University of Kerbala
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Gene Expression and Level Determination of Some Immunological Factors in Patients with Community - Acquired Pneumonia

A Dissertation

Submitted to the Council of College of Science University of Kerbala in Partial Fulfillment of the Requirements for the Degree of Doctorate of Philosophy in Biology

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

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Dedication

To my father's who gave me passion, tenderness and overflowed me with care and love , and to the one who carries the true meaning of love, my dear mother.

To my lovely wife Dr. **Batool Nadum AL-Habeeb** for constantly being on my side, and for all her support, care and love, to my beautiful son and daughter (**Ali & Zumurrud**).

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Summary

Pneumonia is considered a disease that affects the lower respiratory system and occurs as a result of lung infection with bacteria, viruses, parasites, and fungi. It causes serious diseases and complications ,and one of these complications is sepsis, which is a life-threatening organ dysfunction caused by a desregulated host response to infection. It is considered a major cause of morbidity and mortality worldwide, as it affects about 49 million people and causes the death of about 11 million people around the world annually, and as a result of the increase in the number of cases of sepsis as a result of community-acquired pneumonia. It is necessary to find rapid diagnostic methods such as Complete Blood Count(C.B.C.), some of Immunological Factors , and some of Immune Related - Genes for reducing morbidity and mortality.

In this study (120) participants were collected at Imam Al-Sadiq Hospital in Babylon Governorate and Imam Al-Hussein Medical City in Kerbala Governorate from December 2022 to June 2023 and divided into three groups (60) patients with community-acquired pneumonia, (20) patients with sepsis due to community-acquired pneumonia and (40) people as a control groups. They were diagnosed by specialized doctors based on some non-culture methods such as clinical methods (signs , symptoms) and tomographic methods(X-ray or/and Computed Tomography scan).

Average of age groups of participants were (54.9, 72.5 and 51.4 years) for three participants groups (community-acquired pneumonia, sepsis due to community-acquired pneumonia and healthy groups) respectively, and the age range was between 23 to 95 years. Nonetheless, The participants in this study were 30 male and 30 female for the community-acquired pneumonia group, with 7 male and 13 female in the sepsis due to the community-acquired pneumonia group, while there were 18 male and 22 female for the healthy group. The results of the study indicate that the incidence increased

significantly with increasing age, while Sex did not showed any significant in the three groups.

There was no growth in the blood cultures of patients with sepsis due to CAP due to number of samples was low, previous treatment with antibiotics, not used more one bottle at different times, and anaerobic cultivation methods were not used. In contrast the results of sputum culture showed that the bacterial isolates that causes of the infection were belong to the Gram-positive bacteria with 45 isolates and percentage (56.25%) and It was sensitive to Meropenem, Rifampin , and Cefipime. *Streptococcus pneumonia* was the most common of isolated of Gram Posative bacteria. and the number of Gram-negative bacteria was 35 isolates with percnteage(43.75%) and It was sensitive to Cefipim, Amikacin and Meropenem and the results showed that *Hemophilus influenzae* was the most common type Gram negatine bacteria.

The results of the Procalcitonin showed a highly significant difference (*P*. Value $\leq$ 0.000) between the patients and the control groups. The CBC results between the patients and the control groups revealed significant differences (*P*. Value $\leq$ 0.000) in White Blood Cells, Granulocytes , Granulocytes %, and the ratio of Neutrophils to Lymphocytes , while plateletes lymphocytes ratio between the patients and control groups were highly significant difference at *P*. Value $\leq$ 0.007.

The results of the concentrations of Toll Like Receptor 2(TLR2), Toll Like Receptor 4(TLR4), Chemokine(C-X-C motif) ligand 3(CXCL3) and Chemokine(C-C motif) ligand 7(CCL7) in the serum were determined by the ELIZA test. The results showed a highly significant difference in the concentration of TLR2 and CXCL3 between the patient group and the control group at a value of (*P*. Value $\leq$ 0.000), while the results did not show a significant difference in the concentration of TLR4 and CCL7 at a value of *P*. Value $\leq$ (0.585 and 0.673) respectively.

The results of gene expression levels (Fold Chang) of immune-related genes (*TLR2F*,*TLR4F*, Cluster Differentiation 81 (*CD81*F), and Human Leukocyte Antigen (*HLA - DMA*F) showed there was a significant difference in the level of gene expression for the genes (*TLR2F*, *TLR4F*, and *CD81*F) between the patient group and the control group at *P*. Value $\leq$ (0.000), while the results in found the level of gene expression in the group of CAP patients was more than sepsis due to CAP, while the results level of gene expression for the *HLA-DMA*F gene did not appear significantly different (*P*. Value $\leq$ 0.056).

Finally, early detection of causative agents is one of the most essential aspects since , it allows for rapid treatment and reduces mortality rates that may evolve during the time of infection. Even though blood culture represents the gold standard and most used diagnostic method for detecting sepsis , this method is insufficiently sensitive. There are more specific medical laboratory criteria that can be used for early diagnosis of sepsis, especially when the blood culture fails to detect the etiologic agent of sepsis despite the presence of clinical symptoms.

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List of Abbreviation

Abbreviation	Definition
ALRs	**A**im 2 **L**ike **R**eceptors
AP	**A**spiration **P**neumonia
APCs	**A**ntigen-**P**resenting **C**ells
ARDS	**A**cute **R**espiratory **D**istress **S**yndrome
AST	**A**ntibiotic **S**usceptibility **T**est
AUC	**A**rea **U**nder **C**urve
BHI	**B**rain **H**eart **I**nfusion
Bp	**B**ase **p**air
BPM	**B**eat **P**er **M**inute
CALC-1	**C**alcitonin 1 **g**ene
CAP	**C**ommunity -**A**cquired **P**neumonia
CCL7	**C**hemokine CC motif **l**igand 7
CCP-1	**C**alcitonin **C**arboxyl-terminus **P**eptide **1**
CD81	**C**luster **D**ifferentiation **81**
cDNA	**c**omplementary **DNA**
CFU	**C**olony -**F**orming **U**nit
CI	**C**onfidence **I**nterval
CINC-2 alpha	**C**ytokine-**I**nduced **N**eutrophil **C**hemoattractant **2 alpha**
CLRs	**C**-type **L**ectin **R**eceptors
CT	**C**omputerized **T**omography
Ct values	**T**hreshold **C**ycles **values**
CXCL3	**C**hemokine **CXC l**igand motif **3**

Abbreviation	Definition
CXCRs	**C**-**X**-**C** Motif **C**hemokine **R**eceptors
D.C	**D**entric **C**ell
D.M	**D**iabetic **M**ellitus
DAMPs	**D**anger-**A**ssociated **M**olecular **P**atterns
EDTA	**E**thylene**D**iamine **T**etracetic **A**cid
ELISA	**E**nzyme-**L**inked **I**mmunosorbent **A**ssay
FC	**F**old **C**hange
FDA	**F**ood and **D**rug **A**dministration
FETIS	**F**orced **E**xpiratory **T**echnique combined with **I**nduced **S**putum
GRO γ	**G**rowth-**R**elated **O**ncogene **K**ama
GRO3	**G**rowth-**R**elated **O**ncogene 3
H.D	**H**eart **D**isease
HAP	**H**ospital **A**cquired **P**neumonia
HCAP	**H**ealth**c**are –**A**ssociated **P**neumonia
HIV	**H**uman **I**mmunodeficiency **V**irus
HKG	**H**ouse **K**eeping **G**ene
HLA-DMA	**H**uman **L**eukocytes **A**ntigen class II α Para-chain homolog **DMA**
HTN	**H**yper**te**n**s**ion
ICU	**I**ntensive **C**are **U**nit
IFNs	**I**nter**f**ero**n**s
IIP	**I**diopathic Pulmonary **P**neumonia
ITU	**I**ntensive **T**herapy **U**nit
LPS	**L**ipo**P**oly**S**accharide
LRR	**L**eucine-**R**ich **R**epeats
LRTIs	**L**ower **R**espiratory **T**ract **I**nfections
LTA	**L**ipo**T**eichoic **A**cid
MAMPs	**M**icrobe-**A**ssociated **M**olecular **P**atterns
MCP-3	**M**onocyte **C**hemotactic **P**rotein **3**
MD-2	**M**yeloid **D**ifferentiation **P**rotein-**2**
NFkB	**N**uclear **F**actor **K**appa-**B**
NHB ID	*Neisseria* - *Haemophilus* **Id**entification
NKC	**N**atural **K**iller **C**ell
NLRs	**N**ucleotide-binding oligomerization domain-**L**ike **R**eceptors
No. Samp	**N**umber **S**ample
OD	**O**ptical **D**ensity
OR	**Od**ds Ratio
P.Value	**P**robability **v**alue
PAMPs	**P**athogen **A**ssociated **M**olecular **P**atterns

Abbreviation	Definition
RT qRT-PCR	**Q**uantitative **R**eal-**T**ime **R**everse **T**ranscriptase **P**olymer **C**hain **R**eaction
PCT	**P**ro**c**alci**t**onin
PGN	**P**eptido**G**lyca**n**
Pm	**P**ico**m**ole
PMNL	**p**oly**m**orpho**n**uclear **l**eukocytes
PRRs	**P**attern **R**ecognition **R**eceptor**s**
RLRs	**R**etinoic acid-inducible gene-**L**ike **R**eceptors
RNS	**R**eactive **N**itrogen **S**pecies
ROC	**R**eceiver **O**perating **C**haracteristic
ROS	**R**eactive **O**xygen **S**pecies
RPM	**R**evolution **P**er **M**inute
RR	**R**espiratory **R**ate
SCAP	**S**evere **C**ommunity **A**cquired **P**neumonia
SIRS	**S**ystemic **I**nflammatory **R**eaction **S**yndrome
SOB	**S**hortness **O**f **B**reath
SOFA	**S**equential (Sepsis-related) **O**rgan **F**ailure **A**ssessment
SPSS	**S**tatistical **P**ackage for the **S**ciences of **S**ociety
TIR	**T**oll-**I**nterleukin1 (IL-1) **R**eceptor
TLR2,4 con.	**T**oll **L**ike **R**eceptor **2, 4 con**centration
TLR2,4 For	**T**oll **L**ike **R**eceptor **2, 4 For**ward
TLR2,4 Rev	**T**oll **L**ike **R**eceptor **2, 4 Rev**erse
TLR2,4FC	**T**oll **L**ike **R**eceptor **2, 4 F**old **C**hange
TLRs	**T**oll **L**ike **R**eceptors
TNF-a	**T**umors **N**ecrosis **F**actor **α**
TS	Tracheal **S**uction
UPT	**U**p-converting **P**hosphor **T**echnology
VAP	**V**entilations **A**cquired **P**neumonia
WHO	**W**orld **H**ealth **O**rganization
$\chi 2$	**C**hi-**squared**

Chapter

One

Introduction

1.1 Introduction

The lower respiratory tract infections (LRTIs), including pneumonia, are serious health conditions (Cillóniz *et al* .,2020).It is an acute inflammation resulting from infections within the lung tissues.These infections fill the small air sacs inside the lungs with fluid and pus, preventing them from getting the oxygen-rich air that breathe.It kills more than two million people worldwide, most of them are children under five years of age and people who are over 70 years of age (Masud *et al*., 2021) and are the fourth most common cause of mortality globally (Kelli *et al*., 2024)**.**

Community-acquired pneumonia (CAP) is an acute infection of the lungs tissues in the patients who did not contract it from a healthcare system or within the first 48 hours after being hospitalized. Risk factors for CAP include old age, alcohol consumption, smoking, previous history of pneumonia, viral respiratory infections, diabetes, chronic obstructive pulmonary disease, and immunosuppression (Alshammari *et al*., 2023).

The Gram-positive and Gram - negative bacterial pathogen causes CAP such as *Streptococcus pneumoniae, Staphylococcus aureus, Haemophilis influenzae, klebsilla pneumoniae, Moraxella catarrhalis, Escherichia coli , Pseudomonas aeruginosa* and *Acinobacter baumannli*. S. *pneumonia*e is a major global health threat that kills over one million people worldwide (Parveen & Subramanian, 2022).

The effects of pneumonia range from moderate infections to lethal organ failure caused by sepsis, depending on the severity of the condition, the patient's age and the immune system (Kelli *et al* .,2024).

Sepsis is a life-threatening organ dysfunction caused by a deregulated host response to infection (Hammond *et al*., 2024).World Health Organization (WHO) reported sepsis as a global health crisis. A study estimated that there are 48.9 million global incidences of sepsis and 11 million sepsis deaths

across 195 countries and territories. In severe conditions, sepsis will lead to multiple organ dysfunction and death (Ibarz *et al.,* 2024).

There are tests used in diagnosis the patients which included the blood, sputum culture, Complete Blood Count (CBC), Procalcitonin (PCT) (Nasimfar *et al.,* 2018).Cytokines (Toll-Like Receptor2 (TLR2), Toll-Like Receptor4(TLR4)(Behairy *et al.,* 2022) .Chemokine CXC Ligand 3 (CXCL3), Chemokine CC Ligand 7 (CCL7) and some of the immune-related genes such as Toll-Like Receptor2 (*TLR2*), Toll-Like Receptor4 (*TLR4*), Cluster Differenation 81(*CD81*) and Human Leukocytes Antigen DMA (*HLA- DMA*) (Li *et al.,* 2021).

Procalcitonin (PCT) is a biomarker that has shown promise in identifying bacterial etiology in acute infections, including Lower Respiratory Tract infections (Katz *et al* ., 2019). PCT is a known protein biomarker clinically used for the early stages of sepsis diagnosis and therapy guidance (Molinero-Fernández *et al* .,2020).

Toll-like receptors (TLR) play an eminent role in the regulation of immune responses to invading pathogens during sepsis. TLR genetic variants might influence individual susceptibility to developing sepsis.(Behairy *et al.,* 2022). TLR2 and TLR4 are the most critical Pattern Recognition Receptors (PRRs) during bacterial infection and function to promote inflammatory responses upon invasion of bacteria (Xu *et al*., 2024).

CXCL3 is a neutrophil-activating chemokine that belongs to the Growth Related Oncogene (GRO) subfamily of CXC chemokines, which arose as a result of gene duplication events during chemokine evolution. also known as CINC-2 alpha (Cytokine-induced neutrophil Chemoattractant), exerts its functions through several signaling pathways by activating the CXCR2 receptor. It is highly expressed during the number of tumorous and inflammation (Gulati *et al.,* 2018).

Chemokine (C-C motif) ligand 7 (CCL7), known also as monocyte chemotactic protein 3 (MCP-3), CCL7 is expressed in many types of cells under physiological conditions, including in stromal cells, airway smooth muscle cells ,keratinocytes and in tumor cells under pathological conditions. CCL7 is a potent chemoattractant for a variety of leukocytes, including monocytes, eosinophil, basophils, dendritic cells, Natural Killer cells and activated T lymphocytes. (Liu *et al.*, 2018) **.**

Cluster differwnation *(CD81*) is an integral membrane protein of the tetraspanin family and forms complexes with a set of other cell surface membrane proteins and is involved in cell migration and B cell activation (Hosokawa *et al* .,2021). It play major roles in the biological and immunological processes of sepsis-induced Acute Respiratory Distress Syndrome (ARDS) (Chen *et al.*, 2022).

HLA-DM, encoded by *HLA-DMA* and *HLA-DMB*, is a heterodimeric molecule that is important for normal antigen presentation (YU *et al.*, 2023). and play an important role in the regulation and action of the immune system against invading pathogens (Spínola, 2016).

The Research Questions in current study which include:

1- The proinflammatory response not only is beneficial to the clearance of pathogenic agents but also increases the immune damage to the body itself, so immune markers such as TLR2, TLR4, CXCL3, and CCL7 are beneficial to the clearance of pathogenic agents or increases the immune damage to the body itself .

2- Can the TLR2, TLR4, CD81, and HLA-DMA genes identify and explore to susceptibility , severity and prognostic outcomes of CAP and Sepsis due to CAP .

1.2. The Aim of The Study

The aim of the current study was to find potential diagnostic modalities for immune-related genes and immunological Factors , which could provide diagnostic and prognostic value markers for Community-Acquired Pneumonia and sepsis due to this infection and this aim was achieved through the following objectives.

1- Isolation of causative bacteria of blood samples and identification Antibiotic Susceptibility Testing (AST) of patients sepsis with CAP only .

2- Isolation of causative bacteria in sputum samples and identification Antibiotic Susceptibility Testing (AST) of patients with CAP only and sepsis with CAP .

3-Detection of Complete Blood Count (C.B.C.) and Procalcitonin levels of study groups(patients with CAP, and patients sepsis with CAP as well as control groups).

4-Detection some of Immune Factors such as TLR2, TLR4, CXCL3, and CCL7 from blood serum samples by ELISA for study groups.

5- Determination some of Gene Expression Levels such as *TLR2, TLR4, CD81*, and *HLA-DMA* were detected using RT-q RT PCR of study groups.

Chapter

Two

Literature Review

2.0.Literatures Review

2.1.Definition of pneumonia

The word "pneumonia" takes its origin from the ancient Greek word "pneumon," which means "lung" so the word "pneumonia" becomes "lung disease." Medically pneumonia is an acute inflammation of one or both lungs Parenchyma that is more often, but not always, caused by infections. There are many causes of pneumonia include bacteria, viruses, fungi and parasites (Sattar *et al.,* 2021).

The presence of microorganisms in the alveolar space without an accompanying inflammatory response represents colonization and does not constitute pneumonia. A range of other types of infection may also affect the lung and classified according to their principle site of infection (Lim, 2022)**.**

Pneumonia resulted in nearly 2·5 million deaths worldwide in 2019, the fourth highest global cause of death. pneumonia is the single biggest infectious killer of adults and children - claiming the lives of 2.5 million, including Children under five years old and adults over 70 years make up 75 percent of pneumonia deaths. Most pneumonia deaths occur in low and middle-income countries (Liu *et al.,* 2023).

2.1.1. Pathogenesis of Pneumonia

The normal lung microbiome includes bacterial species that may be implicated in the development of pneumonia (Beck *et al.,* 2015).

In most cases, the predominant inflammatory cell involved reflects the inciting pathogen; The systemic cytokine response gives rise to set of the characteristic features of infection, such as fever, myalgia and a rise in C-reactive protein levels. The entry of microorganisms to the lung is most commonly via micro-aspiration. Haematogenous spread from other sites in the body and direct spread from a contiguous source are less common (Wunderink & Waterer, 2017).

There is a balance between pathogen-related factors (e.g., virulence and inoculum size) and host-related factors (e.g., sex, age, and comorbidities) result in the development and severity of pneumonia (Cillóniz et al., 2018).

Bacterial pneumonia is a result of this inflammatory response. These cytokines are essential for the immunity but, the excess can lead to sepsis and multiorgan failure. The body tries to balance the deleterious effects of cytokines by attenuation of several inflammatory mechanisms by IL-10. Microbial virulence factors and predisposing host conditions make a person more vulnerable to pneumonia. The pulmonary system and the airways are continuously exposed to particulate matter and environmental pathogens.The healthy airways normally contain some bacterial species and are not sterile. However, the pulmonary defense mechanisms such as cough reflex, mucociliary clearance system, immune response help maintain low levels of the microbiome. CAP occurs when there is a defect in normal host defense or a virulent pathogen overwhelms the immune response or a large infectious microbial inoculum. The invasion and propagation of these virulent strains of bacteria in the lung parenchyma following micro-aspiration cause the host immune response to kick in leading to a cascade of inflammatory response causing pneumonia.Alveolar macrophage is the predominant immune cell which responds to lower airway bacteria. However, a stronger immune response comes into play when an overwhelming virulent pathogen or a large inoculum causes these alveolar macrophages to recruit polymorphonuclear neutrophils (PMN) to phagocytosis and engulf these bacteria (Pahal *et al.*, 2018).

2.1.2.Types of Bacterial Pneumonia

A. CAP: The acute infection of lung tissue in a patient who has acquired it from the community or within 48 hours of the hospital admission.

B. HAP: The acute infection of lung tissue in a non-intubated patient that develops after 48 hours of hospitalization.

C. VAP: A type of nosocomial infection of lung tissue that usually develops 48 hours or longer after intubation for mechanical ventilation.

D. HCAP: The acute infection of lung tissue acquired from healthcare facilities such as nursing homes, dialysis centers, outpatient clinics, or a patient with a history of hospitalization within the past three months (Stamm *et al.,* 2023) .

2.1.2.1. Community-Acquired Pneumonia (CAP)

Community-acquired pneumonia remains the leading infectious disease cause of death worldwide, with considerable impact on the healthcare system; furthermore, despite several advances in diagnosis, management, and prevention of disease, there remain several unmet needs with regard to this infection. it is the leading cause of death among infectious diseases and an important health problem, having considerable implications for healthcare systems worldwide (Ferreira-Coimbra *et al.,* 2020).

Community-acquired pneumonia (CAP) is a major public health challenge worldwide, with 2.6 million deaths in 2019 (Carugati *et al*., 2020). it is the fourth leading cause of death worldwide, and its high mortality makes continual insight into the management of the condition worthwhile (Davis *et al.,* 2023).

2.1.2.1.1. Causes of Community-Acquired Pneumonia

Etiology of community-acquired pneumonia is an extensive set of agents that include bacteria, viruses, fungi and parasites. Bacteria have classically been categorized into two divisions on the basis of etiology.

A-Typical Pneumonia:can be cultured on standard media or seen on Gram stain and also refers to pneumonia caused *by S. pneumoniae, H. influenzae, S. aureus*, Group A streptococci, *Moraxella catarrhalis*, anaerobes and aerobic Gram-negative bacteria (Çalık *et al.,* 2018).

The most common causes of CAP is *S. pneumonia*e followed by *K. pneumoniae, H.influenzae,* and *P. aeruginosa* **(**Gadsby & Musher, 2022**)**.

B-Atypical Pneumonia: organisms can not be cultured on standard media or seen on Gram stain and it is mostly caused by *Legionella spp, M. pneumoniae, C. pneumoniae,* and *C. psittaci* (Jain *et al.,* 2023).

2.1.2.1.2. Evaluation

The approach to evaluate and diagnose pneumonia depends on the clinical status, laboratory tests and radiological evaluation.

A-Clinical Evaluation: It includes taking a careful patient history and performing a thorough physical examination to judge the clinical signs and symptoms(Lim, 2022).

B-Laboratory Evaluation: This includes lab values such as complete blood count with differentials, inflammatory biomarkers C-reactive protein, blood cultures, sputum culture or Gram staining and/or urine antigen testing, or polymerase chain reaction (Kang *et al* .,2009).

C-Radiological Evaluation: It includes a chest X-ray as an initial imaging test, and the finding of pulmonary infiltrates on plain film is considered a gold standard for diagnosis when the lab and clinical features are supportive (Franquet, 2018).

2.1.2.1.3.Risk factors of Community-Acquired Pneumonia

The Risk factors for CAP include old age, alcohol consumption, smoking, previous history of pneumonia, viral respiratory infections, diabetes, chronic obstructive pulmonary disease and immunosuppression. CAP is a significant contributor to morbidity and death on a global scale as shown in Table(2:1) . (Alshammari *et al.*, 2023);(Kaysin & Viera, 2016).

Table 2.1: Risk Factors and Pathogens in CAP.

Risk factor	Related pathogens
Alcoholism	Anaerobic oral flora, *K. pneumoniae, M. tuberculosis* and *S. pneumoniae*
Aspiration	Anaerobic oral flora
Bioterrorism	*B. anthracis* (anthrax), *Francisella tularensis* (tularemia), *Yersinia pestis* (plague).
Chronic obstructive pulmonary disease or smoking	*C,pneumoniae, H. influenzae, Legionella* species *Moraxella catarrhalis , P.aeruginosa*e or other Gram-negative rods.*S . pneumoniae*
Exposure to bat or bird droppings	*Histoplasma capsulatum*
Exposure to farm animals or parturient cats	*Coxiella burnetii* (Q fever)
HIV infection (early)	*H. influenzae, M. tuberculosis, S. pneumoniae*
HIV infection (late)	*Aspergillus* and *Cryptococcus species, H. capsulatum, H. influenzae, Nocardia species,* Non *tuberculous mycobacteria, Pneumocystis jiroveci*
Hotel or cruise ship travel in past two weeks	*Legionella* species
Influenza active in community	*H. influenzae, influenza* and other respiratory viruses, *S. pneumoniae S. aureus* (including MRSA)
Injection drug use	Anaerobes, *M. tuberculosis, S. aureus* (including MRSA), *S. pneumoniae*
Lung abscess	Anaerobic oral flora, *M. tuberculosis*, non *tuberculous mycobacteria, S. aureus*
Travel to or residence in Middle East	Middle East respiratory syndrome
Travel to or residence in Southeast Asia and East Asia	Avian influenza, severe acute respiratory syndrome
Travel to or residence in southeastern and south central states bordering the Mississippi and Ohio River basins	*Blastomyces dermatitidis*
Travel to or residence in southwestern United States	*Coccidioides* species, *Hantavirus* species

2.1.2.1.4.Complications of Community-Acquired Pneumonia.

The most common bacterial pneumonia complications are respiratory failure, sepsis, multiorgan failure, coagulopathy, and exacerbation of preexisting comorbidities. Other potential complications of bacterial pneumonia include: Lung fibrosis, Destruction of lung parenchyma , Necrotizing pneumonia, Cavitation, Empyema, Pulmonary abscess, Meningitis and Death (Sattar *et al.,* 2021).

2.2. Sepsis

Sepsis is life-threatening organ dysfunction due to a dysregulated host response to infection. It is considered a major cause of mortality and morbidity (Rudd *et al.,*2020).Sepsis affects approximately 49 million people each year, and an estimated 11 million people die of sepsis, accounting for 19.7% of all global deaths (Peng e*t al.,* 2023)**.**

Sepsis is a common systemic disease characterized by various physiological and pathological disorders. It can result from infection by various pathogens, such as bacteria, viruses and fungi (Li *et al* ., 2024).

The consequences of the sepsis syndrome are so severe that the World Health Assembly and the WHO made sepsis a global health priority in 2017.

Septic patients have a higher risk for secondary organ injury, especially those with critical illnesses, and therefore, early identification and appropriate management are crucial for improving outcomes(Chalkias, 2022). Septic patients may present with both hyperinflammatory and immunosuppressive phenotypes (Leijte *et al.*, 2020).

Worldwide, sepsis remains a major cause of mortality, and early stratification of these critically ill patients helps to decrease mortality and disability (Polilli *et al.*, 2021);(Fernando *et al.*, 2019).

2.2.1.Causes of Sepsis

As sepsis is a dysregulated host response in association with a confirmed or strongly suspected infection, it is important to also systematically consider noninfectious causes that can mimic sepsis.

A-Infectious Causes of Sepsis

While a number of infections can result in sepsis, the major causes include:

1. Bacteremia (the major sources of bacteremia are intravascular devices, pulmonary infections, intra-abdominal infections, endovascular infections, or urinary tract infections)
2. Vascular access or intravascular device associated infection
3. Lower respiratory tract infection (e.g., pneumonia or empyema)
4. Intra-abdominal infection (e.g., peritonitis, cholecystitis, diverticulitis/ abscess, pancreatic abscess, septic abortion or Clostridium difficile colitis)
5. Urinary tract infections (e.g., cystitis, pyelonephritis, renal abscess)
6. Endovascular infections (e.g., endocarditis or vascular graft infections)
7. Skin and soft-tissue infections (e.g., necrotizing fasciitis, soft-tissue abscess, or surgical site infection)(Wright, 2018).

B-Noninfectious Causes of Sepsis

1. Trauma, surgery, or burns
2. Myocardial infarction or acute coronary syndrome
3. Severe pancreatitis.
4. Thyroid storm or acute adrenal insufficiency
5. Acute leukemia or tumor lysis syndrome
6. Malignant hyperthermia (e.g., anesthetic-related halothane)
7. Malignant neuroleptic syndrome (e.g., haloperidol)
8. Pulmonary or deep venous thrombosis
9. Intracranial or subarachnoid hemorrhage (or any hematoma)
10. Solid-organ transplantation rejection (Wright, 2018).

2.2.2. Sepsis due to Community-Acquired Pneumonia

Community-Acquired Pneumonia is a common disease and a leading cause of death. CAP may cause local and systemic inflammation, leading to a dysregulated host response and eventually to sepsis, multi-organ dysfunction, septic shock and death. Therefore, early diagnosis and assessment of the risk of poor outcomes is mandatory. the scores developed for CAP predict the risk of death better than Sequential (Sepsis-related) Organ Failure Assessment (SOFA) or qSOFA and that early diagnosis can allow adequate management of patients with sepsis (Ceccato & Torres, 2018).

In Community-Acquired Pneumonia, invading pathogens trigger a host immune response essential for controlling and eliminating pathogens in the lung. However, dysregulation of the initial inflammatory response can lead to tissue damage and excessive systemic inflammation resulting in severe disease and ultimately unfavorable clinical outcomes lead to sepsis and death (Wittermans *et al.*, 2022).

2.3. Diagnostic Biomarkers

Biomarkers defined as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention (Watkins & Lemonovich, 2011).

An ideal diagnostic biomarker for infection should be decrease or absent when infection is absent and increase in the presence of a specific infection. It should, ideally, provide results sooner than conventional culture reports. Furthermore, an ideal biomarker should be of either high specificity or sensitivity and useful for characterizing severity and monitoring response to treatment even in the absence of clinical signs. Preferably, it should not be expensive, should be timely, and should help avoid excess antibiotic use (Karakioulaki & Stolz, 2019).

2.3.1.Biomarkers in CAP and Sepsis

Biomarkers in pneumonia may be the ones that indicate inflammation or may be released specifically after lung infection. The measured levels of biomarkers should be interpreted cautiously and always be correlated with clinical findings as many confounding factors should be taken into consideration for interpretation - Factors like age, antibiotic pretreatment, chronic liver disease, corticosteroids, kidney failure, and viral infection can critically affect some biomarker levels and thus their sensitivity and specificity regarding treatment failure and clinical stability. Hence, results should be interpreted in line with the clinical presentation, and they should never substitute clinical judgment (Karakioulaki & Stolz, 2019).

Biomarkers to diagnose sepsis may allow early intervention which, although primarily supportive, can reduce the risk of death the most commonly used biomarker to identify sepsis include markers of the hyper-inflammatory phase of sepsis, such as pro-inflammatory cytokines and chemokines ; proteins such as C-reactive protein and procalcitonin which are synthesized in response to infection and inflammation; and markers of neutrophil and monocyte activation. These biomarkers may help identify patients who are developing severe sepsis before organ dysfunction develops significantly , thus helping to reduce the mortality rate associated with severe sepsis (Faix, 2013).

Pneumonia and sepsis are diseases of utmost importance to diagnose and treat urgently, since they are responsible for a high worldwide death toll. Although an elaborate history and clinical examination are mainstays for an accurate diagnostic process, certain laboratory adjuncts can comprise invaluable aids in appropriate management. PCT is one of the extensively investigated sepsis biomarkers also employed in pneumonia in a wide range of studies,together with CRP and CBC. It appears to be a reliable diagnostic tool to expedite care of these patients with severe infections in the acute setting (Katz *et al.*, 2019);(Ozbay *et al.*, 2023).

Cytokines as a Biomarker in Sepsis A reliable biomarker for the diagnosis and prognosis of sepsis is critical. The ideal biomarker for sepsis should be easy to determine analytically, highly specific and very sensitive, and assays should be inexpensive and readily available. Using such biomarkers would not only provide early diagnostic accuracy and prognostic information on sepsis but also predict the responsiveness to treatment interventions (Chaudhry *et al.,* 2013).

2.3.1.1. Sputum Cultures During CAP and Sepsis

The sputum samples in lower respiratory tract infection (LRTI) by Gram stain and culture can determine the etiological agent of LRTI and enable targeted antibiotic treatment. American clinical guidelines recommend that laboratories only culture samples of acceptable quality(Miller *et al.,* 2018).

Gram stain is used to assess sputum quality, in general , quality is defined by the number of Squamous Epithelial Cells (SEC) indicating contamination by oropharyngeal microbiota and Polymorphonuclear Leukocytes (PMNL) indicating inflammation .In addition, some studies assess quality by calculating the ratio of PMNL/SEC Gram stain is reported to be highly specific for diagnosing *S. pneumoniae, H. influenzae* , and Gram-negative bacilli and can therefore contribute to clinical decisions before pathogens are verified by culture(Ogawa *et al.*,2020);(Del Rio-Pertuz *et al.,* 2019).

However, the reliability of sputum analysis decreases if a patient has been treated with antibiotics before admission, reducing the clinical usefulness of the results It is unknown and therefore important to investigate how sample type, different sputum quality criteria, and recent antibiotic treatment affect the detection of pathogenic bacteria in CA-LRTI (Cartuliares *et al.,* 2023).

Gram stain of sputum is an inexpensive, noninvasive, readily available test that can provide actionable information for pathogen-directed therapies and also to diagnose *S.pneumonia*e and *H. influenzae* infections in patients with CAP (Ogawa *et al*.,2020). If sample is collected before antimicrobial exposure and/or radiological evidence of pneumonia is present in the patient before sending the sample for culture. Besides these measures rapid transport ,processing and screening of samples(Gunasekaran *et al.,* 2019) .

2.3.1.2Blood Cultures During CAP and Sepsis

The use of blood cultures for the detection of pathogens, can provide information about type of microorganism and susceptibility towards antibiotic therapy. However, only a small part of the analyzed cultures results positive and around 40–90% of patients are negative blood culture, with no growing pathogens (Laukemann *et al*., 2015).Moreover, the long time to results limits initial treatment decision making and contamination leads to suboptimal specificity of the obtained results. In order to improve diagnostic work-up, additional tests are appropriate, which are able to guarantee an early and reliable diagnosis(Gregoriano *et al.,* 2020).

Blood culture is currently the reference standard for diagnosis, but conventional practices have long turnaround times while diagnosis needs to be faster to improve patient care(Dubourg *et al.,* 2018).

Blood cultures are not recommend in adults with CAP managed in the outpatient setting(strong recommendation,very low quality of evidence). Also blood cultures not routinely obtaining in adults with CAP managed in the hospital setting (conditional recommendation, very low quality of evidence).

The yield of blood cultures in most series of adults with non-severe CAP is low, and rarely results in an appropriate change in empiric therapy and blood specimens that include skin contaminants can generate false-positive test results. Growth of organisms such as coagulase-negative staphylococci , which are not recognized as CAP pathogens may lead to inappropriate antimicrobial use that increases the risk for adverse drug effects.

Routinely obtaining blood cultures may generate false positive results that lead to unnecessary antibiotic use and increased length of stay. In severe CAP, delay in covering less common pathogens can have serious consequences. Therefore, the potential benefit of blood cultures is much larger when results can be obtained within 24 to 48 hours. The rationale for the recommendation for blood cultures in the setting of risk factors for MRSA and *P. aeruginosa* is the same as for sputum culture (Metlay *et al.,* 2019).

2.3.1.3. Procalcitonin (PCT)

PCT is the protein (116 amino acid) long precursor of the hormone calcitonin produced primarily by the thyroid gland, Hepatic monocyte cells and macrophages (Yan *et al.,* 2017),(Ghatas & Elfaizy, 2023).

PCT a molecular weight of 14.5 kDa. It consists of three domains ; the amino terminus (57 amino acids), immature calcitonin (33 amino acids) and calcitonin carboxyl-terminus peptide 1 (CCP-1) also known as katacalcin (21 amino acids) Its production is governed by the calcitonin 1 gene (CALC-1) on chromosome 11. This characteristic makes PCT a more specific marker for bacterial infection. In the presence of bacterial infection, the CALC-1 gene is up-regulated (Karakioulaki & Stolz, 2019). It is distributed in multiple organs, such as the brain, heart, lungs, liver, kidney and in the blood vessels of these organs (Kiriyama & Nochi, 2023). and it is not converted to calcitonin but can serve as a useful marker of bacterial infections, especially sepsis syndrome and respiratory tract infections (Fugit *et al.,* 2023).It is used for the early stages of sepsis diagnosis and therapy guidance (Molinero-Fernández *et al.,*2020) **.** It is a cheap and readily available, are by far the most routinely used biomarkers for sepsis(Gregoriano *et al.,* 2020).

2.3.1.3.1. Role of Procalcitonin as Biomarker during CAP and Sepsis

PCT is an inflammatory biomarker that can be useful in distinguishing between bacterial and nonbacterial etiologies of pulmonary infection. It was reported to be more accurate than most other acute phase reactants and indicators as a predictor of pneumonia, bacteremia, sepsis, and poor outcomes. In addition, PCT use is beneficial to judge timing to stop antibiotic therapy in serious infections . PCT may be involved in systemic inflammatory reaction syndrome (SIRS), by affecting the permeability of blood vessels throughout the body. Under normal conditions, vascular permeability remains low. However, under conditions of induced inflammation, inflammatory mediators such as cytokines increase vascular permeability, causing leakage of plasma components and extravasation of leukocytes. However, excessive production of inflammatory mediators further increases vascular permeability and aggravates the pathogenesis (Kiriyama & Nochi, 2023).

PCT can help direct antibiotic decisions for the treatment of acute respiratory infections to minimize antibiotic prescription and orders to improve prognoses in this regard (Katz *et al.*, 2019).

The main indication for PCT measurement is to aid in the diagnosis of bacterial infection and as a marker to guide antibiotic therapy. antibiotics are discouraged if PCT is 0.25-0.5 μg/L (bacterial infection likely) or >0.5 μg /L (bacterial infection very likely).the use of PCT in these three clinical settings will be reviewed. Timely diagnosis and use of antibiotics is an effective measure for reducing morbidity and mortality, whilst minimising the risk of emergence of antibiotic resistance and adverse events(Samsudin& Vasikaran ,2017).The United States Food and Drug Administration (FDA) in 2017, approved the use of PCT as a diagnostic aid to guide the decisions around antibiotic therapy in acute LRTI. Although most of the data supporting the use of PCT for LRTI (Katz *et al.*, 2019).

PCT directed antibiotic treatment has been described to decrease not simply the intake of antibiotic but similarly decrease the antibiotics adverse effects and mortality in patients who had LRTI (Schuetz *et al.*,2013). It is the best biomarker regarding antibiotic stewardship(Gregoriano *et al.*, 2020).

Serum PCT are elevate by responding to bacterial sepsis and bacterial endotoxins and are produced in many organs in humans. The different PCT values induced by Gram-negative bacteria, Gram positive bacteria, and fungi are considered to be due to the fact that each of them stimulate different receptors. The main membrane component of Gram-negative bacteria is LPS and that of Gram-positive bacteria is peptidoglycan (PGN) and lipoteichoic acid (LTA). LPS mainly activates TLR4. In contrast, PGN and LTA mainly activate TLR2(Kiriyama & Nochi, 2023).

2.3.1.4.Role of C.B.C. as Immunological Factors **in CAP and Sepsis**

Complete blood counts are an easy to-perform laboratory test, inexpensive and routine examination technique that provides information about the composition of blood cells. Various types and ratios of blood cells (Karakioulaki & Stolz, 2019).CBC supply a health of information on individual health status. The appropriate interpretation is pivotal for the early detection of several clinical conditions, which should be further investigated by laboratory and clinical test .The CBC parameters can be divided into three categories:(i) White Blood Cells (WBC) that included neutrophil (Neutro.), Lymphocyte **(**Lympho.),Monocyte Mono (.) ,Basophil, Eosinophil, their ratio Neutrophil-to-ymphocyte ratio (NLR) and Monocyte-to-Lymphocyte ratio (MLR) (ii) Red Blood Cells (RBC), (iii) Platelets(PLT) and Platelet-to-Lymphocyte ratio (PLR).These parameters have been proposed as indicators of systemic inflammation and infection such as CAP and Sepsis (Agnello *et al.,* 2021).

2.3.1.4.1.White Blood Cells (W.B.C.)

White blood cells, are also known as leukocytes .They are a heterogeneous population including lymphocytes, monocytes, and granulocytes that include of neutrophils, eosinophils, and basophils. It can be expressed as a percentage or as an absolute value. The WBC absolute value has clinical significance and is more informative than the relative one (percentage) because it indicates the medullary response to inflammatory stimuli. The relative value is helpful for evaluating which WBC population is mainly involved in the inflammatory process, allowing an etiological diagnosis. Commonly, the increase of total WBC count is indicative of inflammation and infection. WBC count could be normal or even reduce in some cases of sepsis(Agnello *et al.,* 2021).

2.3.1.4.1.1. Neutrophils (Neutro.)

Neutrophils are the first line of defense of innate immune system. They represent the most prevalent leukocytes and the most abundant innate cell population in systemic circulation, making up about 40% to 70% of the total leukocyte count (Tian *et al .,* 2021). act as sentinels to eliminate invading pathogens. When infection occurs, neutrophils rapidly migrate to the site of infection and eliminate the invading pathogen by several mechanisms, including phagocytosis and oxidative bursts, neutrophils extracellular traps to execute microbial killing(Witter *et al*., 2016).

During sepsis, neutrophils undergo several functional alterations, including reduced migration, altered antimicrobial activity, and delayed apoptosis, contributing to immune dysfunction and persistent inflammation(Resende *et al.,* 2020),(Gao *et al.,* 2021) Altogether, neutrophil alterations contribute to the worsening of sepsis and the development of secondary complications. During infection, the neutrophil count increases considerably, and it is generally associated with the overall severity of the infection . However, in severe sepsis, the neutrophil apoptosis is delayed, limiting the usefulness of neutrophil count in some cases (Belok *et al.,* 2021).

2.3.1.4.2.Platelets(PLT.)

platelet count used an additional criterion to predict the outcome of hospitalized patients with CAP. Thrombocytopenia in patients with CAP is associated with more severe pneumonia, severe sepsis, septic shock, need for ICU admission, While, thrombocytosis in patients with CAP is associated with more respiratory complications as regard lung abscess, empyema and pleural effusion, and poor outcome (Ghoneim *et al*. , 2020).

During sepsis, multiple factors, including the direct interaction of the pathogen with DAMP receptors expressed on the platelet surface, coagulation system activation, inflammatory response, and endothelial tissue damage, induce the activation of platelets. Upon activation, platelets exert several functions. Activated platelets rapidly aggregate and express multiple receptors on their surface that further enhance their aggregation with nearby platelets and leukocytes or that directly bind to and sequester extracellular pathogens(Guo & Rondina,2019).

2.3.1.4.2.1. Neutroplil/Lymphocytes(NLR)and Platelets/Lymphocytes Ratio (PLR)

The use of combined detection of multiple indicators might potentially be the future development trend, improving early diagnosis and prognostic value of infectious diseases(Riley & Rupert, 2015).

Many studies have shown that NLR can comprehensively reflect inflammation and the immune status of the body. The increase in the two values means an increase in inflammation (Witter *et al.*, 2016). The NLR has emerged as a reliable sepsis biomarker(Agnello *et al.*, 2021). The NLR can be used to assess the severity of stress and systemic inflammation in critical patients. (Resende *et al*., 2020).

The PLR can predict the inflammatory response of patients with sepsis. The PLR can be used as an independent risk factor for the prognosis of patients with sepsis(Resende *et al*., 2020).

2.3.1.5.Cytokines

Cytokines are secreted signaling proteins that play main roles in the initiation, maintenance and resolution of immune responses. Although the unique ability of cytokines to control immune function has garnered clinical interest in the different diseases , the use of cytokine-based therapeutics has been limited. This is due, in part, to the ability of cytokines to act on many cell types and impact diverse biological functions, resulting in dose-limiting toxicity or lack of efficacy (Saxton *et al.,* 2023).

Cytokines are soluble extracellular proteins or glycoproteins that have been shown to be involved in autocrine, paracrine, and endocrine signaling as crucial intercellular immunomodulating regulators and mobilizers of cells. Upon a pathogen attack, cytokines produced at the site where pathogens have entered can drive inflammatory signals that modulate the ability of resident and newly arriving phagocytes to destroy the invading pathogens.They can regulate the antigen-presenting functions of dendritic cells and their migration to lymph nodes to initiate adaptive immune responses (Cao *et al.*,2023).

Cytokines, which include interleukins, interferons, chemokines, colony-stimulating and growth factors, are essential communication molecules involved in cellular cross-talk and signaling. Cytokines shape pro- or anti-inflammatory microenvironment and are involved in a broad number of physiological processes - cell attraction and differentiation, and pathological events - bacterial and viral infections, autoimmunity, metabolic disorders, and cancer (Dukhinova *et al.*,2021).

2.3.1.5.1.Role Cytokines as Immunological Factors in CAP and Sepsis

The innate immune system serves as the first line of defense against foreign pathogens via recognizing their pathogen associated molecular patterns (PAMPs) or microbe-associated molecular patterns (MAMPs). Also, innate immune cells recognize the damage or danger-associated molecular patterns

(DAMPs)generated during the pro-inflammatory conditions disturbing immune homeostasis(Schaefer, 2014).

The recognition of PAMPs or MAMPs and DAMPs involve several pattern recognition receptors (PRRs) , including Toll-like Receptors(TLRs) and multiple germ line encoded receptors [NOD-like receptors (NLRs), retinoic acid inducible gene I (RIG-I)-like receptors(RLRs),C-type lectin receptors(CLRs) and multiple intracellular DNA sensors expressed(cGAS-STING signaling pathway, Aim 2 like receptors(ALRs)](Vijay, 2018); (Atluri *et al.*, 2016).

This induces the pro-inflammatory immune response generating different cytokines, chemokines, interferons (IFNs), and other molecules, including reactive oxygen or nitrogen species (ROS or RNS) for clearing the infection to maintain the immune homeostasis. However, the innate immune response dysregulation during infection may increase its severity via increasing the pathogen load due to the inefficient pathogen clearance or by causing increased and irreversible organ damage in patients succumbed to sepsis.

Hence, a regulated innate immune response during both acute and chronic infections is essential for clearing the infection. The organ-specific innate immune response determines infection severity. For example, the potent innate immune response generation in the lungs during localized pulmonary infections (pneumonia) or its dysregulation as seen in the non-pulmonary sepsis plays a crucial role in the disease outcome(Kumar, 2020).

During the early phases of pneumonia, alveolar macrophages produce a variety of pro-inflammatory cytokines and chemokines whose role is to both attract and activate polymorphonuclear leukocytes necessary for local bacterial defense and clearance (Bordon *et al.*, 2013).

Modulations of cytokine levels remain one of the most important strategies in pneumonia treatment First, cytokines are required for proper antiviral responses (proinflammatory) and further tissue repair (anti-inflammatory). Second, dysregulated cytokine profiles are risk factors for pneumonia predisposition and severity. Improper cytokine signaling may arise from hereditary factors, chronic metabolic and immune disorders, and therapeutic interventions, and consideration of all the listed factors is essential for pneumonia prognosis and successful treatment (Dukhinova *et al.*, 2021).

The cytokine profile in patients with sepsis may be very useful in the diagnosis of disease severity, and prediction of mortality and better patient management. Cytokines in Elderly Patients with Sepsis undergoes defects in T- and B-cell function have been demonstrated in elderly patients. Thus, elderly patients have a considerable reduce in both cell-mediated immune function and decrease humoral immune function. Immune dysfunctions may contribute to increase susceptibility of elderly patients to sepsis. Cytokines in Sepsis Treatment were characterized by the excessive production of cytokines in the circulating blood, leading to a systematic inflammatory response. Therefore, inhibition of excessive cytokine production or removal of cytokines and other inflammatory mediators from the blood may suppress systemic inflammation during sepsis and improve patient outcomes. Sepsis triggers the production of a diverse array of cytokines that are pro-inflammatory and anti-inflammatory .The imbalance of pro- and anti-inflammatory cytokines produced during sepsis may play an major role in pathogenesis(Chaudhry *et al.*, 2013).

The immune response's status during sepsis results from interactions among multiple mechanisms, including cytokines, cell death, and the expression dynamics of cellular biomarkers(Liu *et al.*, 2022).

2.3.1.5.1.1. Chemokines

The chemokines (or chemotactic cytokines) are a large family of small, secreted proteins that signal through cell surface G protein-coupled heptahelical chemokine receptors. They are best known for their ability to stimulate the migration of cells (Hughes & Nibbs, 2018). They are produced by a wide range of leukocyte and non-leukocyte (inducer) cells in the lungs. Four chemokine classes are classified based on conserved cysteine residues (C, CC, CXC, CX3C),and receptors are classified based on their binding to chemokines. Most receptors interact with multiple chemokines, and most chemokines interact with more than one receptor (Russo *et al.*, 2023).

Chemokines are small molecules (8–12 kDa.) that are well known to be essential for leukocyte biology, including cell communication and activation during basal or inflammatory states, and orchestrating immune responses. In addition to immune cells, epithelial and endothelial cells, fibroblasts, and smooth muscle cells can also produce and respond to chemokines . They are crucial inflammatory mediators needed during an immune response to clear pathogens. However, their excessive release is the main cause of hyper-inflammation(Khalil *et al.*,2021).

Chemokines are pleiotropic factors involved in both homeostasis and disease. Indeed, the specific combination of cellular chemokine receptor repertoires determines the migration properties of these cells which respond to chemokine gradients; changes in receptor structure and signaling upon chemokine binding direct cells during various stages of leukocyte maturation and activation(Russo *et al.,* 2023).

Chemokines play a central role in the development and homeostasis of the immune system, and are involved in all protective or destructive immune and inflammatory responses. Classically viewed as inducers of directed chemotactic migration, it is now clear that chemokines can stimulate a variety of other types of directed and undirected migratory behavior. They lies in inflammation and the body’s immune response (Hughes & Nibbs, 2018).

They have a high concentration of peptides in circulation, and they suppress local inflammation by desensitization. the chemokine family is critical in the pathology of sepsis (Doganyigit *et al.*, 2022).

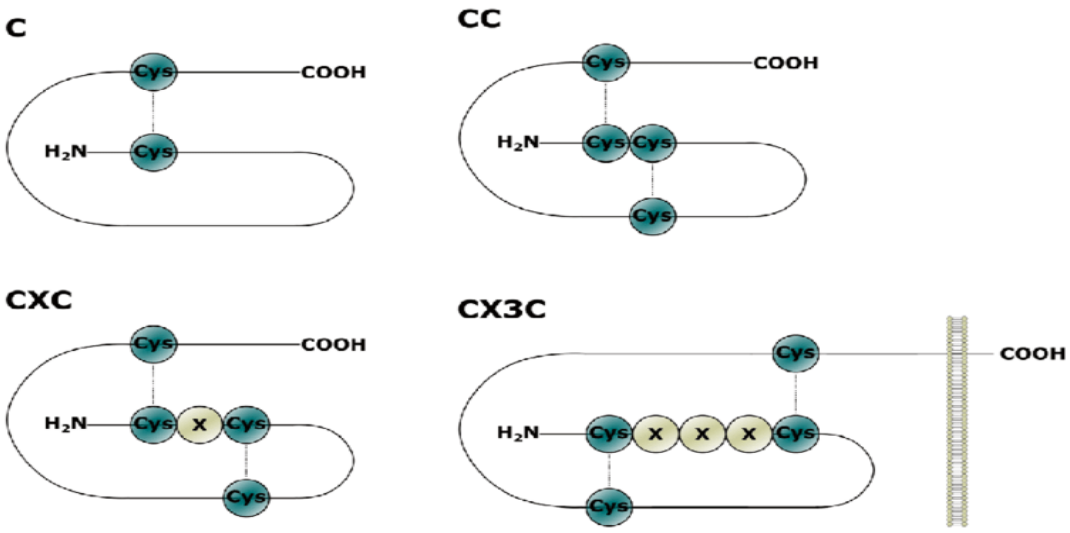


Figure 2.1: Structure of the Four Subtypes of Chemokine Ligand .

Homeostatic and maintenance chemokines are involved in adaptive immune responses. Homeostatic chemokines are expressed in specific tissues, whereas the production of inflammatory chemokines can be mediated by many cell types at multiple sites (Harvanová *et al.*, 2023).

2.3.1.5.1.1.1.Human Chemokine (C-X-C motif) Ligand 3 (CXCL3)

CXC's are soluble cholinesterases that interact with cognate cellular receptors, stimulating cells, and inducing directed chemotaxis. Chemotaxis, migration, and adhesion play essential roles in developing various tumors CXCL3 encoded by the human GRO gene and located within the chromosomal region, 4q13.3. CXCL3 is a granulocyte chemoattractant (GCP-2) and is an ELR + chemokine, similar to other ELR + chemokines that, with alternative splicing, can result in multiple transcript variants. CXCL3 plays a vital role in angiogenesis, tumorigenesis, and cell invasion(Qi *et al.*, 2020); (Xin *et al.*, 2018).

Many of studies have found CXCL3 to participate in the development of many inflammatory and auto-immune diseases and the progression and metastasis of many tumor types (Cui *et al.,* 2022). CXCL3 also called growth-related oncogene γ (GRO-γ) or macrophage inflammatory protein 2 (MIP-2) (Kusuyama *et al.*,2016).

CXCL3 is a neutrophil-activating chemokine that belongs to the growth-related oncogene subfamily of CXC chemokines, which exert their biological roles through the chemokine receptors, C-X-C motif chemokine receptors (CXCRs) 1 and 2 (Reyes *et al.,* 2021); (Gulati *et al.,* 2018).Macrophages, osteoblasts, airway epithelial cells, and dendritic cells secrete CXCL3. In addition, CXCL3 promotes blood vessel formation, tumor cell growth, cancer cell migration, cluster of differentiation (CD) 31 vascular cell infiltration, and smooth muscle cell migration (Qu *et al.*, 2023).

CXCL3 is a member of the CXC chemokine family and is subclassified as a Glu- Leu- Arg (ELR+) CXC chemokine (Zhang *et al.,* 2016). It is a chemotactic factor of neutrophils and assist the recruition and migration of diverse immune cells to different organs or tissues, eventually leading to inflammatory reactions(Zhou *et al.,* 2023). CXCL3 effects on immune and non-immune cells (Reyes *et al.,* 2021).

2.3.1.5.1.1.2.Human Chemokine(C-C motif)Ligand 7 (CCL7)

The chemokine CCL7 (MCP3) is known to promote the recruitment of many innate immune cell types including monocytes and neutrophils to sites of bacterial and viral infection and eosinophils and basophils to sites of allergic inflammation. CCL7 up-regulation has been associated with many inflammatory settings including infection, cardiovascular disease, and the tumor microenvironment.CCL7-blockade or CCL7-deficiency is often marked by decreased inflammation and poor pathogen control(Ford *et al.,* 2019).

CCL7 is expressed at low levels in endothelial cells, fibroblasts and mononuclear cells and up-regulated by various stimuli including viruses, type I or type II interferons (IFNs). CCL7 receptors including CCR1, CCR2, and CCR3 are mainly expressed on the surface of antigen-presenting cells (APCs) such as monocytes, macrophages and dendritic cells (Griffith *et al.,* 2014).

Chemokine (C-C motif) ligand 7 (CCL7), also known as monocyte chemotactic protein 3 (MCP-3), is a group of the CC subfamily that is expressed in many types of cells under physiological conditions, including in stromal cells, airway smooth muscle cells, and keratinocytes, and in tumor cells under pathological conditions. CCL7 is a potent chemoattractant for a variety of leukocytes, including monocytes, eosinophils, basophils, dendritic cells, Natural Killer cells and activated T lymphocytes. As a chemotactic factor, CCL7 recruits a leukocyte subtype to infected tissues to address pathologic invasion and fine-tune the immune response. However, abnormal increase of CCL7 exacerbates the deterioration of various disorders, like lesional psoriasis, acquired immunodeficiency syndrome ,acute neutrophilic lung inflammation and pulmonary fibrosis(Liu , *et al.,* 2018).

CCL7 is a member of the MCP subfamily and also includes CCL2 and CCL8.CCR1, CCR2 and CCR3 are widely acknowledged as the main functional receptors for CCL7 (Palomino & Marti, 2015). high concentrations of CCL7 are found in cultures of idiopathic pulmonary pneumonia (IIP) fibroblasts than in those of non-IIP fibroblasts. Although CCL7 has been demonstrated to bind CCR5 with high affinity, it cannot elicit a functional response. Hence, high levels of CCL7 may act as a natural antagonist of CCR5 by affecting its binding with other ligands (Liu , *et al. ,* 2023).

CCL7 is widely expressed in many cell types and can participate in anti-inflammatory responses through binding to its receptors to mediate the recruitment of immune cells. Abnormal CCL7 expression is associated with certain immune diseases (Brunner *et al.,* 2015).

2.3.1.6.Regulation Immune Related Genes(IRGs) During CAP and Sepsis

The study of IRGs may aid in stratifying risky sepsis patients and evaluating patients' immune (Peng *et al*., 2023).The gene expression profiles presented differences of CAP survivors and non survivors, mainly related to interferon-alpha response, apoptosis, sex hormones, oxidative stress, unfolded protein response, and angiogenesis pathways. These findings may expand our understanding of the immune response in CAP through identification of new candidate pathways and targets for potential intervention. In addition, the differentially expressed genes could potentially be useful as risk-stratification biomarkers that may facilitate healthcare utilization(Viasus *et al.,* 2023).

Sepsis is considered one of the fatal diseases leading to increased mortality. The studies have put an effort to reveal the early molecular markers associated with immunosuppression and severity of sepsis. It is necessary to maintain the homeostasis between the adaptive and innate immune cells for the normal functioning of the body. Dysregulated response of immune cells leads to various disease conditions including sepsis. Immune cell function is impaired in sepsis where genes related to T cell function is down-regulated leading to immunosuppression, and genes associated with neutrophil activation are up-regulated resulting in enhanced inflammatory response (Wang *et al.,* 2024).

2.3.1.6.1.Toll-Like Receptors (TLRs)

Toll-like receptors (TLRs) are a family of transmembrane receptors which play an important role in the host defense against microorganisms. TLRs are mainly expressed in human immune-related cells such as monocytes, neutrophils, macrophages, dendritic cells, T cells, B cells and NK cells. TLRs alert the immune system to infection by recognizing pathogen-associated molecular patterns derived from various microorganisms.Furthermore, functional TLRs are expressed not only in immune cells, but also in cancer cells, thus implicating a role of TLRs in tumor biology (Aref *et al*., 2020).

Toll-like receptors (TLR) play a main role in the regulation of immune responses to invading pathogens during sepsis. TLR genetic variants might influence individual susceptibility to developing sepsis(Behairy *et al.*,2022). to date, 11 functionally different TLRs have been identified in human and categorized into two groups such as transmembrane (TLR1, TLR2, TLR4, TLR5, TLR6 and TLR11) and intracellular (TLR3, TLR7, TLR8 and TLR9). The receptors possess an extracellular domain comprising leucine-rich repeats (LRR) and an intracellular Toll-interleukin1 (IL-1) receptor (TIR) domain. LRR domain is typically involved in the recognition of distinct PAMPs , while the TIR domain plays crucial role in transmitting the signal to elicit inflammatory responses (Mukherjee *et al.*,2019).

TLRs are essential pattern recognition receptors expressed in innate immunocytes such as macrophage and dendritic cells, which initiate innate immune response by recognizing PAMPs, participate in multiple immune reactions including phagocytosis, bacteria killing, antigen presentation, and cytokine production (Shu *et al.*, 2020).

TLRs are stimulate cytokine production through natural immunity, and allow the recognition of PAMPs . TLRs play an major role in inflammatory pathways in response to microbial agents and in the regulation of the natural immune response (Demirci *et al.*, 2020).

2.3.1.6.1.1.Toll-Like Receptor 2 (*TLR2*)

TLR2 gene (ENSG00000137462) is a protein-coding gene located on 4q31.3. It is composed of five exons with a length of 26,564 nucleotides. It is located on Chromosome 4 (Behairy *et al.*, 2022). TLR2 expression is not limited to cells related to inflammation and immune function. The expression of functional TLR2 is found in epithelial cells, while TLR2 is also expressed in many tumor cells and tissues (Meng *et al.*, 2020).

TLR2 has been shown to play a protective role during infection by triggering a strong pro-inflammatory response, which is considered as beneficial for bacterial clearance . However, the excessive inflammation caused by TLR2 can lead to tissue damage and even affect healing of damaged tissues .A better understanding of the mechanisms behind TLR2 regulation of immunity in infectious diseases could be a significant benefit for accelerating the discovery of TLR2-related vaccines or targeted therapeutic treatments (Hu & Spaink, 2022).

2.3.1.6.1.2.Toll-Like Receptor4 (*TLR4*)

TLR4 gene (ENSG00000136869) is a protein-coding gene located on 9q33.1. It is composed of four exons with a length of 20,333 nucleotides. It is located on Chromosome 9 (Behairy *et al.*, 2022). it is the first identified member of TLR family. that can recognize PAMPs and DAMPs. TLR4 expresses not only on immune cells but also on tumor cells (Li. *et al.*, 2017). TLR4 complexed with myeloid differentiation protein-2 (MD-2) recognizes the lipid A portion of the lipopolysaccharide (LPS) present in the Gram-negative cell wall is a well-described inducer of the innate immune response. The LPS structure comprises a lipid A composed of fatty acid (FA) chains linked to a disaccharide backbone, a core saccharide and the O-antigen (Francisco *et al.*, 2022).

TLR4 is a transmembrane protein characterized by an extracellular domain containing leucine-rich repeats (LRRs) where the MD-2 molecule is associated, and a cytoplasmic tail harbouring a conserved region known as Toll/IL-1 receptor (TIR) domain (Ke *et al.*, 2016).The extracellular domain is responsible for ligand binding, receptor dimerization and initiation of intracellular signalling, whereas the intracellular domain shares a significant sequence and structural homology with the interleukin-1 receptor (IL-1R) family (Ding & Liu, 2019).

2.3.1.6.2.Cluster Differentiation 81 (*CD81*)

CD81 is a cell surface transmembrane protein of the tetraspanin family, which critically regulates signal transduction and immune response (Ye , *et al.* ,2024). It is composed of four transmembrane, three intracellular, and two extracellular domains. It is widely expressed in many tissues and is highly conserved in mammals. One distinctive feature of tetraspanins is their ability to interact with other membrane proteins such as integrins,CD81 is forms complexes with a variety of other cell surface membrane proteins and involved in cell migration and B cell activation (Hosokawa *et al.*, 2021).

Tetraspanins constitute a large family of membrane glycoproteins with four transmembrane domains which are widely expressed in human cells. The tetraspanin family comprises 33 different members .These proteins have a role in the regulation of many biological processes such as cell-cell adhesion, fusion, signal transduction, proliferation and differentiation. They probably function in the form of complexes since they interact with each other and with different partners including transmembrane proteins such as adhesion molecules, receptors and intracellular signaling/cytoskeletal proteins, creating a network of interacting proteins called the tetraspanin web (Alvaro-Benito *et al.*, 2015).

2.3.1.6.3.Human Leukocyte Antigen DMA (*HLA - DMA*)

The human leukocyte antigen (HLA) complex has been implicated in key immune responses such as recognition of self-versus nonself , susceptibility to autoimmune disease, drug hypersensitivity, and tolerance to organ transplantation. The peptide-HLA complex serves as the ligand for the antigen-specific T cell receptor (TCR) on T cells. The main function of HLA molecules is to facilitate immune surveillance of the intracellular(class I)and extracellular(class II) environments by presenting antigens to T cells (Bery *et al.*, 2023).

HLA-DM, encoded by HLA-DMA and HLA-DMB, is a heterodimeric molecule that is important for normal antigen presentation. The HLA-DMA and HLA-DMB here immune-related genes may play a key role in the development of sepsis and help improve the immunomodulatory treatment of patients with sepsis (Li ,*et al.*, 2021).

HLA-DMA belongs to the HLA class II α Para-chain homolog. This class II molecule is composed of an α (DMA) chain and a β chain (DMB), both anchored in the membrane. Class II molecules are present on APCs that include (macrophages, dendritic cells, and B lymphocytes). The DMA gene contains five exons. Exon 1 encodes a precursor peptide, exon 2 and exon 3 encode two extracellular domains, and exon 4 encodes a transmembrane domain and a cytoplasmic tail (Baleeiro *et al.*,2022) , (He *et al.*, 2022).

The HLA system harbour a wide set of genes that plays an important role in the regulation and action of the immune system against invading pathogens, making them natural candidates for research on the genetic susceptibility for respiratory infections. and, consequently, several alleles of those loci have been implicated in the ability, more or less effective, to tackle and control invading pathogens in the respiratory system. the main advances in the role of HLA genes for the development and protection against of respiratory infectious disease (Spínola, 2016).

Chapter Three

Materials and Methods

3.1. Materials

3.1.1. Equipments and Instrumentations

All equipment and instruments were utilized in this study, along with their respective countries and other relevant details, were listed in Table (3.1).

Table 3.1: List of Equipment

No.	**Equipment**	**Supplier**	**Origin**
1.	Autoclave	Hirayama	Japan
2.	BacT/Alert	Biomerieux	France
3.	Centrifuge	Hettich	Germany
4.	Computer machine	Dell	USA
5.	ELISA test system	Biotech	USA
6.	Flasks and beakers	Hirschman	Germany
7.	Gas burner	GFL	Germany
8.	Hematology analyzer	Sysmex	Japan
9.	Hotgen Biotech.	Beijing	China
10.	Incubator	Memmert	Germany
11.	Light microscope	Olympus	Japan
12.	Micro centrifuge	Beckman	Germany
13.	Micro pipettes (1-20 µL, 1-200 µL, 100-1000 µL)	Fisher	USA
14.	Micro pipettes tip,10 µL ,100 µL,1000 µL)	Promega	USA
15.	Nano-drop	Scan Drop Biometra	Germany
16.	Oven	Memmert	Germany
17.	Platinum wire loop	Himedia	India
18.	Refrigerator	Concord	Lebanon
19.	Roller	Capp CRR300	Denmark
20.	Sensitive electron balance	A&D	Japan
21.	Shaker	Nanbei	China
22.	Thermocycler Roter gene Q Qiagen	Venlo	Netherland
23.	Vitek 2 system	Biomerieux	France
24.	Vortex	Digsystem	Taiwan
25.	water bath	GFL	Germany

3.1.2. Disposable Materials

The disposable materials used in this study showed in Table (3.2).

Table 3. 2 : List of the Disposable Materials.

S/N	Disposable materials	Company	Origin
1.	Biological safety cabinet	Labogene	Denmark
2.	Disposal Syringes	Meheco	China
3.	Eppendorf tube	Eppendorf	Germany
4.	Gel tubes	Arth Al-Rafidain for labs.	Iraq
5.	Glass slides and cover slides	Bio-zzeik	China
6.	Petri dish	Bio-zzeik	China
7.	Swabs with media	AFMA	Jordan
8.	Tube10ml with screw cap	AFCO	Jordan

3.1.3. Chemicals

The list of the chemicals used in this study as well as their respective company of production and country of origin is presented in Table (3.3).

Table 3.3: List of Chemicals

S/N	Chemical	Company	Origin
1.	Catalase reagent	Himedia	India
2.	Ethanol (70%,95%)	Merk	Germany
3.	Oxidase reagent	Himedia	India

3.1.4. Commercial Kits

Table (3.4) lists the commercial kits used in this study along with their manufacturer company and country of origin.

Table 3.4: List of Commercial Kits

S/N	Kit	Company Origin
1.	Gram stain kit: Crystal violet dye, Iodine, ethanol 95 %, and counter stain (safranin).	Merck (Germany)
2.	Haematology work solution: haemolytic agents, diluents, cleaning solutions, and concentrated cleaning solutions.	Sysmex (Japan)
3.	Human Toll-like Receptor 2, TLR2 ELISA	BT- LAB(China)
4.	Human Toll-like Receptor 4, TLR4 ELISA	
5.	Human C-X-C Motif Chemokine3,CXCL3 ELISA	
6.	Human C-C motif chemokine 7 ,CCL7 ELISA	
7.	Transzol up plus RNA kit	Trans Gen Biotech (China)
8.	TranScript Green One-Step qRT- Super Mix	
9.	Vitek 2 kit: reagent cards (cassette), which have 64 wells for automated biochemical tests and AST kit	Biomerieux(France)

3.1.5.Media

The cultures media used in this study, together with their respective suppliers and country of origin, are listed in Table (3.5).

Table 3.5: List of Culture Media

S/N		Suppliers	Origin
1.	Blood agar	Pronadsia	Spain
2.	Brain- heart infusion broth powder	Oxoid	England
3.	Brain- heart infusion broth vial	Himedia	India
4.	MacConkey agar	Oxoid	England

In accordance with the manufacturer's instructions already - to - use culture media was prepared. The pH was adjusted and sanitized by autoclaving at (15 lbs pressure, 121C°, for 15 minutes) before being distributed into sterile petri dishes or tubes and stored at 4 ºC until use (Paul, 2019).

3.1.6.Solution , Reagents , and Stains

3.1.6.1. McFarland Standard Solution

The 0.5 McFarland bacterial suspension was ready – made . Cards were automatically filled, sealed, and loaded into the VITEK 2 instrument (bioMérieux, France) for incubation and reading by using Gram Positive and Gram Negative cards.

3.1.6.2. Reagents

A-Catalase Reagent: (Hall, 2013).

This reagent composed of 3% hydrogen peroxide (H2O2)

B- Oxidase Reagent: (Prince, 2009).

It was prepared freshly of 0.1g of N,N,N,N-tetramethyl-Pphenylene diamine dihydrochloride was dissolved in 10ml distilled water, stored in dark bottle and used immediately **.**

3.1.6.3. Stains

Gram stain was made – ready .

3.1.7. Material Used in Procalcitonin Test

The materials required of PCT Kit showed in Table (3.6). but not provided : UPT Analyzer; Tubes, tips, etc.

Table 3.6:Components PCT Kit.

Name	Quantity	Mark
Test cassettes	40 set	packed in 40 foil pouches
Sample diluent	6mL×1bottle	
Parameter card	1pcs	
0.5mL centrifuge tube	40pcs	
User manual	1pcs	

3.1.8. Material Used in Gene Expression by RT. q RT-PCR

3.1.8. 1.The Materials Kit Used in RNA Extraction Included

1- TransZol Up**.**

2- Clean Buffer 9 (CB9)**.**

3- Wash Buffer 9 (CB9)**.**

4- RNAse-free water**.**

5- RNAse-free tube (1.5 ml)**.**

6- RNA spin Column with Collection Tubes

3.1.8. 2.The Materials Used in Master Mix Kit shown in Table (3.7).

Table 3.7: The Component of the Master Mix Kit

Component	AQ-01	AQ-02
TransScript® Green One-Step RT/RI Enzyme Super Mix	40 µl	160 µl
PerfectStart™ Green One-step qPCR SuperMix(2×)	1 ml	4×1 ml
Passive Reference Dye (50×)	40 µl	160 µl
RNase-free Water	1 ml	4×1ml

Transcript® Green One-Step qRT-PCR SuperMix

3.1.8. 3.The Materials Used in the Total Reaction Components

TransScript® Green One-Step qRT-PCR SuperMix is designed for one-step qRT-PCR . The Total Reaction Components (20 µl reaction system). See in Table (3.8).

Table 3.8: Reaction Components.

Component	**Volume**
RNA Template with concentration 50-74 ng/dl	2 µl
Forward Primer (10 µM)	0.5µl
Reverse primer (10 µM)	0.5 µl
2xPerfectStartTM Green One - step qPCR SuperMix(2x)	10 µl
Trans Script Green One - Step RT/RI Enzyme Mix	0.4 µl
RNase - free Water	6.6 µl
Total volume	20 µl

Real Time–PCR was performed with the use of the Strata gene Roter gene Q Qiagen(Venlo, Netherland) and computer machine (Dell .U.S.A) .

3.2.Methods

3.2.1. Study Subjects and Design

This study was a case-control study conducted on patients who presented with symptoms of pneumonia, clinically diagnosed as having CAP and hospitalized at the Al-Sadiq Hospital, located in the Province of Babylon as well as Imam Al-Hussain Medical City, located in the Province of Karbala, Iraq, from December 2022 to June 2023. The patients were aged-matched with healthy clinically certified non-pneumonic participants recruited as a control group as mentioned in the inclusion and exclusion criteria in Table (3.9).

The Sputum and Blood samples were collected from the subjects. The sputum samples were subjected to the protocol for the isolation of causative bacteria. The blood samples were also subjected to the protocol for the isolation of causative bacteria, in addition to complete blood count, detection of Procalcitonin, cytokine expression analysis using ELISA as well as gene expression analysis using RT-qPCR. As show in flowchart of the study design in Figure (3.1.)

3.2.1.1. Ethical Considerations

Ethical approval was sought and obtained from the Ethical Committees of the Iraqi Ministry of Health as well as the Iraqi Ministry of Higher Education and Scientific Research in 26/12/2022 of 130 in Babylon Governorate and 7/12/2022 of 223 in kerbala Governorate s show in Appendix (2).The participants were informed about the scope of the study as well as information and analyses that would be conducted and their consent was obtained, before being recruited into the study.

Case - Control study

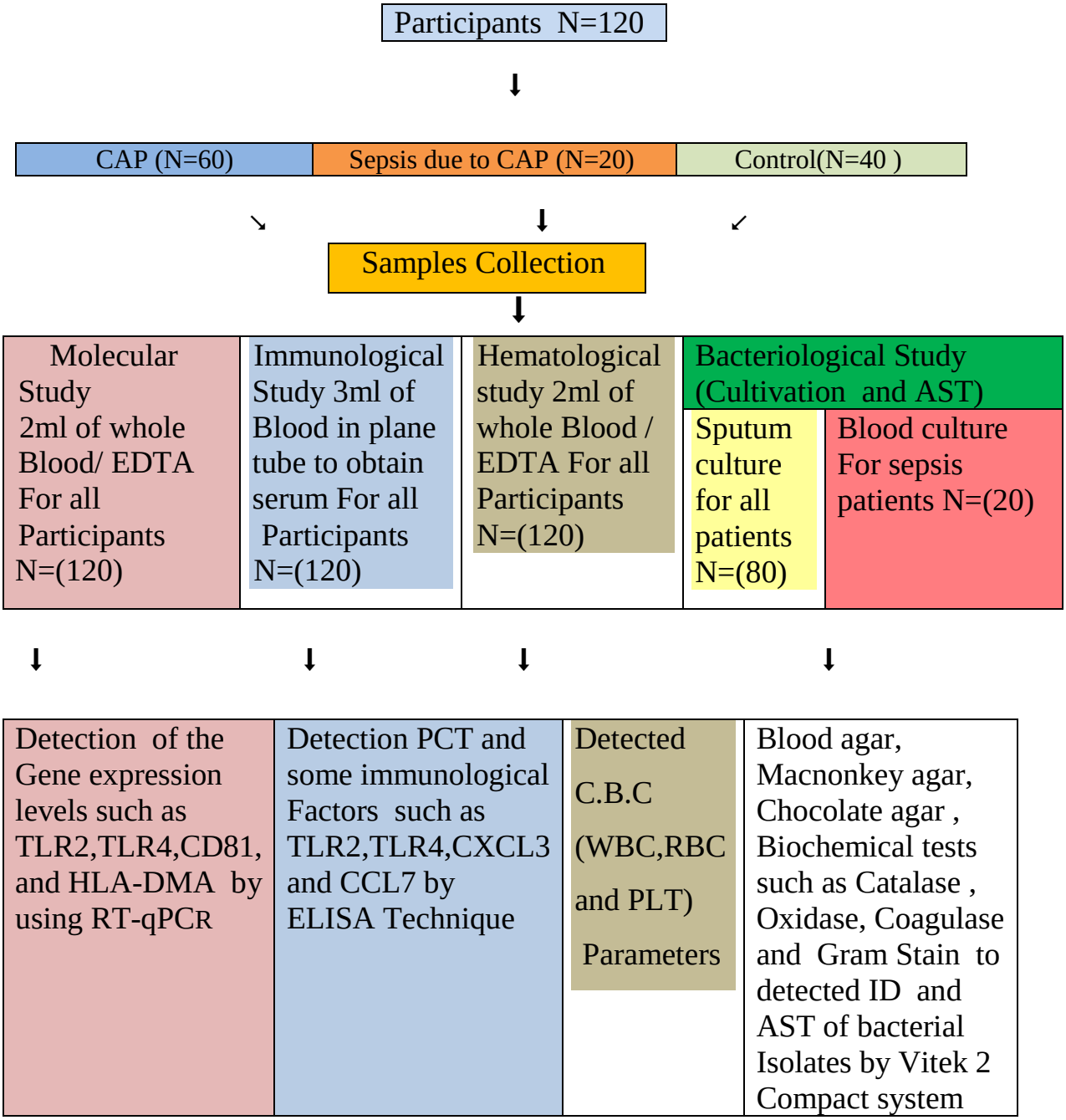


Figure 3.1 : The Study Design.

3.2.1.2.Inclusion and Exclusion Criteria

The inclusion and exclusion criteria of the study are presented in Table (3.9).

Table 3.9: Inclusion and Exclusion Criteria of the Study

S/N	Inclusion Criteria	Exclusion Criteria
Patients		
1.	Clinical diagnosis of bacterial CAP	Other forms of pneumonia (e.g. Hospital-Acquired Pneumonia or Ventilator- Acquired Pneumonia)
2.	Presentation of symptoms such as fever, productive cough (produced sputum), shortness of breath, chest pain and diagnosis by X- Ray or C.T scan	Asymptomatic pneumonia Can not produce sputum
3.	Absence of other immunological disorders	Presence of other immune related disorders such as HIV, cancer etc.
4.	Consented	Not consented
Control		
1.	Clinically certified as not having any form of pneumonia	Presence of pneumonia and other chest or pulmonary infections
2.	Absence of symptoms such as productive cough, shortness of breath, fever, and chest pain	Presence of symptoms, smoking persons and Bird , livestock breeder
3.	Absence of other immunological disorders	Presence of other immune related disorders such as HIV, cancer etc.
4.	Consented	Not consented

3.2.2. Sample/ Data Collection

Patients medical history and Symptoms were obtained from their hospital records while socio-demographical data, family history , and information were obtained from the participants using questionnaire-based interviews were summarized in Appendix (4) .

3.2.2.1 Questionnaire

The questionnaire form used in the study is summarized in Appendix (3).

3.2.2.2 Sputum Sample Collection

Strict aseptic conditions were maintained throughout, and conventional procedures were followed. Sputum samples were collected aseptically in the early morning using a sterile cup at the first day of admmision to the hospital. Each sample was then cultured on Blood Agar, MacConkey Agar, and Chocolate Agar. The growing colonies were stained with Gram staining , along with a few biochemical tests like oxidase, catalase, and coagulase .Vitek 2 was used for diagnosis in order to identify the type of bacteria and detect Antimicrobial Susceptibility Test (AST).

3.2.2.3 Blood Sample Collection

Strict aseptic conditions were maintained throughout, and conventional procedures were followed at the first day of admmision to the hospital.. After sanitizing the injection sites on the patients' anterior surface of the elbow (cubital fossa) with a 70% alcohol and 10% iodine solution, 10 milliliters of blood were taken from them using a sterile syringe and it was split into three sections: 2 ml went into an EDTA tube for the screening test, 3 ml went into a gel tube that had been centrifuged to use the serum in immunological procedures, and 5 ml went into a Brain Heart Infusion (BHI) vial for blood culture. Before being sent to the laboratory, every sample was appropriately labeled with known information. The blood sample-containing BHI broth bottles were incubated at 37°C for a minimum of 48 hours and up to two weeks before being disposed of. To check for bacterial growth or turbidity, if any, during these times, one or two drops of the sample were moved to a particular solid media and re-incubated at 37°C.

3.2.3. Bacteria Isolation and Identification.

A- Isolation of Causative Bacteria from Blood Samples

The blood samples were collected in sterile bottles that contained Brain Heart Infusion Broth (BHI) and transported to the laboratory and put in BacT/ALERT which uses a colorimetric sensor and reflected light to monitor the presence and production of carbon dioxide (CO2) that is dissolved in the culture medium. If microorganisms are present in the blood sample, carbon dioxide is produced as the organisms metabolize the substrates in the culture medium. When the growth of the microorganisms produces CO2, the color of the gas-permeable sensor installed at the bottom of each culture bottle changes to yellow .However, all blood culture results in current study were negative .

B - Isolation of Causative Bacteria from Sputum Samples

The sputum samples were collected in sterile containers and transported to the laboratory. Upon receipt in the laboratory, the samples were first visually inspected for quality and adequacy. Traces of saliva contamination were removed from the sputum samples, and only pure sputum samples were processed further. Each sample was streaked onto appropriate culture media using sterile techniques. Blood agar, MacConkey agar and chocolate were used to culture a wide range of bacteria. The plates were then incubated at the temperature 37c^{o} for 24-48 hours for bacterial growth.

3.2.3.1. Preparation of Culture Media Methods for Isolation of Bacterial Isolates.

The culture media and solutions used in this investigation, together with the procedures used to prepare them, are mentioned below.

A. **Brain Heart Infusion Broth (BHI) – Glycerol Preparation**

The culture media was Prepared according to manufacture information (Tedeschi *et al.,* 2011).

B. **Blood Agar and MacConkey Agar Preparation**

Blood agar and MacConkey agar base agar were prepared according to manufacture information. All media were sterilized by autoclave at 121°C and pressure 15 pound/Inch for 15 min (Paul, 2019).

C. **Chocolate Agar Preparation**

The procedure for preparing CA was the same as that for preparing BA, except for step where red blood cells are lysed to liberate intracellular nutrients. The blood agar base was prepared according to manufactured procedures. 5 percent of defibrinated sheep blood was aseptically added to the solution after it had been autoclaved, and it was then heated in a 75°C water bath. Pour it into sterile petri dishes under aseptic circumstances after allowing it to cool to 50 °C (Casino *et al*., 2023)**.**

3.2.3.2.Preparation Solution and Reagents for Identification of Bacterial.

A. Gram Staining

The colonies that were grown on media were selected and stained by Gram stain to show the morphology and aggregates of the cell

B.Coagulase Test

The slide method was adopted for achieved this test and differentiate among Gram-positive cocci. A sterile loop was used to transfer some colonies from overnight bacterial culture, and they were placed on the glass slide. A sterile dropper covered bacterial colonies on that slide with one or two drops of plasma. This preparation was mixed well with a wooden stick. The test is positive, if it shows clumping (coagulation) within 10 seconds. Additional slides were used for control positive and control negative by utilizing coagulase-positive species (*Sta. aureus*) and coagulase-negative (*Sta. epidermidis*) respectively (Katz ., 2010).

C.Catalase Test

After 24-48 hours of bacterial growth, some colonies were transferred by sterile disposable loop onto the glass slide. On the same glass slide, two drops of 3% H2O2 concerted were placed on these colonies. A wooden stick was used for mixing the colonies with the reagent. Bubbles indicate the existence of catalase enzyme; it is a positive finding. Additional slides were used for both control positive and negative (Hall, 2013).

D. Oxidase Test

Two drops of the reagent were placed on the filter paper. By platinum loop, some colonies were transferred from 24-48 hours of bacterial growth and placed on the prepared filter paper for biochemical testing. The appearance of a purple color within 10 seconds reveals a positive result (Prince, 2009).

3.2.3. 3. Diagnosis of Bacterial Isolates

3.2.3. 3. 1. Identification of Bacterial Isolates Traditionally

The bacterial isolates were diagnosed according to cultural , morphological , and Biochemical properties tests by showing the shape and color of the colonies on the selective media. The bacterial isolates were also stained by Gram stain to detect Gram positive or Gram negative bacteria and by some biochemical tests were performed such as coagulase , catalase , and oxidase tests (Nimer *et al*., 2016).

3.2.3.3.2. Identification of Bacterial Isolates by VITEK-2 Apparatus System

All bacterial isolates present in sputum samples were diagnosed by VITEK-2 . The time between the preparation of the inoculum and the card filling was always less than 30 min .This method is based on a fully automated option for performing about 64 biochemical tests through the presence of reagent cards, which have 64 wells to do this. By manufacture instructions, the procedure was performed as follows (Nimer *et al.,* 2016).

i. With the sterile loop, a single colony was transferred from fresh (24 hours) solid media and inoculated into a tube containing 3 ml of normal saline
ii. The tube was placed into the dens check machine to standardize the colony to McFarland's standard solution (1.5×10^8 cell/ml).
iii. The standardized inoculums were loaded into the cassette, and a barcode was used to enter a sample identification number into the computer.
iv. After being placed in the filler module, the cassette was moved to the reader incubator module once the card had been filled.
v. The VITEK device controls the incubation temperature, reads the card, and sends test results to the computer for analysis.

3.2.3.4.1.Identification Antibiotic Susceptibility Test (AST) by VITEK-2 Apparatus System

The McFarland bacterial suspension was made –ready . Cards were automatically filled, sealed, and loaded into the VITEK 2 instrument for incubation and reading by using Gram Positive and Gram Negative cards.

3.2.4.Immunological Factors **Methods.**

3.2.4.1. Complete Blood Count (C.B.C.) Parameters .

CBC is a common screening test for most diseases. It is detected by a full automated hematology analyzer (Sysmex , Japan). This apparatus was used to measure of white blood cells, , Red blood cells and platelets the blood-containing EDTA tubes.

3.2.4.2.Procalcitonin(PCT) Quantitative Test as Immunological Factors **.**

3.2.4.2.1. Intended Use.

This kit used for quantitative measurement of human Procalcitonin in serum or plasma employing a combination of Up-converting Phosphor Technology (UPT) and immunochromatography as an aiding diagnostic tool for severe bacterial infections and sepsis and differential between viral and bacterial infections.

3.2.4.2.2. Procedure of PCT Test

The PCT test was conducted in the private Baghdad laboratory and under the direct follow of Supervisor Sattar Jabbar Rahi According to the following steps.

1.The UPT analyzer Switched to warm up and stabilize for 20 minutes. Once the test strip, diluent sample and sample have been calibrated to room temperature (20-25 ℃)

2. The setup button on the device screen pressed and chosen to read the PCT test in order for the examination to be performed successfully, then removed the card.

3. The sample ID written on the plastic box of the test strip and then diluted the serum sample using the sample diluent included in the kit by adding 100 µl of Sample to 150 µL of diluted sample and then add 100 µL from the diluted sample to the sample cavity in the PCT strip.

4. Incubated for 20 minutes at room temperature and then inserted and scanned the barcode of the test card to obtain the result. The results see in Table (3.10).

Table 3.10:Value of Procalcitonin (PCT) and Interpretation (Hotgen Biotech , China)

Results	Interpretation	
<0.10ng/mL	Probability Bacterial infection was low	Antibiotic therapy strongly discouraged
0.10ng/mL-0.24ng/mL	Bacterial infection was unlikely	Antibiotic therapy discouraged
<0.25ng/mL	Bacterial infection was unlikely.	Antibiotic therapy encouraged
0.5ng/mL-1.99ng/mL	Local bacterial infection was possible but sepsis is unlikely.	Antibiotic therapy strongly encouraged
2.00ng/mL-9.99ng/mL	Systemic infection (sepsis) was possible and moderate risk of developing to severe sepsis.	
≥10.0ng/mL	High likelihood of severe sepsis or septic shock.	

3.2.4.3. Immunological Factors Methods by Sandwich ELISA Tests.

Measurements were performed in Al-Zahraa Hospital for Obstetrics and Gynecology / Babylon Health Department in Babylon Governorate . ELISA test was conducted in the period from February to June 2023.The following subsections discusses briefly the procedures of different measurements. The level of human TLR2,TLR4,CXCL3 and CCL7 in serum from patients and a healthy population as a control group combined autoantibody were measured using Sandwich ELISA assay.

3.2.4.3.1. Principle of Kit of (TLR2,TLR4,CXCL3 and CCL7) .

ELISA kits were used the Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) principle .The plate has been pre-coated with Human (TLR2,TLR4,CXCL3 and CCL7) antibody. (TLR2,TLR4,CXCL3 and CCL7) present in the sample was added and bound to antibodies coated on the wells. And then biotinylated Human (TLR2,TLR4,CXCL3 and CCL7) Antibody was added and bound to (TLR2,TLR4,CXCL3 and CCL7) in the sample. Then Streptavidin-HRP is added and bound to the Biotinylated (TLR2,TLR4,CXCL3 and CCL7) antibody. After incubation unbound Streptavidin-HRP was washed away during a washing step. Substrate solution was then added and color developed in proportion to the amount of Human (TLR2,TLR4,CXCL3 and CCL7). The reaction was terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

3.2.4.3.2. Procedures

Measurements of studied were performed using specific kit following the manufacture's protocol by following steps which mentioned in the instructions attached to each kit, described in the links **included was shown in Table (3.3).**

3.2.4.3.3. Preparation of Standard Calculation

The concentrations were calculated using a standard curve fitting equations for human immunological Figure (3.2., 3.3., 3.4. and 3.5).

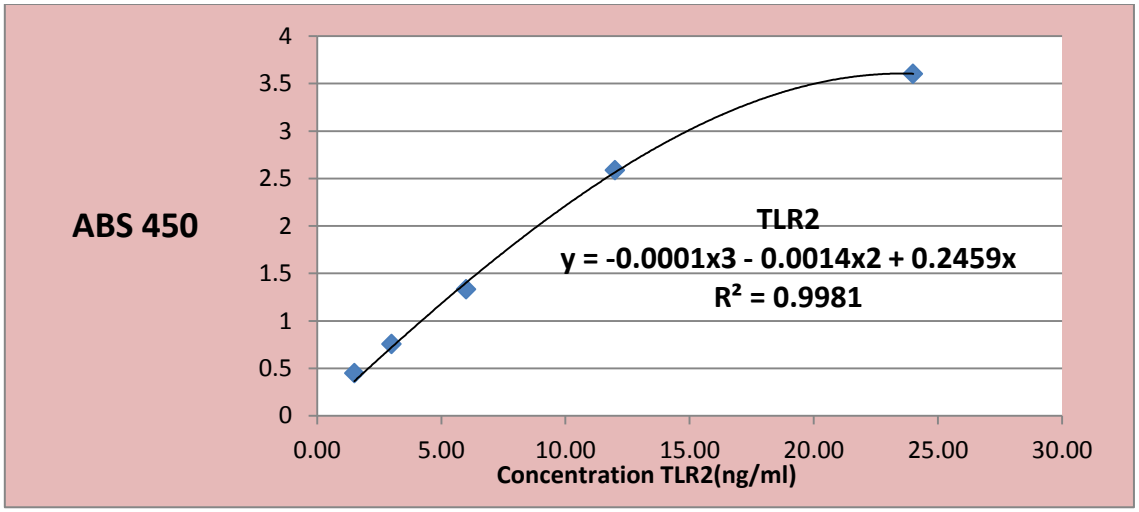


Figure 3.2: Concentration of TLR2 (ng/ml)

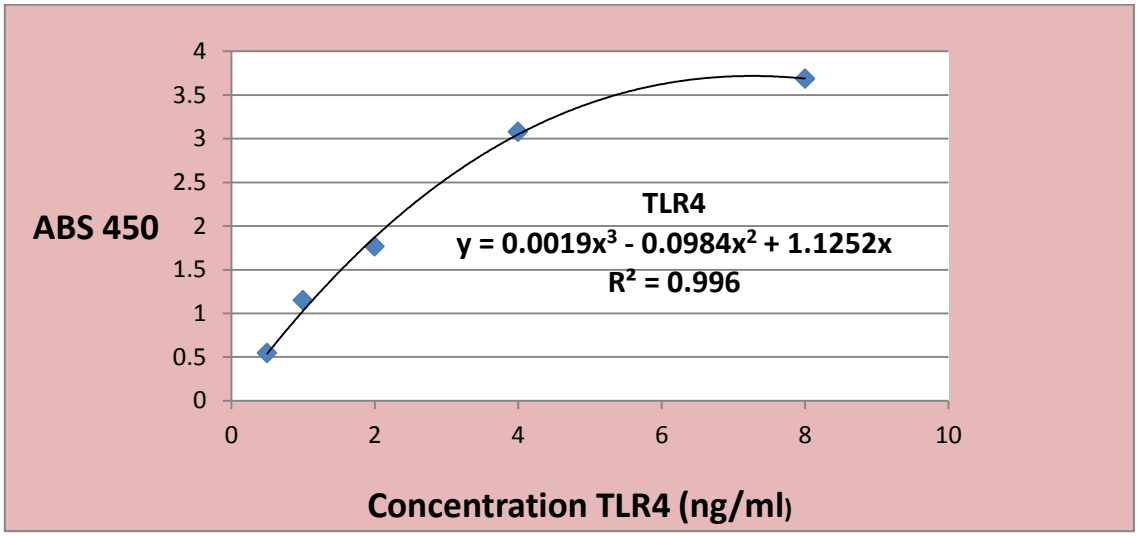


Figure 3.3: Concentration of TLR4 (ng/ml)

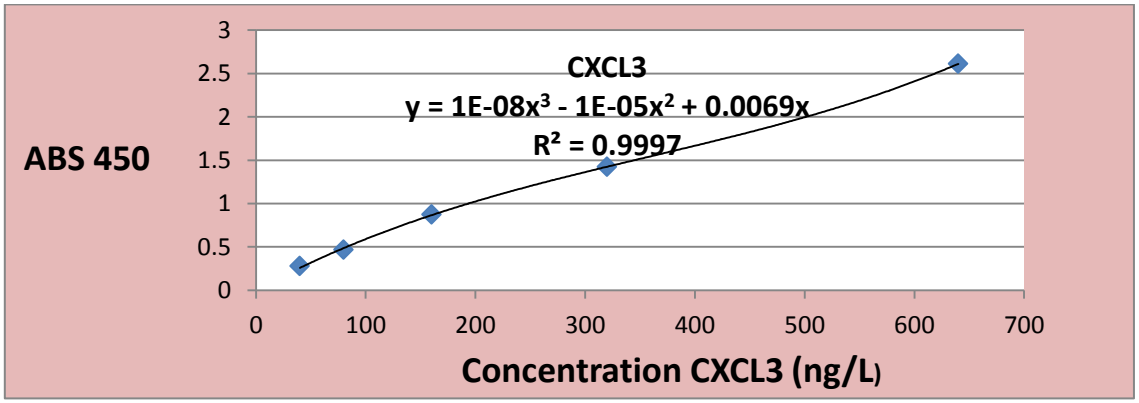


Figure 3.4: Concentration of CXCL3 (ng/L)

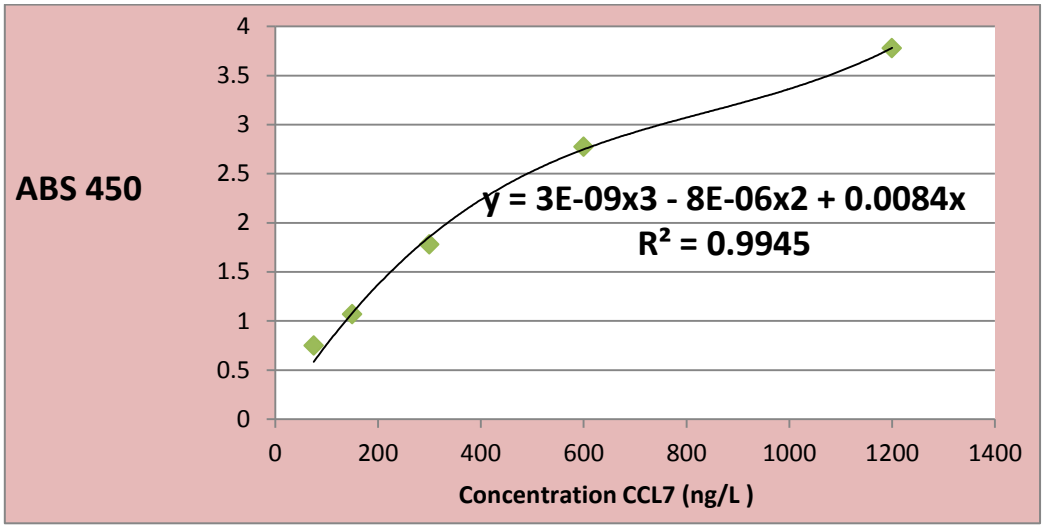


Figure 3.5: Concentration of CCL7 (ng/L)

3.2.4.4. Molecular Study

3.2.4.4.1. RNA Extraction of W.B.C.

For the purpose of obtaining pure RNA was followed some steps.

1- One milliliter of EDTA whole blood was taken and put in polyethylene tube and added 1.5 ml of RBC Lysis with 13.5 ml of Distell water to reach final volume 15ml of RBC lysis and D.W(Brown *et al.*, 2016).

2- The mixture was put in tube roller for 10 minute at room temperature and then in centrifuge was put at 10 minute with speed 3000 rpm and after precipitate was taken and supernatant was removed

3- Two milliliter of RNA later added to the cells and well mixed(Lader, 2001)

4- Then the tube was left for 24 hours at room temperature and then freeze was stored at (-20°C) until use RNA extraction to Measure the gene expression for House Keeping Gene (*H.K.G.) ,TLR2, TLR4, CD81* and *HLA-DMA* By Reverse Transcriptase quantitative Real time Polymerase Chain Reaction (RT. Q Real time PCR) .

Procedure of RNA Purification.

Total RNA Isolation to improve RNA yield and quality by Using TransZol Up Plus RNA kit described by the manufacturer Company (Trans Gen Biotech, China).

3.2.4.4.2.Determination of RNA Purity and Concentration

Total RNA has been successfully extracted from all samples. The RNA quantity and quality was measured by Nano-Drop (scan drop biometra , Germany) employing the scanning ability of diode array from 200 to 700 nm wave length , the absorbance profile Figure (3.7). then processed and analyzed to determine the RNA quantity and quality by calculating the 260/280. If a sample showed 260/280 ratio more than 1.8. Samples RNA quantity ranged 50-74 ng/ul.

Figure 3.6: photo-spectrometry of extracted RNA by Nano drop , the trace represents the absorbance spectrum of one sample at 200-700 nm

3.2.4.4.3. Primers Preparation

All primers(*TLR2,TLR4,CD81,HLA-DMA* and *H.K.G*) used in the Reverse Transcriptase quantitate Real Time Polymerase Chain Reaction (RT.q RT PCR) were prepared for amplification studies genes at form vails containing Forward primers and Reverse primers powder were purchased from (Macrogen ,Korea) see in Table (3.11).

1. Centrifuge at speed 10.000 rpm for 5 minutes and that by dissolved the primers

2. Recommend water quantity was added according to the supplied company instructions (Macrogen, Korea) to obtained final concentration of solution.

3. The tubes were shaken at 10 minutes at room temperature and then the final solution was prepared by diluted 10 µl of primers in 90 µl of Nuclease Free Water to obtained 10 Pm .

4. Preserved in the -20^{o}c until to use.

Table 3.11: The Sequences, Products, and References of Gene.

Genes	**Sequences(5-3)**	Gene Products	Ref.
TLR2	TLR2 Forward TCCGCCTCTCGGT TCGGAA	13 bp	(Keshavarz *et al.*, 2021)
	TLR2 Reverse AAACGGTGGCACAGGACCCC		
TLR4	TLR4 Forward TCAAGCCAGGATGAGGACT GGGT	118bp	
	TLR4 Reverse CAGCAATGGCCACACCGGGA		
CD81	CD81 Forward TTCCACGAGACGCTTGACTG	86bp	(Lasswitz *et al.*, 2022)
	CD81 Reverse CCCGAGGGACACAAATTGTT C		
HLA-DMA	HLA DMA Forward GGGAGATGCTCCTGCCATT	132bp	(Li *et al.*, 2021)
	HLA DMA Reverse GGCTTCAGCGTGAACACTTC		
HKG	Actin Forward GCGAGAAGATGACCCAGAT	88bp	(Eimani *et al.*, 2014)
	Actin Reverse GAGGCGTACAGGGATAGC		

3.2.4.4.4. Gene Expression Estimation by RT-Q-Real Time PCR

Transcript® Green One-Step RT qRT-PCR Super Mix was employed to the accessed the gene expression of (*TLR2*,*TLR4*,*CD81*,*HLA-DMA* and*H.K.G*) by R .T. q RT – PCR by Rotor Gene Q (Germany).

The Reaction Mixer was listed in Table (3.12).

Thermal cycler program condition of real time PCR as shown in Table(3.12).

Table 3.12:Thermal cycler program condition of real time PCR.

Cycle setup	Temp.	Time	Cycle
cDNA synthesis step Denaturation	45°C	10 minutes	1 cycle
	94°C	30 seconds	
Denaturation	95°C	10 seconds	40 cycle
Annealing and Extension at same step.	60°C	30 seconds(Signal reading on green channel)	

Real Time–PCR had been performed with the use of the Strata gene Roter gene Q Qiagen and computer machine (Dell .U.S.A).

3.2.4.4.4.5. Calculating Gene Expression (Fold Change)

Gene fold or RQ calculated firstly by collecting CT (CT-cycle threshold or CQ- cycle quantification) average value from real time PCR device for each triplicated sample then calculate ΔCT value for both patients and control samples as follow:

Δ CT = CT (Target gene) – CT (housekeeping gene)

To calculate ΔΔCT value which found as follow:

ΔΔ CT = Δ CT (case patients) – Δ CT (mean control)

After calculating ΔΔ CT for all samples then take final equation to calculate gene expression or RQ as follow:

Fold change = $2^{-(\Delta\Delta CT)}$ (Livak & Schmittgen, 2001).

3.2.5. Statistical Analysis

Data were collected, entered and analyzed by using the software program Statistical Package for Social Sciences (SPSS) version 26. And Graph Pad prime 8 . All numerical variables were represented by means (a measure of central tendency) and standard deviation (a measure of dispersion) while categorical variables were presented by frequencies and percentages.

The Chi-Square test was used to assess the presence of an association between categorical variables. Kolmogorov-Smirnov test of normality and Levene Statistic test of Homogeneity Variances were used . Mann-Whitney Test used to the Two-Independent-Samples Tests procedure compares two groups of cases on one variable , Kruskal-Wallis test was used to evaluate the difference in mean of numeric variables among more than two groups provided that these numeric variables were abnormally distributed. the Spearman correlation was used correlation when the assumption for Pearson correlation were not met. The level of probability was indicated as *P*. Value $\leq$ 0.05, * *P*. Value $\leq$ 0.01 ** *P*. Value $\leq$ 0.001 and *** *P*. Value $\leq$ 0.0001.

Chapter Four
Results
and
Discussion

4.0. Results and Discussion.

4.1. Demographic Distribution of Patients and Control Groups .

The 60 patients of CAP (30 males and 30 females) which were participated in the study had a mean age of 54.9± 18.5years, with a range of 23-90 years .In addition to that the 20 patients with sepsis due to CAP (7 male and 13 female) had a mean age of 72.5± 14.2years, with a range of 25-95 years . and the 40 healthy controls (18 males and 22 females) had a mean of 51.4± 13.4years, with a range of 23-95 years, they were divided according to Sexs shown in Table (4.1) . They were divided according to age as shown in Table (4.2) Statistical analysis reveals that there are no significant differences between the Sexes (*P.* value ≤ 0.503). while there are highly significant differences between the ages of patients and control (*P.* ≤ 0.000) .

Table 4.1:Demographic Characteristics of Samples According to Sex.

Sex	CAP	Sepsis & CAP	Control	Total	*P.* value
Male N (%)	30(50.0%)	7(35.0%)	18 (45.0%)	55(45.8%)	0.503 n.s
Female N (%)	30(50.0%)	13(65.0%)	22(55.0%)	65(54.2%)	
Total N (%)	60(100.0%)	20(100.0%)	40(100.0%)	120(100.0%)	

The level of probability was indicated as *P ≤ 0.05 , **n. s. : not significant** statistical test: **X^2 test ,** d.f: (2), n = 120.

Table 4.2 :Demographic Characteristics of Samples According to Age

Characteristic	CAP	Sepsis & CAP	Control	Total	*P.* value
Age (Years)					
Mean ±SD	54.9±18.5	72.5± 14.2	51.4± 13.4	56.7± 17.7	
Range	23- 90	25 - 95	23 - 71	23 - 95	
N(%) 21 – 40	16(26.7%)	1(5.0%)	10(25.0%)	27(22.5%)	0.000***
N(%) 41 – 60	21(35.0%)	1(5.0%)	20(50.0%)	42(35.0%)	
N(%) 61 - 80	19(31.7%)	13(65.0%)	10(25.0%)	42(35.0%)	
N(%) 81+	4(6.6%)	5(25.0%)	0(00%)	9(7.5%)	

The significance value was indicated as * between the groups. The level of probability was indicated as *$P.\leq$ 0.05,**$P.\leq$ 0.01 ***, $P.\leq$ 0.001,and n. s. :not significant , Data was presented as mean. S.D:standard deviation ,statistical test: Kruskal Wallis test and Mann-Whitney Test , d.f:(2), n =120.

The results showed the number of people with pneumonia gradually increases with age the high ratio was the age of 41-60 years 21(35.0%) and from 61-80 years 19 (31.7%) with CAP patients and age of 61-80 years 13(65.0%) and age more than81 were 5(25.0%) in sepsis due to CAP.

The current study was consistent with other previous study conducted by Naher & Al-Taa'e ,(2013) documented that risk factors as mentioned in Patients aged more than 49 years were the high incidence of pneumonia.

The high incidence of pneumococcal isolate in the 51-60 age group may be due to impairment of the immune system, and most elderly people are infected with chronic diseases (Motaweq & Naher, 2017).

The study by AL-Hadrawi *et al.*, (2019) of 120 samples were collected from CAP patients in the Najaf governorate. The highest number was 24 patients in the age of more than 50 years. But the lowest number was 12 at 20-30 years old . The advanced age of the person is accompanied by a decrease in the defenses of the physical barriers and a decline in protection against invading pathogens as well as a change in the immune system associated with age, a very important factor for the establishment of pneumonia.

The study Menon *et al.*, (2023) showed that risk factors in Patients. pneumonia participants belonged to the age range of 46–60 years. And as a study Güleç *et al.*, (2023) of 199 patients with CAP Mild Pneumonia (n = 76) with a mean age of 70 years and a range age of (62.25–77) years and Severe pneumonia (n = 123) with a mean age 79 years and a range age of (70–84) years , was observed that high significant differences at p. value <0.001 between age in two groups (Mild and Severe Pneumonia).

The study by Dawood *et al*., (2021) observed that the highest percent of age between 50- 60 years were infected with CAP and study Khan *et al* , (2023) on 60 patients with CAP, the majority of patients with CAP were the middle - aged and the elderly were more than 50 years of age. its incidence rises sharply with extremes of age.

It could be due to the current study showed that the respiratory muscles in human as a result of aging start to weaken and the thoracic wall becomes less elastic due to physiological changes that lead to a decrease in pulmonary function that takes place as the aging process advances and Other effects of aging cause the defense mechanisms of the airways to decrease; it also reduces the efficacy of mucociliary clearance. More importantly, there is a decrease in the efficacy of the cough reflex and Human immune defenses are not only triggered by exposure to pneumonia cells, but also show response to surface proteins, and capsular polysaccharides, Because the human immune system is capable of clearing pneumococcal colonization but the time required for clearance depends on host age and bacterial serotypes.

4.2. Cultural Study

4.2.1. Isolation of Bacteria from Blood Culture .

The results of the blood cultural methods in sepsis due to CAP patients did not exhibit differences when twenty blood samples were inoculated in the BHI vials and not one of them showed any turbidity and growth because low number of samples , previous treatment with antibiotics, it was not used more one bottle at different times, and anaerobic cultivation methods were not used. and this results showed in Table (4.3).

Table 4:3. Blood Culture Findings

Case	Test	Number of samples	Result
Sepsis due to CAP	Blood culture	20	Negative

The current study was consistent with the study Waterer *et al* ., (1999)were reported that blood cultures rarely result in an appropriate change in empirical therapy because blood specimens that include skin contaminants can generate false positive test results.

The study Erdede *et al.*, (2010) showed the use of blood cultures in patients with CAP . Significantly positive results were rare and the majority of the blood cultures revealed negative results. Blood culture tests may not be performed in all patients with CAP.

The study Muro *et al.*, (2023) observed the utility of blood cultures in inpatients with CAP to reduce mortality and length of hospital stay was controversial.

The study Dubourg *et al*., (2018) showed that Blood culture was currently the reference standard for diagnosis, but conventional practices have long turnaround times while diagnosis needs to be faster to improve patient care.

The study Costantini *et al*., (2016) was conducted on adults hospitalized with CAP. The increased use of antibiotics in our study gives false-positive blood culture test results.

The study Nasimfar *et al.* , (2018) showed low sensitivity and a high number of false‑negative blood culture results, indices such as sensitivity, specificity, and positive/ negative predictive values of the examined markers could not be evaluated in comparison with the blood culture test results, as the "gold standard" for the diagnosis of infection.

Many of the researches by Metlay *et al.*, (2019) observed not obtaining blood cultures in adults with CAP managed in the outpatient setting (strong recommendation, very low quality of evidence). And suggest not routinely obtaining blood cultures in adults with CAP managed in the hospital setting (conditional recommendation, very low quality of evidence).

The recommendation for outpatients with CAP is not to obtain blood cultures based on very low - quality evidence. Again, considering the relatively stable clinical status of the patients, this recommendation seems safe and efficient. For the inpatient management of CAP, the recommendation is not to routinely obtain blood cultures based on very low quality of evidence. But in this case, and even as mentioned in the references evaluated by the panel, one large observational study showed lower mortality associated with blood cultures at the time of admission(Shoushtari & Nugent, 2020).

4.2.2. Isolation of Bacteria from Sputum Culture.

The results in Table(4.4) illustrated the bacterial pathogens that cause CAP and sepsis due to CAP obtained in sputum culture belonging to Gram - positive 45(56.25%) % and Gram- negative bacteria 35(46.75%). Among Gram positives *Streptococcus pneumonia*e 32 (40%) , *Staphylococcus aureus* 10(12.5%), Methicillin-resistant *Staphylococcus aureus* (MRSA) 2(2.5%) and *Enterococcus facalis* 1(1.25%), Respectively. while among negative Gram *Haemophilis influenza*e 9(11.25), *klebsilla pneumonia*e 8(10.0%), *Moraxella catarrhalis* 6(7.5%), *Escherichia coli* 4(5.0%) , *Pseudomonas. aeruginosa*e 3(3.75%) , *Acinobacter baumannli* 2 (2.5%), *Morganella morganii* 1(1.25%), *Pantoea agglomeranas* 1(1.25%) and *Enterobacter asburiae*1(1.25%) , Respectively. The results show the Vitek 2 automated report on identification and susceptibility of bacteria in Appendix(5).

Table 4.4:Frequency and Percent of Bacteria Isolated from Sputum in the study groups.

Bacterial Isolates				
Gram positive N=45(56.25%)	Frequency	Percent	CAP	Sepsis /CAP
Streptococcus pneumoniae	32	40.0	23	9
Staphylococcus aureus	10	12.5	10	0
MRSA	2	2.5	2	0
Enterococcus facalis	1	1.25	1	0
Gram negative N=35(43.75%)				
Haemophilis influenzae	9	11.25	8	1
klebsilla pneumoniae	8	10.0	4	4
Moraxella catarrhalis	6	7.5	3	3
Escherichia coli	4	5.0	4	0
Pseudomonas aeruginosae	3	3.75	3	0
Acinobacter baumannli	2	2.5	2	0
Morganella morgagni	1	1.25	0	1
Pantoea agglomeranas	1	1.25	0	1
Enterobacter asburiae	1	1.25	0	1
Total	80	100.0	60	20

The current study agreed with the study Al Ghizawi *et al*., (2007) showed that the most common pathogen caused by CAP was *Streptococcus pneumoniae*. And study Hashemi *et al*.,(2010) showed the prevalence of Gram-positive was more common than Gram-negative bacteria and was evaluated in the elderly the most frequent were S. pneumoniae (12.3%) , *S. aureus*(6.1%) and *P.aeruginosae* (6.1%). And Gupta *et al*., (2012) reported that pneumococcus was the most common organism identified in hospitalized elderly patients with CAP.

The study Khalil *et al.*, (2013) showed that Gram - positive organisms were the most prevalent in CAP especially *S. pneumonia*e followed by *S. aureus*, while *Klebsiella* was the most prevalent Gram - negative organism.

The study Agmy *et al*., (2013) reported *S. pneumoniae* followed by atypical bacteria (*C. pneumoniae and M. pneumoniae*), then *K. pneumoniae* as the causative bacteria of adult CAP at rates of 36%, 30% and , 10%, respectively.

The study Naher & Al-Taa'e, (2013) were Bacterial isolates represented 49 isolates (34.5%) of *Streptococcus pneumoniae,* 26 isolates (18.4%) of *Klebsiella pneumoniae*, 21 isolates (14.8%) of *Neisseria meningitidis*, 4 isolates (2.8%) of *Staphylococcus aureus* , 4 isolates (2.8%) of *Haemophillus influenzae* .

The study Akter *et al*.,(2014) observed of the 105 sputum samples, 55 (52.38%) yielded growth. Among the 55 growths, 23 (21.91%) were Gram- positive cocci, 22 (20.94%) were Gram- negative bacilli and 10 (9.53%) were Gram- negative coccobacilli . *S. pneumoniae* was the predominant Gram positive cocci and *K. pneumoniae* was the predominant Gram negative bacilli .Among the bacteria isolated from sputum, *S. pneumoniae* was isolated in 20 (19.05%), *K. pneumoniae* in 14 (13.33%), *H.influenzae* in 9(8.57%), *P. aeruginosa* in 6 (5.71%), *E. coli* in 3 (1.90%), *S. aureus* in 3 (2.86%) and *A. baumannii* in one (0.96%) sputum samples.

Most bacterial isolates from sputum cultures of patients by Regasa *et al* , (2015) were *S. pneumoniae* (12.8%) and *S. aureus* (10.5%) and from Gram-negative bacteria were *P. aeruginosa* (6.8%), *K. pneumoniae* (5.3%), and *E. coli* 3.8%.

The study Rodrigo-Troyano & Sibila., (2017) showed *S. pneumonia*e and *H. influenza*e most common pathogens that cause CAP . Recently, GNBs such as *P. aeruginosa*, *K. pneumoniae* and *E. coli*, have emerged as causes of CAP with an estimated prevalence ranging from 2% to 30%.

The study El-Sokkary *et al.,* (2018) showed CAP caused by pathogens such as *S. pneumoniae* , *H. influenza*e, and atypical pathogens that are sensitive to the majority of first-line antibiotics.

The reached Bjarnason *et al.,* (2018) showed that *S. pneumoniae* was the most common pathogen (61 of 310 isolated presented 20%).

The study Para *et al.,* (2018) observed that a total of 225 patients (median age: 59 years) were enrolled. *S. pneumoniae* was the most common organism found (30.5%), *K.pneumoniae* (4.8%), MRSA (3.5%), *P.aeruginosa* (3.1%), methicillin-sensitive S. aureus (1.7%), and Acinetobacter sp. (0.8%) with 4% of patients having multiple pathogens etiologies.

The study Temesgen *et al*., (2019) observed the most common pathogens in CAP were *S. pneumoniae, K.pneumoniae, and S.aureus* .

The results Shoar & Musher., (2020) showed that pneumococcus and Haemophilus continue to predominate as the bacterial causes of CAP, followed by *S. aureus* and Enterobacteriaceae. For all the emphasis on Pseudomonas, this organism remained a relatively uncommon cause. On average, Moraxella was implicated in 2–3% of cases, although some series showed this organism to be the third most common cause of CAP.

The study Batool *et al.,* (2021) reported of the 165 patients (43% males and 57% females), 77 (46.6%) patients tested positive for bacterial pathogens out of a total of 165. The most common pathogen was *S. pneumonia*e (34%) followed by *H.influenza*e (16%) *S.aureus* (13%) *Klebsiella* (12%) , *P.aeruginosa* (10%), *Legionella* (6%), *E. coli* (5%), and *Proteus mirabilis* (2%).

The research Do Tran *et al.,* (2022) showed that *S. pneumonia* was the leading factor of infection , followed by *K. pneumonia*e and *P. aeruginosa*e, and showed *S. pneumonia*e was the etiology of 69.6% of the Gram-positive group.

The study Khan *et al*., (2023) observed the predominant pathogen was S. *pneumoniae*, accounting for the highest incidence at 36.4%. followed by *K. pneumoniae* (29%), *S. aureus* (20%), and other Gram-negative bacilli (14.5%) including *H.influenzae* (5.5%), Pseudomonas (1.8%), Acinetobacter (1.8%), Enterobacter (1.8%), E.coli (1.8%),and Citrobacter(1.8%).

The study Menon *et al.,* (2023) showed that *S. pneumoniae* was the most common pathogen in patients with CAP.

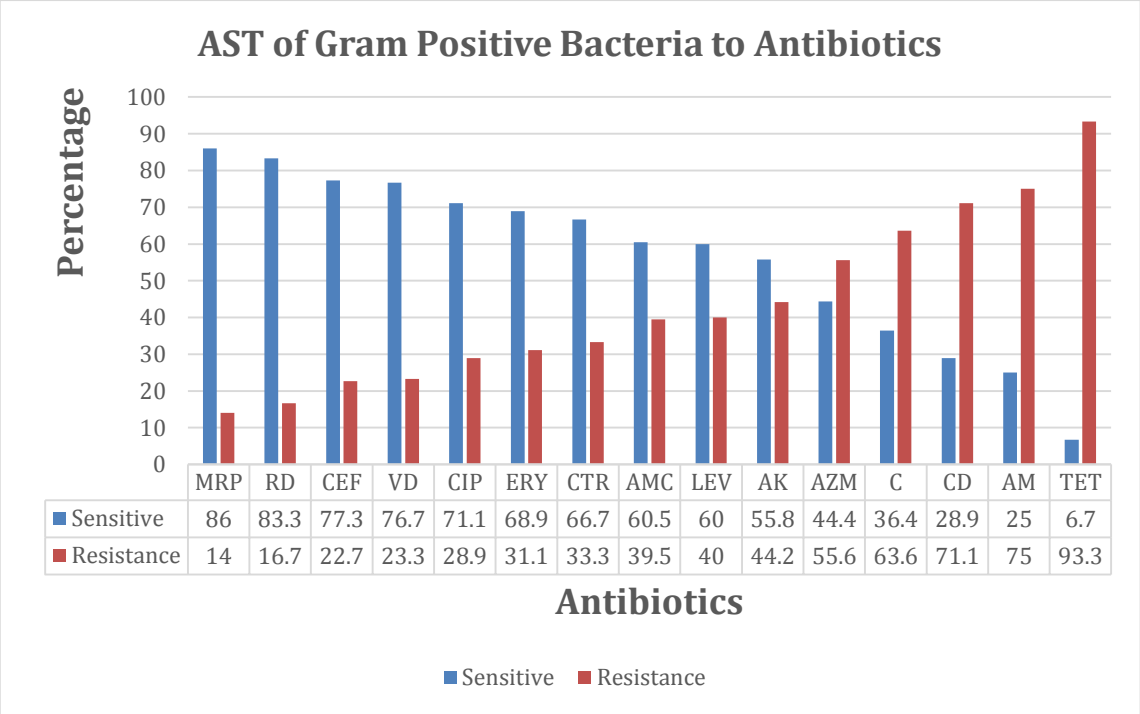
The predominant isolate identification in the study Tilahun *et al.,* (2023) was *S. pneumoniae* 49 (26.3%) followed by *K. pneumoniae* 46 (47.4%) and *P. aeruginosa* 34 (19.4%). others with different geographical regions of the world like India, the United Kingdom and Spain showed that *S. pneumoniae* was the predominant isolate .

The study Hassanzadeh *et al.,* (*2*023) observed that S. *pneumoniae* was the most prevalent bacteria in the CAP and H. *influenzae* was the second most frequent organism identified in 18%

4.2.2.1.Bacterial Antibiotics Susceptibility

4.2.2.1.1.Antibiotics Susceptibility Results of Gram-Positive Bacteria

Most of **Gram-Positive Bacteria** were antibiotic - resistant. Tetracycline , Amoxicillin , Clindamycin , Chloramphenicol , and Azithromycin (93.3%,75%,71.1%, 63.6%, and55.6%, respectively). while the most sensitive percentages were to Meropenem, Rifampin ,Cefipime , Vancomycin, Ciprofloxacin, Erthromycin , Ceftriaxone, Amoxillin-Clavunic Acid, Levofloxacin, and Amikacin (86%,83.3%, 77.3%,76.7%,71.1%,68.9%,66.7%,60.5%,60%,and 55.8%, respectively) . These results are shown in Figure (4.**1).**



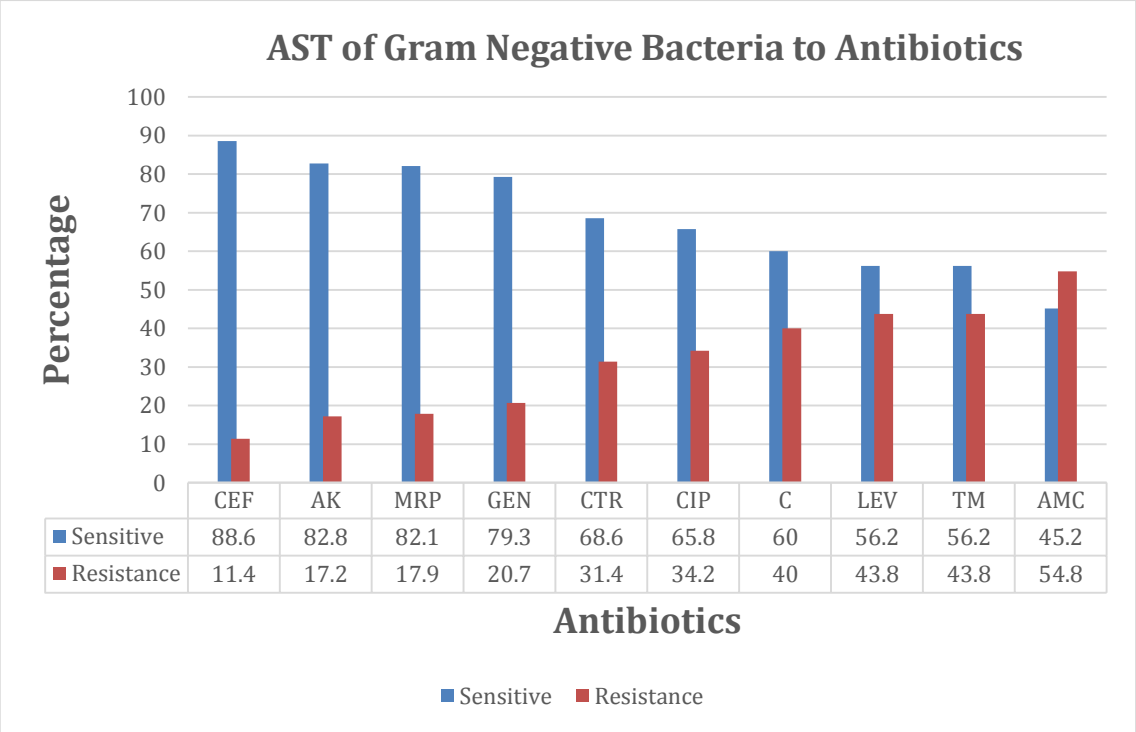
S: Sensitive; R: Resistance; AK: Amikacin ; AM: Amoxillin; AC: Amoxillin-Clananic Acid ; AZM: Azithromycin; CEF: Cefipime ; CTR: Ceftriaxone; C: Chloramphenicol ; CIP.: Ciprofloxacin; CD.:Clindamycin ;ERY:Erthromycin ;LEV:Levofloxacin; MRP: Meropenem; RD: Rifamycin

Figure 4.1: Antibiotic Susceptibility Percentage of Gram Positive Isolates.

4.2.2.2.Antibiotics Susceptibility Results of Gram-Negative Bacteria

Most Gram-Negative pathogens were antibiotic - resistant Amoxillin-Clavunic Acid 54.8% while the most sensitive percentages were to Cefipime, Amikacin , Meropenem, Gentamycin, Ceftriaxone, , ,Ciprofloxacin, Chloramphenicol, Levofloxacin, , and Trimethoprim .(88.6%, 82.8 %, 82.1%, 79.3%,68.6%,65.8% 60%, 56.2% and 56.2% respectively) the results showed in Figure (4.2).

The rate of antimicrobial resistance is still increasing due to the use of empirical antibiotics in the treatment of most hospitalized cases, especially those that have more severe infections with unidentified causative agents.



S: Sensitive; R: Resist; AK: Amikacin; AC: Amoxillin-Clavunic Acid ; CEF: Cefipime ;CTR: Ceftriaxone; C: Chloramphenicol ; CIP: Ciprofloxacin; GEN: Gentamycin; LEV: Levofloxacin; MRP: Meropenem ; TM: Trimethoprim.

Figure 4.2 : Antibiotic Susceptibility Percentage of Gram Negative Isolates .

The results of the current study regarding the AST may agree with some studies and disagree with other studies due to the changes that occur at the genetic and immune levels about the immune responses of people who return to different environments, as well as the differences that occur at the nutritional, social, and cultural levels.

The study by Akter *et al.*, (2014) reported that more than 80% of *S. pneumonia*e were sensitive to ampicillin, amoxycillin‑clavulanate, and ceftriaxone .In contrast, Gram - negative organisms were more sensitive to Meropenem, Ceftriaxone, Amoxycillin‑Clavulanate,and Amikacin .The susceptibility to other antimicrobials ranged from 65% for azithromycin to 70% for levofloxacin.

The study Regasa *et al*., (2015) showed that *S. pneumoniae* isolates were resistant to oxacillin (55%). High resistance rates of S. aureus isolates were observed to tetracycline (100%), penicillin (81.3%), trimethoprim-sulfamethoxazole (81.3%), erythromycin (75%), and doxycycline (50%). Gram-negative bacteria isolates were resistant to tetracycline (66.7-100%), doxycycline (50-100%), trimethoprim-sulfamethoxazole (66.7-100%), and ampicillin (66.7-100%). Resistance to two or more drugs was also observed among 62.7% of bacterial isolates.

The study Temesgen *et al.*, (2019) showed relatively higher proportions of *S. pneumoniae, K.pneumoniae* , and *S.aureus* were identified. Most of the isolates were found susceptible to Ceftriaxone.

The study (Kishimbo *et al.*,2020) observed Gram - negative bacteria resistant to ampicillin, amoxicillin / clavulanic acid and ceftriaxone were the most frequently isolated bacteria among adult patients with CAP.

The study Batool *et al.*, (2021) observed the most common pathogen was *S. pneumonia*e (34%) followed by *H. influenza*e(16%) ,*Klebsiella* (12%),*S. aureus* (13%) , *P.aeruginosa (10%) ,Legionella (6%), E.coli (5%) and P.mirabilis (2%).* the sensitivity pattern of all bacterial isolates was high for cephalosporins (ceftriaxone, 81%; ceftazidime, 80%), penicillins (amoxicillin/sulbactam, 70%), quinolones (levofloxacin, 74%) and aminoglycosides (amikacin, 80.5%). The sensitivity to macrolides (azithromycin, 59.7%; clarithromycin, 53.2%) and tetracycline (55.8%) is somewhat intermediate and low for ampicillin (42.8%) and cotrimoxazole (51.9%).

The study Dawood *et al*.,(2021) observed Amikacin and gentamycin were the most common sensitive drugs for *S pneumoniae, P. aeruginosa* , and *E.coli* with variable antibiotic sensitivity and resistance rates**.**

The study Gebre *et al.,* (2021) showed Gram-positive sensitivity to clindamycin 26(76.5%) and erythromycin 19(55.9%) however, they showed resistance to tetracycline 20(58.8%). *S. aureus* was sensitive to cefoxitin 19(90.6%), gentamycin 16(76.2%) and ciprofloxacin 15(71.4%). *S. aureus* was resistant to tetracycline 13(61.9%) and cotrimoxazole 10(47.6%). *S. pneumoniae* were sensitive to clindamycin 8(88.9%), penicillin 7(77.8%), erythromycin 6(66.7%), but *S. pneumoniae* was resistant to tetracycline 6(66.7%). Gram- negative bacteria Gram-negative bacteria were sensitive to cefepime 93(86.0%), ciprofloxacin 84(77.8%), ampicillin 13(20.3%), augmentin 18(28.1%) and ceftazidime 49(45.4%). *K. pneumoniae* was sensitive to ciprofloxacin 33(91.7%), cefepime 30(83.3%) and cefoxitin 30(83.3%) and K. *pneumonia*e was resistant to augmentin 20(56.6%), ceftazidime 19(52.8%) and ampicillin 19(52.8%). *Pseudomonas* spp. was sensitive to cefepime 23(92.0%), ciprofloxacin 17(68.0%), gentamycin 17 (68.0%), imipenem 16(64.0%) while *Pseudomona*s spp. was resistant to ceftazidime 17(68.0%). *E. coli* were susceptible to cefoxitin 18 (81.1%), cefepime 17(77.3%) and show resistant to ampicillin 19(86.4%).

The study Do Tran *et al.,* (2022) revealed that *S. pneumonia*e was the primary agent responsible for CAP, and the Amoxicillin/clavulanic had the highest rate of resistance among β-lactams. Non- Enterobacteriaceae exhibited greater resistance to carbapenem than Enterobacteriaceae. *S.aureus was* extremely resistant to erythromycin (91.7%) and azithromycin (90%).

The results Hassanzadeh *et al.,* (2023) showed *that S. pneumoniae , S. aureus, P . aeruginosae, H. Influenzae , M. catarrhalis* , and *K. pneumoniae* have been isolated from the CAP patient population with varying frequencies and the cefepime was used to treat patients with CAP with Gram-negative bacteria.

The study Mussema *et al.,* (2023) showed among Gram-positive bacterial isolates, *S. aureus* was high resistance to penicillin (100%), trimethoprim-sulfamethoxazole 88.9%, and tetracycline 77.8%. While 77.8% of isolated *S. pneumoniae* was oxacillin-resistant, and 88.9% of isolated *S. aureus* was methicillin-resistant (MRSA).while Gram-negative isolates tested resistant to ampicillin (100%), cefuroxime (87.2%), and trimethoprim-sulfamethoxazole (89.1%) in antibiogram tests. A significant percentage of resistance to ampicillin (100%), trimethoprim-sulfamethoxazole (78.9%), and doxycycline (73.9%) was demonstrated by *Klebsiella* spp. Additionally , it was shown that third- and fourth-generation cephalosporin resistance was present in 47.4% to 68.4% of isolated *Klebsiella* species. 88.5% of isolated P . aeruginosa were found to be resistant to ceftazidime and cefepime, In addition, 29.4% of the isolated P . aeruginosa were meropenem-resistant.

The study Tilahun *et al*., (2023) showed that Gram-positive bacteria showed high levels of resistance to tetracycline 49 (87.5%), penicillin 48 (85.7%), trimethoprim-sulfamethoxazole 34 (78.6%) and chloramphenicol 37(66.1%). whereas 40 (71.4%) and 42 (75%) of the Gram-positive isolates were sensitive to clindamycin and oxacillin, respectively. Moreover, 57% of *S. aureus* isolates also showed resistance to methicillin.while the Majority of the Gram-negative isolates showed a resistance rate of 67 (88.2%) for tetracycline, 65 (88.2%) for Ampicillin , and 65 (87.8%) for Amoxicillin-Clavulanic acid. Rates of resistance of Gram-negative bacterial isolates against Ceftazidime, Tetracycline, Trimethoprim- Sulfamethoxazole, Chloramphenicol, Amoxicillin-Clavulanic acid, Cefotaxime, Amikacin, Ceftriaxone, Meropenem and Gentamicin ranged from 45 (37.8%)–67 (88.2%). However, Gram-negative bacterial isolates showed relatively low resistance against amikacin 45 (37.8) and meropenem 47 (39.5).

4.3. Immunological Factors

4.3.1. Hematological (C.B.C) Parameters.

Complete Blood count might be extremely useful since it is simple to carry out, accessible in all healthcare facilities, and the first-line laboratory test that is most frequently ordered in all clinical settings(Agnello *et al.*, 2021).

Table (4.5). showed that the WBC levels in the CAP and Sepsis due to CAP groups increased significantly compared to the control group. The mean and Std. Dev. of the three groups were 9.45±4.69, 14.70±7.29, and 6.90±1.16, respectively ($P. \leq 0.000$).

Furthermore, the Granulocytes count increased significantly in the CAP and Sepsis due to CAP patients compared to the control group. The mean and Std. Dev. were 7.27±3.78 for the CAP group, 12.63±5.76 for Sepsis due to CAP, and 4.02±0.79 for the control group ($P. \leq 0.000$).the Granulocytes percentage increased in the CAP, Sepsis due to CAP, and control groups. The mean and Std. Dev. were 77.02±10.22, 83.13±8.41, and 57.42±8.91, respectively ($P. \leq 0.000$).

The NLR increased significantly in the CAP and Sepsis due to CAP groups compared to the control group. The mean and Std. Dev. were 6.30±5.18 for CAP, 15.93±10.77 for Sepsis due to CAP, and 1.91±0.76 for the control group ($P. \leq 0.000$). The PLR also increased significantly in the CAP and Sepsis due to CAP groups compared to the control group. The mean and Std. Dev. were 226.61±189.62 for CAP, 252.74±203.45 for Sepsis due to CAP, and 115.99±38.13 for the control group ($P. \leq 0.007$).

Table 4.5:Comparison among study groups based on the studied factors

Test	Case	No.	Mean	S.D.	Min.	Max.	*P*. Value	LSD
(WBC)	CAP	60	9.45	4.69	1.60	25.10	0.000 ***	0.000
	Sepsis & CAP	20	14.70	7.29	1.60	29.85		0.000
	Control	40	6.90	1.16	4.90	9.70		
	Total	120	9.48	5.17	1.60	29.85		0.001
(GRA)	CAP	60	7.27	3.78	1.40	16.10	0.000***	0.000
	Sepsis & CAP	20	12.63	5.76	4.40	25.45		0.000
	Control	40	4.02	0.79	2.20	5.50		
	Total	120	7.08	4.58	1.40	25.45		0.000
(GRA %)	CAP	60	77.02	10.22	52.00	94.00	0.000***	0.000
	Sepsis & CAP	20	83.13	8.41	62.70	95.10		0.000
	Control	40	57.42	8.91	41.00	70.20		
	Total	120	71.51	13.92	41.00	95.10		0.015
(NLR)	CAP	60	6.30	5.18	4.97	7.63	0.000***	0.000
	Sepsis & CAP	20	15.93	10.77	10.89	20.97		0.000
	Control	40	1.91	.76	1.67	2.15		
	Total	120	6.44	7.35	5.11	7.77		0.000
(PLR)	CAP	60	226.61	189.62	177.62	275.59	0.007**	0.007
	Sepsis & CAP	20	252.74	203.45	157.52	347.95		0.006
	Control	40	115.99	38.13	103.792	128.18		
	Total	120	194.09	167.55	163.80	224.37		0.693

The significance value was indicated as * between the groups. The level of probability was indicated as *$P.\leq 0.05$,**$P. < 0.01$ ***,$P.\leq 0.001$,Data was presented as mean, SD: standard deviation, Min. :Minimum , Max.:Maximum statistical test: Kruskal Wallis test and Mann-Whitney Test , d.f: (2), n =120.

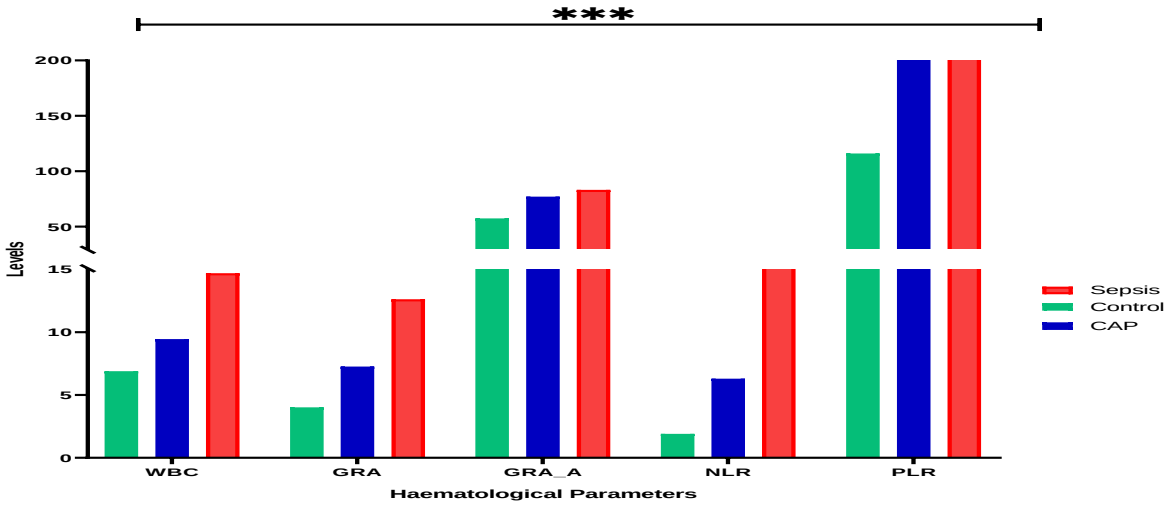


Figure 4.3:Comparison among study groups based on the studied factors.

The current study in Table (4.6) reveals an ROC curve of WBC, GRA, GRA % , NLR, and PLR, where the Area Under the Curve (AUC) of WBC was 0.745 , with a 95% CI of 0.656 - 0.833, The cut-off point for WBC was 8.05 with a sensitivity of 70% and a highly specific value of 90% at $p.\leq$ 0.000. Similarly, the AUC for the granulocyte (GRA) count was 0.850 with a 95% CI of 0.779-0.920. The cut-off point for GRA was 5.55, with a sensitivity of 71% and a strongly specific value of 100% at $p.\leq$ 0.000. The AUC for the percentage of GRA was also 0.938 with a 95% CI of 0.898-0.977. The cut-off point for the GRA percentage was 70.35, with a sensitivity of 76% and a specificity of 100% at $p.\leq$ 0.000.

The AUC for the neutrophil-to-lymphocyte ratio (NLR) was 0.914 with a 95% CI of 0.867-0.962, and the cut-off point for NLR was 3.48. The sensitivity was 69%, and the specificity was 100% at $p.\leq$ 0.000. Finally, the AUC for the platelet-to-lymphocyte ratio (PLR) was 0.674 with a 95% CI of 0.580-0.768. The cut-off point for PLR was 175.96, with a sensitivity of 46% and a specificity of 100% at $p.\leq$ 0.002 Figure (4.4). therefore, in Table (4.6) showed that WBC, GRA, GRA%, NLR and PLR are more diagnostic biomarkers than other C.B.C. parameters in the diagnosis of CAP , sepsis due to CAP patients compared to healthy control.

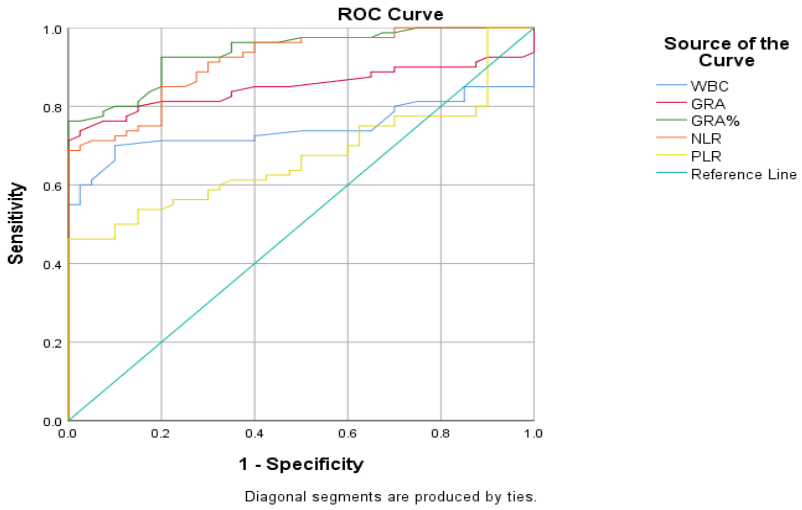


Figure 4.4: Receiver Operating Characteristic (ROC) Curves to the WBC , GRA , GRA% , NLR and PLR.

Table 4.6 : The Cut-off point of to the WBC , GRA , GRA% , NLR and PLR. levels for discrimination patients with CAP and sepsis due to CAP compared healthy groups

Test	Area (95% CI)	Cut off	Sensitivity	Specificity	*P*. value
WBC	0.745 (.656-.833)	8.05	70%	90%	.000***
GRA	0.850 (779- .920)	5.55	71%	100%	.000***
GRA%	0.938 (.898- .977)	70.35	76%	100%	.000***
NLR	0.914(.867-.962)	3.48	69%	100%	.000***
PLR	0.674(.580-.768)	175.96	46%	100%	.002**

The results of the current study agreed with the study Abed *et al*., (2018) observed highly significant differences in WBC , Granulocytes (%) and Lymphocytes(%) at p. value<0.001.between bacterial pneumonia and healthy groups .

The study CİZMECİOGLU *et al*., (2022) observed that WBC the value increased14.07±7.16 in pneumonia patients compared with healthy control 7.29±1.13 at *p*. value $\leq$ 0.00 1.

The study Kadim & AL-Dahmoshi, (2022) that conducted in Babylon Governorate showed the White blood cell (WBC), lymphocyte (LYM) and granulocyte (GRA) were at ($p.\leq$ 0.000), the estimation of WBC, granulocytes, lymphocytes count results may help in the early identification of the causative agent of sepsis. The findings succeeded in translating the overall idea, which relates to constantly updating the methods of sepsis diagnosis, which may lower the death rate and help patients recover quickly .

The study Hwang *et al*., (2017) showed Granulocytes. In the existence of an underlying infection, the response of the host is generally characterized by an increase in the neutrophil count and a decrease in the lymphocytes. Additionally, a change in the NLR may be used as a valuable prognostic marker.

During sepsis, multiple processes, including the activation of the coagulation system, cause the activation of platelets. The surface of activated platelets expresses many receptors that either directly bind to and sequester external pathogens or stimulate the aggregation of neighboring platelets and leukocytes (Guo & Rondina, 2019)

Neutrophil–lymphocyte ratio (NLR) is a simple, cheap ,and easy-to-use marker and its role in predicting the adverse outcome in patients with CAP (Kuikel *et al.*, 2022), .

Neutrophil–lymphocyte ratio is a readily accessible biomarker based on the calculation of the white cell blood count. This ratio has been used previously as a marker for the assessment of patients suffering from several clinical conditions(Ayça *et al*., 2015) ,(Kang ,*et al*.,2014).NLR was powerful prognostic biomarker with great utility in clinical practice, especially in low- and middle income countries with a public health system**(**Cataudella et al.,2017);(Cury *et al*., 2021)**.**

The study Azab *et al.*, (2011) showed NLR promising marker for predicting outcomes in patients with CAP. Its value, either alone or in conjunction with other biomarkers and scoring systems, must be further investigated. and the study Huang *et al.*, (2018) showed 80 patients with CAP and 49 healthy individuals that NLR and MLR were both elevated in the patient group and had a higher diagnostic value for CAP compared to other blood parameters. And the study by Akilli *et al*., (2014) aiming to evaluate the predictive effect of the NLR to mortality. The results concluded that the NLR was more easily accessible tool and warranted to evaluate patients with sepsis. particularly within a few hours after admission, in the critically ill patient group.

The study by Kartal & Kartal, (2017) found that NLR and PLR were significantly increased in CAP and our findings suggest that they can be used as a predictor for the presence of CAP.

4.3.2. Immunological Factors Study

The results in Table (4.7) illustrated that were significant differences in TLR2 ,CXCL3 and PCT at p. Value ≤ 0.000 and also observed not significant differences in TLR4and CCL7 at *p.* value ≤ 0.585 and 0.673 respectively.

Table 4.7:Immunological Factors to TLR2,TLR4,CXCL3,CCL7 and PCT

Test	Case	N	Mean	S.D.	Min.	Max.	*P*. Value	L.S.D.
PCT (ng/ml)	CAP	60	0.38	0.06	0.28	0.48	**0.000*** **	**0.000**
	Sepsis & CAP	20	9.34	13.66	1.23	50.00		**0.000**
	Control	40	0.08	0.06	0.01	0.20		
	Total	120	1.77	6.43	0.01	50.00		**0.000**
TLR2 (ng/ml)	CAP	60	4.36	4.17	0.18	16.23	**0.000*** **	**0.001**
	Sepsis & CAP	20	4.47	2.86	0.45	8.53		**0.000**
	Control	40	1.27	0.21	1.00	2.03		
	Total	120	3.35	3.48	0.18	16.23		**0.345**
TLR4 (ng/ml)	CAP	60	1.91	1.42	0.29	5.71	**0.585 n.s**	**0.944**
	Sepsis & CAP	20	2.10	1.09	0.48	3.47		**0.230**
	Control	40	1.94	1.80	0.27	7.11		
	Total	120	1.96	1.50	0.27	7.11		**0.447**
CXCL3 (ng/L)	CAP	60	355.71	172.86	112.93	705.00	**0.000*** **	**0.000**
	Sepsis & CAP	20	370.42	142.26	177.08	662.26		**0.000**
	Control	40	196.53	121.98	30.78	530.20		
	Total	120	305.10	169.98	30.78	705.00		**0.657**
CCL7 (ng/L)	CAP	60	249.08	301.32	5.02	1253.88	**0.673n.s**	**0.385**
	Sepsis & CAP	20	235.88	248.26	8.38	800.14		**0.826**
	Control	40	147.21	91.34	23.09	397.28		
	Total	120	212.92	244.52	5.02	1253.88		**0.649**

The significance value was indicated as * between the groups. The level of probability was indicated as *P ≤ 0.05,**P ≤ 0.01 *** P ≤ 0.001 , and n.s. : not significant ,Data was presented as mean, SD: standard deviation, L.S.D: Least Significant Differences , statistical test: Kruskal Wallis test and Mann-Whitney Test , d.f: (2), n =120.

Table (4.8) revealed an ROC curve of PCT, TLR2, TLR4, CXCL3, and CCL7, where the Area Under the Curve (AUC) of PCT was 1.0 (95% CI: 1.0-1.0), and the cut-off point for PCT levels was 0.24ng/ml. The sensitivity and specificity of PCT were both 100% at *p*. value ≤ 0.000 and the AUC for TLR2 concentration was 0.718 (95% CI: 0.624 - 0.812) with a cut-off point of 1.57. The sensitivity was 68%, and the specificity was 95% at *p*. value≤ 0.000. In contrast, the AUC for TLR4 concentration was 0.527 (95% CI: 0.419 - 0.635) with a cut-off point of 1.72. The sensitivity was 54%, and the specificity was 70% at *p*. value ≤ 0.000 the AUC of CXCL3 concentration was(95%CI) =0.805(.719-.891) and the cut-off point of CXCL3concentration 220.5 ng/L and the Sensitivity was 79% and the Specificity was 85 % at *p*. value ≤ 0.000).while the AUC of CCL7 concentration was (95%CI) =0.457(.354-.560) and the cut-off point of CCL7 concentration 408.9 ng/L and the Sensitivity was 31% and the Specificity was highly100% at *p*. value ≤ 0.444**.** therefore the PCT ,TLR2 , and CXCL3 concentration more biomarker in diagnostic the patients from TLR4 and CCL7 concentration by using ELISA test Figure (4.5).

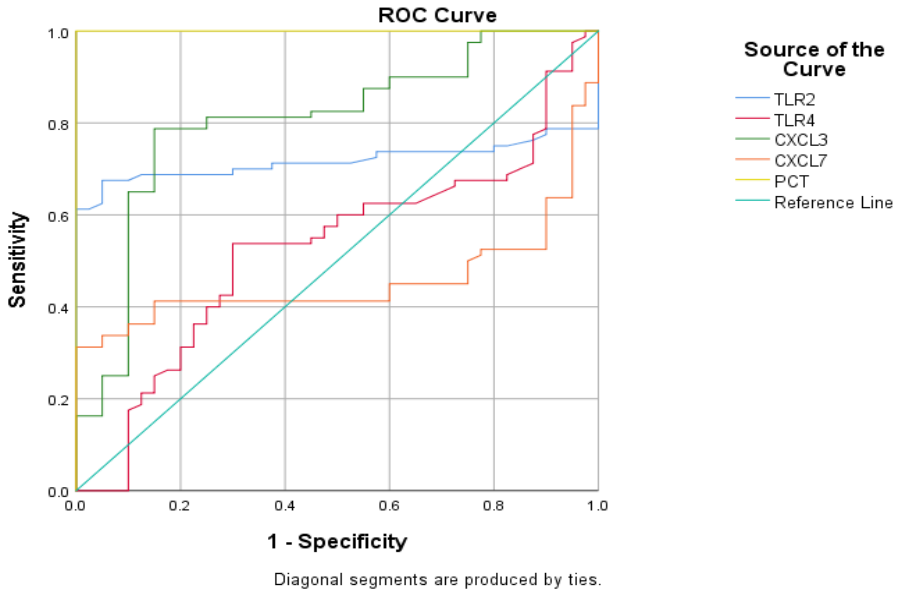


Figure 4.5: ROC Curve of to PCT, TLR2,TLR4,CXCL3 and CCL7

Table 4.8. ROC Curve of PCT ,TLR2, TLR4, CXCL3 and CCL7

Test	Area (95% CI)	Cut off	Sensitivity	Specificity	*P*.Value
PCT	1.0 (1.0-1.0)	0.24	100%	100%	**0.000*** **
TLR2	0.718(.624-.812)	1.57	68%	95%	**0.000*** **
TLR4	0.527(.419-.635)	1.72	54%	70%	**0.630 n.s**
CXCL3	0.805(.719-.891)	220.5	79%	85%	**0.000*** **
CXCL7	0.457(.354-.560)	408.9	31%	100%	**0.444 n.s**

4.3.2.1. Procalcitonin (PCT) Concentration .

According to the presented results which explain by Table (4.7) showed that a significant rising (*p*. value $\leq$ 0.000) in the serum blood mean levels of PCT increased for both patients groups of Sepsis due to CAP and CAP group . The mean was (0.38 ng/mL)± (0.06) for CAP group ,(9.34 ng/mL) ± (13.66) for sepsis due to CAP group and(0.08 ng/mL) ± (0.06) for the control group. and According to the current study that PCT more value biomarker diagnostic than C.B.C parameters and Immunological Factors (TLR2,TLR4, CXCL3 and CCL7) in diagnostic the patients . The results of ROC curve as s showed in Table(4.8).

The current study was agreed many of studies such as the study Güleç *et al.,* (2023) observed there were high PCT value to patients were with severe pneumonia or sepsis compared with mild pneumonia patients at (P. Value <0.001) and this marker used to detect severe CAP patients quickly and accurately**.** also the study Ghatas & Elfaizy, (2023) showed that measurement and detection of (PCT) were perfect in expecting the treatment efficacy and prognosis, alongside in assessment of pneumonia severity consequently, there is a consideration that PCT was as a marker in management of CAP patient and the price of antibiotics treatment may be decreased as antibiotics therapy might be altered or even introduced initially without use and continuance of ineffectual antibiotics**.**

The study by Ito *et al.*, (2020) were showed the majority of the researches concerning the effectiveness of measurement of PCT in CAP have concentrated on evaluation and prediction of mortality and limited researches have explored and assessed anti-microbial usefulness in the short term. And The study Abed *et al*.,(2018) showed the PCT was able to differentiate bacterial pneumonia from other causative pneumonia, and PCT test could be a promising marker for detection of bacterial pneumonia assists and facilitate the early diagnosis of bacterial pneumonia in addition to clinical characteristics and clinician practice.

The study Valencia, (2023) was reported the PCT usage in sepsis workups and antibiotic initiation or cessation. In general, the PCT was a positive predictor for progression to sepsis, more specific than standard lactic acid or CRP tests included in current protocols. moreover, Tian *et al*., (2021) showed the PCT was a high diagnostic value for sepsis patients, and other indicators can be used as an auxiliary diagnostic method for the death of sepsis patients.

Publications reviewing the effectiveness of sepsis biomarkers also noted that early initiation of antibiotics in patients with an elevated PCT helped to reduce mortality and length of hospital stay (Chow *et al*.,2021),(Niederman *et al*., 2021), (De Oro *et al*., 2019).

The general guideline surrounding intervention based on PCT is that antibiotics should be initiated in patients with an elevated PCT and should be discontinued when values begin to decline or normalize (Bartoletti *et al*.,2018),(Neeser *et al*.,2019),(Schuetz *et al*., 2019),(Gauer *et al*.,2020),(Kyriazopoulou *et al*.,2021),(Velissaris *et al*., 2021).

4.3.2.2 Toll Like Receptor 2 (TLR_2) Concentration(ng/ml) .

In Table(4.7) the mean of TLR_2 in CAP patients 4.36 ng/ml and SD (4.17) and the mean of TLR_2 in sepsis due to CAP patients 4.47 ng/ml and SD (2.86) while the mean of TLR_2 in Healthy group1.27 ng/ml and SD (0.21) therefore there were high significant differences between CAP and sepsis due to CAP Patients and healthy control at (*p*. value $\leq$ 0.000).

4.3.2.3. Toll Like Receptor 4 (TLR_4) Concentration(ng/ml) .

According to the presented results which explain by Table(4.7) showed that a significant rising (*p*. value $\leq$ 0.585) in the serum blood mean levels of TLR_4 increased for both patients groups of Sepsis due to CAP and CAP group compared with healthy group. The mean was in CAP patients 1.91 ng/ml and SD (1.42) and the mean of TLR_4 in sepsis due to CAP patients 2.10 ng/ml and SD (1.09) while the mean of TLR_4 in Healthy group 1.94 ng/ml and SD (1.80)**.**

Toll-like receptors (TLRs) are a recently described family of immune receptors involved in the recognition of PAMPs. The central role of TLR-2 and TLR-4 in microbial responses they may be implicated in the pathogenesis of human sepsis(Armstrong *et al*.,2004).

TLR2 has been reported to have very broad functions in infectious diseases and also in other diseases. TLR2 has been shown to have an important function in innate immunity, its usefulness as a therapeutic target in clinical application (Hu & Spaink, 2022)**.**

The increased concentration of TLR2 occurred as a result of an increase in the immune response as a result of the body's exposure to pathogenic factors such as bacteria, where the body's cells secrete receptors to recognize these pathogenic factors, and according to the results obtained from our study, there was an increase in Gram-Positive Bacteria compared to Gram- Negative Bacteria , while the cell wall of Gram-positive bacteria contains a layer of peptidoglycan, which recognizes the TLR2 receptor,

while the cell wall of Gram-Negative Bacteria contains a layer of LPS, which recognizes TLR4.

4.3.2.4. Chemokine X C Ligand 3 ($CXCL_{3)}$ Concentration(ng/L).

In Table(4.7)the mean of $CXCL_3$ in CAP patients 355.71 ng/L and SD (172.86) and the mean of $CXCL_3$ in sepsis due to CAP patients 370.42 ng/L and SD(142.26) while the mean of $CXCL_3$ in Healthy group 196.53 ng/L and SD (121.98) therefore there were high significant differences between CAP and sepsis due to CAP Patients and healthy control at (*p*. value $\leq$ 0.000).

4.3.2.5. Chemokine C Ligand 7 (CCL_7) Concentration(ng/L).

According to the presented data which explain by Table (4.7) showed that a significant rising (*p*. value $\leq$ 0.673) in the serum blood mean levels of CCL_7 increased for both patients groups CAP and of Sepsis due to CAP group compared with healthy group. The mean was in CAP patients 249.08 ng/L and SD 301.32 and the mean of CCL_7 in sepsis due to CAP patients 235.88 ng/L and SD 248.26 while the mean of CCL_7 in Healthy group 147.21 ng/L and SD 91.34 .

Chemokines are crucial inflammatory mediators needed during an immune response to clear pathogens. However, their excessive release is the main cause of hyper-inflammation(Khalil *et al.*, 2021). Also they play a central role in the development and homeostasis of the immune system, and are involved in all protective or destructive immune and inflammatory responses. Classically viewed as inducers of directed chemotactic migration, it is now clear that chemokines can stimulate a variety of other types of directed and undirected migratory behavior .The key role of chemokines lies in inflammation and the body's immune response (Hughes & Nibbs, 2018). They have a high concentration of peptides in circulation, and they suppress

local inflammation by desensitization. the chemokine family is critical in the pathology of sepsis (Doganyigit *et al.*, 2022).

The current study was agreed with the study Al-Alwan *et al.*, (2013) were found that CXCL3 may regulate the remodeling of airway smooth muscle in asthmatic subjects. Moreover, Qu *et al.*, (2023) were showed that Macrophages, osteoblasts, airway epithelial cells, and dendritic cells secrete CXCL3. In addition, CXCL3 promotes blood vessel formation, tumor cell growth, cancer cell migration, cluster of differentiation (CD31) vascular cell infiltration, and smooth muscle cell migration.

The study Choi *et al.*, (2004)showed that CCL7 was highly expressed in biopsies and pulmonary fibroblast lines obtained from patients with upper lope(ULP) pneumonia relative to patients with other lower lope pneumonia (LLP) and patients without IIP, and that this CC chemokine may have a major role in the progression of fibrosis in this IIP patient group**.** Moreover, Mercer *et al*., (2014) reported CCL7 exacerbates the deterioration of various disorders, like acute neutrophilic lung inflammation and pulmonary fibrosis.

The study Liu *et al*., (2018) showed CCL7was expressed in many types of cells under physiological conditions, including in stromal cells, airway smooth muscle cells, and keratinocytes, and in tumor cells under pathological conditions. CCL7 is a potent chemoattractant for a variety of leukocytes, including monocytes , eosinophil, basophils, dendritic cells (DCs), NK cells and activated T lymphocytes. As a chemotactic factor, CCL7 recruits a leukocyte subtype to infected tissues to address pathologic invasion and fine-tune the immune response. However, abnormal increase of CCL7 exacerbates the deterioration of various disorders, like lesional psoriasis, acquired immunodeficiency syndrome.

4.3.3.Molecular study of Immune-Related Gene (IRG)

The results in Table(4.9) and Figure (4:7,4:8,4:9,4:10,4:11,4:12,4:13,4:14, 4:15 , and 4:7) showed there were significant differences in *TLR2* , *TLR4* and *CD81* at (P≤0.000, 0.000 and 0.000) respectively. and also observed not significant differences in *HLA-DMA* at(P ≤ 0.056).

Table 4.9: Immune Related - Genes estimated by RT. RT-qPCR.

Test	Case	N	Mean	S. D.	Min.	Max.	*P*. Value	L.S.D.
TLR2F	CAP	60	71.13	100.10	0.40	329.99	**0.000*** **	**0.000**
	Sepsis & CAP	20	49.60	43.22	0.33	146.02		**0.000**
	Control	40	0.66	0.31	0.25	0.99		
	Total	120	48.69	79.63	0.25	329.99		**0.851**
TLR4F	CAP	60	19.11	30.38	0.43	133.56	**0.000*** **	**0.000**
	Sepsis & CAP	20	18.29	17.67	0.24	78.25		**0.000**
	Control	40	0.53	0.29	0.20	0.97		
	Total	120	14.38	24.56	0.20	133.56		**0.198**
CD81F	CAP	60	191.88	338.53	0.19	1845.00	**0.000*** **	**0.000**
	Sepsis & CAP	20	93.15	122.15	0.75	439.59		**0.000**
	Control	40	0.68	0.32	0.04	0.94		
	Total	120	121.16	260.54	0.04	1845.00		**0.928**
HLA-DMAF	CAP	60	16.26	56.85	0.00	263.87	**0.056n.s**	**0.026**
	Sepsis & CAP	20	1.18	1.24	0.00	3.76		**0.718**
	Control	40	0.73	0.30	0.03	0.98		
	Total	120	8.80	41.17	0.00	263.87		**0.112**

The significance value was indicated as * between the groups. The level of probability was indicated as *$P. \leq 0.05$,**$P. \leq 0.01$ *** $P. \leq 0.001$, and n.s. : not significant Data was presented as mean, SD: standard deviation, L.S.D: Least Significant Differences , statistical test: Kruskal Wallis test and Mann-Whitney Test , d . f: (2), n =120.

Table (4.10) reveals an ROC curve of TLR2F, TLR4F, CD81F , and HLA-DMA , where the AUC for gene expression TLR2F level was 0.965 (95% CI: 0.926-1.0) with a cut-off point of 1.23. The sensitivity was 95%, and the specificity was 100% at *P*. value ≤ 0.000. On the other hand, the AUC for gene expression TLR4F level was 0.973 (95% CI: 0.939-1.0) with a cut-off point of 1.35. The sensitivity was 95%, and the specificity was 100% at *P*. value ≤ 0.000 .

The AUC of CD81 F was(95%CI) =0.939(.885-.993)and the cut-off point of CD81F level 2.57 and the Sensitivity was 92% and the Specificity was 100% at *P*. value ≤ 0.000).while the AUC of gene expression HLA-DMAF level was (95%CI) =0.631(.510- .751)and the cut-off point of 1.0 and the Sensitivity was 60% and the Specificity was 100% at *P*. value ≤ 0.080 Figure (4.6).

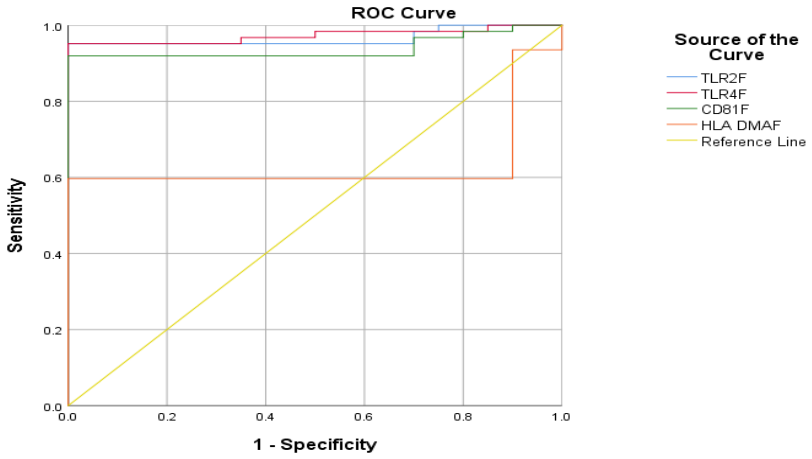


Figure 4.6: Immune Related Genes(IRG) estimated by Reverse Transcriptase RT-qPCR

Table 4.10:ROC of Immune Related - Genes estimated by Reverse Transcriptase RT-qPCR.

Test	Area (95% CI)	Cut off	Sensitivity	Specificity	P.Value
TLR2F	0.965(.926-1.0)	1.23	95%	100%	**0.000*** **
TLR4F	0.973(.939-1.0)	1.35	95%	100%	**0.000*** **
CD81F	0.939(.885-.993)	2.57	92%	100%	**0.000*** **
HLA-DMAF	0.631(.510- .751)	1.0	60%	100%	**0.080***

4.3.3.1Gene Expression of Toll - Like Receptor2(TLR2F) Fold Change.

According to the presented results which explain by Table (4.9) showed that a significant rising (*P*. value $\leq$ 0.000)in the mean levels of TLR_2F increased for both patients groups of CAP and Sepsis due to CAP compared with healthy group. The mean was in CAP patients (71.13) and SD (100.10) and the mean of TLR_2F in sepsis due to CAP patients (49.60) and SD (43.22)while the mean of TLR_2F in Healthy group (0.66) and SD (0.33).

Amplification and Melting Curve of House Keeping Gene (*H.K.G*) by RT -q RT- PCR

a- Amplification

b- - Melting Curve

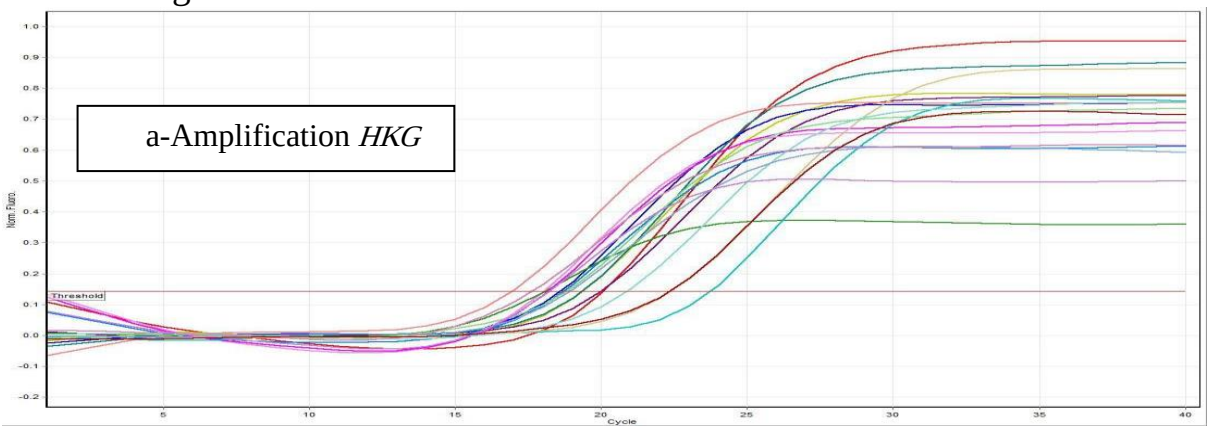


Figure 4.7 :Amplification Analysis by RT -q RT- PCR to *HKG*.

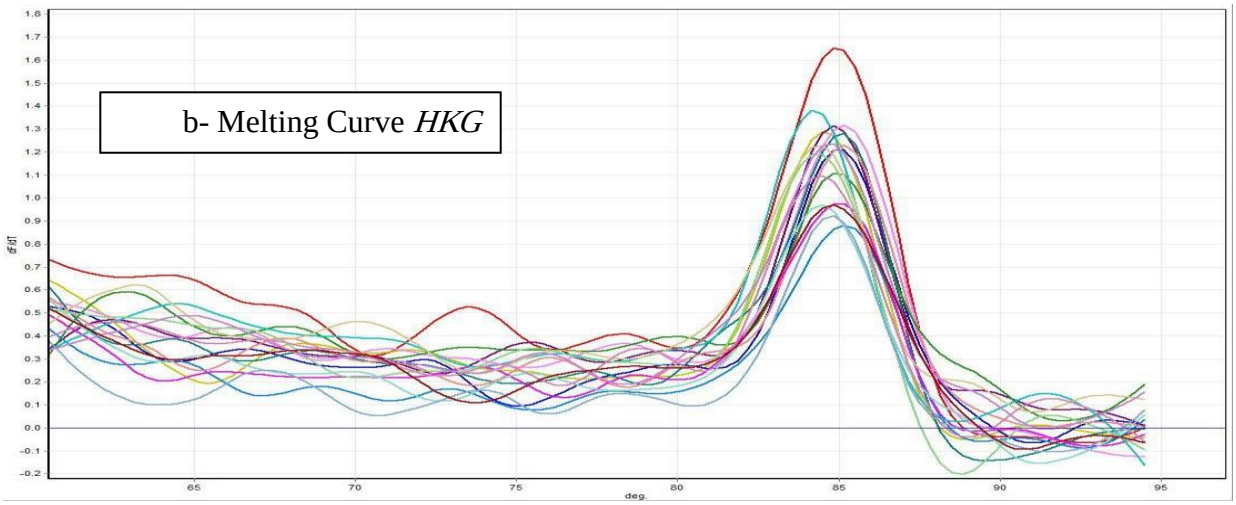


Figure 4.8: Melting Curve by RT –q RT -PCR to *HKG*.

Amplification and Melting Curve of Toll Like Receptor2 (*TLR2*) by RT-q RT - PCR

a- Amplification

b- Melting Curve

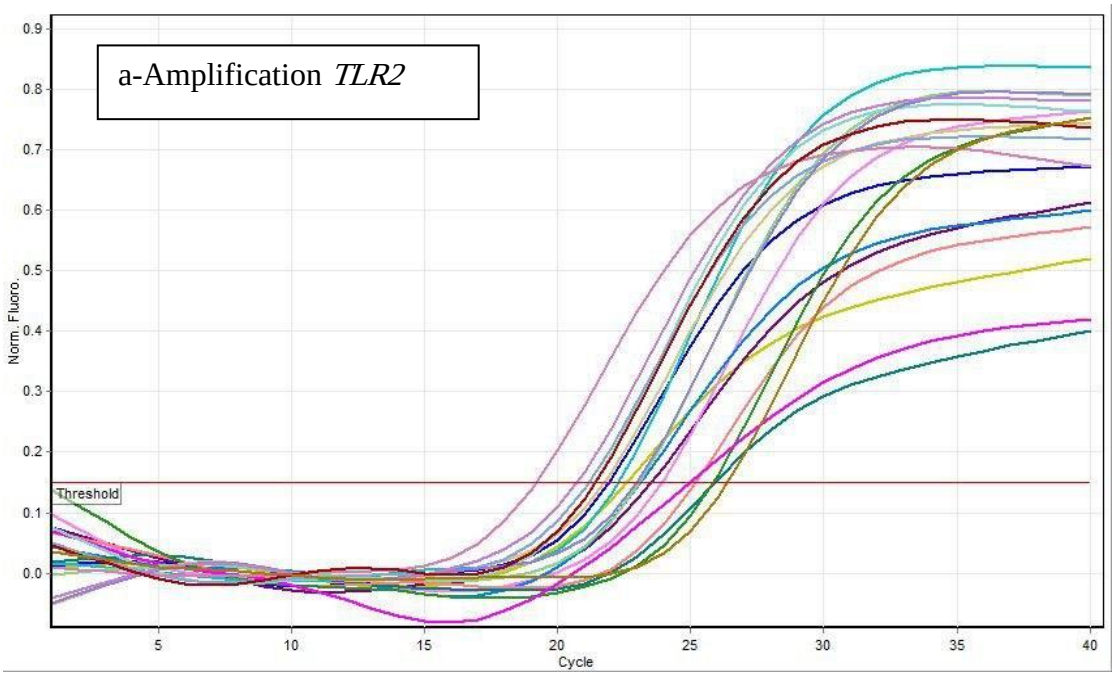


Figure 4.9: Amplification analysis to *TLR2*by RT- q RT- PCR.

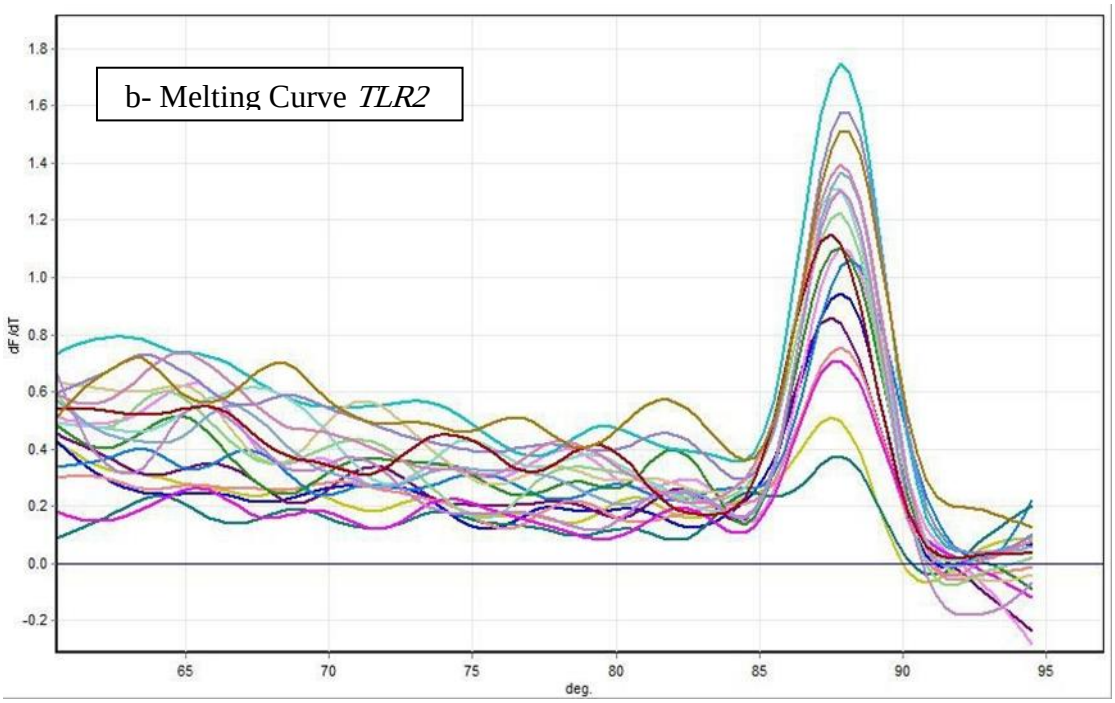


Figure 4.10: Melting Curve to *TLR2* by RT- q RT –PCR

The current study results was agreed with number of studies. where, the study Knapp *et al*., (2004) showed the contribution of *TLR2* in Gram-positive pneumonia and investigated the requirement for *TLR2* during infection with *S. pneumonia*e. Also the study Muzio *et al.*, (2000) detected the highest *TLR2* mRNA levels in the Gram-positive population where LPS presence, although unlikely to be absent, was minimal, suggesting that some other factor or a combination of factors in the sepsis. And results Takeuchi *et al.*, (1999) showed the *TLR2* plays a major role in Gram-positive bacterial recognition.

The study Tang *et al.*, (2014) showed that Increased *TLR2* and *TLR4* expression levels were detected in elderly patients with severe pneumonia compared with healthy control . Moreover, The study by Armstrong *et al*., (2004) showed the *TLR2* mRNA was significantly up-regulated on monocytes from subjects with both Gram-positive and increased levels of *TLR2* protein on the surface of monocytes from sepsis ,Therefor. The expression and regulatory response of monocyte *TLR2*, and to a lesser extent *TLR4* may be abnormal in human sepsis. also the study Severino *et al*., (2014) showed that Patients admitted with sepsis secondary to CAP exhibit a gene induction profile when compared to healthy controls. Specifically genes clustered in host defense and inflammatory response ontology were up-regulated during sepsis, consistent with the needs for a host response to infection.

4.3.3.2.Gene Expression of Toll-Like Receptor4(TLR4F) Fold Change.

In Table (4.9)the mean of TLR_4 F in CAP patients (19.11) and SD (30.38) and the mean of TLR_4 F in sepsis due to CAP patients 18.29 and SD 17.67 .while the mean of TLR_4F in Healthy group (0.53) and SD (0.29) therefore there are significant differences between sepsis due to CAP patients and CAP Patients and healthy at (*P*. value ≤0.000). This results illustrated in Figure(4.13).

therefore, the results explained in Table (4.10) showed that gene expression levels of *TLR2* F and *TLR*4 F by using (qRT–PCR) were more valuable diagnostic markers than *TLR2* and *TLR4* concentration by using ELISA test for the diagnosis the patients with CAP and Sepsis due to CAP that explained previously in Table (4.9).

Amplification and Melting curve of Toll Like Receptor4 (*TLR4*) by RT-q RT-PCR

a- Amplification

b- Melting Curve

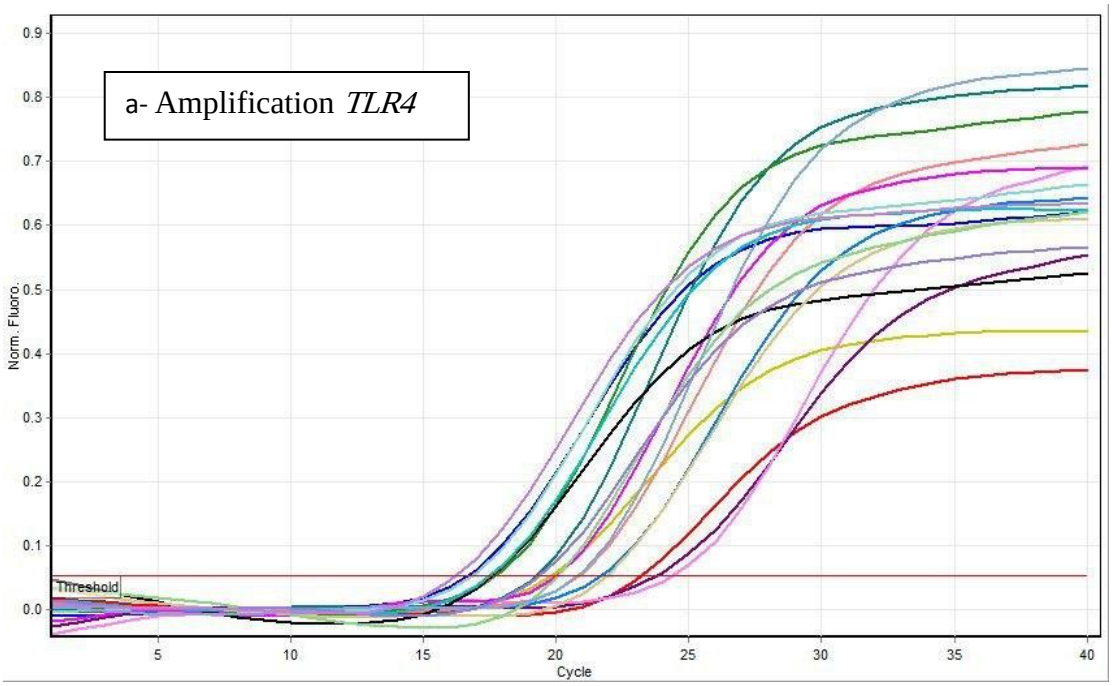


Figure 4.11 Amplification Analysis to *TLR4* by RT -q RT- PCR

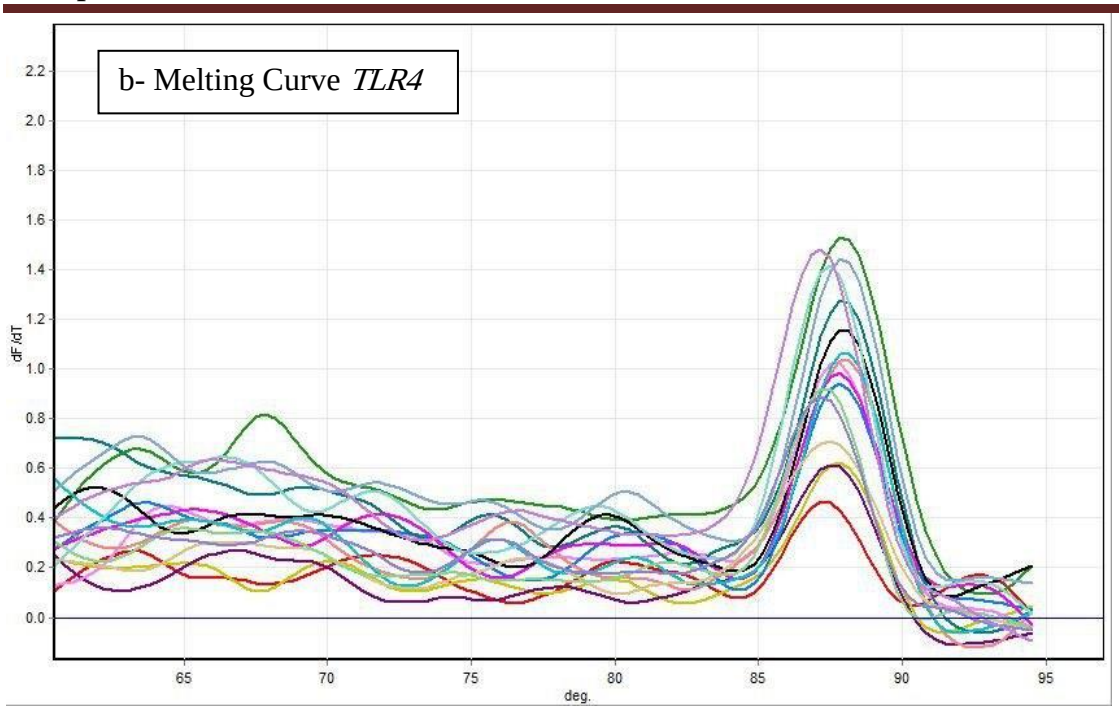


Figure 4.12:Melting Curve to *TLR4* by RT -q RT- PCR

The current study results was agreed with number of studies. the study by Ding & Liu, (2019) showed the *TLR4* role in GNB pneumonia by signaling was involved in a variety of physiological and pathological processes in human body, and thus, the specificity of *TLR4*-targeted therapy may be the biggest challenge in the clinical . Gram-negative bacteria (GNB) emerge as important pathogens causing pulmonary infection, which can develop into sepsis due to bacterial resistance to antibiotics. GNB pneumonia poses a huge social and economic burden all over the world. During GNB infection in the lung, Toll-like receptor 4 (*TLR4*) can form a complex with MD2 and CD14 after recognizing lipopolysaccharide of GNB.

The result by Skjesol *et al.,* (2019) were showed the Toll-like receptor 4 (*TLR4*) was expressed on the surface of macrophages and recognizes Gram-negative bacteria. Moreover, *TLR4* has been suggested to play a role in the phagocytosis of Gram-negative bacterial . *TLR4* is a key sensing receptor of lipopolysaccharide on Gram negative bacteria.

The study Bhan *et al.,* (2010) showed that *TLR4* was distinct time dependent and interactive functions during the development of protective innate antibacterial immunity in the lung.

In the current study, it was demonstrated that gene expression levels of TLR2F and TLR4F, assessed using quantitative Real-Time Polymerase Chain Reaction (qRT-PCR), were found to serve as more valuable diagnostic markers compared to determining TLR2 and TLR4 concentrations through an Enzyme-Linked Immunosorbent Assay (ELISA) for diagnosing patients with Community-Acquired Pneumonia (CAP) and Sepsis due to CAP. The findings presented in Table (4.10) highlight that the gene expression of the TLR4 gene, estimated using RT- Real-Time PCR, showed a highly significant p-value of 0.013. In contrast, the quantitative estimation of TLR4 in blood serum using the ELISA technique did not show significant results with a p-value of 0.889. This discrepancy may be attributed to the direct impact of the causative agent responsible for the infection on the gene expression of TLR4. The infection may lead to an increase in the gene expression of TLR4, which would be responsible for instigating the infection.

The study revealed that a majority of patients who developed the infection had Gram-positive bacterial origins, eliciting a higher response from TLR2. In contrast, a smaller percentage of patients presented with Gram-negative bacterial infections, showing a heightened response from TLR4. Additionally, it is worth noting that the measurement of TLR4 was conducted in its soluble form rather than its bound form, which may have resulted in a lower concentration in blood serum compared to its bound form.

4.3.3.3.Gene Expression of Cluster Differentiation 81(CD81F)Fold Change.

According to the presented data which explain by the Table (4.9) showed that a significant rising at (*P*. value $\leq$ 0.000) in the mean levels of *CD81*F increased for both patients groups of Sepsis due to CAP and CAP group compared with healthy group. The mean of *CD81*F was in CAP patients 191.88 and SD 338.53 and the mean of CD81F in sepsis due to CAP patients 93.15 and SD(122.15) while the mean of *CD81*F in Healthy group 0.68 and SD(0.32) .

Amplification and Melting Curve of *CD81* by RT- q RT -PCR

a- Amplification

b- Melting Curve

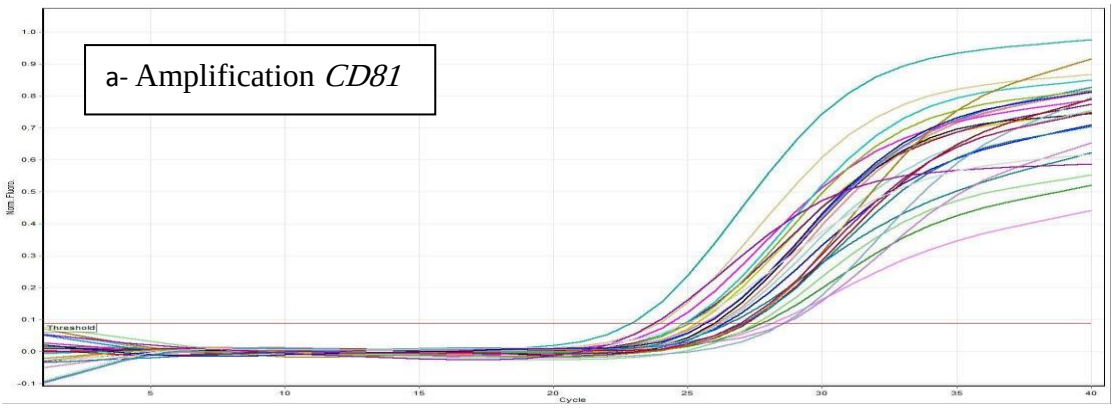


Figure 4.13:Amplification Analysis to *CD81* by RT -q RT- PCR

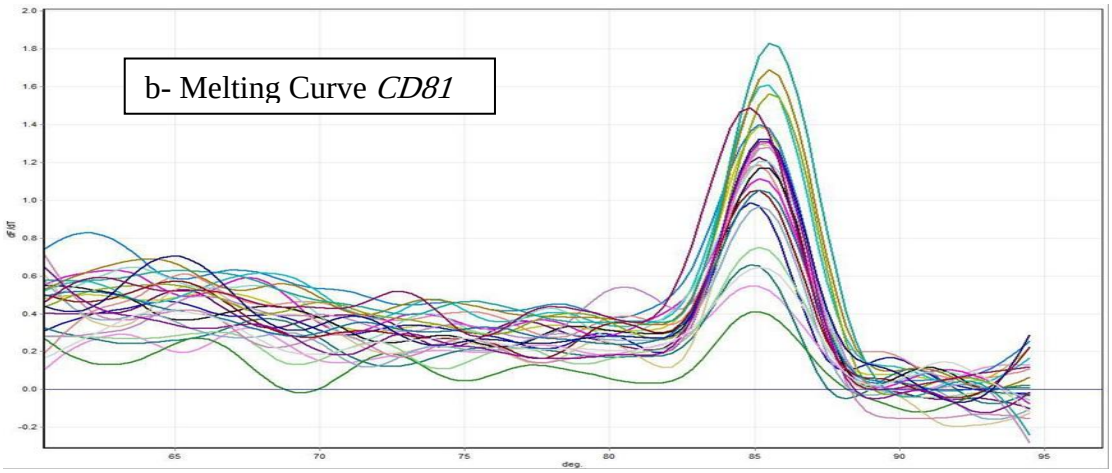


Figure 4.14: Melting Curve to *CD81* by RT- q RT- PCR

The study by Chen *et al.*, (2022) Regarding the extremely high mortality caused by sepsis-induced acute respiratory distress syndrome (ARDS) and showed that *CD81* may play critical roles in the biological and immunological processes of sepsis-induced ARDS. and this result agreed with our study.

4.3.3.4 Gene Expression of Human Leukocytes Antigen DMA(HLA - DMA)Fold Change.

In Table (4.9) the mean of *HLA- DMA* F. in CAP patients 16.26 and SD 56.85 and the mean of *HLA- DMA* F. in sepsis due to CAP patients 1.18 and SD 1.24 while the mean of *HLA- DMA* F.in Healthy group 0.73 and SD 0.30 therefore there are not significant differences between sepsis due to CAP patients and CAP Patients and healthy at (*P*. value $\leq$ 0.056).

Amplification and Melting curve of *HLA- DMA* by RT q RT- PCR

a- Amplification

b- Melting Curve

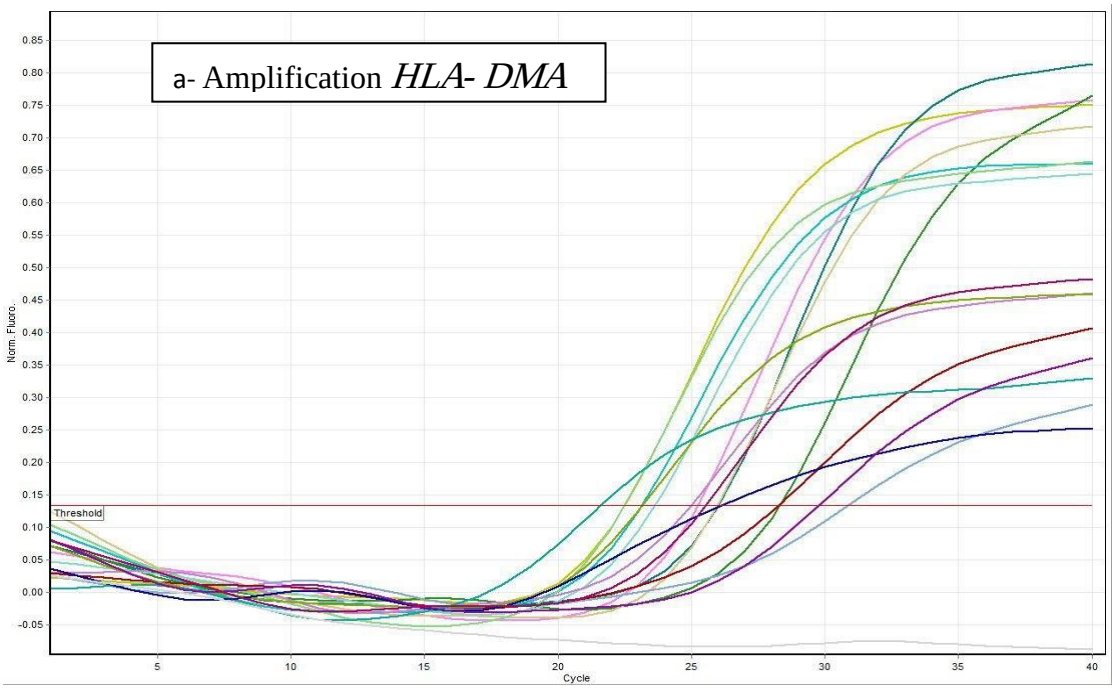


Figure4.15Amplification Analysis to *HLA- DMA* by RT- q RT –PCR.

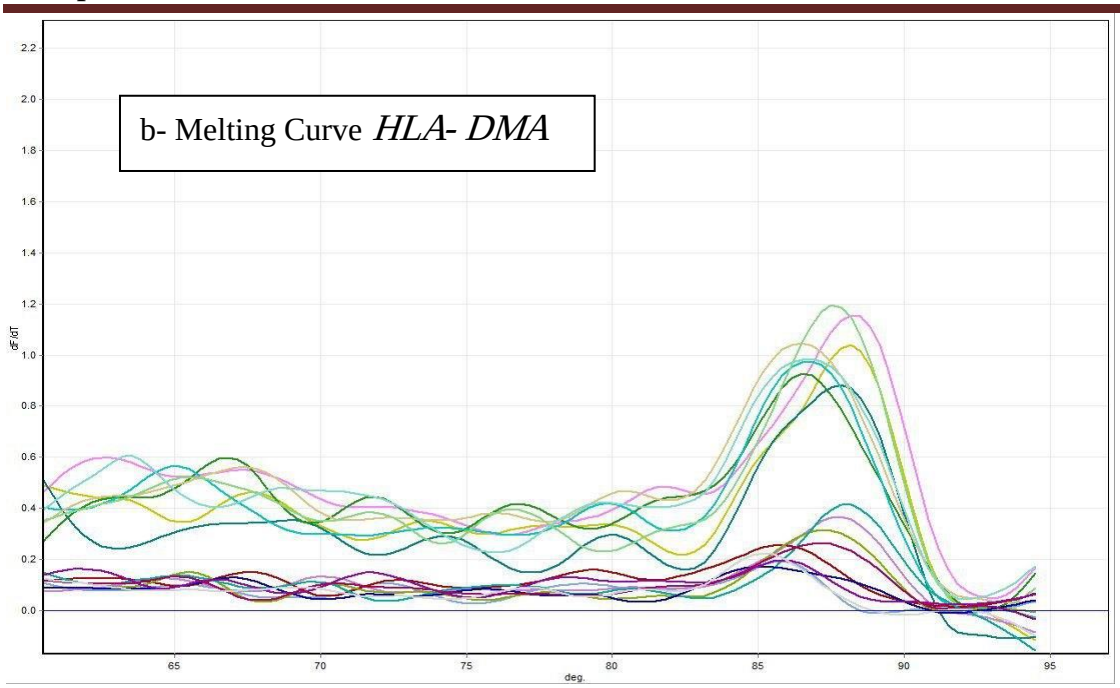


Figure 4.16: Melting Curve to *HLA- DMA* by RT q RT -PCR

The study by Li *et al.*, (2021) were showed the *HLA-DM*, encoded by *HLA-DMA* and *HLA-DMB*, is a heterodimeric molecule that is important for normal antigen presentation. and the low level of PDE4D expression was associated with *HLA-DMA* and *HLADMB. HLA-DMA* might participate in the mechanism of Community Acquired Pneumonia (CAP) with sepsis. and this result agreed with our study.

Conclusions and Recommendations

Conclusions

The present study has reaveled the following conclusions:

1- CAP and sepsis due to CAP patients could be multifactorial , whether G+ ve Bacteria or G-ve Bacteria mainly *Streptococcus pneumoniae* was the most common bacterial pathogen in patients with CAP and sepsis due to CAP. Thus, Gram-positive bacteria are the most common infection in these patients

2- Blood culture failed to detect the cusative agent of sepsis due to CAP patients due to low number of samples , previous treatment with antibiotics, it was not used more one bottle at different times, and anaerobic cultivation methods were not used.

3- Most CBC parameters were significant elevated .Thus,it can be considered as marker for Diagnosis,Managment and Prognostic of the patients.

4- Procalcitonin level was an important tool in diagnosting sepsis patients

5- PCT was more excellent diagnostic Biomarkers than C.B.C. parameters and Immunological Factors in diagnosis of patients.

6- Immunological Factors TLR2, and CXCL3 can be used as excellent diagnostic Biomarkers for CAP and sepsis due to CAP patients.

7- Immunr – Related Genes such as (TLR2F, TLR4F and CD81F) can be used as excellent diagnostic Biomarkers for CAP and sepsis due to CAP patients.

Recommendations

The study recommended the following :

1- Another study may done to detect causative microorganisms such as fungal, viral, parasites , and atypical pneumonia
2- Use of molecular methods to detect bacterial agents in diagnosis of CAP and sepsis due to CAP.
3- Recombined comparative study in causative microbial, some of immune related genes and immunological Factors between Community - Acquired Pneumonia (CAP), Hospital - Acquired Pneumonia (HAP) and Ventilation - Acquired Pneumonia (VAP).
4- Use many other immunological Factors related to CAP and sepsis due to CAP
5- Further studies are needed to identify other immune related genes.
6- Finding effective ways to prevent disease

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APPENDICES

Appendix (1):Comprehensive Examination Committee

Republic of Iraq
Ministry Of Higher Education and Scientific Research
College of Science – University of Karbala
Division of Postgraduate Studies

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة كربلاء – كلية العلوم
شعبة الدراسات العليا

العدد/ع/6/ 2416
التاريخ/ 8/ 8 /2022

أمـــر إداري

استناداً إلى مصادقة السيد رئيس الجامعـــة المحترم على أصـــل محضـــر الجلسة السادسة عشر لمجلس كليتنا للعـــام الدراسي 2021-2022 والمنعقدة بتاريخ 2022/7/7 والمرسل إلينا بكتاب امانة مجلس الجامعة ذي العدد /ج/ 978 بتاريخ 2022/7/27 والمبلغ الينا بكتاب امانة مجلس الكلية ذي العدد م/ك/ 306 بتاريخ 2022/7/28 واستنادا للصلاحيات المخولة لنا تقرر:
تشكيل لجنة من التدريسيين المدرجة أسماؤهم في الجدول أدناه لاجراء الامتحان الشامل لطالب الدراسات العليا/ الدكتوراه/ قسم علوم الحياة **(جعفر سلمان جاسم).**

على ان يكون موعد الامتحان التحريري في يوم الاثنين الموافق 2022/10/10 والامتحان الشفهي في يوم الاثنين الموافق 2022/10/24.

ت	اسم التدريسي	اللقب	المادة الامتحانية المقترحة	المنصب	مكان العمل
1	د.حيدر هاشم محمد علي	استاذ	Advanced Immunology	رئيساً	كلية العلوم/جامعة كربلاء
2	د.جواد كاظم طراد	استاذ	Respiratory tract infections	عضواً	كلية الطب/جامعة بابل
3	د.اسماعيل حسين عزيز	أستاذ مساعد	Gene Expression	عضواً	معهد الهندسة الوراثية/جامعة بغداد
4	د.ابتسام عباس ناصر	أستاذ مساعد	Blood physiology	عضواً	كلية العلوم/جامعة كربلاء
5	د.رحاب محمد جاسم	أستاذ مساعد	Medical enzymes	عضواً	كلية التربية للعلوم الصرفة/جامعة كربلاء

أ. م.د. جاســـم حنون هاشـــم العوادي
العمـــــيد وكالة
2022 / 8 / 7

نسخة منه إلى
- رئاسة الجامعة / قسم الدراسات العليا / للعلم.. مع التقدير..
- مكتب السيد العميد / للتفضل بالعلم مع التقدير..
- جامعة كربلاء/كلية العلوم/ للتفضل بالموافقة على إشراك الأستاذ الدكتور حيدر هاشم محمدعلي ... مع التقدير..
- جامعة بابل/ كلية الطب / للتفضل بالموافقة على إشراك الاستاذ الدكتور جواد كاظم طرد ...مع التقدير..
- جامعة بغداد/ معهد الهندسة الوراثية/ للتفضل بالموافقة على إشراك الأستاذ المساعد الدكتور إسماعيل حسين عزيز... مع التقدير..
- جامعة كربلاء/ كلية العلوم / للتفضل بالموافقة على إشراك الأستاذ المساعد الدكتور ابتسام عباس ناصر ...مع التقدير..
- جامعة كربلاء/ كلية التربية للعلوم الصرفة/ للتفضل بالموافقة على اشراك الأستاذ المساعد الدكتور رحاب محمد جاسم...مع التقدير
- حسابات الكلية /لاجراء اللازم فيما يخص المتعلقات المالية.
- السيد رئيس قسم علوم الحياة مع التقدير.
- الدراسات العليا مع الأوليات.
- الرقابة والتدقيق الداخلي.
- الصادرة

أ.م.د. زمان حميد كريم
مسؤول شعبة الدراسات العليا
E-mail science@uokerbala.edu.iq :

طباعة :A.Khalaf 2022/08/04

العنوان : العراق- محافظة كربلاء المقدسة – المدينة الجامعية – كلية العلوم ص.ب. 1125

Appendix (2): Ethical Approval of Study

جمهورية العراق
وزارة الصحة والبيئة
دائرة صحة محافظة بابل
المدير العام
مركز التدريب والتنمية البشرية
لجنة البحوث

Ministry Of Health
Babylon Health Directorate
Email:-
Babel_Healthmoh@yahoo.com
Tel:282628 or 282621

استمارة رقم :-٠٣/ ٢٠٢١

رقم القرار :- ١٢٠
تاريخ القرار :- ٦/١٢/ ٢٠٢٢

وزارة الصحة
دائرة صحة بابل
مركز التدريب والتنمية البشرية
لجنة البحوث

قرار لجنة البحوث

تحية طيبة ...

درست لجنة البحوث في دائرة صحة بابل مشروع البحث ذي الرقم (٢٠٢٢/٠١٣٨ بابل)
المعنون:- (study of some immune-related genes and immunological factors in patients with sepsis due to community –acquired pneumonia)

والمقدم من الباحث(جعفر سلمان جاسم) إلى وحدة ادارة البحوث والمعرفي مركز التدريب والتنمية البشرية في دائرة صحة بابل بتاريخ ٢٠٢٢/١٢/٦ وقررت :

قبول مشروع البحث أعلاه كونه مستوفيا للمعايير المعتمدة في وزارة الصحة والخاصة بتنفيذ البحوث ولا مانع من تنفيذه في مؤسسات الدائرة .

مـــــع الاحتـــرام

وزارة الصحة
دائرة صحة بابل
مركز التدريب والتنمية البشرية

الدكتور / محمد عبد الله عجرش
رئيس لجنة البحوث
٢٠٢٢/ /

نسخة منه إلى:
• مكتب المدير العام / مركز التدريب والتنمية البشرية / وحدة إدارة البحوث ... مع الأوليات.

babiltrainning@gmail.com دائرة صحة محافظة بابل / مركز التدريب والتنمية البشرية // ايميل المركز

وزارة الصحة
دائرة صحة كربلاء
مركز التدريب والتنمية البشرية
لجنة البحوث

استمارة رقم ٢٠٢١/٠٣
رقم القرار ٢٤٢
تاريخ القرار ٧/١٢/٢٠٢٢

قرار لجنة البحوث

درست لجنة البحوث في دائرة صحة كربلاء مشروع البحث ذي الرقم (٢٠٢٢.٢٢٣/كربلاء) المعدون
لإنجاز بحثه الموسوم
(دراسة بعض الجينات المرتبطة بالمناعة والعوامل المناعية لدى مرضى الالتهاب الرئوي المكتسب من المجتمع)
والمقدم من الباحثة :- (جعفر سلمان جاسم)

الى شعبة ادارة المعرفة / وحدة ادارة البحوث في مركز التدريب والتنمية البشرية في دائرة صحة كربلاء
بتاريخ ٢٠٢٢/١٢/٧ وقررت:

قبول مشروع البحث اعلاه كونه مستوفيا للمعايير المعتمدة في وزارة الصحة والخاصة بتنفيذ البحوث ولا مانع من تنفيذه في مؤسسات الدائرة.

مقرر لجنة البحوث
07/12/2022

دائرة صحة كربلاء لجنة البحوث

المرفقات:
Choose an item.

ملاحظات:
- تم تخويل عضو لجنة البحوث (د.تقوى خضر عبد الكريم) او مقرر اللجنة (د.نعيم عبيد طلال) للتوقيع على هذا القرار استنادا الى النظام الداخلي للجنة البحوث.
- الموافقة تعني ان مشروع البحث قد استوفى المعايير الاخلاقية والعلمية لاجراء البحث والمعتمدة في وزارة الصحة. اما التنفيذ فيعتمد على التزام الباحث بتعليمات المؤسسة الصحية التي سينفذ فيها البحث.

جمهورية العراق
وزارة الصحة والبيئة
دائرة صحة محافظة بابل
المدير العام
مركز التدريب والتنمية البشرية
وحدة ادارة البحوث

Ministry Of Health
Babylon Health Directorate
Email:-
Babel_Healthmoh@yahoo.com
لأهل عراق افضل مستقبل ..ستتحمل معنا لترشيد استهلاك الطاقة الكهربائية والمحافظة على البيئة من التلوث

العدد :
التاريخ: ٢٠٢٣/ /

إلى / جامعة كربلاء/ كلية العلوم
م / تسهيل مهمة

تحية طيبة . .
بناءاً على الطلب المقدم من قبل الباحث طالب الدراسات العليا (جعفر سلمان جاسم) نود أعلامكم بان احد مشرفي بحث الموما أليه هو الطبيب الاستشاري أمراض الصدر والجهاز التنفسي (منقذ عبد المحسن كاظم الجنابي)

للتفضل بالاطلاع .... مع الاحترام .

وزارة الصحة
دائرة صحة بابل
مركز التدريب والتنمية البشرية

للطباعة بالاختصاص
الدكتور محمد عبد الله عجرش
مدير مركز التدريب والتنمية البشرية
٢٠٢٣/ /

نسخة منه إلى:
• مركز التدريب والتنمية البشرية / وحدة ادارة البحوث .... مع الأوليات .

جمهورية العراق
وزارة الصحة كربلاء المقدسة
مركز التدريب والتنمية البشرية
شعبة ادارة المعرفة / وحدة ادارة البحوث

Holy Karbala governorate
Karbala Health Department
General manager's office
Training and Human Development Center

العدد ٦١٠٧
التاريخ / ٢٠٢٢
١٤/١١

الى / جامعة كربلاء / كلية العلوم
الموضوع /تسهيل مهمة

تحية طيبة...

كتابكم المرقم ع/٦/٤٣٤٥ في ٢٠٢٢/١١/٤
نود إعلامكم بأنه لا مانع لدينا من تسهيل مهمة طالب الدكتوراه(جعفر سلمان جاسم) لإنجاز بحثها الموسوم:
(دراسة بعض الجينات المرتبطة بالمناعة والعوامل المناعية لدى مرضى الالتهاب الرئوي المكتسب من المجتمع (تسمم الدم) النائج عن الالتهاب الرئوي المكتسب من المجتمع)
في مؤسساتنا الصحية وبأشراف الدكتور (حميد الحيالي) على ان لا تتحمل دائرتنا اي نفقات مادية مع الاحترام .

الدكتورة
تقوى خضر عبد الكريم
مدير مركز التدريب والتنمية البشرية
٢٠٢٢/ /

نسخة منه الى
مدينة الامام الحسين (عليه السلام)الطبية /لجراء اللازم مع الاحترام .
مستشفى الامام الحسن المجتبى (عليه السلام) /لجراء اللازم مع الاحترام
مدينة الامام الحسين (عليه السلام)الطبية / العيادة التخصصية للامراض الصدرية والتنفسية

Appendix (3) : Questionnaire Form

S/N	ID	
1.	Name	
2.	Sex	
3.	Age	
4.	Level of Education	
5.	Smoking Status	
5.	Marital Status	
6.	Name of Hospital Admitted	
7.	Date and Time	
8.	Duration of Disease	
9.	Social History	
10.	Personal History	
11.	Vaccination	
12.	Other Notes	

Appendix(4): Demographics, Vital Signs, and Medical History of Study Population

Variable		C.A.P.	Sepsis&C.A.P.	Control	P.Value
Temperature(n)%	Low	14(23.3)%	4(20.0)%	0(0.0)%	**0.000*** **
	Normal	22(36.7)%	8(40.0)%	40(100.0)%	
	High	14(23.3)%	4(20.0)%	0(0.0)%	
	Afebrile	10(16.7)%	4(20.0)%	0(0.0)%	
Pulse Rate(n)%	Low	2(3.3)%	0(0.0)%	0(0.0)%	**0.000*** **
	Normal	49(81.7)%	10(50.0)%	40(100.0)%	
	High	9(15.0)%	10(50.0)%	0(0.0)%	
RR SPO2(n)%	Low	22(36.7)%	14(70.0)%	0(0.0)%	**0.000*** **
	Normal	38(63.3)%	6(30.0)%	40(100.0)%	
Blood Pressure(n)%	Low	8(13.3)%	0(0.0)%	0(0.0)%	**0.000*** **
	Normal	15(25.0)%	17(85.0)%	40(100.0)%	
	High	37(61.7)%	3(15.0)%	0(0.0)%	
Married Slate(n)%	No	1(1.7)%	0(0.0)%	4(10.0)%	**0.074ns**
	Yes	59(98.3)%	20(100.0)%	36(90.0)%	
Pa. Occupation(n)%	Employed	44(73.3)%	12(60.0)%	12(30.0)%	**0.000*** **
	Ployed	16(26.7)%	8(40.0)%	28(70.0)%	
Academic Ache.(n)%	Primary	44(73.4)%	13(65.0)%	6(15.0)%	**0.000*** **
	Secondary	8(13.3)%	2(10.0)%	12(30.0)%	
	BAC	8(13.3)%	5(25.0)%	22(55.0)%	
Place(n)%	Rural	31(51.7)%	8(40.0)%	6(15.0)%	**0.001** **
	City	29(48.3)%	12(60.0)%	34(85.0)%	
SOB(n)%	No	2(3.3)%	1(5.0)%	40(100.0)%	**0.000*** **
	Yes	58(96.7)%	19(95.0)%	0(0.0)%	
Fever(n)%	No	0(0.0)%	0(0.0)%	40(100.0)%	**0.000*** **
	Yes	60(100.0)%	20(100.0)%	0(0.0)%	
Pro. Cough(n)%	No	0(0.0)%	0(0.0)%	0(0.0)%	**0.000*** **
	Yes	60(100.0)%	20(100.0)%	40(100.0)%	
Chest Pain (n)%	No	1(1.7)%	7(35.0)%	40(100.0)%	**0.000*** **
	Yes	59(98.3)%	13(65.0)%	0(0.0)%	
Pre. Hops. Hist.(n)%	No	31(51.7)%	9(45.0)%	40(100.0)%	**0.000*** **
	Yes	29(48.3)%	11(55.0)%	0(0.0)%	
Same illness(n)%	No	25(43.1)%	8(40.0)%	40(100.0)%	**0.000*** **
	Yes	35(56.9)%	12(60.0)%	0(0.0)%	
Surgical History(n)%	No	43(71.7)%	7(35.0)%	40(100.0)%	**0.000*** **
	Yes	17(28.3)%	13(65.0)%	0(0.0)%	
Drug Sensitive(n)%	No	56(93.3)%	15(75.0)%	40(100.0)%	**0.002** **
	Yes	4(6.7)%	5(25.0)%	0(0.0)%	
Covid Vaccines(n)%	No	44(73.3)%	13(65.0)%	14(35.0)%	**0.001** **
	Yes	16(26.7)%	7(35.0)%	26(65.0)%	
Pneumonia Vac.(n)%	No	49(81.7)%	19(95.0)%	34(85.0)%	**0.351ns**
	Yes	11(18.3)%	1(5.0)%	6(15.0)%	
Smoker(n)%	No	29(48.3)%	7(35.0)%	40(100.0)%	**0.000*** **
	Yes	31(51.7)%	13(65.0)%	0(0.0)%	
Social His umber(n)%	(1-3)	9(15.0)%	6(30.0)%	6(15.0)%	**0.269ns**
	(4-6)	21(35.0)%	9(45.0)%	18(45.0)%	
	6>	30(50.0)%	5(25.0)%	16(40.0)%	
Bird livestock(n)%	No	42(70.0)%	12(60.0)%	40(100.0)%	**0.000*** **
	Yes	18(30.0)%	8(40.0)%	0(0.0)%	
Travel His.(n)%	No	41(68.3)%	14(70.0)%	40(100.0)%	**0.000*** **
	Yes	19(31.7)%	6(30.0)%	0(0.0)%	
D.M(n)%	No	29(48.3)%	10(50.0)%	40(100.0)%	**0.000*** **
	Yes	31(51.7)%	10(50.0)%	0(0.0)%	
HTN(n)%	No	33(55.0)%	7(35.0)%	40(100.0)%	**0.000*** **
	Yes	27(45.0)%	13(65.0)%	0(0.0)%	
H.D(n)%	No-	50(83.3)%	14(70.0)%	40(100.0)%	**0.003** **
	Yes	10(16.7)%	6(30.0)%	0(0.0)%	

Appendix 5 :

Vitek 2 Automated Report on Identification and AST of Bacteria (1).

Baghdad Lab

Name:Mortada جعفر سلمان ث

Gender:Male

AGE: Specimen:Sputum

REMARK:

Sp. code:155

Dr. Name:

Diagnosis:

Sampling Data:2023/04/

Culture results

Concentration:% A.baumannii ()Acinetobacter

Antibiotic Susceptibility Testing

(Group A) First choice for allergic reactions				(Group B) Choose,when Group.A Resistant/Useless			
Drug Name	Range	MIC	Result	Drug Name	Range	MIC	Result
Gentamicin		<=1	S	Amikacin		<=4	S
Meropenem		<=0.06	S	Trimethoprim/Sulfa		<=2/38	S
Levofloxacin		<=0.06	S	Piperacillin/Tazobac		<=2/4	S
Imipenem		<=0.25	S	Cefotaxime		=1	S
Tobramycin		<=1	S	Minocycline		=4	S
Ampicillin/Sulbactam		<=8/4	S	Cefepime		=1	S
Ciprofloxacin		=0.06	S				
Ceftazidime		=4	S				

(Group C) Substitute,when Group B Useless				(Group U) For urinary system infection only			
Drug Name	Range	MIC	Result	Drug Name	Range	MIC	Result

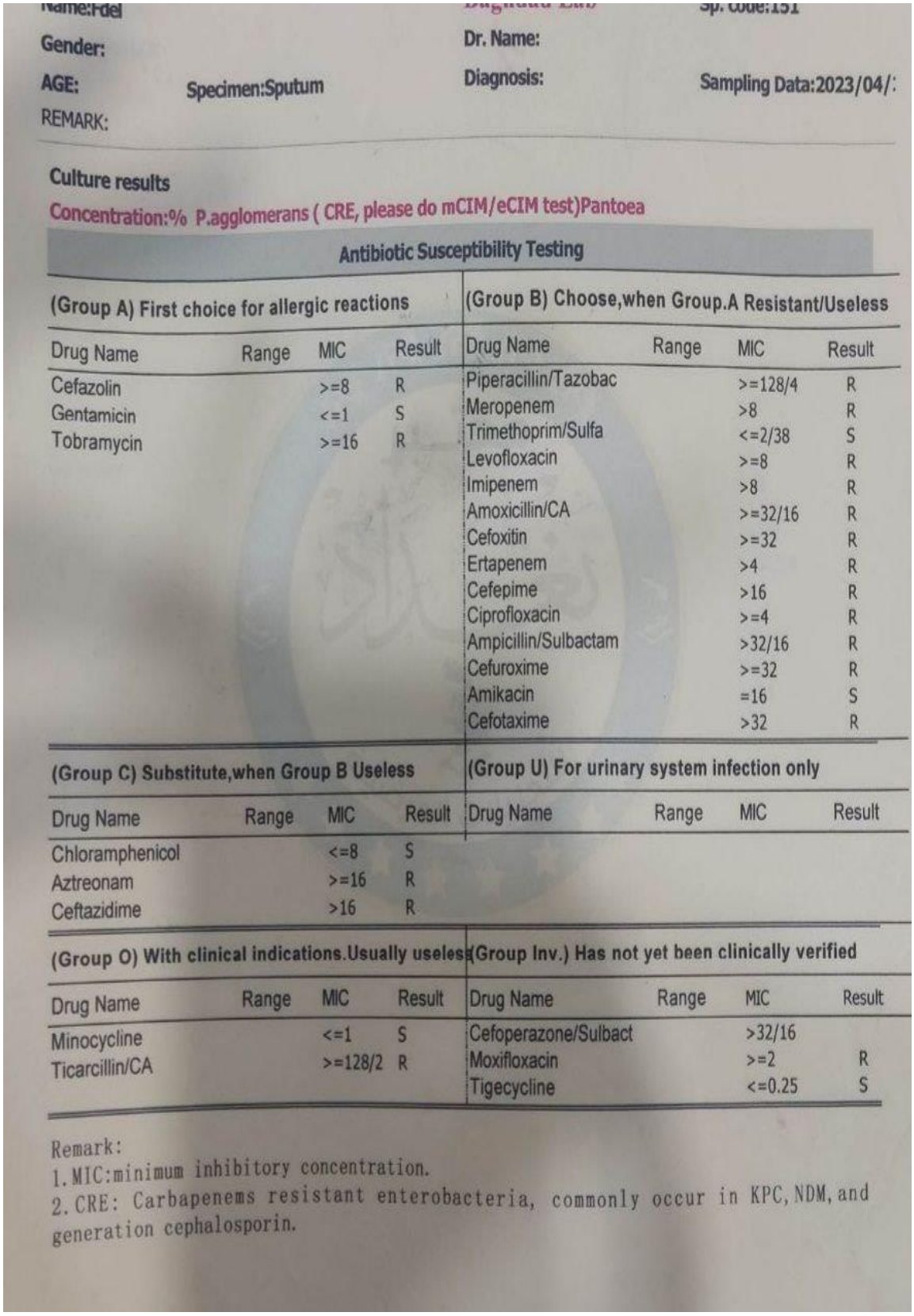
(Group O) With clinical indications.Usually useless				(Group Inv.) Has not yet been clinically verified			
Drug Name	Range	MIC	Result	Drug Name	Range	MIC	Result
Polymyxin B		<=1	S	Cefoperazone/Sulbact		<=16/8	
Ticarcillin/CA		<=16/2	S				

Remark:

1.MIC:minimum inhibitory concentration.

Appendix 5 :

Vitek 2 Automated Report on Identification and AST of Bacteria (2)



Name:Fdel Sp. Code:151

Gender: Dr. Name:

AGE: Specimen:Sputum Diagnosis: Sampling Data:2023/04/:

REMARK:

Culture results

Concentration:% P.agglomerans (CRE, please do mCIM/eCIM test)Pantoea

Antibiotic Susceptibility Testing

(Group A) First choice for allergic reactions

Drug Name	Range	MIC	Result
Cefazolin		>=8	R
Gentamicin		<=1	S
Tobramycin		>=16	R

(Group B) Choose,when Group.A Resistant/Useless

Drug Name	Range	MIC	Result
Piperacillin/Tazobac		>=128/4	R
Meropenem		>8	R
Trimethoprim/Sulfa		<=2/38	S
Levofloxacin		>=8	R
Imipenem		>8	R
Amoxicillin/CA		>=32/16	R
Cefoxitin		>=32	R
Ertapenem		>4	R
Cefepime		>16	R
Ciprofloxacin		>=4	R
Ampicillin/Sulbactam		>32/16	R
Cefuroxime		>=32	R
Amikacin		=16	S
Cefotaxime		>32	R

(Group C) Substitute,when Group B Useless

Drug Name	Range	MIC	Result
Chloramphenicol		<=8	S
Aztreonam		>=16	R
Ceftazidime		>16	R

(Group U) For urinary system infection only

Drug Name	Range	MIC	Result

(Group O) With clinical indications.Usually useles

Drug Name	Range	MIC	Result
Minocycline		<=1	S
Ticarcillin/CA		>=128/2	R

(Group Inv.) Has not yet been clinically verified

Drug Name	Range	MIC	Result
Cefoperazone/Sulbact		>32/16	
Moxifloxacin		>=2	R
Tigecycline		<=0.25	S

Remark:

1. MIC:minimum inhibitory concentration.

2. CRE: Carbapenems resistant enterobacteria, commonly occur in KPC, NDM, and generation cephalosporin.

Appendix 5 :

Vitek 2 Automated Report on Identification and AST of Bacteria (3).

Name:Kadmea Dr. Name:

Gender:Female Diagnosis: Sampling Data:2023/04/:

AGE: Specimen:Sputum

REMARK:

Culture results

Concentration:% E.asburiae (CRE, please do mCIM/eCIM test)Enterobacter

Antibiotic Susceptibility Testing

(Group A) First choice for allergic reactions

Drug Name	Range	MIC	Result
Ampicillin		>32	R
Gentamicin		>=16	R
Tobramycin		>=16	R
Cefazolin		>=8	R

(Group B) Choose,when Group.A Resistant/Useless

Drug Name	Range	MIC	Result
Imipenem		>8	R
Cefepime		>16	R
Cefoxitin		>=32	R
Meropenem		>8	R
Levofloxacin		>=8	R
Piperacillin/Tazobac		>=128/4	R
Trimethoprim/Sulfa		=4/76	R
Amoxicillin/CA		>=32/16	R
Ertapenem		>4	R
Ciprofloxacin		>=4	R
Ampicillin/Sulbactam		>32/16	R
Cefuroxime		>=32	R
Amikacin		>=64	R
Cefotaxime		>32	R

(Group C) Substitute,when Group B Useless

Drug Name	Range	MIC	Result
Chloramphenicol		>=32	R
Aztreonam		>=16	R
Ceftazidime		>16	R

(Group U) For urinary system infection only

Drug Name	Range	MIC	Result

(Group O) With clinical indications.Usually useless

Drug Name	Range	MIC	Result
Minocycline		=8	I
Ticarcillin/CA		>=128/2	R

(Group Inv.) Has not yet been clinically verified

Drug Name	Range	MIC	Result
Cefoperazone/Sulbact		>32/16	
Moxifloxacin		>=2	R
Tigecycline		>=4	R

Remark:

1.MIC:minimum inhibitory concentration.

2.CRE: Carbapenems resistant enterobacteria, commonly occur in KPC, NDM, and generation cephalosporin.

Appendix 5 :

Vitek 2 Automated Report on Identification and AST of Bacteria (4).

Name: Shahan

Gender:Male

AGE:

REMARK:

Dr. Name:

Specimen:Sputum

Diagnosis:

Sampling Data:2023/04

Culture results

Concentration:% M.morganii ()Morgancella

Antibiotic Susceptibility Testing

(Group A) First choice for allergic reactions				(Group B) Choose,when Group.A Resistant/Useles			
Drug Name	Range	MIC	Result	Drug Name	Range	MIC	Result
Tobramycin		>=16	R	Amikacin		>=64	R
Gentamicin		>=16	R	Trimethoprim/Sulfa		>4/76	R
				Piperacillin/Tazobac		>=128/4	R
				Meropenem		>8	
				Levofloxacin		>=8	R
				Imipenem		>8	
				Cefoxitin		>=32	R
				Cefepime		>16	R
				Ertapenem		>4	R
				Cefotaxime		>32	R
				Ciprofloxacin		=2	I
				Ampicillin/Sulbactam		>32/16	R

(Group C) Substitute,when Group B Useless				(Group U) For urinary system infection only			
Drug Name	Range	MIC	Result	Drug Name	Range	MIC	Result
Chloramphenicol		<=8	S				
Ceftazidime		>16	R				
Aztreonam		>=16	R				

(Group O) With clinical indications.Usually useless				(Group Inv.) Has not yet been clinically verified			
Drug Name	Range	MIC	Result	Drug Name	Range	MIC	Result
Ticarcillin/CA		>=128/2	R	Cefoperazone/Sulbact		>32/16	
				Moxifloxacin		=1	I

Remark:

1. MIC:minimum inhibitory concentration.

Appendix 5 :

Vitek 2 Automated Report on Identification and AST of Bacteria (5).

Name:Sajad

Gender:

Dr. Name:" "

AGE: Specimen:Sputum Diagnosis: Sampling Data:2023/04/1

REMARK:

Culture results

Concentration:% S.aureus (MRSA)Staphylococcus

Antibiotic Susceptibility Testing

(Group A) First choice for allergic reactions				(Group B) Choose,when Group.A Resistant/Useless			
Drug Name	Range	MIC	Result	Drug Name	Range	MIC	Result
Clarithromycin		>=8	R	Rifampin		=2	I
Penicillin		>2	R	Doxycycline		>=16	R
Cefoxitin		>8	Positive	Tetracycline		>=16	R
Erythromycin		>=8	R	Vancomycin		=16	
Clindamycin		>4	R	Linezolid		<=1	S
Oxacillin		>4	R	Minocycline		>=16	R
Trimethoprim/Sulfa		=2/38	S				
Azithromycin		>=8	R				

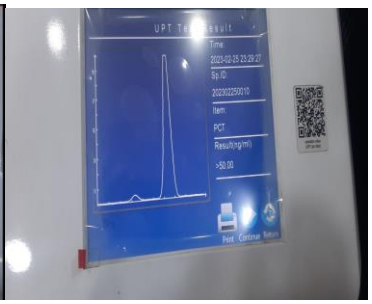
(Group C) Substitute,when Group B Useless				(Group U) For urinary system infection only			
Drug Name	Range	MIC	Result	Drug Name	Range	MIC	Result
Levofloxacin		>=4	R				
Moxifloxacin		>=2	R				
Gentamicin		=8	I				
Ciprofloxacin		>=4	R				
Chloramphenicol		>=32	R				

(Group O) With clinical indications.Usually useless				(Group Inv.) Has not yet been clinically verified			
Drug Name	Range	MIC	Result	Drug Name	Range	MIC	Result
Tobramycin		>=16	R	Teicoplanin		=2	S
Amikacin		=16	I	D Test		>0.5/4	Positive
				Tigecycline		=0.5	S

Remark:

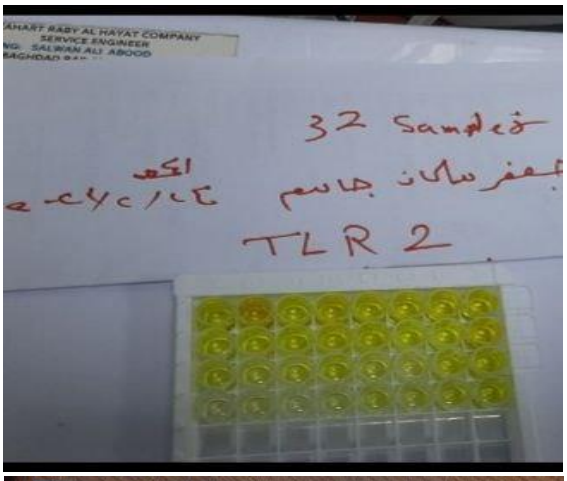
1. MIC:minimum inhibitory concentration.
2. MRSA: Methicillin resistant S. aureus, MDR strains, resistant to all beta-lactamase except anti-MRS cephalosprins, including penicillins, carbapenems, beta-lactam/enzyme inhibitor, and cephalosprins. nether test as resistant nor susceptable, but no clinical effects.

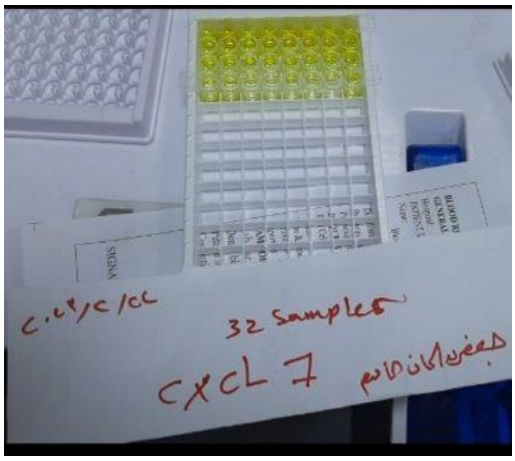
Appendix (6) : Some Results PCT Test .

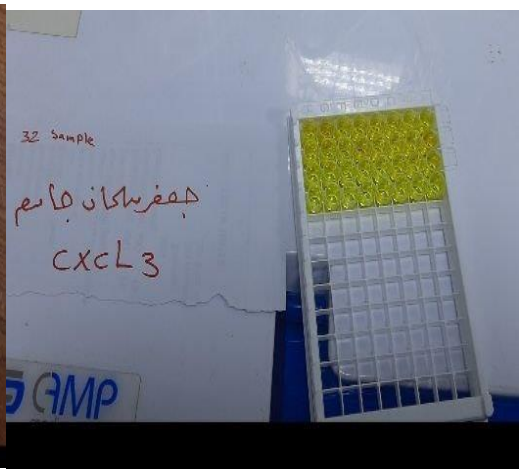


Appendix(7):

ELISA Kits and Results Some of Samples to TLR2,TLR4 , CXCL3 and CCL7.

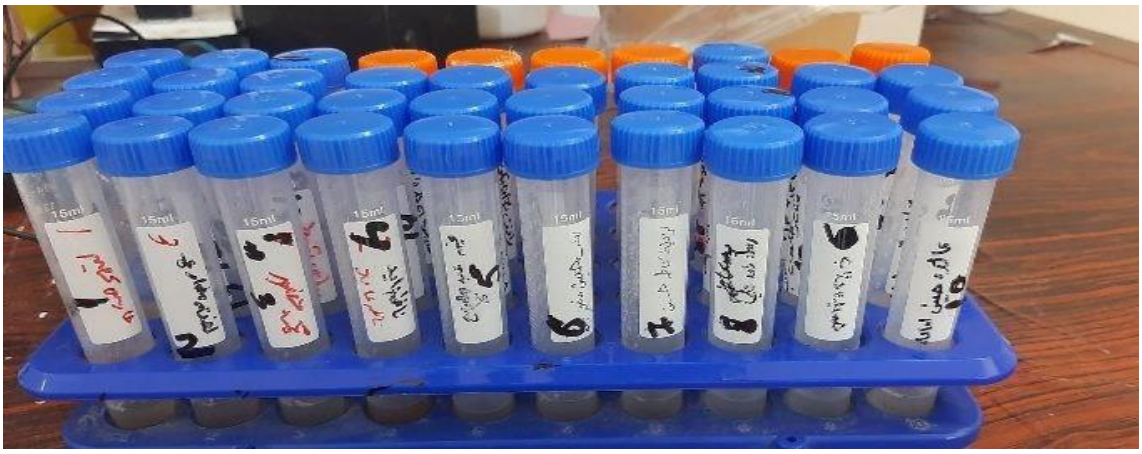


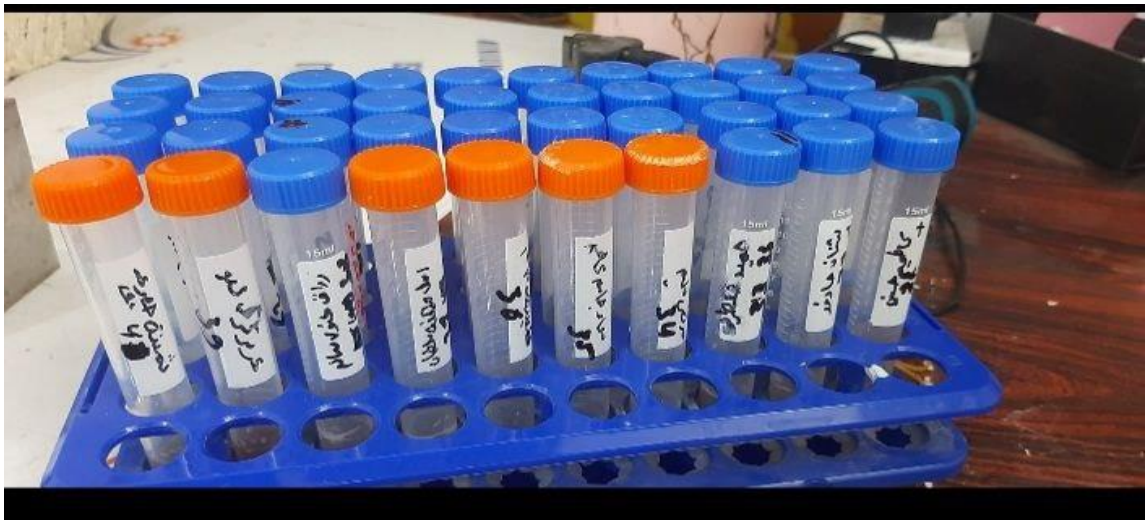


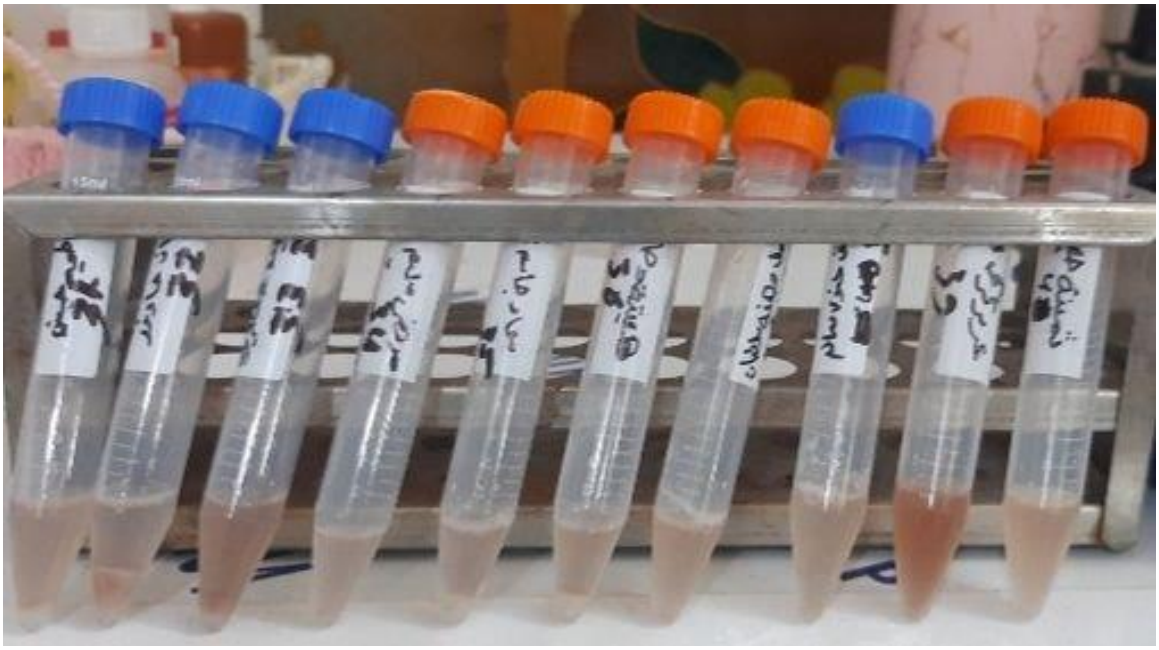


Appendix : (8)

Preparation samples to Extraction RNA from WBC in RT qPCR .

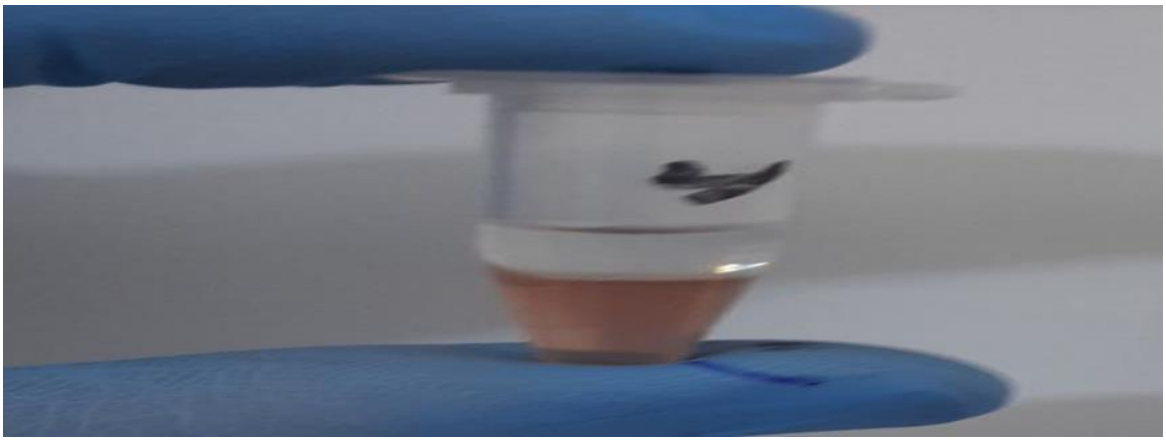


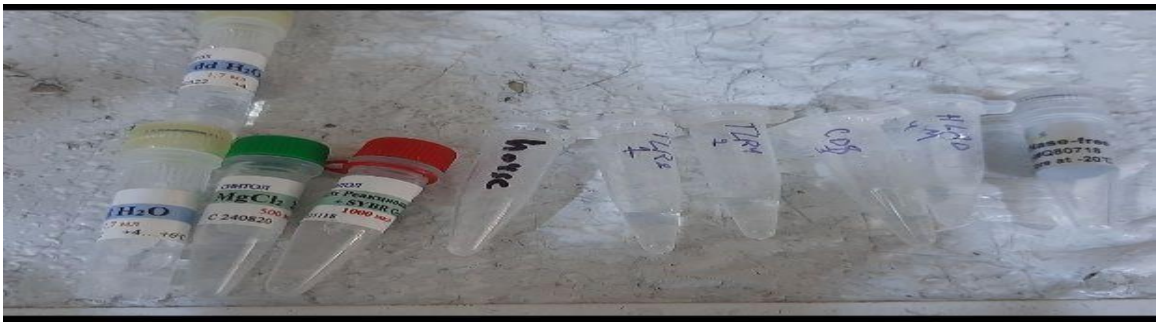




Appendix (9) :

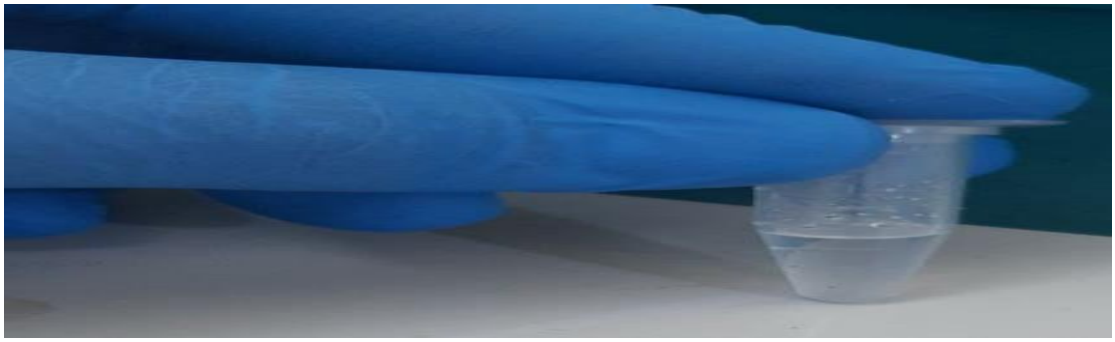
Steps Gene Expression by RT qPCR (1).

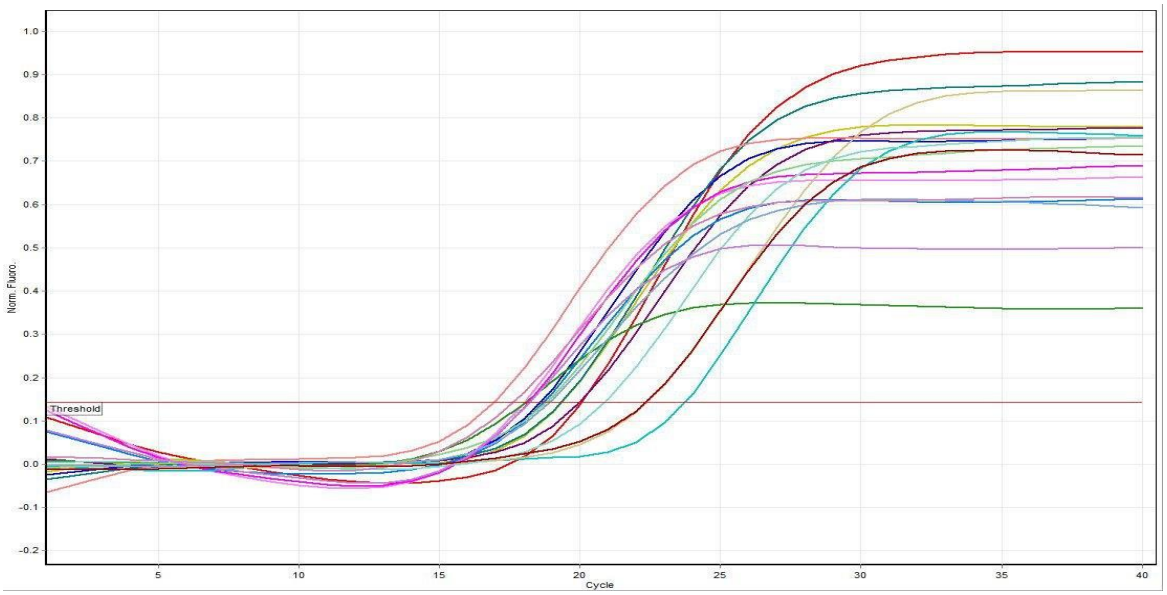




Appendix (9):

Steps Gene Expression by RT- qPCR (2)





الخلاصة

يعتبر الالتهاب الرئوي Pneumonia من الامراض التي تصيب الجهاز التنفسي السفلي والتي تحدث نتيجة اصابة الرئة بالبكتريا ،الفايروسات ،الطفليات و الفطريات و يسبب امراضا ومضاعفات خطيرة و من هذه المضاعفات الانتان الدموي Sepsis والذي هي اصابة تتمثل بخلل وظيفي يهدد حياة المرضى نتيجة لاستجابة المضيف غير المنتظمة للعدوى البكتيرية ويعتبر سبباً رئيسيا للموت حيث يكون مسؤول عن اصابة حوالي 49 مليون شخص و يسبب بوفاة 11 مليون شخص سنويا حول العالم ونتيجة لزيادة حالات تطور الاصابة بالإنتان الدموي بعد الاصابة بالالتهاب الرئوي المكتسب من المجتمع لذلك من الضروري ايجاد طرق تشخيصية سريعة معتمده على فحص الدم الشامل وبعض الجينات والعوامل المناعية ذات العلاقة لتقليل من حالات الاصابة من الانتان الدموي والموت .

في هذه الدراسة تم جمع (120) مشاركاً من المرضى الراقدين في مستشفى الامام الصادق (عليه السلام) التعليمية في محافظة بابل ومستشفى مدينة الامام الحسين(عليه السلام) الطبية التعليمية في محافظة كربلاء للفترة من كانون الاول 2022 الى حزيران 2023 وبعد ذلك تم تقسيم المشاركين الى ثلاث مجموعات (60) مريضا من المرضى الذي يعانون من الالتهاب الرئوي المكتسب من المجتمع، (20) مريضاً من المرضى المصابين بالإنتان الدموي و الالتهاب الرئوي المكتسب من المجتمع و (40) شخصاً من الاصحاء (كمجموعة سيطرة) . وقد تم تشخيص المشاركين في هذه الدراسة من قبل أطباء متخصصين بالاعتماد على بعض الطرق غير المعتمدة على الزراعة مثل الطرق السريرية (العلامات والأعراض) وطرق التصوير المقطعي (الأشعة السينية و/أو التصوير المقطعي المحوسب).

بلغ متوسط الفئات العمرية للمشاركين (54.9، 72.5، 51.4 سنة) للمجاميع الثلاثة (الالتهاب الرئوي المكتسب من المجتمع، والإنتان الدموي الناتج عن الالتهاب الرئوي المكتسب من المجتمع، ومجموعة السيطرة من الاصحاء) على التوالي، وكان المدى العمري للمشاركين بين 23 إلى 95 سنة. ومع ذلك، كان المشاركون في هذه الدراسة 30 ذكراً و30 أنثى لمجموعة الالتهاب الرئوي المكتسب من المجتمع، مع 7 ذكور و13 أنثى من مرضى الانتان الدموي نتيجة الالتهاب الرئوي المكتسب من المجتمع، بينما كان هناك 18 ذكراً و22 أنثى كمجموعة سيطرة من الأصحاء.. وتشير نتائج الدراسة إلى أن معدل الإصابة يزداد بشكل ملحوظ مع تقدم العمر، في حين لم يظهر الجنس أي تأثير معنوي على المجموعات الثلاث.

كما اظهرت نتائج الدراسة الحالية عدم ظهور نمو لمزارع الدم لمرضى الانتان الدموي و الالتهاب الرئوي المكتسب من المجتمع بسبب قلة عدد العينات المشاركة في هذه الدراسة (20 عينه) ، الاستخدام المرضى المسبق للمضادات الحيوية، وعدم استخدام أكثر من زجاجة مخصصه للزرع خلال أوقات مختلفة من الاصابة ، وكذلك لم يتم استخدام طرق وظروف الزراعة اللاهوائي في زرع العينات ، في المقابل أظهرت نتائج زراعة عينات البلغم أن ظهور العزلات البكتيرية المسببة للإصابة تعود إلى البكتيريا الموجبة لصبغة غرام بواقع 45 عزلة وبنسبة (56.25%) وكانت جميع هذه العزلات حساسة لمضادات الميروبينيم والريفامبسين والسيفيبيم. و تعتبر العقدية الرئوية *Streptococcus pneumoniiae* اكثر انواع البكتريا المعزولة المسببة للإصابة والذي تنتمي الى الانواع الموجبة لصبغة غرام بينما عدد البكتيريا السالبة لصبغة غرام كانت 35 عزلة بنسبة (43.75%) وكانت جميع هذه العزلات حساسة للسيفيبيم والأميكاسين والميروبينيم وأظهرت النتائج أن المستدمية النزلية *Hemophilus influenzae* هي أكثر أنواع البكتيريا سلبية الجرام شيوعا.

كما أظهرت نتائج البروكالسيتونين وجود فرق معنوي كبير (*P.* Value ≤0.000) بين المرضى ومجموعة السيطرة. وكذلك أظهرت نتائج فحص الدم الشامل (CBC) وجود اختلافات معنوية (*P.* Value ≤ 0.000) بين المرضى ومجموعات السيطرة في متوسط نسبة خلايا الدم البيض ومنها كل من الخلايا المحببة، النسبة المئوية للخلايا المحببة٪، نسبة العدلات إلى الخلايا الليمفاوية. بينما كانت (*P.* Value ≤ 0.007) بالنسبة لمتوسط الصفائح الدموية الى الخلايا الليمفاوية .

تم تحديد تراكيز TLR2 , TLR4, CXCL3 وCCL7 في مصل الدم لجميع المشاركين في هذه الدراسة بواسطة اختبار ELIZA حيث اظهرت النتائج وجود اختلاف عالي المعنوية في تركيزTLR2 وCXCL3 بين مجموعة المرضى ومجموعة السيطرة عند قيمة (*P.* Value ≤0.000) وكذلك اظهرت زيادة في تركيزهما في مرضى الالتهاب الرئوي المكتسب من المجتمع و مرضى الانتان الدموي و الالتهاب الرئوي المكتسب من المجتمع مقارنة مع مجاميع السيطرة ، بينما لم تظهر النتائج اي اختلاف معنوي في تركيز TLR4و CCL7 عند قيمة (0.585 و 0.673) ≤ *P.* Value على التوالي .

اما نتائج التعبير الجيني للجينات المرتبطة بالمناعة (*TLR2, TLR4, CD81 وHLADMA)*اذ اظهرت وجود اختلافا معنوياً في مستوى التعبير الجيني للجينات *(TLR2,TLR4وCD81)* بين مجموعه المرضى ومجموعة السيطرة عند (P. ≤0.000)، بينما اوجدت النتائج زياده في مستوى التعبير الجيني في مجموعة مرضى الالتهاب الرئوي المكتسب من المجتمع عن مرضى الانتان الدموي و الالتهاب الرئوي المكتسب من المجتمع بينما لم يظهر هناك أي اختلاف معنوي (P. value ≤0.056) في مستوى التعبير الجيني لجين *HLA-DMA* في مجاميع الدراسة .

لذلك يعد الكشف المبكر عن الالتهاب الرئوي المكتسب من المجتمع والانتان الدموي نتيجة الالتهاب الرئوي المكتسب من اهم الخطوات العلاجية لأنه يساعد بالعلاج السريع ويقلل من معدل الوفيات التي قد تتطور خلال وقت الإصابة . على الرغم من أن زراعة الدم تمثل المعيار الذهبي والطريقة التشخيصية الأكثر استخداما للكشف عن الانتان الدموي ، إلا أن هذه الطريقة غير حساسة بما فيه الكفاية . لذلك تم ايجاد معايير مخبرية طبية أكثر تحديدا يمكن استخدامها للتشخيص المبكر ، خاصة عندما تفشل مزارع الدم في اكتشاف العامل المسبب للإنتان الدموي رغم وجود أعراض سريرية.

جامعة كربلاء
كلية العلوم
قسم علوم الحياة

التعبير الجيني لبعض العوامل المناعية وتحديد مستوياتها في مرضى الالتهاب الرئوي المكتسب من المجتمع

اطروحة مقدمة الى

مجلس كلية العلوم – جامعة كربلاء

وهي جزء من متطلبات نيل درجة الدكتوراه فلسفة في علوم الحياة

من قبل الطالب

جعفر سلمان جاسم عباس البناء

بكالوريوس قسم علوم الحياة / كلية العلوم / جامعة كربلاء ، 2009

ماجستير قسم علوم الحياة/ كلية العلوم / جامعة كربلاء ، 2013

بأشراف

الاستاذ الدكتور
ناجح هاشم كاظم

الاستاذ المساعد الدكتور
ستار جبار راهي الكريطي

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