 1X TBE buffer, and the wells were thoroughly washed with running buffer. The core and the gel sandwiches linked to it were placed in the electrophoresis tank, allowing the core to lock in place.

3.10.2: Preparing the Sample

Given 2.5 ml of PCR product was combined with 2.5 ml of SSCP gel loading buffer and heated at 90^{o}C for 5 minutes before being placed on ice.

3.10.3: Electrophoresis

1-After the running buffer reached the correct temperature, the pre-run was terminated, the wells were washed with running buffer, and 5 ml of the samples were put into the wells using long tips. The gel was run for 6 hours at 5-15 seconds at a steady power of 125 volts.

2-After the electrophoresis was finished, the power supply and water chiller system were switched off, the electrodes were detached, and the core was gently removed from the electrophoresis tank.

3.10.4: Silver Staining

1-To absorb buffer leaks, the core and gel sandwich were placed on a cushioned surface. The gel sandwich was taken out of the core. The gel was gently removed from the plates and briefly washed in deionized water.

2-The gel was dipped in a tray containing the first solution (90 ml of deionized water, 10 ml of ethanol, 0.2 gram silver nitrate, and 500 ml of

acetic acid), and the tray was put on top of a shaker for at least 20 minutes to combine.

3-Solution 1 was drained away, and the gel was washed briefly with deionized water. The second solution was placed in a 55^{o}C water bath. After the first solution was withdrawn, the second solution (three grams of NaOH, 100 ml of formaldehyde, and 100 ml of deionized water) was added.

4-Once the bands were clearly visible, the second solution was discarded, and the third solution (10 ml of ethanol, 90 ml of deionized water, and 500 ml of acetic acid) was added for 5 minutes. After placing the gel on top of the LED light, the bands were read, the findings on the gel were recorded, and a snapshot was taken.

3.11: DNA Sequencing

The resolved PCR amplicons were commercially sequenced from both forward and reverse termini according to the sequencing company's instruction manuals (Macrogen, South Korea). Only clean chromatograms acquired from sequence files were examined further, verifying that the annotation and variances were not the result of PCR or sequencing errors. The virtual locations and other information of the obtained PCR fragments were determined by matching the observed DNA sequences of the NCBI Blast engine.

3.12: Interpretation of Data for Sequencing

Using Bio Edit Sequence Alignment Editor Software Version 7.1, the sequencing results of various PCR products were edited, aligned, and assessed as long as they matched the appropriate sequences in the reference database (DNASTAR, Madison, WI, USA). The detected differences in each sequenced sample were numbered in both PCR amplicons and their matching locations within the reference genome.

3.13: Examining The Originality of SNPs

The detected SNP was submitted to the single nucleotide polymorphism database dbSNP for verification. Each SNP was re-positioned based on its location in the reference genome. Following that, the presence of a prior SNP was determined by viewing its associated dbSNP location. The identified SNP's dbSNP position was recorded.

3.14: Statistical data

The SPSS application was used to analyze all of the data (adaptation 17 for Windows 7) The T-test, odds ratio, and P-value (0.001) with a 95% confidence interval were used to assess the significant level of difference in genotypes and allele frequency, and the mean was used to determine IL-17A and IL-1β serum levels for each genotype.

4: Results and Discussion

4.1: Demographic Information

A total of 263 blood samples were collected from patients with typhoid fever, with the current study only including those only with symptoms of typhoid fever during the period from February to August 2021. Only 50 (19%) of 263 people tested were positive for *S typhi* infection using blood culture, biochemical tests, and the VITEK-2 compact system. The demographic data included the age and gender distribution of typhoid fever patients. This study comprised 50 seemingly healthy control individuals who appeared well and disease-free individuals who had a Widal test and blood culture negative.

4.1.1: Patient Distribution based on Age

The mean ± standard deviation of the patients age was (36.66 ± 12.32) years old, with a range of (15-65) years old, and (50) control individuals were included in this study, with the age mean ± standard deviation for healthy control (34.84 ± 11.51). According to the findings of the current study, the age group most likely to have typhoid fever infections was 25–35 years old, which was reached at (34%), followed by (15–25) years old, which was reached at (24%), and less that the group of (35–45) years old reached (20%) and the group of (45–55) years old reached (12%). Finally, the group of (55-65) year olds reached (10%) as can be seen in figure (4-1).

The disease was more frequent in the patient group (25-35) years old in this study. The possible reason may be their unhygienic lifestyle and eating contaminated junk food, which was the major source of infection in young

adults, along with increased number of social gatherings and the interaction with certain environmental factors or lifestyle (Luby *et al*., 1998).

Patients at these ages were more active and met more people, potentially predisposing them to infections, according to the findings of (Jaafar *et al*.; 2013 and Al-ameri and Saif, 2014). The result of this study was agreed with Rasul *et al.* (2017), when they found the age group 21–30 was highly affected by typhoid infection.

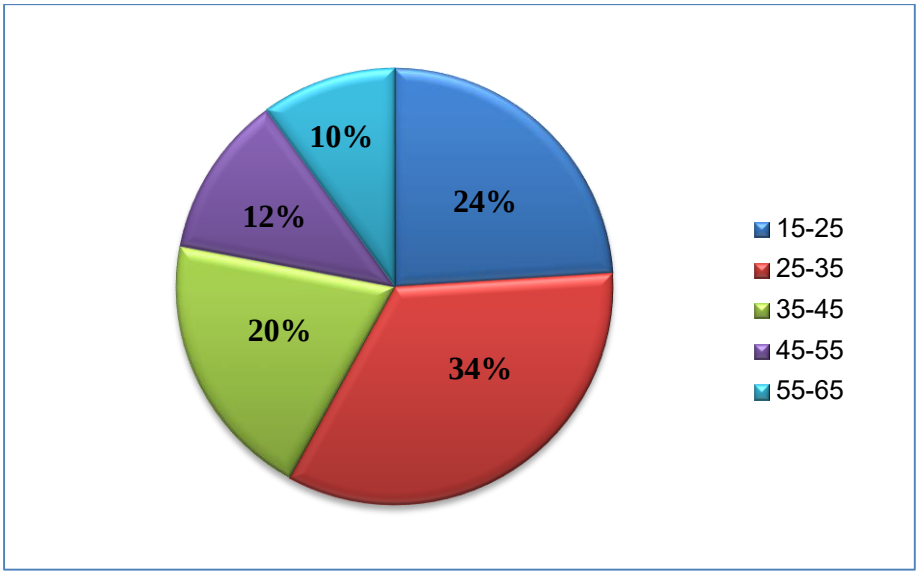


Figure (4-1): Distribution of patients according to age

4.1.2: Patient Distribution based on Gender

The study shows the percentage distribution of the two study groups based on gender that there were 32 (64%) of cases for males and 18 (36%) of cases for females' with typhoid fever infection as shown in Figure (4-2). while the control group had 30 (60%) for males and 20 (40%) for females.

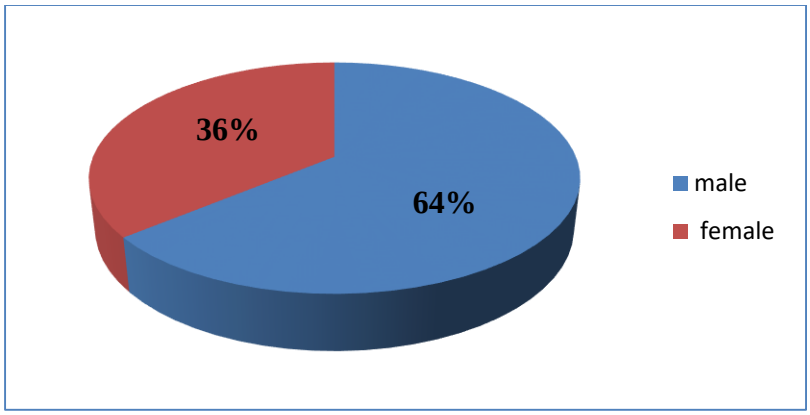


Figure (4-2): Distribution of patients according to gender

Males were found to be more infected than females, which is consistent with prior findings (AL-Khafaji *et al*., 2006; Saleh, 2013; Abd, 2019). The explanations might be that most men were out-doored, in which case they could be viewed as food eating and handling or contact with other patients (Michael and AL-Wan., 2008). Males' greater exposure to polluted food and water outside the house may be a cause of higher infection rates because *S typhi* spread by the fecal oral route (Jerrold and Tunner, 2010).

4.2: Bacteriological Study

4.2.1: Serologically Test

According to the findings, clinical samples were divided into 50 (19%) positive blood cultures for *S typhi* and 213 (81%) negative cultures for *S typhi*. The Widal test, which measures antibody responses titer to *S typhi* H and O antigens, revealed that the blood specimens were positive. Misleading results when using the Widal test may keep one from making the correct diagnosis due to cross reaction of antigen from other infections with *Salmonella* species antibody. *S typhi* has cross-reacting epitopes with other Enterobacteriaceae, which can lead to false-positive results and is not reliable for typhoid fever diagnosis (Mawazo *et al*., 2019). Widal test

methods are inexpensive and widely available, making them ideal for poor under developing countries, but they have low sensitivity and specificity (Andualem *et al*., 2014).

Detect the agglutination of the antigens and antibodies found in the patient's serum needed for confirmation of results with a second test method (Elvis *et al*., 2019). *S typhi* is most commonly isolated from blood during the first week of illness, but it can also be found during the second and third weeks. Blood culture diagnosis of *S typhi* is a more standard and rapid method than stool culture because blood culture sensitivity is higher in the first week of illness. Stool culture is less than 50% sensitive on its own, and urine is even less so (Fadil, 2019).

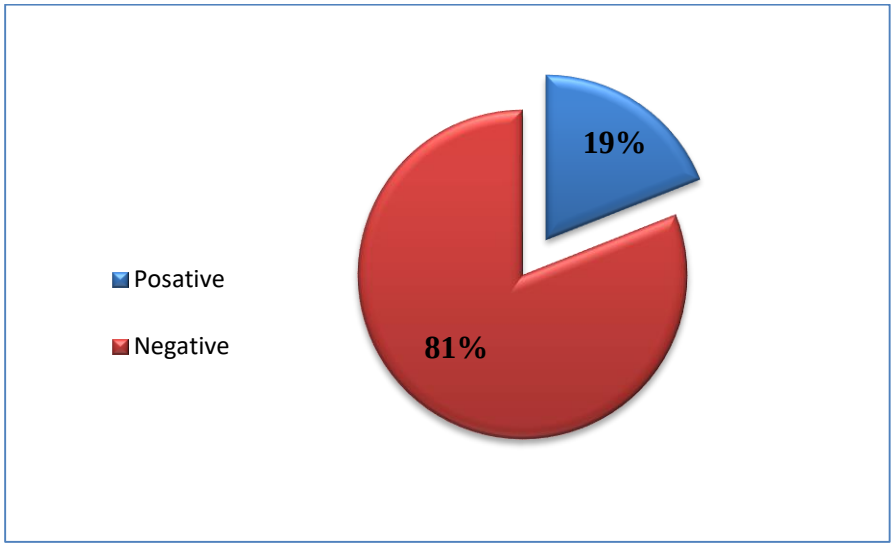


Figure (4-3): Percentage of blood cultures with *S typhi* for patients with typhoid fever

4.2.2: Isolation and Identification of *S typhi*

Brain Heart Infusion broth has been used to inoculate the blood samples. As evidence of microbial growth, several changes in the broth have been observed, including turbidity, gas formation, color change, and foul odor. Identification was done by sub culturing from on MacConkey agar, Blood agar, *Salmonella Shigella* agar and Xylose Lysine Deoxycholate agar figure (4-4). Gram-negative bacteria, bacilli in shape, were discovered under the microscope to be motile by peritrichous flagella around the cell body (Fàbrega and Vila, 2013).

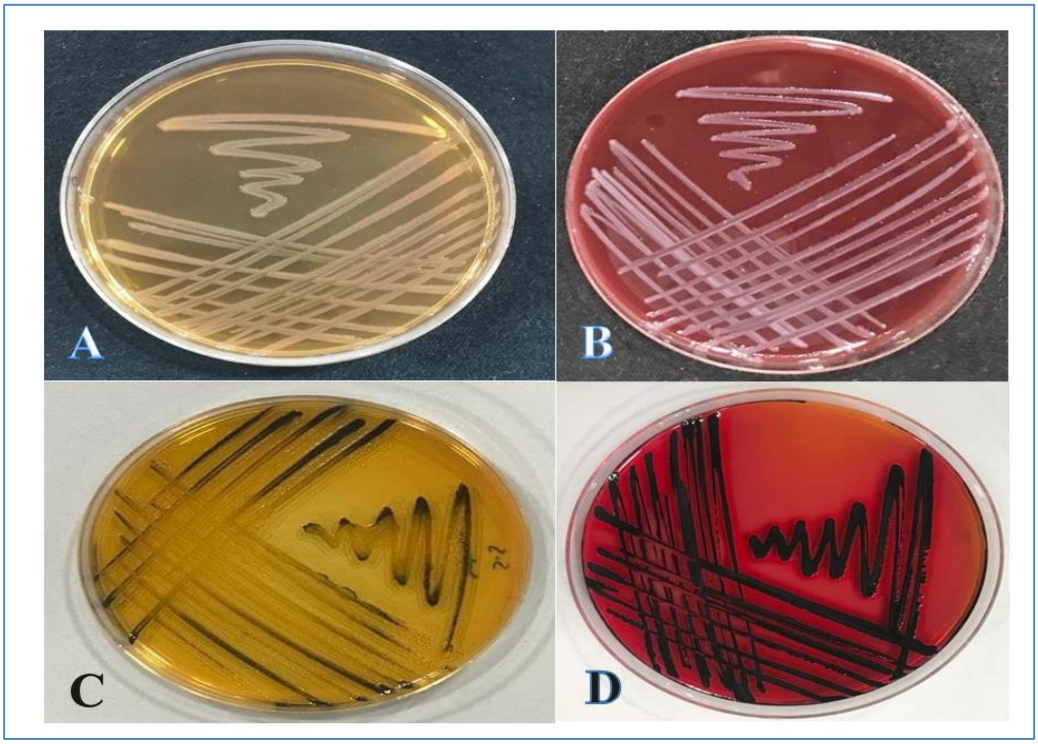


Figure (4-4): *Salmonella typhi* in different culture media

A: On Macconkey agar *S typhi* appeared as pale colonies (non-lactose fermentation).

B: On blood agar, *S typhi* appeared as white colonies (non-hemolytic).

C: On S.S agar, *S typhi* formed black colonies with H_2S production.

D: On XLD Agar, *S typhi* appeared as red colonies with black centers.

Biochemical tests that are used as complimentary to the identification of *S typhi* isolates are shown in Table (4-1). *S typhi* tested negative for oxidase, indole and Voges-Proskauer test but positive for methyl red, catalase test and citrate test. In triple sugar iron, *Salmonella.typhi* isolates produced hydrogen sulfide.

Table (4-1): Biochemical test for *S typhi*

Test	Catalase	Indole	Oxidase	Methyl-red	Voges-Proskauer	Citrate	Kligler
Result	+	-	-	+	-	+	Alk / acid+H_2S production

+: positive , - :negative ,TSI: triple sugar iron

4.2.3: Identification of *S typhi* by VITEK-2 Compact System

The automated VITEK-2 compact system with GN-ID cards was used for the final identification, which included 47 biochemical tests and one negative control well. As shown in the appendix, the results show that the blood isolates are *S typhi*.

The isolates were identified using the VITEK-2 compact system auto analyzer system, and the results were analyzed using compact software, which may provide accurate microbial identification (Pincus, 2010) demonstrates that a VITEK-2 compact system phenotypic confirmatory tool with faster results, higher specificities, demonstrated confidence, and less training time than manual microbial identification techniques.

4.2.4: Antibiotic Sensitivity by VITEK-2 Compact System

A unique kit of VITEK-2 compact system AST-N222 cards for Enterobacteriaceae was utilized in this investigation, which contains a variety of antibiotics. For all isolates, the study proved that the isolates have high resistance rate for Penicillins: Ticarcillin, Ticarcillin/Clavulanic Acid, Piperacillin were (100%) and Piperacillin/Tazobactam was (28%) ; for Monobactam: Aztreonam was (76%) ; for Aminoglycosides: Amikacin was (80%), Gentamicin was (100%) and Tobramycin (92%) ; for Carbapenems: Meropenem was (24%) and Imipenem was (8%) ; for (Quinolones and fluoroquinolones): Ciprofloxacin was (100%) ; for Cephalosporins: Cefepime and Ceftazidime were (100%) and for Sulfonamides: Trimethoprim/Sulphamethoxazole was (36%). The isolates' results revealed multidrug resistance because the *S typhi* isolates were resistant to more than three groups of antibiotics, as shown in Figure (4-5).

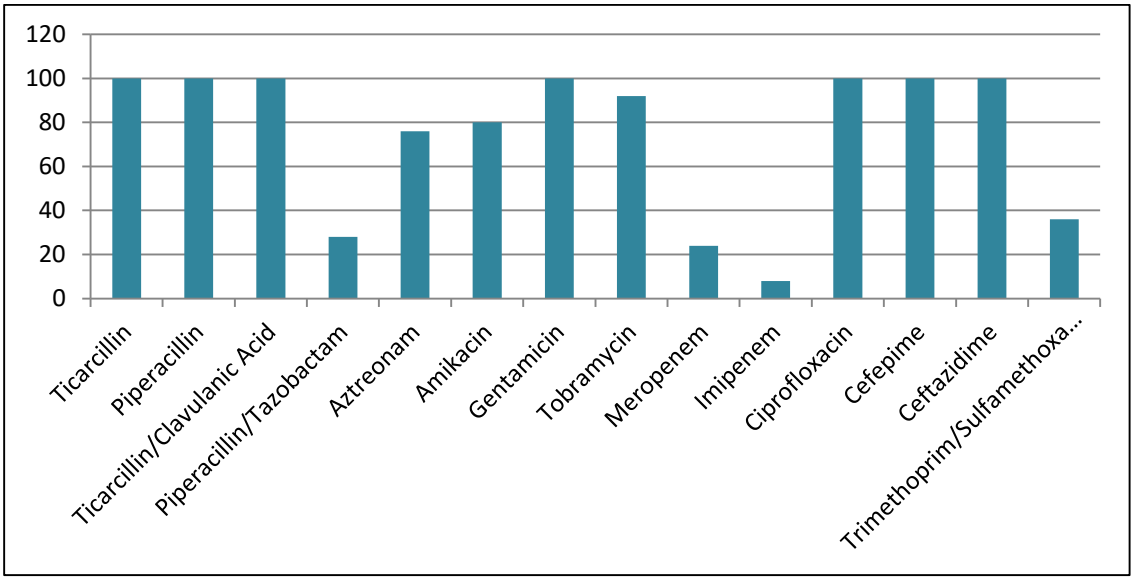


Figure (4-5): Rates of antibiotic resistance for *S typhi* isolates

Testing was carried out with VITEK-2 compact system using AST-N222 cards as shown in the Appendix. Ticarcillin, Ticarcillin/Clavulanic Acid, and Piperacillin were all resistant with MIC ≥ (128) µg/ml. Gentamicin, Cefepime, Ceftazidime, Ciprofloxacin, Tobramycin and Piperacillin / Tazobactam had resistance to MIC ≥ (16) µg/ml. The MIC value for trimethoprim/Sulfamethoxazole resistance was ≤ (20) µg/ml. Amikacin found that isolates were resistant to it with MIC value ≥ (64) µg/ml. While the MIC value for Aztreonam resistance was ≤ (4) µg/ml. Imipenem, Meropenem appeared to have low resistance to *S typhi* isolate with MIC value were ≤ (1) as shown in table (4-2).

Table (4-2): Antibiotic susceptibility by the VITEK-2 Compact System

Antibiotics	*Salmonella typhi* (25)		MIC µg/ml
	Sensitive (%)	Resistance (%)	
Ticarcillin	----------	25 (100%)	≥ 128
Ticarcillin/Clavulanic acid	----------	25 (100%)	≥ 128
Piperacillin	----------	25 (100%)	≥ 128
Piperacillin/Tazobactam	18 (72%)	7 (28%)	≤ 16
Aztreonam	6 (24%)	19 (76%)	≤ 4
Cefepime	----------	25 (100%)	≥ 16
Ceftazidime	----------	25 (100%)	≥ 16
Ciprofloxacin	----------	25 (100%)	≥ 1
Trimethoprim/Sulfamethoxazole	16 (64%)	9 (36%)	≤ 20
Gentamicin	----------	25 (100%)	≥ 16
Amikacin	----------	25 (100%)	≥ 64
Tobramycin	2 (8%)	23 (92%)	≥ 16
Imipenem	23 (92%)	2 (8%)	≤1
Meropenem	19 (76%)	6 (24%)	≤1

The results showed that the antimicrobial resistance rate to DNA synthesis inhibitors; Ciprofloxacin may be due to the use of Fluoroquinolones for the treatment of typhoid has become more common in developing countries. Previous research found an increase in *S typhi* resistance to ciprofloxacin (Mutai *et al*., 2018).

Reduced ciprofloxacin sensitivity increases the chance of typhoid fever medicine failure, especially in underdeveloped nations. Iraq has the largest ciprofloxacin sensitivity drop among Middle Eastern countries (Rahman *et al*., 2014). That was consistent with the findings of the current investigation. Furthermore, the current investigation is analogous to a study achieved in Baghdad which *S typhi* was resistant to ciprofloxacin (Rasool *et al*., 2020).

Protein synthesis inhibitor showed the rate of resistance to Gentamicin 100% and Amikacin 80% Tobramycin 92%, Aminoglycosides can be inactivated in several ways, Lo *et al*. (2014) reported the low activity of aminoglycosides against intracellular *S typhimurium*. This study agreement with a study conducted in Baghdad-Iraq, which demonstrated the resistance of *S typhi* to Tobramycin (Rasool *et al*., 2020).

Some aminoglycoside modifying enzymes catalyze the addition of hydroxyl groups for phosphates or adenyl groups. Aminoglycosides binding to the 30S ribosomal subunit inhibiting protein translation. *Salmonella* resistance to aminoglycosides by an enzymatic modification of the compound (Fadil, 2019; Abood, 2020).

Carbapenems, are synthetic β-lactam antibiotics that differ in structure from the Penicillin in that the sulfur atom of the thiazolidine ring has been externalized and replaced by a carbon atom. For Meropenem and Imipenem,

the most bacterial isolates in this study were susceptible to them, because of the most resistance to β-lactams is conferred by β-lactamases that enzymatically cleave the β-lactam ring and prevent it from bonding to and inactivating cell wall enzymes where as a new β-lactams were synthesized through modification of the chemical groups around the β-lactam ring to produce β-lactams that are resistant to the β-lactamases (Giguere *et al*, 2013; Fadil, 2019).

Aztreonam from Monobactams group are considered critically important antimicrobials in medicine according to the World Health Organization 24% (WHO, 2006), which disrupt bacterial cell wall synthesis, are unique because the β-lactam ring is not fused to another ring. Modifications can improved their activity on specific bacteria or accessibility to certain infection sites. That resistance 100%, were reported to the Cephalosporins: Cefepime and Ceftazidime (Giguere *et al*., 2013). *S typhi* may not have any effect as a result of the isolates already being resistant to broad spectrum Cephalosporins (AL-Edhari, 2015).

Several investigations have revealed *Salmonella spp*. resistance to Ticarcillin (Johnson *et al*., 2005). Piperacillin is frequently used in conjunction with Tazobactam, which increases piperacillin's efficacy by blocking beta-lactamase enzymes in several species. The current investigation found that *S typhi* isolates were responsive to piperacillin/Tazobactam 28% which is consistent with previous findings (Salman *et al*., 2021). Piperacillin/Tazobactam remains a suitable antibiotic against Gram-negative bacteria (Lowman, 2013). Resistance to Trimethoprim is because mutations in the chromosomal gene that encodes dihydrofolate reductase, the enzyme that reduces dihydrofolate to

tetrahydrofolatem which reduces the binding affinity of the drug (Lutterloh *et al*., 2012; Chand *et al*., 2014). The high prevalence of multi drug resistance may was because of the extensive use of antibiotics without consulting doctors, which is very common in developing countries particularly in Iraq (Omulo, 2015).

4.3: Immunological Study

4.3.1: Concentration of Interleukin-17A in Serum

The cytokines IL-17A was measured in patients and controls using an Enzyme-Linked Immunosorbent Assay (ELISA) based on the manufacturing company principle. In this study, the levels of IL-17A in the serum of patients were investigated and compared them with control group. The mean ± standard deviation of IL-17A concentration in the serum of patients was 27.06±8.76 pg/ml while the mean ± standard deviation for concentration in control group was 13.41±5.27 pg/ml .There were significant differences ($P \leq 0.000$) as shown in figure (4-6).

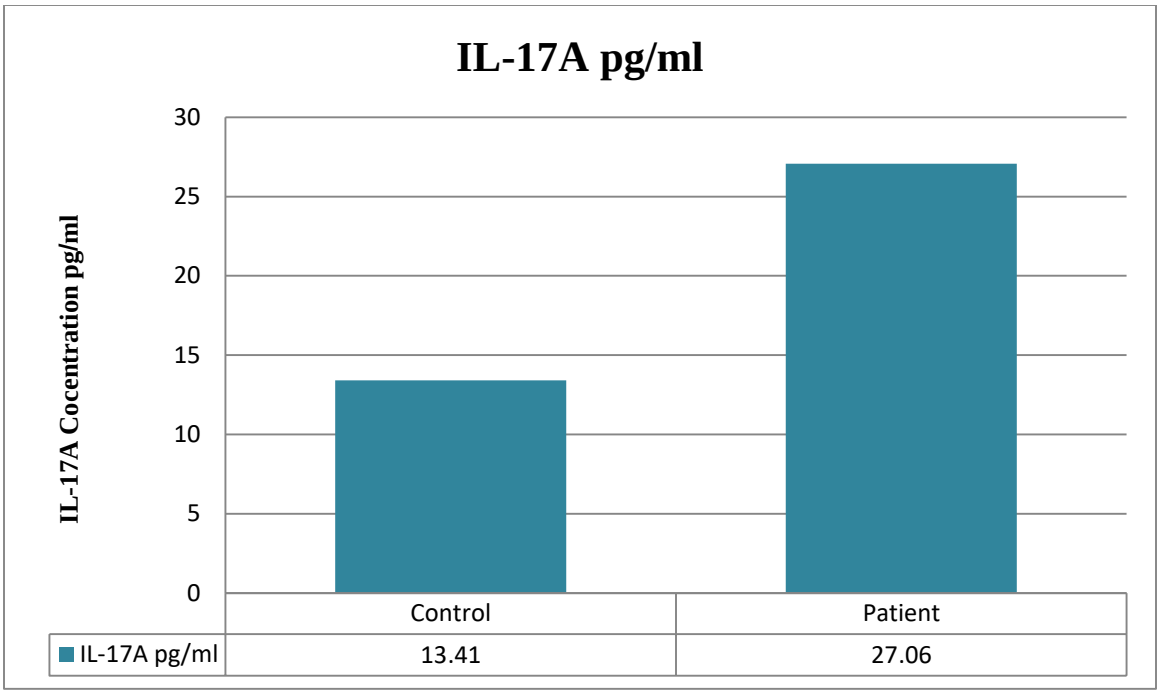


Figure (4-6) :Concentration of IL-17A in patients and control groups

Th17 cells produce IL-17, which acts as a mediator in the inflammation associated with host defense against bacterial and fungal pathogens, particularly at mucosal surfaces (Ouyang *et al*., 2008). Th17 $CD4^{+}$T cells provide protection against bacteria and fungi by recruiting acute inflammatory cells to infection sites (Weaver *et al*., 2007). Other cytokines, such as IL-6, are also produced by Th17 cells. It is also necessary for the production of cytokines and antimicrobial peptides in the gastrointestinal tract (Song *et al*., 2011).

Khader and Gopal (2010) describe IL-17 as an inflammatory cytokine that protects against infection and is increasingly connected to host defenses against intracellular infections. Antigen-specific IL-17A cells were found in response to *Salmonella enteriditis* infection, and IL-17A knockout animals

exhibited a greater bacterial load in the liver and spleen when compared to wild-type mice (Schulz *et al*., 2008).

It was important to begin studies to determine whether IL-17A might play a role in *S typhi* protection because the gastrointestinal mucosa is the first point of contact for *S typhi* and mucosal immune responses are likely to play a role in protection (Ktsoyan *et al*., 2013) showed elevated level of IL-17 in patients with acute salmonellosis caused by two serotypes of *S enterica, S enteritidis* and *S typhimurium* compared to healthy control subjects.

According to a study utilizing the intracellular pathogen *S typhimurium*, IL-17 and IL-22 are also induced in the ileal mucosa in response to infection (Raffatellu *et al*., 2008). In the absence of IL-23 and IL-17R found the signaling, chemokine, macrophage, and anti-microbial neutrophil recruitment to the ileal mucosa was decreased, but bacterial spread to the lymph nodes was enhanced (Godinez *et al*., 2009).

The findings of this study are consistent with those of Bhuiyan *et al*. (2014), who discovered a significant increase in IL-17 during typhoid fever infection. Because *S yphi* infection can persist intracellularly, protective immune responses are complex and involve both humoral and cellular immune responses. Cell-mediated immunity is essential for infection clearance (Levine *et al*., 2001; Sztein, 2007).

In IL-17R-deficient mice infected with *Klebsiella pneumoniae*, lower IL-17A levels are associated with an increased risk of bacteremia (Zhao *et al*., 2016). The findings of this study agree with those of Sivick *et al*. (2010), who discovered that IL-17A deficient mice are more susceptible to *E. coli* infection. Following cecal ligation and puncture, IL-17R deficient mice

showed decreased neutrophil recruitment, increased infection spread, and exacerbated inflammatory responses (Zhao *et al*., 2016).

IL17A also induces antimicrobial responses in epithelial cells against intracellular bacteria such as *S typhimurium* (Broz *et al*., 2012). In clinical studies, low IL-17A levels are associated with impaired host immunity when infected with bacteria such as *Bordetella pertussis* and *Citrobacter rodentium* (Levy *et al*., 2016). IL-17A was also found to be important in orchestrating early inflammatory responses during *S typhimurium* colitis in another study (Schulz *et al*., 2008 ; Keestra *et al*., 2011).

The expression of an antimicrobial peptide induced by IL-17A at the apical site of the intestinal mucosa led to the killing of invading pathogens at the epithelial surface immediately after infection with *S typhimurium* (Mayuzumi *et al*., 2010).

Interleukin-17 works in concert with other mediators such as IL-1, IL-6, and TNF-α to stimulate tissue-infiltrating neutrophils, which aid in the successful clearance of invading germs (Schwarzenberger *et al*., 2000). Production of Human IL-17A enhanced against bacteria or their toxins (Niebuhr *et al*., 2011).

4.3.2: Concentration of Interleukin-1β in Serum

The cytokines IL-1β was measured in patients and controls using an Enzyme-Linked Immunosorbent Assay (ELISA) based on the manufacturing company principle. In this study, It has been investigated the levels of IL-1β in the serum of patients and compared them with control group.

The mean ± standard deviation of IL-1β concentration in the serum of patients was 15.38±6.45 pg/ml while the mean ± standard deviation for concentration of control group was 7.23±3.85 pg/ml .There were significant differences ($P \leq 0.000$) as shown in figure (4-7).

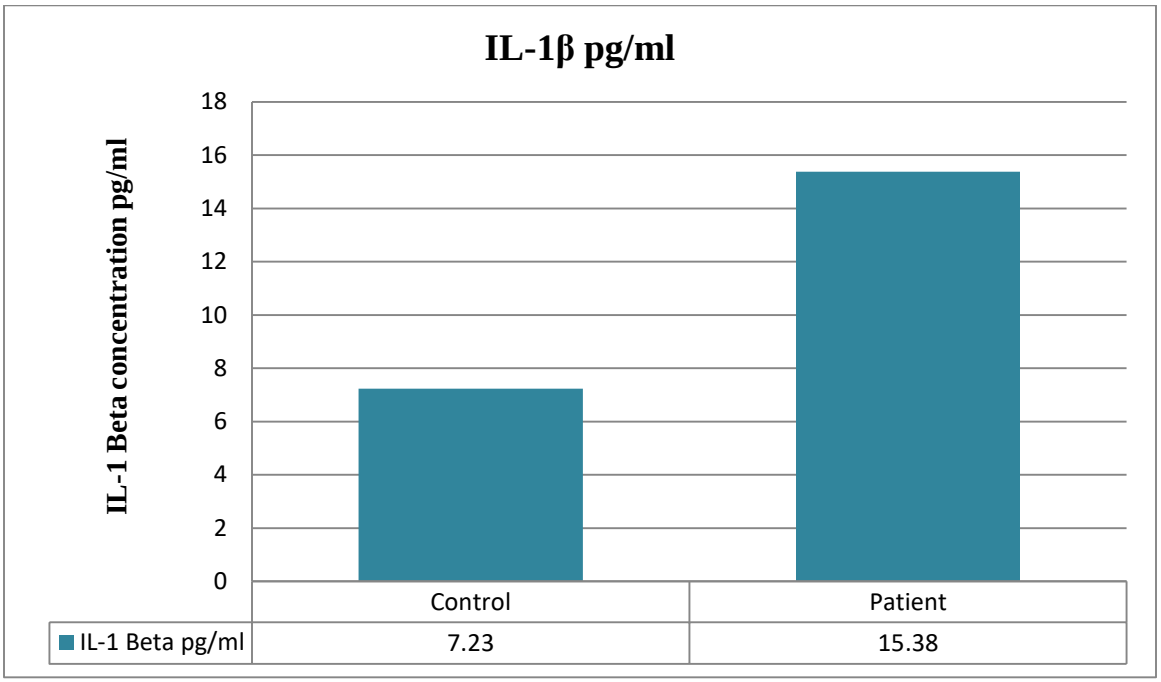


Figure (4-7) :Concentration of IL-1β in patients and control groups

Correlation among IL-17A, IL-1β, age and gender, the results of statistical analysis shown that the IL-17A was significantly differences at ($P \leq 0.05$), positive correlation with age (r =0.688**), and its positively correlation with gender (r = 0.502*), IL-1β was significantly differences at ($P \leq 0.05$) and positively correlation with age and the value of r was reached to (0.438*) and its positive correlation with gender (r = 0.863**).

Table (4-3): Correlation among IL-17A, IL-1β, Age and Gender

Correlation					
		IL-17A	**IL-1β**	**Age**	**Gender**
IL-17A	**Pearson Correlation**	1	-0.18	0.688**	0.502*
	Sig. (2-tailed)		0.865	0.05	0.30
	N	50	50	50	50
IL-1β	**Pearson Correlation**	-0.18	1	0.438*	0.863**
	Sig. (2-tailed)	0.862		0.42	0.23
	N	50	50	50	50
Age	**Pearson Correlation**	0.688**	0.536	1	0.7.1**
	Sig. (2-tailed)	0.005	0.041		0.000
	N	50	50	50	50
Gender	**Pearson Correlation**	-0.205	0.863**	0.703**	1
	Sig. (2-tailed)	0.130	0.021	0.000	
	N	50	50	50	50
**. Correlation is significant at the 0.01 level (2- tailed).					

Cytokines from the IL-1 family are expressed and excreted from many types of cells in response to infectious diseases. Blocking IL-1 activity could play a significant role in controlling a wide range of diseases (Dinarello *et al*., 2012). The results are in agreement with findings that show the increased IL-1β levels in humans and mice result in increased Th17 dominant immunopathology (Meng *et al*., 2009).

Intracellular pathogenic bacteria trigger immune responses that are mainly recognized by toll like receptor. Reports have indicated that cytosolic recognition of *Salmonella* flagellin mediates caspase-1 activation and IL-1β maturation (Franchi *et al*., 2006). The host protein Ipaf is required for the activation of caspase-1 and IL-1β processing as well as for the inducement of rapid cell death through the sensing of intracellular flagellin during *Salmonella* infection (Miao *et al*., 2006).

SipB, an IpaB homolog, has been proposed to interact directly with caspase-1 and mediate its activation (Hersh *et al*., 1999). Induced caspase-1 activation induced by *Salmonella* depends on the sensing of intracellular flagellin by Ipaf but not SipB, in that flagellin mutants do not induce caspase-1 activation even though their SipB function is intact (Franchi *et al*., 2006).

The secreted protein SipB is the effector molecule responsible for the induction of apoptosis in macrophages infected by *S typhi*. Macrophages undergo cell death when microinjected with purified SipB, demonstrating that this molecule is sufficient to induce apoptosis. Cytoplasmically-distributed SipB directly engages a key component of the macrophage's apoptotic machinery. This interaction that presumably leads to the activation of caspase-1. Then IL-1β undergoes maturation to its biologically active form, which could initiate an inflammatory response (Hersh *et al*., 1999).

These two sensory pathways result in different responses, resulting in the secretion of many cytokines and other transcriptional responses, including pro-IL-1 expression but not mature IL-1 secretion. Ipaf, in contrast, initiates IL-1β processing and stimulates the secretion of mature IL-1β.

By using two sensory pathways for bacterial flagellin, the innate immune system may be able to modulate the intensity or quality of its response according to the virulence characteristics of the pathogen. The presence of less-virulent flagellated commensal bacteria in tissues would warrant a less-vigorous response, with responses to flagellin mediated by TLR5 but not Ipaf (Franchi *et al*., 2006; Miao *et al*., 2006).

4.4: Molecular Study

4.4.1:Purity and Concentration of DNA

The extraction of DNA from samples of whole blood was successfully extracted and the concentration of DNA was ranged between (20–195 µg/ml) and the purity of DNA was ranged between (1.7–2.0) as shown in figure (4-8).

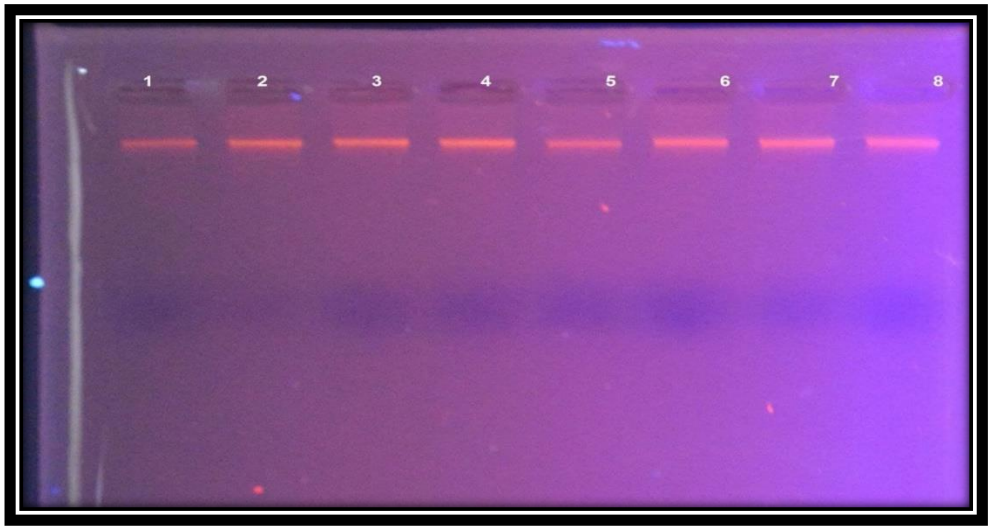


Figure (4-8): Electrophoresis of DNA extracted from blood samples, 2% agarose gel, 5 volts/cm for 1 hr.

4.4.2: PCR detection of IL-17A and IL-1β genes

All the 50 confirmed positive cases and the 50 control subjects were submitted to PCR for the detection of IL-17A and IL-1β by using specific primers.

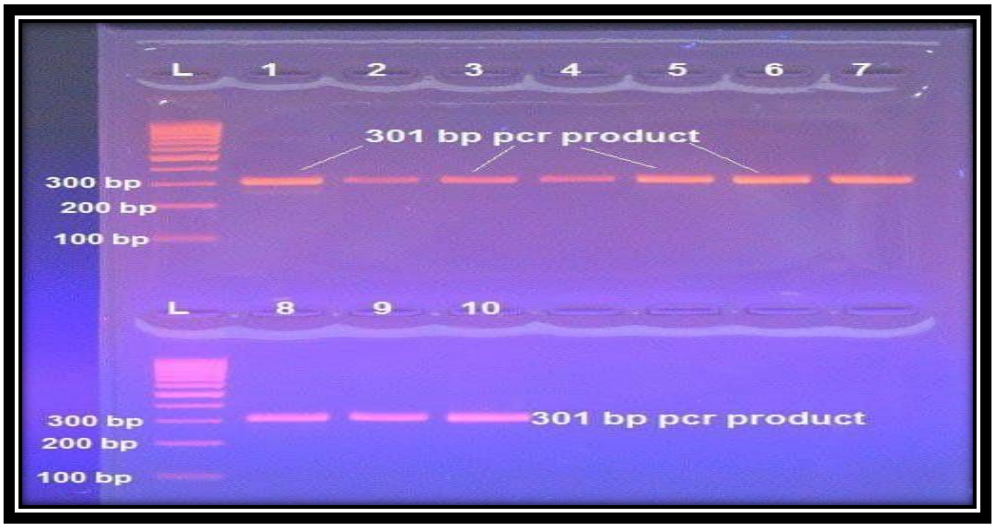


Figure (4-9): Agarose gel electrophoresis of IL-17A1. 2% agarose,75 volts/20 mA, for 1 hour.

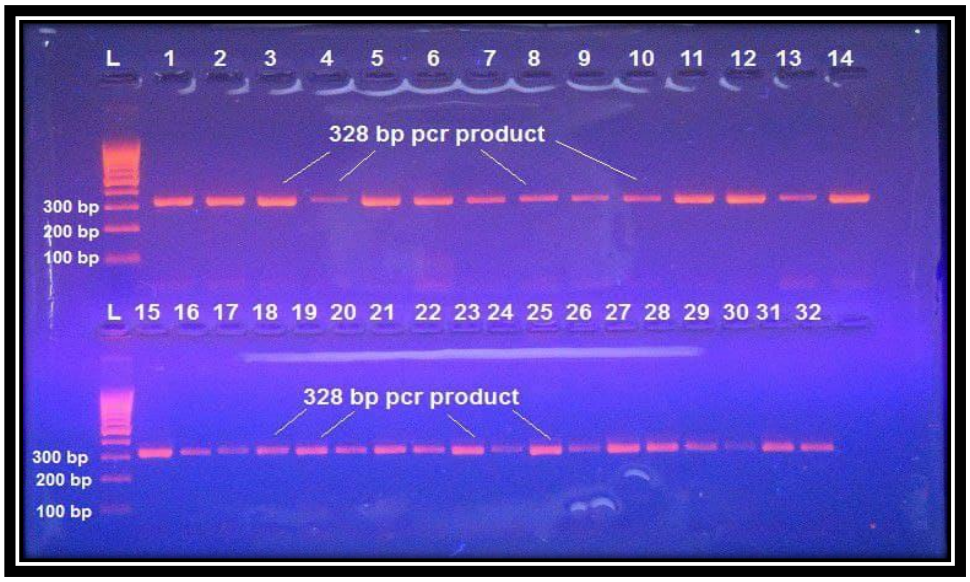


Figure (4-10): Agarose gel electrophoresis of IL-17A2. 2% agarose, 75 volts/20 mA, for 1 hr.

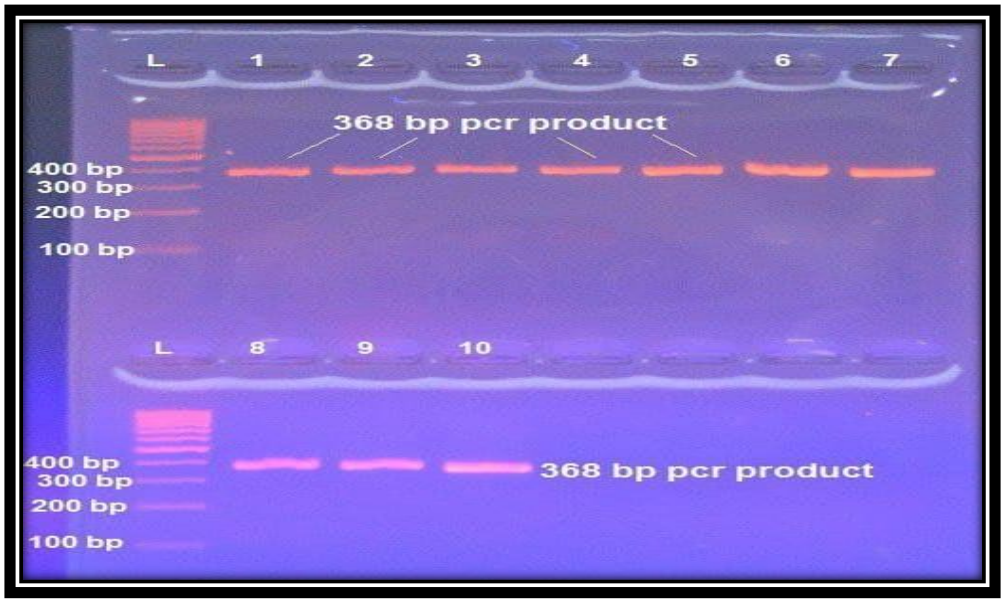


Figure (4-11): Agarose gel electrophoresis of IL-1β1. 2% agarose, 75 volts/20 mA, for 1 hr.

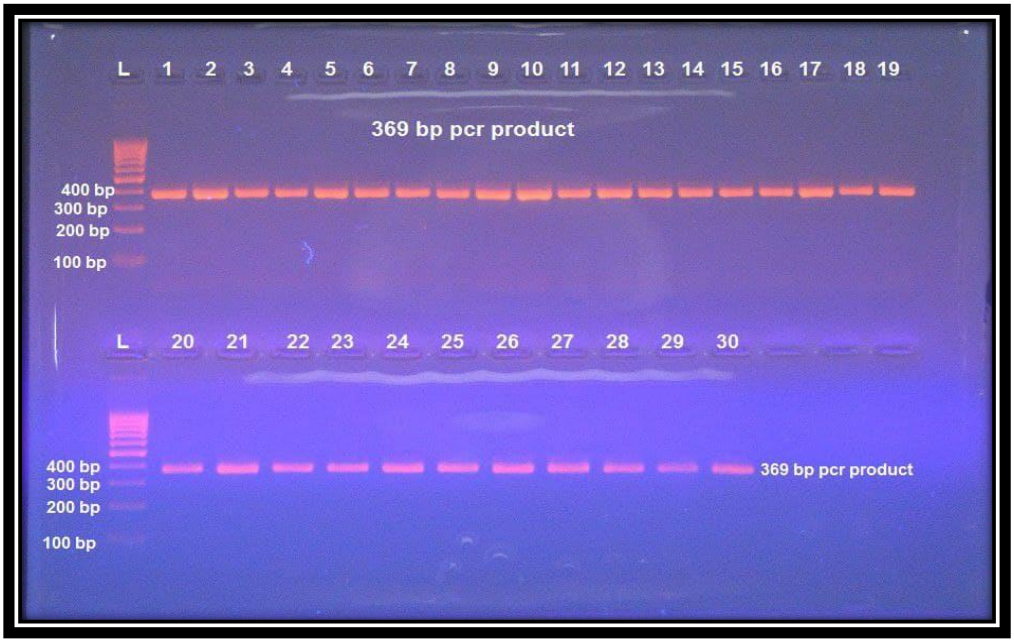


Figure (4-12): Agarose gel electrophoresis of IL-1β2. 2% agarose, 75 volts/20 mA, for 1 hr.

4.4.3: Detection of IL-17A and IL-1β Genotyping Using PCR - SSCP

Lane L DNA ladder, 100 bp, 9, 11, and 13 in an A pattern. Lane 16 has a C pattern, while the other lanes have a B pattern. The DNA sequence analysis reveals that pattern A, B, and C represent the CT, CC, and TT genotypes of rs1974226, respectively.as shown in figure (4-13).

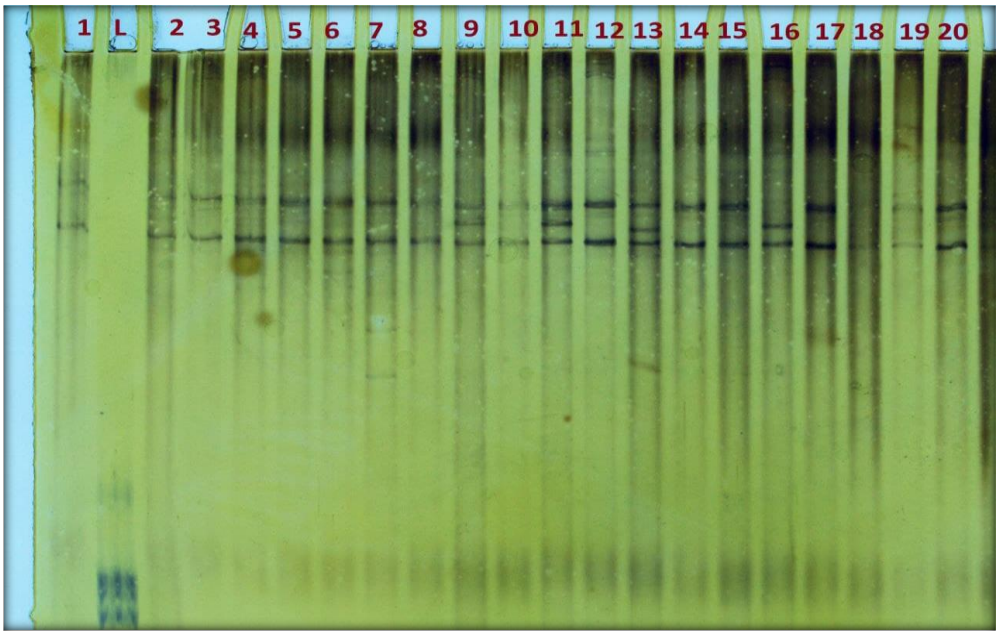


Figure (4-13): Silver stained polyacrylamide gel of PCR-SSCP for rs1974226 genotyping, 12% gel, 125 volts/cm, for 6 hours.

Lane L DNA ladder 100 bp. lanes 1,2,5,7,8,9,11,13,14,15,18,19,20, and 21 A pattern lanes 3,4,6,10,12, and 17 B have pattern lanes 16, and 22 C pattern. The DNA sequence analysis reveals that patterns A, B, and C represent the GA, AA, and GG genotypes of rs1143629, respectively as shown in figure (4-14).

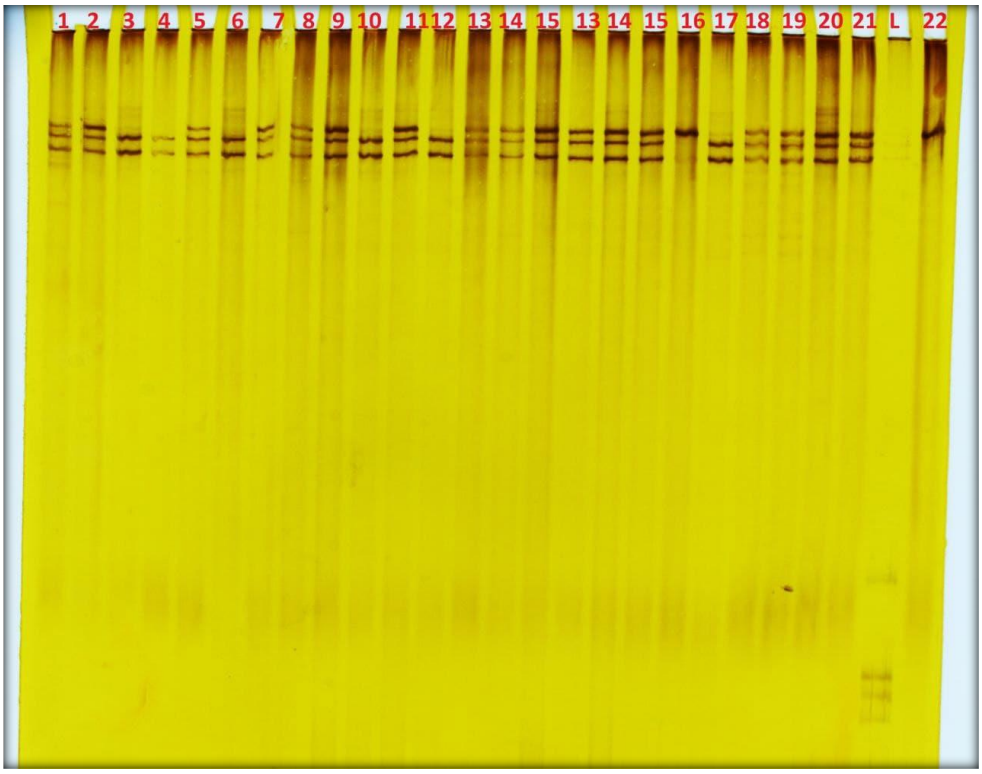


Figure (4-14): Silver stained polyacrylamide gel of PCR-SSCP for rs1143629 genotyping, 12% gel, 125 volts/cm, for 6 hours.

4.4.4: Association of rs1974226 with Genotypes and Allele Frequency

According to the results, individuals with two T alleles (homozygous for the TT) were significantly represented among the patients with typhoid fever: 14 (28%), as compared with healthy control subjects, 6 (12%), and had an increased risk of developing typhoid fever infection. The IL-17A (C) allele was less frequent among patients (28%, n=28) than control, and the (T) allele was less frequent among patients than control.

The result that are obtained from equilibrium of Hardy-Weinberg of exact test that are revealed that the frequency of control healthy group genotype had been flowed equilibrium of the Hardy-Weinberg; while the distribution

of typhoid fever patients genotype (P value > 0.0061) that are recorded a significant deviation from equilibrium of hardy-Weinberg as shown in table (4-6).

Alleles frequency for patients with typhoid fever and control healthy group that had been listed in table (4-4), the results that had been shown that there was significant difference in allele frequency between patients and control groups.

Table (4-4): Genotypes and allele frequency for rs1974226 in typhoid fever patients and controls

Genotype variation	Healthy (n=50)	Patient (n=50)	P-value	Odd ratio (C.I 95%)
CC	28 (56%)	26 (52%)	0.67	0.538 (0.350-1.150)
CT	16 (32%)	10 (20%)		
TT	6 (12%)	14 (28%)	0.0061	1.576 (0.870-2.856)
Allele Frequency				
Allele type	**Healthy (n=50)**		**Patient (n=50)**	
C	72 (72%)		28 (28%)	
T	62 (62%)		38 (38%)	

C.I : confidence interval

The data has been further examined for each genotype correlated with typhoid fever under different inheritance models. The results showed that there was no significant association between any genotype and typhoid fever under most of studies inheritance models, except inheritance of recessive model that had significant associated with typhoid fever patients, T allele represents as a risk factor for patients with typhoid fever and inheritance of

recessive model as risk factor. The TT genotype has a related odd ratio (TT) of 2.85 (C.I.95% 1.10-17.18) as shown in table (4-5).

Table (4-5): Association of rs1974226 genotypes under different models of inheritance

Model	Genotype	Control	Case	OR (C.I 95%)	P-value
Codominant	C/C	28 (56%)	26 (52%)	1.00	0.92
	C/T	16 (32%)	10 (20%)	0.67 (0.26-1.75)	
	T/T	6 (12%)	14 (28%)	2.51 (0.84-7.51)	
Dominant	C/C	28 (56%)	26 (52%)	1.00	0.69
	C/T-T/T	22 (44%)	24 (48%)	1.17 (0.53-2.58)	
Recessive	C/C-C/T	44 (88%)	36 (72%)	1.00	0.043
	T/T	6 (12%)	14 (28%)	2.85 (1.10-17.18)	
Over dominant	C/C-T/T	34 (68%)	40 (80%)	1.00	0.17
	C/T	16 (32%)	10 (20%)	0.53 (0.21-1.32)	

The findings appear to support the link between the T/T genotype and susceptibility to typhoid fever. Th17 cells express IL-17A and IL-17F, which are involved in coordinating local tissue inflammation (Park *et al*., 2005; Lee *et al*., 2013). Polymorphisms in IL-17 cytokines alter interleukin activity and may alter cytokine function, resulting in dysregulation of IL-17 expression (Ishigame *et al*., 2009).

Because IL-17 is thought to be an important pro-inflammatory factor, many studies of IL-17 SNPs and susceptibility have focused on inflammation-related diseases (Zhang *et al*., 2013). A few studies have looked into the role of IL-17 SNPs in breast cancer susceptibility (Slattery *et al*., 2014), and studies of gastric cancer have mostly focused on rs2275913

but have yielded contradictory results (Wu *et al*., 2010; Qinghai *et al*., 2014;).

Other studies have found that the IL-17 polymorphism plays an important role in many autoimmune diseases Shen *et al*. (2015) found that the IL-17 rs2275913 and rs3819024 variant alleles were associated with a lower risk of rheumatoid arthritis, while the IL-17 rs3819025 and rs8193036 variant alleles were associated with an increased risk of rheumatoid arthritis. The rs1974226 SNP was linked to an increased vulnerability to gram-positive infections. In two sepsis cohorts, patients with the rs1974226 GG genotype were more vulnerable to gram-positive infection than patients with the AG/AA genotype, and in the subgroup with lung infection, the IL17A rs1974226 G allele was related with greater 28 day mortality of severe sepsis (Nakada *et al*., 2011).

Previous research indicated that the T allele of rs1974226 in IL17A was related to asthma, and that other genetic variations in the IL17 pathway genes were connected with both protection and risk for asthma development with IgE levels (Silva *et al*., 2019).

According to Ahmed Ali *et al*. (2018), no significant association was found between IL-17 rs1974226 genotypes and related serum cytokine levels, implying that elevated serum IL-17 may increase the susceptibility to septic complications in polytrauma patients and thus could be a useful biomarker for trauma patient management. There is mounting evidence that the IL-17 gene single nucleotide polymorphism rs1974226 is linked to gram positive bacterial resistance and susceptibility to infectious and non-infectious illnesses (Schwarzenberger *et al*., 2000).

4.4.5: Association of rs1143629 with Genotypes and Allele Frequency

According to the results, individuals with genotype GA were significantly represented among the patients with typhoid fever: 33(66%), as compared with healthy control subjects 18(36%), and had an increased risk of developing typhoid fever infection. The IL-1β (G) allele was less frequent among patients (51%, n=51) than control, and the (A) allele was more frequent among patients than control (49%, n=49). As shown in table (4-6).

Alleles frequency for patients with typhoid fever and control healthy group that had been listed in table (4-6), the results that had been shown that there was no significant difference in allele frequency between patients and control groups.

Table (4-6): Genotypes and allele frequency for rs1143629 in typhoid fever patients and controls

Genotype variation	Healthy (n=50)	Patient (n=50)	P-value	Odd ratio (C.I 95%)
GG	20 (40%)	9 (18%)	0.0067	1.754 (0.431-10.317)
GA	18 (36%)	33 (66%)		
AA	12 (24%)	8 (16%)	0.32	1.327 (0.759-4.318)
Allele Frequency				
Allele type	**Healthy (n=50)**	**Patient (n=50)**		
G	58 (58%)	51 (51%)		
A	42 (42%)	49 (49%)		

C.I : confidence interval

The result that are obtained from equilibrium of Hardy-Weinberg of exact test that are revealed that the frequency of control healthy group frequency of genotype that had been flowed equilibrium of the Hardy-Weinberg; while the distribution of typhoid fever patients genotype ($P < 0.0067$) that are recorded a significant deviation from equilibrium of hardy-Weinberg as shown in table (4-6).

The data had been further examined for each genotype correlated in typhoid fever under different inheritance models; The results showed that there was significant association between GA genotype and typhoid fever under most of studies inheritance models. A allele represents a risk factor for patients with typhoid fever and inheritance of Codominant, Dominant and Overdominant model as risk factor. The GA genotype has a related odd ratio of 4.07 (1.54-10.79) in the model of inheritance Codominant, Dominant model has a related odd ratio 3.04 (1.21-7.60) and Overdominant model has a related odd ratio 3.45 (1.52-7.85) as shown in table (4-7).

Table (4-7): Association of rs1143629 genotypes under different models of inheritance

Model	Genotype	Control	Case	OR (95% CI)	P-value
Codominant	G/G	20 (40%)	9 (18%)	1.00	0.0084
	G/A	18 (36%)	33 (66%)	4.07 (1.54-10.79)	
	A/A	12 (24%)	8 (16%)	1.48 (0.45-4.88)	
Dominant	G/G	20 (40%)	9 (18%)	1.00	0.014
	G/A-A/A	30 (60%)	41 (82%)	3.04 (1.21-7.60)	
Recessive	G/G-G/A	38 (76%)	42 (84%)	1.00	0.0032
	A/A	12 (24%)	8 (16%)	0.60 (0.22-1.63)	
Over dominant	G/G-A/A	32 (64%)	17 (34%)	1.00	0.0025
	G/A	18 (36%)	33 (66%)	3.45 (1.52-7.85)	

Interleukin-1β is a proinflammatory cytokine produced by monocytes and macrophages that plays an important role in the innate immune response to infection (Halle *et al*., 2008). A recent study with IL-1β rs1143629 in HCV patients found that the AA genotype was significantly more common and that the polymorphisms had no significant association with response to interferon treatment in Egyptian Chronic Hepatitis C Patients (Estfanous *et al*., 2019).

Rs1143629 has been linked to malaria, which is consistent with findings (Sortica *et al*., 2012) discovered that the rs1143629 G allele was more common in patients than in controls, and that the G/G and A/G genotypes confer an increased risk of developing juvenile systemic lupus erythematous compared to the main genotype A/A. Other studies conclude that polymorphisms in proinflammatory genes such IL-1β do not contribute to typhoid fever susceptibility and, in light of previous findings, show that the polymorphism is more likely connected to the severity of existing disease than to vulnerability in general (Ali *et al*., 2007). These findings differ from those of recent Korean research in which SNPs in the IL-1β rs1143629 gene were not linked to an increased incidence of acute pancreatitis (Park *et al*., 2018).

Patients with dilated cardiomyopathy, the IL-1β rs1143629 G/G genotypes work together to increase the risk of atrial fibrillation (Ogimoto *et al*., 2018). The GG genotype in IL-1β rs1143629 was related to dental caries and gingivitis more often (Reis *et al*., 2021). The findings of this study correspond with those of Mario-Vásquez *et al*. (2020), who discovered a link between IL-1β rs1143629 and malaria. A single-nucleotide variation in interleukin-1β was related to significant levels of child distress (Ersig *et al*., 2017).

4.4.6: Sequencing Analysis

The genotypes observed in this study were validated by sequencing data. The 6 samples were sequenced using PCR-sequences to determine the nucleotide alterations responsible for the findings of the alignment analysis. They were compared with the relevant reference DNA sequences. The alignment findings of the 328 bp samples indicated the presence of only one difference in some of the tested samples as shown in figure (4-15). In this work, a particularly intriguing nucleic acid polymorphism (SNP) was discovered in the studied samples, in which cytosine was substituted with thymine at position 226, specifically C226T.

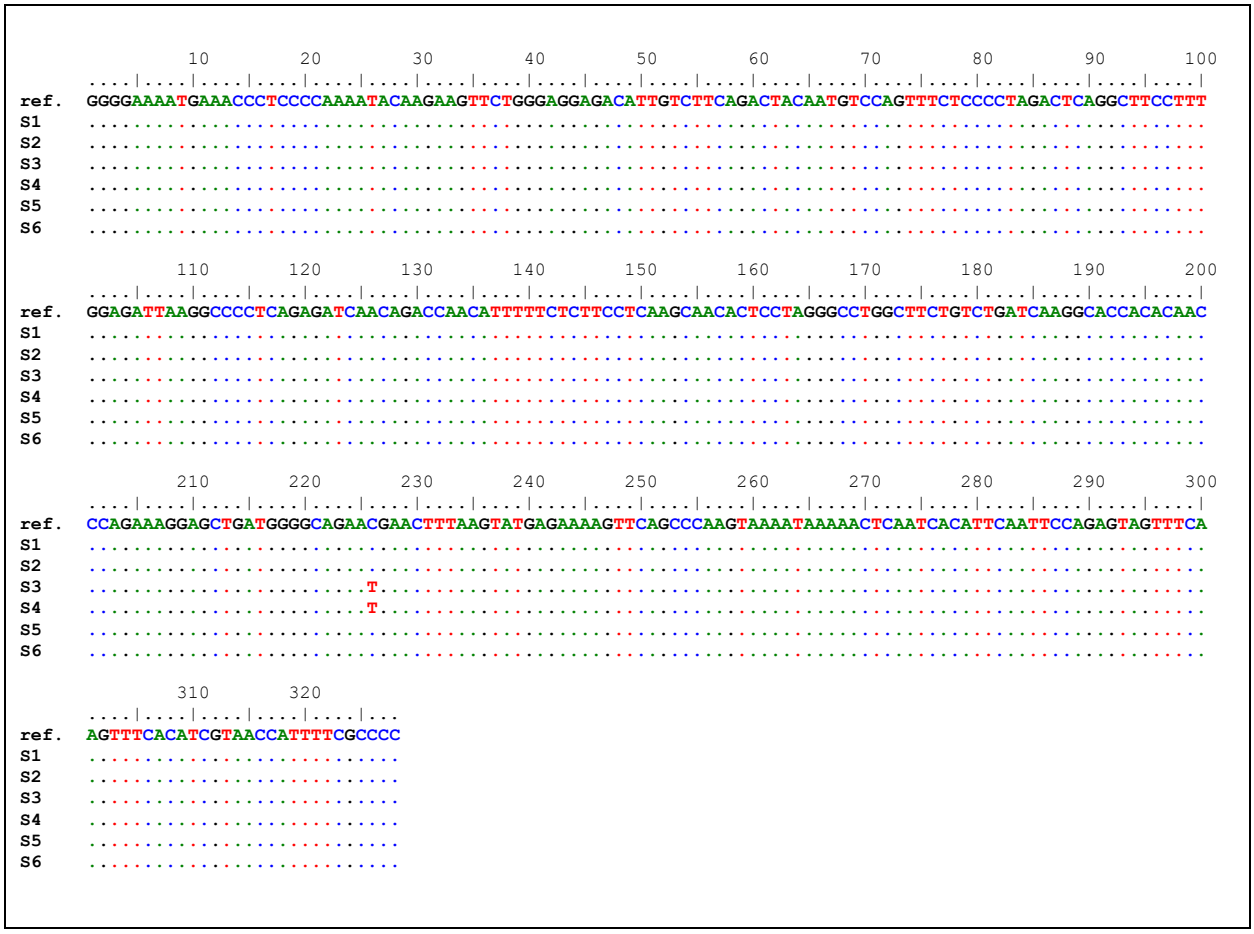


Figure (4-15): DNA sequences alignment of 6 genotyped samples with their corresponding reference sequences of the 328 bp amplicons of the coding sequences of the IL-17 gene

The detected variation's sequencing chromatogram, as well as its extensive annotations, were noted, and the chromatogram of this sequence was exhibited according to its location in the PCR amplicon. However, as indicated in the figure, this SNP was found in heterozygous C/T status in S1 and S2, homozygous T/T status in S3 and S4, and homozygous C/C status in S5 and S6 as shown in figure (4-16).

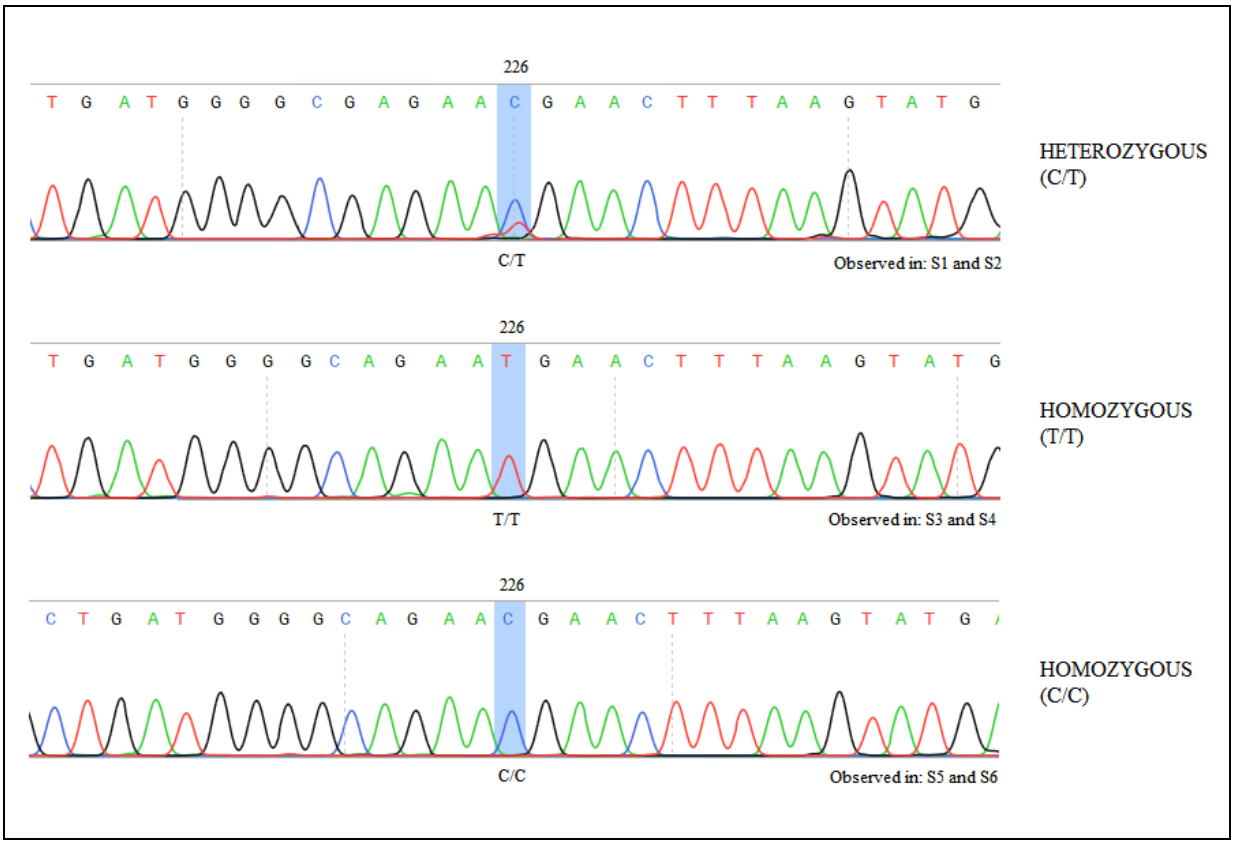


Figure (4-16): The pattern of the detected C226T SNP within the DNA chromatogram of the targeted 328 bp amplicons of the IL-17 gene

The newly discovered SNP was highlighted in the PCR amplicons based on its location. In the indicated polymorphic locus, S1/S2, S3/S4, and S5/S6 samples showed the C/T, T/T, and C/C states, respectively. The matching location of the IL-17 gene was downloaded from the dbSNP website (https://www.ncbi.nlm.nih.gov/projects/SNP/) to reveal the position of the targeted SNP in their deposited SNP database of the sequenced 328 bp fragment. To determine the nature of this SNP, a graphical depiction of the IL-17 dbSNP database on chromosome 6 (Gen Bank Acc. No. NC 000006.11) was created. By checking the dbSNP engine, it was discovered that this identified SNP was originally known as rs1974226 Figure (4-17). However, this SNP was located in the 3′-UTR sequences of the targeted IL-17 gene (https://www.ncbi.nlm.nih.gov/snp/rs1974226). A relatively high frequency of the deposited rs1974226 SNP in the dbSNP database was seen, which was estimated to be 0.152 for allele A according to the GnomAD database and 0.146 for allele T according to the TOPMED database. However, this SNP was reported in many publications regarding the potential effect of the IL-17 locus on many variations associated with immunological and metabolic issues (https://www.ncbi.nlm.nih.gov/snp/rs1974226#publications).

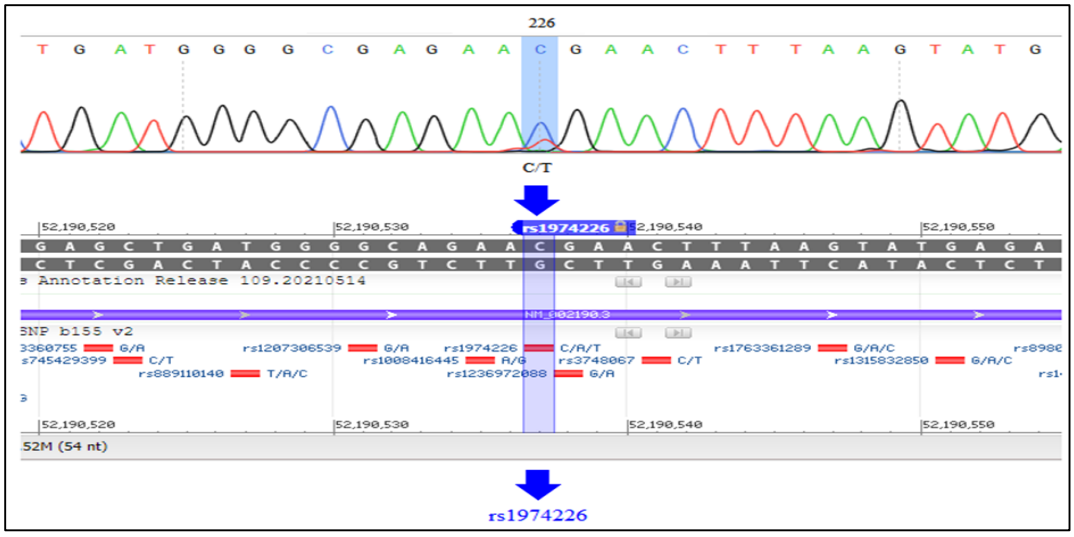


Figure (4-17): The SNP's novelty checking of IL-17 genetic single nucleotides polymorphisms using the dbSNP server

The detected C226T SNP was highlighted in blue. The marked replacement SNP was positioned using the Gen Bank account number NC 000006.11. The targeted sequences were discovered in the positive strand.

```
                10        20        30        40        50        60        70        80        90       100
      ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ref.  GGTGCTCCCTGTTGGATCTTGAGGCCTAACCTCTAGCCCAGCAGAGTCAGCTAAAATCTGAGCTCTCCCTCTCCCTCCAAGCCACACTTTGCAAAGGGAT
S1    ....................................................................................................
S2    ....................................................................................................
S3    ....................................................................................................
S4    ....................................................................................................
S5    ....................................................................................................
S6    ....................................................................................................

              110       120       130       140       150       160       170       180       190       200
      ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ref.  TCCTTGTATTGTGGGCTTGGAGTCTTTTCTCCCCATTTGCCTCTGCAGGAAGCCCTTGCAACAACACATCTGGATAGCCTCCAGGTCCCAAGGCTGGAGG
S1    ....................................................................................................
S2    ....................................................................................................
S3    ....................A...............................................................................
S4    ....................A...............................................................................
S5    ....................................................................................................
S6    ....................................................................................................

              210       220       230       240       250       260       270       280       290       300
      ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ref.  GACTTGTAATGGGAAAGTAGTCTTTAAATCAGATTTACTTGGCACCCTGTTTGCCACTGAAAGAGGCAATTTAGGGGAAAAATCTGGTCTCCAAGCACAG
S1    ....................................................................................................
S2    ....................................................................................................
S3    ....................................................................................................
S4    ....................................................................................................
S5    ....................................................................................................
S6    ....................................................................................................

              310       320       330       340       350       360
      ....|....|....|....|....|....|....|....|....|....|....|....|
```

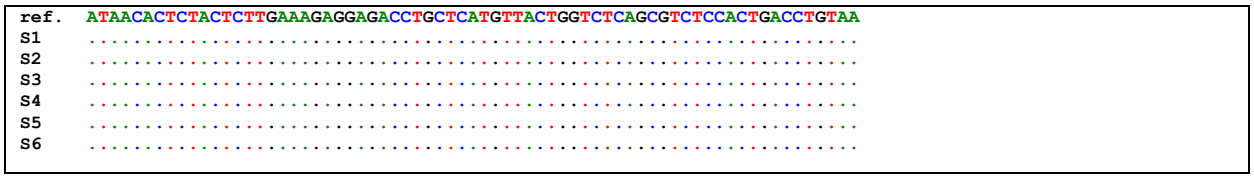


Figure (4-18): DNA sequences alignment of 6 genotyped samples with their corresponding reference sequences of the 369 bp amplicons of the coding sequences of the IL-1β gene

The detected variation's sequencing chromatogram, as well as its extensive annotations, were noted, and the chromatogram of this sequence was exhibited according to its location in the PCR amplicon. This SNP, on the other hand, was found in heterozygous G/A in S1 and S2, homozygous A/A in S3 and S4, and homozygous G/G in S5 and S6, as shown in figure (4-19).

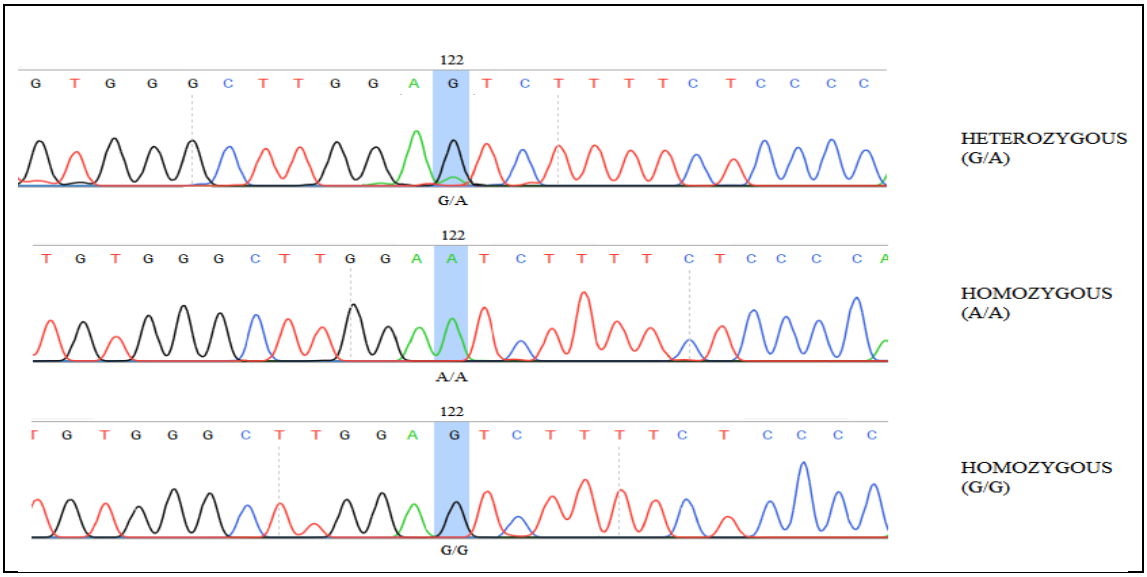


Figure (4-19): The pattern of the detected G122A SNP within the DNA chromatogram of the targeted 369 bp amplicons of the IL-1β gene

The newly discovered SNP was highlighted in the PCR amplicons based on its location. In the highlighted polymorphic locus, S1/ S2, S3/S4, and S5/S6 samples had the G/A, A/A, and G/G states, respectively. The relevant

location of IL-1β gene was downloaded from the dbSNP service to reveal the position of the targeted SNP in their deposited SNP database of the sequenced 369 bp segment (https://www.ncbi.nlm.nih.gov/projects/SNP/). To determine the nature of this SNP, a graphical depiction of the IL-1 db SNP database on chromosome 2 (Gen Bank Acc. No. NC 000002.12) was created. By checking the dbSNP engine, it was discovered that this identified SNP was originally known as rs1143629 figure (4-20). This SNP, on the other hand, was discovered in the intron-2 sequences of the targeted IL-1 gene (https://www.ncbi.nlm.nih.gov/snp/rs1143629).

The deposited rs1143629 SNP has a reasonably high frequency in the dbSNP database, which was assessed to be 0.39 for allele G according to the GnomAD database and 0.40 for the same allele according to the TOPMED database. This SNP, however, has been documented in several articles on the possible influence of the IL-1β locus on various variants related to immunological and metabolic disorders (https://www.ncbi.nlm.nih.gov/snp/rs1143629#publications).

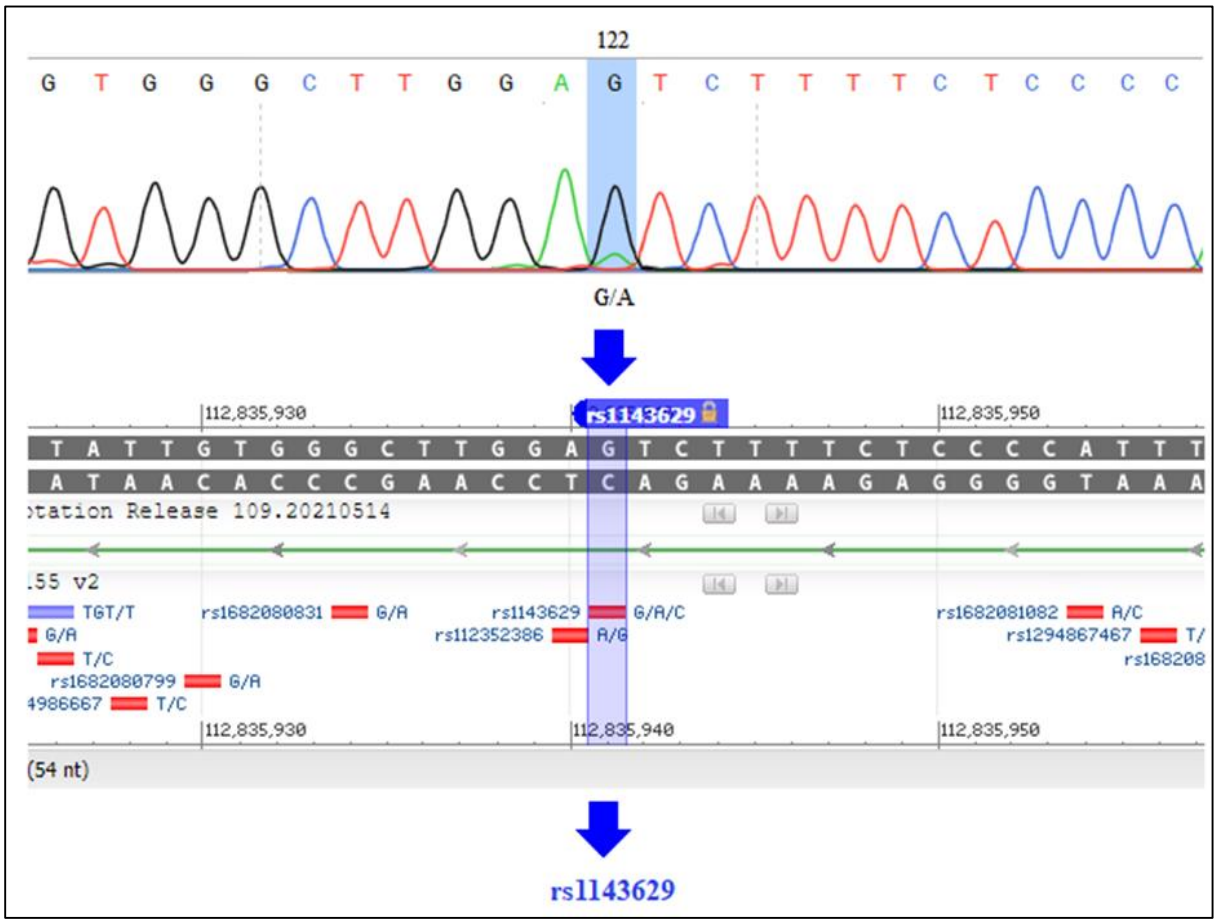


Figure (4-20): The SNP's novelty checking of IL-1B genetic single nucleotides polymorphisms using the dbSNP server

The detected G122A SNP was highlighted in blue. The marked replacement SNP was positioned using the Gen Bank account number NC 000002.12. The targeted sequences were discovered in the negative strand.

- Alvi, A.; Ahmed, A.; Butt, A.; Nawaz, A. and Hanif, A. (2014). Assessment of dengue fever severity through liver function tests. J. Coll. Physicians Surg. Pak. 24(9):640-644.
- Ahmed Ali, M.; Mikhael, E. S.; Abdelkader, A.; Mansour, L.; El Essawy, R.; El Sayed, R.; Eladawy, A. and Mukhtar, A. (2018). Interleukin-17 as a predictor of sepsis in polytrauma patients: a prospective cohort study. European journal of trauma and emergency surgery : official publication of the European Trauma Society. 44(4), 621-626.
- Alber, G. and Kamradt, T. (2007). Regulation of protective and pathogenic Th17 responses. Curr. Immunol. Rev. 3: 3-16. 26.
- Akalin, E. and Murphy, B. (2001). Gene polymorphisms and transplantation. J. Curr. Opin. Immunol; 13(5):572-6.
- Akira, S.; Uematsu, S. and Takeuchi, O. (2006). Pathogen recognition and innate immunity. Cell. 124(4), 783-801.
- Al-ameri, G., & Saif, N. (2014). A prevalence study of typhoid fever in Taiz and Al-Hodiedahcities in Yemen. Microbiology Research. J. International. 4(2), 214-223.
- AL-Edhari, A. S. Y. (2015). Preparation of polyvalent diagnostic antisera for *Salmonella* serovares typhi and typhimurium. (3-3-2):56.
- Ali Awad Obeis Abd. (2019). Genotypic and phenotypic characterization of Salmonella typhi isolated from typhoid patients in Babylon Province. MSc. Thesis, Babylon University.
- Alikhan, N.; Zhou, Z.; Sergeant, M. and Achtman, M. (2018). A genomic overview of the population structure of Salmonella. PLoS. Genet. 14(4):e1007261.

- Ali musa kadhim Abood. (2020). Immunogen preparation of Salmonella typhi virulence factors isolated from typhoid fever patients, MSc. Thesis, Babylon University.
- Ali, S.; Vollaard, A. M.; Kremer, D.; de Visser, A. W.; Martina, C. A.; Widjaja, S.; Surjadi, C.; Slagboom, E.; van de Vosse, E. and van Dissel, J. T. (2007). Polymorphisms in proinflammatory genes and susceptibility to typhoid fever and paratyphoid fever. Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research. 27(4), 271-279.
- AL-Khafaji, J. K.; Yasarri, H. F. and Altaei, M. H. (2006). Prevalence of typhoid fever among pediatric patients at Al musaib District. Med. J. Babylon. 3:1-2.
- Al-zwghaibi, A.; Yahyaraeyat, R.; Fasaei, B.; Langeroudi, A. and Salehi, T. (2018). Rapid molecular identification and differentiation of common Salmonella serovars isolated from poultry, domestic animals and foodstuff using multiplex PCR assay. Arch. Microbiol. 200(7): 1009-1016. Amatya, N.; Garg, A. V. and Gaffen, S. L. (2017). IL-17 signaling: the yin and the yang. Trends Immunol. 38:310-22.
- Anderson, R. C. and Ziprin, R. L. (2001). Bacteriology of Salmonella. In: Hui, Y. H.; Pierson, M. D. and Gorham, J. R. editors. Foodborne disease handbook, bacterial pathogens. New York: Marcel Dekker. pp. 247-263.
- Andrei, C.; Margiocco, P.; Poggi, A.; Lotti, L. V.; Torrisi, M. R. and Rubartelli, A. (2004). Phospholipases C and A2 control lysosome-mediated IL-1 beta secretion: Implications for

inflammatory processes. Proceedings of the National Academy of Sciences of the United States of America. 101(26), 9745-9750.

- Andrews, J. R. and Ryan, E. T.(2015). Diagnostics for invasive Salmonella infections: current challenges and future directions. Vaccine 33:C8-C15.
- Andualem, G.; Abebe, T.; Kebede, N.; Gebre-Selassie, S.; Mihret, A. and Alemayehu, H. (2014). A comparative study of Widal test with blood culture in the diagnosis of typhoid fever in febrile patients. B.M.C. research notes. 7, 653.
- Antillón, M.; Warren, J.; Crawford, F.; Weinberger, D. Kürüm, E.; Pak, G.; Marks, F. and Pitzer, V. (2017). The burden of typhoid fever in low and middle income countries: A meta-regression approach. PLoS neglected tropical diseases. 11(2), e0005376.
- Appiah. G.; Chung, A.; Bentsi-Enchill, A.; Kim, S.; Crump, J.; Mogasale, V.; Pellegrino, R, Slayton, R. and Mintz, E. (2020). Typhoid outbreaks, 1989-2018: Implications for prevention and control. Am. J. Trop. Med. Hyg. 102(6):1296-1305.
- Arango, M. T.; Jaramillo, C.; Montealegre, M. C.; Bohórquez, M. H. and Delgado, M. (2010). Genotipificación de los polimorfismos -511, -31 y +3954 del gen de la interleucina-1 β humana en una población colombiana con cuadro de dispepsia [Genetic characterization of the interleukin 1 b polymorphisms -511, -31 y +3954 in a Colombian population with dyspepsia]. Biomedica : revista del Instituto Nacional de Salud. 30(2), 199-206.
- Arisawa, T.; Tahara, T.; Shibata, T.; Nagasaka, M.; Nakamura, M.; Kamiya, Y.; Fujita, H.; Nakamura, M.; Yoshioka, D.; Arima, Y.; Okubo, M.; Hirata, I. and Nakano, H. (2008). The influence of

polymorphisms of interleukin-17A and interleukin-17F genes on the susceptibility to ulcerative colitis. J. clinical immunology. 28(1), 44-49.

- Baker, S.; Holt, K. and Clements, A. (2011). Combined high-resolution genotyping and geospatial analysis reveals modes of endemic urban typhoid fever transmission. Open Biol. 1:110008.
- Barton, A.; Hill, J.; Blohmke, C. and Pollard, A. (2021). Host restriction, pathogenesis and chronic carriage of typhoidal Salmonella. FEMS. Microbiology Reviews. Vol. 45, No. 5.
- Bergsbaken, T.; Fink, S. L. and Cookson, B. T. (2009). Pyroptosis: host cell death and inflammation. Nature reviews. Microbiology. 7(2), 99-109.
- Brenner, F.; Villar, R., Angulo, F., Tauxe, R. and Swaminathan, B. (2000). *Salmonella* nomenclature. J. Clin. Microbiol. 38:2465-2467.
- Bhuiyan, M. S.; Sayeed, M. A.; Khanam, F.; Daniel, T.; Bhuiyan, T. R.; Sheikh, A.; Salma, U. and et al. (2014). Cellular and cytokine responses to Salmonella enterica serotype typhi proteins in patients with typhoid fever in Bangladesh. Am. J. Trop. Med. Hyg. 90(6):1024-1030.
- Bhutta, Z. A. (2006). Current concepts in the diagnosis and treatment of typhoid fever. B.M.J. 333(7558):78-82.
- Bierschenk, D.; Monteleone, M.; Moghaddas, F.; Baker, P. J.; Masters, S. L.; Boucher, D. and Schroder, K. (2019). The Salmonella pathogenicity island-2 subverts human NLRP3 and NLRC4 inflammasome responses. J. leukocyte biology, 105(2), 401-410.

- Blohmke, C.; Darton, T. and Jones, C. (2016). Interferon-driven alterations of the host's amino acid metabolism in the pathogenesis of typhoid fever. J. Exp. Med. 213:1061-1077.
- Brands, D. A.; Inman, A. E.; Gerba, C. P.; Maré, C. J,; Billington, S. J.; Saif, L. A,; Levine, J. F. and Joens, L. A. (2005). Prevalence of *Salmonella spp*. In oysters in the United States. Appl. Environ. Microbiol. 71(2):893-897.
- Brooks, G.; Carroll, K.; Butel, J. and Morse, S. (2007). Jawetz, Melnick, & Adelberg. Medical microiology. 21th ed. McGrow-Hill. Appleton & Lange. New York.
- Brough, D. and Rothwell, N. J. (2007). Caspase-1 dependent processing of pro-interleukin-1beta is cytosolic and precedes cell death. J. cell science. 120(5), 772-781.
- Broz, P.; Ohlson, M. B. and Monack, D. M. (2012). Innate immune response to *Salmonella typhimurium*, a model enteric pathogen. Gut Microbes. 3:62-70.
- Pui, C.; Wong, W.; Chai, L.; Tunung, R.; Jeyaletchumi, P.; Noor Hidayah, M.; Ubong, A.; Farinazleen, M.; Cheah, Y. and Son, R. (2011). Review article Salmonella: A foodborne pathogen. International Food Research Journal 18: 465-473.
- Burki, T. (2018). Typhoid conjugate vaccine gets WHO prequalification. Lancet. Infect. Dis. 18(3):258.
- Camargo, M. C.; Mera, R.; Correa, P.; Peek, R. M.; Fontham, E. T.; Goodman, K. J.; Piazuelo, M. B.; Sicinschi, L.; Zabaleta, J. and Schneider, B. G. (2006). Interleukin-1beta and interleukin-1 receptor antagonist gene polymorphisms and gastric cancer: a meta-analysis. Cancer epidemiology, biomarkers & prevention : a

publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 15(9), 1674-1687.

- Cantor, M. J., Nickerson, P. and Bernstein, C. N. (2005). The role of cytokine gene polymorphisms in determining disease susceptibility and phenotype in inflammatory bowel disease. Am. J. Gastroenterol.100:1134-1142.
- Chand, H. J.; Rijal, K. R.; Neupane, B.; Sharma, V. K. and Jha, B. (2014). Re-emergence of susceptibility to conventional first line drugs in Salmonella isolates from enteric fever patients in Nepal. J. Infect. Dev. Ctries. 8(11): 1483-1487.
- Chiodini, J. (2017). The CDC Yellow Book app. Travel Med. Infect. Dis. 19:75-77.
- Crump, J. A.; Luby, S. P. and Mintz, E. D. (2004). The global burden of typhoid fever. 1. Bull World Health Organ. 82:346-53. PMID:15298225.
- Dai, Z. M.; Zhang, T. S.; Lin, S.; Zhang, W. G.; Liu, J.; Cao, X. M.; Li, H. B.; Wang, M.; Liu, X. H.; Liu, K.; Li, S. L. and Dai, Z. J. (2016). Role of IL-17A rs2275913 and IL-17F rs763780 polymorphisms in risk of cancer development: an updated meta-analysis. Scientific reports. 6, 20439.
- Darton, T.; Meiring, J. and Tonks, S. (2017). The STRATAA study protocol: a programmed to assess the burden of enteric fever in Bangladesh, Malawi and Nepal using prospective population census, passive surveillance, serological studies and healthcare utilization surveys. B.M.J. Open. 7:e016283.

- Devilee, P., & Rookus, M. A. (2010). A tiny step closer to personalized risk prediction for breast cancer.
- Dinarello, C. (2006). Interleukin-1 and interleukin-18 as mediators of inflammation and the aging process. Am. J. Clin. Nutr. 83:447-455.
- Dinarello, C. A.; Simon, A. and van der Meer, J. W. (2012). Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. Nature reviews. Drug discovery. 11(8), 633-652.
- Dhaouadi, T.; Chahbi, M.; Haouami, Y.; Sfar, I.; Abdelmoula, L.; Ben Abdallah, T. and Gorgi, Y. (2018). IL-17A, IL-17RC polymorphisms and IL17 plasma levels in Tunisian patients with rheumatoid arthritis. PloS. One. 13(3), e0194883.
- Dong, C. (2008). Regulation and pro-inflammatory function of interleukin-17 family cytokines. J. Immunol. 226:80-86.
- Dougan, G. and Baker, S. (2014). *Salmonella enterica* serovar *typhi* and the pathogenesis of typhoid fever. Ann. Rev. Microbiol. 68:317–336.
- Dufresne, K. and Daigle, F. (2017). Current Topics in *Salmonella* and Salmonellosis. London: In Tech Open;. Salmonella fimbriae: What is the clue to their hairdo? pp. 59-79.
- El-Omar, E. M.; Carrington, M.; Chow, W. H.; McColl, K. E.; Bream, J. H.; Young, H. A.; Herrera, J.; Lissowska, J.; Yuan, C. C.; Rothman, N.; Lanyon, G.; Martin, M.; Fraumeni, J. F., Jr, and Rabkin, C. S. (2000). Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature, 404(6776), 398-402.

- Elvis, C.; Christabel, N.; Arrey, Leonard, F.; Sama, Lucy, A.; Agyingi, N. (2019). Comparative study on the use of Widal test to stool culture in the laboratory diagnosis of typhoid fever in Holy Family Hospital Akum, North West Region of Cameroon. DOI: The Open Microbiology Journal 10.2174/1874285801913010073. 13, 73-80.
- Ersig, A. L.; Schutte, D. L.; Standley, J.; Leslie, E.; Zimmerman, B.; Kleiber, C.; Hanrahan, K.; Murray, J. C. and McCarthy, A. M. (2017). Relationship of genetic variants with procedural pain, anxiety, and distress in children. Biological research for nursing. 19(3), 339-349.
- Esmailpour, N.; Rasoolinejad, M. and Abdolbaghi, M. (2006). Cardiopulmonary manifestations of typhoid fever: a prospective analysis of 65 cases in Iran. Trop. Doct. 36(2):118-9.
- Estfanous, S., Ali, S. A., Seif, S. M., Soror, S., & Abdelaziz, D. (2019). Inflammasome genes' polymorphisms in egyptian chronic hepatitis C patients: Influence on vulnerability to infection and response to treatment. Mediators of inflammation. 3273645.
- Everest, P.; Wain, J. and Roberts, M. (2001). The molecular mechanisms of severe typhoid fever. Trends. Microbiol. 9:316-320.
- Fàbrega, A. and Vila, J. (2013). Salmonella enterica serovar Typhimurium skills to succeed in the host: Virulence and regulation. Clinical Microbiology Reviews. 26(2), 308-341.
- Farhana, K.; Alaullah, S. and Sayeed, M. (2013). Evaluation of a typhoid/paratyphoid diagnostic assay (TPTest) detecting anti-

Salmonella IgA in secretions of peripheral blood lymphocytes in patients in Dhaka, Bangladesh. PLoS. Negl. Trop. Dis. 7:e2316.

- FDA. (2010). Food and Drug Administration. National Antimicrobial Monitoring System-Enteric Bacteria (NARMS):2007 Executive Summary Report. Rokville, MD: U.S. Department of Health and Human Services.
- Forbes , B. A. and et al. (2007). Bailey and Scott's Diagnostic microbiology . 12th ed., Mosby Elsevier company , USA Carlone, G. M.; Thomas , M.; Rumschlag, H.; and Sottnek, F. (1986). Rapid micro procedure for isolation detergent insoluble OMP. from Hib. Clin. Microb. J. 24(3): 330-332.
- Founou, L. L.; Founou, R. C. and Essack, S. Y. (2016). Review antibiotic resistance in the food chain: A developing country-perspective. Front Microbiol. 7:1881.
- Franchi, L.; Amer, A.; Body-Malapel, M.; Kanneganti, T. D.; Özören, N.; Jagirdar, R.; Inohara, N.; Vandenabeele, P.; Bertin, J.; Coyle, A.; Grant, E. P. and Núñez, G. (2006). Cytosolic flagellin requires Ipaf for activation of caspase-1 and interleukin-1beta in salmonella-infected macrophages. Nature immunology. 7(6), 576–582.
- Gaffen, S.L. (2009). Review Structure and signaling in the IL-17 receptor family. Nat Rev Immunol. 9(8):556-67.
- Gala´n, J. E. (2016). Typhoid toxin provides a window into typhoid fever and the biology of Salmonella typhi. Proc. Natl. Acad. Sci. U.S.A. 113:1-7.
- Gasser, R. B. (2006). Molecular tools – advances, opportunities and prospects. Vet. Parasitol. 36,69-89.

- Ge, Y.; Huang, M. and Yao, Y. (2020). Biology of interleukin-17 and its pathophysiological significance in sepsis. Front. Immunol. 11:1558.
- Giguere, S.; Prescott, J. F. and Dowling, P. M. (2013). Antimicrobial Therapy Veterinary Medicine .5th .Wiley J. P.704.
- Goay, Y. X.; Chin, K. L.; Tan, C. L.; Yeoh, C. Y.; Ja'afar, J. N.; Zaidah, A. R.; Chinni, S. V. and Phua, K. K. (2016). Identification of five novel Salmonella typhi-specific genes as markers for diagnosis of typhoid fever using single-gene target PCR Assays. Biomed. Res. Int.:8905675.
- Godinez, I.; Raffatellu, M.; Chu, H.; Paixão, T. A.; Haneda, T.; Santos, R. L. and et al. (2009). Interleukin-23 orchestrates mucosal responses to *Salmonella enterica* serotype *Typhimurium* in the intestine. Infect Immun. 77:387-98.
- Gu, D.; Wang, Z.; Tian, Y.; Kang, X.; Meng, C.; Chen, X.; Pan, Z. and Jiao, X. (2020). Prevalence of *Salmonella* isolates and their distribution based on whole-genome sequence in a chicken slaughterhouse in Jiangsu, China. Front. Vet. Sci. 7:29.
- Gunn, J.; Marshall, J.; Baker, S.; Dongol, S.; Charles, R. and Ryan, E. (2014). Trends. Microbiol. 22:648-55.
- Hadeel Abbas Hassan Fadil. (2019). Bacteriological and immunological study of patients with enteric fever, MSc Thesis, Babylon University.
- Halle, A., Hornung; V., Petzold, G. C.; Stewart, C. R.; Monks, B. G.; Reinheckel, T.; Fitzgerald, K. A.; Latz, E.; Moore, K. J. and Golenbock, D. T. (2008). The NALP3 inflammasome is involved

in the innate immune response to amyloid-beta. J.Nature immunology. 9(8), 857-865.

- Hart, P. J.; O'Shaughnessy, C. M. and Siggins, M. K. (2016). Differential killing of *Salmonella* enterica Serovar typhi by antibodies targeting Vi and lipopolysaccharide O: 9 antigen. PLoS. One. 11:e0145945.
- Hersh, D.; Monack, D. M.; Smith, M. R.; Ghori, N.; Falkow, S. and Zychlinsky, A. (1999). The *Salmonella* invasin SipB induces macrophage apoptosis by binding to caspase-1. Proceedings of the National Academy of Sciences of the United States of America. 96(5), 2396-2401.
- Heymans, R.; Vila, A.; Van-Heerwaarden, C.; Jansen, C.; Castelijn, G.; Vander-Voort, M. and Biesta-Peters E. (2018). Rapid detection and differentiation of *Salmonella* species, *Salmonella Typhimurium* and *Salmonella* Enteritidis by multiplex quantitative PCR. PLoS. One. 13:e0206316.
- House, D.; Bishop, A. and Parry, C. (2001). Typhoid fever: pathogenesis and disease. Curr. Opin. Infect. Dis. 14:573-578.
- Huang, W.; Fidel, P. and Schwarzenberger, P. (2004). Requirement of interleukin-17A for systemic anti-Candida albicans host defense in mice. J. Infect. Dis. 190: 624-631.
- Ishigame, H.; Kakuta, S.; Nagai, T.; Kadoki, M.; Nambu, A.; Komiyama, Y. and Sudo, K. (2009). Differential roles of interleukin-17A and-17F in host defense against mucoepithelial bacterial infection and allergic responses. Immunity. 30(1), 108-119.

- Islam, K.; Sayeed, A. and Hossen, E. (2016). Comparison of the performance of the TPTest, Tubex, Typhidot and Widal immunodiagnostic assays and blood cultures in detecting patients with typhoid fever in Bangladesh, including using a bayesian latent class modeling approach. PLoS. Negl. Trop. Dis. 10:e0004558.
- Iwamoto. M.; Reynolds, J.; Karp, B. Tate, H. Fedorka-Cray, P.; Plumblee; J.; Hoekstra, R.; Whichard, J. Mahon, B. (2017). Ceftriaxone-resistant non typhoidal *Salmonella* from humans, retail meats, and food animals in the United States, 1996-2013. Foodborne Pathog. Dis. 14(2):74-83.
- Jaafar, N. J.; Yuan, X. G.; Nur, F. M. Z.; Heng, C. L.; Hani, M. H.; Wan, M. H.; Subhash, J. B.; Prabha, B. Asma, I. and Kia, K. P. (2013). Epidemiological analysis of typhoid fever in Kelantan from a retrieved registry. Malay. J. Microbiol. 9: 147-151.
- Jackson, B.; Iqbal, S. and Mahon, B. (2015). Centers for Disease Control and Prevention (CDC). Updated recommendations for the use of typhoid vaccine-Advisory Committee on Immunization Practices, United States, 2015. MMWR. Morb. Mortal. Wkly. Rep. 64(11):305-8.
- Janeway, C. A. and Medzhitov, R. (2002). Innate immune recognition. Annual review of immunology. 20, 197-216.
- Jawetz, E.; Melnick, J. and Adelbergs, E. (2007). "Enterobacteriaceae" In: Review of medical microbiology. 24th ed. by Geo, F.; Janet S. and Stephen, McGraw–Hill companies, U.S.A. pp.256-261.
- Jerrold, R. and Turner. (2010). The gastrointestinal tract: Salmonellosis, in Kumar, V.; Abbas, A. K.; Fausto, and Aster, J.

C. editors, Robins and Cotran Pathologic Basis of Disease, 8th edition India, Elsevier, 801.

- Jorgensen, J. H. and Yolken, R. H. (2003). Biochemical test for identification of medical bacteria. 8th Ed., Manual of clinical American Society for Microbiology, Washington, D. C.
- Johnson, J. M.; Rajic, A. and McMullen, L. M. (2005). Antimicrobial resistance of selected *Salmonella* isolates from food animals and food in Alberta. Can. Vet. J. 46(2): 141-146.
- Kaiser, P. and Hardt, W. (2011). *Salmonella typhimurium* diarrhea: switching the mucosal epithelium from homeostasis to defense. Curr. Opin. Immunol. 23:456-463.
- Karkey, A.; Arjyal, A.; Anders, K.; Boni, M. and Dongol, S. (2010). The burden and characteristics of enteric fever at a healthcare facility in a densely populated area of Kathmandu. PloS. One. 5: e13988.
- Kawaguchi, M.; Adachi, M.; Oda, N.; Kokubu, F. and Huang, S. K. (2004). IL-17 cytokine family. J. Allergy and Clinical Immunology. 114(6), 1265-1273.
- Kawaguchi, M.; Takahashi, D.; Hizawa, N.; Suzuki, S.; Matsukura, S.; Kokubu, F.; Maeda, Y.; Fukui, Y.; Konno, S.; Huang, S. K.; Nishimura, M. and Adachi, M. (2006). IL-17F sequence variant (His161Arg) is associated with protection against asthma and antagonizes wild-type IL-17F activity. J. allergy and clinical immunology. 117(4), 795-801.
- Keestra, A. M., Godinez, I., Xavier, M. N., Winter, M. G., Winter, S. E., Tsolis, R. M., & Bäumler, A. J. (2011). Early MyD88-

dependent induction of interleukin-17A expression during *Salmonella* colitis. Infection and immunity. 79(8), 3131–3140.

- Kelly-Hope, L. A.; Alonso, W. J.; Theim, V. D.; Anh, D. D. and Canh, D. G. (2007). Geographical distribution and risk factors associated with enteric diseases in Vietnam. Am. J. Trop. Med. Hyg. 76: 706-712.
- Keshavarz, M.; Mirzaei, H.; Salemi, M.; Momeni, F.; Mousavi, M.; Sadeghalvad, M.; Arjeini, Y.; Solaymani-Mohammadi, F.; Sadri Nahand, J.; Namdari, H.; Mokhtari-Azad, T. and Rezaei, F. (2019). Influenza vaccine: Where are we and where do we go?. Reviews in medical virology. 29(1), e2014.
- Khader, S. A.; Gaffen, S. L. and Kolls, J. K. (2009). Th17 cells at the cross roads of innate and adaptive immunity against infectious diseases at the mucosa. Mucosal Immunol. 2:403-411.
- Khader, S. A. and Gopal, R. (2010). IL-17 in protective immunity to intracellular pathogens. Virulence. 1: 423-427.
- Klemm, E.; Shakoor, S.; Page, A.; Qamar, F.; Judge, K.; Saeed, D.; Wong, V.; Dallman, T.; Nair, S.; Baker, S.; Shaheen, G.; Qureshi, S.; Yousafzai, M.; Saleem, M.; Hasan, Z.; Dougan, G. and Hasan, R. (2018). Emergence of an extensively drug-resistant *Salmonella enterica* Serovar typhi clone harboring a promiscuous plasmid encoding resistance to fluoroquinolones and third-generation cephalosporins. mBio. 9(1): e 00105-18.
- Kolls, J. K. and Khader, S. A. (2010). The role of Th17 cytokines in primary mucosal immunity. Cytokine Growth Factor Rev. 21: 443-448.

- Koshiol, J.; Wozniak, A. Cook, P.; Adaniel, C.; Acevedo, J.; Azócar, L.; Hsing, A.; Roa, J.; Pasetti, M.; Miquel, J.; Levine, M. and Ferreccio, C. (2016). Gallbladder Cancer Chile Working Group. *Salmonella* enterica serovar typhi and gallbladder cancer: a case-control study and meta-analysis. Cancer Med. 5:3310-3235.
- Kothari, A.; Pruthi, A. and Chugh, T. (2008). The burden of enteric fever. J. Infect. Dev. Ctries. 2: 253-259.
- Kozak, M.; Dabrowska-Zamojcin, E.; Mazurek-Mochol, M. and Pawlik, A. (2020). Cytokines and their genetic polymorphisms related to periodontal disease. J. clinical medicine. 9(12), 4045.
- Ktsoyan, Z.; Ghazaryan, K.; Manukyan, G.; Martirosyan, A.; Mnatsakanyan, A.; Arakelova, K. and et al. (2013). Inflammatory responses to *Salmonella* infections are serotype-specific. Hindawi Publishing Corporation International J. of Bacteriology, Article ID 168179, 7.
- Kumwenda, M. and Iroh, P. (2019). An adolescent with multi-organ involvement from typhoid fever. Malawi. Med. J. 31(2):159-160.
- Kurtz, J.; Nieves, W.; Bauer, D.; Israel, K.; Adcox, H.; Gunn, J.; Morici, L. and McLachlan, J. (2020). Salmonella persistence and host immunity are dictated by the anatomical microenvironment. Infect. Immun. 88.
- Lee, D. Y.; Hong, S. W.; Chang, Y. G.; Lee, W. Y. and Lee, B. (2013). Clinical significance of preoperative inflammatory parameters in gastric cancer patients. J. of gastric cancer. 13(2), 111-116.

- Lee, K. Runyon, M.; Herrmanm, T., Phillips, R. and Hsieh, J. (2015). Review of *Salmonella* detection and identification methods: Aspects of rapid emergency response and food safety. Food Control. 47:264–276.
- Lee, E.; Park, J.; Kim, D.; Song, M.; Sahastrabuddhe, S.; Kim, H.; Chon, Y. and Yang, J. (2020). Comparison of anti-Vi IgG responses between two clinical studies of typhoid Vi conjugate vaccines (Vi-DT vs Vi-TT). PLoS. Negl. Trop. Dis. 14(3):e0008171.
- Le Feuvre, R. A.; Brough, D.; Iwakura, Y.; Takeda, K. and Rothwell, N. J. (2002). Priming of macrophages with lipopolysaccharide potentiates P2X7-mediated cell death via a caspase-1-dependent mechanism, independently of cytokine production. The J. of biological chemistry. 277(5), 3210-3218.
- Levine, M. M.; Tacket, C. O. and Sztein, M. B. (2001). Host-*Salmonella* interaction: human trials. Microbes Infect. 3: 1271–1279.
- Levinson, W. (2004). Review of medical microbiology and immunology. 9th ed. McGrow- Hill. Appleton & Lange. New York.
- Levy, R.; Okada, S.; Beziat, V.; Moriya, K.; Liu, C.; Chai, L. Y. A. and et al. (2016). Genetic, immunological, and clinical features of patients with bacterial and fungal infections due to inherited IL-17RA deficiency. Proc. Natl. Acad. Sci. U.S.A. 113:E8277-85.
- Lianou, A.; Nychas, G. and Koutsoumanis, K. (2017). Variability in the adaptive acid tolerance response phenotype of *Salmonella enterica* strains. Food Microbiol. 62:99-105.

- Lo, J. H.; Kulp, S. K.; Chen, C. S. and Chiu, H. C. (2014). Sensitization of intracellular *Salmonella enterica serovar Typhimurium* to aminoglycosides in vitro and in vivo by a host-targeted antimicrobial agent. Antimicrob. Agents Chemother. 58(12): 7375-7382.
- Lowman, W. (2013). Re-evaluating the role of Piperacillin - Tazobactam in the treatment of hospital-acquired infections: Original research. Southern Afr. J. Epidemiol. Infect.. 28(1): 16-21.
- Lutterloh, E., Likaka, A., Sejvar, J., Manda, R., Naiene, J. and Monroe, S.S., Khaila, T. (2012). Multidrug-resistant typhoid fever with neurologic findings on the Malawi Mozambique border. Clin. Infect. Dis. 54(8): 1100-1106. 46. Majid, A.; Bin Waqar, S.; Rehan, A. and Kumar, S. (2019). From gut to heart: havoc in a young patient with typhoid-associated cardiomyopathy. Cureus. 11(7):e5049.
- Luby, S. P.; Faizan, M. K., Fisher-Hoch, S. P.; Syed, A. and Mintz, E. D. (1998). Risk factors for typhoid fever in an endemic setting, Karachi, Pakistan. Epidemiol. Infect.; 120: 129-138.
- Mandal, S.; Deb Mandal, M. and Pal, N. (2012). Antibiotic resistance of *Salmonella* enterica serovar typhi in Kolkata, India, and in vitro experiments on effect of combined chemotherapy. The scientific world journal. https:// doi. org/ 10. 1100/2012/ 454059.
- Man, S. M.; L. J.; Hopkins, E.; Nugent, S.; Cox, I. M.; Glu¨ck, P.; Tourlomousis, J. A.; Wright, P.; Cicuta, T. P.; Monie, and C. E. Bryant. (2014). Inflammasome activation causes dual recruitment

of NLRC4 and NLRP3 to the same macromolecular complex. Proc. Natl. Acad. Sci. U.S.A. 111: 7403-7408.

- Matsuda, K. (2017). PCR-based detection methods for single-nucleotide polymorphism or mutation: real-time PCR and its substantial contribution toward technological refinement. In Advances in clinical chemistry. Elsevier. 80, 45-72.
- Mariathasan, S.; Weiss, D. S.; Newton, K.; McBride, J.; O'Rourke, K.; Roose-Girma, M.; Lee, W. P.; Weinrauch, Y.; Monack, D. M. and Dixit, V. M. (2006). Cryopyrin activates the inflammasome in response to toxins and ATP. Nature. 440(7081), 228-232.
- Mariathasan, S. and Monack, D. M. (2007). Inflammasome adaptors and sensors: intracellular regulators of infection and inflammation. Nature reviews. Immunology. 7(1), 31-40.
- Mawazo, A.; Bwire, G. and Matee, M. (2019). Performance of Widal test and stool culture in the diagnosis of typhoid fever among suspected patients in Dar es Salaam, Tanzania. B.M.C. Res. Notes. 12(1):316.
- McFadden, J. (2000). Biochemical tests for the identification of aerobic bacteria. Clinical Microbiology Procedures Handbook, 3rd Edition, 503-642.
- McGeachy, M. J.; Cua, D. J. and Gaffen, S. L. (2019). The IL-17 family of cytokines in health and disease. Immunity. 50(4), 892-906.
- McQuiston, J. R.; Fields, P. I.; Tauxe, R. V. and Logsdon, J. M. (2008). Do *Salmonella* carry spare tyres? Trends. Microbiol. 16(4):142-148.

- Meiring, J.; Gibani, M. and Basnyat, B. (2017). The typhoid vaccine acceleration consortium (TyVAC): vaccine effectiveness study designs: accelerating the introduction of typhoid conjugate vaccines and reducing the global burden of enteric fever. Report from a meeting held on 26-27 October 2016, Oxford. Vaccine. 35:5081-5088.
- Mellon, G.; Eme, A.; Rohaut, B.; Brossier, F.; Epelboin, L. and Caumes, E. (2017). Encephalitis in a traveller with typhoid fever: efficacy of corticosteroids. J. Travel. Med. 24(6).
- Merican, I. (1997). Typhoid fever: present and future. Med. J. Malay. 52: 299-308.
- Mayuzumi, H.; Inagaki-Ohara, K.; Uyttenhove, C.; Okamoto, Y. and Matsuzaki, G. (2010). Interleukin-17A is required to suppress invasion of *Salmonella* enterica serovar typhimurium to enteric mucosa. Immunology, 131(3), 377-385.
- Matsuda, K. (2017). PCR-based detection methods for single-nucleotide polymorphism or mutation: real-time PCR and its substantial contribution toward technological refinement. In Advances in clinical chemistry (Vol. 80, pp. 45-72). Elsevier.
- Meng, G. F.; Zhang, I.; Fuss, A.; Kitani, and W. Strober. (2009). A mutation in the Nlrp3 gene causing inflammasome hyper-activation potentiates Th17 cell-dominant immune responses. Immunity. 30:860-874.
- Miao, E. A.; Alpuche-Aranda, C. M.; Dors, M.; Clark, A. E.; Bader, M. W.; Miller, S. I. and Aderem, A. (2006). Cytoplasmic flagellin activates caspase-1 and secretion of interleukin 1beta via Ipaf. Nature immunology. 7(6), 569-575.

- Miao, E. A.; Andersen-Nissen, E.; Warren, S. E. and Aderem, A. (2007). TLR5 and Ipaf: dual sensors of bacterial flagellin in the innate immune system. Seminars in immunopathology. 29(3), 275-288.
- Michael, S. and AL-Wan, H. (2008). Evaluation of Single Widal test in Microbial. 16(10): 496-506.
- Mario-Vásquez, J. E.; Naranjo-González, C. A.; Montiel, J.; Zuluaga, L. M.; Vásquez, A. M.; Tobón-Castaño, A.; Bedoya, G. and Segura, C. (2021). Association of variants in IL1B, TLR9, TREM1, IL10RA, and CD3G and Native American ancestry on malaria susceptibility in Colombian populations. Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases. 87, 104675.
- Mogasale, V.; Maskery, B. and Ochiai, R. (2014). Burden of typhoid fever in low income and middle-income countries: a systematic, literature-based update with risk-factor adjustment. Lancet Glob Heal 2:e570-e580.
- Mogasale, V.; Ramani, E.; Mogasale, V. and Park, J. (2016). What proportion of *Salmonella* typhi cases are detected by blood culture? A systematic literature review. Ann. Clin. Microbiol. Antimicrob. 15:32.
- Murray, P. R. and et al. (2003). Manual of Clinical Microbiology. 8 th Edition. Washington. D.C.
- Muruve, D. A.; V. Petrilli, A. K; Zaiss, L. R; White, S. A; Clark, P. J; Ross, R. J; Parks and J. Tschopp. (2008). The inflammasome recognizes cytosolic microbial and host DNA and triggers an innate immune response. Nature. 452:103-107.

- Mutai, W. C.; Muigai, A. W. T.; Waiyaki, P. and Kariuki, S. (2018). Multi-drug resistant *Salmonella* enterica serovar typhi isolates with reduced susceptibility to ciprofloxacin in Kenya. BMC Microbiol. 18(1): 187.
- Nakada, T. A.; Russell, J. A.; Boyd, J. H. and Walley, K. R. (2011). IL17A genetic variation is associated with altered susceptibility to Gram-positive infection and mortality of severe sepsis. Critical care (London, England), 15(5), R254.
- Näsström, E.; Jonsson, P.; Johansson, A.; Dongol, S.; Karkey, A.; Basnyat.; B.; Tran, Vu Thieu, N.; Trinh Van, T.; Thwaites, G.; Antti, H. and Baker S. (2018). Diagnostic metabolite biomarkers of chronic typhoid carriage. PLoS. Negl. Trop. Dis. 12(1):e0006215.
- Nicholson, T. L. and Baumler, A. J. (2001). *Salmonella enterica* serotype typhimurium elicits cross-immunity against a *Salmonella* enterica serotype enteritidis strain expressing LP fimbriae from the lac promoter. Infect. Immun. 69:204-212.
- Niebuhr, M.; Gathmann, M.; Scharonow, H.; Mamerow, D.; Mommert, S.; Balaji, H. and Werfel, T. (2011). Staphylococcal alpha-toxin is a strong inducer of interleukin-17 in humans. Infect Immun.;79:1615-22.
- Nordang, G. B.; Viken, M. K.; Hollis-Moffatt, J. E.; Merriman, T. R.; Førre, Ø. T.; Helgetveit, K.; Kvien, T. K. and Lie, B. A. (2009). Association analysis of the interleukin 17A gene in Caucasian rheumatoid arthritis patients from Norway and New Zealand. Rheumatology (Oxford, England). 48(4), 367-370.
- Ochiai, R.; Acosta, C.; Danovaro-Holliday, M.; Baiqing, D. and Bhattacharya, S. (2008). A study of typhoid fever in five Asian

countries: disease burden and implications for controls. Bull World Health Organ. 86: 260-268.

- Ogimoto, A.; Suzuki, J. Inoue, K.; Nishimura,K.; Nagai, T. Shigematsu, Y.; H.; Okayama, and J. Higaki. (2018). Interaction between polymorphisms in inflammation-related genes and atrial fibrillation in patients with dilated cardiomyopathy. Circulation. 2011;124:A16747.
- Ohka, S.; Nishizawa, D.; Hasegawa, J.; Takahashi, K.; Nakayama, K.; Ebata, Y.; Fukuda, K. I. and Ikeda, K. (2018). Association between rs2275913 single-nucleotide polymorphism of the interleukin-17A gene and perioperative analgesic use in cosmetic orthognathic surgery. Neuropsychopharmacology reports, 38(2), 67-74.
- Omoshaba, E. O.; Olufemi, F. O.; Ojo, O. E.; Sonibare, A. O. and Agbaje, M. (2017). Multidrug-resistant *Salmonella* isolated in Japanese quails reared in Abeokuta, Nigeria. Trop. Anim. Health Prod. 49(7):1455–1460.
- Omulo, S.; Thumbi, S. M.; Njenga, M. K. and Call, D.R. (2015). A review of 40 years of enteric antimicrobial resistance research in Eastern Africa: What can be done better? Antimicrob. Resist. Infect. Control. 4(1): 1-1.
- Ouyang, W.; Kolls, J. K. and Zheng, Y. (2008). The biological functions of T helper 17 cell effector cytokines in inflammation. Immunity. 28(4), 454-467.
- Pacheco, A. G. and Moraes, M. O. (2009). Genetic polymorphisms of infectious diseases in case-control studies. Disease markers. 27(3-4), 173-186.

- Park, H.; Li, Z.; Yang, X. O.; Chang, S. H.; Nurieva, R.; Wang, Y. H. and Dong, C. (2005). A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nature immunology. 6(11), 1133-1141.
- Park, J. W.; Choi, J. S.; Han, K. J.; Lee, S. H.; Kim, E. J. and Cho, J. H. (2018). Association of a genetic polymorphism of IL1RN with risk of acute pancreatitis in a Korean ethnic group. The Korean journal of internal medicine. 33(6), 1103-1110.
- Parry, C.; Hien, T.; Dougan, G.; White, N. and Farrar, J. (2002). Typhoid fever. N. Engl. J. Med. 28;347(22):1770-82.
- Parry, C. M. (2006). Epidemiological and clinical aspects of human typhoid fever. In: Matroeni, P. And Maskell, D. (Eds.). *Salmonella* infections: Clinical, Immunological And Molecular Aspects, P: 1-18. New York: Cambridge University Press.
- Pegues, D. A.; Michael, E. O. and Miller, S. I. (2002). *Salmonella*, including *Salmonella* typhi. In Blaser, M. J.; Smith, P. D. and Ravdin, J. I.; Greenberg, H. B. and Guerrant, R. L., ed. Infections of the Gastrointestinal Tract. Philadelphia: Lippincott Williams and Wilkins:669-7.
- Phetsavanh, C.; Mayfong, M.; Phouthapanya, X.; Tamalee, R.; Sayaphet, R.; Manivanh, V.; Paul, N. and John, A. (2020). Estimation of incidence of typhoid and paratyphoid fever in Vientiane, Lao People's Democratic Republic. Am. J. Trop. Med. Hyg. 102(4), pp. 744-748.
- Pincus, D. (2010). Microbial Identification Using the bioMérieux VITEK® 2 System. Encyclopedia of Rapid Microbiological, Bethesda, MD: Parenteral Drug Association.

- Pinto, L. A.; DE Azeredo Leitão, L. A.; Mocellin, M.; Acosta, P.; Caballero, M. T.; Libster, R.; Vargas, J. E.; Polack, F.; Comaru, T.; Stein, R. T. and DE Souza, A. P. (2017). IL-8/IL-17 gene variations and the susceptibility to severe viral bronchiolitis. Epidemiology and infection. 145(4), 642-646.
- Pontillo, A.; Reis, E. C.; Liphaus, B. L.; Silva, C. A., and Carneiro-Sampaio, M. (2015). Inflammasome polymorphisms in juvenile systemic lupus erythematosus. Autoimmunity. 48(7), 434.437.
- Popoff, M. and Le Minor, L. (2005). In: *Salmonella*. Bergey's manual of systematic bacteriology. 2nd ed. Brenner, D. J.; Kreig, N. R. and Staley, J. T. editors. New York. U.S.A. pp. 764-799.
- Pouzol, S.; Tanmoy, A.; Ahmed, D.; Khanam, F.; Brooks, W.; Bhuyan, G.; Fabre, L.; Bryant, J.; Gustin, M.; Vanhems, P.; Carman, B.; Weill, F.; Qadri, F.; Saha, S. and Endtz, H. (2019). Clinical evaluation of a multiplex PCR for the detection of *Salmonella* enterica serovars typhi and paratyphi A from blood specimens in a high endemic setting. Am. J. Trop. Med. Hyg. 101(3): 513-520.
- Poyet, J.; Razmara, M.; Datta, P.; Zhang, Z. and Alnemri, E. (2002). The PYRIN-CARD protein ASC is an activating adaptor for caspase-1. Srinivasula. S. M, J. Biol. Chem. 277:21119-22.
- Prantner, D.; Darville, T.; Sikes, J. D.; Andrews, C. W.; Brade, H.; Rank, R. G. Nagarajan, U. M. (2009). Critical role for interleukin-1beta (IL-1beta) during Chlamydia muridarum genital infection and bacterial replication-independent secretion of IL-1beta in mouse macrophages. Infection and immunity. 77(12), 5334-5346.

- Qamar, F.; Yousafzai, M.; Khaliq, A.; Karim, S., Memon, H.; Junejo, A.; Baig, I.; Rahman, N. Bhurgry, S.; Afroz, H. and Sami, U. (2020). Adverse events following immunization with typhoid conjugate vaccine in an outbreak setting in Hyderabad, Pakistan. Vaccine. 38(19):3518-3523.
- Qinghai, Z.; Yanying, W.; Yunfang, C.; Xukui, Z. and Xiaoqiao, Z. (2014). Effect of interleukin-17A and interleukin-17F gene polymorphisms on the risk of gastric cancer in a Chinese population. Gene. 537(2), 328-332.
- Qu, Y.; Franchi, L.; Nunez, G. and Dubyak, G. R. (2007). Non classical IL-1 beta secretion stimulated by P2X7 receptors is dependent on inflammasome activation and correlated with exosome release in murine macrophages. Journal of immunology (Baltimore, Md. : 1950). 179(3), 1913-1925.
- Qu, Y.; Misaghi, S.; Newton, K.; Maltzman, A.; Izrael-Tomasevic, A.; Arnott, D. and Dixit, V. M. (2016). NLRP3 recruitment by NLRC4 during *Salmonella* infection. The Journal of experimental medicine, 213(6), 877-885.
- Raffatellu, M; Santos, R.; Verhoeven, D.; George, M. and Wilson, R. (2008). Simian immunodeficiency virus-induced mucosal interleukin-17 deficiency promotes Salmonella dissemination from the gut. Nat. Med. 14: 421-428.
- Raffatellu, M.; George, M.; Akiyama, Y.; Hornsby, M.; Nuccio, S. and Paixao, T. (2009). Lipocalin-2 resistance confers an advantage to *Salmonella enterica* serotype *Typhimurium* for growth and survival in the inflamed intestine. Cell Host Microbe. 5:476-86.

- Rahman, B.; Wasfy, M.; Maksoud, M.; Hanna, N.; Dueger, E. and House, B. (2014). Multi-drug resistance and reduced susceptibility to ciprofloxacin among *Salmonella* enterica serovar typhi isolates from the Middle East and Central Asia. New Microbes New Infect. 2(4): 88-92.
- Rasool, K.H., Hussein, N.H. and Taha, B.M. (2020). Molecular detection of gyrA gene in *Salmonella* enterica serovar typhi isolated from typhoid patients in Baghdad. Pak. J. Biolog. Sci. 23(10): 1303-1309.
- Rasul, F.; Sughra, K.; Mushtaq, A.; Zeeshan, N.; Mehmood, S. and Rashid, U. (2017). Surveillance report on typhoid fever epidemiology and risk factor assessment in district Gujrat, Punjab, Pakistan. Biomedical Research (India), 28(16), 6921-6926.
- Rathinam, V. A. and Fitzgerald, K. A. (2016). Inflammasome complexes: emerging mechanisms and effector functions. Cell. 165(4), 792-800.
- Reis, C., Barbosa, M., Machado, B., Baratto, S., de Lima, D. C., Paza, A. O., Filho, F. B., Brancher, J. A., Küchler, E. C., & de Oliveira, D. (2021). Genetic polymorphisms in interleukin-6 and interleukin-1-beta were associated with dental caries and gingivitis. Acta. Odontologica. Scandinavica. 79(2), 96-102.
- Robertson, S. E.; Young, J. D.; Kitson, S.; Pitt, A.; Evans, J. Roes, J.; Karaoglu, D.; Santora, L.; Ghayur, T.; Liew, F. Y.; Gracie, J. A. and McInnes, I. B. (2006). Expression and alternative processing of IL-18 in human neutrophils. Eur J. Immunol. 36:722–31.
- Rogo, L. D., Rezaei; F., Marashi, S. M.; Yekaninejad, M. S.; Naseri, M.; Ghavami, N. and Mokhtari-Azad, T. (2016). Seasonal

influenza A/H3N2 virus infection and IL-1β, IL-10, IL-17, and IL-28 polymorphisms in Iranian population. J. of medical virology. 88(12), 2078-2084.

- Rohilla, R.; Bhatia, M.; Gupta, P.; Singh, A.; Shankar, R. and Omar, B. (2019). *Salmonella* osteomyelitis: A rare extra intestinal manifestation of an endemic pathogen. J. Lab. Physicians. 11:164-170.
- Rolandelli, A.; Hernández Del Pino, R. E.; Pellegrini, J. M.; Tateosian, N. L.; Amiano, N. O.; de la Barrera, S.; Casco, N.; Gutiérrez, M.; Palmero, D. J., and García, V. E. (2017). The IL-17A rs2275913 single nucleotide polymorphism is associated with protection to tuberculosis but related to higher disease severity in Argentina. Scientific reports. 7, 40666.
- Rotimi, V.; Jamal, W.; Pal, T.; Sovenned, A. and Albert, M. (2008). Emergence of CTX-M-15 type extended-spectrum β-lactamase-producing *Salmonella* spp. in Kuwait and the United Arab Emirates. J. Med. Microbiol. 57:881–886.
- Ross, E.; Coughlan, R.; Flores-Langarica, A.; Lax, S.; Nicholson, J.; Desanti, G.; Marshall, J.; Bobat, S.; Hitchcock, J. and White, A. (2012) Thymic function is maintained during *salmonella*-induced atrophy and recovery. J. Immunol. 189: 4266-4274.
- Sabbagh, S.; Forest, C.; Lepage, C. and Leclerc, J. (2010). Review So similar, yet so different: uncovering distinctive features in the genomes of *Salmonella* enterica serovars Typhimurium and Typhi. Daigle. FFEMS. Microbiol. Lett. 305(1):1-13.
- Safia Sultana. (2012). Comparison Of different test methods including polymerase chain reaction for early and reliable

diagnosis of typ Saleh, M. A. D. (2013). Comparison of Widal agglutination test with ELISA typhi test for serological diagnosis of typhoid fever in some Iraqi Patients in Diyala Governorate. 1(5):59-64.hoid fever. Department of microbiology. Mymensingh. Medical college Mymensingh. Bangladesh.

- Saleh, M. A. D. (2013). Comparison of Widal agglutination test with ELISA typhi test for serological diagnosis of typhoid fever in some Iraqi Patients in Diyala Governorate. 1(5):59-64.
- Salman, H. A.; Abdulmohsen, A. M.; Falih, M. N., & Romi, Z. M. (2021). Detection of multidrug-resistant *Salmonella enterica* sub spp. *enterica* serovar typhi isolated from Iraqi subjects. Veterinary world, 14(7). 1922-1928.
- Schirmer, M. A.; Lüske, C. M.; Roppel, S.; Schaudinn, A.; Zimmer, C.; Pflüger, R. and Hackert, T. (2016). Relevance of Sp binding site polymorphism in WWOX for treatment outcome in pancreatic cancer. JNCI: J. of the National Cancer Institute. 108(5).
- Schroder, K. and Tschopp, J. (2010). The inflammasomes. Cell, 140(6), 821–832.
- Schulz, S. M.; Köhler, G.; Holscher, C.; Iwakura, Y. and Alber, G. (2008). IL-17A is produced by Th17, gamma delta T cells and other CD4+ lymphocytes during infection with *Salmonella* enterica serovar Enteritidis and has a mild effect in bacterial clearance. Int. Immunol. 20: 1129-1138.
- Schwarzenberger, P.; Huang, W.; Ye, P.; Oliver, P.; Manuel, M.; Zhang, Z.; Bagby, G.; Nelson, S. and Kolls, J. K. (2000). Requirement of endogenous stem cell factor and granulocyte-

colony-stimulating factor for IL-17-mediated granulopoiesis. Journal of immunology (Baltimore, Md. : 1950), 164(9), 4783-4789.

- Sellge, G.; Magalhaes, J.; Konradt, C.; Fritz, J. and Salgado-Pabon, W. (2010). Th17 cells are the dominant T cell subtype primed by Shigella flexneri mediating protective immunity. J. Immunol. 184: 2076-2085.
- Silva, M. J.; de Santana, M.; Tosta, B. R.; Espinheira, R. P.; Alcantara-Neves, N. M.; Barreto, M. L.; Figueiredo, C. A. and Costa, R. (2019). Variants in the IL17 pathway genes are associated with atopic asthma and atopy makers in a South American population. Allergy, asthma, and clinical immunology: official journal of the Canadian Society of Allergy and Clinical Immunology. 15, 28.
- Sivick, K. E.; Schaller, M. A.; Smith, S. N; Mobley, H. L. (2010). The innate immune response to uropathogenic Escherichia coli involves IL-17A in a murine model of urinary tract infection. J. Immunol. 184:2065-75.
- Sharma, P.; Ramakrishnan, R.; Hutin, Y.; Manickam, P. and Gupte, M. D. (2009). Risk factors for typhoid in Darjeeling, west Bengal, India: evidence for practical action. Trop. Med. Int. Health. 14: 696-702.
- Sharma, D. and Kanneganti, T. D. (2016). The cell biology of inflammasomes: Mechanisms of inflammasome activation and regulation. The Journal of cell biology, 213(6), 617-629.
- Shen, H.; Yu, R. And Chou, C. (2007). Acid adaptation affects the viability of *Salmonella* typhimurium during the lactic fermentation

of skin milk and product storade. International Journal of Food Microbiology.114 (3): 380-385.

- Shen, L; Zhang, H.; Yan, T.; Zhou, G. and Liu, R. (2015). Association between interleukin 17A polymorphisms and susceptibility to rheumatoid arthritis in a Chinese population. Gene .566: 18-22.
- Shibata, K.; Yamada, H.; Hara, K.; Kishihara, K. and Yoshikai, Y. (2007). Resident Vδ1+λδ T cells control early infiltration of neutrophils after Escherichia coli infection via IL-17 production. J. Immunol. 178: 4466-4472.
- Slattery, M. L.; Herrick, J. S.; Torres-Mejia, G.; John, E. M.; Giuliano, A. R.; Hines, L. M.; Stern, M. C.; Baumgartner, K. B.; Presson, A. P. and Wolff, R. K. (2014). Genetic variants in interleukin genes are associated with breast cancer risk and survival in a genetically admixed population: the Breast Cancer Health Disparities Study. Carcinogenesis. 35(8), 1750-1759.
- Smith, S.; Seriki, A. and Ajayi, A. (2016). Typhoidal and non-typhoidal *Salmonella* infections in Africa. Eur. J. Clin. Microbiol. Infect. Dis. 35(112):1913-1922.
- Song, E. J.; Kang, M. J.; Kim, Y. S.; Kim, S. M.; Lee, S. E.; Kim, C. H.; Kim, D. J. and Park, J. H. (2011). Flagellin promotes the proliferation of gastric cancer cells via the Toll-like receptor 5. Int. J. Mol. Med. 28:115-119.
- Sortica, V. A.; Cunha, M. G.; Ohnishi, M. D.; Souza, J. M.; Ribeiro-Dos-Santos, A. K.; Santos, N. P.; Callegari-Jacques, S. M.; Santos, S. E.; and Hutz, M. H. (2012). IL1B, IL4R, IL12RB1 and

TNF gene polymorphisms are associated with Plasmodium vivax malaria in Brazil. Malaria journal. 11, 409.

- Stanaway, J. D.; Reiner, R. C. and Blacker, B. F. (2019). The global burden of typhoid and paratyphoid fevers: a systematic analysis for the global burden of disease study 2017. Lancet. Infect. Dis. :19:369-81.
- Sultana, S.; Al Maruf, M. and Sultana, R. (2016). Laboratory diagnosis of enteric fever: a review update. Bangladesh Journal of Infectious Diseases. 3:43-51.
- Sur, D.; Ali, M.; Von-Seidlein, L.; Manna, B. and Deen, J. (2007). Comparisons of predictors for typhoid and paratyphoid fever in Kolkata, India. BMC Public Health. 7: 289.
- Sutterwala, F. S.; Ogura, Y.; Szczepanik, M.; Lara-Tejero, M.; Lichtenberger, G. S.; Grant, E. P.; Bertin, J.; Coyle, A. J.; Galán, J. E.; Askenase, P. W. and Flavell, R. A. (2006). Critical role for NALP3/CIAS1/Cryopyrin in innate and adaptive immunity through its regulation of caspase-1. Immunity. 24(3), 317-327.
- Suzuki, T.; Franchi, L.; Toma, C.; Ashida, H.; Ogawa, M.; Yoshikawa, Y.; Mimuro; H.; Inohara. N.; Sasakawa, C. and Nuñez, G. (2007). Differential regulation of caspase-1 activation, pyroptosis, and autophagy via Ipaf and ASC in Shigella-infected macrophages. PLoS pathogens. 3(8), e111.
- Sztein, M. B. (2007). Cell-mediated immunity and antibody responses elicited by attenuated *Salmonella* enterica serovar typhi strains used as live oral vaccines in humans. Clin Infect Dis. 45 (Suppl 1): S15-S19.

- Tiwaskar, M. (2019). Cefixime-ofloxacin combination in the management of uncomplicated typhoid fever in the Indian community setting. J. Assoc. Physicians India. 67(3):75-80.
- Thompson, L.; Dunstan, S. and Dolecek, C. (2009). Transcriptional response in the peripheral blood of patients infected with *Salmonella* enterica serovar typhi. Proc. Natl. Acad. Sci. U.S.A. 106:22433–22438.
- Townsend, S.; Kramer, N. and Edwards, R. (2001). *Salmonella* enterica serovar typhi possesses a unique repertoire of fimbrial gene sequences. Infect. Immun. 69:2894-2901.
- Tran, H.; Bjune, G.; Nguyen, B.; Rottingen, J. and Grais, R. (2005). Risk factors associated with typhoid fever in Son La province, northern Vietnam. Trans. R. Soc. Trop. Med. Hyg. 99: 819-826.
- Ulaganathan, V. K.; Sperl, B.; Rapp, U. R. and Ullrich, A. (2015). Germline variant FGFR4 p. G388R exposes a membrane-proximal STAT3 binding site. Nature. 528(7583), 570-574.
- Vande-Vosse, E.; Ali, S.; De-Visser, A.; Surjadi, C.; Widjaja, S.; Vollaard, A. and Van-Dissel, J. (2005). Susceptibility to typhoid fever is associated with a polymorphism in the cystic fibrosis trans-membrane conductance regulator (CFTR). Hum. Genet. 118(1):138-40.
- Vollaard, A.; Ali, S.; Van-Asten, H.; Widjaja, S. and Visser; L. (2004). Risk factors for typhoid and paratyphoid fever in Jakarta, Indonesia. JAMA. 291: 2607-2615.
- Voss-Rech, D.; Potter, L.; Vaz, C.; Pereira, D.; Sangioni, L.; Vargas ,Á. and de Avila, B. (2017). Antimicrobial resistance in

non-typhoidal *Salmonella* isolated from human and poultry-related samples in Brazil. 20-year meta-analysis. Foodborne Pathog. Dis. 14(2):116–124.

- Wallace, H. A.; Andrew, J. and Thomas, H. (2011). Bacteriological Analytical Manual. Chapter 5. USDA/FSIS Microbiological Laboratory Guidebook, 3rd Edition. U.S. Food and Drug Administration.
- Wang, L. X.; Li, X. J.; Fang, L. Q.; Wang, D. C.; Cao, W. C. and Kan, B. (2012). Association between the incidence of typhoid and paratyphoid fever and meteorological variables in Guizhou, China. Chinese medical journal., 125(3), 455-460.
- Wang, N.; Yang, J.; Lu, J.; Qiao, Q.; Bao, G.; Wu, T. and He, X. (2014). IL-17 gene polymorphism is associated with susceptibility to gastric cancer. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 35(10), 10025-10030.
- Weaver, C. T.; Hatton, R. D. Mangan, P. R, Harrington, L. E. (2007). IL-17 family cytokines and the expanding diversity of effector T cell lineages. Annu. Rev. Immunol. 25:821-52.
- Wen, S.; Best, E. and Nourse, C. (2017). Non-typhoidal *Salmonella* infections in children: Review of literature and recommendations for management. J. Paediatr. Child. Health. 53(10):936-941.
- Wijedoru, L.; Mallett, S. and Parry, C. (2017). Rapid diagnostic tests for typhoid and paratyphoid (Enteric) fever. Meta-analysis of RDTs for typhoid and paratyphoid fever. Cochrane Database Syst. Rev. CD008892.

- Winstanley, C. and Hart, A. (2001). Type III secretion systems and pathogenicity islands. J . Med. Microbial. 50:116-126.
- Whitaker, J.; Franco-Pardes, C.; del-Rio, C. and Edupuganti, C. (2009). Rethinking typhoid fever vaccines: implications for travelers and people living in highly endemic areas. J. Travel. Med. 16: 46-52.
- World Health Organization. (2006). Typhoid vaccine (Initiative for Vaccine Research). Geneva.
- World Health Organization. (2018). Typhoid vaccines: WHO position paper, Recommendations. Vaccine. 07;37(2):214-216.
- Zorgani, A. and Ziglam, H. (2014). Typhoid fever: misuse of Widal test in Libya. Journal of infection in developing countries. 8(6), 680-687.
- Wu, Q.; Martin, R.; Rino, J.; Breed, R.; Torres, R. and Chu, H. (2007). IL-23-dependent IL-17 production is essential in neutrophil recruitment and activity in mouse lung defense against respiratory Mycoplasma pneumoniae infection. Microbes Infect. 9: 78-86.
- Wu, X.; Zeng, Z.; Chen, B.; Yu, J.; Xue, L.; Hao, Y. and Hu, P. (2010). Association between polymorphisms in interleukin-17A and interleukin-17F genes and risks of gastric cancer. International Journal of Cancer. 127(1), 86-92.
- Yang, J.; Zhao, Y.; Shi, J. and Shao, F. (2013). Human NAIP and mouse NAIP1 recognize bacterial type III secretion needle protein for inflammasome activation. Proceedings of the National Academy of Sciences of the United States of America, 110(35). 14408–14413.

- Ye, P.; Garvey, P.; Zhang, P.; Nelson, S. Bagby, G. and Summer, W. (2001). Interleukin-17 and lung host defense against Klebsiella pneumoniae infection. Am. J. Respir. Cell Mol. Biol. 25:335-40.
- Yen, D.; Cheung, J; Scheerens, H.; Poulet, F.; McClanahan, T.; McKenzie, B.; Kleinschek, M.; Owyang, A.; Mattson, J.; Blumenschein, W. (2006). IL-23 is essential for T cell-mediated colitis and promotes inflammation via IL-17 and IL-6. J. Clin. Invest. 116: 1310-1316.
- Yue, M. and Schifferli, D. (2014). Review Allelic variation in *Salmonella*: an underappreciated driver of adaptation and virulence. Front Microbiol. 4:419.
- Zahraa Hameed Oda AL-Quraishi. (2018). Bacteriological and molecular study on *Salmonella* typhi isolated from gallbladder chronic infections in Al-Najaf city. A thesis submitted to the council of college of Science/University of Kufa.
- Zhao, Y.; Yang, J.; Shi, J.; Gong, Y. N.; Lu, Q.; Xu, H.; Liu, L. and Shao, F. (2011). The NLRC4 inflammasome receptors for bacterial flagellin and type III secretion apparatus. Nature. 477(7366), 596-600.
- Zhao, Y.; Zhang, X.; Song, Z.; Qi, D.; Deng, X.; Xia, J. and et al. (2016). Rapamycin attenuates acute lung injury induced by LPS through inhibition of Th17 cell proliferation in mice. Sci. Rep. 6:20156.
- Zhang, X.; Yu, P.; Wang, Y.; Jiang, W.; Shen, F.; Wang, Y. and Zhang, H. (2013). Genetic polymorphisms of interleukin 17A and interleukin 17F and their association with inflammatory bowel

disease in a Chinese Han population. Inflammation Research. 62(8), 743-750.

- Zhang, G.; Zhou, B.; Li, S.; Yue, J.; Yang, H.; Wen, Y.; Zhan, S.; Wang, W.; Liao, M.; Zhang, M.; Zeng, G.; Feng, C. G.; Sassetti, C. M. and Chen, X. (2014). Allele-specific induction of IL-1β expression by C/EBPβ and PU.1 contributes to increased tuberculosis susceptibility. PLoS. Pathogens. 10(10), e1004426.
- Zheng, Y.; Valdez, P.; Danilenko, D.; Hu, Y.; Sa, S. and Gong, Q. (2008). Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. Nat. Med. 14:282-9.
- Zhou, L. and Pollard, A. (2010). A fast and highly sensitive blood culture PCR method for clinical detection of *Salmonella* enterica serovar typhi. Ann. Clin. Microbiol. Antimicrob. 9:14.

Conclusions and Recommendations

Conclusions

1-When the Vitek-2 compact system found numerous antibiotic resistance in *Salmonella typhi* isolates, it was necessary to enhance the degree of awareness about the use of antibiotics.

2-Interleukins IL-17A and IL-1β levels in serum can be employed as biomarkers to identify the typhoid fever infection.

3-The findings suggested that bearers of rs1143629 for IL-1β had a nearly doubled probability of contracting typhoid fever.

Recommendations

1-More research is needed to assess the levels for another interleukins of patients with typhoid fever.

2-Examining the polymorphism in IL-17A at different locations in the gene.

3-Determining the levels of IL-17A and IL-1β receptors in patients with typhoid fever.

4-Research can be conducted into additional cytokine genes polymorphism associated with typhoid fever, such as IL-8 and IL-4.