University of Kerbala

College of Applied Medical Sciences

Department of Clinical Laboratories

Study of Soluble Triggering Receptor Expressed on Myeloid Cells 1 ,Chitinase 3-Like Protein 1 and Soluble Urokinase Plasminogen Activator Receptor in Pediatric Bacterial Pneumonia

A thesis

Submitted to the Council of the College of Applied Medical Sciences – University of Kerbala in Partial Fulfillments of the Requirements for the Degree of Master in Clinical Laboratories

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B.Sc. Medical Laboratory Techniques / 2021

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Supervisor's certification

I certify the thesis entitled (**Study of Soluble Triggering Receptor Expressed on Myeloid Cells 1 ,Chitinase 3-Like Protein 1 and Soluble Urokinase Plasminogen Activator Receptor in Pediatric Bacterial Pneumonia**) was prepared under my supervision by **(Zainab Muhsin Mohammed**) at the department of Clinical Laboratories\ College of Applied Medical Sciences\ University of Kerbala, in partial fulfillment of the requirements for the degree of Master in Clinical Laboratories.

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Head of Department Recommendation

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/ /2024

Committee Certification

We, the examining committee, certify that we have read the thesis entitled " **Study of Soluble Triggering Receptor Expressed on Myeloid Cells 1 ,Chitinase 3-Like Protein 1 and Soluble Urokinase Plasminogen Activator Receptor in Pediatric Bacterial Pneumonia** " and have examined the student **(Zainab Muhsin Mohammed Hassan)** in its content and that in our opinion it is accepted as a thesis for degree of Master of **Clinical Laboratories.**

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Approval Certification

We certify that the thesis entitled **"Study of Soluble Triggering Receptor Expressed on Myeloid Cells 1 ,Chitinase 3-Like Protein 1 and Soluble Urokinase Plasminogen Activator Receptor in Pediatric Bacterial Pneumonia"** fulfills partial requirements of the degree of Master in Clinical Laboratories.

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Dedication

To the one who was and still is my support during dark nights, calm my heart with an abundance of reassurance and contentment and be kind to my affairs, the one who is most gracious to me, Imam Aba Al-Fadl (peace be upon him).

To my family, who supported me with everything and worked hard for this achievement and for my name to be raised high and to my life partner , my husband I offer them this in the hope that it will cover part of what they have made.

zainab

2024

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Zainab

2024

Summary

Respiratory tract infections (RTIs) are any upper or lower respiratory disease that affect children all over the world and are associated with significant morbidity and mortality. RTIs are usually triggered by viruses, though bacterial infections are also common. sTREM1 (soluble Triggering Receptor Expressed on Myeloid cells-1), suPAR (soluble Urokinase-type Plasminogen Activator Receptor), and CHI3L1 (Chitinase-3-like protein 1) these biomarkers can be useful in assessing the severity and prognosis of pneumonia, potentially guiding treatment decisions.

The study was a cross-sectional and was conducted at the Babil Teaching Hospital for Maternity and Children and AL nour hospital for children in Babylon governorate, from the beginning of November 2023 to April 2024, blood and sputum samples were obtained from 120 suspected respiratory tract infections patients and divided into three groups: mild, moderate and severe after diagnostic by pediatrics, their age ranged from less than 1 years to 14 years. This study aimed to investigate the most common types of bacterial isolates responsible about upper and lower bacterial infections in children and investigate the role of biomarkers in prognostic respiratory tract infections in children.

Sputum samples were cultivated on culture media and manual biochemical tests were used to identification of bacteria species. Creactive protein and white blood Cells were measured . The biomarkers Chitinase 3-like protein 1, sTREM-1(soluble triggering receptor expressed on myeloid cell 1) and suPAR (soluble urokinase plasminogen activator receptor) were measured by using ELISA system.

The results showed that out of 127 sputum samples, 7 had no bacterial growth ,and 120 were found with significant bacterial growth only them were include in the present work.

The results of bacterial isolates were *Haemophils influenza* 21.67% ,*Klebsiella pneumonia* 20% *Streptococcus Pneumonia* 19.17%, *Staphlococcus aureu*s 15%, *Streptococcus Pyogenes* 14.16% *Pseudomonas aeruginosa* 5%, and *Moraxella catarrhalis* 5%.

The comparison study revealed that there was increase in concentrations of all biomarkers in the severe cases of disease. The mean sTREM-1 concentrations 2841.981 ± 2816.161 ng/mL in severe cases, mean CHI3L1 concentration 2025.397±1016.498 ng/mL and suPAR concentrations 21.123±10.812 ng/mL .

Statically a significant difference was found in some parameters among the severe infections. As a result, there is a significant negative correlation between Human sTREM-1 and Human CH13L1, at the pvalue< 0.05. Furthermore , there is a significant positive correlation between WBC and ESR (p-value= 0.389). There is a negative correlation among WBC with Human sTREM-1, Human CH13L1, and Human suPAR p-value< 0.05.

moderate case of infection was a significant positive correlation between Human suPAR and Human sTREM-1, at the p-value< 0.05. on the other hand, there is a significant positive correlation among Human suPAR with CRP , at the p-value< 0.01. while there is a negative correlation among ESR with Human sTREM-1, Human CH13L1, and Human suPAR, at the p-value< 0.05.In addition there is strong positive correlation between WBC and CRP (0.436**) .

mild case of infection there is a significant positive correlation among Human CH13L1 with Human sTREM-1 and Human suPAR, at the p-value< 0.05. In addition , there is a significant positive correlation between Human suPAR and Human sTREM-1 , at the p-value< (0.05).While found significant negative correlation between Human suPAR and ESR ,at the p-value< 0.01 .

This study showed that the elevated level of CRP, WBC and ESR in children with less than 1 year is much more than other age group, and serum level of Human sTREM-1, Human CH13L1, and Human suPAR were much greater in severe cases in comparison with moderate and mild cases.

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List of Abbreviations

Symbols	Definition
AECOPD	Acute Episodes of Chronic Obstructive Pulmonary Disease
ALRTI	Acute Lower Respiratory Tract Infections
AOM	Acute Otitis Media
ARI	Acute Respiratory Infection
BAL	bronchoalveolar lavage
CAP	Community-Acquired Pneumonia
COPD	Chronic Obstructive Pulmonary Disease
CRP	C-reactive Protein
CSOM	Chronic Suppurative Otitis Media
DALYs	Disability Adjusted Life Years
GAS	Group A Streptococci
HAP	Hospital-Acquired Pneumonia
Hib	Haemophilus influenzae type b
HIV	Human Immunodeficiency Virus
hRV	human Rhinoviruses
ICU	Intensive Care Unit
IFN	Interferon
IL	Interleukin
IP	Inducible Protein
LRIs	Lower Respiratory Tract Infections
LRT	Lower Respiratory Tract
MPV	Metapneumovirus
NPA	nasopharyngeal aspirate
NTHi	Non-Typeable Haemophilus influenzae
OME	Otitis Media with Effusion

PF	**Pleural Fluid**
PRRs	**Pattern-Recognition Receptors**
RSV	**Respiratory Syncytial Virus**
RSV	**respiratory syncytial virus**
RTIs	**Respiratory Tract Infections**
Th-1	**T helper 1**
TNF	**Tumor Necrosis Factor**
URIs	**Upper Respiratory Tract Infections**
URT	**Upper Respiratory Tract**
VAP	**Ventilator-Associated Pneumonia**
WHO	**World Health Organization**

Chapter one Introduction

1.1 Introduction

Respiratory tract infections (RTIs) are a major global health concern since they are the primary cause of death for children, especially in developing country. RTIs include upper respiratory tract infections(URTIs) otherwise referred to as common cold and lower respiratory tract infections(LRTIs) . The upper respiratory tract infections (URTIs) that are most frequently detected are tonsillitis, otitis media, laryngitis, pharyngitis, and the common cold (Assane *et al*., 2018). LRTIs include pneumonia and bronchitis (Shrestha *et al*., 2013).

Gram-negative bacterial infections are the predominant causal agents of LRTIs. Many investigations into lower respiratory tract infections (LRTIs) have revealed that gram-negative bacteria account for a significant fraction of infections in the modern era, particularly in hospital-acquired infections and community-dwelling patients (Karaiskos and Giamarellou, 2014). It usually occurs when pathogenic microorganisms get past the mechanical and other nonspecific barriers in the upper respiratory tract and enter the parenchyma pulmonary airway. Infection may occur due to inhaling infectious aerosols, aspiration of oral or gastric contents, or heterogeneous spread (Mahon and Lehman, 2022).

The prevalence of respiratory tract infections is highest in children under the age of five and much higher in newborns and undernourished children(Safiri *et al*., 2023).

Antibiotics are not necessary for the self-treatment of the majority of URTIs because they are viral, self-limiting infections (Peters *et al*., 2011). The empirical medication of choice is typically used to treat community-acquired infections. Antibiotics are thought to be harmless; nevertheless, this belief causes strains of bacteria to become resistant to them quickly, which complicates the process of

choosing antimicrobial chemicals (Uzoamaka *et al*., 2017). Current antibiotics are not effective against bacteria that are multidrug-resistant (MDR) ,the management of infectious diseases that cause high rates of morbidity and mortality in the population is impacted globally by bacterial resistance (Dahiya and Purkayastha, 2012).

There is an increasing demand in biochemical investigations to assess the severity of RTIs and identify potential prognostic and diagnostic biomarkers. These biomarkers are soluble triggering receptor expressed on myeloid cells , soluble urokinase plasminogen activator receptor and Chitinase 3-like protein 1could be a biomarkers in pneumonia (Kofoed *et al*., 2007).

Soluble urokinase plasminogen activator receptor, which is found on a variety of immune and nonimmune cells. It is released when this membrane protein is cleaved, resulting in a plasma concentration that represents the level of immune activation during bacterial or viral infections, cancer, or autoimmune disease (Ulaj *et al*., 2024). Elevated levels of suPAR can indicate an ongoing inflammatory process, which is often seen in severe pneumonia (Song *et al*.,2020). Studies have shown that higher levels of suPAR in serum are associated with more severe forms of pneumonia in children. It may reflect the degree of lung injury, systemic inflammation, and the overall severity of the infection (El-Mekkawy *et al*., 2022).

TREM-1 are mainly expressed in neutrophile granulocyte, mature monocyte, and the surface of macrophages (Zysset *et al*., 2016).The cascade reaction induces the production of pro-inflammatory cytokines, such as tumor necrosis factor alpha, monocyte chemoattractant protein 1, and interleukin-1β, after bacterial infection (Kany *et al*., 2019). Furthermore, TREM-1 can affect the pathological process of septic shock (Qian *et al*., 2014). Hence, it plays an

important role in the inflammatory reaction triggering and amplification process (Yang *et al*., 2015). sTREM-1 are the acute phase product of bacterial infection ,detection of sTREM-1 in the bodily fluids of a living body has a great application value for bacterial infection diagnosis (Su *et al*., 2016). Overall, sTREM-1 levels correlate with the severity of pneumonia in children, with higher levels indicating more severe disease and potentially worse outcomes. Measuring sTREM-1 can assist clinicians in assessing the severity of pneumonia, guiding treatment strategies, and monitoring the response to therapy (Karakioulaki and Stolz , 2019).

Chitinase 3-like protein 1 is a member of glycoside hydrolase family 18 and is synthesized and secreted by many cells, including macrophages, neutrophils, synoviocytes, smooth muscle cells, and tumor cells (Zhao *et al* .,2020).CHI3L1 has been reported to promote cancer growth, production of pro-inflammatory cytokines, and microglial activation (Yeo *et al*.,2019). It is strongly associated with diseases such as asthma, arthritis, sepsis, diabetes, liver fibrosis, and coronary artery disease (Kamle *et al*.,2021) . CHI3L1 levels may correlate with the severity of the inflammatory response to bacterial infections. Higher levels could indicate a more severe infection and greater lung injury (Zhao *et al*., 2020). Some studies have proposed that measuring serum CHI3L1 levels might be useful as a biomarker for diagnosing and assessing the severity of pneumonia in pediatric patients (Chandna *et al* ., 2023) .

1.2 Aim of study

This study aimed to investigate the most common types of bacterial isolates that are responsible about upper and lower bacterial infections in children and investigate the role of biomarkers in prognostic pneumonia.

1.3 Objective

1. Evaluated of CRP, CBC, ESR in children suffering from upper and lower respiratory tract infections.
2. Bacteria were isolated from children who were infected with upper and lower respiratory bacterial infections..
3. The study examined the antibiotic sensitivity patterns of the isolated bacteria
4. The study examined the correlation between biochemical biomarkers (Chitinase 3-like protein 1, sTREM-1 (soluble triggering receptor expressed on myeloid cell 1), and soluble urokinase plasminogen activator receptor) and pneumonia.

Chapter Two Literatures review

2.1 Respiratory Tract Infections

Respiratory tract infection constitutes a major load in pediatric outpatient services and hospitalizations, especially in developing countries (Walker *et al.*,2013) and there of recurrent respiratory tract infection is commonly seen among young children (Marengo *et al*., 2020). Respiratory infection is categorized into two distinct classifications: upper respiratory infection and lower respiratory infection. Generally, acute respiratory infections stem from the common cold, a condition that frequently impacts the sinuses, air passages, or pulmonary system. The manifestations of respiratory ailments vary depending on the specific type of infection afflicting a child. While certain infections present mild symptoms, others manifest more severe indications. Typically, the onset of respiratory infection is marked by symptoms such as nasal congestion, headache or sometimes fever. However, these manifestations may go unnoticed in infants and toddlers below the age of two, as they lack the capacity to articulate or exhibit signs of discomfort. Consequently, this oversight could potentially culminate in pneumonia for a significant number of children (Kharel and Bhandari, 2020).

2.1.2 Epidemiology

Acute respiratory infections are the main reason for morbidity and mortality among children below 5 years and present a significant burden on the healthcare system (Accinelli *et al*.,2017) .Worldwide, acute respiratory infections account for approximately 1.3 million deaths annually among children below 5 years (WHO , 2013).Respiratory infections account for 6% of the entire global burden of diseases, according to the World Health Organization estimation, which is higher than the burdens of ischemic heart disease, HIV infection, cancer, malaria, or diarrheal . Due

to the direct impact on tissue oxygenation that acute respiratory infections have on children, which leads to complications and worse consequences like raised death and morbidity, these infections frequently qualify as medical emergencies. Annually, more than 12 million children under 5 years old are hospitalized because of acute respiratory infections (Correia *et al*.,2021).

2.1.3 Pathogenesis of Respiratory Tract Infection

The pathogenesis of bacterial respiratory tract infections involves a series of steps and interactions between the bacteria and the host's immune system. These infections can be categorized based on the affected area: the upper respiratory tract (URT) and the lower respiratory tract (LRT) (Pérez-Cobas *et al*., 2023).

Bacteria enter the URT through inhalation of respiratory droplets. They adhere to the mucosal surfaces using adhesions, which are surface proteins that bind to host cell receptors. Bacteria employ various strategies to evade the immune system, such as producing capsules to prevent phagocytosis, secreting enzymes that degrade host tissues, and producing toxins that impair immune cell function. The presence of bacteria and their toxins triggers an inflammatory response. This includes the recruitment of immune cells like neutrophils and macrophages to the site of infection, leading to symptoms like swelling, redness, and pain. Bacterial toxins and the immune response itself can cause damage to the mucosal lining, leading to symptoms like sore throat, sinus congestion, and coughing (Li *et al*., 2023) .

LRTIs organisms enter the distal airway by inhalation, aspiration or by hematogenous seeding. The pathogen multiplies in or on the epithelium, causing inflammation, increased mucus secretion, and impaired mucociliary function; other lung functions may also be affected. In severe bronchiolitis, inflammation and

necrosis of the epithelium may block small airways leading to airway obstruction (Di Simone *et al*., 2023).

2.1.4. The Immune System

The human immune system comprises of the two broad categories of immunity, which include the innate and adaptive immunity. The innate immunity is composed of various physical barriers and chemical barriers, as well as specialized immune response cells that readily recognize pathogens and respond to them within a short time without prior exposure and sensitization .The adaptive immunity encompasses two classes of T and B lymphocytes that are capable of specifically recognizing pathogens that have been encountered before and launching a more vigorous immune response .Some of these mechanisms are generalized, while others pertain to specific types of immune cells. It is therefore, evident that the defense mechanisms are categorized in to innate and adaptive depending on the cellular receptor's immunity (Mukherjee *et al*., 2023).

While innate receptors are programmed as real single genes in the genome, adaptive receptors are encoded in the genome as gene segments of an array that rearrange at random when the T or B cell is being developed (Hira, 2022). Eosinophils act to fore off foreign antigens or microbes circulating in the blood and tissues as a principal responsibility of these immune cells (Parija,2023).

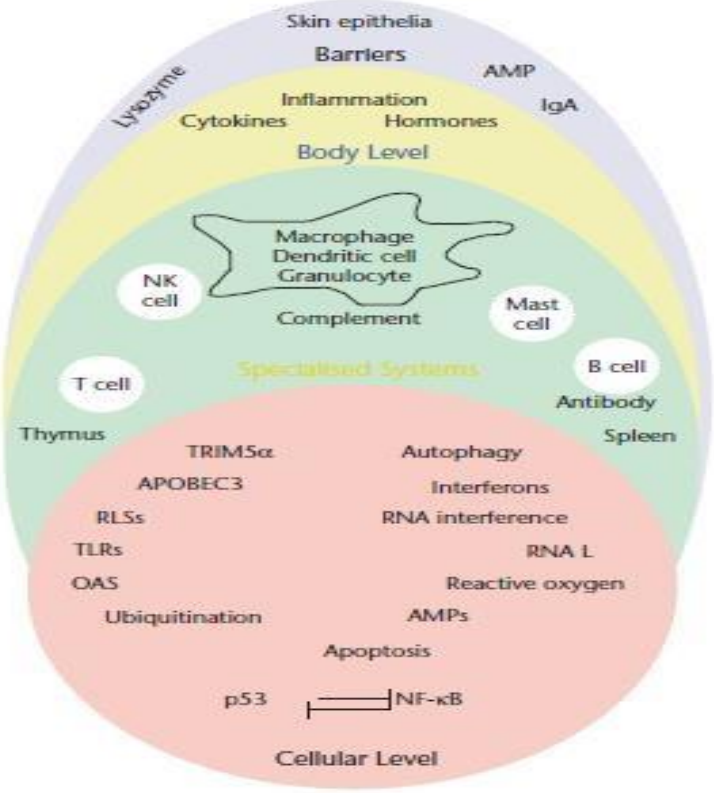


Figure (2.1): Human immune system (Danilova, 2008)

2.1.4.1. Host immune response to respiratory tract

For various reasons, the immunological defense is viewed as a complex phenomenon which divides the human organism's immune response into two categories – innate and adaptive immunity. The initial cells that come into contact with respiratory pathogens in the air are the linings of the nasal airway cells. During an episode of acute respiratory infection (ARI), nasal epithelial cells produce an influx of inflammatory cytokines following the invasion of microbial pathogens into the epithelial cells (King and sharma ,2015; Dembic, 2015). However, the detection of cytokine in nasal wash or nasopharyngeal specimens is not frequently assessed in children with ARI (Breindahl *et al*., 2012, Faber *et al*., 2012, Bertrand *et al*., 2015).

Host immune response chemotactic cytokines, also known as chemokines, are tiny proteins secreted by diverse host immune cells in order to regulate the immune

system. It is noteworthy to mention that cytokines are created by T lymphocytes and coagulate in response to inflammatory and immunomodulatory stimuli, as previously stated. Cytokines, which are produced by T lymphocytes, have the ability to function as both inflammatory and immunoregulating agents. They play a crucial role in balancing the pathogenesis of diseases and the clearance of pathogens. Some commonly reported cytokines that are released during respiratory infections include interleukin (IL)-1ß, interleukin -2, interleukin -5, interleukin -6, interleukin -8, interleukin -10, interleukin -12, interleukin -13, interleukin -17,

Inducible protein (IP)-10, Tumor Necrosis Factor (TNF)-α, and Interferon (IFN)-γ .Cytokines (IL-2, TNF and IFN –γ) are produced by T helper 1 (Th-1) type immune cells and contribute to cell-mediated immunity against intracellular bacteria and viruses by activating macrophages (as shown in figure 2.2). Furthermore, these cytokines play a pivotal role in the phagocytic-dependent immune response (Calbo and Garau, 2010; Turner *et al*., 2014; Dembic, 2015; Russell *et al*., 2017).

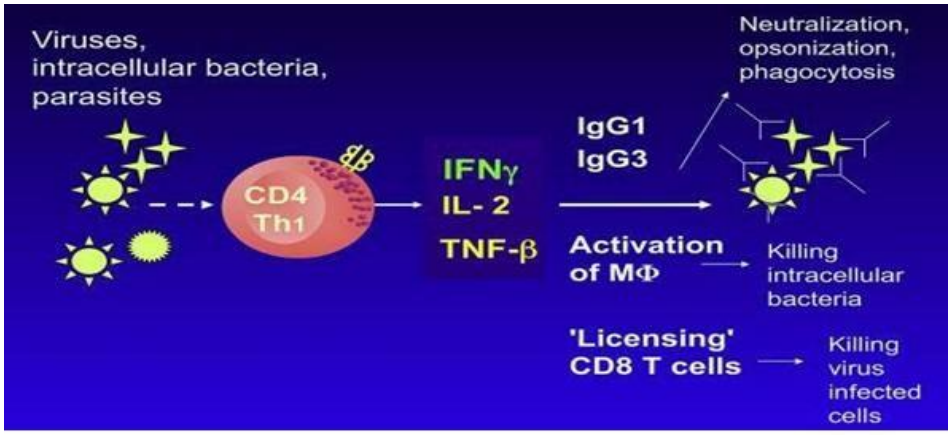


Figure (2.2): Th1 Lymphocytes step in the immune response (Bhuiyan, 2019).

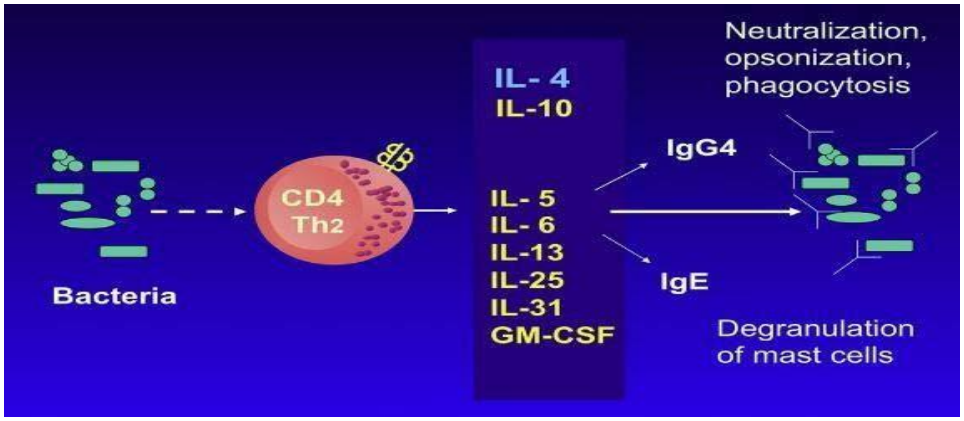


Figure (2. 3): Th2 Lymphocytes step in the immune response (Bhuiyan, 2019).

Cytokines such as interlukine-4, interlukine-5,interlukine-6, interlukine-10 and intrelukine-13 are generated by immune cells of the Th-2 phenotype (as shown in figure 23). These Th-2 cytokines facilitate the production of immunoglobulin E (IgE), thereby eliciting a robust antibody response. Furthermore, they suppress the phagocytosis performed by Th1-type immune cells (Turner *et al*., 2014; Dembic, 2015). These findings suggest that IP-10 a chemokine being induced by IFN- γ is a chemo attractant which attracts activated T cells (Liu *et al*., 2011).

Cytokines are mainly strain-specific pro-inflammatory immunomodulating proteins, which are mainly released at the site of infection. However, it is identified that potential could occur when the cytokines levels reach a higher threshold than normal, spilling over into the systemic circulation (Bhuiyan, 2019). It is well documented that the cytokines levels increase in the systemic circulation of patients with pneumonia (de Brito *et al*., 2016 ;Hoffmann *et al*., 2016). Based on the type of pathogen, the severity of ARI and immuno-physiological maturation, and lung injury, various cytokines predominate in these adversities (Bhuiyan, 2019). Pro-inflammatory cytokines include IL-1ß, IL- β, TNF-α, and

IFN-γ , which are prevalent in the early stages of infection. However, there are alternative markers such as interleukin-4 (IL-4), Interleukin-10 (IL-10), and Interleukin-13 (IL-13) in the process and are referred to as inflammation markers (Turner *et al*., 2014; Dembic, 2015).

The pro-inflammatory marker known as cytokine IL-6 has gained considerable recognition; however, recent reports have highlighted its immunosuppressive effect with anti-inflammatory properties (Scheller *et al.*, 2011). Anti-inflammatory agents' primary function is to reduce inflammation by suppression pro-inflammatory agents, which in the most severe case can cause serious diseases and organ malfunction (Schulte *et al*., 2013). For example, in cases of pneumonia, the inflammatory response within the lungs requires proper coordination to maintain a delicate equilibrium between pro-inflammatory and anti-inflammatory markers. Any insufficiency or excessive production of either type of inflammatory marker may result in more severe health consequences, such as septic shock and mortality (Moldoveanu *et al*., 2008).

2.1.5. Upper Respiratory Tract Infections

URIs are a group of diseases that affect the mucous membrane of the upper airway passages including the nostrils, paranasal sinuses, the throat, and the larynx (Calderaro *et al*., 2022). Now, according to the International Statistical Classification of Diseases, a URI can be classified to acute nasopharyngitis, acute sinusitis, acute pharyngitis, acute tonsillitis, acute laryngitis, and laryngotracheitis or laryngotracheobronchitis (as shown in the figure 2. 4).

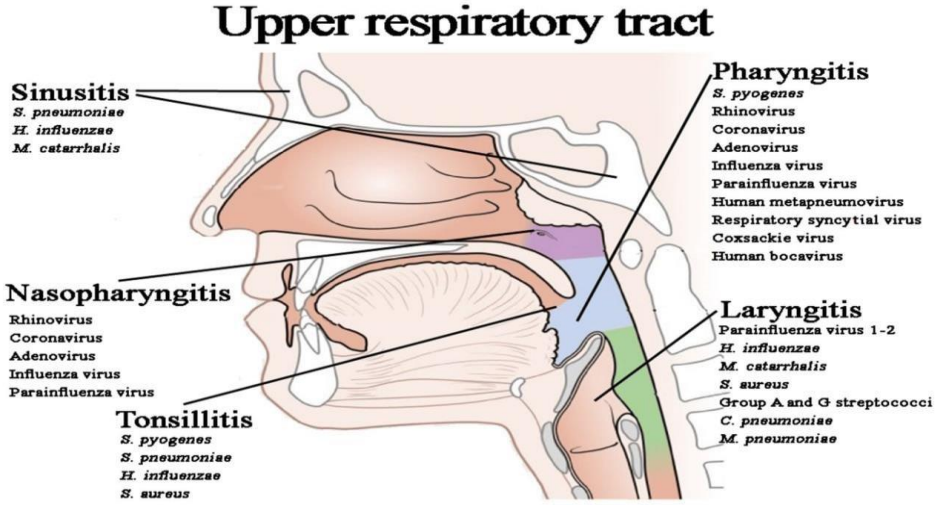


Figure (2.4) categorizing the causative agents of the URIs (Calderaro *et al*.,2022)

Human rhinoviruses (hRV) are the main source of (URTIs) and are thought to account for the majority of cases (Thomas and Bomar, 2018; Bosch *et al*., 2016). Sneezing, coughing, pharyngitis, and nasal obstruction and discharge are signs of URIs. It is also possible for irritability to arise, particularly in young children (Brealey, 2017). These infections can range in severity from mild, self-limiting illnesses like the seasonal flu to serious illnesses like epiglottitis. (Peroš-Golubičić and Tekavec-Trkanjec, 2015).

Human respiratory viruses, which are responsible for the majorities of cases of URIs, are classified as following: human rhinoviruses (hRVs), respiratory syncytial virus (RSV), parainfluenza viruses (PIVs), influenza viruses, metapneumoviruses (MPV) and to a lesser extent, respiratory enteroviruses. The results of morbidity tied to viral URIs, for instance, bacterial sinusitis and bacterial otitis media are identified. The three principal bacteria that can cause such complications include *Streptococcus pneumoniae*, nontypeable *Haemophilus influenzae*, and *Moraxella catarrhalis* (Butler 2010; Marom *et al*., 2014; Nokso-Koivisto *et al*.,2015).

In addition , it is noted that in general, the prognosis of URIs is quite favorable and there, on average, their duration is individually calculated to be 7 to 10 days (Peroš Golubičić and Tekavec-Trkanjec, 2015).

Various bacterial and viral communities within the upper respiratory tract (URT) and the development of respiratory syncytial virus (RSV) infection in children. In contrast to other body sites, the URT microbiome shows a clear link between microbiota composition and immune system, the severity of the disease, and the number of wheezing episodes (Rosas-Salazar *et al*. ,2022)

2.1.5.1. Common Cold (Nasopharyngitis)

The common cold is an informal term for a viral infection of the upper respiratory tract that is officially known as nasopharyngitis. It is manifested by such signs as having a stuffy or blocked nose, sore throat, cough, sneezing, and low-grade fever. The main representative of the common cold is the rhinovirus which leads to these symptoms through viral infection (Pappas, 2018; Rahmadiena *et al*.,2021).

Colds are common since people do not have long-term immunity after getting infected with certain viruses and because there are different serotypes within certain viruses. For instance, respiratory syncytial virus (RSV), parainfluenza viruses (PIVs), and human coronaviruses (HCoVs) are examples of cold viruses that are not lifelong. On the other hand, viruses including rhinoviruses, adenoviruses, influenza viruses and enteroviruses have multiple serotypes, but they elicit serotype specific immune response after infection though long lasting (Wilson *et al*., 2021).

2.1.5.2. Pharyngotonsillitis

Acute pharyngotonsillitis which involves redness in the pharynx and tonsils can be categorized as acute or chronic (Adam *et al*., 2023). This is a common childhood illness and is caused by a variety of pathogens such as viruses and bacteria (Rhumaid *et al.,* 2023). Most cases of pharyngotonsillitis are caused by viral infections, contributing to about 60% of all cases (Stjernquist_Desatnik and Orrling, 2009). Pneumonia can be caused by various bacteria, including Group A β-hemolytic streptococci (which contribute to 15-40% of patients), *H.influenzae*, *staph.aureus*, *Mycoplasma pneumoniae*, and anaerobic bacteria living in the oral cavity. Some of the well-known viruses that are known to be potentially fatal are Adenovirus, the influenza virus, parainfluenza virus, Epstein-Barr virus (EBV), and enterovirus. The true cause of pharyngotonsillitis is difficult to identify due to its clinical appearance; therefore, laboratory testing and culture methods should be utilized to identify the pathogen (Schlossberg, 2015; Rhumaid *et al*., 2023).

2.1.5.3. Acute otitis media

Acute otitis media can be described as an infection that occurs in the airfilled cavity of the middle ear. This category of diseases includes Acute Otitis Media (AOM), Chronic Suppurative Otitis Media (CSOM), and Otitis Media with Effusion (OME). Acute otitis media is the second most common diagnosis in childhood after upper respiratory tract infections (Meherali *et al*.,2019).

The causes of the otitis media infection include viral, bacterial, or a combination of two. Among the bacterial pathogens, *Strep. pneumoniae* is said to be the most frequently isolated, then non-typeable *H. influenzae* (NTHi) and *Moraxella catarrhalis*. The introduction of conjugate pneumococcal vaccines has led to changes in the pneumococcal organisms and the emergence of non-vaccine serotypes. In terms of viral etiology, the respiratory syncytial virus (RSV),

coronaviruses, influenza viruses, adenoviruses, human metapneumovirus, and picornaviruses are the most frequently encountered pathogens associated with otitis media (Ubukata *et al*., 2018; Ubukata *et al*., 2019; Protasova *et al*., 2017).

The occurrence of otitis media reaches its highest point between the ages of six and twelve months and decreases after the age of five. It is estimated that approximately 80% of all children will experience a case of otitis media at some point in their lives, and between 80% and 90% of all children will experience otitis media with an effusion before reaching school age. Otitis media is less prevalent in adults compared to children, although certain sub-populations, such as those with a history of recurrent OM during childhood, cleft palate, immunodeficiency or immunocompromised status, and others, have a higher incidence (Usonis *et al*., 2016; Schilder *et al*., 2016).

Acute otitis media is one of the most frequent diseases in the world and may cause different acute complications and hearing impairments and bacterial biofilms are involved in the development of chronic adenoiditis and middle ear infections in children. (Torretta *et al*.,2019)

2.1.6. Lower Respiratory Tract Infections

LRIs are acute infectious illnesses involving the bronchi, bronchioles, alveoli, and lungs. The term LRIs is a broad definition that refers to a variety of infectious inflammatory diseases of the lower respiratory airways, among which acute bronchitis , acute bronchiolitis and pneumonia are major matters of concern (Figure 2.5).

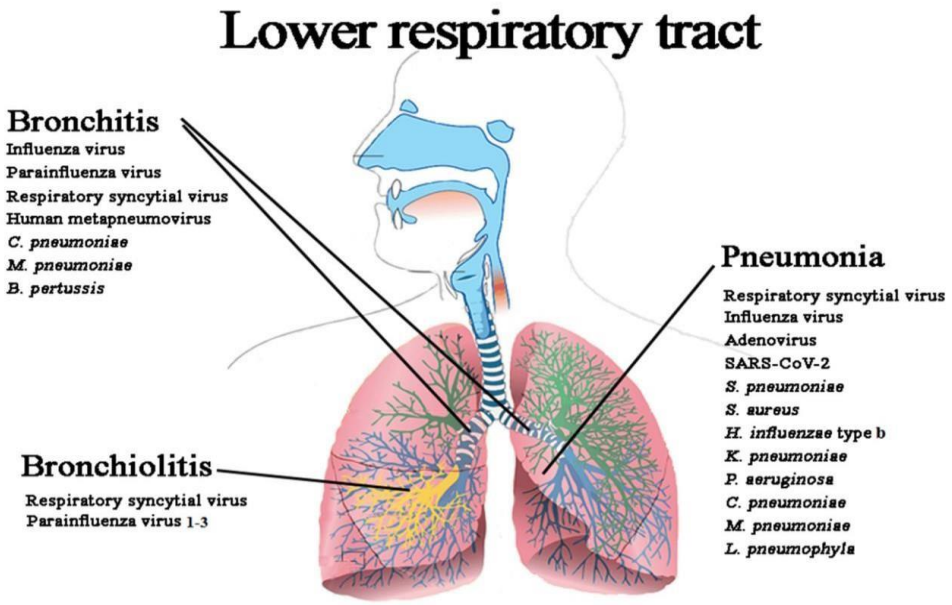


Figure (2.5) categorizing the causative agents of the URIs with the associated most relevant causative agents (Calderaro *et al*.,2022)

Lower respiratory tract infection (LRTIs) is the predominant etiology of fatal infectious ailments globally, ranking fifth overall in terms of mortality, and constituting the second principal contributor to disability adjusted life years (DALYs). Though they are known to be mainly avoidable causes of ailments and death, their occurrence continues to be high (Al-Haifi *et al*., 2020; Troeger *et al*., 2018; Edin, 2018; Liu *et al*., 2015). Changes in the epidemiological profile of lower respiratory tract infections (LRTIs) have occurred over the past decade. These changes are defined by a reduced number of cases among children younger than five years and an increase in caseload among the geriatric population. Also, the rate of viral diseases has increased in the recent past (Troeger *et al*., 2018). However, it was suggested that such inconsistency in categorization of the (LRTIs) prevents from understanding its actual epidemiological role (Feldman and Shaddock, 2019; Troeger, *et al*., 2018). From an epidemiological view, most definitions of LRTI include influenza, pneumonia, acute bronchitis, including AECOPD, and bronchiolitis as important diseases (Al-Haifi *et al*., 2020; Fair and

Tor, 2014; Nowicki and Murray, 2020). The most common bacteria implicated in LRTIs include Gram-positive cocci especially *Staphylococcus aureus* and Enterococci. In addition to Gram-negative bacteria like Acinetobacter spp. *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and H.influenza (Gebre *et al*., 2021; Singh *et al*., 2020; Santella *et al*., 2021).

viral infection is not often the leading reason for acute lower respiratory tract illness. A significant percentage of patients are linked to bacteriologic and atypical organisms, which include Chlamydia pneumonia (Vos *et al*.,2021).

RSV is the predominant viral cause of acute lower respiratory tract infections (ALRTI) in children aged less than 5 years, particularly in infants younger than 6 months of age. It exhibits a higher prevalence during the winter season and is associated with the development of severe infection (Fattouh *et al*., 2011).

2.1.6.1. Pneumonia

Pneumonia, a respiratory infection known for its acute nature and impact on the lungs, results in the accumulation of inflammatory fluid in the alveoli. This fluid obstructs oxygen absorption, leading to breathing difficulties. The development of pneumonia is attributed to the invasion and excessive growth of microorganisms in lung tissue, although the precise pathophysiological mechanisms remain unclear (as shown in figure 2.6) (Bhuiyan, 2019) . The agents that cause pneumonia, such as *Strep pneumoniae*, *H. influenzae*, or *Chlamydophila pneumoniae*, can be used to categorize cases of pneumonia . Pneumonia is also categorized into three subcategories based on the clinical circumstances in which the patient manifests symptoms of infection: community-acquired pneumonia (CAP), hospital-acquired pneumonia, and ventilator-associated pneumonia (Dueck *et al*., 2021).

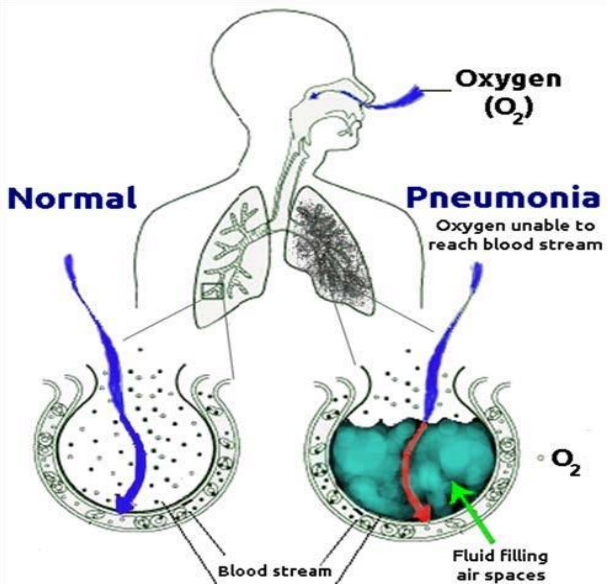


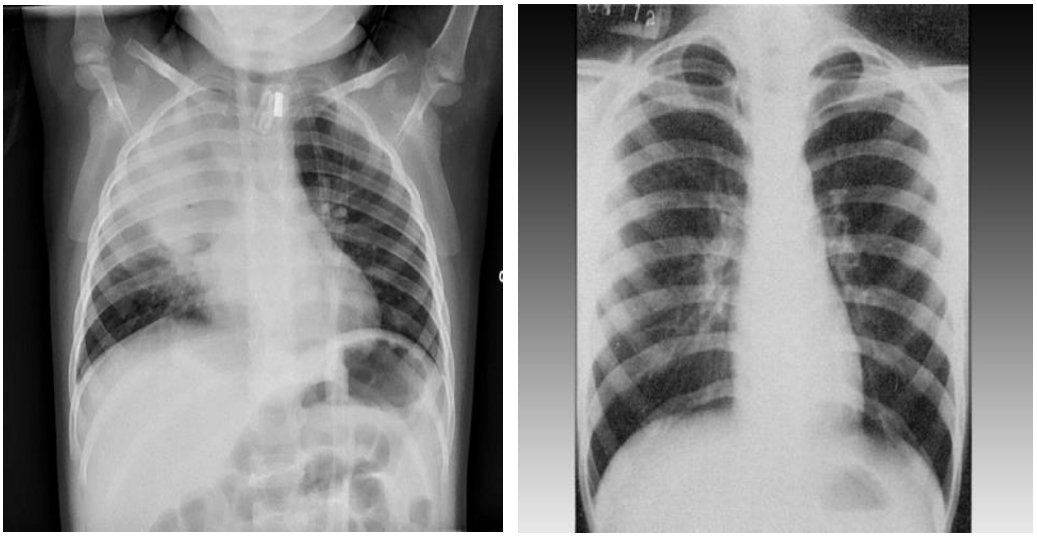
Figure (2.6): Pathogenesis of Pneumonia (Bhuiyan, 2019). community-acquired pneumonia is characterized as a respiratory tract infection that occurs in individuals outside of a hospital environment. This type of infection is accompanied by acute infection symptoms and the presence of new opacities on a chest radiograph, if one is performed (Dueck *et al*., 2021; Kaysin and Viera, 2016).

Hospital-acquired pneumonia (HAP) represents the prevailing infectious condition within the intensive care unit (ICU). This particular ailment encompasses two distinct entities: pneumonia associated with mechanical ventilation (ventilatorassociated pneumonia or VAP) and the manifestation of severe pneumonia during the duration of hospitalization (Leone *et al*., 2018; Lanks *et al*., 2019).

The World Health Organization (WHO) formulated a straightforward clinical algorithm for the diagnosis of community-acquired pneumonia (CAP) based on available evidence and guidelines. This algorithm focuses on identifying the presence of cough and rapid or labored breathing. Moreover , the presence of other symptoms such as feeding difficulties, elevated respiratory rate, chest wall indrawing, fever, and rapid heartbeat provide further evidence of the severity of the disease (World Health Organization (WHO) 1991; World Health Organization

(WHO) 1994). The WHO definition has proven to be highly sensitive and appropriate for capturing the majority of pneumonia cases, especially in resourcelimited and inadequately equipped healthcare settings.

However, this definition lacks specificity as there are several other childhood illnesses that share similar signs and symptoms. Although the WHO radiological definition, which involves the detection of lobar pulmonary consolidation, has improved specificity for the two most significant bacterial pathogens associated with pneumonia, *Strep. pneumoniae and H. influenzae* type b (Figure 2.7, A), it lacks sensitivity in identifying cases caused by other pathogens, including viral pathogens .These pathogens are commonly associated with interstitial infiltrate (Figure 2.7, B) (Cherian *et al*., 2005).



A **B**

Figure (2.7): A. Chest Radiograph of Lobar Consolidation, B. Chest radiograph of interstitial consolidation (Bhuiyan, 2019).

In relation to community-acquired pneumonia caused by bacterial communities, it is noteworthy that *Strep. pneumoniae* is the predominant causative pathogen in children (Bénet *et al*., 2017, Wahl *et al*., 2018). In addition to this, pneumonia caused

by *H. influenzae* type b should also be considered (Jullien, 2021; Scott and English, 2008). *Staph. aureus and Strep. pyogenes*, although less frequent causes of bacterial pneumonia in children, are often associated with severe disease (O'Brien *et al*., 2019, Harris *et al*., 2011). A significant proportion of causative agents of bacterial pneumonia include Mycoplasma pneumoniae and Chlamydia pneumoniae. While these agents are more frequently detected in children older than five years of age, they should not be disregarded in younger children (Jain, *et al*., 2015; O'Brien, *et al*., 2019).

It is important to note that hospital-acquired pneumonia is caused by different microorganisms compared to community-acquired pneumonia. Gram-negative bacteria, such as *Klebsiella pneumoniae and Pseudomonas aeruginosa*, are prominent causal pathogens (Scott *et al*., 2012). Other pathogens, including *Bordetella pertussis* and *Legionella pneumophila*, may also result in clinical episodes that are often indistinguishable from more classical cases of bacterial pneumonia. On the other hand, viruses are the primary etiological factor of pneumonia in children below the age of five years, with the respiratory syncytial virus (RSV) being universally recognized as the most prevalent pathogen in this particular age cohort (Rudan *et al*., 2013; Jullien, 2021). Additional notable viral origins of pneumonia encompass influenza viruses, parainfluenza viruses, human rhinovirus, and human metapneumovirus (Shi *et al*., 2015; O'Brien, *et al*., 2019; Benet *et al*., 2017).

Despite the highly sensitive nature of the radiological definition in diagnosing pneumonia cases, many developing countries still rely on the WHO clinical case definition due to the lack of appropriate radiological equipment in healthcare facilities and the need to capture a wide range of pneumonia cases with varying causes (Scott *et al*., 2012). Another reason is to be able to capture as many pneumonia cases as possible across the causative agents. Systematic review and

meta-analysis of clinical manifestation of pneumonia in children under five years revealed making it clear that there is no specific clinical sign or symptoms that is diagnostic of pneumonia. The authors revealed that lower chest wall indrawing and rapid breathing did not have high potential in contributing to the classification process while nasal flaring, grunting, respiratory rate greater than 50 breaths per minute, and chest indrawing were more informative. As a result, pneumonia appreciations based on various factors may improve the accuracy of diagnosing this disease in children (Rambaud-Althaus *et al*., 2015; Rhedi *et al*., 2019). For instance, in 2019, it was found that increase in mortality of pneumonia was 14% of the total child deaths under age of 5 to be precise 740,180 children died from pneumonia before turning 5 years (Tekam *et al*., 2023).

2.1.6.2 Bronchitis and bronchiolitis

Bronchitis and bronchiolitis are lower respiratory tract diseases that are caused by inflammation of bronchial tissues. Bronchitis is caused by inflammation of the bronchi. It manifests as a chronic cough, fever, and wheezing (Polack *et al*., 2019).

The condition is typically classed as either acute or chronic, acute bronchitis can be caused by viruses such rhinovirus, coronavirus ,adenovirus, or influenza virus, as well as bacteria like *Bordetella pertussis, Mycoplasma pneumoniae,* and *Chlamydophila pneumonia*. Laryngo-trachea bronchitis is also prevalent, resulting from inflammation of the larynx and trachea. It begins with a regular cold, which causes fever, coughing, and upper airway blockage symptoms. Bronchiolitis is defined as bronchiole blockage caused by mucus collection, followed by bronchiole plugging, which causes wheezing. The primary signs and symptoms include fever, dry wheezy cough, and nasal discharge with increased respiratory effort. Bronchiolitis is a significant cause of hospitalization for newborns.

(Antonucci and Oggiano, 2015; Polack *et al*., 2019).

2.1.7. Infections in Immunosuppressed Children

Immunocompromised children are notably susceptible to both upper and lower respiratory tract infections, leading to increased rates of morbidity and mortality. The fatality rate among children with primary immunodeficiency is approximately between 13.6 to 17.5%. Specifically, lower respiratory tract infections account for the majority of mortality cases at 44%, with prevalent pathogenic microorganisms being *Pseudomonas* and *Staphylococcus*. The mortality rate associated with viral lower respiratory infections in this cohort was 0.7%. Various factors have been identified as predictors of mortality in children with pneumonia, including age less than 12 months, malnutrition, tachycardia, anemia, initial lymphocyte count equal to or less than 800/μL, and hypoxemia. In the context of immunocompromised children with respiratory tract infections, these findings are crucial for understanding and managing the associated risks (Sutrisna *et al*., 2022).

2.1.8. Bacterial Respiratory Pathogens

It is commonly accepted that individuals have created relationship with their symbiotic bacteria, which are essential for good health. However, local alterations that disrupt the symbiotic relationship, resulting dysbiosis, cause a variety of disorder, including respiratory infections, allergies, and asthma. This disorder can be attributed, in part, to the initial colonization process, which is primarily impacted by the type of delivery (cesarean section or normal birth) and also by breastfeeding (Curtis *et al*., 2012, Ranucci *et al*., 2017) .

Nasopharyngeal infections are prevalent worldwide and are associated with significant morbidity. In contrast, lower respiratory tract infections are relatively rare but have a higher mortality rate. The nasal and oral cavities play a unique role

in determining the bacterial composition and development. As an individual age, the microbiome of the mouth and pharynx becomes increasingly similar. Depending on the specific location of pathogenic bacteria growth, it can result in local dysfunction, such as pharyngitis, or a more widespread disease, such as pneumonia (Bassis *et al*., 2014; de Steenhuijsen *et al*., 2016).

The rhino-pharynx harbors numerous potential pathogenic bacteria, including *S. pneumoniae, H. influenza, and S. aureus*, which are considered part of the normal microbiota. However, the rhino-pharynx also serves as a reservoir for germs associated with acute respiratory infections. The spread of *S. pneumoniae* from the rhino-pharynx can lead to pneumonia, meningitis, or sepsis. In young adults, colonization is less frequent and shorter in duration due to a robust immune response, resulting in a lower incidence of disease unless there are other factors or a concurrent flu-like infection (Bassis *et al*., 2014; Huang and Boushey, 2014 ; Gallacher and Kotecha, 2016). In the elderly, transmission rates are lower, but there is a higher incidence of pneumonia. Some studies suggest that changes in the elderly microbiome contribute to increased susceptibility to respiratory infections. nevertheless, it remains unknown whether specific bacterial species or the overall dynamics of the bacterial community make the elderly be more vulnerable to respiratory infections. Changes in the immune system associated with aging contribute to the increased incidence of respiratory infections (Bassis *et al*., 2014; de Steenhuijsen Piters *et al*., 2016 and Teo *et al*., 2015).

The sinuses are cavities surrounding the nose where inflammations, such as acute or recurrent sinusitis, may occur.

Sinusitis can affect the ethmoidal, sphenoidal, and frontal sinuses. While the sinuses typically have their own distinct microbiome, they can also be influenced by the nasal and oral cavity microbiota, particularly when there is an accumulation of

secretions. The main bacteria involved in sinusitis are *Pneumococcus, Haemophilus,* and *Streptococcus*.

Lastly, rhinitis, pharyngitis, and tonsillitis are caused by a heterogeneous group of pathogens, including viruses and bacteria. Hemolytic streptococcus is the most common bacterial cause, accounting for 15% of cases (Bokulich *et al*., 2016; Marsland and Gollwitzer, 2014).

Otitis is an acute infection of the middle ear that primarily affects children and is mainly caused by Pneumococcus, Haemophilus, and Staphylococcus (Rogers *et al*., 2014; Di Serio *et al*., 2016, Johnston and Douglas, 2018). Several factors can either increase or decrease the risk of developing a lung disease.

The inflammation of the lungs resulting from infection during early childhood is associated with the development of asthma. Therefore, the dysbiosis of the airway microbiota could serve as the foundation for the vulnerability and progression of chronic lung disease. Initially, most newborns are colonized by Staphylococcus or Corynebacterium before acquiring stable colonization with Moraxella. The correlation between bacterial colonization of the airways in children and the emergence of asthma later in life has been observed. Infants whose pharynx has been colonized by *Streptococcus pneumoniae, Haemophilus influenzae, or Moraxella catarrhalis* since birth have an increased risk of asthma. These same bacteria are consistently associated with the exacerbation of asthma, including chronic obstructive pulmonary disease (Santacroce *et al*., 2020).

2.1.8.1. Gram-Positive Bacteria of Respiratory Pathogens

The predominant bacterial pathogens responsible for lower respiratory tract infections (LRTIs) are Gram-positive bacteria, specifically *S.aureus and Enterococcus* spp. (Singh *et al*., 2020 ; Gebre *et al*., 2021). Regrettably, the ongoing dissemination of extended-spectrum β-lactamases and carbapenems has initiated a

constriction in the clinical effectiveness of β-lactam agents. This inclination is most likely attributed to the empirical administration of antibacterial therapy (Aslan and Akova, 2019). The situation is further complicated by the emergence of multi-resistant pathogens, such as *K. pneumoniae* carbapenemase and *H. influenzae* β-lactamase (Ferreira *et al*., 2019). Consequently, the current knowledge pertaining to bacterial etiology and their antimicrobial susceptibility pattern would facilitate the selection of antimicrobial therapy for bacterial LRTIs, thereby curbing the development of antimicrobial resistance and diminishing overall management expenditures (Llor and Bjerrum, 2014; Brusaferro *et al*., 2018).

2.1.8.2. Gram Negative Bacteria of Respiratory Pathogens

The immune resistance of children is lower, making respiratory tract infections as a prevalent occurrence among paediatric diseases (Rebolledo *et al*., 2023). These infections are caused by various pathogens, including bacteria. Among these bacteria, Gram-negative bacteria are known to be particularly pathogenic in children (Castagnola *et al*., 2022; Bamshmous, *et al*., 2021).

Gram negative bacteria are characterized by their pink color when stained with Gram stain, due to the thin peptidoglycan layer in their cell wall (Paray *et al*., 2023). They also have an outer membrane that contains lipopolysaccharides, which are important virulence factors that can cause inflammation and damage to host tissues (Giordano *et al*., 2020, Chen *et al*., 2023).

Some common examples of Gram negative bacteria include: *H. influenza* (is a major cause of bacterial pneumonia in young children) (Slack, 2021; Tripathi and Mukherjee , 2024), *Pseudomonas aeruginosa* (Although less common in healthy children, it can cause respiratory tract infections, especially in those with underlying conditions like cystic fibrosis or compromised immunity) (Bhagirath *et al*., 2016;

Dong *et al*., 2022*), K. pneumonia* (is known to cause severe respiratory infections in immunocompromised children) (Zar *et al.*, 2022), *Moraxella catarrhalis* (often responsible for otitis media and respiratory tract infections, especially in younger children) (Bair and Campagnari, 2020) and Acinetobacter baumannii (more frequently associated with hospital-acquired infections, but it can also cause respiratory tract infections in children, particularly those with significant medical conditions) (Ayobami *et al*., 2019).

Children are especially susceptible to infections caused by Gram-negative bacteria in the respiratory tract which is due to their developing immune systems and close proximity to other children in school and daycare settings. Of these bacteria, those classified as Gram negative are known to cause infections of the respiratory tracts in children and lead to myriad respiratory disorders including bronchitis, bronchiolitis, otitis media, sinusitis and pneumonia (Zhu *et al*., 2022; Rodrigo_Troyano and Sibila, 2017).

2.1.9. Biomarkers

The fundamental elucidation of a biomarker may appear straightforward at first glance The following trait definition applies: A property that is measured as an index of normal biological processes, disease processes, or responses to a stimulus or intervention. These diverse characteristics may be derived from molecular, histological, radiological, or physiological properties in conjunction with medical interventions(Califf, 2018).

2.1.9.1. Soluble Triggering Receptor Expressed on Myeloid Cells 1 (sTREM-1)

Myeloid cell trigger receptor-1 (TREM-1) is a member of the immunoglobulin super receptor family mainly expressed on the surface of myeloid cells such as neutrophils and monocytes/macrophages. Soluble TREM-1 (sTREM1),

is a TREM-1 subtype that has been reported as a novel and strong indicator of pneumonia (Zhao *et al*., 2020) . It has been used as a marker for identifying infection and assessing inflammation severity in adult patients with ventilatorassociated pneumonia (Yang *et al*., 2021) .

Soluble Triggering Receptor Expressed on Myeloid Cells 1 (sTREM-1) plays a crucial role in pneumonia in children, particularly in severe cases like Severe Mycoplasma Pneumoniae Pneumonia (SMPP) and ventilator-associated pneumonia (VAP). Studies have shown that sTREM-1 levels are positively correlated with the severity of pneumonia, indicating its potential as a diagnostic biomarker for SMPP (Xu et al., 2024). TREM-1 is a receptor expressed on the surface of neutrophils and monocytes. When triggered by microbial products, it amplifies the immune response by promoting the release of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 , during infections like pneumonia, the membrane-bound TREM-1 can be cleaved, resulting in the release of its soluble form, sTREM-1, into the circulation. The level of sTREM-1 correlates with the severity of the inflammatory response (Sigalov, 2014) . sTREM-1 can further amplify the inflammatory response by potentiating the effects of other inflammatory mediators. This is crucial in combating infections but can also lead to tissue damage if not properly regulated , elevated levels of sTREM-1 in body fluids (e.g., blood, bronchoalveolar lavage fluid) have been associated with bacterial infections and severe pneumonia. In children, sTREM-1 can serve as a biomarker to distinguish bacterial from viral pneumonia and to assess the severity of the infection (Abdelgawad *et al* ., 2024).

2.1.9.2. Chitinase-3 Like-Protein-1 (CHI3L1)

CHI3L1 from glycoside hydrolase family 18 recognize chitin, a Polysaccharide made of repeating N-acetylglucosamine units and a polymer of important structure in arthropod and other group (Zhao *et al*., 2023) . CHI3L1 is

primarily produced by macrophages, neutrophils, epithelial cells, and fibroblasts in response to inflammatory stimuli, such as infection or tissue injury. Its expression is upregulated in conditions of acute and chronic inflammation , CHI3L1 influences the activity of various immune cells, including macrophages and neutrophils, which are crucial in the defense against respiratory pathogens. It modulates the balance between pro-inflammatory and anti-inflammatory responses, helping to regulate the intensity and duration of inflammation (Mizoguchi and Yorioka, 2024).

In the lungs, CHI3L1 is involved in the inflammatory response to infectious agents such as bacteria, viruses, and fungi. It contributes to the recruitment and activation of immune cells at the site of infection, promoting the clearance of pathogens beyond its role in acute inflammation, CHI3L1 is implicated in tissue remodeling and repair processes. In the context of pneumonia, this can be beneficial by promoting healing, but excessive or prolonged CHI3L1 expression may lead to fibrosis and impaired lung function, particularly in severe or chronic cases , CHI3L1 also plays a role in maintaining epithelial barrier function and regulating mucus production in the airways, which are critical in protecting against respiratory infections , dysregulation of these processes can contribute to disease severity and complications in pneumonia CHI3L1 is known to mediate inflammation and macrophage polarization, which are crucial processes in the pathophysiology of pneumonia (Yu *et al*., 2024).

2.1.9.3. Soluble Urokinase Plasminogen Activator Receptor

Soluble urokinase plasminogen activator receptor (suPAR) is the soluble form of the cell membrane-bound protein uPAR, secreted from cells of immune defense, endothelial and smooth muscle origin. uPAR is expressed on various immune cells, including neutrophils, monocytes, and macrophages. Upon activation, particularly during inflammation or infection, uPAR can be cleaved to

release suPAR into the bloodstream. suPAR interacts with several receptors and signaling pathways, influencing immune cell behavior. It modulates the immune response by affecting cell adhesion, migration, and cytokine production, which are essential in the body's defense against respiratory pathogens (Saleh, *et al*., 2024).

Soluble urokinase plasminogen activator receptor plays a crucial role in determining the severity of pneumonia in both children and adults beyond its role in immune cell regulation, suPAR is involved in processes related to tissue remodeling and repair. In the lungs, this can affect the repair of damaged tissue following infection but may also contribute to fibrosis and other complications if the response is dysregulated , suPAR has been associated with endothelial dysfunction, which is important in the pathogenesis of severe pneumonia. Endothelial cells, which line the blood vessels in the lungs, can become activated by suPAR, leading to increased vascular permeability, edema, and potentially contributing to acute respiratory distress syndrome (El-Mekkawy *et al*., 2022).

suPAR is increasingly recognized as a biomarker that correlates with the severity of pneumonia and other infectious diseases. Elevated suPAR levels have been linked to poor outcomes, including prolonged hospitalization, the need for intensive care, and increased mortality risk. This makes suPAR a useful tool for risk stratification and guiding treatment decisions (Eefsen *et al*., 2023).

Chapter Three Materials and Methods

3.1. Materials

3.1.1. Equipment analysis and apparatus

Equipment that used in this work shown in the table (3-1).

Table (3-1): The equipment.

No.	Instruments	Company	Origin
1.	100-1000µl, 10-50 µl tips	Dolphin	Syria
2.	5ml syringe	Dolphin	Syria
3.	Centrifuge tube racks	Truslab	China
4.	Cover slide	Supertek	India
5.	Distillwator (Water distiller)	GFL	Germany
6.	EDTA tube	Vacuum blood collection tube	China
7.	Eppendorf (1.5ml tube)	Vitrex, Meae	China
8.	Gel tube	Xinle	China
9.	Hitachi cup	Driui	China
10.	Micropipette 10-100µL	Slamed	Germany
11.	Petri dish	Plasilab	Lebanon
12.	Standard loop	Himedia	Lebanon
13.	Sterile Container	Deltalab	Italy
14.	Tourniquet for blood	Yyfmed	China
15.	Transport swab	AFCO	Jordan
16.	Westergren tube	Yyfmed	China

Table (3-2): The apparatus.

No.	Apparatus	Company	Origin
1.	Absorbance ELISA microplate reader	Biotech	USA
2.	Autoclave	Hirayama	Japan
3.	Benson burner	Amal	Germany
4.	Centrifuge	Heraeus	Germany
5.	Deep freezer	Kirsh	Germany
6.	Elisys Uno	Human	German
7.	Incubator	Memmert	Germany
8.	Laminar flow safety cabinet	Labtec	UK
9.	Microscope	Olympus	Japan
10.	Refrigerator	Kiriazi	Egypt
11.	Sensitive balance	Denver	Germany
12.	Water bath	Memmert	Germany

3.1.2.Culture media

The culture media that used in this study were presented in table (3-3).

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

No.	Type of media	Company	Origin
1.	MacConkey agar	HIMEDIA	INDIA
2.	Blood agar	HIMEDIA	INDIA
3.	Urea base agar	HIMEDIA	INDIA
4.	Simmon citrate Agar	HIMEDIA	INDIA

5.	Muller –Hinton agar	Oxoid	USA
6.	Kligler Iron Agar	HIMEDIA	INDIA
7.	Chocolate Agar	HIMEDIA	INDIA
8.	Triple sugar Iron agar	HIMEDIA	INDIA
9.	Mannitol salt agar	HIMEDIA	INDIA
10.	Brain Heart Infusion broth	Oxoid	UK

3.1.3 Chemical and biological materials

The primary biological and chemical components utilized in this study are shown in the table (3-4). **Table (3-4) Chemical and biological materials used in the study.**

No.	Materials	Company	Origin
1.	Gram stain	Panreak	India
2.	Sodium chloride (Nacl)	BDH	UK
3.	Hydrogen peroxide	Himedia	India
4.	Kovacs reagent	Himedia	India
5.	Glycerol	Panreak	Spain

3.1.4. Kits

The kits were used according to the manufacturer and origin, as shown in the following table (3-5).

Table (3-5): The Kits.

No.	Kits	Company	Origin
1.	Human sTREM-1(soluble Triggering Receptor Expressed on Myeloid Cells-1) ELISA Kit	ELK Biotechnology	USA
2.	Human CHI3L1(Chitinase-3-Like Protein 1) ELISA Kit	ELK Biotechnology	USA
3.	Human suPAR (soluble urokinase-type plasminogen activator receptor) ELISA Kit	ELK Biotechnology	USA
4.	C-Reactive Protein (CRP)	Dirui CS-T180	China

3.2. Methods

3.2.1. Study design

The study design of the present study is a cross-sectional study, and the samples collection and investigations were descripted in figure (3-1).

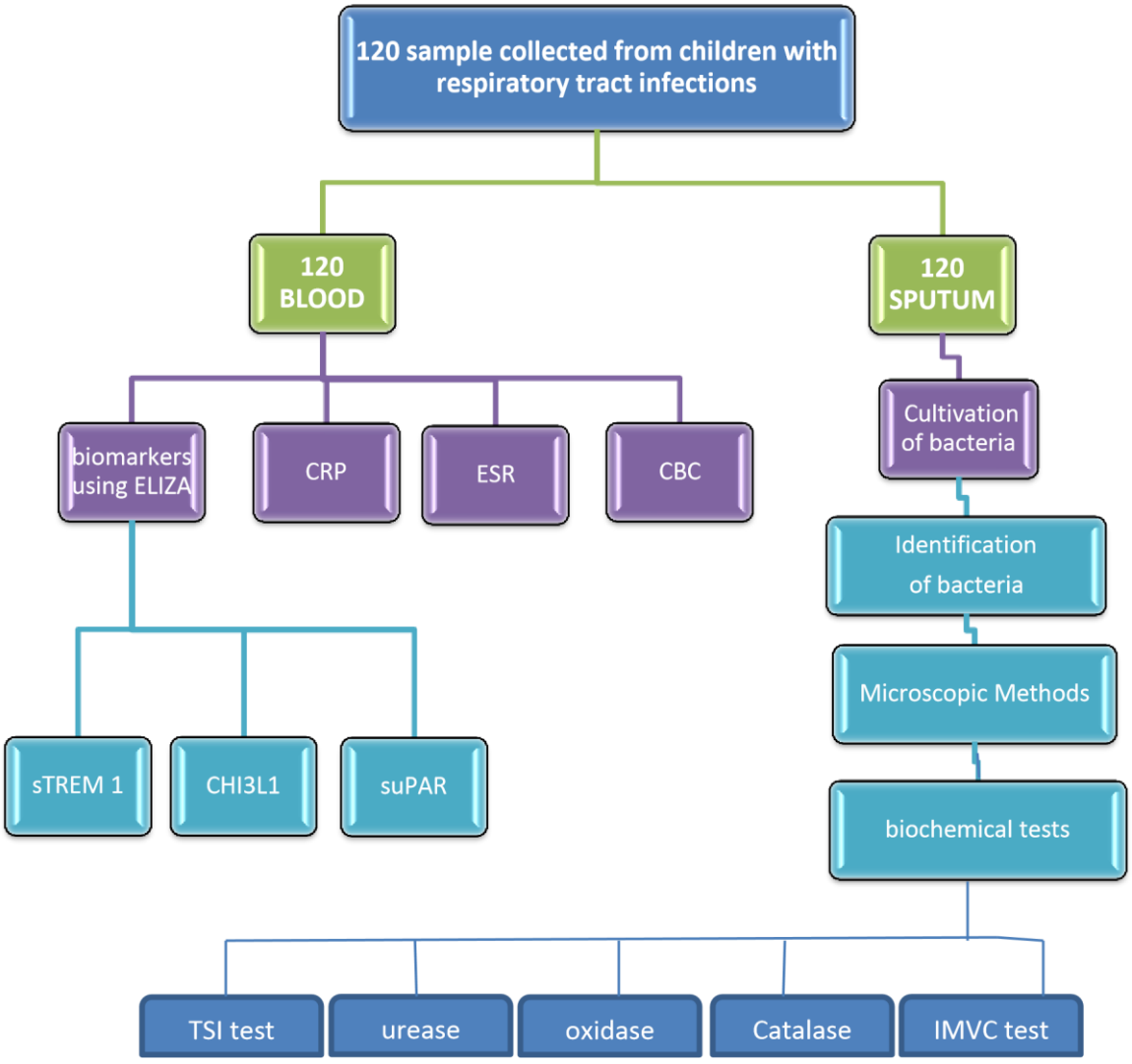


Figure (3-1): The study design and sample collection methods of the present study.

3.2.2. Sample collection

Collection of samples carried out in two places (Babil Teaching Hospital for Maternity and Children and AL nour hospital for children) during the period extend between November 2023 to April 2024 . Blood and sputum samples were obtained from 120 suspected respiratory tract infection (RTI) patients. They were between the ages of less than one to fourteen. Based on factors including :gender, age, site of infections, and kind of bacteria, these

individuals were divided into three groups: sever, moderate and mild according to (Thokngaen and Karoonboonyanan,2019). After being identified by pediatricians, patients were deemed to have a positive RTI based on the results of tests such as a chest x-ray (CXR), complete blood count (CBC), erythrocyte sedimentation rate (CRP), C-reactive protein (ESR), and sputum culture. In the current study, only bacteria with positive cultures were used; those with negative cultures were excluded

3.2.2.1 Collection of blood and serum preparation

Each patient provided five ml of venous blood, of which two ml was collected using sterile, disposable syringes and put into an anticoagulant tube containing ethylene-diamine-tetra acetic acid (EDTA). The blood samples were directly tested for (CBC) using the Sysmex XP-300 and measure ESR . Furthermore, three ml of blood were drawn into a gel tube, and the serum was separated by centrifuging for five minutes at 1000 rpm to measuring CRP by DIRUI CS-T180 and measure the biomarkers in the serum using ELISA.

3.2.1.2. sputum preparation

Sputum or tracheal aspirates samples were collected from patients who were clinically diagnosed with (LRTIs) and put sample in the sterile container which was labelled and add few drop of normal slain to maintain the fluidity of the sputum. For tracheal aspirates samples, they were taken by suction tube tip was cut with a scalpel during the bronchial aspiration in order to collect the sample. Whereas from patients who were suspected (URTIs) throat specimens were collected, the handle of a spoon was used to depress the tongue was used to flatten the tongue in such a way that it remains extended so as to provide a clear view of the mouth and throat, the sample was then collected from the posterior pharynx, tonsils, and any inflamed area. Samples were transported immediately into biological laboratory in the hospital for analysis . All sample

streaked into chocolate medium, blood medium and MacConkey medium with the help of sterilized loop for inoculation of bacteria. Any sample with less sputum volume than the amount necessary was rejected.

Blood, Mannitol salt agar, and MacConkey mediums were incubated in the incubator at 35°C for 18-24 hours while the chocolate agar was incubated in a 5% CO2 at 35°C for the same period. Bacterial identification all bacterial isolates were identified using microscopic, morphological, and biochemical techniques. Identification of morphologies following incubation, colony morphology was assessed on positive agar plates in accordance with Clinical Laboratory Standards Institute (CLSI) recommendations to provide a preliminary estimate of the type of bacteria that grow on agar plates). The bacteria were preliminary identified based on their colonial morphology, presence or absence of hemolysis on blood agar, fermenter or nonfermenter.

Microscopically identification: Gram's staining was done to confirm whether gram-positive or gram-negative cocci or bacilli. Small colonies, Gram positive cocci arranged in chain, forming complete hemolysis on blood agar and both coagulase and catalase negative were taken as S. pyogenes isolates. S. pneumoniae isolates were identified by gram positive alphahemolytic small colonies on blood agar and were susceptible to optochin. S. aureus isolates were identified by gram positive cluster forming glistering golden yellow colonies on blood agar and mannitol salt agar (MSA) which were coagulase, catalase and oxidase positive. Moraxella catarrhalis were identified by large kidney shaped diplococci gram negative grey to white hemispheric colonies on blood agar with both oxidase and catalase positive.

Pure cultures of Moraxella yielding oxidase, catalase test, no growth on nutrient agar at room temperature, failure to ferment glucose, lactose, mannitol and sucrose were considered significant Various essential tests identified the bacterial species include bile solubility and optochin sensitivity

test for *S.pneumoniae*, bacitracin sensitivity test for *S. pyogenes*, and appropriate biochemical tests including TSI (Triple Sugar Iron), Citrate, MIU (Motility Indole Urea) media, for Enterobacteriaceae like *Klebsiella pneumoniae*.

3.2.2.3 Antibiotic Susceptibility Testing

The antibacterial susceptibility testing was done using the Kirby-Bauer disc diffusion method on Muller Hinton agar. Simply, for every isolate a small number of bacteria was mixed with 3 mL of normal saline in a sterile manner. The density of the solution was then compared to a barium chloride standard at a concentration of 0. 5 McFarland. A sterile cotton tipped swab was first immersed in the prepared bacterial culture standard and streaked uniformly on to MH agar plates (Oxoid, USA) were left open to dry. After that put antibiotic disc on surface of agar (Gajic et al., 2022).

The plates were incubated at 37 for 24 hours, and the diameters of the zone of inhibition were measured compared with recorded diameters of the reference strain. In case of susceptibility to the antibiotic, a clear zone was produced and test organism was not grown around the disk. The depth of the agar, the sensitivity of microbe to the antibiotic and the rate of diffusion of the antibiotic through the agar are the main factors responsible for the size of this zone. No zone of inhibition or a relatively small zone was given by microorganisms resistant to that antibiotic. Results were reported as either Susceptible (S), Intermediate (I), or Resistant (R) following interpretation according to the CLSI guidelines (CLSI, 2019). At last the percentage of sensitivity and resistant pattern of different antibiotics against different pathogens was calculated.

The following antimicrobial agents that use: Ampicillin, Cefotaxime, Amoxcillin, Gentamycin, Cefuroxime, Ciprofloxacin, amoxicillin-clavulanic,

Colsitin ,Ceftriaxone, Azithromycin, Meropenem , Ceftriaxone, Levofloxacin and Imipenem.

3.2.3 Exclusion Criteria

1. Patients with incomplete data.
2. Patients with tumors.
3. Patients with autoimmune diseases.
4. Patients took antibiotics.

3.2.4 Inclusion Criteria

1. Patients aged (less than 1-14 yrs.).
2. Patients who are suffering from upper and lower respiratory tract infection.
3. Patients with complete data.

3.2.5. Ethical approval

In the present study, it was ensured that all the specimens were collected from children only after obtaining permission from the parents. Both parents of patient agreed in order to take samples of this patient into this study. Due consideration was given to the ethical issues with regard to human subject research and this study was conducted with the consent of the respective hospitals and clinics.

3.2.6. Culture Media preparation

Table (3-3) displays the media utilized in this study. The media were prepared following the guidelines specified by manufacturers attached to their containers(Steven Obenauf and Susan Finazzo ,2021).

3.2.6.1 Blood agar

To prepare, 40 gram of blood agar was suspended in 1 liter of distilled water (DW), brought to a boil, and sterilized at 121°C for 15 minutes. The agar was then cooled to 45-50°C and mixed with 7% sterilized defibrinated, this media was used to culture streptococci and other picky microbes can be isolated and

their hemolytic activity detected. Beef extract and peptone are present in the base medium. To complete the medium, sheep blood is added; however, for the characterization of some hemolytic organisms, such as Haemophilus spp., rabbit and horse blood may be used in lieu of sheep blood (James and Michael, 2015).

3.2.6.2 MacConkey Agar

This medium was prepared by dissolving 40gm of agar in 1000 ml of D.W and then sterilizing in autoclave at 121C°for 20 minutes. After cooling, it was poured to the plates, this type of media used selective gram-negative media (Jung and Hoilat ,2022).

3.2.6.3 Simmon Citrate Agar

Ammonium salts provide nitrogen, citrate serves as the only source of combined carbon, and bromothymol blue serves as a pH indicator in Simmons citrate medium. On this medium, only organisms capable of importing and using citrate will be able to thrive. The medium's pH rises as a result of ammonium and citrate metabolism. In an alkaline environment, the bromthymol blue pH indicator turns from green to vivid blue. This medium was prepared by dissolving 24.28 grams of medium in 1000 ml of distilled water. It was then autoclaved for 20 minutes at 121°C to ensure sterilization(Steven *et al*., 2021).

3.2.6.4. Methyl Red and Voges-Proskauer Broth

One liter of distilled water should contain 17 grams of. Thoroughly combine, transfer into 10-milliliter test tubes, and autoclave for 15 minutes at 121°C to achieve sterilization. Two tubes of Methyl Red Voges Proskauer Broth should be inoculated with one microbe each (Nordin *et al*., 2020).

3.2.6.5 Muller Hinton Agar

Weighing 38 g of media and dissolving it in 1000 ml D. W then autoclaving at 15 min, was performed according to a company instruction (Åhman *et al*., 2022).

3.2.6.6 Chocolate Agar

This is an enriched medium used for the cultivation of nutritionally fastidious microorganisms. the primary components of medium are peptone, starch, digest of beef heart, and sheep blood. Dissolve the powdered nutrient agar in distilled water according to the manufacturer's instructions. Heat the mixture to ensure complete dissolution. Autoclave the nutrient agar base at 121°C for 15 minutes to sterilize it. Cool the sterilized agar base to about 5056°C. Add the defibrinated blood slowly while stirring gently to avoid frothing. The high temperature will lyse the red blood cells, giving the medium a chocolate-brown color. Ensure that the blood is evenly distributed throughout the medium. Pour the medium into sterile Petri dishes and allow it to solidify (Breuil *et al*., 2022) .

3.2.7 Preparation of Reagents

3.2.7.1 Catalase test:

This test is helpful in differentiating between gram-positive cocci that are clinically significant. Enterococcus and Streptococcus are catalasenegative bacteria that are distinct from Staphylococcus and Micrococcus, which are both catalase-positive. In order to do this test, use a loop to scrape off a portion of the colony cells from the plate surface. Next, transfer the cells to a glass slide and give them a drop or two of H2O2. Keep an eye out for bubbles, which are a sign that the catalase enzyme is producing oxygen. Soon after adding H2O2 to the slide, bubbles ought to start to form. As an alternative, H2O2 can be applied to the colonies on the dish's surface to test for catalase. The size of the bubbles varies depending on how strong the interaction is. Any bubbles

suggest that there is. Depending on the strength of the interaction, the bubbles may be relatively small or large. Any bubbles suggest that the response is favorable. If there are no bubbles at all, the response is negative(Steven *et al*., 2021). **3.2.7.2 Kovac´s Reagent (Barritt's Reagent):**

Kovac's reagent is made by slowly adding 50 ml of concentrated hydrochloric acid after dissolving 10 gm of p-dimethyl aminobenzaldehyde in 150 ml of isoamyl alcohol (MacFaddin, 2000).

3.2.7.3 Oxidase Reagent:

This reagent was made by Tetramethyl p-phenyl diaminedihydrochloride (0.1 gm) was dissolved in 10 ml D.W. in a dark bottle to create this reagent. It was employed to ascertain the capacity of bacteria to generate oxidase (Forbes *et al*., 2007).

3.2.8 Biochemical tests:

3.2.8.1 catalase test:

A small amount of an organism from a well-isolated 18–24 hour colony was introduced onto a microscope slide using a sterile inoculating loop or wooden applicator stick. On the microscope slide, the organism received a drop of 3% H2O2. Positive responses are shown by instantaneous effervescence (bubble formation). The catalase enzyme in bacteria can be found using the catalase test. It is required to differentiate catalase negative Streptococcaceae from catalase positive Staphylococcus ssp (Reiner, 2010).

3.2.8.2 Free Coagulase Test:

0.1 ml of bacterial suspension was added to 0.4 ml of the diluted human plasma, and 0.2 ml of human plasma was diluted with 1.8 ml of normal saline in a simple tube. For four hours, the tube was incubated at 37 °C. The results were rechecked every 30 minutes to ensure that Staphylococcus aureus

(positive), which produces the enzyme coagulase, was distinguished from *S. epidermis* and *S. saprophyticus* (negative), which do not. (Becker *et al*., 2014).

3.2.8.3 Methyl Red-Voges Proskauer Test:

The fresh bacterial culture was added to Methyl red Voges Proskauer broth and cultured for 24 hours at 37°C. The result was read immediately after mixing five drops of methyl red solution. Bright red was the color of a positive test (MacFadden, 2000). **3.2.8.4 Novobiocin Susceptibility Test:**

A Mueller-Hinton agar plate is heavily seeded with the test organism to produce a confluent growth on the agar surface. After the seeding, a novobiocin antibiotic disc is applied to the agar surface. Following incubation, the sensitivity of an organism to the antibiotic is determined by the Kirby-Bauer method. Novobiocin test is used to differentiate coagulasenegative staphylococci (CONS) and presumptively identify the isolate as *Staphylococcus* saprophyticus which is resistant to Novobiocin (Steven *et al*.,2021).

3.2.8.5 Oxidase Test:

This procedure is used to distinguish among gram-negative rods. Membersof the enterics, which are facultative anaerobes, do not utilize cytochrome oxidase and are considered oxidase-negative. Aerobic, gramnegative *Pseudomonas aeruginosa* is oxidase-positive. The substrate, tetramethyl-p-phenylenediamine dihydrochloride, was soaked onto a filter paper. Sterile distilled water was used to wet the paper. Using a platinum or wooden loop, the newly formed colonies were selected for testing and then smeared over the filter paper. In 5 to 10 seconds, the hue changes to dark purple, indicating an oxidase positive. While the color of delayed oxidasepositive turns to purple in 60 to 90 seconds, the color of delayed oxidasenegative does not change (Steven *et al*.,2021).

3.2.8.6 Simmons Citrate Test:

The test is primarily used to distinguish between coliform bacteria like *Escherichia coli*, which do not utilize citrate, and bacteria like *Enterobacterand Klebsiella*, which do. A Simmons citrate slant was filled with a new bacterial culture, and it was cultured for 48–72 hours at 37°C. A growing streak with a blue tinge signify a positive test (Chauhan *et al*., 2020). **3.2.8.7 Triple Sugar Iron (TSI) Test:**

This test a differential test used to identify gram-negative enteric bacteria based on their ability to ferment sugars and produce hydrogen sulfide. It is particularly useful in differentiating members of the family Enterobacteriaceae .The tested bacteria were grown thickly on the slope's surface, punctured into the bottom, and cultured for a whole day at 37°C to assess the organism's ability to ferment glucose, lactose, and sucrose as well as create hydrogen sulfide (Paul *et al*., 2020).

3.2.8.8 Urease Production Test

This test used to determine an organism's ability to produce the enzyme urease, which hydrolyzes urea into ammonia and carbon dioxide. The bacteria were introduced into the full urea agar slop and left to grow at 37°C for 48 hours. The results were examined a day later. The urease test was positive if the indicator's color shifted from medium to purple-pink (Mekonnen *et al*., 2021).

3.2.8.9 Gram Stain Preparation

Gram staining is the differential staining procedure most commonly used for microscopic examination of bacteria. The majority of clinical laboratories employ the traditional Gram stain method, which involves first flooding the slide with a primary stain of crystal violet in 100% methanol. The mordant Gram's iodine is added to the slide after at least 15 seconds, increasing the primary stain's affinity for the bacterial cell. The slide is then rinsed with water. After 15 seconds, the slide is cleaned with water and the decolorizing

solution acetone-alcohol. From a Gram-negative cell, the decolorizing agent will eliminate the main stain. Cells that are Gram-positive for bacteria keep the original stain. The slide is promptly cleaned and counterstained with safranin for at least 15 seconds. After that, this slide is cleaned, dried, and magnified × 1,000 for light microscopy examination (James and Michael, 2015).

3.2.8.10 McFarland Standard Solution Preparation

Preparation of 0.5 McFarland standard: Solution A is prepared by adding barium chloride ($BaCl_2$, $2H_2O$) to 100ml distilled water. Solution B is prepared by adding 1ml of sulphuric acid (H_2S04 (0.36N) to 100 ml of distilled water. The turbidity is adjusted to match that of a 0.5 McFarland standard (approximately 10^8 CFU/ml). The cap is closed tightly to avoid evaporation. The mixture is stored in the dark. The solution is agitated vigorously before using it. This standard used for determination antibiotic sensitivity (Gayathiri *et al*., 2018). **3.2.9 Preservation of Bacterial Isolate**

The organisms were suspended in a 15% glycerol brain heart infusion broth. The suspension was frozen and kept at a temperature of -15 to -30°C.

3.2.10 Isolation and identification of Microorganisms:

The isolated microorganisms were differentiated and identified by various conventional biochemical methods (biochemical analysis) such as triple sugar iron (TSI), citrate utilization, urease, oxidase, coagulase testes. Catalase activity analysis to distinguish among Streptococcus and Staphylococcus species (Wu *et al*., 2020) , a deoxyribonuclease (DNase) activity test to differentiate between *Staphylococcus aureus* and *Staphylococcus epidermidis* and bile esculin test to distinguish between Streptococcus and Enterococcus were performed.

3.2.11 Measurement of the hematological parameter:

Approximately 3 ml of blood was taken into an EDTA anticoagulant tube to measure ESR and WBC. The WBC count was determined using the Sysmex XP-300.

3.2.12 Measurement of The Immunological Parameter

3.2.12.1 Concentration of C-reactive Protein Test

The principle of C-reactive protein identification in serum was measured by using auto-chemistry analyzer in accordance with the criteria mentioned in the Kits instructions by the manufacturer mentioned in the (appendix 2).

3.2.12.2 Determination Human suPAR (soluble urokinase-type plasminogen activator receptor) by ELISA.

principle of the test

The testing principle applied in this group was sandwich enzyme immunoassay. The microtiter plate provided in this kit was pre-coated with an antibody specific for Human suPAR in accordance with the criteria mentioned in the Kits instructions by the manufacturer mentioned in the(appendix 3)

3.2.12.3 Estimation of Human s-TREM1(soluble triggering receptor expressed on myeloid cells-1) by ELISA.

The test principle applied in this group was sandwich enzyme immunoassay. The microtiter plate provided in this kit was pre-coated with an antibody specific for Human s-TREM-1 in accordance with the criteria mentioned in the Kits instructions by the manufacturer mentioned in the(appendix 4).

3.2.12.4 Estimation of CHI3L1(chitinase -3-like protein-1) by ELISA.

Principle test of CHI3L1

The test principle applied in this group was sandwich enzyme immunoassay. The microtiter plate provided in this kit was pre-coated with an antibody

specific to Human CHI3L1 in accordance with the criteria mentioned in the Kits instructions by the manufacturer mentioned in the(appendix 5).

3.3 Statistical Analysis

All data were analyzed using SPSS software (V.28 Inc., Chicago, USA) and Microsoft Excel 2019. Pearson's correlation was used to determine associations between variables all values are expressed as ± the mean value of the standard deviation, P- value of less than (0.05), the independent t-test was used to compare two independent groups. Chi square was used to examine categorical data, and compared two groups as well as to see if there was a relationship between them was used One-way ANOVA test.

Chapter four Results and discussion

4. Results and Discussion

4.1 Sociodemographic Characteristics of Study Participants

A total of 120 children were involved in the present investigation, resulting in a response rate of 100%. For matching objectives, the study participants were grouped into four age groups; <1 year, 1-4 years, 5-9 years and 10-14 years and divided into three group according to severity of infection (Thokngaen and Karoonboonyanan,2019) . The sociodemographic characteristics of participated children are summarized in Table 4-1.

Table 4-1 Sociodemographic Characteristics of Children with Respiratory Tract Infection in Current Study.

Variables	Frequency	Percent %
Age:		
<1 Y	30	25
1-4 Y	39	32.5
5-9 Y	31	25.83
10-14Y	20	16.67
Sex:		
Male	71	59.2
Female	49	40.8
Respiratory tract infections:		
URTI	34	28.3
LRTI	86	71.7
Severity of disease:		
Mild	32	26.67
Moderate	40	33.33
Severe	48	40

*Y=Years

4.1.1 Distribution of Patients Based on Age:

In this study found the highest percentage of RTIs were in patients with age < 5 (57.5%), followed by age 5-9 years (25.80%) , whereas the lowest percentage with RTIs patients ages is 10-14 (16.60%) .as shown in table (4.1).

Children under five years of age were more susceptible to respiratory infections compared to other age groups. This is especially true for lower respiratory infections such as pneumonia, which is the leading cause of death among children in this age group. A study at Bamenda Regional Hospital in Cameroon showed that 54.7% of children who visited the hospital who were under five years old developed acute respiratory infections (ARIs). These infections were distributed between mild, moderate, and severe infections (Tazinya *et al* ., 2018).

In another study, among 436 children with ARIs in Nigeria, the most affected age group was 10 to 19 months (Ujunwa and Ezeonu, 2014). While other study which was conducted in Iran among 182 children, the mean age of patients was 4.7 years and the most common age of the disease was older than 5 years (Boloorsaz *et al*., 2007).

The reasons why children under 5 years old are more susceptible to RTIs infection were Poor education, poverty, malnutrition, exposure to secondhand smoke, poor ventilation, HIV, traditional cooking stoves, unclean fuel usage, poor sanitation facilities and unclean drinking water make children under five more vulnerable to acute lower respiratory infections(ALRTIs) (Sarfo *et al.*,2023).

4.1.2 Distribution of Pediatric Based on Sex

In the present study where it was observed that the number of patients with respiratory infection 71 (59.2%) were male, while around 49 (40.8%) were female .As shown in table 4.1.

These results were similar to other study in Iran it was conducted on 303 children the number of male 180 was higher than female 123(Mirkarimi *et al*., 2020). Which was also in agreement with the study in Erbil city that show the prevalence of lower respiratory tract infection caused by bacterial infection is higher in males than females (Saydoka *et al*., 2021).

Other study conducted in Nepal among 188 children, 51% were males whereas 49%were females (Koirala, 2019).

The high RTIs incidence in male can be attributed to many the reasons such as phagocytic activity of macrophages is greater in females than in males owing to the suppressive effects of androgens on male macrophage activity (Klein and Flanagan , 2016).

4.1.3 Prevalence of Signs and Symptoms of RTIs Among Participants

From the total participating children, 82.50 % had a history of cough, 85 % had fever, 10.83 % had nasal discharge, 65.83%% had difficulty breathing and 10 % had sore throat at the time of the study as shown in the Table 4-2.

Table 4-2 Clinical Profile of RTIs in Pediatrics

Clinical Profile	Frequency	Percent %
Cough	99	82.50
Fever	102	85.00
Nasal Discharge	13	10.83
Difficulty Breathing	79	65.83
Sore Throat	12	10.00

Table 4-2 showed that the fever (85 %) ,cough (82.5 %,) and difficulty Breathing (65.83) were the most symptoms of RTIs among the infected children .The results of study agreed with other study conducting in India that was observed cough (98.15%), fever (91.67%), difficulty in breathing (76.39%) (Bathla *et al*., 2020).

4.1.4 Distribution Pediatric According to Bacterial Isolates

Table (4-3) show the percentage of each bacterial isolate from all patients ; *H. influenzae* where (21.66%) was the most prevalence bacteria that identified in RTIs patients, followed by *Klebsiella pneumonia* (20%), *Strep. Pneumonia (*19.16*%), Staph. aureus(*15*%), Strep. Pyogen*es (14.16%) , *Moraxella catarrhalis (*5*%)* and *Pseudomonas aeruginosa* (5%).

Table 4-3 Distribution of Bacterial Isolates from Upper and Lower Respiratory Tract Infections in Paediatrics.

Bacterial isolates	Number of bacteria	Percentage
Haemophlius influenza	26	21.67%
Klebsiella pneumonia	24	20%
Streptococcuc Pneumonia	23	19.17%
Stapthalococcus aureus	18	15%
streptococcus Pyogenes	17	14.16%
Moraxella catarrhalis	6	5%
Pseudomonas aeruginosa	6	5%

The pattern and the frequency of bacterial isolates obtained in this study were comparable with different study findings done in different countries. For example, A study conducted in China that found most frequent species were

H.influenzae (35.7%), *Strep.pneumoniae* (12.9%), *M. catarrhalis* (11.5%), *Escherichia coli* (8.9%), *Staph.aureus* (8.9%), *Klebsiella pneumoniae* (8.2%), *Acinetobacter baumannii* (3.1%), *P.aeruginosa* (2.8%) (Mai *et al*.,2023).

Another study in Indonesia that showed prevalence was 49.5% for *S. pneumoniae*, 27.5% for *H. influenzae*, 42.7% for *M. catarrhalis*, and 7.3% for *S. aureus* (Dunne *et al*., 2018).

4.1.5 Distribution of Patients According to Site of Infections

The present study indicated that among 120 patients with RTIs divided into lower respiratory tract infections (LRTI) and upper lower respiratory tract infections (URTI), where the LRTI was 71.7%, and URTI was 28.3%. As shown in (figure 4.4)

Table (4-4) Distribution Bacterial isolates in respiratory tract

Bacterial Isolates	No. of Bacterial Isolates in:	
	URTI	LRTI
StreptococcusPneumonia	2	21
Moraxella catarrhalis	0	6
Staphlococcusaureus	6	12
Heamophlius influenzae	9	17
Streptococcus pyogenes	17	0
Pseudomonas aeruginosa	0	6
Klebsiella pneumonia	0	24

This result was agreement with other study conducted in Thailand it was 30.2 % had upper respiratory tract infection, whereas 66.3 % had lower respiratory tract infection (Wanlapakorn *et al*., 2023).

While disagreement with present work noticed by (Turyasiima *et al*., 2024) in western Uganda who showed that upper respiratory tract infections with a prevalence of 24.8% were more common than lower respiratory tract infections 12.2% .

Bacteria that were isolated from the upper respiratory tract in this study are : *H. influenza* , *Strep. pneumonia*, *Staph. aureus* and *Strep. Pyogenes* . This results similar with other study conducted in Nepal that observed *Streptococcus pneumoniae* (16.6%) was the most common bacterial pathogen recovered, followed by *Staphylococcus* aureus (14.7%), βhaemolytic streptococci (non-Group A) (8.8%), *Streptococcus pyogenes* (5.3%),Corynebacterium diphtheriae (3.4%) , *Klebsiella pneumoniae* (4.9%), *Haemophilus influenzae* (3.4%) and *Neisseria meningitidis* (1.4%) (Thapa *et al*., 2017). While Bacteria that were isolated from the lower respiratory tract *H. influenza* , *Strep. Pneumonia*, *Staph. Aureus* , *Klebsiella pneumonia* , *Moraxella catarrhalis and Pseudomonas aeruginosa*. These results are almost similar to a study conducted in China the most common species are *Haemophilus influenzae* (35.7%), *Streptococcus pneumoniae* (12.9%), *Moraxella influenzae* (11.5%), Escherichia coli (8.9%), and *Staphylococcus aureus* (89, 8.9%), *Klebsiella pneumoniae* (8.2%), *Acinetobacter baumannii* (3.1%), *Pseudomonas*

aeruginosa (2.8%), and *Streptococcus agalactiae* (1.3%) (Mai *et al*., 2023).

4.1.5.1 Distribution of Upper Respiratory Tract Infections in Study Participants

In the URTIs tonsillitis infection was common among the study participants with percent of 15.83% , followed by pharyngitis infection with 8.3% , and finally sinusitis infection with 4.1% (as present in Table 4.5)

Table (4-5) Distribution of URTIs infections in Paediatrics

URTI	Frequency	Percent (%)
Tonsillitis	19	15.83
Sinusitis	5	4.1
Pharyngitis	10	8.3

It was observed that tonsillitis was more significant infection of upper respiratory tract than pharyngitis and sinusitis. This outcome consistent with a study conducted in Pakistan ,who reported that pharyngitis and tonsillitis were a common upper respiratory tract infection (URTI) among pediatric populations (Ahmed *et al*., 2022). Because of the tonsils, located in the pharynx, act as a defense mechanism against pathogens entering the body, making them susceptible to infections (Brugha *et al*., 2013)**.**

4.1.5.2 Distribution of Infection in The Lower Respiratory Tract

Bronchitis and pneumonia were common lower respiratory tract infections. Pneumonia (44.1 %) was more prevalent than bronchitis (27.5%) among the study paediatrics (as shown in table 4.6).

Table 4.6 Distribution of LRTIs in Paediatrics

LRTI	Frequency	Percent %
Pneumonia	53	44.1
Bronchitis	33	27.5

These result was compatible with the results conducted in Iran showed Pneumonia (74.6%) and bronchitis (17.2%) were the most common LRTIs among patients(Mirkarimi *et al*., 2020).

Other study conducted in western Uganda that found Pneumonia was (37%), while bronchiolitis (16.2%) (Turyasiima *et al*., 2024).

4.1.6 Study Antibiotic Sensitivity Pattern of Bacteria Isolate from Respiratory Tract

In the current study different antibiotics were checked against the isolated bacterial species. The bacterial strains were evaluated as resistant and sensitive by measuring the inhibitory zones around antibiotics disks. All the bacterial isolates showed resistance against ampicillin as shown in table 4-7.

Table (4-7) Antibiotic Sensitivity Pattern of gram Negative Bacteria Isolate from Respiratory Tract

Antibiotic	***p.aeurgenosa*** **n=6**		***K.pneumonia*** **n=24**		***H.influenza*** **n=26**		***M.catarhalas*** **n=6**	
	R	S	R	S	R	S	R	S
Ciprofloxacin	33.33	66.66	87.5	12.5	34	65.38	83.33	16.66
Levofloxacin	--	--	29.1	70.8	34	65.38	33.33	66.66
Cefotaxime	83.33	16.66	87.5	12.5	26	73	--	--
Cefuroxime	66.66	33.33	83.3	16.6	80	20	83.33	16.66
Ceftriaxone	--	--	79	20	11.5	88.46	33.33	66.66
amoxicillin-clavulanic	83.33	16.66	91.6	8.3	85	15	16.6	83.33
Ampicillin	100	0	100	0	100	0	100	0
Meropenem	33.33	66.66	16.66	83.33	23	76.9	--	--
Azithromcin	--	--	--	--	--	--	16.6	83.33
Gentamicin	--	--	79	20	20	80	--	--
Colsitin	0	100	--	--	--	--	--	--
Amikacin	16.66	83.33	--	--	--	--	--	--
Imipenem	33.33	66.66	--	--	--	--	--	--

R= resistance , S= sensitive , n= number of bacterial isolate

H. influenza was most common bacterial isolates that found higher resistance 100% to ampicillin followed by amoxicillin-clavulanic 85% and

Cefuroxime 80% while higher sensitive to ceftriaxone 88.46% followed by gentamycin 80% ,meropenem 76.9% ,cefotaxime 73% and 65.38% to levofloxacin and ciprofloxacin. Similar results that conducted in China that found *H. influenza* sensitivity rate of 100% to ceftriaxone , meropenem ,cefotaxime and levofloxacin (Su *et al*., 2021) .

Wang *et al*., (2019) found that resistance rates of the *H. influenza* isolates to cefuroxime, Cefotixime were 31.2%, 5.9%, respectively and suggested that the main mechanism used by *H. Influenza* to resist ampicillin is the production of β-lactamase to break antibiotics. Another mechanism is the mutation of penicillin-binding protein 3 (PBP3), which decreases the antibiotic susceptibility of *H. influenza* towards ampicillin and other *β*lactam antibiotics (Li *et al*., 2017).

Klebsiella pneumonia was the second common gram negative bacterial isolate (24) cases and was sensitive to , meropenem 83.33% and levofloxacin 70.8%. These are same as findings in study conducted in China that found *K.pneumonia* sensitivity rate to carbapenem antibiotics (meropenem, ertapenem, and imipenem) is 90% (Su *et al*., 2021) . In the current study *K. pneumoniae* was observed 100 % resistance to Ampicillin followed by amoxicillin-clavulanic 91.6%, Cefotaxime 87.5%, Ciprofloxacin 87.5%, and 79% to gentamicin and Cefuroxime and ceftriaxone 71% .The current findings were compared with the work done by (Amin *et al*., 2023) that observed *K.pneumonia* 100 resistance to ampicillin and amoxicillin-clavulanic followed by 98%, cefotixime 85%, cefuroxime 90%, ciprofloxacin and Gentamycin 78% and 75% respectively. *M.catarhalas* showed resistance 100% to ampicillin and

83.33% to cefuroxime and ciprofloxacin while sensitive with 83% to amoxicillin-clavulanic and azithromycin, followed with 66.66% to ceftriaxone and levofloxacin. This results agree with study in Japan that found most of

M.catarhalas isolates were susceptible for amoxicillinclavulanic ,azithromycin, ceftriaxone and levofloxacin (Nagai *et al*., 2019).

Pseudomonas aeurgenosa was sensitive 100% to colsitin followed by amikacin 83.33%, and with 66.66% for each of the ciprofloxacin , meropenem , and imipenem. This results agree with study in Iran that found *pseudomonas aeruginosa* high susceptibility to colistin (100%) and amikacin (81.8%) followed by tobramycin (69.2%), ciprofloxacin (68.5%), meropenem (67.2%), cefepime (65.7%), ceftazidime (64.3%), and imipenem (63.3%) (Esfahani *et al*.,2024) while *p.aeurgenos* was resistance to ampicillin (100%) , cefuroxime (88%) , amoxicillin-clavulanic (66%) and Cefotaxime (66%). Similar finding were observed by (Saha *et al*., 2018 ; Singh *et al* ., 2020) .

Table(4-8) Antibiotic Sensitivity Pattern of Gram Positive Bacteria Isolate from Respiratory Tract in pediatric.

Antibiotic	***Strep.pneumonia n=23***		***Staph.aureus n=18***		***Strep.pyogen n=17***	
	R	S	R	S	R	S
Ciprofloxacin	22	78	12	88	18	82
Levofloxacin	17	82.6	--	--	18	82
Ceftriaxone	--	--	12	88	--	--
amoxicillin-clavulanic	--	--	12	88	--	--
Meropenem	13	86.9	--	--	--	--
Gentamycin	13	86.9	--	--	--	--
Azithromycin	5	95	6	94	6	94
Cefotaxime	86	13	23	77	88	12
Ampcilin	91	9	100	0	100	0
Amoxcilin	--	--	--	--	82	18

R= resistance , S= sensitive , n= number of bacterial isolate

Streptococcus. Pneumonia resistance to ampicillin 91% and cefotaxime 86% while sensitivity were determined against azithromycin 95%,meropenem 86.9%, levofloxacin 82.6% and ciprofloxacin 78%. This results agree with

other study conducted in Pakistan was found that *S. pneumonia* resistance to ampicillin, penicillin, co-amoxyclav, cefotixime, cefuroxime, 99%, 98%, 85%, 78%, 82% respectively, while low resistivity were determined against ciprofloxacin 30%, levofloxacin 25% and gentamycin18% (Amin *et al*., 2023).

Staphylococcus aureus were sensitive to azithromycin 94%, followed by 88% for each of the ciprofloxacin ,ceftriaxone , amoxicillin-clavulanic and 77% to cefotaxime while higher resistance 100% against ampicillin .

Streptococcus pyogenes were noted in higher resistance 100% against ampicillin ,88% against Cefotixime and amoxcilin 82% while sensitive 94% to azithromycin and 82% ciprofloxacin and levofloxacin. In a study conducted in Kenya found *Strep. pyogenes* resistance percentages against amoxicillin, ampicillin, ciprofloxacin, ceftazidime, cephalexin, gentamicin, amikacin, and cefuroxime were 97.6%, 100.0%, 100.0%, 91.7%, 83.3%, 0.0%, 0.0% and 0.0%, respectively (Miriti *et al*., 2023).

The results of antibiotic sensitivity patterns of gram positive bacteria agree with study conducted in Iraq that found ceftriaxone, azithromycin and amoxiclav were effective against *S. aureus*, whereas ciprofloxacin, azithromycin, levofloxacin and amoxicillin were effective against *S. pyogenes.* Ciprofloxacin, levofloxacin, meropenem and azithromycin were found to be effective against *Streptococcus* spp (Hussein, 2024).

Variations in antibiotic susceptibility against pathogenic bacteria have been observed in the studies. These variations can be attributed to various factors such as the type and structure of antibiotics, dosages administered, origin of industrial companies, geographical differences, use of antibiotics without a prescription, use of antibiotics without laboratory guidance, misuse of the drug through incorrect dosage schedules or concentrations, and

differences in the study areas and bacterial types under investigation (Hussein, 2022).

4.1.7 The Relationship Between the Severity of Respiratory Tract Diseases and Ages Groups in Study Participants.

The severity of respiratory tract diseases among investigated children ranged from mild to severe cases (as shown in table 4.9).

Table 4-9 Distribution of the severity of respiratory tract diseases with ages groups in paediatrics

Age groups	Frequency of Mild Cases	Frequency of Moderate Cases	Frequency of Severe Cases
<1 Y	0	0	30
1-4 Y	0	21	18
5-9 Y	12	19	0
10-14Y	20	0	0
Total	32	40	48

*Y=Years

The current study proved that in severe cases younger children under 5 years are more likely to have RTIs than older children. This results agree with the study conducted at a regional hospital in Cameroon found that 54.7% of children under five years of age experienced acute respiratory infections, of which 16% were severe cases requiring special attention (Tazinya *et al*.,2018). Other study found that children under five years old have the highest probability of infection (Arguedas *et al*.,2019).

Moderate cases of disease were distributed between the age groups (1-4) years and the age group (5-9) years with a frequency of 21 and 19 cases, respectively. However, moderate respiratory diseases were common in

children under 10 years old, with the highest burden in those under 5 years of age, this result is proved by (El-Koofy *et al*., 2022).

The mild cases of diseases were most prevalent in the age group (10-14) years with a frequency of 20 cases, while they were least prevalent in the age group (5-9) years with a frequency of 12 cases.

4.1.8 The Relationship Between Bacterial Isolates and the Severity of Respiratory Tract Diseases in Study Participants

Respiratory diseases exhibit a spectrum of severity, ranging from mild to moderate to severe as shown in table (4-10). There exists an association between the type of bacteria and the severity of the disease. In mild cases, *Staph. pyogen* is the most prevalent bacterium, followed by *Staph. aureus ,H. influenza*, *Moraxella catarrhalis*, *Strep. Pneumonia* , *Klebsiella pneumonia* .

The moderate cases of infection found *H. influenzae* emerges as the most common bacterium, followed by *Staph. aureus* , *Strep. Pyogen* ,*Strep. Pneumonia* , *Klebsiella pneumonia* and *Moraxella catarrhalis* .

For severe cases, *Klebsiella pneumonia,* followed by most common , followed by *Strep. Pneumonia* , *H. influenza*, *p.aeruginosa* and *S. aureus*.

Table 4-10 Relationship Between Bacterial Isolates and the Severity of Respiratory Tract Diseases in Paediatrics

Bacterial Isolates	No. of Bacterial Isolates in:		
	Mild Cases	Moderate Cases	Severe Cases
Streptococcus pneumonia	4	6	13
Moraxella catarrhalis	4	2	0
Staphloccous aureus	6	10	2
Heamophlis influenza	5	12	9
Streptococcus. Pyogenes	10	7	0
Pseudomonas aeruginosa	0	0	6
Klebsiella pneumonia	2	4	18

It appears that numerous bacteria could cause severe respiratory diseases, such as *K. pneumonia* and *Strep. pneumonia* , where they were the most frequent bacteria in severe cases with frequencies of 18 and 10. This result was agree with study conducted in Vietnam who reported those bacteria were the most significant in severe respiratory diseases (Dung *et al*.,

. 2024)

Other study conducted in Western Rajasthan that showed *Klebsiella pneumonia* and *Strep. pneumonia* were the commonest comments bacteria causes severe respiratory diseases (Singh *et al*., 2020).

Whereas, *H. Influenzae*, *Staph. aureus* were more repeated bacteria associated with moderate respiratory diseases. The present study was agreement with study performed by (Yoo *et al* ., 2022).

However, in mild cases, *Strep. pyogen*es was the most prevalent bacterium, with 10 counts.

4.2 The Relationship Between The Age Groups and Biomarkers

Age played a crucial role in influencing biomarkers across various health conditions, the mean and standard deviation of biomarkers concentrations across age groups as presented in table 4-11.

Table 4-11 Relationship Between The Age Groups and Biomarkers in Pediatris with RTIs.

Age groups	Biomarkers					
	sTREM-1 pg/mL **Mean ±SD**	**suPAR** ng/mL **Mean ±SD**	**CHI3L1** pg/mL **Mean ±SD**	**WBC** (x 103 µl) **Mean ±SD**	**ESR** (mm/h) **Mean ±SD**	**CRP** (mg/l) **Mean ±SD**
<1 Y	2418.269±2145.870	21.678±11.320	2094.274±927.587	20.828±3.154	43.480±3.254	110.040±7.865
1-4 Y	2956.508±2994.722	5.239±4.933	893.412±1062.873	16.321±3.464	39.242±5.285	72.484±30.776
5-9 Y	2132.287± 2550.73	19.763±8.261	294.050±606.037	13.889±2.291	36.730±4.600	56.346±10.921
10-14 Y	2630.721± 3499.048	1.554±2.203	57.775±143.597	11.150±2.694	36.805±4.509	38.972±4.053
P value	0.001	0.098	0.16	0.005	0.00001	0.02

p-value <0.05 ; Y=Years ;SD=standard deviation ; sTREM-1 =soluble triggering receptor expression myeloid cell ; suPAR=soluble uorokinase plasminogen activator receptor ; CHI3L1 =chitinase 3-like protein 1; WBC =white blood cell ; ESR =erythrocyte sedimentation rat ; CRP= c- reactive protein

The average of sTREM-1 concentration in age of less than 1 year were 2418.269±2145.870 pg/ mL, 2956.508±2994.722 pg/ mL in the second age group of 1-4 years, 2132.287± 2550.733 pg/ mL, in the third age group of 59 years and 2630.721± 3499.048 pg/ mL in the age of 10-14 years. There were significant differences among the four age groups (p=0.001).

SuPAR concentration showed the following average values across age groups: 21.678±11.320 ng/mL (less than 1 year), 5.239±4.933 ng/mL (1-4 years),

19.763±8.261 ng/mL (5-9 years), and 1.554±2.203 ng/mL (10-14 years). Statistical analysis no significant differences between the groups (p=0.098).

CHI3L1 concentration exhibited no statistically significant differences (p=0.16) between age groups. The average values were: 2094.274±927.587 ng/mL (less than 1 year), 893.412±1062.873 pg/mL (1-4 years), 294.050±606.037 pg/mL (5-9 years), and 57.775±143.597 pg/mL (10-14 years).

WBC concentration showed significant differences (p=0.005) across age groups. The average concentration in the following age groups were: less than 1 year (20.828±3.154), 1-4 years (16.321±3.464), 5-9 years (13.889±2.291), and 10-14 years (11.150±2.694).

ESR concentration exhibited significant differences across age groups (p=0.00001). The mean concentrations were as follows: less than 1 year (43.480±3.254), 1-4 years (39.242±5.285), 5-9 years (36.730±4.600), and 10-14 years (36.805±4.509).

Analysis of CRP concentration revealed significant differences across age groups (p=0.02). Mean concentrations were: 110.040±7.865 mg/L (less than 1 year), 72.484±30.776 mg/L (1-4 years), 56.346±10.921 mg/L (5-9 years), and 38.972±4.053 mg/L (10-14 years).

The initial mean sTREM-1 serum concentration was statistically significantly higher among newborns with sepsis compared to the control group (Smok *et al*., 2020).

Soluble triggering receptor expressed by myeloid cells-1 (sTREM-1) showed an excellent predictive accuracy to identify bacterial community acquired pneumonia (CAP) in 110 patients with lower respiratory tract infections (Hogendoorn *et al*., 2022).

Chitinase 3-like protein 1 (CHI3L1), concentrations were higher levels were associated with increased disease severity in children with communityacquired pneumonia in Egypt (Konrad *et al*., 2023).

WBC, CRP are increasingly used in the early diagnosis of bacterial respiratory tract infection in children (Hu and Wang , 2018) .

4.3 The relationship between the severity of respiratory tract diseases and biomarkers

The severity of respiratory tract diseases is closely linked to specific biomarkers that can indicate disease progression and outcome. Table 4.12 present the analysis of mean and standard deviation for biomarker concentrations across the spectrum of respiratory tract diseases severity.

Table 4-12 Relationship between the severity of respiratory tract diseases and biomarkers in paediatrics.

Severity	Biomarkers					
	sTREM-1(pg/ml) **Mean ±SD**	**sUPAR** (ng/ml) **Mean ±SD**	**CHI3L1** (pg/ml) **Mean ±SD**	**WBC** (x 103 µl) **Mean ±SD**	**ESR** (mm/h) **Mean ±SD**	**CRP** (mg/l) **Mean ±SD**
Mild	2256.952±3309.643	2.027±2.797	48.553±132.783	11.105±2.507	37.418±4.536	39.162±3.878
Moderate	2649.117±2514.206	13.066±9.825	363.429±505.272	14.534±1.403	35.900±3.894	55.975±9.205
Severe	2841.981±2816.161	21.123±10.812	2025.397±1016.498	20.637±2.878	43.702±3.471	110.486±7.065
P value	0.004	0.161	0.316	0.031	0.004	0.086

p-value <0.05 ;SD=standard deviation ; sTREM-1 =soluble triggering receptor expression myeloid cell ; suPAR=soluble uorokinase plasminogen activator receptor ; CHI3L1 =chitinase 3-like protein 1; WBC =white blood cell ; ESR =erythrocyte sedimentation rat ; CRP= c- reactive protein

The mean sTREM-1 concentrations in mild cases were 2256.952±3309.643 ng/mL, 2649.117±2514.206 ng/mL in the moderate cases

and 2841.981±2816.161 ng/mL in the severe cases, indicating significant variations among the cases (p=0.004).

sUPAR concentrations , there were no significant differences (p=0.161) among severity of disease .Children with severe or moderate case of disease had higher value than those with mild disease . Mean concentrations (ng/mL) were: 21.123±10.812 ng/mL in severe case , 13.066±9.825 ng/mL in the moderate cases and 2.027±2.797 ng/mL in the mild cases.

These results agree with study conducted by (Louka *et al*.,2024) that found children with acute COVID -19 and a severe or moderate clinical presentation had higher values than those with mild symptoms: 5.79 ± 1.58 versus 5.40 ± 1.94 versus 3.19 ± 0.73 ng/mL, respectively ($P < 0.001$).

Other results found suPAR level was significantly higher in children with severe pneumonia compared with those having non-severe pneumonia (Khatab *et al*., 2021).

suPAR levels increased in patients with LTRIs and suPAR values were higher in patients with severe pneumonia than mild pneumonia (Citlenbik *et al*., 2019).

(Savva *et al*.,2011) observed a group of 180 patients with ventilatorassociated pneumonia (VAP): 26% had sepsis, 27% severe sepsis and 47% were in septic shock. suPAR was a reliable marker of the patients' condition and could discriminate between these critical conditions (higher values were observed in patients with severe sepsis, and even higher in septic shock).

Serum levels of IL-18 and sTREM-1 are high value in predicting the severity and prognosis of VAP and can provide reference for clinical treatment (Wang *et al*., 2021).

Regarding CHI3L1 concentration, there were no statistically significant differences (p=0.316) among cases. The mean concentrations were 48.553±132.783 pg/mL (mild cases), 363.429±505.272 pg/mL (moderate cases) and 2025.397±1016.498 pg/mL (severe cases).

CHI3L1 has been regarded as a promising biomarker for evaluating severity of interstitial lung disease and predicting disease prognosis (Jiang *et al*., 2019).

Chitinase-3-like protein 1 (CHI3L1) in serum is a biomarker of inflammation associated with the activity and mortality of lung chronic diseases, diseases that are characterized by inflammation (Huang *et al*., 2021)

WBC concentration unveiled significant variations (p=0.031) across cases with mean concentrations of 11.105±2.507 (mild cases), 14.534±1.403 (moderate cases) and 20.637±2.878 (severe cases).

ESR concentration displayed notable differences across all cases (p=0.004) with mean concentrations per case as follows: 37.418±4.536 (mild cases), 35.900±3.894 (moderate cases) and 43.702±3.471 (severe cases).

The examination of CRP concentrations indicated no significant differences among all cases (p=0.086) with mean concentrations of 110.486±7.05 mg/L (severe cases), 55.975±9.205 mg/L (moderate cases) and 39.162±3.878 mg/L (mild cases).

Statically found a significant correlation in some parameters among the severe infections, as in table (4-13). As a result, there is a significant negative correlation between Human CH13L1 and Human s TREM-1, at the p-value< 0.05. Also, there is a significant positive correlation between WBC and ESR, at the p-value< 0.05.

There is a negative correlation among WBC with Human sTREM-1, Human CH13L1, and Human suPAR p-value< 0.05.

Table (4-13) Correlation coefficient among different parameters for severe infections.

Pearson Correlation Coefficient	Human CH13L1	Human suPAR	Human sTREM-1	WBC	ESR	CRP
Human CH13L1	1					
Human suPAR	0.043	1				
Human sTREM-1	-0.100*	-0.004	1			
WBC	-0.040	--0.029	-0.186	1		
ESR	-0.197	-0.229	0.125	0.389*	1	
CRP	-0.069	0.131	-0.004	0.234	0.116	1

*. Correlation is significant at the 0.05 level

**. Correlation is significant at the 0.01 level

Positive Correlation.

Negative Correlation.

While in moderate cases statically found a significant correlation in some parameters, as in table (4-14). As a result, there is a significant positive correlation between Human suPAR and Human sTREM-1 , at the p-value< 0.05. While, there is a significant positive correlation among Human suPAR with CRP , at the p-value< 0.01. On the other hand , there is a significant positive correlation among WBC with CRP , at the p-value< 0.01.

There was positive correlation among Human sTREM-1 with Human CH13L1, and Human suPAR p-value < 0.05 ; and there was negative correlation with ESR.

As a result, there is a positive correlation among WBC with Human sTREM-1, Human CH13L1, and Human suPAR, at the p-value< 0.05.

Implicated sTREM-1 and CHI3L1 in the effect of severe acute malnutrition on mortality, suggesting that enhanced activation of these inflammatory pathways is associated with the increased mortality in under nourished children with pneumonia (Weckman *et al*., 2023)

This study was disagreed with other study that indicated sTREM-1 was positively correlated with the WBC count, neutrophile granulocyte absolute count (Yang *et al*., 2021).

Other study agreed with the present study that was revealed that ESR with significantly increased sTREM-1 levels (Badiee *et al*., 2022).

Table (4-14) Correlation coefficient among different parameters for moderate infections.

Pearson Correlation Coefficient	Human CH13L1	Human suPAR	Human sTREM-1	WBC	ESR	CRP
Human CH13L1	1					
Human suPAR	-0.207	1				
Human sTREM-1	-0.062	0.269*	1			
WBC	0.262	0.212	0.216	1		
ESR	-0.169	-0.006	-0.023	0.163	1	
CRP	0.214	0.685**	0.222	0.436**	0.046	1

*. Correlation is significant at the 0.05 level

**. Correlation is significant at the 0.01 level

Positive Correlation.

Negative Correlation.

In mild case statically found a significant correlation in some parameters, as in Table (4-15). As a result, there is a significant positive correlation among Human CH13L1 with Human sTREM-1 and Human suPAR, at the p-value< 0.05. While, there is a significant positive correlation between Human suPAR and Human sTREM-1, at the p-value<

(0.05).

There is a significant negative correlation between Human suPAR and ESR p-value< 0.01.

As a result, there is a positive correlation among CRP with Human sTREM-1, Human CH13L1, and Human suPAR, at the p-value< 0.05, while there is a negative correlation among ESR and WBC with Human sTREM-1, Human CH13L1, and Human suPAR, at the p-value< 0.05.

Table (4-15) Correlation coefficient among different parameters for mild infections.

Pearson Correlation Coefficient	Human CH13L1	Human suPAR	Human sTREM-1	WBC	ESR	CRP
Human CH13L1	1					
Human suPAR	0.260*	1				
Human sTREM-1	0.269*	0.276*	1			
WBC	-0.139	-0.108	-0.187	1		
ESR	-0.052	-0.495**	-0.249	0.137	1	
CRP	0.163	0.059	0.211	-0.185	-0.222	1

*. Correlation is significant at the 0.05 level

**. Correlation is significant at the 0.01 level

Positive Correlation. Negative Correlation.

Elevated CRP and Procalcitonin serum levels provide superior markers to sTREM-1 in pneumonia patients (Shirani and Hajzargarbashi, 2019).

CHI3L1 and IL-6 exhibited a significant positive correlation with CRP, ESR, D-dimer, ferritin, LDH, and lymphocytopenia levels in COVID19 patients (Schoneveld *et al*., 2021).

Suggested that the clearance of suPAR is low and/or that the production and release persists over a longer time compared to the other inflammatory markers (Koch *et al* ., 2011).

Plasma levels of inflammation-associated proteins CRP and CHI3L1 were significantly higher in children with end-point pneumonia compared to the other groups (Erdman *et al*., 2015).

Conclusions and Recommendations

Conclusions

1. The present study observed that frequency of (RTIs) bacteria in children with severe case caused by *Klebsiella pneumonia* and *streptococcus pneumonia* , while , *Haemophilus influenzae* and *Staphlococcus aureus* were more repeated bacteria associated with moderate case, and in mild cases, *streptococcus pyogenes* is the most prevalent bacterium.
2. Children under 5 years are more likely to have RTIs than older children.
3. LRTI is more prevalence in investigated children than URTI. It was found that tonsillitis is more significant infection of than pharyngitis and sinusitis while in LRTI was discovered that pneumonia is more prevalent compared to bronchitis.
4. The results of antibiotic sensitivity patterns of gram positive bacteria that found ceftriaxone, azithromycin and amoxiclav were effective against *S. aureus*, whereas ciprofloxacin, azithromycin, levofloxacin and amoxicillin were effective against *S. pyogenes.* Ciprofloxacin, levofloxacin, meropenem and azithromycin were found to be effective against *Streptococcus* spp.
5. Serum level of Human sTREM-1 , Human suPAR and Human CH13L1 were much greater in severe case comparison with moderate and mild case of infection.
6. Statically, it was found a significant correlation in some parameters among the severe ,moderate and mild infections .

Recommendations for future studies

1. Add to investigations into different cytokines may yield important information about the processes behind the start and development of pediatric respiratory illnesses.
2. Study gene polymorphisms of some genes contribute in respiratory tract infections in children.
3. Investigation the cluster of differentiation markers by Flow Cytometry contribute in respiratory tract infections in children.

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Appendices

Appendix 1:

Assessment Questionnaire

Name:…….……. Age……. Date:…….…….

Length:……..Weight:…….OFC……

Modes of delivery……Gestational age…….....

GPA. G…. P……A….. Gender :……Single ……or Twin…….

Breast feeding…… Bottle feeding…….Mix……..

Ward…… Emergency…… PICU……HDU……Fate …….

Address:……. City:…….. Urban……Rural……

PULMONARY DISEASES

Respiratory distress yes…….. Grade……. No…….

Duration of hospitalization…….

Medical history

COVID 19……. Measles…….. Atopy……..Upper air way infection ……..

Lower air way infection……

OTHRES……..

History of previous hospitalization ……..Why……

Drugs history……..

Surgical history . chest tube …… surgery …… bronchoscopy ……..

Family history

Current respiratory infection ……..Atopy …… …..Others……

Tests :

CBC	**WBC …… N%.....L%.....Other%.....** **Hb……. MCV…….. Platelets ……**
ESR	
CRP	
CXR	

Type of microbe ..Bacterial ……...viral……. Others …….

Appendix 2:

C-Reactive Protein kit

Kit component of CRP

Component	Quantity
Diluent (R 1)	Tris buffer 20 mmol/L , PH 8.2
Latex (R 2)	Latex particles coated with goat anti-human CRP , PH 7.3
Calibrator (CAL)	Human serum. CRP concentration is stated on the label vial and it is traceable to the certified reference material ERM-DA470(IRMM)

Principle

The latex particles coated with anti- CRP are agglutinated when they react with samples that contain C-reactive protein (CRP). The latex particles agglutination is proportional to the concentration of the CRP in the sample and can be measured by turbidimetry. **Reagents preparation**

All reagents are ready to use. **Sample collection**

Fresh serum stables 7 days at 2-8ºC or 3 months at -20ºC. Samples with presence of fibrin should be centrifuged before testing. Hemolyzed or contaminated samples are not suitable for testing.

Analytical procedure

1- Distilled water used to set the instrument to 450 nm

2- 5 µl of sample, calibrator was added to 1 ml of working reagent and distilled into a vessel

3- Mix well and record the absorbance immediately (A1) and after 2 minutes (A2) of adding the sample.

Appendix 3 :

Determination Human suPAR (soluble urokinase-type plasminogen activator receptor) by ELISA.

principle of the test

The testing principle applied in this group was sandwich enzyme immunoassay. The microtiter plate provided in this kit was pre-coated with an antibody specific for Human suPAR. Standards or samples were added to the wells of the appropriate microtiter plate and then using a biotin-conjugated antibody specific for Human suPAR. Next, Avidin conjugated to horseradish peroxidase (HRP) was added to each microplate well and incubated. After addition of the TMB substrate solution, only those wells containing Human suPAR, biotin-conjugated antibodies, and enzyme-conjugated avidin showed a color change. The reaction of the enzyme with the substrate was terminated by adding a sulfuric acid solution and the color change was measured spectrophotometrically at a wavelength of 450 nm ± 10 nm. The concentration of Human suPAR in the samples was determined by comparing the OD of the samples to the standard curve, and the group compounds are listed in Table (3-6).

Table (3-6) Components kit of Human suPAR (soluble urokinase-plasminogen activator receptor).

Item	Suppliers
Pre-coated Microtiter Plate	ELK Biotechnology
Standard (Lyophilized)	
Biotinylated Detection Ab 100×	

Streptavidin-HRP (100×)	(USA)
Standard/Sample Diluent Buffer	
Biotinylated Antibody Diluent	
HRP Diluent	
Wash Buffer (25×)	
TMB Substrate Solution	
Stop Solution	
Plate Covers	

Preparation of Reagents

The kits' reagents were manufactured in accordance with the manufacturing instructions below:

Wash Buffer

1. The wash solution was diluted 25× in 1× wash buffer using double distilled water.
2. Standard working solution - standard centrifuge at 1000 x g for 1 minute. The standard was reconstituted with 1.0 ml of standard dilution solution, kept for 10 min at room temperature, and gently shaken (do not foam). The concentration of the standard in the stock solution is 10 ng/mL. Seven tubes containing 0.5 ml of the diluted standard were prepared, and the diluted standard was used to produce the double dilution series shown in Figure (3-2). To mix each tube well before the next transfer, the solution was passed up and down several times. 7 drops of standard diluent such as 10 ng/ml, 5 ng/ml, 2.5 ng/ml, 1.25 ng/ml, 0.63 ng/ml, 0.32 ng/ml, 0.16 ng/ml were prepared, and the last EP tubes were prepared with diluent. Standard is 0 ng/ml. In order to confirm the validity of the experimental results,

a new standard solution was used for each experiment. The pipette tip was replaced for each dilution when the standard was diluted from high to low concentration.

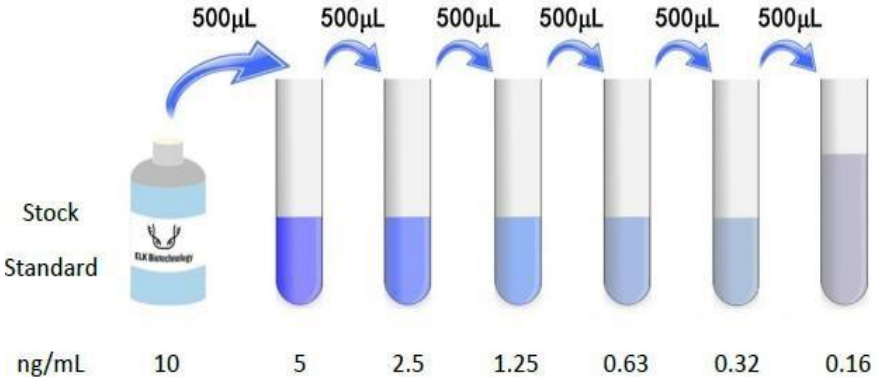

Figure (3-2) Human suPAR standard solution

Assay procedure of Human suPAR

1. Wells were designated for diluted, blank, and sample standards. Prepare 7 wells for standard, 1 well for blank. 100 µL of each working standard, or 100 µL of samples were added into the appropriate wells. Put plate cover and incubation at 37°C for 80 minutes.
2. Liquid was poured from each well. The solution was withdrawn and washed with 200 µl of 1× wash buffer per well and left for 1–2 min. Residual liquid was removed from all wells completely by picking up the plate on absorbent paper. It was washed completely 3 times. After the final wash, any washing buffer was removed by aspiration or decanting. The plate was flipped over and wiped on absorbent paper.
3. Add 100 µL of biotinylated antibody working solution to each well, cover the wells with a plate coverslip and incubate for 50 minutes at 37°C.

4. The suction and washing process was repeated 3 times as in step 2.
5. 100 µl of Streptavidin-HRP working solution was added to each well, and the wells were capped using a stopper plate and incubated for 50 min at 37°C.
6. The suction and washing process was repeated 5 times as in step 2.
7. 90 µL of TMB substrate solution was added to each well. Cover with a new cover plate. Incubate for 20 minutes at 37 °C (not to exceed 30 minutes) in the dark. The liquid turned blue by adding the TMB substrate solution. The microplate reader was heated for approximately 15 min before measuring the OD.
8. 50 µl of stop reagent was added to each well. The liquid turned yellow by adding the stop reagent. The liquid was mixed by tapping on the side of the dish.
9. Wipe off any drop of water and print the fingerprint on the bottom of the plate. Make sure there is no bubble on the surface of the liquid. The microplate reader was turned on and the measurement at 450 nm was performed immediately.

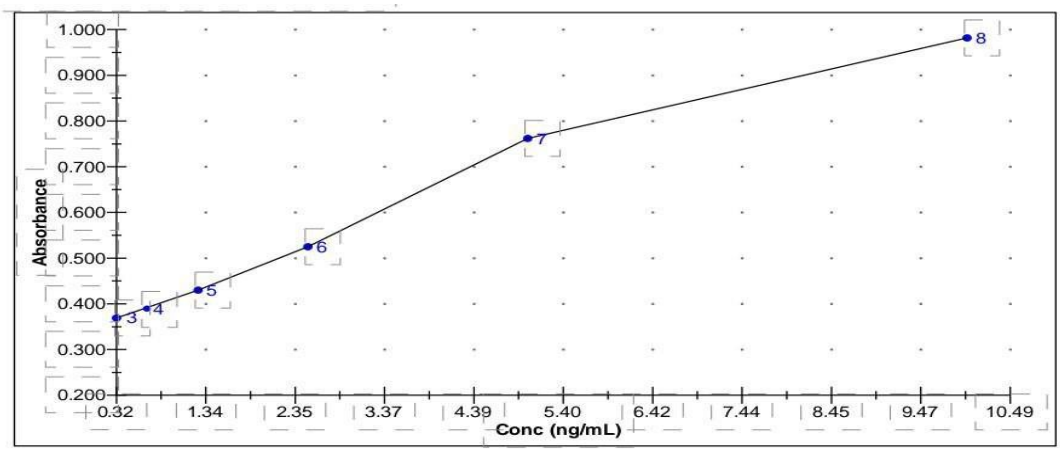

Figure (3-3) standard curve of suPAR.

Appendix 4:

Estimation of Human s-TREM1(soluble triggering receptor expressed on myeloid cells-1) by ELISA.

Principal test of s-TREM -1

The test principle applied in this group was sandwich enzyme immunoassay. The microtiter plate provided in this kit was pre-coated with an antibody specific for Human suPAR. Standards or samples were added to the wells of the appropriate microtiter plate and then using a biotin-conjugated antibody specific for Human suPAR. Next, Avidin conjugated to horseradish peroxidase (HRP) was added to each microplate well and incubated. After addition of the TMB substrate solution, only those wells containing Human s-TREM, biotin-conjugated antibodies, and enzyme-conjugated avidin showed a color change. The reaction of the enzyme with the substrate was terminated by adding a sulfuric acid solution and the color change was measured spectrophotometrically at a wavelength of 450 nm ± 10 nm. The concentration of Human s-TREM in the samples was determined by comparing the OD of the samples to the standard curve.

Reagent preparation of s-TREM -1

The kits' reagents were manufactured in accordance with the manufacturing instructions below:

Wash Buffer : -

1. The wash solution was diluted 25× in 1× wash buffer using double distilled water.
2. Standard working solution - standard centrifuge at 1000 x g for 1 minute. The standard was reconstituted with 1.0 ml of standard dilution solution, kept for 10 min at room temperature, and gently shaken (do not foam). The concentration of the standard in the stock solution is 10 ng/mL. Seven tubes containing 0.5 ml of the diluted

standard were prepared, and the diluted standard was used to produce the double dilution series shown in Figure (3-4). To mix each tube well before the next transfer, the solution was passed up and down several times. 7 drops of standard diluent such as 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.25 pg/ml were prepared, and the last EP tubes were prepared with standard diluent is the Blank as 0 pg/ml. In order to confirm the validity of the experimental results, a new standard solution was used for each experiment. The pipette tip was replaced for each dilution when the standard was diluted from high to low concentration.

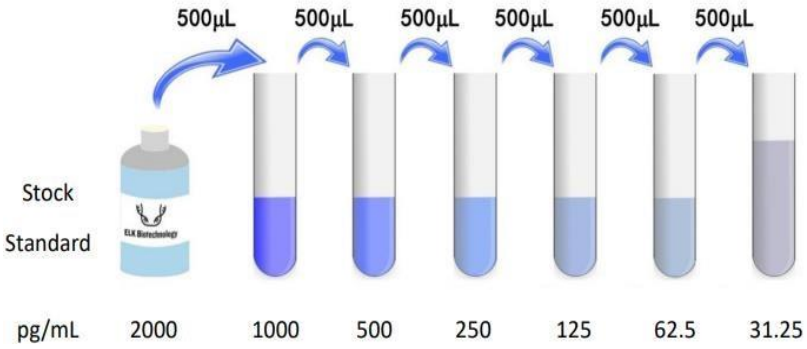

Figure (3-4) standard solution of s-TREM.

Assay Procedure of Human s-TREM1

1. Wells were designated for diluted, blank, and sample standards. Prepare 7 wells for standard, 1 well for blank. 100 µL of each working standard, or 100 µL of samples were added into the appropriate wells. Cover with a plate cover. Incubate for 80 minutes at 37 °C.
2. Liquid was poured from each well. The solution was withdrawn and washed with 200 µl of 1× wash buffer per well and left for 1–2 min. Residual liquid was removed from all wells completely by picking up the plate on absorbent paper. It was washed completely

3 times. After the final wash, any washing buffer was removed by aspiration or decanting. The plate was flipped over and wiped on absorbent paper.

3. Add 100 µL of biotinylated antibody working solution to each well, cover the wells with a plate coverslip and incubate for 50 minutes at 37°C.
4. The suction and washing process was repeated 3 times as in step 2.
5. 100 µl of Streptavidin-HRP working solution was added to each well, and the wells were capped using a stopper plate and incubated for 50 min at 37°C.
6. The suction and washing process was repeated 5 times as in step 2.
7. 90 µL of TMB substrate solution was added to each well. Cover with a new cover plate. Incubate for 20 minutes at 37 °C (not to exceed 30 minutes) in the dark. The liquid turned blue by adding the TMB substrate solution. The microplate reader was heated for approximately 15 min before measuring the OD.
8. 50 µl of stop reagent was added to each well. The liquid turned yellow by adding the stop reagent. The liquid was mixed by tapping on the side of the dish.
9. Wipe off any drop of water and print the fingerprint on the bottom of the plate. Make sure there is no bubble on the surface of the liquid. The microplate reader was turned on and the measurement at 450 nm was performed immediately.

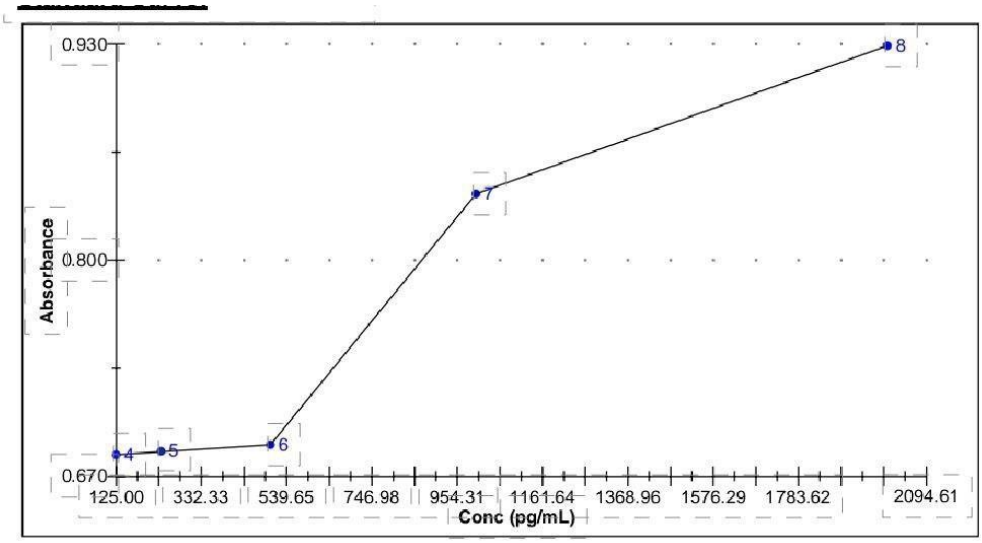

Figure (3-5) standard curve of s-TREM-1 Appendix 5:

Estimation of CHI3L1(chitinase -3-like protein-1) by ELISA.

Principle test of CHI3L1

The test principle applied in this group was sandwich enzyme immunoassay. The microtiter plate provided in this kit was pre-coated with an antibody specific to Human CHI3L1. Standards or samples were added to the wells of the appropriate microtiter plate and then using a biotin-conjugated antibody specific for Human CHI3L1. Next, Avidin conjugated to horseradish peroxidase (HRP) was added to each microplate well and incubated. After addition of the TMB substrate solution, only those wells containing Human CHI3L1, biotin-conjugated antibodies, and enzyme-conjugated avidin showed a color change. The reaction of the enzyme with the substrate was terminated by adding a sulfuric acid solution and the color change was measured spectrophotometrically at a wavelength of 450 nm ± 10 nm. The concentration of Human CHI3L1in the samples was determined by comparing the OD of the samples to the standard curve.

Preparation reagent of CHI3L1

The kits' reagents were manufactured in accordance with the manufacturing instructions below:

Wash Buffer

1. The wash solution was diluted 25× in 1× wash buffer using double distilled water.
2. Standard working solution - standard centrifuge at 1000 x g for 1 minute. The standard was reconstituted with 1.0 ml of standard dilution solution, kept for 10 min at room temperature, and gently shaken (do not foam). The concentration of the standard in the stock solution was 4000 pg/mL. Seven tubes containing 0.5 ml of the diluted standard were prepared, and the diluted standard was used to produce the double dilution series shown in Figure (3-6). To mix each tube well before the next transfer, the solution was passed up and down several times. 7 drops of standard diluent such as 4000 pg/ml, 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml were prepared, and the last EP tubes were prepared with standard diluent is the Blank as 0 pg/ml. In order to confirm the validity of the experimental results, a new standard solution was used for each experiment. The pipette tip was replaced for each dilution when the standard was diluted from high to low concentration.

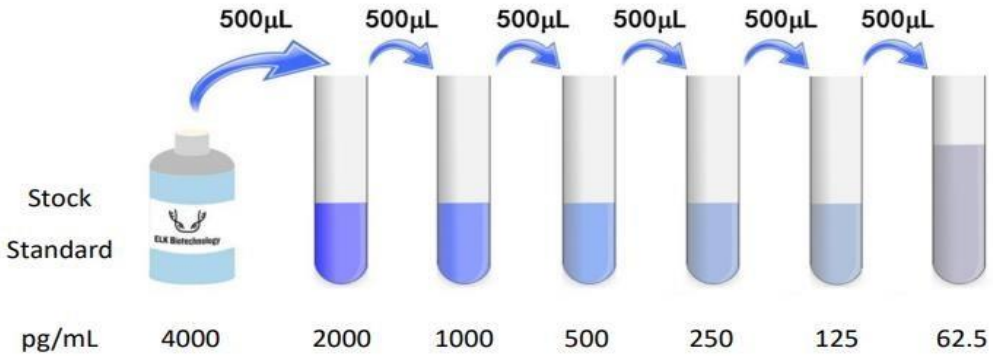

Figure (3-6) the standard solution of CHI3L1

Procedure of Human CHI3L1

1. Wells were designated for diluted, blank, and sample standards. Prepare 7 wells for standard, 1 well for blank. 100 µL of each working standard, or 100 µL of samples were added into the appropriate wells. Cover with a plate cover. Incubate for 80 minutes at 37 °C.
2. Liquid was poured from each well. The solution was withdrawn and washed with 200 µl of 1× wash buffer per well and left for 1–2 min. Residual liquid was removed from all wells completely by picking up the plate on absorbent paper. It was washed completely 3 times. After the final wash, any washing buffer was removed by aspiration or decanting. The plate was flipped over and wiped on absorbent paper.
3. Add 100 µL of biotinylated antibody working solution to each well, cover the wells with a plate coverslip and incubate for 50 minutes at 37°C.
4. The suction and washing process was repeated 3 times as in step 2.
5. 100 µl of Streptavidin-HRP working solution was added to each well, and the wells were capped using a stopper plate and incubated for 50 min at 37°C.
6. The suction and washing process was repeated 5 times as in step 2.
7. 90 µL of TMB substrate solution was added to each well. Cover with a new cover plate. Incubate for 20 minutes at 37 °C (not to exceed 30 minutes) in the dark. The liquid turned blue by adding the TMB substrate solution. The microplate reader was heated for approximately 15 min before measuring the OD.

8. 50 µl of stop reagent was added to each well. The liquid turned yellow by adding the stop reagent. The liquid was mixed by tapping on the side of the dish.
9. Wipe off any drop of water and print the fingerprint on the bottom of the plate. Make sure there is no bubble on the surface of the liquid. The microplate reader was turned on and the measurement at 450 nm was performed immediately.

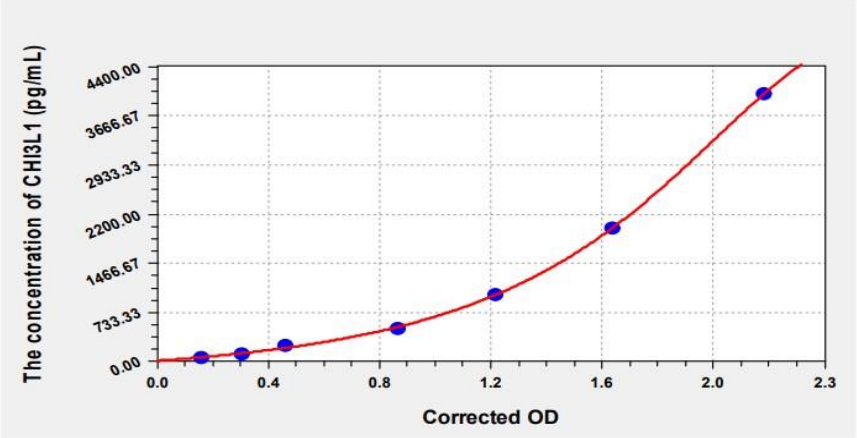

Figure (3-7) standard curve of CHI3L1

الخلاصة

التهابات الجهاز التنفسي هي أي مرض تنفسي علوي أو سفلي يؤثر على الأطفال في جميع أنحاء العالم، ويرتبط بمعدلات مرض ووفيات مهمة. تحدث العدوى في المسالك التنفسية عادةً بواسطة الفيروسات، على الرغم من أن العدوى البكتيرية شائعة أيضًا. مستقبلات التحفيز القابلة للذوبان المعبر عنها على الخلايا النخاعية-1، مستقبلات منشط البلازمينوجين القابلة للذوبان من نوع يوروكيناز وبروتين شبيه بالكيتيناز-3- 1 يمكن أن تكون هذه المؤشرات الحيوية مفيدة في تقييم شدة الالتهاب الرئوي وتوقعاته، مما قد يساعد في توجيه قرارات العلاج.

كانت الدراسة مقطعية وأجريت في مستشفى بابل التعليمي للأم والطفل ومستشفى النور للأطفال في محافظة بابل، من بداية تشرين الثاني 2023 إلى نيسان 2024، وتم الحصول على عينات الدم والبلغم من 120 حالة مريض مشخص من قبل اطباء الاطفال بالتهابات الجهاز التنفسي . وتم تقسيمهم حسب شدة الاصابة إلى ثلاث مجموعات: خفيفة، متوسطة وشديدة ، وتراوحت أعمارهم بين أقل من سنة إلى 14 سنة.

هدفت هذه الدراسة إلى دراسة أكثر أنواع العزلات البكتيرية شيوعاً والمسؤولة عن الالتهابات البكتيرية العلوية والسفلية لدى الأطفال والتحقيق في دور المؤشرات الحيوية في تشخيص التهابات الجهاز التنفسي لدى الأطفال. تم زرع عينات البلغم على أوساط زرعية واستخدمت اختبارات الكيمياء الحيوية اليدوية لتحديد أنواع البكتيريا. تم قياس C-reactive protein وWBC .تم قياس المؤشرات الحيوية Chitinase 3-like protein 1 و sTREM-1 وsuPAR باستخدام نظام ELISA .

أظهرت النتائج أن من بين 127 عينة من البلغم، كانت 7 خالية من النمو البكتيري، وتم العثور على 120 عينة تحتوي على نمو بكتيري ملحوظ، وتم تضمينها فقط في العمل الحالي. وكانت نتائج العزلات البكتيرية هي : وكانت نتائج العزلات البكتيرية هي *Haemophilus influenza* 21.67%، *Klebsiella pneumoniae* 20%، *Streptococcus Pneumoniae* 19.17%، *Staphylococcus aureus* 15%، *Streptococcus Pyogenes* 14.16%، *Pseudomonas aeruginosa* 5%، و *Moraxella catarrhalis* 5%.

وكشفت الدراسة المقارنة عن زيادة في تركيزات جميع المؤشرات الحيوية في الحالات الشديدة من المرض. وبلغ متوسط تركيزات sTREM-1 2841.981 ± 2816.161 بيكوغرام/مل في الحالات الشديدة، ومتوسط تركيز CHI3L1 2025.397 ± 1016.498 بيكوغرام/مل وتركيزات suPAR 21.123 ± 10.812 نانوغرام/مل.

ووجد إحصائيًا فرقًا كبيرًا في بعض المعايير بين حالات العدوى الشديدة. ونتيجة لذلك، يوجد ارتباط سلبي بين sTREM-1 وCH13L1 ، عند قيمة $p < 0.05$. وعلاوة على ذلك، يوجد ارتباط إيجابي كبير بين WBC ومعدل ESR (قيمة $p = 0.389$). هناك ارتباط سلبي بين WBC مع sTREM-1 ، CH13L1, suPAR قيمة $p < 0.05$.

اما في الحالة المعتدلة، كان هناك ارتباط إيجابي كبير بين Human sTREM-1 و Human suPAR، عند القيمة $p <0.05$. من ناحية أخرى، هناك علاقة إيجابية كبيرة بين Human suPAR و CRP، عند القيمة $p <0.01$. هناك علاقة سلبية بين ESR مع sTREM-1 ,CH13L1 و suPAR عند والقيمة $p <0.05$. وبالتالي، هناك ارتباط إيجابي بين WBC مع CRP، عند قيمة $p <0.01$. بالإضافة إلى ذلك هناك علاقة إيجابية قوية بين WBC وCBC (**0.436) .

اما بالنسبة للإصابة في الحالة الخفيفة، يوجد ارتباط إيجابي كبير بين CH13L1 و sTREM-1 و suPAR ، عند قيمة $p < 0.05$. بالإضافة إلى ذلك، يوجد ارتباط إيجابي كبير بين suPAR و sTREM-1 البشري، عند قيمة $p < 0.05$). في حين وجد ارتباط سلبي كبير بين suPAR وESR، عند قيمة $p < 0.01$.

اظهرت هذه الدراسة إلى أن المستويات المرتفعة لـ CRP وWBC وESR لدى الأطفال الذين تقل أعمارهم عن سنة واحدة هو أكثر بكثير من الفئات العمرية الأخرى، وكان مستوى مصل Human sTREM-1 وHuman CH13L1 وHuman suPAR في الحالات الحادة من المرض أكبر بكثير مقارنة مع الحالات المتوسطة والخفيفة.

جامعة كربلاء

كلية العلوم الطبية التطبيقية

قسم التحليلات المرضية

دراسة مستقبلات التحفيز القابلة للذوبان المعبر عنها في الخلايا النخاعية 1، بروتين شبيه الكيتيناز 3 ومستقبل منشط البلازمينوجين اليوروكيناز القابل للذوبان في الالتهاب الرئوي البكتيري عند الأطفال.

رسالة مقدمة

الى مجلس كلية العلوم الطبية التطبيقية-جامعه كربلاء

وهي جزء من متطلبات نيل شهادة الماجستير في التحليلات المرضية

كتبت بواسطة

زينب محسن محمد حسن

بكالوريوس تحليلات المرضية (2021)

بأشراف

أ.د. حسن علي حسين السعدي

١٤٤٦هـ **٢٠٢٤م**