**University of Kerbala**

# Biomedical Study of Idiopathic Nephrotic Syndrome with Improvement of Nano hybrid Antibiotic against Bacterial Resistance

**A thesis**

Submitted to the council of the

College of Applied Medical Sciences-University of Kerbala

In Partial of Fulfillment of Requirement for the Degree of

Master in Clinical Laboratories

Submitted by


**Fatimah Abdul Hasan Ali**

**B.Sc. Applied Medical Sciences/University of kerbala,2016**


Supervised by

**Assist. Prof.**

**Dr. Alaa Abdul Hussein Al-Daamy**

**June (2022)** **Thul Qi'dah(1444)**

{ بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ }

{ شَهِدَ ٱللَّهُ أَنَّهُۥ لَآ إِلَٰهَ إِلَّا هُوَ وَٱلۡمَلَٰٓئِكَةُ وَأُوْلُواْ ٱلۡعِلۡمِ قَآئِمَۢا بِٱلۡقِسۡطِۚ لَآ إِلَٰهَ إِلَّا هُوَ ٱلۡعَزِيزُ ٱلۡحَكِيمُ (١٨) إِنَّ ٱلدِّينَ عِندَ ٱللَّهِ ٱلۡإِسۡلَٰمُۗ وَمَا ٱخۡتَلَفَ ٱلَّذِينَ أُوتُواْ ٱلۡكِتَٰبَ إِلَّا مِنۢ بَعۡدِ مَا جَآءَهُمُ ٱلۡعِلۡمُ بَغۡيَۢا بَيۡنَهُمۡۗ وَمَن يَكۡفُرۡ بِـَٔايَٰتِ ٱللَّهِ فَإِنَّ ٱللَّهَ سَرِيعُ ٱلۡحِسَابِ (١٩) }

صدق الله العلي العظيم

[سُورَةُ آلِ عِمْرَانَ: ١٨-١٩]

# Dedication

***I would like to dedicate this thesis to the station of the owner of the age and time, Imam Al-Mahdi, whom I seek to pave the way for his appearance, even if by any small work, in order to develop the country of Islam and strengthen it for his presence with all our knowledge and work wherever I am.***

***Also I dedicate my thesis to my dear family who all supported me. I dedicate the fruits of my efforts to those who, thanks to them, we lived and studied with all security and tranquility, enhanced and honored in our country, and we did not submit to a usurper to the righteous martyrs,***

***and I ask God to accept this little from me***

# Acknowledgments

Praise be to God Almighty for success and facilitating my affairs to complete this work and cross this stage of my life.

Praise to my college Applied medical science university of Karbala which studied and graduated from and thanks to Department of Clinical Laboratories.

A heartfelt thank due to supervisor, Dr. Alaa Al-Daamy , who was the best supervisor and the irreplaceable Assist. professor who helped me a lot in all stages of the thesis with dedication.

Thanks and appreciation are to the supervisor, Dr. Qahtan Al-Obaidy, who supported me in completing my samples and what I needed for my thesis.

Praise to Dr.Zaid Hasan Al-Aaragy and Dr. Ali Al Ghanimy whom helped me in Nano Technology analyses .

Finally I would like to thank my dear family father ,mother ,husband and my children ,also thanks to my good friends and sisters who supported me a lot in my work .

# Supervisors certification

We certify that this entitle (Biomedical Study of Idiopathic Nephrotic Syndrome with Improvement of Nano hybrid Antibiotic against Bacterial Resistance) was prepared under our supervision at the department of Clinical Laboratories, for College of Applied Medical Sciences, University of Karbala, as a partial requirement for the degree Master in Clinical Laboratories.

Signature

Dr. Alaa Abdul Hussein Al-Daamy

Scientific order: Assist. Prof.

Date 25/10/2022

Department Head of Clinical Laboratories Recommendation

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

Signature

Name: Prof. Dr. Hadi Rasool Hassan

Address Head of Clinical Laboratories college of Applied Medical Sciences/ University of Kerbala

Date: / /2022

# Languifical Certification

I certify that the master's thesis tagged: (Biomedical Study of Idiopathic Nephrotic Syndrome with Improvement of Nano hybrid Antibiotic against Bacterial Resistance) by the master's student (Fatimah Abdul Hasan Ali), in the Department of Clinical Laboratories - College of Applied Medical Sciences - University of Karbala, has been reviewed from the linguistic point of view, and correcting the grammatical and stylistic errors in it, and the thesis became eligible for discussion.

Signature:

Linguistic component: Prof .Dr .Hussain Mousa Al-Nasravy

Academice title:

College of:

Date: / / 2022

## Discussion Committee Certification

We are members of the discussion committee certify that we read this thesis entitled "**Biomedical Study of Idiopathic Nephrotic Syndrome with Improvement of Nano hybrid Antibiotic against Bacterial Resistance**" and we discussed Mr. student **Fatimah Abdul-Hasan Ali Ibrahim** in its contents. It is adequate for the award of the certification of Master of Clinical Laboratories with excellent grade.

Signature
**Prof. Dr. Qasim Najim Thewaini**
(chairman)
2022/10/31

Signature
**Prof. Dr. Suhad Hadi Mohammed**
(member)
2022/10/25

Signature
**Prof. Dr. Ali Galeel Ali**
(member)
2022/10/25

Signature
**Assist. Prof. Dr. Alaa Abdul Hussein Al-Daamy**
(member & Supervisor)
2022/10/25

Signature
**Dr. Qahtan Mohammed Ali Al-Obaidy**
(Member and Consultant Supervisor)
2022/10/25

**I have certified upon the discussion of the examining committee.**

Signature
**Assist. Prof. Dr. Jawdat Noori Ghaaib**
Dean of the College of Applied Medical Sciences/ University of Kerbala
2022/11/7

# List of Contents

# List of Tables

# List of figures

# List of Abbreviations

| Abbreviations | Item |
|---|---|
| µg | Microgram |
| µl | Microliter |
| Acb | *Acinetobacter baumannii* |
| AFM | Atomic Force Microscope |
| Ag | Silver |
| $Ag_2O$ | Silver oxide |
| Al | Aluminum |
| ALP | Alkaline phosphatase |
| ALT | Alanine aminotransferase |
| AST | Aspartate aminotransferase |
| (AST) | Antibiotic susceptibility test |
| Au | Gold |
| BCID | Blood Culture Identifying Ianel |
| CaO | Calcium oxide |
| CFU | Colony forming units |
| CHNS | precise analysis of C,H,N&S elements |
| CNIs | Calcineurin inhibitors |
| CNS | Coagulase-negative staphylococci |
| CO2 | Carbon dioxide |
| CRP | C-reactive protein |
| CTX | Ceftriaxone |
| Cu | Copper |
| CuO | Copper oxide |
| D.Bilirubin | Direct Bilirubin |
| DNA | Deoxyribonucleic acid |
| DW | Distilled water |
| ESBLs | Extended spectrum β-lactamases |
| ESR | Erythrocyte sedimentation rate |
| EUCAST | European Committee on Antimicrobial Susceptibility Testing |
| fL | Femtoliters |
| FRNS | Frequent relapse nephrotic syndrome |
| FSGS | Focal segmental glomerulosclerosis |
| FT-IR | Fourier transform infrared spectroscopy |
| GGT | Gamma-glutamyl transferase |
| gm | Gram |
| HAIs | Hospital Acquired Infections |
| HDL | High density lipoprotein |
| hr | Hour |
| ICU | Intensive care unit |
| IgG | Gama Immunoglobulin |
| INS | Idiopathic Nephrotic syndrome |

| | |
|---|---|
| KBr | potassium bromide |
| L | Liter |
| (LDH) | Lactate dehydrogenase |
| LDH | Layer double hydroxide |
| LDL | Low density lipoprotein |
| LYM | Lymphocyte |
| MALDITOF-MS | Matrix assisted laser desorption ionization time of flight mass spectrometry |
| MCD | Minimal change disease |
| MCH | Mean Corpuscular Hemoglobin |
| MCHC | Mean Corpuscular Hemoglobin Concentration |
| MCNS | Minimal change nephrotic syndrome |
| MCV | Mean Corpuscular volume |
| MDRAB | Multidrug-Resistant *Acinetobacter baumannii* |
| MesPGN | Mesangioproliferative glomerulonephritis |
| Mg | Magnesium |
| mg | Milligram |
| Mg-AL-$No_3$-LDH | Magnesium -aluminum nitrate layer double hydroxide |
| MgO | Magnesium oxide |
| MIC | Minimum Inhibitory Concentration |
| min | Minute |
| ml | Milliliter |
| MPV | Mean platelet volume |
| MRSP | Multiple antibiotic-resistant S. pneumoniae |
| NaCl | Sodium chloride |
| NaOH | Sodium Hydroxide |
| NEUT | Neutrophil |
| NGS | Next generation sequencing |
| $NO_3$ | Nitrate |
| NPs | Nanoparticles |
| NS | Nephrotic Syndrome |
| NVs | Nano-sized vehicles |
| OXA | Oxacillinase gene |
| PBPs | Penicillin-binding proteins |
| PCR | Polymerase chain reaction |
| PCT | Plateletcrit |
| P-LCR | Platelet Large Cell Ratio |
| PLT | Platelet |
| PMNL | Polymorphonuclear leukocyte |
| PNA FISH | Peptide Nucleic Acid Fluorescent In Situ Hybridization |
| PRSP | Penicillin-resistant *S. pneumoniae* |
| qPCR | quantitative PCR |
| RBC | Red blood cells |
| RDW-CV | Red cell Distribution Width-Coefficient of Variation |
| RDW-SD | Red cell Distribution Width-Standard Deviation |
| Rh D | Rhesus D |
| RNA | Ribonucleic acid |
| rpm | Round per minute |

| | |
|---|---|
| SBI | Sever blood infections |
| SBP | Spontaneous bacterial peritonitis |
| SBP | Spontaneous bacterial peritonitis |
| SDNS | Steroids dependent nephrotic syndrome |
| Si | Silicon |
| SRNS | Steroids responded nephrotic syndrome |
| SRNS | Steroid resistance nephrotic syndrome |
| SSNS | Steroid sensitive nephrotic syndrome |
| T. Bilirubin | Total Bilirubin |
| T.p | Total serum protein |
| TC | Total Cholesterol |
| TEM | Temoniera gene |
| temp | Temperature |
| TG | Triglycerides |
| Ti | Titanium |
| TiO2 | Titanium dioxide |
| US | United State |
| USA | United States of America |
| UTI | Urinary tract infection |
| VDBP | Vitamin D-binding protein |
| WBC | White blood cell count |
| WT1 | Wilms' tumor suppressor gene |
| XRD | X-ray diffraction |
| ZnO | Zinc oxide |

## Summary

This study aims to training and utilization of nano method by two ways to improve the antibiotic use in treatment of blood infections in children who have nephrotic syndrome, determine bacteremia in this group of children and study bacterial isolate to susceptibility pattern by phenotypic method as well as detect isolated bacteria resistance genes to antibiotics .Also detect relationship between some biological and immunological parameters in pediatric nephrotic syndrome with different ages .

The study was done between October 2021 to May 2022 , At the Pediatric teaching hospital Center for Nephrology /Karbala Health Directorate. A five -milliliter sample of venous blood was collected from 116 Nephrotic syndrome children patient and 42 healthy children. There are significant blood culturing procedures , and several analyses have been performed as a result including biochemical parameters ; Vitamin $D_3$, Lipid profiles (TC, LDL, HDL and TG),Liver function tests (Albumin, T. bilirubin , D. bilirubin ,TP, ALT, AST, ALP, GGT, LDH),Immunological parameters (WBC, CRP, IgG),Hematological parameters CBC ,Physiological parameters (Glucose, ABO),General Parameters (Age, Weight ,Gender) , and Microbial examination (Bacterial Identification and Antibiotic Susceptibility Test ).

Preparation of nanohybrid Zno- ceftriaxone ( drug conjugate with Zno by soll gel method ) ,nanohybrid LDH-ceftriaxone (drug conjugate with layers of Mg-AL-$No_3$ ) , were tested then by FTIR ,SEM,XRD,AFM analysis . The effect of these nano drugs was examined in isolated bacteria .The resistant genes for ceftriaxone were also identified.

The results of parameters examined for nephrotic children patients compared with healthy children showed : decreased Vit $D_3$ levels , increased (S. Cholesterol , LDL, HDL, and TG )levels , decreased Albumin and Total serum protein levels, also increased T. bilirubin and D. bilirubin levels ,increased (ALT, AST, ALP) levels ,increased GGT and LDH levels , increased WBC and CRP levels and decreased IgG

levels . In children with Nephrotic syndrome there were some correlation between some parameters such as CRP and WBC (0.509) , also CRP and Albumin(0.515) ,as well as WBC with both Gran and Mid (0.856,0.727) respectively ,which all of them was positive correlation. In addition there were positive correlation between ; ALP and D. bilirubin (0.566) ,GGT and LDH (0.640), T.C and LDL (0.808) ,and TG with both LDL and T.C (0.526,0.539) respectively.

In children with nephrotic syndrome , B blood group was more than in healthy. Bacteria isolated was *Acinetobacter bumanii* complex , *Staphylococcus warnerii* , and *Bacillus cerus* . All of them had resistance genes for β-lactamase drug.

The nanohybrid ceftriaxone prepared with Zno was not effective .while nano hybrid ceftriaxone with layers of Mg-Al-$No_3$ was effective and its conjugation was successful .

The study concluded that more blood infection in pediatric Nephrotic syndrome came from bacteria of nosocomial infections. The nano method of Zno soll gell method for ceftriaxone was not successful and not effective on bacteria. Precipitation ceftriaxone with Mg-Al-$No_3$ was effective and successful. These isolated Bacteria had β-lactamase genes.

# Chapter One

# Introduction

## 1.1. Introduction

Nephrotic syndrome remains a common chronic illness marked by changes in perm capability on the glomerular capillary wall، result in an incapacity to control protein loss in the urine. Proteinuria in the nephrotic range stays definite as more than one thousand $mg/m^2$ per daytime or a random urine protein-to-creatinine ratio of more than Two mg/mg. In children with pediatric nephrotic syndrome, proteinuria remains usually selective, consisting mostly of albumin **(Bagga and Mantan, 2005).**

The two most prevalent idiopathic kidney sicknesses are Minimal Change Nephrotic Syndrome (MCNS) & Focal Segmental Glomerulosclerosis (FSG) which result in childhood nephrotic syndromes (FSGS). In children, a third kind of nephropathy known as membranous nephropathy is uncommon. The next are two species of other causes of isolated nephrotic syndrome: Infections, neoplasia, and medications are all instances of rare genetic illnesses and secondary diseases .While the cause for idiopathic nephrotic syndrome is inexact, evidence suggests that it is caused by a main T-cell disease that growths to glomerular podocyte dysfunction. Mutations popular genes that code for essential podocyte protein have been situated identified in kids with familial nephrotic syndrome, according to genetic investigations**(Eddy and Symons, 2003) .**

The global incidence of pediatric Nephrotic syndrome is reported to be 4.7 per 100,000 children (range 1.15–16.9), with sub regional diversity due to country background and geographic location**(Downie *et al.*, 2017).**

Nephrotic syndrome may commence nearly as 6 months of age, that can be previous until adulthood. Children under the age of six are the most common wounded of minimal change nephrotic syndrome (MCNS); The typical age at diagnosis for MCNS is 2.5 years, however the median age at diagnosis for focal segmental glomerulosclerosis (FSGS) is six years .Males are more respected than females in small children (ratio 2:1)**(Alhares *et al.*, 2020).**

The most frequent primary glomerular disorders in Iraq are FSGS, which are followed by mesangial glomerular nephritis and minimum change disease (MCD) **(Reshi *et al.*, 2008).**

The popular of nephrotic kids with mild variation lesions will either license away on their own or remit after 3 years (in two-thirds of pateints) or will acclimate to corticosteroids (CS) or cytotoxic medicines faster speedily and with fewer problems (95 %).Children with focal segmental glomerulosclerosis with simple or chronic proteinuria, on the other side, are at a greater risk of problems. In the nearly kids, filled NS can lead to kidney failure & also dialysis, requiring renal replacement . In NS, complications might occur as a result of the disease or as a result of the rectally used to treat it. Depletion of serum proteins inside the urine causes NS problems as a direct result of changes in plasma protein concentrations or as a direct cause of cellular dysfunction. Several infections, thrombosis, circulatory disease, anemia (loss of Hb), hypovolemic crisis & acute renal failure are disorder complications. In nephrotic children, long-term therapy frequently includes corticosteroid, alkylating drugs, Calcineurin inhibitors in addition mycophenolate mofetil (MMF)**(Park and Shin, 2011).**

Patients with NS may experience urinary loss of transferrin, erythropoietin, ceruloplasmin, transcobalamin, iron, and trace elements in addition to the loss

of albumin and vitamin D binding protein in the urine. Although the precise mechanism of these losses is still unknown, these shortages are more common in individuals with therapy-resistant disease and may cause anemia to develop, which may continue despite adequate iron and erythropoietin supplementation. There is ample evidence of transferrin urinary loss in children with NS **(Hampson *et al.*, 2021).**

The aim of INS therapy in affected role stays to found and keep complete reduction with the resolution of proteinuria besides edema while limiting thoughtful side properties. Aside from INS-specific therapies, helpful healings are frequently cast-off to pleasure edema (loop diuretic drug, aldosterone rivals, albumin brews, Angiotensin-converting enzyme inhibitors), lessens infection risk (antibacterial, pneumococcal immunization), thromboembolism also regulates hyperlipidemia (aspirin ,acetylsalicylic acid,)**(Hodson, 2003).**

Infection is a major cause of mortality and death in children of renal disease. Patients with SSNS are more prone to bacterial infections, and various infections can trigger relapses, steroid resistance, and even disease onset **(Moorani, 2011).**

*Streptococcus pneumoniae* puts children having nephrotic syndrome (NS) at risk for invasive infections, mainly peritonitis and/or bacteremia. Recent reports from the US and other countries have documented the incidence of dangerous infections in children caused by penicillin-resistant *S. pneumoniae* (PRSP) & multiple antibiotic-resistant S. pneumoniae (MRSP)**(Ilyas *et al.*, 1996).**

Antibiotic resistance is a major public health concern, and diagnostic bacteriology labs are essential for finding resistant isolates fast. The use of phenotypic tests to detect susceptibility or resistance is a "gold standard"

against which subsequent technologies are measured of performance, cost, and ease of use. There are several molecular methods for identifying resistance, and they are widely used in academia and reference laboratories, Gaining a foothold in diagnostic laboratories, on the other side, is proving more difficult. If widely used in a diagnostic situation, however, these techniques would have a wider impact on patient care and is useful infection control measures, eg, by rapidly detecting patients who were colonized by resistant bacteria. Rapid detection of a particular resistance mechanism through a molecular testing would clearly allow clinicians to avoid potentially ineffective treatment options at the start **(Woodford and Sundsfjord, 2005).**

Despite of the fact that today live in an era where advanced and new tools for elucidating disease underlying mechanisms and molecularly building new drugs are possible. We have a long way to go, infectious diseases remain one of the world's greatest serious health issues ,the rise of multi - drug resistance and unpleasant side effects are the main drawbacks of conventional antibacterial agents. Antibiotics are often administered at high doses due to the drug resistance, resulting in intolerable toxicity, requests for significant financial, labor, and time investments in the development of new antibiotics. Nontraditional antibiotic drugs have recently piqued authors' interest as a means of combating pathogenic bacteria' resistance to most commonly used antibiotics. Several classes including antimicrobial nanoparticles (NPs) & nanosized carriers for antibiotic delivery, in especially, have shown their efficacy in treating of infectious diseases **(Huh and Kwon, 2011).**

The study aims to isolate the resistance bacteria from nephrotic syndrome patients and improve of the antibiotic that bacteria resist by nano-methods.

### 1.1.2.Objectives:

1. General information for both patients and healthy in the proposal study.
2. Collected the blood samples from nephrotic syndrome patients.
3. Conducted the clinical and laboratory tests that include: CBC, CRP, IgG, Serum Albumin, GLU,ABO, liver functions (ALT, AST, ALP, GGT, Albumin, Total protein, Total bilirubin, Direct bilirubin), (LDH), lipid profile, D3.
4. Determined the common bacteria by identification of the blood culture results by Vitik System.
5. Made the antibiotic susceptibility tests of the bacteria that was identified.
6. Prepared nanohybrid of the antibiotic and test their antimicrobial activity against resistance bacteria.

# Chapter Two

# Literatures Review

# 2.1. Literatures Review

## 2.1.1. Nephrotic Syndrome

### 2.1.1.1. The Overview of Nephrotic Syndrome

Nephrotic Syndrome(NS) is one of the kidney diseases associated with increasing the permeability across the glomerular purification barrier. It is considered by heavy proteinuria (>3.5 g/24 hr in adults or 40 mg/$m^2$ caused by hr in children), Hypoalbuminemia (2.5 g/dl) , edema and hyperlipidemia all seem to be markers of hypoalbuminemia***(*Alhares *et al.*, 2020).**

At the turn of the century, clinicians noted a nephrotic disorder of fiery origin and a nephrotic syndrome progressive origin. Today these ideas are outdated, but the term "Nephrotic syndrome" is clinically valuable and has persevered, because hefty proteinuria, regardless of its cause, is related with various of clinically important impacts, particularly NaCl holding, thromboembolic and infectious problems, and also hyperlipoproteinemia. The description however, the following is arbitrary, and no special significance should be agreed upon the criteria used to differentiate nephrotic versus non-nephrotic albuminuria**(Orth and Ritz, 1998).**

It's mainly an illness of youthful 15 times further than an adult**(Alhares *et al.*, 2020).** Childhood NS could be congenital, with problems seeming in the first three years of life, then in these locations, operation is typically effective individuals, a podocyte or glomerular basement membrane-related genetic mutation ,is usually present. Apart from the congenital form, nephrotic syndrome can be caused by a variety of underlying etiologies, like glomerular diseases, vasculitis, poisons, contaminants, spite, hereditary problems, and,

maximum generally, unidentified. Minimal change disease (MCD) or focal segmental glomerulosclerosis (FSGS) — the quintessential podocyte diseases, or podocytopathies — are the most common histological classifications for idiopathic child NS.MCD refers to glomeruli that give the impression normal on light microscopy and is more common in infantile NS than FSGS , by evidence of podocyte foot process effacement with Electron microscope. On electron microscopy, FSGS looks like to MCD, but on light microscopy, it has segmented destruction of the glomerular capillaries by adhesions, unlike MCD. Amid the sclerotic segments beside Bowman's capsule, synechiae form. Injury to these foot processes reasons podocytes to alteration shape and their cellular cytoskeleton to reorganize, As a result, serum protein is lost through the glomerulus. In MCD, this shape change is usually upturned with corticosteroid treatment, but in FSGS, it is often resistant and progressive**(Downie *et al.*, 2017).** NS in the first year constitute a small percentage and it had a poor prognosis, Finnish type represent the highest proportion of biopsy in congenital nephrotic syndrome and focal segmental glomerulosclerosis highest proportion in infantile type**(Mohammed et al., 2019b).**

## 2.1.2. Epidemiology

Global occurrence of pediatric NS was stated to be 4.7 per 100,000 children (range 1.15–16.9), with sub regional diversity due to country background and geographic location**(Downie *et al.*, 2017).**

South Asian kids were exposed a higher prevalence of NS than European population in several European studies **(McKinney *et al*., 2001),**

and historical data from US studies suggests that African children have a higher incidence than children of European descent **(Srivastava *et al.*, 1999).**

NS is currently the most common chronic childhood glomerular disorder seen in Nigeria. Glucocorticoids have remained the mainstay of its treatment since their introduction in the 1950s, and steroid responsiveness is regarded as its most important prognostic indicator. Earlier studies on childhood NS were at Ibadan and Kaduna both in Nigeria and other parts of tropical Africa**(Asinobi *et al.*, 2019).**

Primary nephrotic syndrome epidemiology in Egyptian youngsters; the average onset age was 4.43- 2.7 years. Thirty-four percent of patients were steroid resistant, whereas 66 percent had a good steroid response at the start; while 46 of the latter were steroid-reliant. A nephron biopsy is done on forty patients, with minimal change nephrotic syndrome occurring in 30% in mesangial proliferative patients.37.5 percent had glomerulonephritis, and 30% had focal segmental glomerulosclerosis. In 9% of cases, chronic renal insufficiency developed**(Kaddah *et al.*, 2012)**.

Created on originated epidemiologic evidence, children in Asia get higher prevalence for NS versus kids as in Western world **(Al-Bahrani, 2017).**

Idiopathic NS, which is linked to primary glomerular disease, impacts nearly > 90% of children with NS**(Vogt and Avner, 2007).**

Hoseini *et al.* study demonstrates an increasing trend in FSGS incidence in Iranian children. However, kidney survival rates of our patients were similar to those reported by others in different countries**(Hoseini *et al.*, 2012).** The etiology in 67.5%, 49.8%, and 27.8% of the cases were in Sweden, Japan, and Iraq **(Al-Mosawi, 2002)** respectively.

Study in Iraq gives a detailed information about the primary nephrotic syndrome in childhood a most common type was minimal change disease type , which was consequently with focal segmental glomerulosclerosis, but frequency of focal segmental glomerulosclerosis and to lesser extent the membranoproliferative glomerulonephritis had been increasing in Iraqi children with Idiopathic nephrotic syndrome ( INS). Clinical feature, age, sex and respond to steroid are similar to most parts of world**(Naif and Mohammed, 2019).**

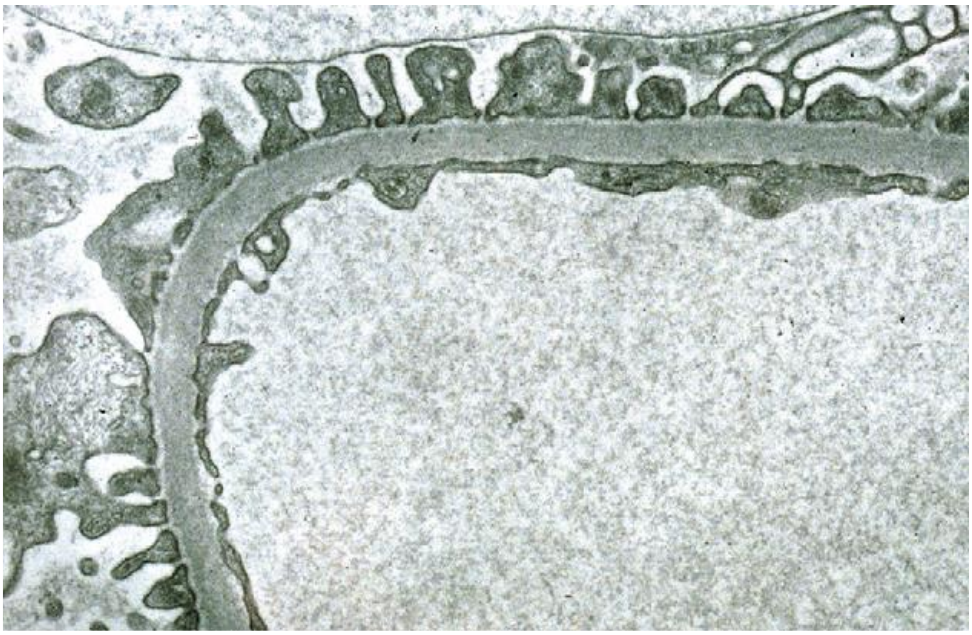
## 2.1.3. Causes of Nephrotic Syndrome in children

Systemic lupus erythematosus is an fundamental disorder which may be recognized in fewer than 5 % in some cases , amyloidosis, HIV infection, parvovirus B19, hepatitis B and C viruses, and Henoch Schoenlein purpura amyloidosis .On light microscopy, extra than 80% of affected role with nephrotic syndrome display MCD (minimal change disease), is categorized by usual kidney histology. The cash comes from focal segmental structure glomerulosclerosis (FSGS), and mesangial proliferative glomerulonephritis (Mes PGN) MCD and FSGS are usually measured to be the semi pathophysiological progression. Membranoproliferative glomerulonephritis & membranous nephrosis are unusual situations through childhood **(Bagga and Mantan, 2005).**

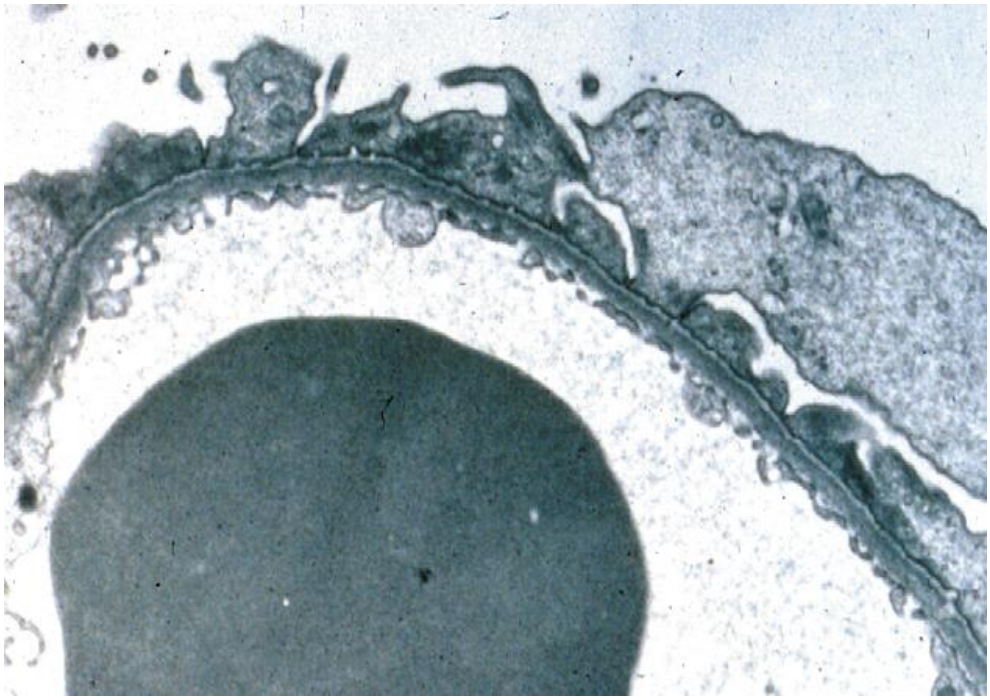
Immune dysregulation, circulating substances, or hereditary podocyte structural abnormalities are all thought to have a role in pathogenesis of idiopathic NS .Children with steroid-resistant disease are more likely to have a genetic risk**(Noone *et al.*, 2018).**

The glomerular filtration barrier is a complex filtration system used by the kidney (GFB ).A glomerular basement membrane is sandwiched between a fenestrated endothelium and an epithelial layer made up of podocytes and their foot processes, with filtration slits and a slit diaphragm interposed.

Water and tiny solutes can pass through the system's pore and into the urinary space for its size and charge specific design. On electron microscopy, there is an effacement of the podocyte foot procedures in nephrotic syndrome. Proteinuria is seen in nephrotic syndrome when this barrier is damaged .The immune system and podocytes production are a major part in genesis of idiopathic NS, as per majority of ongoing research. T-cell dysfunction causes cytokine announcement, that touches glomeruli and reasons for increase permeability, based to Immune system disfunction principals to the creation of mingling factors (one example is solvable urokinase plasminogen activator receptor) that modify podocyte building and/or role, resultant of proteinuria. Remission after rituximab, and an anti-CD20 antibody treatment has also been linked to B-cell involvement. However, there is currently no definitive proof of the underlying mechanism of action**(Andolino and Reid-Adam, 2015).**



**Figure(2-1):Electron microscopic appearance of normal glomerular epithelial cells in normal epithelial cells(Eddy and Symons, 2003)**



**Figure(2-2): Electron microscopic appearance of Nephrotic Syndrome(Eddy and Symons, 2003)**

## 2.1.4. Risk factors for pediatric Nephrotic syndrome

Patients with nephrotic syndrome are vulnerable to infections and potentially fatal thromboembolic events. The long-term consequences of persistent hyperlipidemia and steroid medication are becoming more well-known. Proteinuria reduction after corticosteroid treatment has a better prognostic price than the precise renal histology in terms of the long outcome **(Bagga and Mantan, 2005).**

**A-**T cell subset and/or function abnormalities have been reported in a variety of patients with MCD9-11. The most of the functional abnormalities described are not specific and it may represent a disease effect rather than a cause **(Grimbert *et al.*, 2003).**

**B-** Cytokine bias: To understand better the pathogenesis of nephrotic syndrome, The use of current understanding on functional divisions of the immune reaction. Antigen presentation to T cells causes a separated immune comeback that may be type I (subjugated by g-interferon and interleukin (IL)2) or type II (dominated by interleukin (IL) 3). (IL4, IL10 or IL13) , In cell-mediated immunity, typeI cytokines predominate, but typeII cytokines are used in some features for humoral immunity. Atopy and B cell class switching for IgG4 and IgE1 production are strongly linked to type II cytokines**(Prete, 1992).**

**C-**Increased plasma IgE levels, comparatively standard IgG4 (with less IgG1 & IgG2), and a connotation with atopy propose type II cytokine bias in patients MCD have been reported that system production of representative cytokines, including IL4, is increased**(Vanden Berg *et al.*, 2000).**

**D-** The role of a systemic circulating element, may result in increased glomerular permeability, has been proposed in patients with MCD and FSGS.

**E-** The lack of inflammatory alterations in the renal parenchyma, and the clinical responsiveness of nephrotic syndrome to immunosuppressive medications, suggest the proteinuria is produced by something other than the renal.

**F-** Several vascular permeability factors, especially vascular endothelial growth factors, heparinase, & hemopexin, have been implicated **(Brenchley, 2003) .**

**G-** Normal glomerular podocytes produce the permeability factor vascular endothelial growth factor *in vivo*, and receptors for the factor are found on glomerular endothelial and mesangial cells.

Recessively inherited FSGS is caused by mutations in another gene, the NPHS2 gene, that code for podocin. This mutation is to found in 10- 3 percent steroid-resistant FSGS individuals having sporadic onset**(Ruf *et al.*, 2004).**

On chromosome 19, the alpha-actinin-4 gene for autosomal dominant FSGS has been recognized. WT1 (Wilms' tumor suppressor gene), FSGS2, and LMX1B are some of the other genes implicated (nail patella syndrome). WT1 mutations are linked to Denys-Darsh syndrome (categorized with male pseudo hermaphroditism, Nephrotic Syndrome, & also Wilms' tumor) .The steroid-sensitive nephrotic syndrome (SSNS) gene was discovered on chromosome 1q25, similar to but separate from the podocin gene**(Fuchshuber *et al.*, 2001).**

## 2.1.5.Management

Oedema in nephrotic syndrome for severe or symptomatic oedema, salt & fluid limit with the inclusion of a loop diuretic is one of the key techniques in oedema management, created on the etiology of edema in NS. **(Teoh *et al.*, 2015).**

Pharmacologic and dietary treatments are needed for accurate management. Fluid restriction, as well as corticosteroids, albumin, and diuretics, are used to manage edema. Patients with recurrent relapses or steroid-refractory disease may require steroid-sparing therapy such alkylating agents and calcineurin inhibitor, as well as dietary changes to avoid dairy and gluten.**(Hampson *et al.*, 2021)**.

Diuretics unaccompanied may worsen hypovolemia and intravascular reduction, needing use of albumin. Albumin should be given with care in children who do not have hypovolemic symptoms, as a rise in intravascular fluid may cause pulmonary oedema **(Teoh *et al.*, 2015).**

## 2.1.6. Initial therapy

Standard treatment consists of at least 4–6 weeks of day steroids (prednisone or prednisolone), As according clinical study data, a least of 6 weeks of alternating day treatment must be followed .The variability in the prednisone taper is noted in numerous published procedures by guide, center, or republic. Until , a recent report endorsed that month of care was larger to 2–3 calendar month of therapy, due to large drop in reversions afterward the early performance of NS. Over 700 children will be included in the meta-analysis of controlled tri+-als **(Hodson *et al.*, 2005).**

Since 2013, three randomized trials have shown Extending therapy to 6 months instead of 2–3 months had no result on the rate of relapse, the risk of relapses, steroid dependency, or the requirement for a further immunosuppressive drug **(Sinha *et al.*, 2015).**

Obesity, striae, ocular problems such as cataracts and glaucoma, metabolic abnormalities, musculoskeletal features including such osteoporosis & avascular necrosis of the head of the femur, plus behavioral traits are all associated with excessive steroid usage**(Fakhouri *et al.*, 2003).**

In individuals with Frequently Relapsing Nephrotic Syndrome (FRNS) / Steroid Dependent Nephrotic Syndrome (SDNS), the following agents can reduce the requirement for steroids:

**1-**Levamisole : As compared to placebo or no treatment, it reduces the risk of relapse**(Vaziri, 2016)**. However, there was a lot of variation in the meta-analysis**(Pravitsitthikul *et al.*, 2013)**.

**2-**Cyclophosphamide: Effective for FRNS**(Pravitsitthikul *et al.*, 2013)**,

However, this is less true for steroid-dependent individuals with nephrotic disorder**;** there is, though, some overlay in arrangements.

**3-**Ciclosporin: critical in FRNS or SDNS, nonetheless many patients report relapses if ciclosporin therapy is removed (ciclosporin dependence) **(El-Husseini *et al.*, 2005).**

**4-**Tacrolimus: Tacrolimus definitely helps for FRNS or SDNS, by several studies**(Sinha *et al.*, 2012)** but, no randomized trials exist.

**5-**Mycophenolate mofetil: Fewer real than ciclosporin**(Gellermann *et al.*, 2013)** Then it has a short risk of complications.

**6-**RituximabIn children who are depending on both drugs, Lower dosages of prednisone & calcineurin inhibitors are not inferior to standard doses in sustaining short-term remission, allowing children to simply stop treatment**(Ravani *et al.*, 2011).**Despite effective for children having complex FRNS / SDNS, all kids had declines 19 months later received rituximab**.** In SDNS, it is quasi to steroids in order to maintain remission**(Ravani *et al.*, 2015).**

**7-**Mizoribine: Nevertheless non active for SDNS, a subclass analysis of kids aged ten and fresher showed the prevalence of patients who lapsed in the mizoribine group was significantly lower than in placebo group**(Yoshioka *et al.*, 2000).**

## 2.1.7.Complications:

Complications in NS might arise as a result of pharmacological treatment or from the disease itself. Because of altered cellular processes or as a direct consequence of altered plasma protein composition, the waste of plasma in the urine causes NS complications; thromboembolism, cardiac illness, hypovolemic shock, anemia, and acute renal disaster are some of the symptoms that can happen. Problems of prolonged therapy in Nephrotic kids are usually related to CS, alkylating drugs, calcineurin inhibitor also mycophenolate mofetil (MMF)**(Park and Shin, 2011).**

## 2.1.8. Hyper urine albumin

In patients with Nephrotic Syndrome, enlarged urine loss of proteins also protein-bound molecules pay to a range of complications. The decrease of protein and thyroid bound globulin may lower total $T_3$and thyroxine and thyroxine binding capacity. Overt hypothyroidism, on the other hand, is rare due to a surge in thyroid follicle stimulating (TSH)**(Shantha *et al.*, 2011).**

## 2.1.9 .Edema

Nephrotic syndrome (NS) is defined by edema, a urine protein/creatinine ratio with less as 2 mg/mg, and hypoalbuminemia of less than 2.5 g/dl, as per the nephron **(Pal and Kaskel, 2016).** Edema is a disease which the fluid accumulated improperly in the extracellular compartment of the body's tissues. This condition is commonly seen in dependent parts as legs in nephrotic syndrome, but could also cause significant accretion in other places, lead to pulmonic congestion, ascites or anasarca **(Kallash and Mahan, 2021).**

### 2.1.9.1.Mechanisms of edema

Various mechanisms planned to drive the formation of Edema in a variety of disorders, including:

First: Oncotic pressure within vessels is reduced (hypoalbuminemia as in NS, other causes of low serum albumin and other proteins) Hydrostatic pressure is increased (kidney insufficiency, heart failure).

Second: Increased tissue oncotic pressure causes high blood vessel wall permeability (capillary escape condition as inflammation and anaphylaxis). Swelling can cause significant morbidity and lower quality of life in kids with

active Nephrotic Syndrome due to obstruction of the lymph system (masses, lymphangiomatosis).

The pathophysiology for edema in this syndrome is complex, and identifying underfill , overcrowd mechanisms in exaggerated children may aid in finding the optimum medical help. While many diuretics are now available to treat edema in children NS, there are rare high-quality clinical hearings also reports to guide us. Even clear knowledge of the best ways to use albumin infusions in the care of NS in children was focused on a limited body of evidence. Some exciting novel abscess agents are currently being built**(Kallash and Mahan, 2021)**

## 2.1.10. Nephrotic Syndrome and 25-hydroxyVitamin D.

Due to a decrease of Vitamin D compulsory protein in the urine, less blood ionized calcium levels can arise due of lower free serum calcitriol so perchance lead to secondary hyperparathyroidism, and in rare cases, severe problems such osteocalcin and osteitis fibrosis . When vitamin D deficiency is detected, cholecalciferol as well as ergocalciferol supplementation could be begin. Thromboembolic events are more common among patients with nephrotic syndrome **(Mahmoodi *et al.*, 2008).**

Elevated procoagulants, such as fibrin, von Willebrand factor, and plasmin activity factor-1, and reduced anticoagulants, such as antithrombin, plasminogen, and protein S, are associated to such events**(Singhal and Brimble, 2006).**

## 2.1.11. Thromboembolism

Thrombotic events are known to be more prevalent in children with hereditary nephrotic syndrome and membranous nephropathy**(Kerlin *et al.*, 2009)**.In this case, the role of preventive anticoagulation is still being disputed . Some have advised primary pharmacologic prophylaxis with correctly dosed low-molecular weight heparin for Nephrotic patients at the highest risk of thromboembolic events,or the other anticoagulant .Patients with additional thrombotic risk variables, such as surgery, cancer, or pregnant, as well as those who had a prior thromboembolism or have membranous nephropathy, should be examined. **(Cadnapaphornchai *et al.*, 2014).**

## 2.1.12.Hyperlipidemia:

Hyperlipidemia is among the common features of nephrotic syndrome, per a study performed in Iraq. On the edge of therapy in NS patients, the levels of cholesterol in blood returns to normal. Furthermore, maintaining a healthy lipid levels in the body is vital for overall health . Somewhat irregularities in lipid amounts, such those seen in relapse, cause atheromatous cardiovascular disease, blood clot embolic problems, and Atherosclerotic vascular disease, thrombus embolic issues, and lipid accumulation in glomeruli and renal tubule epithelial cells are all caused by fat accumulation inside nephrons and proximal tubular epithelium. As a result of these negative consequences, renal failure occurs **(Al-Bahrani, 2017).**

Hyperlipidemia is normally transitory in persons by steroid-sensitive Nephrotic Syndrome and has no extensive-term implications. Though, in patients with steroid resistance nephrotic syndrome (SRNS), elevated lipid levels in the blood may persist, contributing to cardiovascular mortality and

glomerulosclerosis development Patients are encouraged to maintain weight for their height, and saturated fats should be limited in their diet. While there are no specific standards for the use of statins, there are a few things to keep in mind, children it has been shown that they are effective and safe in the short run . Simvastatin and atorvastatin both reduce total & low density lipoprotein (LDL), cholesterol, and triglycerides while slightly increasing HDL cholesterol **(Bagga and Mantan, 2005).**

## 2.1.13.Anemia

Patients with NS may also lose transferrin, erythropoietin, ceruloplasmin-min, iron, and trace minerals in the urine, in addition to Albumin also Vitamin D binding protein **(Hampson *et al.*, 2021).**

Although the cause of these shortages is unclear, there are more common in those who have therapy-resistant conditions and can lead to anemic that persists despite appropriate iron and erythropoietin supplements. The fall in transferrin level in the urine of NS kids has been well documented. Iron stays attached to transferrin in the alkaline environment of the urine, enabling both to be excreted **(Iorember and Aviles, 2017)** .Decreased erythropoietin amounts also found in kids with NS, spite of the fact that iron loss alone can create Anemia. For these patients, iron replacement treatment is required to correct iron deficiency anemia, and individuals with poor erythropoietin levels should get erythropoietin **(Iorember and Aviles, 2017).**

These treatments should raise iron and erythropoietin levels, but not transferrin until the proteinuria is resolved**. (Hampson *et al.*, 2021).**

## 2.2. Infections

Infections are more common in patients with NS. Infections with NS takes reduced in advanced nation state, although they continued to be a major issue in developing nation states. One of the top sources of mortality in kids had NS is sepsis.

kids who are given cytotoxic medicines have a bigger rate of clinical infections than rather than who are treated just prednisolone. Streptococcus pneumoniae is the most common pathogen in primary peritonitis in children with NSSimvastatin and atorvastatin both reduce total & low density lipoprotein (LDL), cholesterol, and triglycerides while growing slowly HDL cholesterol. Fluid collection in cavities and edema drop of local humoral defenses, as well as immunological factors such as low serum immunoglobulin G level, factor B & factor I in alternate route components, transferrin, and low T-cell function, may all contribute to Nephrotic patients' susceptibility to infections **(Park and Shin, 2011).**

Reasons of an Increased Infection Risk despite the fact that death rates have dropped to 3%, infection continues to be a problem. Lipid abnormalities management is a major cause of deaths in kids with Idiopathic nephrotic syndrome, bacteremia, urinary tract infection and also peritonitis. In Tuberculosis, lipid irregularities and morbidity are public, as is cellulitis. In an Australian study of 92 Nephrotic Syndrome kids with INS, eight (8.7%) industrialized bacterial infections through the first year of their diagnosis; those with high levels of very low density lipoprotein (VLDL)-coltsfoot had primary peritonitis . Sterol, triglycerides, and low - density lipoproteins (LDL),cholesterol .Urinary wounded of Ig, diminished opsonization of bacteria come from urine wounded of complement elements B and D, skin

breakdown associated with acute edema, and immunosuppressive therapy, those also factors, increase susceptibility to infection **(Hodson, 2003).**

## 2.2.1.Bacterial infection

## 2.2.1.The Overview of Bacterial Infections

Bacteria are prokaryotic single-celled bacteria that may be found practically wherever on Earth. They didn't have a nucleus that was attached to the membrane. Bacteria have traditionally been classed based on their morphologies (e.g., spheres, rods, and spirals) and their ability to take up color stains (e.g., the Gram stain) that define bacterial cell wall characteristics under a light microscope.

When bacteria are refined, their nutrient needs also chemical metabolic features is used to determine them further. Despite genotypic classification, which includes 16s ribosomal. Even if RNA sequencing and whole-genome sequencing allow us to more accurately group genus, identify various species and sub-species, and in some instances alter genera classification, nomenclature based on pigment staining properties is still helpful in clinical repetition. The ability of many popular antibiotics, such penicillin, to pierce bacterial cell wall and goal the synthesis of bacterial cell wall components is critical to their effectiveness**(Berkley, 2021).**

Bacteremia mentions to the expression of alive bacteria. Asymptomatic bacteremia can arise after normal lives such as washing one's mouth and after modest medicinal procedures. When the immune system's response mechanisms fail or become overwhelmed, bacteremia progresses to septicemia, a bloodstream infection, which can cause a variety of clinical

symptoms. Symptoms of untreated and clinically common bacteremia are chronic inflammatory reaction , sepsis, septic shock, and multi-organ failure syndrome **(Smith and Nehring, 2020).**

All bacterial infections depend on the host's immune system, which is affected by their genetic profile as well as acquired and genetic defects . Active germs in the circulating blood are filtered by hepatic and spleen, while cell adaptive and innate and adaptive immune responses are essential for first microbe clearance. Bacteria then begin to colonize their first source of site in their most basic form. The bacteria might be transient and clinically unimportant at this point, or they may escape the cellular response, multiply, and develop a resident infection which can travel to other body regions. The infection may clear up on its own or progress to septicemia if the bacteria still are living and infect the bloodstream. The skin and mucosal surfaces are first line defense in bacterial invasion. Medical treatments that pass through the skin, and also anatomical lumina, commonly interfere with these normal defense barriers. Trauma, wounds, ulcers, and the normal aging process can all help to the breakdown of defense**(Smith and Nehring, 2020).**

## 2.2.2. Bacterial Infections Associated with Nephrotic Syndrome

Infections are a frequent and sometimes fatal adverse effect of NS. The increased risk of infection is multifaceted, with immunoglobulin deficiency, complement components in urine, mechanical factors such as edema and ascites, & immunosuppressive therapy all playing a role. Vaccination and antibiotic prophylaxis are two strategies for limiting serious infectious diseases (SBIs) in children with NS. While the former has a lot of support, the latter is a bit more controversial. Furthermore, there is no consensus on how to treat a

febrile nephrotic child, and there are currently no well established guidelines for identifying and treating febrile nephrotic children. The bulk of published studies on infectious problems in children with NS have focused on the kinds or outcomes of infections. Only a few studies investigated the risk factors for SBI, and there was a lot of variation in infection frequency. Also, none of the research examined into the efficacy of infection prevention strategies**(Lebel et al., 2020).**

### 2.2.2.1.Septicemia

Fever with systemic chronic symptoms, prostration, or lethargy, and a harmful bacterium grown in clinical specimens, with or without evidence of organ failure. Septicemia, along with peritonitis and pneumonia, is a leading source for death in children had nephrotic syndrome, but it is infrequently reported. In this study, it accounted for a significant portion of infections (16.7 %)**(Krishnan *et al.*, 2017).** Similar results were found in other studies by **(Chang-Ching wei *et al*).** The pathogens that causing septicemia in study, MRSA and CONS, were not the most frequently reported pathogens of nephrotic syndrome**(Krishnan *et al.*, 2017).**

### 2.2.2.2. Spontaneous bacterial peritonitis (SBP)

Abdominal pain, tenderness, distension, diarrhea, or vomiting, as well as an ascitic study with >100 leukocytes/$mm^3$ and at least 50% neutrophils &/or a positive culture. With a 2-6 percent incidence, spontaneous bacterial cystitis is a serious complication of nephrotic syndrome, and overwhelming infection has a 15% mortality risk. It usually occurs within first two years of a nephrotic syndrome diagnosis**(Hingorani *et al.*, 2002).**

Peritonitis is caused by low serum albumin, ascites, and a weak immune system, and it is often multifactorial in origin. Encapsulated gram-positive organisms, primarily Streptococcus pneumoniae, cause the majority of peritonitis, but gram-negative organisms also can cause it**(Uncu *et al.*, 2010).**

In one study, both pneumococcal strains were penicillin resistant**(Krishnan *et al.*, 2017)**The *E coli* isolate was multidrug resistant.

## 2.2.3. Bacterial Resistance

Resistance to existing antibiotics in bacterial infections is currently a global concern, as the number of strains resistant to many classes of antibiotics has grown considerably each year and spread worldwide. To solve this issue, a 'antibiotic adjuvant' in conjunction with an antibiotic is currently being used **(González-Bello, 2017).**

Over the last few decades, dangerous, antibiotic-resistant bacteria have become progressively public. **(Fair and Tor, 2014).**

There are several harmful Gram-positive and Gram-negative bacteria species present .Methicillin (MRSA) resistance with vancomycin (VRSA) resistance Gram-positive bacteria became a significant danger in the 1990s **(Fair and Tor, 2014).**

Antimicrobial resistance has been found all over the world is one of most significant threats to global community health today, and the situation has gotten worse. It is now well accepted that the misuse and overuse of antimicrobials have played a significant role in the progress of antimicrobial resistant (AMR) in bacteria that harm humans and animals in recent decades for a variety of reasons. **(O'Neill, 2015).** While AMR can arise naturally as

microbes adapt to their surroundings, it is exacerbated while antimicrobials are used incorrectly or excessively . Lack of regulation, poor treatment adherence, non-therapeutic usage, and over-the-counter selling, as well as poor farming, hygiene, and agriculture waste management, all have contributed to this. Antimicrobial resistance patterns are constantly changing, and multi drug resistance (MDR) organisms develop progressive antimicrobial resistance ; as a result, to have speed data about antimicrobial susceptibility profiles is critical for ensuring reliable and effective empiric therapies**(Abass *et al.*, 2017).**

The fact that genes conferring resistance were carried in plasmids or other highly genetic elements which are independently replicated and passed across bacterial cells and species is one of the main reasons for the rapid spread of AMR among bacterial populations. Clinically significant resistance often develops in months to years after a newly discovered antimicrobial agent is proven to be effective and approved for therapeutic use **(Elshamy and Aboshanab, 2020).**

Antimicrobial Resistance is really a worldwide thing. Antibiotic-resistant bacteria are responsible for an estimated 23,000 fatalities in the U.S. each year as well as 25,000 deaths in the European Union Antibiotic use is rising in low- and middle-income countries, owing to rising incomes, reduced antibiotic costs, a lack of antimicrobial usage management in hospitals, and over-the-counter drug sales, among other factors these conditions are thought to be promoting the growth and spread of germs resistant to multiple antibiotics in countries such as China, India, Indonesia, & Thailand. However, a lack of information makes assessing the scale of the problem and then monitoring how antibiotic and multidrug resistant evolves with these and similar low- and middle-income countries difficult**(Lim *et al.*, 2016).**

Gram-positive bacteria are still a major cause of death in the United States, accounting for the vast majority of bacterial-related deaths. **(Roder and Thomson, 2015)** According the European Disease Surveillance Network, resistance rates for numerous of them, such MRSA and VRE, have stabilized or decreased in recent years (EARS-Net)**(Shallcross and Davies, 2014)**

In some respects, the new resistant gram-negative infections are much more concerning since their outer membranes are more difficult to penetrate and efflux pumps are more plentiful, making them theoretically resistant to numerous medicines. Carbapenem-resistant $G^-$ strains, mainly bacteria related to a family Enterobacteriaceae (CRE), are more common. Multi drug resistance (MDR) and involves several components *Acinetobacter*, *Klebsiella pneumoniae* ,*Pseudomonas aeruginosa*, *Escherichia coli*, *Enterobacter*, & most newly, *Neisseria gonorrhoeae* are the main gram-negative threats**(Kumarasamy *et al*., 2010).**

## 2.2.4.Hospital Acquired Infections (HAIs)

Bacteria cause approximately 90% of all HAIs **(Sievert *et al.*, 2013).** HAIs are critical problematic in the industrial world, taking incidence rates of 5% and 7.1 % in the US and EU, respectively. The problem is much worse in developing states, where sterilized applies are less severe, with an estimated incidence at 15.5 percent**(Allegranzi *et al*., 2011).**

Furthermore, the patients who are often immunocompromised and are the target of these infections have higher rates of death than those who have a healthy immune system. Infections caused by resistant bacteria have a much higher risk of fatality as infections caused by antibiotic-sensitive bacteria In the majority of instances, this is due to prolonged bacterial contact as a result

of delayed treatment or an absence of a suitable therapy, instead of increased virulence **(Paphitou, 2013).**

## 2.2.5. Antibiotics Sensitivity

Antibiotic susceptibility testing (AST) often is performed in a laboratory**(Giuliano *et al*., 2019).** A susceptibility test requires administering antibiotics to microorganisms and seeing how they react (phenotypic testing). Methods employed can be qualitative, such as identifying whether or not resistance exists, or quantitative, such as determining the concentration of antibiotics with which a bacteria is sensitive using a minimum inhibitory concentration (MIC)**(van Belkum *et al*., 2019).**

## 2.2.6. Phenotypic methods

Agar plates or dilution in broth or agar are utilized in studies that require bacteria to be treated to antibiotics **(Pulido *et al*., 2013)**. Antibiotics could be chosen based on the microorganism be cultured & the antibiotic available in the area**(Reller *et al*., 2009).** To guarantee reliable findings, the amount of bacteria added to the agar or broth (the inoculum) should be constant.The turbidity of bacteria distributed in saline or broth is measured to the turbidity of a McFarland standard solution with the same turbidity as a solution containing only a specified concentration of bacteria . The inoculum is added to the growth media till it reaches the required concentration, which can be determined visually or by photometry **(Hombach *et al*., 2015)**. Choosing a bacteria type, putting it on an agar plate, and observing the bacteria to grow around antibiotic-impregnated discs is the disc diffusion method **(Syal *et al*., 2017).** While altered methods are also utilized , the Kirby-Bauer method is the most common. On a plate where bacteria are growing, small paper discs containing antibiotics are put. A clear ring, or zone of inhibition, is seen around

the disk if the antibiotic inhibits microbial growth. By measuring the diameter of the inhibition zones to defined thresholds that correlate to MICs, the size of the zone of inhibition may be calculated, bacteria are classified as sensitive, intermediate, or resistant to an antibiotic. Mueller-Hinton agar is frequently used in this antibiotic susceptibility test**(Jorgensen and Turnidge, 2015)**. Here are standards for conducting the tests and interpreting the results**(Reller *et al.*, 2009)**. Values for agar kind and depth, incubation conditions, and practice of analysis are established by European Committee on Antimicrobial Susceptibility Testing (EUCAST)**(Hombach *et al.*, 2015)**. The simplest and least costly way for determining susceptibility is disc diffusion; however, the major disadvantage of this method is that it requires human processing and lacks automation. Moreover, this process produces only qualitative instead of quantitative outcomes**(Behera *et al.*, 2019).**

Furthermore, disk diffusion is used to study AST in a variety of ways. Khatoon *et al.* used disk diffusion to study AST using silver nanoparticles (AgNPs) instead of conventional drugs**(Khatoon *et al.*, 2017)**.

## 2.2.7. Causes of Antibiotics Resistance

## 2.2.7.1 Natural resistant

Antibiotic resistance (ABR) is the ability of a microorganism to resist antibiotic contact, which would usually kill or halt its growth **(Li and Webster, 2018)**. To mention a few, the degree of resistance expression and the microorganism's capacity to withstand resistance mechanisms, as well as mutations in chromosome (cross-resistance) or The formation of an antimicrobial-resistant phenotype is influenced by gene transfer among bacteria through plasmids, transposons, integrons, and bacteriophages.

Microorganisms that are resistant to antibiotics. Resistance factors are present on plasmids, thus they rapidly spread **(Giedraitienė *et al.*, 2011).**

Bacteria can use a number of biochemical resistance mechanisms to protect themselves versus various agents, the most important of which include enzyme degradation, target change, reduced absorption, and active efflux protein overexpression **(Gajdács, 2019)**. Despite the fact that it has been a common occurrence in clinical practice since the introduction of first-generation antibiotics **(Monserrat-Martinez *et al.*, 2019)**.

Because of the exponential growth and spread of resistance, and the lack of new medicines to address it, ABR is now regarded a global public health crisis **(O'Neill, 2016)**.

## 2.2.7.2. Antibiotic resistance mechanisms

### 2.2.7.2.1.The modifications of target site

Drug-related receptor changes plus the location of the antibiotic relationship's target areas varies, and enzymes and ribosomes may be implicated**(Prashanth *et al.*, 2012)**. The most common kind of resistance associated with ribosomal target changes is resistance to macrolide antibiotics **(Shaikh *et al.*, 2007).** Penicillin resistance have evolved in strains of *Neisseria meningitides* , *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Enterococcus faecalis* due to mutations in penicillin-binding proteins beta-lactamase enzymes**(Southon *et al.*, 2020).**

### 2.2.7.2.2.Enzymatic Inactivation of Antibiotics

Antibiotic degradation enzymes are produced by a majority of bacteria, and enzymatic deactivation is among the most active antibiotic resistant mechanisms **(Pérez-Llarena and Bou, 2016)**. The most common examples are beta-lactamases, amino glycosidase, chloramphenicol, as well as erythromycin modifying enzymes **(Suleiman *et al.*, 2020)** .

#### 2.2.7.2.3. Reduced Permeability of the Inner and Outer Membranes

Changes in the permeability of the internal and external membranes result in reduced drug absorption into the cell or fast expulsion from the pump systems **(Santajit and Indrawattana, 2016)**. A reduction of outer membrane permeability can also cause porin mutations in resistant strain proteins, like Quinolone resistance , aminoglycoside resistance **(Li *et al*., 2012).**

#### 2.2.7.2.4.Active Pumps System

Among antibiotics in the tetracycline group, the active pump mechanism is the most common source of resistance. Tetracyclines are scared out and cannot focus inside the cell in an energy-dependent active pumping mechanism. **(Li et al., 2020)**. This mechanism of resistance is regulated by plasmids and chromosomes. Quinolones, 14-membered macrolides, chloramphenicol, and beta-lactams are all resistant to active pumping systems **(Guo et al., 2020b)**.

#### 2.2.7.2.5. Using a Different Metabolic Route

Unlike some of the target alterations within bacteria, the most recent drug-susceptible pathway does not need impartial progress**(Fatahi-Bafghi, 2019).** Bacteria may prepare Folic acid from the environment instead of synthesize it, making it resistance against trimethoprim and sulfonamide **(Tan *et al.*, 2020)**. Resistance mechanisms in clinical infections reflect a major issue in the treatment of pathogenic microbes. Infections are caused by fungi and bacteria are becoming more well recognized as major causes of disease and mortality**(Abdu-Allah and Mussa, 2015).**

## 2.2.8. Bacteria associated with Nosocomial infections

### *2.2.8.1.Acinetobacter baumannii*

When Acinetobacter spp. infections were first discovered in the 1970s, this pathogen was thought to be a benign commensal opportunist with very little clinical importance. These bacteria have become important nosocomial infections. Acinetobacter infections are estimated to infect 45 000 persons in United States and 1 million people global each year **(Wong *et al.*, 2017).***Acinetobacter baumannii* is a multidrug-resistant, opportunistic, nosocomial pathogen for which a new line of treatments is desperately needed **(Ahmad *et al.*, 2022).** It is the most medically significant of over 60 bacterium species, including *Acinetobacter baumannii*, *Acinetobacter nosocomialis*, and *Acinetobacter pittii*, as well as *Acinetobacter calcoaceticus*, an environmental species. Because they are difficult to detect using traditional identification methods, these species have been grouped together as the *Acinetobacter calcoaceticus* or *Acinetobacter baumannii* (Acb) complex **(Peleg *et al.*, 2008).***Acinetobacter baumannii* is a common opportunistic bacteria seen in hospitals, particularly in intensive care. The emergence and rapid spread of multidrug-resistant *A. baumannii* strains have become a major public health concern throughout the world, limiting the pathogen's treatment options**(Alsaadi, 2014)**.

For all antimicrobial agents, significant chronological reductions in susceptibility rates among Acb complex isolates were seen in all regions. The highest prevalence of widely drug resistant *A.baumannii* complex was found in Europe (66.4%), followed by Latin America (61.5%), Asia-Pacific (56.9%), and North America (38.8%)**(Gales *et al.*, 2019).**

Gram-negative, strictly aerobic, nonfermenting, non - fastidious, nonmotile, catalase-positive, oxidase-negative bacteria with a DNA G+C content of 39 percent to 47 % make up the genus Acinetobacter**(Peleg *et al.*, 2008).**

The genus Acinetobacter contains a wide range of bacteria that can be found in soil, surface water, vegetables, animals, and humans**(Zordan *et al.*, 2011)** . *A.baumannii's* natural reservoirs are unclear, and the species has been isolated mainly from the hospital environment . Although Acinetobacter spp. may invade healthy people's skin and respiratory tract, *A. baumannii* infection are uncommon**(Wong *et al.*, 2017)**.

*A. baumannii* may persist inside the nosocomial environment, being transmitted by the hands of health care workers and/or contaminated medical equipment because of its ability to thrive on very little food and resist desiccation **(Peleg *et al.*, 2008).** Although the mortality directly related to A. baumannii infections is debatable, these infections had crude death rates ranging from 26.0 to 61.6 percent, with insufficient empirical treatment playing a significant role in these high rates **(Kwon *et al.*, 2007).** *A. baumannii* has gained genes that make it resistant to almost all antibiotics that treat Gram negative bacteria , like cephalosporins, aminoglycosides , fluoroquinolones, and is intrinsically resistant to penicillin**(Wong *et al.*, 2017).**Almost all antibiotics, including beta-lactams, carbapenems, tigecycline, and now colistin, a last-resort antibiotic, have developed resistance in *A. baumannii* .The world is on the verge of entering the post-antibiotic age, and the emergence of multi-, extreme-, and pan-drug-resistant *A. baumannii* strains is an obvious forerunner**(Singh *et al.*, 2022).**

## 2.2.8.1.1.Ribosome structure

The antibiotics amikacin and tigecycline, which target ribosomes, are among the few options for treatment such infections. Cryoelectronic microscopy

revealed high-resolution structures of the 70S ribosome from *A. baumannii* in complex with these antibiotics. When ribosomes from other bacteria are compared, they reveal several unique structural features at functionally important locations, such as the exit of the polypeptide tunnel and the periphery of the subunit interface. The structures also reveal how and where these drugs interact with the ribosome **(Nicholson *et al.*, 2020)**.

### 2.2.8.2. *Staphylococcus warneri*

Staphylococci other than *Staphylococcus aureus* (SOSA traditionally referred to as coagulase-negative staphylococci, but not all are technically coagulase negative) are standard microbiota of the human skin and mucosal surfaces and important opportunistic pathogens, mostly associated with infection in the setting of indwelling/implanted foreign bodies and devices **(Humphries et al., 2020).**Bacteria with a Gram positive *Staphylococcus warneri* is a common type commensal that colonizes human and animal skin & mucosal membranes as part of the normal flora. It belongs to the Bacillus's order and is a facultative anaerobe, nonmotile, coagulase-negative staphylococcus (CNS). *S. warneri* has been discovered as an opportunistic etiological agent in immunocompromised individuals with bacteremia, sepsis with multiple abscesses, orthopedic infections, spinal osteomyelitis & ventricular shunt infections in clinical reports over the last three decades**(Liu *et al.*, 2020).**Virulence factors of this bacteria , like other pathogenic staphylococci, has been proposed to be multifactorial, including adhesins, exoenzymes, capsule, iron absorption routes, and virulence regulators all implicated. A recent study on the pathogenesis of *S. warneri* infections found that isolates from blood samples are able of sticking to epithelial cells and creating biofilms with several activated antibiotic resistance genes inside **(Liu *et al.*, 2020).** In

those who do not have risk factors, such as endovascular prosthetic devices or catheters, *Staphylococcus warneri* rarely causes infection **(Ivić *et al.*, 2013).**

### 2.2.8.3. *Bacillus cereus*

*Bacillus cereus* is a gram positive rod shape that is ubiquitous, facultatively anaerobic, spore-forming, and spore-forming. *B. cereus* is widely disturbed in nature and contaminates all agricultural products; it has also been isolated from animal hair, cereal crops, dust, vegetation, fresh water, and sediments, although being isolated from fish in several cases. Motile bacteria, which can be found in plant material, hay, raw and processed food, and pasteurized milk, cause spoilage via releasing lipases and proteases**(Jessim *et al.*, 2017).**

Bacillus cereus is most commonly associated with food poisoning and severe gastrointestinal infections. The combined effects of a number of virulence factors that enhance intestinal cell destruction and/or resistance to the host immune system are mainly responsible for this microorganism's intestinal and non-intestinal pathogenicity. The various emetic toxin, which effects may overlap in the development of human disease.(Saeed et al.)2019.

Because of its ability to cause food spoiling and infection by producing numerous toxins, it has a strong interest in food safety and public health**(Al-Jobory and Abdulaal, 2020).** Blood infections, also known as sepsis, can be produced by Bacillus cereus. Sepsis affects over 18 million individuals worldwide each year, with a mortality rate of 28-50 percent. As a reason, it is regarded as a major global issue. When septic patients are diagnosed early in the disease, their chances of survival improve dramatically even when a person with sepsis begins to show a clinical response, the concentration of bacteria current in the blood ranges from 1 to 100 colony forming units (CFU)/mL for

adults and 10 CFU/mL for newborns, making early detection challenging**(Park *et al.*, 2018).**

*B. cereus* induces local and systemic infections in addition to food poisoning. Septicemia, endophthalmitis, pneumonia, endocarditis, meningitis, and encephalitis are most commonly described conditions, especially in immunocompromised individuals such as neonates, with about 10% of cases resulting in patient death. In addition, several cases of fulminant anthrax-like infections affecting healthy people have been reported Intravenous drug use, surgical or traumatic wounds, intravascular catheters, and prematurity due to an immature immune response and the presence of indwelling devices in the intensive care environment of newborns are all predisposing factors**(Glasset *et al.*, 2018)**. Immunosuppressed people, patients receiving continuous intravenous medication, patients with underlying malignancies, and neonates have all been found to have *Bacillus cereus* BSIs. Antimicrobial use for more than 3 days during the 3-month period prior to *B. cereus* isolation was significantly higher in the BSI group compared to the contaminated blood culture group in these study **(Horii *et al.*, 2011).**

## 2.3.Cefitraxione

Ceftriaxone (CTX) is a broad-spectrum cephalosporin with strong antibacterial action against Gram-negative and Gram-positive bacteria. It falls inside the biopharmaceutical classification system's class III. It has a high CNS penetration and a good safety record in neonates. CTX evicts far more slowly than penicillin and other third-generation cephalosporins, allowing for a single daily dose**(Maghrabia *et al.*, 2021).**

Its widespread use is most likely due to its effectiveness in susceptible organisms in difficult and uncomplicated urinary tract infections, skin, respiratory tract infections, bone and joint infections, , soft tissue, and bacteremia/septicemia**(Petri, 2011)** .

Ceftriaxone vials are one of the most often used antibiotics, with the highest mortality rate from vial injection. Diarrhea, elevated liver enzymes, blood urea nitrogen, eosinophilia, thrombocytosis, and other local reactions are also common adverse effects of ceftriaxone. Given the inevitability of using this antibiotic in today's health-care system, developing new prodrugs is essential**(Ebrahimi *et al.*, 2020).**

However, the unavoidable consequence of widespread antimicrobial use with lengthy period of usage, and the administration of suboptimal quantities and duration of hospital stay, have all donated to the formation also spread of antimicrobic resistance**(Gururaja, 2013).**

Antibiotic Resistance is a main factor to enlarged patient and also mortality, as well as increased medical costs. It is named, for instance, in effort of **(Lee *et al.*, 2009),** antimicrobial drug resistance is projected to increase healthcare

costs by 100$ million to 30 $billion each year. According this, the misuse of ceftriaxone result in an annual cost of 4-5 million dollars due to infections with antibiotic-resistant microorganisms in all over the world. **(Ayinalem *et al.*, 2013).**

## 2.4.1. Diagnosis of Bloodstream Infections in Children

Bloodstream infection identification is one of the most important tasks achieved by clinical microbiology labs. While the criteria for obtaining an appropriate volume of blood culture material are clearly defined in adults, there is considerable uncertainty in pediatric populations **(Dien Bard and McElvania TeKippe, 2016).**

Blood cultures are still the gold standard of diagnosing bloodstream infections (BSIs) in newborns and children in the lab. The recovery of a pathogen is beneficial so it validates the diagnosis of bacteremia & allows the organism to be diagnosed and then tested for susceptibility, so allowing for the most effective antimicrobial therapy and duration. A negative blood culture is just as important, as it eliminates the possibility of bacteremia and allows for the further investigation of other infectious or noninfectious causes, as well as the termination of needless experiential antimicrobial treatment . The pathogens that cause pediatric Blood Sever Infection BSI vary greatly depend on the child's age, presenting symptoms, and immune status **(Dien and Mcelvania, 2016).**

## 2.4.2.Blood Culture Collection

The timing for blood collection, the number of groups collected, and volume of blood all are factors that could affect pathogen recovery**(Isaacman *et al.*,**

**1996).** Numbers of studies have supported the observation that the quantity of blood cultured is by far the most important factor in increasing pathogen recovery rates from blood**.** It is because there is a rich association between both the amount of blood cultured and microbial recovery rates. Microbial recovery rates for children also increase as the quantity of blood cultured rises. Volume is still important for younger children, especially newborns, but the amount of blood that can be taken for culture is limited by body mass and total blood volume. The overall volume of blood drawn from these individuals must not exceed 1% of the total body weight**(Wilson, 2020).**

In recent years, the advance of a variety of rapid diagnostic tools has changed how BSIs are identified in all patient populations. The BacT/ALERT® Bacterial Detection Method (bioMérieux, Marcy l'Etoile, France) was used to screen bacteria found in the blood cultures. While VITEK® 2 system (bioMérieux) has been used to identify and test bacteria grown in conventional aerobic blood culture bottles for antimicrobial susceptibility. Both VITEK 2 ID & AST systems, respectively, gave results in 3 & 2.5- 16.25 hours. **(Chen *et al.*, 2008).**

Peptide Nucleic Acid Fluorescent In Situ Hybridization (PNA FISH), FilmArray, Blood Culture Identifying Ianel (BCID), and Verigene Gram-Positive Blood Culture (BC-GP) & Gram-Negative Blood Culture (BC-GN) panels are also FDA-approved for in vitro diagnostic usage on positive blood cultures. As well as use of matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) **(Vickers, 2017).**

Available commercially molecular platforms can uncover pathogen identification and gene resistance markers in a matter of a few hours, providing preliminary data **(Höring *et al*., 2019).**

## 2.4.3. Polymerase Chain Reaction (PCR)Technique

The polymerase chain reaction (PCR) is a technique that is widely used in basic and biomedical sciences. PCR is a laboratory method used to amplify particular DNA segments for scientific and/or clinical purposes. Mullis and coworkers developed PCR in the early 1980s, expanding on Panet and Khorana's successful in-vitro DNA amplification, and were given the Nobel Prize a decade later. It is useful in a number of applications, include gene cloning, infectious disease diagnostics, and prenatal newborn screening for harmful genetic disorders, since it allows for billion-fold replication of specified target areas**(Ghannam and Varacallo, 2018).**The digital polymerase chain reaction (dPCR) is a new method for quantifying target nucleic acids in absolute terms. The random distribution of molecules in many partitions follows a Poisson distribution, which makes dPCR quantification possible . Fluorescence detects amplified target sequences in partitions, and each partition functions as an independent PCR microreactor. The fraction of PCR-positive divisions is sufficient to determine the concentration of the target sequence without the requirement for calibration. Microfluidics advances enabled the current digital quantity revolution with providing effective partition methods **(Quan *et al.*, 2018)**.

Its sensitivity is based on enzyme-based amplification, and its specificity is based on sequence hybridization. A sequence of temperature cycles is typically repeated 20 to 40 times in PCR .Each cycle begins with the denaturation of

DNA duplexes, followed by the hybridization of two DNA primers with a DNA Polymerase. Each cycle separately doubles the number of target DNA molecules (Exponential Amplification), and then after n cycles, 2n copies might theoretically be created . In practice, once the PCR reagents are depleted and the accumulated PCR products self-anneal, the amplification procedure permeates & reaches a plateau, inhibiting further amplification. In conventional PCR, gel electrophoresis (end-point analysis) is used to analyze amplified product at the conclusion of the process **(Quan *et al.*, 2018).**

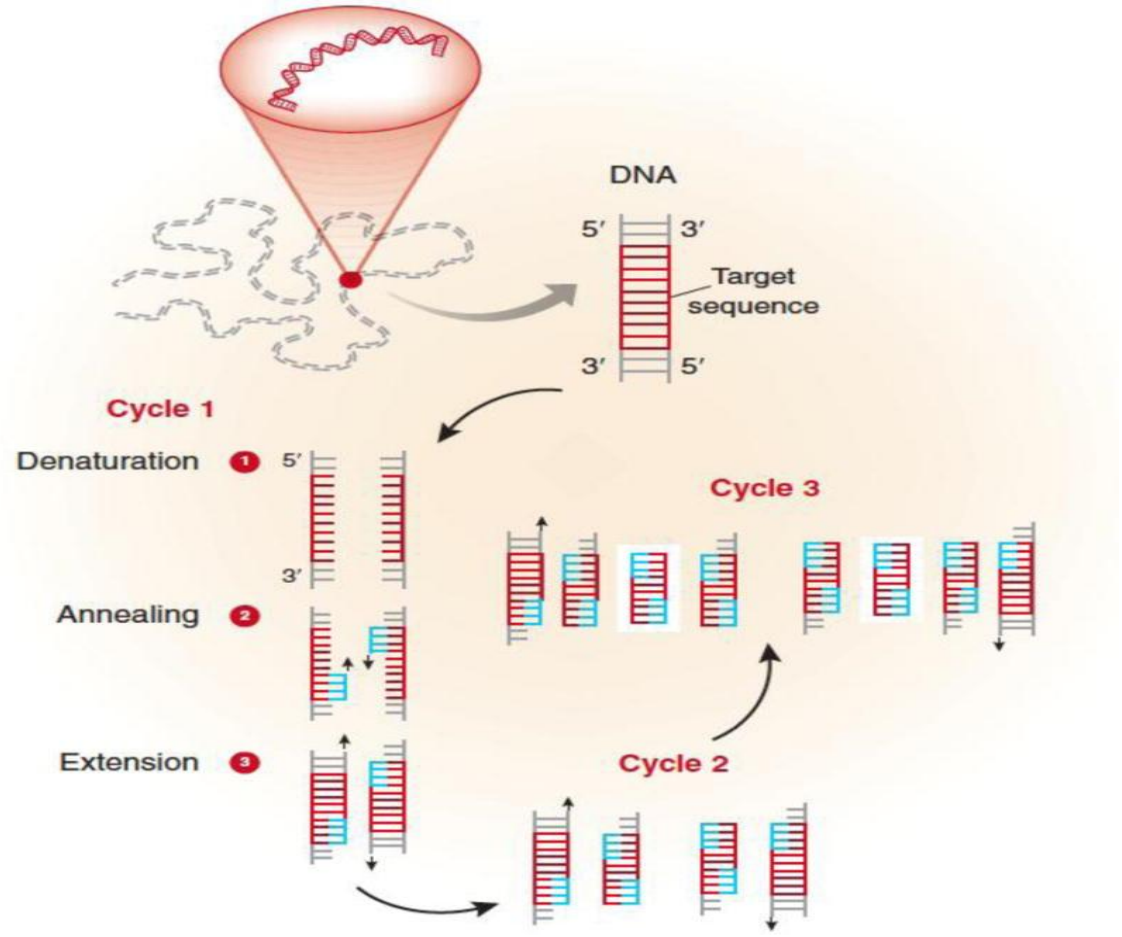



**Figure (2-3): Schematic presentation of PCR principle (Garibyan and Avashia, 2013)**

## 2.4.3.1.Analysis of the PCR product

One of two ways can be used to visualize the PCR products:

1- Staining the amplified product using a chemical dye that intercalates here between duplex's two strands, like ethidium bromide or

2-Prior to PCR amplification, fluorescent dyes (fluorophores) are used to label PCR primers or nucleotides .The labels can be directly incorporated in the PCR product using the latter approach **(Garibyan and Avashia, 2013).**

Agarose gel electrophoresis is the most extensively used technique for evaluating PCR products. Separate, gaging, identifying Electrophoresis on agarose or polyacrylamide gels is used to separate and purify DNA fragments. The approach is easy to apply, quick to execute, and effective of resolving DNA fragments that would be difficult to separate using other techniques, including such density gradient centrifugation. Staining using small amounts of fluorescent intercalating dye, such as ethidium bromide or SYBR Gold, can also be used to directly detect the position of DNA bands in the gel. Direct UV (ultraviolet) light examination of the gel can then reveal bands having as low as 20 pg of the double DNA. If necessary, these DNA bands can be removed from the gel**(Green and Sambrook, 2019).**

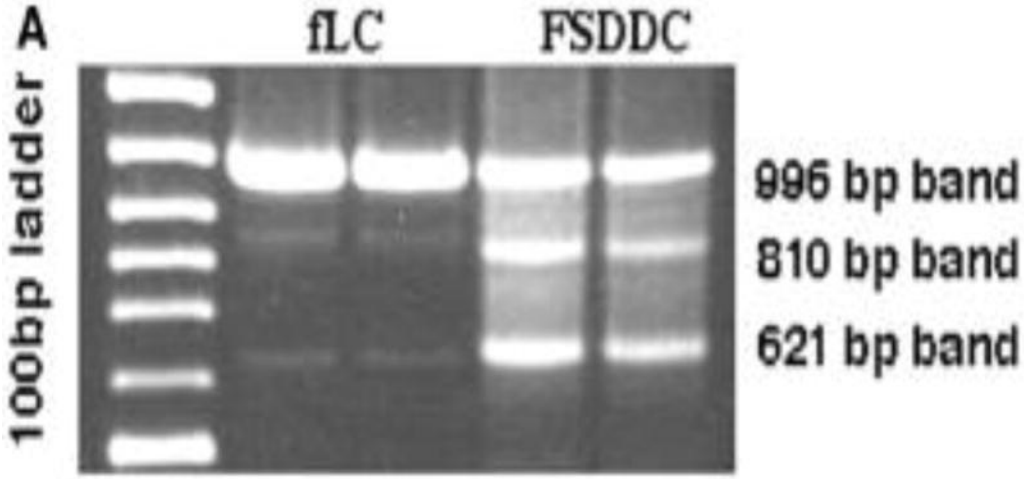


**Figure (2-4):Visualation of the PCR product on an agarose gel (Garibyan and Avashia, 2013)**

Qualitative PCR is utilized when PCR is performed to identify the presence or absence of a particular DNA product. When it comes to cloning or detecting pathogens, qualitative PCR is a good tool to utilize **(Riedl and Shi, 2004).**

The following are the two common methods for measuring the product.

1-fluorescent dyes that intercalate non-specifically with the double DNA and

2-fluorescently labeled reports that are sequence specific DNA probes.

These only allow detection after the probe has been hybridized with its complementary DNA target . Real-time PCR can be used in concert with reverse transcription to transform messenger RNA into cDNA (i.e. reverse transcription), and then the cDNA to be quantified using qPCR**(Valasek and Repa, 2005)**

End-point PCR, which includes analyzing the results of the last PCR cycle, has problems that can be overcome with measuring the desired gene during exponential amplification. The use of PCR in tumor analysis is an excellent example .It may be used to isolate tumor inhibitor or proto-oncogene DNA and amplify it. The amount of a specific gene isolated can then be determined by quantitative PCR . It, on the other hand, may be used to assess and quantify any combination of DNA, mRNAs, and proteins in single cells **(Stahlberg *et al.*, 2012).**

## 2.4.3.2. Advantages and Limitations of PCR

There are numerous benefits of using PCR. To begin with, it is a simple technique to grasp and implement, and it produces rapid results**(Bolognia *et al.*, 2008).** It is a precise technology that enables for the creation of millions to billions of copies of a single product for sequencing, cloning, & analysis. Although qRT-PCR offers the advantage of being able to quantify the synthesized product, this is also true**.** As a result, it may be used to investigate changes in gene expression in cancers, bacteria, and other diseases**.** PCR is a valuable tecqnique, it is not without problems. Since PCR is a sensitive technique, False findings can be caused by even the least amount of DNA contaminating in the sample**(Bolognia *et al*, 2008).**

As well as , some of prior sequence information is needed in order to design primers for PCR. As a reason, PCR can only assess if a pathogen and gene is found or not. Another drawback is also that PCR primers might anneal to sequences that really are similar but just not similar to the target DNA non-specifically Furthermore, at a very low rate, false nucleotides may be incorporated into the PCR sequence by the DNA polymerase **(Garibyan and Avashia, 2013).**

## 2.4.3.3. Detection Multidrug Resistant *Acinetobacter bumannii* (MDRAB) genes

Infections with *A. baumannii* are more common in intensive care unit (ICU) patients, per a growing some of reports. While studies show that *A. baumannii* causes a higher rates of nosocomial infections in both European & Asian hospitals, however *A. baumannii* isolated from Asian and European ICUs had a more widespread antibiotic resistance distribution than isolates from US ICUs **(Wang *et al.*, 2021).**

Antibiotic resistance is already on the rise globally, posing a severe threat to public health, threatens to make modern medicine ineffective in the near future. A substantial number of resistance genes were found in the genomes of sensitive & resistant *A. baumannii* strains**(Fournier *et al.*, 2006)** .

Due to the fact that the mechanism of antibacterial activity carbapenems is unclear, The formation of β-lactamase is generally recognised as the most common pathway of antibiotic resistance **(Hamidian and Nigro, 2019).** Class A extended spectrum β -lactamase gene, class B metal lactamase gene, and class C cephalosporinase gene (AmpC) of -lactamase are all encoded by the Temoniera gene (TEM), multidrug-resistant *A.baumannii* has been related to the oxacillinase gene (OXA-23 )& (OXA-51) of Benz oxacillin for class D, among other genes**(Perez et al., 2007).**

Because multidrug-resistant *A. baumannii* is difficult to control and treat **(Butler *et al.*, 2019)** , Traditional approaches for obtaining strain as well as phenotype antibiotic susceptibility information take at least 48 hours, which remains to be a bottleneck for prompt anti-infection therapy **(Maurer *et al.*, 2017).**

As a result, techniques that enable the quick identification of drug-resistant genes are highly desired in order to improve antibiotic treatment efficacy, reduce morbidity, and restrict the spread of drug resistant bacteria. In clinical microbiology laboratories, novel technologies such like Matrix Assisted Laser Desorption Ionization Time Of Flight Mass Spectrometry (MALDITOF-MS), Next-Generation Sequencing (NGS), & molecular biology-based approaches have evolved in recent years, which have improved the specificity and

sensitivity of pathogen analysis while also reducing turnaround time**(Mei *et al.*, 2018).**

NGS (next generation sequencing) has developed from study tool toward a diagnostic technology in the last five years , It is becoming more popular in clinical microbiology laboratories. NGS was used to detect severe community-acquired pneumonia induced by *A.bumannii* in a recent research, and the test was completed in less than 48 hours **(Xu *et al.*, 2020)** .

Multiplex PCR was also used to genotype multidrug-resistant bacteria such as *Acinetobacter baumannii* as a cost effective & highly prognostic method of identifying multiple resistance genes in bacteria **(Karmostaji *et al.*, 2013).**

## 2.5.Nano Technology

It was necessary to develop a new and more effective drug delivery technique that improves the therapeutic index of currently used antimicrobial drugs while avoiding local or systemic side effects and delays the development of resistant bacteria to antibiotics. Antimicrobial medications delivered on nanosized transporters (nanomedicine) now can overcome the limitations of standard therapy and delivery technologies **(Gupta *et al.*, 2019) .**

Nanotechnology is a ground-breaking technological development technique which deals with material management at the nanoscale (1 billion times smaller than a meter). It's a catch-all term for any nanoscale technologies with a variety of real-world applications. Nanotechnology entails the creation and use of chemical, physical, & biological systems at sizes ranging from personal molecules or atoms into submicron dimensions, and the integration of nanomaterials into larger systems . It has the potential to alter our expectations and perceptions while also giving us with the skills to address global concerns.

Carbon nanomaterials' discovery and use has paved the way for several other revolutionary nanomedicine, biosensor, and bioelectronics technologies. Nanotechnology has expanded into a multidisciplinary study in recent years, with a fundamental understanding of the electrical, optical, magnetic, and mechanical properties of nanostructures promising to deliver the next generation of functional materials with a wide range of applications. Nanostructures can aid with in resolution of technological and environmental difficulties in areas such as catalysis, medicine, solar energy conversion, and water treatment **(Nasrollahzadeh *et al.*, 2019).**

Metal nanoparticles play an important role in a variety of applications due to their superior physicochemical properties (optical, catalytic activity, magnetic, electronic, and antibacterial properties) owing to their small size and large surface area. This case distinguishes nanoscale and extends its horizons in a variety of applications, where it can penetrate and damage microorganism cell walls. Furthermore, it easily binds with any material on the Nano molecule's surface to produce new results**(Atwan and Hayder, 2020).**

Nano-sized vehicles (NVs) of various dimensions are used in nanomedicine to load and transport antimicrobial drugs. These novel NVs have distinct properties such as decreased chances of microorganism resistance, enhanced therapeutic efficacy, elevated systemic drug circulation times, long - lasting therapeutic efficacy, reduced adverse side effects on healthy tissues/cells, and the ability to deliver multiple drugs on the same site-specific cell utilizing combination therapy**(Zhang *et al.*, 2010).** NVs are vesicles or particles with at least one dimension between 1 and 100 nanometers. It is have certain advanced physicochemical properties as contrasted to materials, particular atoms, or molecules. These properties are mostly due to the quantum properties with significantly larger surface area-to-volume ratio. In 2000, the National

Institutes of Health launched the Nationwide Nanotechnology Initiative to organize, integrate, and promote nanoscale research and development. Working at the nanoscale allows scientists to benefit from the unique physical, chemical, mechanical, & optical features of materials that occur naturally at that size **(Weissig *et al*., 2014).**

Metals such as zinc (Zn), gold (Au), titanium (Ti), copper (Cu), and silver (Ag) have been used for medicinal purposes since prehistoric times due to its broad antibacterial properties **(Malarkodi *et al*., 2014).** The relevance of these metals has been confirmed by recent advances in nanotechnology, and antimicrobial Nanoparticles (NPs) have gained wide spread scientific recognition as effective disease inhibitors .Nanoparticles (NPs) had multiple functions in the battle to microorganism drug resistance, such boosting antimicrobial agent intracellular storage then preventing biofilm formation **(Huhand and Kwon, 2011).**

Antimicrobial properties of various metal based nano particles( NPs) have been investigated, such (magnesium oxide MgO ,titanium dioxide TiO2, silver oxide Ag2O, copper oxide CuO , zinc oxide ZnO, silicon (Si), gold (Au), and calcium oxide CaO) **(Muzammil *et al*., 2018)**.

The antibacterial efficacy of metal oxide nanoparticles is due to their large surface region, which allows for a variety of bio-organic interactions on the cell surface **(Mukherjee *et al*., 2011)**. The greater the surface area to volume ratio of a particle, the better; thus, a larger area of contact between a metal and a microbe can elevated its chemical and biological activities. The use of nanoscale metals has resulted in a 100-fold reduction in concentration while simultaneously increasing antibacterial capabilities; particle size reduction from 10 m – 10 nm improves interaction surface area by 109 times **(Pal *et al*., 2007).**

# Chapter Three

# Materials & Methods

# Chapter Three : Materials and Methods

## 3.1.The materials

### 3.1.1.Kits and Chemicals

**Table(3-1): Kits and Chemicals**

| NO. | Kits and chemicals | Company | Source |
|---|---|---|---|
| 1 | 25 OH Vitamin D Elisa Kit | EUROIMMUN Medizische Laborrdiagnostika AG | Germany |
| 2 | ABO Reagents | AFCO | Jorden |
| 3 | Bacterial Genomic DNA Extraction Kit | AddPrep | Korea |
| 4 | Agarose | Intron | Korea |
| 5 | ALP kit | DIRUI | China |
| 6 | ALT kit | DIRUI | China |
| 7 | Al $(No_3)_3$ | BDH | |
| 8 | AST For Gram Positive | BIONMERIEUX | France |
| 9 | AST For Gram Negative | BIONMERIEUX | France |
| 10 | AST kit | DIRUI | China |
| 11 | Ceftriaxone | Barcelona | Spain |
| 12 | Cholesterol kit | DIRUI | China |
| 13 | CRP kit | Boditech | Korea |
| 14 | Direct and Total bilirubin | DIRUI | China |
| 15 | Ethanol | Teeba | Iraq |
| 16 | Ethidium bromide | Intron | Korea |
| 17 | GGT kit | DIRUI | China |
| 18 | GN card | BIONMERIEUX | France |
| 19 | GP card | BIONMERIEUX | France |
| 20 | Gram Staining kit | VSI | Iraq |
| 21 | HDL | DIRUI | China |
| 22 | Immunoglobulin G kit | GESAN | Italy |

| | | | |
|---|---|---|---|
| 23 | Iodine | Areej Bagdad | Iraq |
| 24 | LDH kit | DIRUI | China |
| 25 | LDL kit | DIRUI | China |
| 26 | Manual Bacterial Genomic DNA Extraction Kit | Addbio | Korea |
| 27 | Master Mix | Promega | USA |
| 28 | Mg(No3)2 | BDH | |
| 29 | NaOH | Schorlau- European Union | |
| 30 | Primers kit | macrogen | Korea |
| 31 | Random blood sugar kit | DIRUI | China |
| 32 | Serum Albumin kit | DIRUI | China |
| 33 | Total serum protein kit | DIRUI | China |
| 34 | Triglyceride | DIRUI | China |
| 35 | Zinc oxide | Fluka | |

### 3.1.2. Instruments & Tools

**Table( 3-2): The Devices & Tools**

| NO. | Inustruments &Tools | Company | Source |
|---|---|---|---|
| 1 | Auto-chemistryAnalyzer | DIRUI | China |
| 2 | Autoclave | Labtech | Korea |
| 3 | BACT/ALERT 3D | BIONMERIEUX | France |
| 4 | Centrifuge | Gallen Kamp | England |
| 5 | Cooling centerfuge | Bio base | China |
| 6 | Electrophoresis | Bio base | China |
| 7 | Electrophoresis apparatus | Bio-Rad | Italy |
| 8 | ELISA Auto Reader | Meridian | American |
| 9 | Elisa UNO Human | Human | Germany |
| 10 | FTIR-8400S | Shimadzu | England |
| 11 | Fume Hood | Faster Bio 4s | Italy |
| 12 | Glass wears | AFCO | Jorden |
| 13 | Hot plate magnetic stirrer | Labtech | Korea |

| | | | |
|---|---|---|---|
| 14 | Ichroma | Biotech | Korea |
| 15 | Incubator | Gallen Kamp | England |
| 16 | Magnetic Stirrer | Heidolph | Korea |
| 17 | Micro centrifuge (1.5 ml tube) | Hettich | Germany |
| 18 | Micropipettes | Micropipette | Germany |
| 19 | Micropipettes | Slamid | Germany |
| 20 | Microscope | Olympus | Japan |
| 21 | Oven | Memmert | Germany |
| 22 | Refrigerator | LG | Korea |
| 23 | Sensitive Balance | Kern | Germany |
| 24 | Shaker incubator | Gallen Kamp | England |
| 25 | Thermo cycler | Bio base | China |
| 26 | Uv transmilloter | Bio base | China |
| 27 | Vitek 2-System | BIONMERIUX | France |
| 28 | Vortex | Scientific Industries | Korea |
| 29 | Water Distilling | GEL | Germany |

### 3.1.3. Primers used in PCR

**Table (3-3) Primers used in PCR.**

| Organism | Target gene | Primer Sequence 5´- 3´ | Size (bp) | Temp $^0$C | Reference |
|---|---|---|---|---|---|
| *Acinetobacter bumani complex* | blaSHV-F blaSHV-R | TTATCTCCCTGTTAGCCACC GATTTGCTGATTTCGCTCGG | 795 | 59.1 58.6 | Priyadharsini ) (*et al*., 2018 |
| | blaTEM-F blaTEM-R | ATGATGATTCAACATTTCCG CCAATGCTTAATCAGTGAGG | 858 | 52.2 55.2 | *Priyadharsini*) ( *et al*., 2018 |

| | | | | | |
|---|---|---|---|---|---|
| | blaCTX-M-F blaCTX-M-R | CGCTTTGCGATGTGCAG ACCGCGATATCGTTGGT | 550 | 58.5 58 | Priyadharsini ) (*et al*., 2018 |
| *Staphylococcus Warneri* | blaZ-F blaZ-R | ACTTCAACACCTGCTGCTTTC TGACCACTTTTATCAGCAACC | 173 | 59.6 57.2 | (Duran *et al*., 2012) |
| *Bacillus cereus* | blaA1-F blaA1-R | CATTGCAAGTTGAAGCGAAA TGTCCCGTAACTTCCAGCTC | 680 | 52.2 56.7 | (Chen *et al*., 2004) |

### 3.1.4. The Media

#### 3.1.4.1.Blood Agar

40 g of blood agar was suspended in 1liter of Distilled water( DW). It was bringed to a boil to completely dissolve it. Then sterilized by sterilizing at 121°C for fifteen minutes. This agar was cooled to 45-50$^0$C add 7%from sterilized defibrinated blood , this media was used to culture and activated bacteria that was collected from samples. activated bacteria that was collected from samples**(Yeh *et al.*, 2009).**

#### 3.1.4.2. MacConkey Agar

prepared according to the manufacturer's directions, 51.55 g of media was dissolved in 1000 ml of distilled water, bringed it to the boil while gently stirred to fully dissolved the agar. 15 minutes of autoclave sterilization at 15 pounds for pressure 121$^0$C.Without the risk of overheating. It was cooled to 45-50°C before puttied into sterile petri dishes. The surface of the medium should be dry when inoculated ,this media was used to culture and activated bacteria that was collected from samples and specially selective for Gram negative bacteria.

#### 3.1.4.3. Brain Heart Infusion Broth:

Brain heart infusion broth served as the base medium, which was enhanced by 35 % (v/v) glycerol. It was autoclaved, cooled, & kept at 4 ° C till it was used in 5 mL tubes. This medium was utilized to maintain the isolate alive for an lengthy period of time at 20°C **(Torres *et al.,* 2017)**.

**3.1.4.4.Nutreint agar**

prepared according to the manufacturer's directions **.**

**3.2.1. Patients**

From November 2021 to April 2022, a case control study for pediatric patients which have Nephrotic syndrome was done. In Karbala teaching hospital for children, Karbala pediatric hospital/Karbala Health Directorate, one hundred and sixteen pediatric patients were diagnosed with Nephrotic Syndrome and forty-two as healthy pediatric persons. All of the patients were children, age up to fifteen , of both sexes, with Nephrotic Syndrome.

**3.2.1.1. Collection the data**

Name, age, gender, weight, edema, fever, drugs, and blood group were among the important information and data collected from the patients.

**3.2.1.2.Ethical approvals**

After acquiring the necessary authorization from the hospital administration and parents of children patients ,the samples of this investigation were taken from patients at Karbala pediatric hospital/Karbala Health Directorate.

**3.2.1.3.Collection Samples**

After visiting the pediatric hospital, about 5 ml of venous blood samples were collected from NS children patients.

Nutusi *et al.* performed an important blood culturing procedure which should be followed**(Ntusi *et al.,* 2010)** in children with fever and high CRP, similar :

1- The patient's identification had been requested, and the process of validating the identity of the patient had begun .It was wrote on the wall above its bed, or even in the patient notes, confirmed identification.

2- Children patient was informed about the surgery as well as the specifics of the plans. Verbal approval was often obtained.

3- Among the items collected were; blood culture bottles, a 6 mL syringe, a tourniquet, sterile gloves , an adhesive strip, alcohol solution ,or a povidone iodine (or any disinfection), a sterile pack containing cotton/gauze swabs, as well as a sharps waste disposal bucket.

4-A tourniquet was applied, and a suitable vein was selected, hands were disinfected by alcohol or cleansed (by the soap & water). It is must then cleansed or rubbed till they were completely dry. It was tried to use sterile gloves.

5-The puncture area cleansed by alcohol or povidone solution in an aseptic process. For 1 to 2 minutes, the disinfectant was allowed to dry. A yellow sterile cover with an aperture was placed over the blood culture site.

The 6ml needle was put into the children patient' blood vein to care, giving at minimum of 3 ml of blood. If a vacutainer device was used, the first blood specimen obtained would be a blood culture.

7-The tourniquet had been removed. The syringe and needle were removed from the puncture wound. Inoculate blood into the culture bottle after disinfecting the cap of the blood culture bottle with an alcohol swab if blood was not derived directly into the culture bottle using vacutainer system. Before collecting blood for further tests, inoculate the blood culture tube. Between drawing blood samples and inoculating the blood culture vial.

8-The blood culture container was gently rotated to mix the blood and culture medium (Avoided shake vigorously).

9-The blood culture vial was delivered to the laboratory as soon as possible. Approximately 3 mL of blood was placed into a gel tube (5 mL) at room temp and allowed to clots for at least 15 minutes before being centrifuged about 2500 rpm. Pindrop's serum was then isolated from the main of the serum. The leftover blood sample (1ml) was put in an EDTA tube and agitated for at minimum 15 minutes.

### 3.2.2. Estimation of Lipid Profile

The Auto-Chemistry Analyzer CS-T180 assessed Triglycerides (TG), Cholesterol (TC), Low density lipoprotein (LDL) and High density lipoprotein using an automatic technique**. (Young, 1997)**.

### 3.2.3. Estimation of Liver function analyses

The Auto-Chemistry Analyzer CS-T180 measured AST ,ALT,ALP ,GGT ,Total Bilirubin, Direct Bilirubin, and Total serum protein automatically **(Young, 1997).**

### 3.2.4.Estimating of Lactate dehydrogenase (LDH)

Using the estimated method, Lactate Dehydrogenase (LDH) was evaluated automatically in the CS-T180 Auto-Chemistry Analyzer **(Young, 1997)**

### 3.2.5. Identification for Nephrotic Syndrome

Clinical symptoms, hypoalbuminemia, and glomerular biopsy are used to diagnose nephrotic syndrome. A specialist doctor in the pediatric hospital identified children with Nephrotic syndrome based on the clinical symptoms, hypoalbuminemia, and kidney biopsy.

### 3.2.6. Estimation for C- Reactive Protein

1- A sample collector that was empty was inserted throughout a puncture above top part of the detecting buffer tube.

2- A sample collector was used to collect a 10-microliter sample ( for both children whole blood, plasma, serum & control).

3- To make one tube, the sample collector & tubing were joined together.

4-The material was shaken ten times or more till it was inverted out of the sample collection. Within 30 seconds, the buffer and sample combination were used.

5- The top cap of the assembled tube was removed. Prior to actually adding the reagents to the cartridge, 2 drops were started pouring onto a paper towel.

6- Only 2 drops from the mixture were put on the well of cartridge sample .

7- For ichroma measurements, the device was implanted into the device's holder. Before fully inserting the cartridge into the cartridge holder, it was checked to avoid alignment. As a result, the cartridge now includes a special arrow.

8-The device's( 'Select' or 'START' ) key was pushed for ichroma checks.

9-For ichroma checks, cartridges have been put into the instrument, and the device can begin scanning the sample-loaded cartridge after 3 minutes.

10- The test result for ichroma checks was displayed on the instrument's show screen **(Wei *et al.*, 2000).**

### 3.2.7. Gama Immunoglobulin Estimation (IgG)

In the Auto-Chemistry Analyzer CS-T180, was measured automatically by using method from **(Young, 1997).**

## 3.2.8. Estimation fo25-OH-Vitamine D

### Manual test performance

**First step**: Incubation of the sample: 200 microliters of diluted sample , Each microplate well received a pipette in biotin/sample buffer & incubated to 2 hours at room temp (+18$^0$C to +25$^0$C).

Note: The sample was diluted by adding 0.5ml of working strength biotin to 20 microliters of sample (serum) and mixed thoroughly (vortex). The mixture was incubated for at least 10 minutes on room temp (+18$^0$C to +25$^0$C).Using 300 l from the working Wash buffer three times, the wells were emptied & washed.

**Second step Incubation of enzyme conjugate:**100 microliters of enzyme conjugate were pipetted into each microplate well and incubated at room temperature for 30 minutes .The wells were emptied and washed as described above.

**Third step Substrate incubation** : A 100 microliter chromogen(substrate) solution was pipetted into the microplate wells and incubated for 15 minutes at room temperature (*was protected from direct sunlight). 100 microliters of stop solution were pipetted into microplate wells in the same order and speed.

**The Measurement**: 30 minutes after adding the stop solution, a photometric measurement of the color intensity was taken at 450 nm and a reference wavelength of 620 nm to 650 nm. The microplate was vigorously shaken before measuring to ensure that the fluid was evenly distributed.

### 3.2.9.Physiological Parameters Estimation

#### 3.2.9.1. Assessment of Complete Blood Counts

The procedure was followed b **(Blum ,2018)**

1. In the first step, the samples were all at room temp. It was ten times spun by hand until it was suspended.

2. If the samples are barcoded, it was run as if it were a normal patient (Caps lock was disabled).

3. After placing the sample on the analyzer, the RUN button was pressed. After all of the samples had been examined, the results were printed. " To print the information, "Stored Data" was selected.

4.The output button was pushed.

5. Clear symbols by pushed "Mark," "All Clear," and "Cancel" **(Blum,2018).**

#### 3.2.9.2. Assessment of Glucose

In the Auto-Chemistry Analyzer CS-T180, glucose was measured automatically using the method from **(Young, 1997).**

**3.2.9.3. Assessment of Rh& ABO Blood Group.**

In this method, A drop from donor or recipient blood is mixed separately with anti-A, anti-B, & anti-D antibodies for each part of the white porcelain support. By visually viewing the reaction or blood clump pattern, the ABO & rhesus D (RhD) blood types may be determined. The test takes 5 to 10 min to complete and costs little if just little amount of blood types reagents are used **(Mujahid and Dickert, 2016).**

**3.3.1. The Diagnoses of Bacteria**

**3.3.1.1. Blood culture specimens**

Blood (3-4 ml)was drawn from an arm vein of child and placed in blood culture bottles. For the initial testing of the blood cultures, BacT/ ALERT® 3D system (bioMeriéux, Marcy l'Etoile, France) utilized . After collecting the samples, they were inoculated on blood agar (BAP; Asan Pharmaceutical Co., Ltd., Seoul, Korea) and MacConkey agar (Becton Dickinson, Sparks, MD, USA) before being incubated at 35°C in a 5% CO2 atmosphere for 48 hours **(Ha *et al*., 2018)**.

**3.3.1.2.The Positive Blood Culture**

After a positive signal from the BacT/ALERT® 3D Device, Gram staining was completed first, then growth on the a pieces fit agar medium . So after an overnight incubation period, the colonies on the agar plates were used for identification and also antibiotic susceptibility testing (AST) and used a commercial Automated Vitek2 System (bioMeriéux) **(Ha *et al*., 2018).**

1. The traditional technique's ID and AST results were used as the institution's protocol for comparison.
2. Put the inoculum in the VITEK® 2 Cassette in the Smart Carrier Station TM.
3. A barcode connects the (VITEK® 2 Card and sample).

4. When the Cassette is loaded, the instrument will manage the incubation and printout of the results.

The VITEK® 2 Compact is still a biochemically based automated microbial detection tool with 48 biochemical characteristics that is often used in clinical laboratories. VITEK® 2 Compact may identify microorganisms up to four hr. Each well assesses the metabolic performance of a strain, including its capacity to acidify, alkalize, and bacterial growth in the presence of inhibitors, as well as enzymatic hydrolysis of substrates. Bacterial growth and metabolic changes in the microwells are monitored utilizing fluorescence-based sensors.

Bacteria incubating conditions, such as medium composition and pH, influenced the results of biotyping & biochemical-based methods**(Gherardi *et al.*, 2012).**

A sterilized micro loop was used to extract a few colonies from the a pure culture that had grown on blood or macconkey agar for 18 to 24 hours. Using the VITEK® 2 DensiChek, a bacteria was calibrated to a McFarland turbidity Standard of 0.5–0.63 using 3 mL of a 0.45 percent sodium chloride solutionIf the gram stain was negative, the GN card was only placed on tape and placed in the instrument; if the gram stain was positive, the GP card was only placed on the cassette and placed in the instrument.

To avoid turbidity changes, the time among suspension preparation and card filling was kept at 30 minutes. These cards were incubated for 1 hour at 35.5 1 °C. After each card was removed from the incubator, colorimetric measurements were made every 15 minutes. After 10 to 18 hours of incubation, the results are read **(Morka *et al.*, 2018)**.

### 3.3.2. Antibiotic Susceptibility Determination

The antibiotic susceptibility test is used to determine the antibiotic susceptibility of a bacterial isolate. After being inoculated, the cards were placed in the Vitek 2 automated reader-incubator. Identification and susceptibility cards were infected &

evaluated in accordance with the manufacturer's instructions, as explained below. To ensure that the number and density of bacteria inoculated into the Vitek cards were correct, colony counts were used **(Gherardi *et al.*, 2012).**

• Antibiotics have been administered to the microorganism, as well as the examination will determine whether it can grow in although presence of the antibiotics. The clinician is given the Minimum Inhibitory Concentration (MIC), which is a measure of how sensitive or resistant a microorganism is to an antibiotic.

### 3.4.1. Nano hybrid LDH- Ceftriaxone Preparation

The nanohybrid antibiotic was made using the layered double hydroxide method, as detailed by Klemkaite *et al.* **, (Klemkaite *et al.*, 2011).**

**1-Magnesium nitrates solution Mg(NO3)2.6H2O :** This solution was made by dissolving 32.05 gram of Mg(No3)2 in 250 mL of unionized distilled water.

**2- Aluminum nitrates solution Al(No3)3.9H2O** : This solution was made by dissolving 11.72 gm of aluminum nitrates Al(No3)3 in an amount of unionized distilled water, and after that, the volume was increased to 250 ml using unionized distilled water.

**3-Sodium Hydroxide Solution:** A solution was made by dissolving 4 gm of Sodium Hydroxide NaOH inside a volume of unionized distilled water, and dilute to 50 ml using the same unionized distilled water.

**A .Training of Mg-AL-No3-LDH by co-precipitation:**

This training was prepared according to the process prescribed in **(Klemkaite *et al.*, 2011),**with some modulation; by mixed Magnesium nitrates solution with Aluminum nitrates solution, the precipitation was 10.5 PH ,by added drops of Sodium Hydroxide (2molar), Before placing the mixture inside the incubator, magnetically stir it for two hours at room temperature. Incubate solution at 37°C for 18 hr . After an hour, distinguish the precipitate by centrifuging it for 20 minutes at

5000 rpm. The precipitate was then rinsed many times with deionized water before being dry at 60°C. Then after, it was ground inside a ceramic mortar & then kept.

**B. Ceftriaxone solution**: This solution was made by melting 1 g of commercial vial ceftriaxone in 50% ethanol and added to 100 ml of ethanol.

**C. Training of Nanohybrid Ceftriaxone from Mg-AL-No3-LDH layers by sol-gel- method**

The techniques used previously published by Kolekar *et al.*,with some modification , drop 100 ml of Ceftriaxone solutions (both at the same time) into Mg-AL-No3-LDH solution The mixture should then be magnetically stirred for two hours at room temperature before being put in the incubator. Vibrations were performed at 37°C for 18 hours before being put in a 40°C incubator for an additional 24 hours. Separate the precipitate after an hour by centrifuging it for 20 minutes at 5000 rpm. Then after, the precipitate was rinsed with distilled water many times before even being dried at 40°C temperature . It was then ground inside a ceramic mortar & kept **( Kolekar *et al* ).**

**3.4.2. Preparation of Nanohybrid Zno-Ceftriaxone**

**A. Zinc Oxide solution :** To make this solution, melt 2 gm Zinc Oxide in 50 percent ethanol, and dilute to 100 ml by (ethanol).

**B. Ion exchange method using Ceftriaxone Gel Sol for training nanohybrids made from zinc oxide layers:** The methods used formerly published by **(Kolekar *et al* )**with some modification drop by drop into zinc oxide solution 100 ml of the above prepared solutions (both at the same time) then Before put the mixture inside the incubator, magnetically stir it for two hours at room temperature. Vibrations were carried out for 18 hours at 37°C before being incubated for 24 hours at 40 ° C. After an hour, separate the precipitate through centrifuging it for 20 minutes at 5000 rpm.

The precipitate was then cleaned with distilled water several times before being dried at 40°C. It was then ground in a ceramic mortar and stored.

### 3.4.3. Nutrient Agar Preparation

According to the design instructions: **(Niederstebruch and Sixt, 2013)**

In 1000 mL of deionized water, 28 g of lab-prepared media be added to a beaker.

1. The suspension was then brought to a boil to completely dissolve the media.
2. The dissolved medium was then autoclaved for 15 minutes at 15 lbs of pressure (121°C).
3. After the autoclaving process was finished, the beaker was removed and cooled to about 40-45°C.
4. Under sterile conditions, the media was put into sterile Petri dishes.
5. Before using the plates, they were put in the drying oven on a low thermal setting in a little minutes to reduce any moisture.

### 3.4.4. Concentrate and petri dish preparation

The series of concentrations used were prepared according to the results of the initial experiment in this study, and the following concentrations were made.

1. Forty-two from petri dishes were equipped for each type from bacteria isolated, 14 of them are nano ceftriaxone (zno), 14 for nano (LDH), and 14 of them were free ceftriaxone (for each type of our bacteria).
2. Any of the free and nano (Zno, LDH) dishes were assigned a number based on the concentrations (0, 0.25, 0.50, 1, 2, 4, 8)mg/ml with a duplicate for each concentration.
3. For each petri dish, two wells with a diameter of 5 mm were drilled into the media.

4. 18 tube were prepared from stock solutions, 6 of which were Free-ceftriaxone, 6 of which were Nano-ceftriaxone(zno), and 6 of which were for Nano ceftriaxone (LDH) as explained in Table (3-4).

### 3.4.5. Stock Solution Training

Free-Ceftriaxone(commercial) and nano-Ceftriaxone stock solutions were prepared separately, Using 0.2 gm of the drug in a conical flask and 5 ml distilled water added to make a stock solution with a concentration of 40 mg/ml , that will be used to prepare the concentrations utilized in this study in the following phases.

### 3.4.6. Antibiotic Concentration :

The concentrations of Ceftriaxone for both (free and Nano )used in this test were made separately using the methods listed in Table (3-4).

**Table (3-4): Antibiotic Concentration Training**

| No. of tube | Distilled water (ml) | Stock Solution (ml) | End volume (ml) | End concentration (mg/ml) |
|---|---|---|---|---|
| 1 | 800 | 200 | 1000 | 8 |
| 2 | 900 | 100 | 1000 | 4 |
| 3 | 950 | 50 | 1000 | 2 |
| 4 | 975 | 25 | 1000 | 1 |
| 5 | 987.5 | 12.5 | 1000 | 0.5 |
| 6 | 993.75 | 6.25 | 1000 | 0.25 |
| 7 | 1000 | 0 | 1000 | 0 |

### 3.4.7. Description of the Nanohybrid Antibiotic

(FT-IR); (XRD); (AFM); as well as detailed analysis of C, H, and N elements were used to characterize the nanohybrid antibiotic under study.

**1.FT-IR** (Fourier transform infrared spectroscopy): By grinding a disk of the compound under investigation with potassium bromide (KBr), as well as measuring the infrared spectrum in the (400-4000) $cm^{-1}$ wave number range, the infrared spectrum of Nanohybrid-Ceftriaxone, Ceftriaxone in free form, and zinc oxide (ZnO), LDH, Nano-Zno Ceftriaxone, and Nano-LDH-Ceftriaxone was measured.

**2**.**XRD( X-ray diffraction** ): The Nanohybrid- Ceftriaxone was characterized to use a diffraction spectrum. Using Brack's low (n = 2dSin), XRD explains the difference in layer thickness before and after the intercalation process for ceftriaxone antibiotic.

**3.Atomic Force Microscope (AFM)**: The Nanohybrid-Ceftriaxone samples were examined by AFM to determine the nanoparticles' diameters, sizes, and aggregation.

**4.Detailed analysis of elements C, H& N**: The percentage of C, H, and N in the ceftriaxone-free and Nanohybrid-Ceftriaxone samples were compared.

**5.Scaning Electronic Microscope (SEM )**: also used to examine the Ceftriaxone nanocomposite's outer surface and layers of free LDH.

**3.4.8. Measurement of Free-Ceftriaxone and Nano-Ceftriaxone Antimicrobial Activity:** The well agar diffusion method was used to test their antibacterial activity against the ESBL-producing bacteria that were studied**(El-Rab *et al.*, 2018)**

**3.4.9. The media**

**3.4.9.1. Media for nutrient broth:** was made according to company instructions, called for weighing 13 g of media and diluting it in 1 L distilled water before autoclaving it for 15 minutes. The bacteria were activated to use this medium.

**3.4.9.2. Muller Hinton ager media**: 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. The antibacterial action of Free- Ceftriaxone as well as Nano-Ceftriaxone compared with *Acinetobacter bumani*, *Staphylococcus Warnerii,* and *Bacillus cereus* was investigated using this media **(Murray and Zeitinger, 1983).**

**3.4.9.3. Activation of bacteria**: Before 1 hr of culturing, *Acinetobacter bumanii* complex, *Staphylococcus warneri, and Bacillus cerus* were activated on nutrient broth.

**3.4.10. Assay for Antimicrobial Bioactivity:** A 2 well (with a diameter of 5 mm) were created in each plate (Muller Hinton agar) after bacteria had been activated, and 100µl of antibiotic concentration was added to each well, and 50 µL of active bacteria solution was placed on each petri plate and incubated for one day at 37°C. A diameter of the inhibitory zone surrounding the well was measured using a ruler, and growth was observed **(Padmavathy and Vijayaraghavan, 2008).**

**3.5. Diagnoses of un identified Bacteria isolated by PCR Technique**

**3.5.1.DNA Extraction**

According to manual **AddPrep Bacterial Genomic DNA Extraction Kit** instructions.

**3.5.1.1. Lysis protocol for Gram-negative bacteria :**

1. The overnight cultured cells (1m ~ 2ml) were harvested by centrifuge for 30 seconds on 13,000 rpm. with a 1.5 ml tube .

2.The supernatant was discarded.

3. A 200µl of lysis solution and 20µl proteinase K solution (20 mg/ml) was added then resuspend the cell bit with pipetting or vortexing.

4.It was incubated into 56℃ water bath for 10 minutes and Vortexed occasionally during incubation to disperse the sample. Optional RNase treatment :If RNA-free genomic DNA is required, 20µ RNase A Solution (10 mg/ml) was added.

5. A 200µl of binding solution and 200µl from absolute ethanol were added then mixed well with pulse-vortexed for fifteen seconds.

6. It was centrifuged at 13,000 rpm, for 3 minutes.

7. 500-600 l of surplus was transferred aseptically without pellet into to the top reservoirs of the spin column using a 2.0 ml collecting tube without wetting the rim,

then was continued with step 2. According to manual **AddPrep Bacterial Genomic DNA Extraction Kit** instructions.

**3.5.1.2.Lysis protocol for Gram-positive bacteria**

1.The overnight cultured cell 1 ml ~ 2ml were harvested with centrifuge at 13,000 round per minute for thirty second. with 1.5 ml tube.

2.The supernatant was discarded.

3.A 500µl of lysozyme buffer and 20µl from lysozyme (fifteen mg/ml)were added then resuspend the cell pellet with pipetting or vertexing.

4.It was incubated into 37℃ water bath for 60 minutes and mixed well occasionally during incubation to disperse the sample.

5.The supernatant was removed after 3 minutes of centrifugation at 13,000 rpm.

6.A total of 200µl of Lysis Solution and 20µl Proteinase K Solution (20 mg/ml) were added and resuspended the cell pellet by pipetting or vertexing.

7. It was incubated into 56℃ water bath for 10 minutes and vortexed occasionally during incubation to disperse the sample.

Optional RNase A treatment :The 20 microliter of RNase A Solution (10 mg/ml) was added if RNA-free genomic DNA was required.

8. A total of 200 microliter of Binding Solution and 200 microliter of ethanol were added and well mixed using a pulse-vortexer for 15 sec.

9.Then was centrifuged at 13,000 rpm , for 3 minutes.

10. With a 2.0 ml collection tube, 500- 600 l of supernatant was carefully transferred without pellet into the upper reservoir of the spin column without wetting the rim was continued with step 2.

**3.5.1.3.Continued steps of extraction for two types of bacteria**

**1.** It was centrifuged at 13,000 rpm in 1 minute , With the 2.0 ml collection tube, the flow was poured through and the spin column was assembled..

**2.** After a 500 microliter of Wash 1 Solution was poured to the spin column using collection tube and spun at 13,000 rpm for 1 minute, the flow was drained through and spin column was formed with the 2.0 collection tube.

**3.** After a 500 microliter of Cleaning 2 Solution was put to the spin column using collection tube and spun at 13,000 rpm , for 1 min, the flow was drained through and spin column was built with 2.0 collection tube.

**4.** Additional centrifugation of 13,000 rpm for 1 min was used to dry the spin column & remove any residual ethanol.

**5.** The spin column was placed in new 1.5 ml mini-centrifuge tube

**6.** 100-200 l of Elution Solution was then added to the spin column via a micro-centrifuge tube and left to exist for at least 1 minute.

**7.** The genomic DNA was eluted by centrifugation around 13,000 rpm for 1 min.

According to manual **AddPrep Bacterial Genomic DNA Extraction Kit** instructions.

### 3.5.2. PCR Product analysis

The following steps were used to analyze the PCR products by agarose gel electrophoresis:

1. 1 percent agarose gel was ready by dissolving 1X TBE in a water bath at $100^0$ C for 15 min., and cooling at $350^0$ C. Ethidium bromide stain (5 microliter) was then added to the agarose gel solution.
2. After the comb was positioned, the agarose gel solution was then poured into the tray & dried for 15 min at room temperature .The comb was carefully extracted from the tray , and 5 µl of DNA sample, as well as a 100 bp DNA size marker, were added to each comb well.
3. 1XTBE buffer was added to the gel tray in the electrophoresis chamber. Then after, the electric current was applied at 45 degrees for 1 hour (5V/cm between electrodes).

4. UV Transilluminator was used to see PCR products.

### 3.5. 3.Measurement the purity of DNA

Nanodrop was used to test the extracted DNA purity by measuring absorbance around (260/280 nm) and quantifying the DNA content **(Adams and Otárola-Castillo, 2013).** The following steps to assess the purity of DNA:

1. The Nanodrop program was launched, and a fit application was selected (Nucleic acid, dsDNA).
2. All device parts were cleansed with a dry wipe, especially those related to measurements, and the system was completely wiped by carefully pipetting 2 microliter free nuclease water on the surface of the bottom measurement plat forms.
3. After cleaning the measuring pedestal, 1 microliter of DNA was put on it after closing the sampling cover and pressing the okay button to start the Nanodrop process.
4. All of the primers used in this study were prepared by dissolving lyophilized primers in an appropriate quantity of nuclease-free water to form a stock solution of 100 pmol/microliter, as specified by the manufacturer. To create a workable solution with such a concentration of 10 pmol/microliter, dilution methods were used. **(Adams and Otárola-Castillo, 2013)**

## 3.6.Identification of resistance genes to ceftriaxone in isolated bacteria .

### 3.6.1.Molecular detection

The thermal lysis method was used to extract the DNA for monoplex-PCR**. *(Abrar et al.,* 2017)**. As previously stated, 2 whole cell lysates DNA were used separately in 25 PCR-master mix with amplification primers for each isolate in monoplex-PCR. The PCR amplification conditions were as follows: Denaturation at 95°C for five minutes was followed by 35 cycles of denaturation at 95°C for one minute , The

primers were prepared by annealing at 56°C during 1.5 min, extension for 95°C for 1 min, and final extension at 95°C for 10 min ,primers were mentioned in Table 3.

### 3.6.2. In-silico analysis and amplicon sequencing

For direct sequencing, PCR amplicons were sent to an ABI 3730 XL DNA analyzer (Bioneer Corporation, Daejeon, South Korea). All sequences were trimmed at the 5′ and 3′ ends before being analyzed with Chromas software version 2.6 and similarity determined with the NCBI BLAST program. ((**Ranjbar and Farahani, 2019).**

### 3.7. Statistical Analysis

Data were analyzed using SPSS (Version 22) to determine the Chi-square,(One-way) T-test at significance levels of (0.01 and 0.05), and Correlation (r).

# Chapter Four

# Results & Discussion

# 4.Results and Discussin

## 4.1.General parameters of the study samples

### 4.1. 1. The Age ,Gender and Weight Distribution

The results of the statistical analysis for children samples at age (0.5-15) years from healthy and Nephrotic in the Table (4-1) found that there were significant differences (*P< 0.001*) between female patients and controls and male and female groups, as well as significant differences (*P< 0.001*) across patients and controls in all patients and controls. Despite the reality, there were no significant differences (*P <0.05*) between male and female patients , the number of males is higher than the number of females. The results in the same Table showed that no significant differences ($P < 0.05$) between patients and controls in age of both female and male children with mean of age was (7.45 and 7.62)years for controls &cases respectively. Weight factor result showed that there were no significant differences ($P < 0.05$) between male & female patients with mean of weight was 28.17 kg.

**Table(4-1) : General Parameters of(healthy and NS )children age at (0.5 -15)years**

| Factor | Gender | Control | Patients | *P value* |
|---|---|---|---|---|
| **Gender (mean ±SD)** | **Female** | 16 ( 38 %) | 48 (41.38 %) | **0.00006 **** |
| | **Male** | 26 (62 %) | 68 (58.62 %) | **0.00001 **** |
| | **Total** | **42 (100 %)** | **116 (100 %)** | **0.00001 **** |
| | ***P value*** | **0.12282** | **0.06332** | |
| **Age(Years) (mean ±SD)** | **Female** | 5.87 ± 2.80 | 7.14 ± 3.86 | **0.2304** |
| | **Male** | 8.42 ± 3.54 | 7.96 ± 3.65 | **0.5830** |
| | **Total** | **7.45 ± 3.47** | **7.62 ± 3.74** | **0.7974** |
| | ***P value*** | **0.0093 *** | **0.2470** | |
| **Weight(kg) (mean ±SD)** | **Female** | 23.25 ± 13.83 | 26.45 ± 14.55 | **0.4437** |
| | **Male** | 28.00 ± 11.81 | 29.39 ± 14.09 | **0.6565** |
| | **Total** | **26.19 ± 12.67** | **28.17 ± 14.29** | **0.4296** |
| | ***P value*** | **0.2427** | **0.2771** | |

* significance differences (*P <0.05*), ** high significances differences (*P <0.001*)

The result according to gender factor agreement with study of Frank *et al.* and Anigilaje and Adesina, and Isah and Abdulazeez showed that, male had higher frequency of nephrotic syndrome patients than the female, This may be due to increase progression of disease in men with the exhibition of greater decrements in renal function and raised glomerular sclerosis which occurs more in male than women **(Anigilaje and Adesina, 2019), (Franke *et al.*, 2019), (Isah and Abdulazeez, 2021).**

Childhood nephrotic syndrome is frequently characterized by a relapsing course. The frequency of relapse has been noted to be higher in children<3-4 years at onset of NS, who had delayed time to remission (after 7-9 days) and who had occurrence of an early relapse (in the first six months after initial treatment)**(Anochie *et al.*, 2006).**

This agreement with study that was reported no statistically significant difference between the three groups as regard to , age this group was control children ,steroid sensitive nephrotic syndrome(SSNS), and steroid resistance nephrotic syndrome (SRNS) all children aged 4-18years **(Amin et al., 2022).** Also another study was showed no differences in ages in both male and female in patients and controls with mean of age in patient 8.3 years and mean of age in controls 8.19. Also in these study the average age for children on patient and control both were close 7.45 year for control and 7.62 year for patient **(Guha *et al.*, 2009)** .

In Amin *et al*. and Gooding *et al*. concluded that NS can affect children of any age, from infancy to adolescence. As regard comparison between the two studied groups according to duration of steroid intake (years), there was no statistically significant difference between groups in duration of steroid intake**(Gooding *et al.*, 2020), (Amin et al., 2022).**

The result of weight factors was Similar to study Basu *et al*. which studied 30 children and have mean Body weight (Kg) 16.5±4.7 , which show no significance differences among NS children body weight, matched in this study only current weight of children with NS and compared with control healthy children in different age under 15 years **(Basu *et al.*, 2020)** .

Also in another study for Konstantelos *et al.*, reported that Low birth weight and prematurity are important clinical factors to consider in the history of children with nephrotic syndrome. Their conclude that LBW/premature children with nephrotic syndrome are more likely to be steroid resistant, and thus should be followed closely to ensure prompt assessment and suitable treatment. LBW/prematurity can be used by physicians as indicator for increased risk of steroid resistance**(Konstantelos *et al.*, 2019).**

## 4.2. Immunological Parameters

### 4.2.1. WBC, CRP , IgG Distribution

The results in Table (4-2) showed that there were significance increased ($P < 0.05$) in number of white blood cells for cases Compared with controls for both female and male children separately with mean (8.73, 11.624) Thousand/cubic millimeter respectively, and high significant increased ($P < 0.001$) in total , Also there was showed significance differences ($P < 0.05$) in WBC among male and female patients , male had more number of WBC.

The results in table (4-2) also showed there are significant increased ($P < 0.05$) between cases than control male patients in CRP. Although there were no significant differences in female children alone , but there were high significance increased ($P < 0.001$) in total of cases compared with controls in amount of CRP in blood. A significance decreased ($P < 0.05$) between cases than controls in total of male and

female children in the IgG number was appeared ,with mean (1770.71, 3137.63) IU/ml respectively.

**Table( 4-2) : Immunological Parameters of (healthy and NS )children age at (0.5 -15)years**

| Factor | Gender | Control | Patients | *P value* |
|---|---|---|---|---|
| **WBC** | **Female** | 8.51 ± 1.59 | 10.541 ± 3.74 | **0.0397 *** |
| | **Male** | 8.86 ± 2.27 | 12.388 ± 5.612 | **0.0026 *** |
| | **Total** | **8.73 ± 2.02** | **11.624 ± 4.992** | **0.0004**** |
| | ***P value*** | **0.5925** | **0.0492 *** | |
| **CRP** | **Female** | 1.373 ± 0.536 | 10.428 ± 18.885 | **0.0611** |
| | **Male** | 2.319 ± 2.422 | 11.999 ± 24.421 | **0.0473 *** |
| | **Total** | **1.959 ± 1.975** | **11.349 ± 22.222** | **0.0001**** |
| | ***P value*** | **0.1333** | **0.7094** | |
| **IgG** | **Female** | 3003.81 ± 1626.90 | 1827.12 ± 2281.21 | **0.0616** |
| | **Male** | 3219.36 ± 2019.33 | 1730.89 ± 3776.43 | **0.0600** |
| | **Total** | **3137.63 ± 1861.68** | **1770.71 ± 3230.78** | **0.0106 *** |
| | ***P value*** | **0.7204** | **0.8753** | |

* significance differences ($P$ <0.05) ** high significances differences ($P$ <0.001)

This result in agreement with recent study conducted by Abdel-Salam *et al.*, who conducted a significant increase in WBCs levels in the patient group, compared to the control group, while there is no significant difference in the CRP level between both the groups**(Abdel-Salam *et al.*, 2020).**

In one study showed levels of Total Leukocyte TLC (cells/mm3 ) in healthy children is 7960.71 (±2587.7) and nephrosis children is 7892.11 (±1807.8) with p value= 0.7,which is no significance differences appeared**(Rai *et al.*, 2017).**

Infection is one of the most common complications of idiopathic nephrotic syndrome in kids, and it can lead to relapses, treatment failures, or even death. Due to the main disease and therapeutic drugs, children having INS are more prone to have a poor prognosis post infection. Most research on children's INS today focus

on the study of infection risk factors, but there are few large-scale studies on the risk and outcome variables for severe infection .CRP was defined as one of the symptoms of acute infection in kids with INS, based on the definition of serious infection in adults and the signs of serious infection in children**(Zhang et al., 2021).** Besides the liver, renal tubular epithelial cells are also apparently capable of producing CRP in response to inflammatory processes The result of this study also indicated increased in CRP levels in children with nephrotic syndrome in Karbala Teaching Hospital for Children , agreements with studies of Isah and Abdulazeez, and Racki et al. , reported that , Serum CRP was significantly higher in patients' group when compared with the controls **(Isah and Abdulazeez, 2021), (Rački et al., 2006).**

On other hand , patients with the nephrotic syndrome had unusual susceptibility to encapsulated bacterial infection. Studies showed that there are changes in the serum IgG, IgM levels associated with proteinuria in nephrotic patients. According to studies, changes in the serum Ig levels can be attributed to either T-cell dysfunction and/or increased urinary excretion of albumin . However Kaysen and Al Bander ,reported that in nephrotic syndrome, there is increased urinary excretion of albumin and IgG accompanied by a decrease in their serum concentration **(Kaysen and Al Bander, 1990)**

This study also agreed with study of Mohammed *et al,* showed a highly statistically significant difference between groups (SSNS ,SRNS, and controls ) as regard IgG. This study also agreement with this result**(Mohammed et al., 2019a).**

## 4.3.Biochemical Parameters

### 4.3.1.Alb,T.Protein,Liver function test ,Glu, Vit D and Lipid Profile Distribution

The result in Table (4-3) for children showed High significance decreased ($P < 0.001$) in patients with nephrotic syndrome than healthy children in both serum albumin and total serum protein levels, with means (30.54, 46.03) g/l for S. Albumin and (50.23, 71.09) g/l for Protein respectively. Also it was appeared high significant decreased (*P<0.001*) in levels of Vitamin $D_3$ among patients than control children with mean(12.89, 40.180) ng/ml respectively. In liver function test results were showed significance decreased (*P<0.05*) in levels of both ALT and ALP in cases than controls with mean (17.7 , 20.68) U/l for ALT and (164.27 , 213.23)U/l for ALP. While in other parameters like GGT and ( LDH) levels found high significance increased (*P<0.001*) in case than control children as mean (101.59 ,28.15) U/l respectively for GGT and (577.98 ,241.48) U/l respectively for LDH. In state of Direct bilirubin it was significance increased *(P<0.05*) with mean (0.175 ,0.082) mg/dl respectively.

In Lipid profile parameters were resulted with high significant increased (*P<0.001*) in levels of all TG ,LDL and Cholesterol between case and control children with mean as (308.67 , 103.44) mg/dl ,(204.27 ,83.37) mg/dl and (330.62,148.89) mg/dl for each of them respectively. The results of table (4-3) also showed no significance decreased in levels of AST among cases and controls with mean (27.83 ,29.37)U/l respectively. Total bilirubin was showed no significance increased between patient and healthy as mean (0.389 ,0.342 ) mg/dl . As well as HDL had no significant increased in case than control children with mean respectively (58.51, 46.48) mg/dl. The last parameter glucose was also showed increased un significantly in cases than controls ,which mean was(115.27 ,106.21) mg/dl correspondingly .

**Table(4-3) : Biochemical Parameters of (healthy and NS )children age at (0.5 -15)years**

| The parameter | Control (N=42) | Patients (N=116) | *P value* |
|---|---|---|---|
| **Serum Albumin** | 46.03 ± 3.87 | 30.54 ± 4.36 | **0.0001 **** |
| **T. Serum Protein** | 71.09 ± 3.59 | 50.23 ± 16.35 | **0.0001 **** |
| **ALT** | 20.68 ± 8.24 | 17.17 ± 10.18 | **0.0464 *** |
| **AST** | 29.37 ± 9.88 | 27.83 ± 18.21 | **0.6036** |
| **ALP** | 213.23 ± 65.84 | 164.27 ± 93.58 | **0.0022 *** |
| **T. Bilirubin** | 0.342 ± 0.062 | 0.389 ± 0.311 | **0.3333** |
| **D. Bilirubin** | 0.082 ± 0.013 | 0.175 ± 0.251 | **0.0178 *** |
| **GGT** | 28.15 ± 16.23 | 101.59 ± 90.85 | **0.0001 **** |
| **(LDH)** | 241.48 ± 44.39 | 577.98 ± 477.48 | **0.0001 **** |
| **Glucose** | 106.21 ± 12.10 | 115.27 ± 32.25 | **0.0782** |
| **D3** | 40.18 ± 17.44 | 12.89 ± 12.95 | **0.0001 **** |
| **TG** | 103.44 ± 70.89 | 308.67 ± 233.77 | **0.0001 **** |
| **HDL** | 46.48 ± 10.54 | 58.51 ± 70.49 | **0.2733** |
| **LDL** | 83.37 ± 23.64 | 204.27 ± 115.72 | **0.0001 **** |
| **S. Cholesterol** | 148.89 ± 29.97 | 330.62 ± 158.32 | **0.0001 **** |

* significance differences *(P< 0.05)* , ** high significance differences (*P<0.001*)

Proteinuria and malnutrition have a usually beneficial effect. Malnutrition could come from a lack of protein. Children with nephrotic syndrome, on the other hand, may develop hypoproteinemia and vitamin deficiencies, that can lead to chronic hypoalbuminemia. This low serum albumin and micronutrients could cause edema and an ineffective immune response, leading in proteinuria repeat **(Gebrehiwot *et al.*, 2020).** Patients with a low serum albumin concentration may be at risk of losing protein like IgG & factors I and B, which have been observed to be low in the blood of patients having NS in active disease. These proteins are essential components of the complement system's alternative pathway, that contribute in opsonization, phagocytosis, & host defense. In compared to patients with NS solely and healthy controls, patients with NS or a history of infection of NS with ongoing infection

have significantly higher urinary excretion for factors I & B and lower blood levels of these factors. The low serum levels correlate with increased urinary excretion of these factors in those patients with a history of infection. There's also a positive correlation amongst factor I & albumin levels in patients with relapsing and infection **(Hingorani *et al.*, 2002).**In this study which appeared high significance decrease in levels of albumin agreement with the study of Rahul et al. **(Rahul *et al.*, 2015)** .Also in the study Rai et al., showed significance decrease in levels of serum albumin and total serum protein between nephrotic and healthy children **(Rai *et al.*, 2017)**.

Primary hepatic amyloidosis is a rare disease but an important one for a clinician to recognize. Hepatic amyloidosis should be suspected in cases of hepatomegaly, elevated ALP, and proteinuria, especially after ruling out other common liver diseases**(Doukas *et al.*).** NS may lead to a variety of complications, include dyslipidemia and coagulation disorders. The compensatory increased protein synthesis in the hepatic in response to a loss of numerous proteins, including albumin (Alb), into the urine, can explain most of these complications, though the pathophysiology still unknown. The enzyme cholinesterase (ChE) is formed in the hepatic and belongs to the serine hydrolase family. ChE is widely used in clinical practice as a valuable marker for assessing liver health, particularly protein synthesis. This enzyme, on the other hand, can cause an increase in NS. Based with one study, almost all people with NS showed hypercholesterinemia (HC)***(Goto et al.*, 2022).** A drop in colloid osmotic pressure, produced by a decrease of various proteins, is thought to trigger protein production in the liver as one of the mechanisms of enhanced protein synthesis. In reality, it has been shown that the quantity of Alb lost in the urine is proportional to the amount of Alb synthesized in the liver**(Goto *et al.*, 2022).** Although the reports and articles which measure these parameters NS patient it is not much or rare ,one study Zhu et al., which took these tests for adult nephrotic patients and compared them with patient have other kidney

disease ,showed no significances differences in levels of ALT ,AST ,ALP ,GGT ,and Total Serum Bilirubin but only founded significance differences for Direct serum Bilirubin **(Zhu *et al.*, 2018).**Because Lactate dehydrogenase enzymes is released into the blood after cell lesioning, measuring total serum (LDH) activity is an essential diagnostic parameter in many diseases with tissue damage. Only a few studies have studied at the function of total serum (LDH) activity in various kidney diseases. Filtrate proteins overflow the proximal tubules in nephrotic syndrome, where tubular cells reabsorb proteins by endocytosis. Tubular cells may become tired and damaged as a result of this high-energy procedure Large amounts of protein have a toxic effect upon tubular cells, increasing tubular cell turnover by raising cell proliferation and death .Serum proteins are normally protected from filtering into the urine by the glomerular barrier some serum protein (e.g. albumin and transferrin) are thought to migrate into the proximal tubules and cause inflammation, resulting in damage to the epithelial cells' apical & basolateral membranes in nephritic syndrome , so LDH can freely pass out of the cytoplasm into to the urine and serum as a result**(Mohás *et al.*, 2008)**.

In the study of Mohas *et al*., conclusive showed the higher (LDH) activities in the NS might be due to kidney damage. Their observation further strengthens the hypothesis of Remuzzi *et al.* , whereas the filtered excessive quantities of protein reach the proximal tubules and the protein over-reabsorption contributes to renal injury by activating inflammation **(Remuzzi, 2000)** . Their findings in mentioned study of Mohas et al. show that high serum total (LDH) activities in nephrotic syndrome can be used as a disease marker, and that routine testing mixed with isozyme analysis provide useful information about the illness's activity and severity. It cannot, however, take the place of quantitative protein determination**(Mohás *et al.*, 2008).**

The serum (LDH) activity and (LDH) isozymes in different groups of pediatric children with nephrotic syndrome were measured by Murdock et al (patients in relapse & patients in remission with or without steroid treatment) In compared to the other groups, relapse patients had significantly higher urine and serum total LDH activity **(Murdock *et al.*, 1981).** The researchers, on the other side, did not make any obvious conclusion between (LDH) isozymes and nephrotic syndrome**.** Theses agreement with this study and also agreement to study of Zhu *et al*., which found levels of Lactate dehydrogenase (LDH) with significance increase in nephrotic patient **(Zhu *et al.*, 2018).**

This study showed no differences and the levels of Random Blood Sugar under limited normal range ,this agreement with pervious study of Ningsih *et al.* **(Ningsih *et al.*, 2021).**

The majority of serum 25(OH)D is tightly linked to Vitamin D-binding protein (VDBP), while the other 10% to 15% is loosely bound to albumin, and in the market, a small portion, 0.03 to 0.04 %, circulates. The physiologically inactive fraction of 25(OH)D bound to VDBP. The bioavailability of albumin-bound fractions is poorly understood. In NS patients, both of these fractions are lost in the urine**(Banerjee *et al.*, 2020)**.

In the study of Banerjee *et al.* ,the total 25(OH)D levels were significantly different (lowest in NS relapse and highest in controls), and our data supported these results. Acute or chronic tubular damage may also prevent the proximal tubule from reabsorbing VDBP- and albumin-25(OH)D complexes, leading in lower levels of total 25(OH)D**(Tsuprykov *et al.*, 2018)**.

Children with high triglyceride levels at the time of diagnosis of nephrotic syndrome were 3.37 times more likely to relapse than those with borderline triglyceride

levels**(Sarker *et al.*, 2012)**. The finding of this study was in agreement with the study done in India Sarker *et al*., The podocyte biology explanation of proteinuria pathogenesis suggests that elevated triglyceride levels change the glomerular filtration barrier, which could explain this. Furthermore, the main infection's plasma protein loss will result in lipoprotein synthesis & low albumin levels. In response to the drop in serum albumin levels, the liver begins to produce more albumins. At the same time, the liver makes more cholesterol and triglycerides, which lead to the reappearance of nephrotic syndrome signs and symptoms. In a study by Gebrehiwot *et al*., 88 percent of children with a high triglyceride level and a blood albumin level of the less than 1.5 g/dl had relapse**(Gebrehiwot *et al.*, 2020).** HDL is a protective factor against atherosclerosis, according the Framingham risk score, which has been widely used in predicting the risk of disease related to atherosclerosisHDL particles are complex molecules that carry lipid from of the artery to the liver, where that is excreted by bile duct. HDL particles also contain various amounts of oxidants and antioxidants, that help in controlling systemic inflammation **(Astuti *et al.*, 2015).** Except for HDL, the values above are still above normal, according the National Cholesterol Education Program (NCEP), namely total cholesterol≥200 mg/dL, LDL> 130 mg/dL, triglycerides ≥100 mg/dL, and HDL≤ 40 mg/dL. Abnormalities These findings suggest that NS patients are likely to have atherosclerotic lesions, though this is debatable. While some clinical studies have found an increased risk of heart disease in older NS patients, others do not**(Ningsih *et al.*, 2021).**

In one study also similar to this study showed, TC , LDL and TG were significantly high in patients of nephrotic syndrome in comparison of control children. Further, HDL was significantly low in nephrotic syndrome group in comparison of control group **(Aslam and Sharma, 2018).** Corticosteroids may have an impact on lipid metabolism. The very first line of treatment for children in Indonesia, according to the NS consensus, should be corticosteroids. Because corticosteroid usage in

subjects varies in length and number of doses depending on the kind of NS being treated, lipid levels were significantly affected by how often kid has a relapse **(Astuti *et al.*, 2015).**

## 4.4.Hematological Parameters of study sample

### 4.4.1.WBC Message

The results in the Table(4-4) for differentiated WBC types showed significances higher (*P <0.05)* between cases and controls in both male and female children in levels of each Granulocytes (Gran) , Monocytes(Mid) ,Percent of Granulocytes (Gran%),Percent of Monocytes (Mid %).

Lymphocytes had not significance higher in both male and female patients than controls , however percent of Lymphocytes (Lym%), have significance lower(*p<0.05*) levels among cases than control ,also these levels had high significances decrease (*P <0.001*) in total numbers of cases and controls.

The tendency of nephrotic syndrome patients to bacterial infections has long been known. Infection was a common cause of mortality among these patients, especially before the advent of antibiotics. The higher risk of infection can be described in a number of ways . Excessive fluid collections, fragile nephrotic skin, edema producing dilution of local humoral immune factors, losses of IgG & complement factor B in urine, urinary zinc, & transferrin losses all contribute to lymphocyte dysfunction in nephrotic syndrome patients **(Floege and Feehally, 2018).** On the other hand, leukocyte phagocytic function has been found to be impaired. The phagocytosis functions of polymorphonuclear leukocytes (PMNLs) and monocytes were also evaluated in this study. In terms of PMNL phagocytic functions, there was no statistically significant difference in nephrotic syndrome patients and healthy controls**(Akyol *et al.*, 2007).**

**Table(4-4) : Hematological Parameters (WBC Message) of (healthy and NS )children age at (0.5 -15)years**

| Factor | Gender | Control | Patients | *P value* |
|---|---|---|---|---|
| **WBC** | **Female** | 8.51 ± 1.59 | 10.541 ± 3.74 | **0.0397 *** |
| | **Male** | 8.86 ± 2.27 | 12.388 ± 5.612 | **0.0026 *** |
| | **Total** | **8.73 ± 2.02** | **11.624 ± 4.992** | **0.0004**** |
| | ***P value*** | **0.5925** | **0.0492 *** | |
| **Lym** | **Female** | 4.33 ± 1.22 | 3.97 ± 2.32 | **0.5561** |
| | **Male** | 3.86 ± 0.94 | 4.29 ± 2.16 | **0.3308** |
| | **Total** | **4.04 ± 1.07** | **4.16** ± 2.23 | **0.7225** |
| | ***P value*** | **0.1681** | **0.4476** | |
| **Gran** | **Female** | 3.87 ± 0.84 | 6.10 ± 2.89 | **0.0036 *** |
| | **Male** | 4.58 ± 1.84 | 7.38 ± 4.66 | **0.0038 *** |
| | **Total** | **4.31 ± 1.56** | **6.85 ± 4.06** | **0.0001**** |
| | ***P value*** | **0.1553** | **0.0944** | |
| **Mid** | **Female** | 0.36 ± 0.15 | 0.67 ± 0.33 | **0.0006**** |
| | **Male** | 0.41 ± 0.28 | 0.70 ± 0.43 | **0.002 *** |
| | **Total** | **0.39 ± 0.24** | **0.69 ± 0.39** | **0.0001**** |
| | ***P value*** | **0.5152** | **0.6854** | |
| **Lym %** | **Female** | 49.78 ± 7.70 | 37.71 ± 14.08 | **0.0018*** |
| | **Male** | 44.63 ± 9.96 | 38.03 ± 13.63 | **0.0270*** |
| | **Total** | **46.6 ± 9.41** | **37.90 ± 13.76** | **0.0002**** |
| | ***P value*** | **0.0850** | **0.9024** | |
| **Gran %** | **Female** | 45.27 ± 8.14 | 56.05 ± 14.77 | **0.0073*** |
| | **Male** | 50.23 ± 10.76 | 55.93 ± 14.78 | **0.0766** |
| | **Total** | **48.34 ± 10.04** | **55.98 ± 14.71** | **0.0022*** |
| | ***P value*** | **0.1212** | **0.9657** | |
| **Mid %** | **Female** | 4.93 ± 1.14 | 6.57 ± 2.05 | **0.0035*** |
| | **Male** | 5.12 ± 2.40 | 6.29 ± 2.24 | **0.0288*** |
| | **Total** | **5.05 ± 2.00** | **6.41 ± 2.16** | **0.0005**** |
| | ***P value*** | **0.7689** | **0.4938** | |

*significance differences *(P< 0.05)* ,** high significance differences (*P<0.001*)

Guo et al. retrospectively reviewed patients at Jinling Hospital (a tertiary center in Nanjing, China) during 2000 to 2019. If a case had a prior medical history of NS, they were included in research Ten patients had a fever, with a body temp of 38.9 1.0 °C on average. Nine of the 11 patients had high white blood cell (WBC) levels, and all were lymphopenic, with a mean lymphocyte percent of 7.84.7 percent (normal range 20–40 percent) **(Guo et al., 2020a).**

In a study by Yousefichaijan *et al*., white blood cell component counting showed that monocytes in the steroid resistance group were 4% lower than normal, 96 % normal, and perfect (100 percent) normal in the other groups, Also, lymphocyte responses to steroids were 8% lower than normal and 92 % normal, compared to 33 % lower than normal, 3% higher than normal, and % normal in three other groups; thus, the difference was significant. Also, there was a difference in neutrophil count between two groups (SRNS against FRNS, SDNS and SRNS). But, when it came to basophil and eosinophil counts, the researcher found that they were normal in all four groups and that there was no difference between them **(Yousefichaijan *et al.*, 2016).**

The most common type of leukocyte, granulocytes, constitutes the initial line defense against pathogen invasion. However, granulocytes' versatility and plasticity have been increasingly revealed, particularly in terms of their versatile roles in coordinating adaptive immune responses. Under pathogenic or inflammatory conditions, granulocytes may acquire the role of antigen-presenting cells, according to a large body of recent evidence**(Lin and Loré, 2017).**

## 4.4.2.RBC Message

The results in the Table(4-5) showed there were no significance differences in levels of RBC ,HGB,HCT,MCV,MCH, and MCHC between cases and controls in both

male and female children .However there was high significance increases(*P <0.001*) in levels of RDWa and RDW% among cases and controls in both sexes ,with mean (44.13, 53.63) fL for RDWa and (11.05 , 13.06) % for RDW% respectively.

Urinary loss of iron, erythropoietin, transcobalamin, transferrin, & soluble transferrin receptors can induce anemia. Data on iron deficiency & iron deficiency anemia in nephrotic syndrome are rare, especially in iron-deficient endemic places like India, where the prevalence is as high about 70% in children under the age of 5, complicating or adversely impacting outcome in this subgroup of children**(Sreekanth *et al.*, 2021).**

According to Prinsen *et al*., there seems to be a rise in transferrin synthesis, that correlates with albumin production, but it is insufficient to ease the effects**(Prinsen *et al.*, 2001).**

Since nephrotic syndrome is a pro-inflammatory condition, serum ferritin alone as an iron storage marker could be falsely raised as an acute phase reactant, this strict definition of iron shortage in nephrotic syndrome is used**(Sreekanth *et al.*, 2021).**

The levels of Red blood cells (RBC )in this study also is not significantly higher in patients with NS ,similar to study of Abdel-Salam et al. **(Abdel-Salam *et al.*, 2020).** As well as they noted levels of Hemoglobin (HGB) and Hematocrit(HCT) was less higher in patients than control children and no differences significantly found similar to this study.

**Table(4-5) : Hematological Parameters (RBC Message) of (healthy and NS )children age at (0.5 -15)years**

| Factor | Gender | Control | Patients | *P value* |
|---|---|---|---|---|
| **RBC** | **Female** | 4.50 ± 0.36 | 4.55 ± 0.77 | **0.8036** |
| | **Male** | 4.61 ± 0.30 | 4.75 ± 0.67 | **0.3672** |
| | **Total** | **4.57 ± 0.33** | **4.66 ± 0.72** | **0.4367** |

| | | | | |
|---|---|---|---|---|
| | ***P value*** | **0.5496** | **0.1395** | |
| **HGB** | **Female** | 12.03 ± 0.64 | 11.97 ± 2.25 | **0.9169** |
| | **Male** | 12.11 ± 0.67 | 12.38 ± 2.34 | **0.5649** |
| | **Total** | **12.08 ± 0.65** | **12.21 ± 2.30** | **0.7190** |
| | ***P value*** | **0.7044** | **0.3471** | |
| **HCT** | **Female** | 34.95 ± 2.52 | 34.98 ± 6.94 | **0.9866** |
| | **Male** | 35.31 ± 2.32 | 35.81 ± 6.71 | **0.7119** |
| | **Total** | **35.17 ± 2.37** | **35.47 ± 6.79** | **0.7800** |
| | ***P value*** | **0.6390** | **0.5190** | |
| **MCV** | **Female** | 77.7 ± 1.73 | 76.55 ± 7.89 | **0.5670** |
| | **Male** | 76.58 ± 4.00 | 74.05 ± 9.63 | **0.3090** |
| | **Total** | **77.00 ± 3.34** | **75.08 ± 9.00** | **0.1799** |
| | ***P value*** | **0.2969** | **0.1413** | |
| **MCH** | **Female** | 26.85 ± 1.35 | 26.26 ± 2.68 | **0.4028** |
| | **Male** | 26.36 ± 1.76 | 26.40 ± 3.87 | **0.9597** |
| | **Total** | **26.55 ± 1.62** | **26.34 ± 3.42** | **0.7029** |
| | ***P value*** | **0.3464** | **0.8290** | |
| **MCHC** | **Female** | 34.53 ± 1.27 | 34.27 ± 1.17 | **0.4539** |
| | **Male** | 34.43 ± 1.15 | 34.59 ± 1.44 | **0.6130** |
| | **Total** | **34.47 ± 1.18** | **34.46 ± 1.34** | **0.9660** |
| | ***P value*** | **0.7939** | **0.2062** | |
| **RDWa** | **Female** | 44.73 ± 2.32 | 53.76 ± 10.68 | **0.0014 *** |
| | **Male** | 43.76 ± 3.19 | 53.54 ± 9.38 | **0.0001**** |
| | **Total** | **44.13 ± 2.90** | **53.63 ± 9.89** | **0.0001**** |
| | ***P value*** | **0.2979** | **0.9067** | |
| **RDW%** | **Female** | 11.08 ± 0.66 | 13.15 ± 1.96 | **0.0001**** |
| | **Male** | 11.03 ± 0.68 | 13.00 ± 2.50 | **0.0002**** |
| | **Total** | **11.05 ± 0.67** | **13.06 ± 2.28** | **0.0001**** |
| | ***P value*** | **0.8162** | **0.7292** | |

* significance differences *(P< 0.05)* , ** high significance differences (*P<0.001*)

The diversity in size of erythrocytes in the circulation is represented by the red blood cell distribution width (RDW), which is routinely recorded as a parameter of set of standardized complete blood count (CBC). RDW has traditionally been used to differentiate between different types of anemia. RDW has been linked to cardiovascular, hepatic, and renal problems in various studies in recent years. RDW has also been identified as an unique inflammatory marker in a variety of

inflammatory diseases, like septic shock, inflammatory bowel disease, & acute appendicitis **(Xu *et al.*, 2017).**

## 4.4.3.PLT Message

The outback of the Table(4-6) showed there were no significance differences for parameters : PLT ,PDWa ,PDW% ,and PCT among patients and healthy children in both sexes. However there was significance decreases (*P <0.05*) in levels of Mean Platelet Volume (MPV) among cases and control kids in both male and as total with means (7.55 ,7.82 ) fL, respectively .

**Table (4-6): Hematological Parameters (PLT Message) ) of (healthy and NS )children age at (0.5 -15)years .**

| Factor | Gender | Control | Patients | *P value* |
|---|---|---|---|---|
| **PLT** | **Female** | 377.62 ± 77.20 | 383.42 ± 160.62 | **0.8902** |
| | **Male** | 340.23 ± 40.70 | 372.32 ± 152.76 | **0.2948** |
| | **Total** | **354.47 ± 59.40** | **376.91 ± 155.47** | **0.3642** |
| | ***P value*** | **0.0462 *** | **0.7066** | |
| **MPV** | **Female** | 7.48 ± 0.50 | 7.59 ± 0.74 | **0.5826** |
| | **Male** | 8.03 ± 0.78 | 7.52 ± 0.62 | **0.0013 *** |
| | **Total** | **7.82 ± 0.73** | **7.55 ± 0.67** | **0.0304*** |
| | ***P value*** | **0.0161*** | **0.5817** | |
| **PDWa** | **Female** | 10.175 ± 0.60 | 10.21 ± 1.09 | **0.9033** |
| | **Male** | 10.91 ± 1.07 | 11.52 ± 7.88 | **0.6959** |
| | **Total** | **10.63 ± 0.98** | **10.98 ± 6.08** | **0.7114** |
| | ***P value*** | **0.0163 *** | **0.2556** | |
| **PDW%** | **Female** | 39.43 ± 1.13 | 39.27 ± 6.51 | **0.9228** |
| | **Male** | 40.41 ± 1.40 | 38.12 ± 6.95 | **0.0999** |
| | **Total** | **40.04 ± 1.38** | **38.60 ± 6.76** | **0.1734** |
| | ***P value*** | **0.0231 *** | **0.3696** | |
| **PCT** | **Female** | 0.28 ± 0.06 | 0.30 ± 0.10 | **0.4539** |
| | **Male** | 0.27 ± 0.04 | 0.27 ± 0.10 | **0.9999** |
| | **Total** | **0.27 ± 0.04** | **0.28 ± 0.10** | **0.5302** |
| | ***P value*** | **0.5199** | **0.1143** | |

*significance differences *(P< 0.05*) , ** high significance differences (*P<0.001*)

Platelets are acute-phase reactants which are often elevated in infection. Some researchers have attempted to link platelet count to splenic function, hypothesizing

that reduced splenic function increases infection susceptibility in NS patients, The authors of one study hypothesized that a high platelet count may be used as a surrogate indicator for splenic dysfunction **(Hingorani *et al.*, 2002).**

In a study by Yousefichaijan *et al.* ,about PLT, they found a significant differences between the two groups (SRNS versus FRNS, SDNS and SRNS), with 92 % normal, 8% higher than normal, and no one lower than normal in the steroid-responding group, but only 26 % normal, 70 percent higher than normal, and 4 percent lower than normal in the other tree groups. MPV, PCT, PDW, RDW, MCHC, MCH, MCV, HCT, RBC, and HGB blood biomarkers were also shown by Yousefichaijan *et al.*, who observed no significant differences between four groups **(Yousefichaijan *et al.*, 2016)** .

## 4.4.4. Blood groups of study sample

The results of Table (4-7) showed there was significance differences *(P <0.05)* in ABO group between controls and between patients alone which higher was O group in both .While in comparison with cases and controls in ABO group there were no significance differences in all groups except B group ,which had significance differences (*P <0.05)* among case and control children. B groups in patients children are more than in healthy .

**Table (4-7) : ABO Blood groups of (healthy and NS )children age at (0.5 -15)years .**

| Blood Group | Control | Patients | *P value* |
|---|---|---|---|
| **A** | 10 ( 23.81 %) | 25 (21.55 %) | **0.68028** |
| **B** | 6 (14.28 %) | 33 (28.45 %) | **0.02365 *** |
| **AB** | 6 (14.28 %) | 17 (14.66 %) | **1.0000** |
| **O** | 20 ( 47.62 %) | 41 ( 35.34 %) | **0.15304** |
| **Total** | **42 (100%)** | **116 (100 %)** | |
| ***P value*** | **0.00592 *** | **0.01154 *** | |

*significance differences *(P< 0.05*) ,**high significance differences (*P<0.001*)

Blood group antigen are genetically encoded proteins that can increase the risk of some diseases whilst lowering the risk of others. Several diseases have been linked to the ABO blood group in studies, such as coronary artery disease, depression, type 2 diabetes mellitus, chronic kidney failure, gastroduodenal ulcers, Crohn's disease, hepatitis B infection, Covid-19, thyroiditis, and several cancer types, including brain, breast, skin, pancreatic, and small cell lung cancers, as well as rheumatological diseases like Systemic Lupus Erythematosus **(Oruç *et al.*, 2021).** Many ideas (inflammation, infection) have been proposed to explain the propensity or protection associated with blood types, and while no clear mechanism has been found, they are still considered possible causes. However, a link among blood group and illness has not been shown in other studies. The findings of this study confirm those of Abbas *et al.*, who found no link between ABO blood type antigens as well as kidney function tests**(Abbas *et al.*, 2019).**

In another study by Oruc *et al*., no link between NS and blood group was found. The results of this study support their results.

### 4.4.5. The correlation between parameters (that have significance effects ) in nephrotic patients.

Table (4-8) showed the correlation is significant at 0.01 level ( 2-tailed ) in Nephrotic patients between WBC and Gran that was positive correlation (0.856) ,WBC and Mid with positive correlation (0.727) , LDL and S.Chol. also positive correlation (0.808).Also found significant correlation at 0.05 level ( 2-tailed ) in Nephrotic patient between WBC and CRP , CRP and Serum Albumin ,ALP and Direct Bilirubin ,GGT and LDH ,TG and LDL ,TG and S.Chol. which all was positive correlation at (0.509, 0.515, 0.566, 0.640, 0.526, 0.539) respectively.

To assess the risk of serious bacterial infection, the total white blood cell count (WBC) plus serum C-reactive protein (CRP) level are commonly used .The significant variance in WBC and CRP levels in both children with acute and non-serious bacterial infections, however, reduces the discriminating ability of these markers . The variability of the both groups in terms of microbial etiology might be a major factor in this difference**(Peltola *et al.*, 2006).**

**Table (4-8): The correlation between parameters (that have significance effects) in nephrotic patients.**

| | WBC | CRP | IgG | Serum Albumin | T.S. Albumin | ALT | ALP | GGT | LDH | Direct Bilirubin | D3 | TG | LDL | S. Chol. | Gran | Mid | RDWa | MPV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WBC | 1 | 0.509* | -0.02 | 0.282 | 0.121 | 0.015 | -0.05 | -0.126 | 0.252 | -0.018 | 0.253 | 0.112 | -0.203 | -0.254 | 0.856** | 0.727** | 0.003 | 0.023 |
| CRP | - | 1 | -0.06 | 0.515* | -0.105 | -0.13 | -0.06 | -0.020 | 0.107 | 0.138 | 0.072 | -0.118 | -0.093 | -0.249 | 0.442 | 0.380 | -0.287 | 0.018 |
| IgG | - | - | 1 | 0.058 | 0.292 | 0.134 | -0.03 | -0.105 | -0.17 | -0.061 | 0.222 | -0.103 | -0.104 | -0.042 | 0.037 | -0.108 | 0.011 | 0.110 |
| S. Albumin | - | - | - | 1 | 0.179 | 0.061 | 0.034 | -0.080 | -0.03 | -0.028 | 0.175 | -0.221 | -0.311 | -0.360 | 0.207 | 0.060 | -0.169 | 0.061 |
| T.S. Albumin | - | - | - | - | 1 | 0.395 | 0.081 | 0.127 | 0.189 | -0.105 | 0.453 | -0.178 | -0.392 | -0.351 | 0.176 | 0.057 | 0.035 | 0.373 |
| ALT | - | - | - | - | - | 1 | -0.004 | -0.093 | -0.01 | -0.034 | 0.108 | -0.197 | -0.354 | -0.229 | 0.010 | 0.041 | 0.068 | 0.334 |
| ALP | - | - | - | - | - | - | 1 | -0.126 | 0.094 | 0.566* | 0.108 | -0.097 | -0.037 | -0.081 | -0.054 | 0.118 | 0.127 | 0.003 |
| GGT | - | - | - | - | - | - | - | 1 | 0.640* | 0.027 | -0.019 | 0.397 | 0.292 | 0.219 | -0.058 | -0.129 | -0.089 | 0.298 |
| LDH | - | - | - | - | - | - | - | - | 1 | -0.071 | 0.066 | 0.385 | 0.071 | 0.102 | 0.345 | 0.157 | 0.137 | 0.293 |
| D. Bilirubin | - | - | - | - | - | - | - | - | - | 1 | -0.103 | 0.031 | 0.179 | 0.069 | -0.016 | 0.167 | -0.169 | -0.09 |
| D3 | - | - | - | - | - | - | - | - | - | - | 1 | -0.249 | -0.448 | -0.450 | 0.339 | 0.139 | 0.097 | 0.128 |
| TG | - | - | - | - | - | - | - | - | - | - | - | 1 | 0.526* | 0.539* | 0.107 | 0.019 | 0.139 | -0.18 |
| LDL | - | - | - | - | - | - | - | - | - | - | - | - | 1 | 0.808** | -0.192 | -0.136 | 0.132 | -0.05 |
| S. Chol. | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 | -0.282 | -0.095 | 0.077 | -0.17 |
| Gran | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 | 0.460 | 0.122 | 0.131 |
| Mid | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 | -0.037 | -0.01 |
| RDWa | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 | -0.005 |
| MPV | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 |

*The correlation is significant at 0.05 level (2-tailed)

**The correlation is significant at 0.01 level (2-tailed)

In study of Peltola *et al.* , invasive bacterial infections had greater median WBC and CRP levels than viral infections, but values were disappeared **(Peltola *et al.*, 2006).**

Serious infection, particularly cellulitis and spontaneous bacterial peritonitis (SBP), can follow idiopathic nephrotic syndrome (INS)) Peritonitis, that has a percentage case fatality rate of 2–6% in INS, is a common illness. Hypoalbuminemia in the INS has been linked to a greater risk of developing SBP, implying that intravenous administration of human albumin solutions to patients with SBP caused by liver cirrhosis may be beneficial. The discovery revealed that serum albumin, as one of the most important and abundant antioxidants in vivo, plays a vital part in the host defense system **(Kaneko *et al.*, 2012).**

In the same study of Kaneko *et al.* , hypoalbuminemia samples were found to have weakly positive CRP.

The positive correlation among WBC and mid conforms with previous studies showing WBC is the most often used measure to evaluate infection, but it is also the least helpful. Leukocytosis or leukopenia can occur as a result of septic shock. Between these two extremes, many septic patients with a normal WBC occur (such patients usually develop leukocytosis in a delayed way). For example, 50% of patients with bacteremia who arrive to the hospital may have had a normal WBC **(Seigel *et al.*, 2012)**. As a result, while a significantly abnormal WBC may indicate infection, a normal WBC tells little. If the WBC is abnormally low, the absolute neutrophil count must be evaluated. Neutropenia is defined as a neutrophil count of less than 500/microliter or a decreasing count with in range of 500–1,000/microliter. Neutropenia patients frequently fail to show focal indications of infection.

Granulocytes, as key components of the innate immune system, have long been thought of as fast responder in first lines of defense against pathogens. Granulocytes

have long been suspected to only have a role in the first phase of the host defense**(Lin and Loré, 2017)**. This agreement with positive correlation of study with WBC so both have important role in defenses and immunity of the body.

The positive correlation of WBC and Mid was in agreement with the role of mono cytes in body, Polymorphonuclear leucocytes & mononuclear phagocytes are well-known for their role in host defense, however, as far as we know, their role in IMCNS (Idiopathic Minimal Change Nephrotic Syndrome) has not been investigated. In study of Estevez *et al.* ,when compared with healthy controls, the monocyte lytic activity of people with IMCNS was decreased. Using either *C. pseudotropicalis* or *C. albicans* as target cells, the differences were statistically significant**(Estevez *et al.*, 1989).**

In every day clinical routine, total serum LDH activity is a routinely used laboratory parameter. The enzyme is released into the circulation when cells are damaged. In a variety of conditions, like hepatological, cardiological, even hematological disease, measuring enzyme activity is quite useful. Due to the overall high nephrotic proteinuria, the proximal tubular cells are severely damaged**(Mohás *et al.*, 2008).**

GGT (gamma-glutamyl transferase) is a broadly distributed enzyme involved in amino acid absorption that is found in the liver, small bowel, and kidney. It is considered a sensitive but not specific indicator of liver disease .GGT levels rise by aging, diabetes, obesity, and congestive heart failure. GGT has also been utilized as a marker for transient elevations in GGT after acute myocardial infarction. Several population - based studies have recently shown strong correlations between GGT & blood pressure & lipid metabolism **(Wannamethee *et al.*, 1995).**

On the other hand series of epidemiological studies have suggested serum gamma glutamyl transferase (GGT) within its normal range might be an early marker of

oxidative stress. Oxidative stress appears to be a key component of many reactions associated with chronic inflammation**(Lee and Jacobs Jr, 2005)**.

The nephrotic state is associated with markedly increased total cholesterol and LDL cholesterol levels, due to both impaired clearance and increased production**(Hari *et al.*, 2020).**

Positive correlation between TG and serum cholesterol and between TG and LDL and also correlation among LDL and Serum cholesterol all of them were in agreement with pervious study such Ningsih *et al.* , It was shown that in individuals with NS, an atherogenic lipid profile is linked to an increased risk of cardiac disease like myocardial infarction.

Several reports of myocardial infarction have also been documented in children. However, persistent lipid abnormalities in patients with NS, even in remission, the risk of long-term cardiovascular events for children with NS remains unclear**(Ningsih *et al.*, 2021).**

The lipid metabolism disorders in NS can improve with treatment, but can persist during periods of remission**(Kniażewska *et al.*, 2009).**

## 4.5.Nanotechnology Study

### 4.5.1. Infrared spectrum (FTIR)

#### 4.5.1.1 . FTIR ( Infrared Spectrum) of zinc oxide (ZnO)

FT-IR spectrum of free-ceftriaxone antibiotic figure (4-1): The doublet band at 3444 attributed to ($NH_2$) stretching. The absorption band at 3252 for (O-H) groups stretching. The band around 3117 attributed to the amidic (N-H) stretching. The band at 3047 assigned to the aromatic (C-H) stretching of thiazole ring. The bands at 2935 due to aliphatic (C-H) stretching. The strong band around1735 due to carboxylic (C=O) stretching. The band at 1647 for amidic (C=O) stretching. The bands at 1604,

1535 and 1500 assigned to (C=N) groups stretching. The band around 1396 assigned to ($CH_3$) bending. The band at 1365 for (C-N) stretching. The band at 1284 for (C-O) stretching of carboxylic group. The band around 1184 for (C-OH) stretching. The band around 1107 for (C-$OCH_3$) stretching. The band at 802 due to aromatic (C-H) bending.

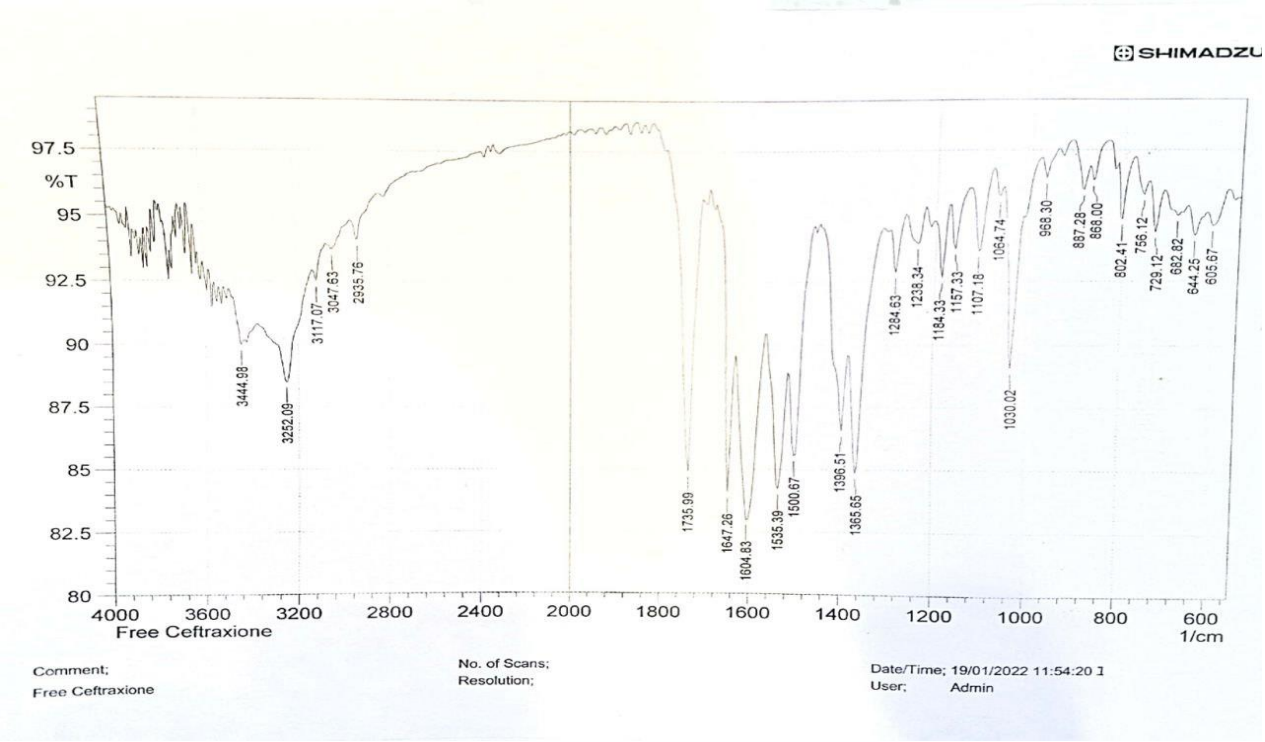



Figure (4-1) :FTIR for free-ceftriaxone

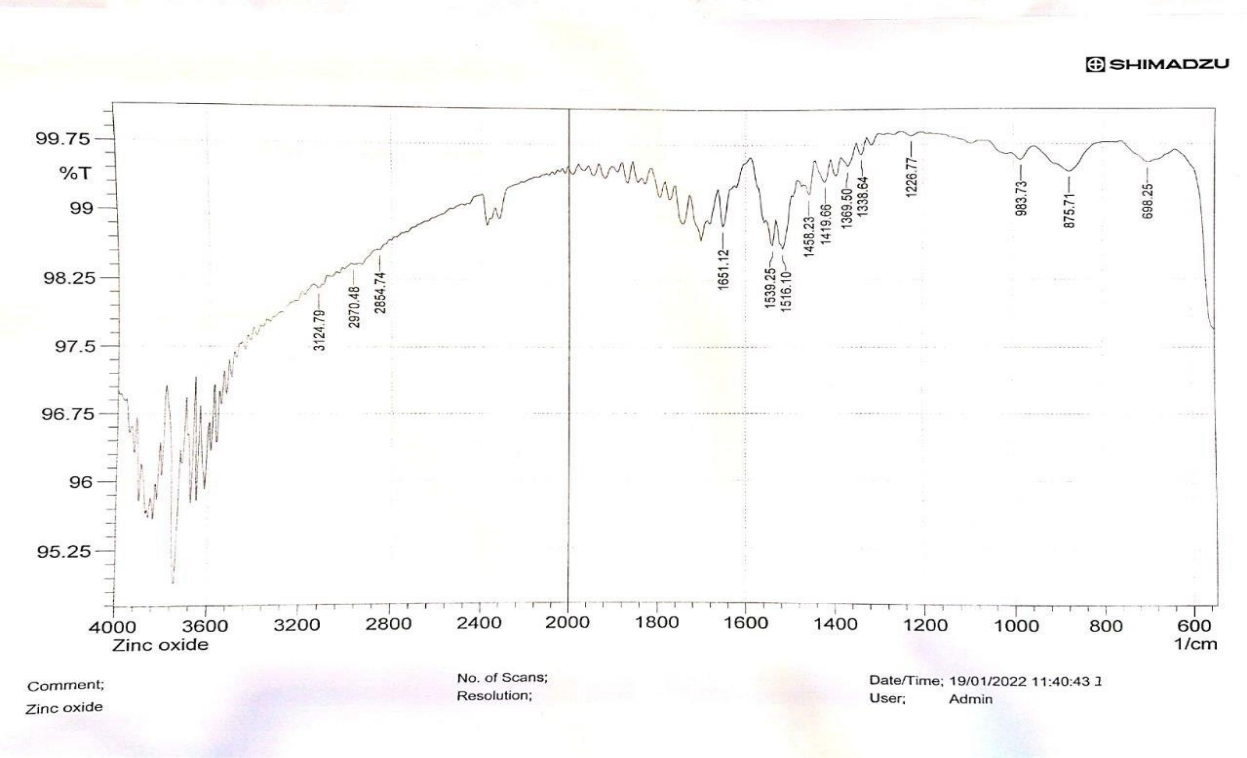



Figure (4-2) :FTIR for free-Zno

FT-IR spectrum of nano Nano-Zno-Ceftriaxone figure (4-3): The doublet band around 3440 attributed to ($NH_2$) stretching. The absorption band at 3279 for (O-H) groups stretching which was shifted to higher frequency. The band around 3180 attributed to the amidic (N-H) stretching. The band at 3028 assigned to the aromatic (C-H) stretching of thiazole ring which was shifted to lower frequency. The bands at 2947 due to aliphatic (C-H) stretching which was shifted to higher frequency. The strong band around1739 due to carboxylic (C=O) stretching which was a little shifted to higher frequency. The band at 1651 for amidic (C=O) stretching which was also a little shifted to higher frequency. The bands at 1604, 1543 and 1516 assigned to (C=N) groups stretching which were shifted to higher frequency. The bands around 1454 and 1419 assigned to ($CH_3$)groups bending. The band at 1369 for (C-N) stretching. The broad band at 1215 for (C-O) groups

stretching (overlapped). The broad band at 879 due to aromatic (C-H) bending and (Zn-O) stretching (overlapped).

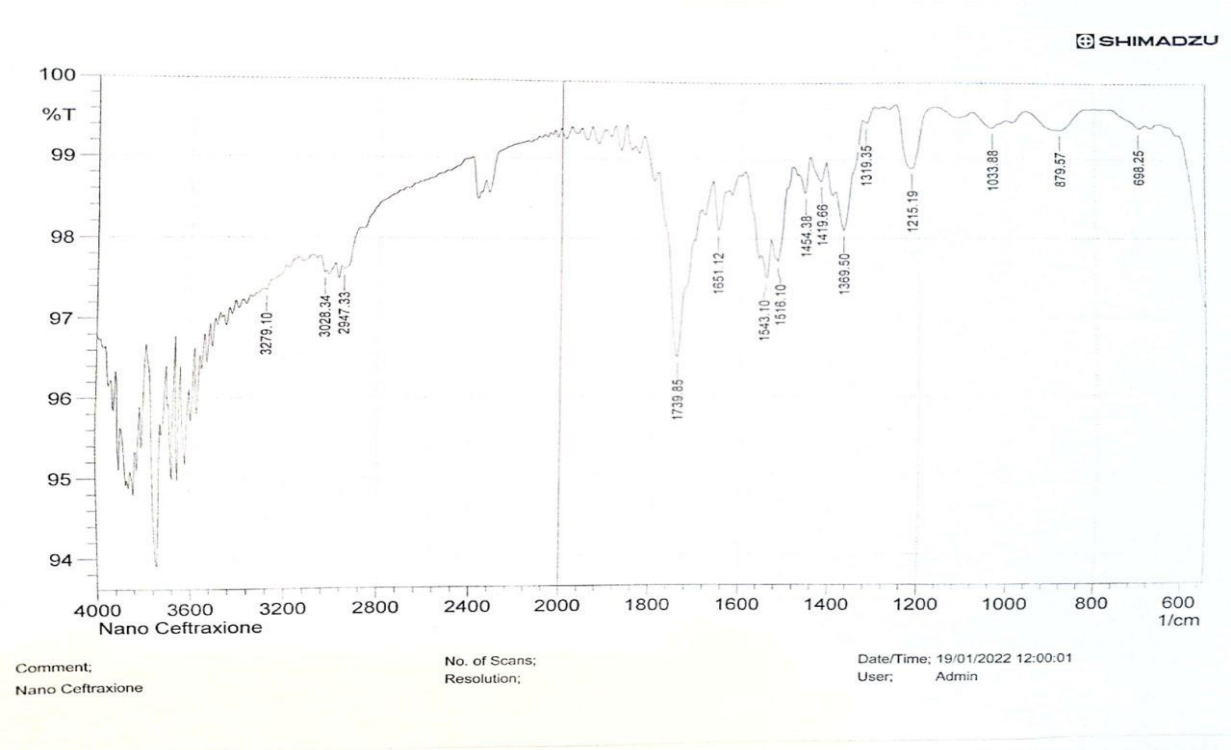



Figure (4-3):FTIR for Nano-Zno-Ceftriaxone

### 4.5.1.2.Fourier Transform Infrared Spectroscopy (FT-IR) for LDH

FT-IR spectrum of free LDH figure (4-4): The doublet band at 3375 and 3252 attributed to ($NH_2$) stretching. The strong band around1735 due to carboxylic (C=O) stretching. The band at 1647 for amidic (C=O) stretching. The band at 1346 for (C-N) stretching**(Duceac *et al.*, 2018)**.

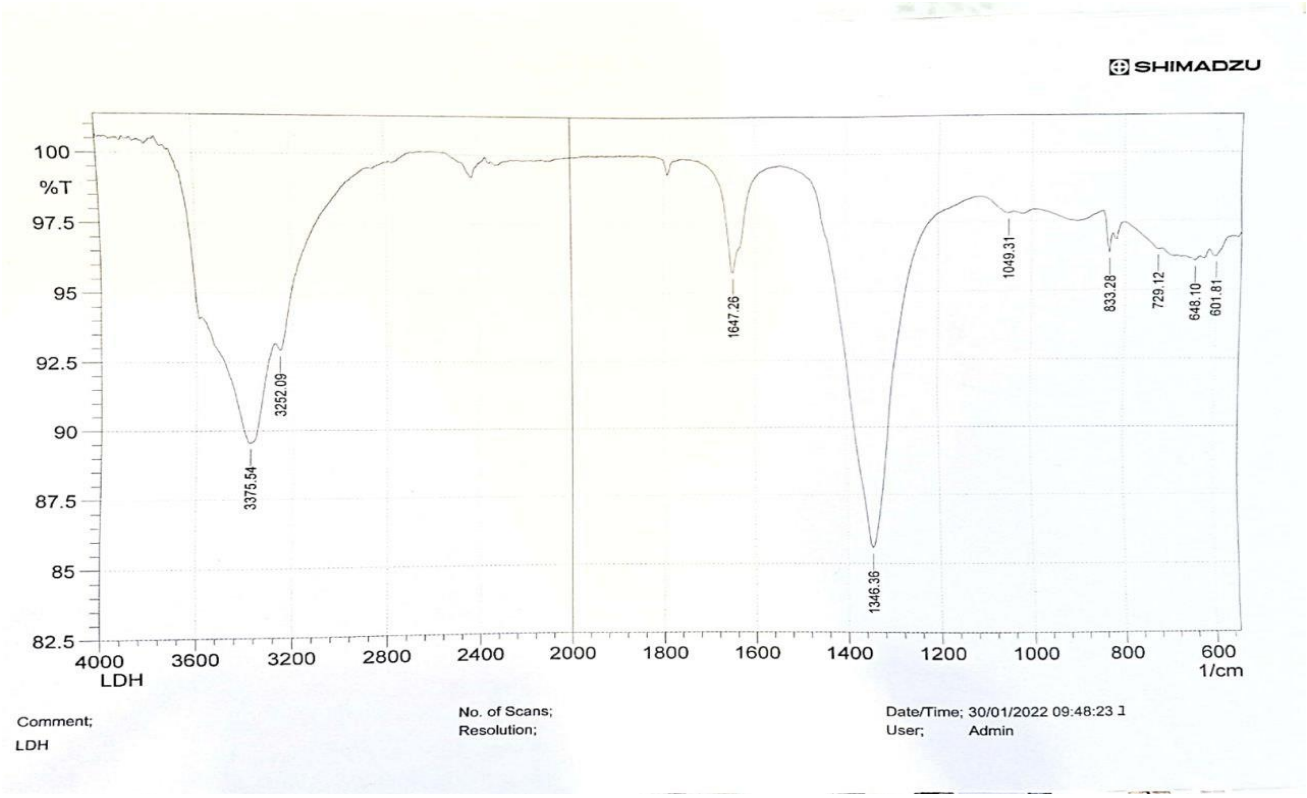



Figure (4-4): FT-IR for free LDH

FT-IR spectrum of Nano-LDH-Ceftriaxone antibiotic figure (4-5) : The band at 3460 attributed to ($NH_2$) stretching which was shifted to higher frequency. The absorption band at 3383 for (O-H) groups stretching and amidic (N-H) stretching (overlapped) which was also shifted to higher frequency. The band at 3050 assigned to the aromatic (C-H) stretching of thiazole ring. The bands at 2935 due to aliphatic (C-H) stretching. The strong band around1735 due to carboxylic (C=O) stretching. The band at 1643 for amidic (C=O) stretching which was a little shifted to lower frequency. The broad band at 1562 assigned to (C=N) groups stretching (overlapped). The band around 1396 assigned to ($CH_3$) bending. The band at 1365 for (C-N) stretching. The broad band at 1211 for (C-O) groups stretching of (overlapped). The band near 840 due to aromatic (C-H) bending.

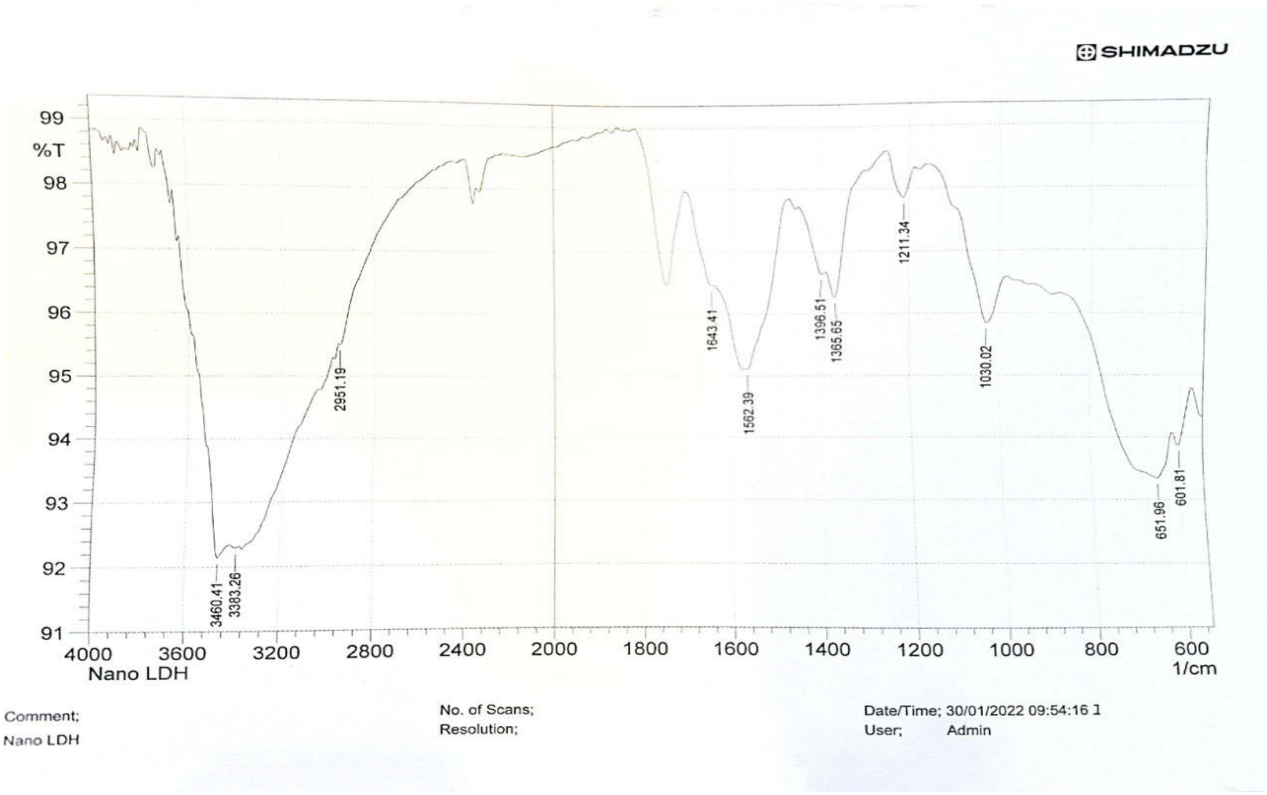
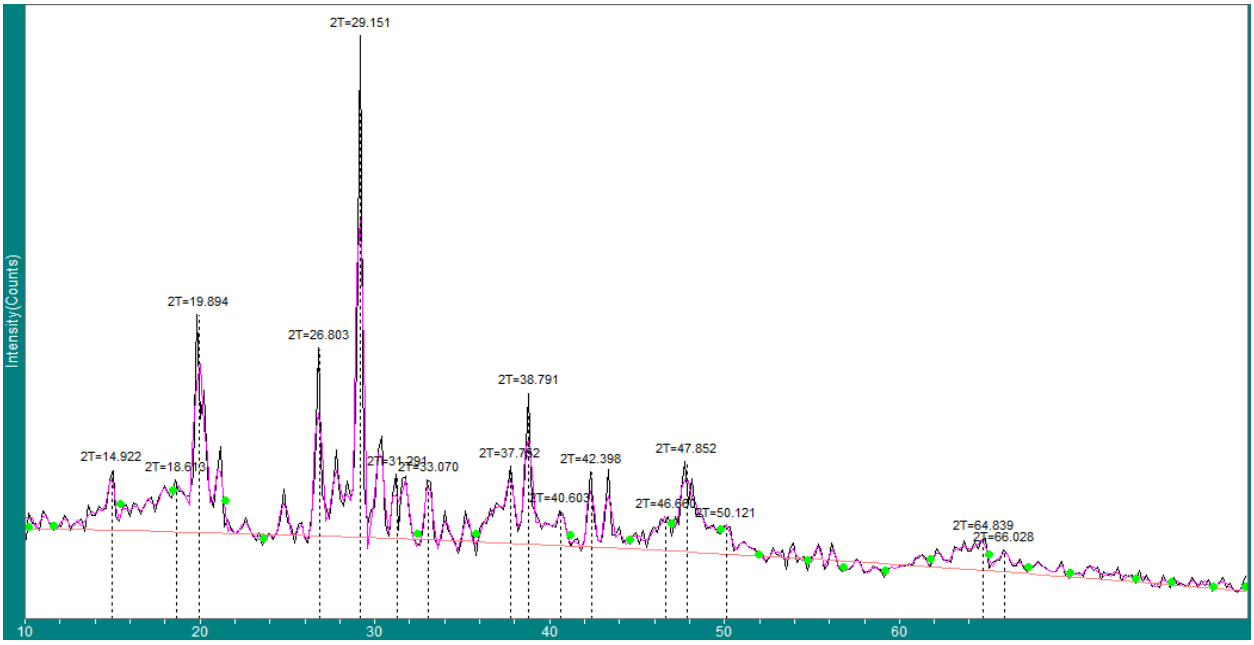


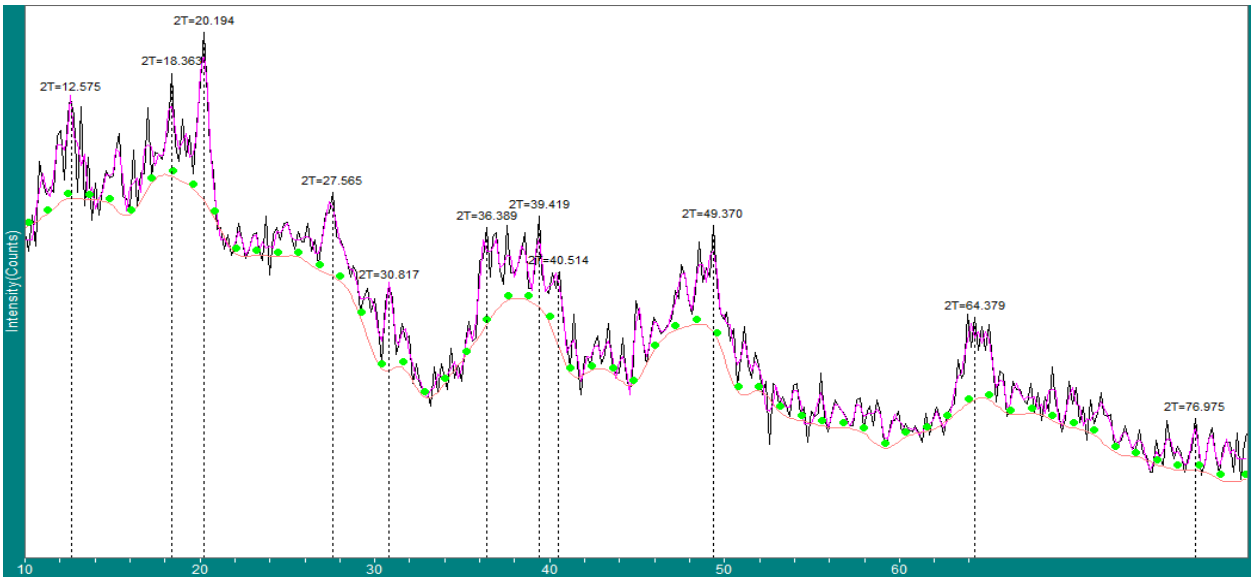

Figure (4-5) : FTIR for Nano-LDH-Ceftriaxone

### 4.5.2. (XRD)

Figure(4-6) shows the X-ray diffraction spectrum of the magnesium/aluminum Di hydroxide layer (Mg/Al-NO3-LDH) noting the crystalline levels (003), (006), and (009). The plane (003) appears at an angle of 19.8° and a crystal distance of 0.44 nm as for the level (006), it appears at the angle of 29.1° with a crystal distance of 0.30 nm while the plane (009) appears at an angle of 38.7° and a crystalline distance equal 0.23 nm. And by observing the X-ray diffraction spectrum of hybrid Nano LDH Ceftriaxone Mg/Al-$No_3$-CT , it shows diffraction spectrum of the plane (003) appears at an angle of 12.5° with a crystal distance 0.7 nm ,it also shows the diffraction of the plane (006) appears at an angle of 20.1° with a crystal distance 0.43 nm in addition to the appearance of the plane (009) appears at an angle of 39.4° with a crystal distance of 0.22 nm as shown in the figure (4-7).




Figure(4-6): XRD for free LDH

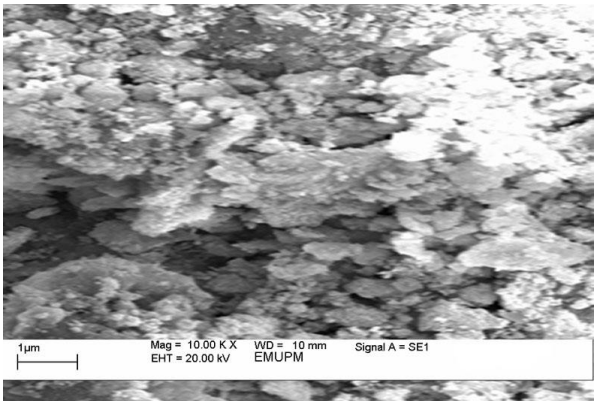



Figure(4-7): XRD for Nano LDH

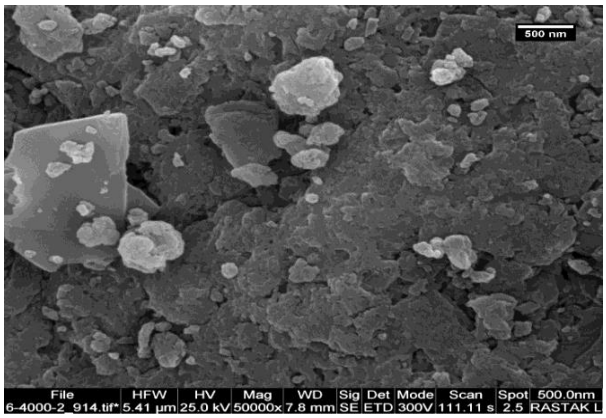
### 4.5.3. Scanning Electronic Microscope Characterization (SEM)

Figure(4-8)shows a scanning electron microscope image of theDi hydroxide layers is observed, in which plate-like structures with few pores and irregular shapes &sizes are observed **(Wang and Zhang, 2012).** In scanning electron microscope picture of ceftriaxone layers, where it is observed scanning electron microscopy examination

showed ,the rough and irregular surfaces appeared in Ceftriaxone powder **(Cho *et al.*, 2004).** It is evident in the scanning electronic microscope of the hybrid Nano ceftriaxone in figure(4-9) that the plate-like structures have turned into irregular shapes as well as the absence of pores, which indicates the success of the process of inserting the drug between the two hydroxide layers.



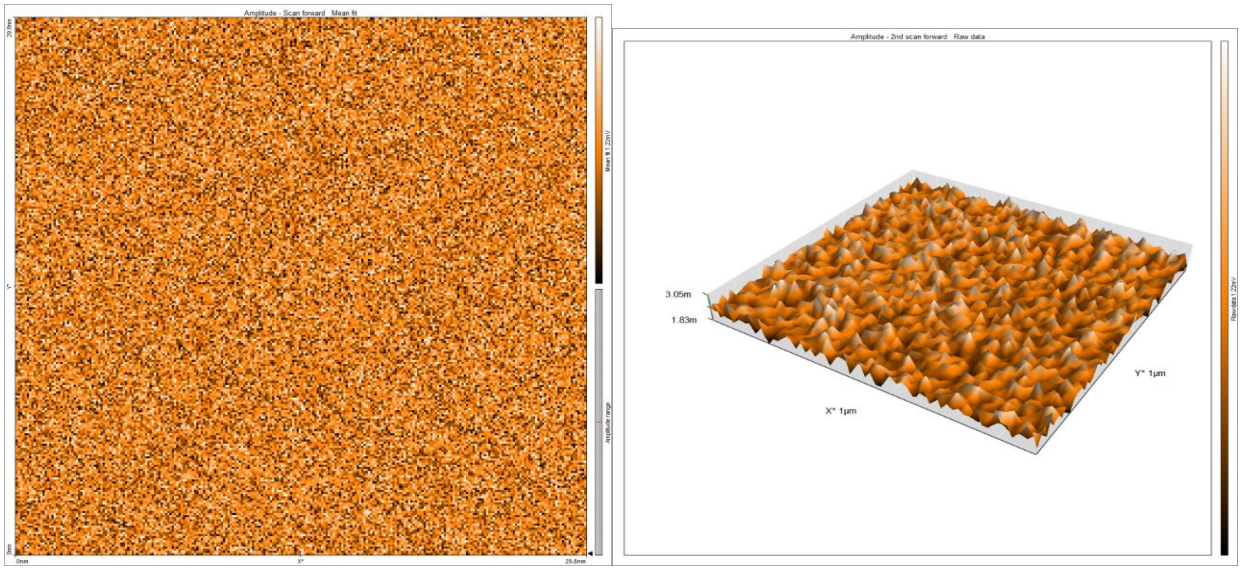

Figure(4-8): SEM for (Mg/Al-$NO_3$-LDH) layers




Figure(4-9): SEM for ceftriaxone layers

### 4.5.4. Atomic Force Microscope Characterization (AFM)

The exterior surface of the nanohybrid-ceftriaxone was studied by AFM.Figure (4-10) depicts semispherical shapes of Nano LDH ceftriaxone in two dimensions. The Figure(4-11)depicted a three-dimensional picture of the surface section of the Nano hybrid antibiotic, indicating that the elevation of molecular assemblies reaching 3.05 nm. This indicates the manufacture of a hybrid drug compound of ceftriaxone and hydroxide layers Mg-Al-No3-Ceftraxione.




Figures(4-10):AFM for Nano LDH (Two dimensions),Figures(4-11):AFM for Nano LDH(Three dimensions).

**Table (4-9): Granularity Accumulation Distribution Report**

| | |
|---|---|
| **Sample:**Sample Name | **Code:**Sample Code |
| **Line No.:**lineno | **Grain No.:**373 |
| **Instrument:**SPM | **Date:**2022-03-28 |

| | |
|---|---|
| **Avg. Diameter:2.43 nm** | **<=10% Diameter:1.20 nm** |
| **<=50% Diameter:2.20 nm** | **<=90% Diameter:3.80 nm** |

| Diameter(nm) < | Volume(%) | Cumulation(%) | Diameter(nm) < | Volume(%) | Cumulation(%) | Diameter(nm) < | Volume(%) | Cumulation(%) |
|---|---|---|---|---|---|---|---|---|
| 0.60 | 0.80 | 0.80 | 2.60 | 9.65 | 64.88 | 4.60 | 0.80 | 96.78 |
| 0.80 | 0.80 | 1.61 | 2.80 | 6.97 | 71.85 | 4.80 | 0.54 | 97.32 |
| 1.00 | 1.61 | 3.22 | 3.00 | 5.09 | 76.94 | 5.00 | 0.54 | 97.86 |
| 1.20 | 4.02 | 7.24 | 3.20 | 4.02 | 80.97 | 5.20 | 0.80 | 98.66 |
| 1.40 | 7.51 | 14.75 | 3.40 | 2.41 | 83.38 | 5.40 | 0.54 | 99.20 |
| 1.60 | 6.17 | 20.91 | 3.60 | 3.49 | 86.86 | 5.60 | 0.27 | 99.46 |
| 1.80 | 5.63 | 26.54 | 3.80 | 2.41 | 89.28 | 5.80 | 0.27 | 99.73 |
| 2.00 | 10.46 | 37.00 | 4.00 | 2.95 | 92.23 | 6.20 | 0.27 | 100.00 |
| 2.20 | 10.19 | 47.18 | 4.20 | 2.68 | 94.91 | | | |
| 2.40 | 8.04 | 55.23 | 4.40 | 1.07 | 95.98 | | | |

It is clear from Table(4-9)that the average diameters of the anti-hybrid nanoparticles amounted to a 2.43 nanometer. The process of preparing this hybrid antibiotic led to obtaining Nano antibiotic confined between 0.6 to 6.20 nanometers and that the highest percentage of these nanoparticles reached 10.46% for the particles with a diameter 2.00 nm, while the lowest percentage of minutes was 0.27% in diameters (5.6, 5.8, 6.2 )nm .

### 4.5.5. CHNS elemental analysis

The results in this analysis showed that contents of these elements carbon, nitrogen, hydrogen, and sulfur in (Mg/Al-NO3-LDH) ceftriaxone (nano antibiotic) were(10.51, 4.628, 1.997, 4.294) respectively . The contents of free ceftriaxone antibiotic for same elements were(24.19, 12.09, 1.74, 10.02) respectively as in Table(4-10).

**Table(4-10): Elemental analysis of nano-ceftriaxone**

| NO. | Name | Weight (mg) | $O_2$ | C/N Ratio | Content [%] | Peak Area |
|---|---|---|---|---|---|---|
| 1 | Nano(LDH) Ceftriaxone | 7.7010 | Index 2 | 2.271 | N: 4.628<br>C: 10.51<br>S: 4.294<br>H: 1.997 | 11432<br>18279<br>3275<br>11364 |
| 2 | Free Ceftriaxone | 9.1460 | Index 1 | 2.000 | N: 12.09<br>C: 24.19<br>S: 10.02<br>H: 1.740 | 35042<br>49923<br>9177<br>11793 |

From these results appeared the percentage of ceftriaxone which conjugated with LDH layers was 43.447.(by divided Carbone of Nano ceftriaxone on Carbone of free then multiply with 100 % ; 10.51/24.19 *100% =43.447% )

This result explained if use of nano LDH ceftriaxone was successful or useful as treatment .which showed from carbon elements content that 43%of ceftriaxone found in nano drug (Mg/Al-NO3-cefetriaxone) and in this percentage it remain high activity on tested bacteria in this study also nano LDH ceftriaxone can cause less side effects by increasing intracellular delivery that was similar to **(Duceac *et al.*, 2018).**

## 4.6.Bacterial Identification

**Table (4-11) :The bacteria isolated from Nephrotic Syndrome patient children**

| Genus | Species | Number |
|---|---|---|
| *Acinetobacter* | *Bumanii* complex | 2 |
| *Staphylococcus* | *Warnerii* | 2 |
| *Bacillus* | *Cerus* | 2 |

### 4.6.1.1. Identification of Gram Positive Bacteria

Diagnosis Gram positive bacteria *Staphylococcus warneri* were isolated from two children with Nephrotic syndrome whom had infection after surgery . It was identified by VITEK® 2 Compact devices by using many deference biochemical tests for Gram positive bacteria .

### 4.6.1.2. Identification of Gram Negative Bacteria

Diagnosis Gram Negative bacteria *Acinetobacter bumani complex* were isolated from two child with Nephrotic syndrome. It was identified by VITEK® 2 Compact devices by using many deference biochemical tests for Gram Negative bacteria .

### 4.6.2. Identification by Polymerase Chain Reaction Technique

Two positive blood culture with unknown bacteria isolation which was not identified by VITEK® 2 Compact devices was diagnosed by PCR technique

### 4.7.1.Disgnosis no identified bacteria by PCR

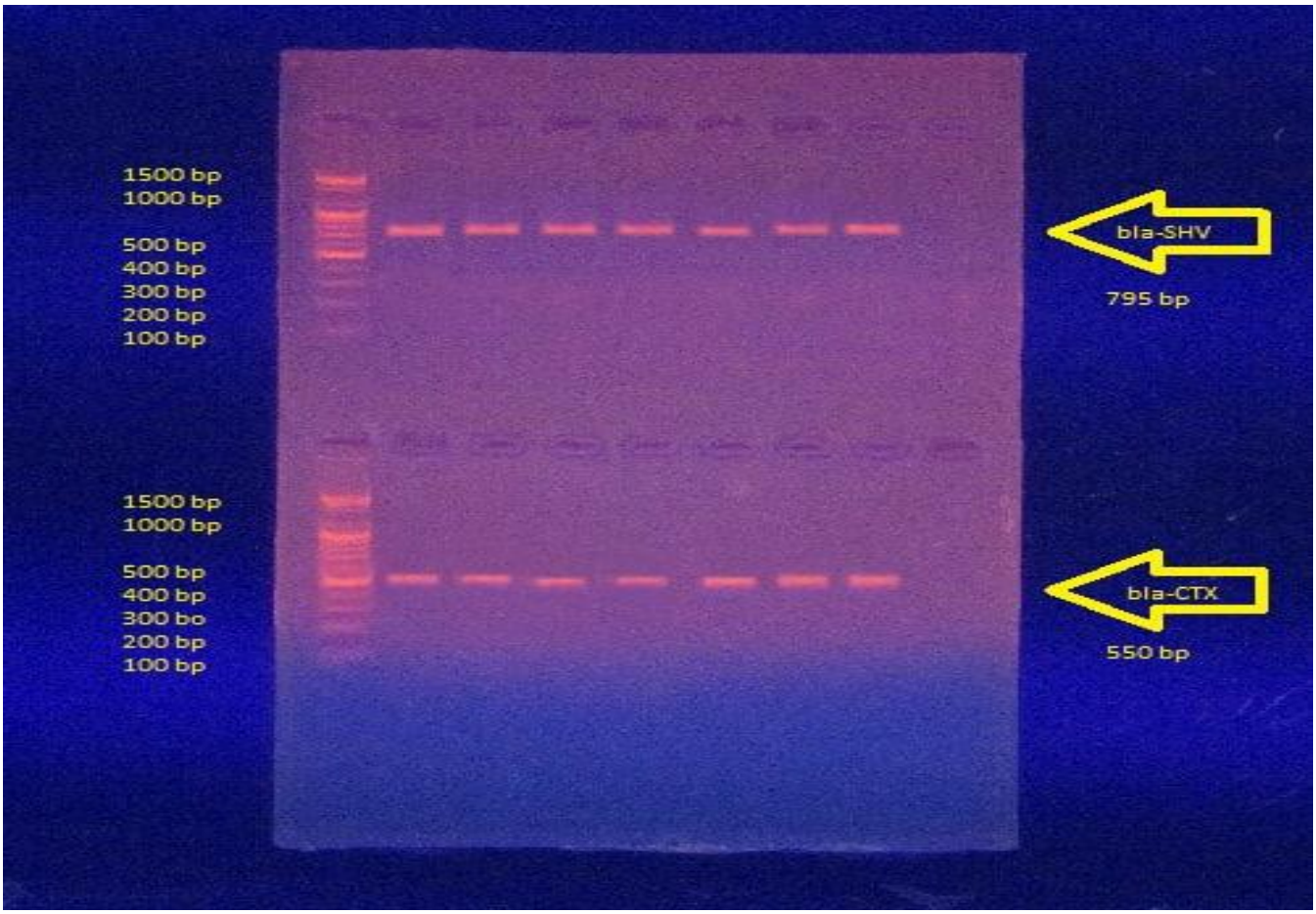
The results of PCR product for non diagnosed isolated bacteria showed as sequences in Figure(4-12) ,which obtained and after checked in BLAST tool showed them are associated with *Bacillus cerus* species bacteria.


Figure (4-12): results of sequencing genes for *Bacillus cerus*

## 4.7.2.Diagnoses resistant genes

### 4.7.2.1.*Accinitobacter bumanei* complex resistance genes for ceftriaxone

In figure(4-13), the results of detected genes of *Acinetobacter bumani* which resistance to ceftriaxone by PCR then appeared by gell electrophoresis showed that $bla_{SHv}$ and $bla_{CTX\_M}$ were found and appeared in band 795bp and550 bp respectively in this species of *Acinetobacter bumani* complex isolated from child female with nephrotic syndrome who had bacteremia.




Figure(4-13) :Ethidium bromide stained agarose gel of PCR products amplified bla-CTX and bla-SHV primers from *Acinetobacter bumanei* extracted DNA (550 bp,795 bp)

*Acinetobacter baumannii*, which is linked to nosocomial infections, is one of the top six drug-resistant bacteria. The widespread use of the -lactam antibiotic group

has resulted in the evolution of drug resistance as well as a severe clinical dilemma. Among the newer β -lactamases, extended spectrum β -lactamases (ESBLs) have emerged as a critical cause of cephalosporin resistance **(Smiline *et al.*, 2018).**

The most significant β-lactamase genes are variants of CTX-M, SHV, TEM, VEB, GES, PER, TLA and OXA which have broadened the substrate specificity against ceftazidime, cefotaxime and ceftriaxone . Moreover, many clinical pathogens harbor more than one β-lactam genes . Plasmid association of these genes makes them easily spreadable**(Abrar *et al.*, 2019).**

Asian countries are highly affected by extended spectrum-β-lactamase-producers inducing multidrug-resistant phenotype Several studies have reported the community-association of ESBL-producers**(Jean and Hsueh, 2011).**

Mutations in penicillin-binding proteins (PBPs), changes in membrane permeability, and chromosomal or plasmid-borne cephalosporinases generated by -lactamases (bla-genes), blaTEM, blaSHV, and blaCTX-M have all been implicated in resistance in *A.baumannii***(Smiline *et al.*, 2018).**

The result in this study was similar to study of Abrar *et al.* , which also did not find blaTEM encode gene in 8 isolated *Acinetobacter bumani* species in Pakistan.

Smiline *et al.* found that all 73 *A.baumannii* isolates were resistant to more than one cephalosporin antibiotic, and molecular analysis of blaTEM, blaSHV, and blaCTX-M revealed PCR positivity of 57.5 % (n = 42) for blaTEM, 6.8 % (n = 5) for blaSHV, and 6.8 % (n = 5) for blaCTX-M , However, none of the strains had blaCTX-M. Three isolates (4.1 percent) included blaTEM & blaSHV. DDST positive isolates had 30.1 percent (n = 22) and 1.4 percent (n = 1) blaTEM & blaSHV, respectively, whereas CDM verified strains had 32.9 percent (n = 24) and 2.7 percent (n = 2) blaTEM and blaSHV positive isolates **(Smiline *et al.*, 2018)**.

### 4.7.2.2.*Staphylococcus warneri* resistance gene for ceftriaxone

In figure(4-14) the results of detected genes of *Staphylococcus warneri* which resistance to ceftriaxone by PCR then appeared by gell electrophoresis showed that blaZ and was found and appeared in band 173 bp in this species of *Staphylococcus warneri* isolated from female child with nephrotic syndrome that had bacteremia after appendix surgery.

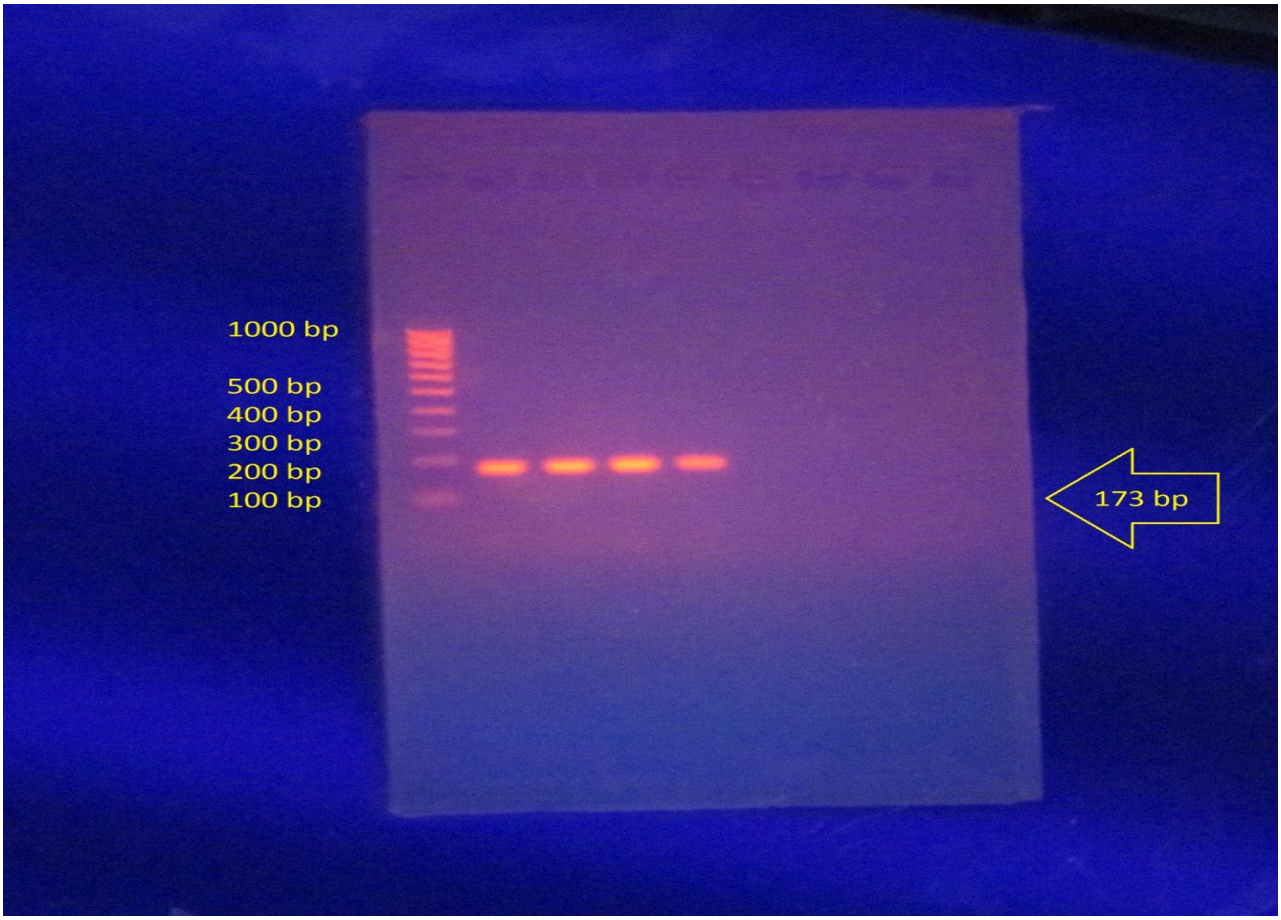



Figure (4-14): Ethidium bromide stained agarose gel of PCR products amplified bla-Z primers from *Staphylococcus warneri* extracted DNA (173 bp)

Coagulase-negative staphylococci (CNS) have emerged as among the most common germs causing nosocomial infections globally, especially in

immunocompromised individuals .Infections vary from superficial wound involvement to deeper soft tissue infections. Clinically, these infections are milder than those caused by Staphylococcus aureus & Candida sp. However, the treatment has become more difficult due to higher in antimicrobial resistance during the last few decades. The number of resistant strains to penicillin, oxacillin/methicillin, ciprofloxacin, clindamycin, erythromycin, & gentamicin has increased dramatically **(Obajuluwa *et al.*, 2017).** CNS may easily obtain antibiotic resistance genes via conjugative plasmids, that can spread these determinants between genera and species. In this way, CNS are a problematic group in hospitals because they can be pathogenic to humans or reservoir genes for more deadly bacteria. Enzymatic antibiotic inactivation (e.g., expressed by the genes blaZ, ermA, ermB, ermC, & aac apD), active antibiotic removal from the cell (e.g., efflux mechanisms pumps), as well as reduction of antibiotic binding affinity to the drug are the most commonly identified resistance mechanisms in Staphylococcus**(Pedroso *et al.*, 2018)**.

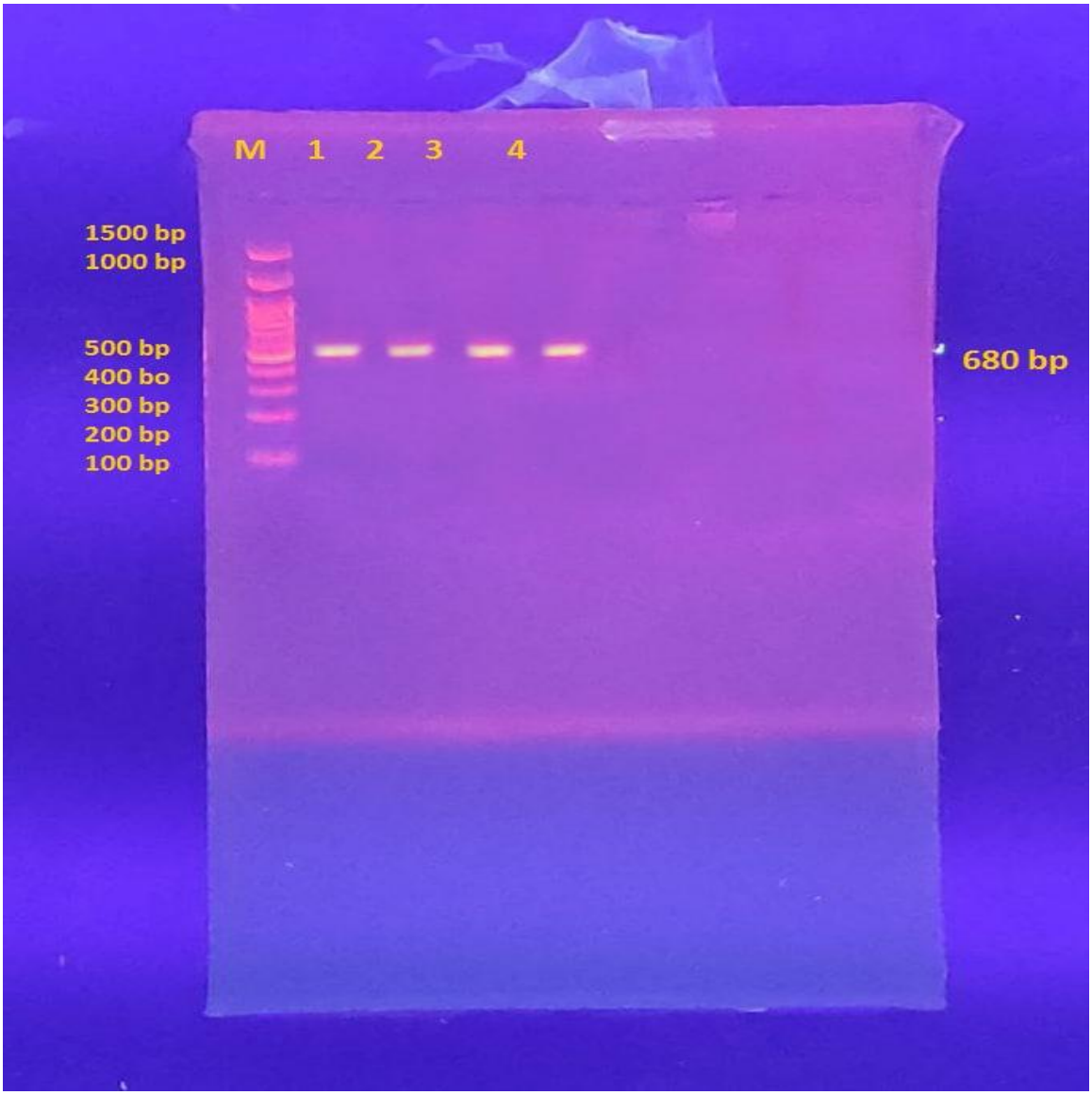
Martineau *et al.* found that two CNS strains (one *S. epidermidis* & one *S. warneri*) were resistant to oxacillin but lacked mecA. Nitrocefin testing was used to further characterize these two strains. The presence of blaZ and a positive response to the nitrocefin test for both bacteria revealed that they were -lactamase producers**(Martineau *et al.*, 2000).**

In the research of Pedrose *et al.*, which analyzed some coagulase negative staphylococci for resistance genes showed *S. warneri*: blaZ (71.4%), mecA (42.8%); vanA (0%).This agreement with this study results **.**

### 4.7.2.3.*Bacilus cerus* resistance genes for ceftriaxone

In figure(4-15) the results of detected genes of *Acinetobacter Bacillus cerus* which resistance to ceftriaxone by PCR then appeared by gell electrophoresis showed that

genes $bla_{A1}$ was found and appeared in band 680 bp in this species of *Bacilus cerus* which isolated from male child blood with nephrotic syndrome who had bacteremia. (These bacteria isolate after give positive culture was don't identified by VITEK apparatus even after repeated test .so diagnosed it by PCR).




Figure(4-15): Ethidium bromide stained agarose gel of PCR products amplified bla-$A_1$ primer from *Bacillus cerus* extracted DNA (680 bp).

*Bacillus cereus* members are spore-forming organisms that are usually connected with food poisoning & intestinal infections. Also, some strains of the group can

produce bacteremia in humans, particularly in immunocompromised individuals**(Bianco *et al.*, 2021).**

Bianco *et al* study's of 17 isolated *Bacillus cerus* of blood patients with bacteremia found beta-lactamase resistance genes: BLA-1, BLA-2, and blaZ 12 in 100 percent (17/17) and 6 percent (1/17) of isolates, respectively . Eight isolates from beta-lactam antibiotic class were resistant to ceftriaxone, while nine isolates exhibited intermediate resistance **(Bianco *et al.*, 2021).** This agreement with this study which found blaA1 gene in isolated *Bacillus cerus* which also intermediate resistance to ceftriaxone after susceptibility test.

### 4.8.1.Antibiotics susceptibility Test

Resistance to antibiotics has emerged recently due to the misuse of antibiotics and is a threat to the health-care system, especially in developing countries like Iraq where there are no antimicrobial stewardship programs in most intensive care units (ICU)**(Shareef *et al.*, 2021).**

**Tables(4-12) :Antibiotic susceptibility profile for Gram negative bacteria isolated from NS by Vitek system**

| **Antibiotics** | *Acinetobacter bumanii complex (1)* | *Acinetobacter Bumanii complex (2)* | R [%] | I [%] | S [%] |
|---|---|---|---|---|---|
| +Amoxicilin | R | R | **100** | **0** | **0** |
| +Cefazolin | R | R | **100** | **0** | **0** |
| +Cefixime | R | R | **100** | **0** | **0** |
| Cefotaxime | R | R | **100** | **0** | **0** |
| Ceftazidime | S | S | **0** | **0** | **100** |
| +Ceftizoxime | R | R | **100** | **0** | **0** |

| | | | | | |
|---|---|---|---|---|---|
| +Ceftriaxone | R | R | **100** | **0** | **0** |
| Cefepime | S | S | **0** | **0** | **100** |
| +Aztreonam | R | R | **100** | **0** | **0** |
| Imipenem | S | S | **0** | **0** | **100** |
| Meropenem | S | S | **0** | **0** | **100** |
| Gentamicin | S | S | **0** | **0** | **100** |
| Ciprofloxacin | S | S | **0** | **0** | **100** |
| Trimethoprim/ Sulfamethoxazole | R | R | **100** | **0** | **0** |

(R=resistance ,I=intermediate ,S=sensitive )

*Acinetobacter bumanii* (A) species are widespread Gram-negative coccobacilli that frequently cause nosocomial infections, especially ventilator-associated pneumonia and catheter-associated bacteremia, as well as urinary tract and soft tissue infections **(Wong *et al.*, 2017).**

In other studies they reported these bacteria were resistance to Ceftriaxone ,Cefotaxime ,Ceftazidime ,Piperacillin , Piperacillin-tazobactam, Ticarcillin ,Ticarcillin-Clavulanate, Imipenem , Ciprofloxacin ,Gentamicin and Levofloxacin and Tobramycin. While they appeared sensitive to Doxycycline ,Amikacin and Trimethoprim and its only intermediate to Tetracycline **(Lahmidi *et al.*, 2020).**

In study of Ioannou *et al.* reported *Acinetobacter bumanii* was resistant to Penicillin 100%, Ampicillin 100%, Piperacillin/tazobactam 83.3%, Carbapenems 66.7% ,Sulbactam 71.4% ,Quinolones 50% ,Aminoglycosides 36.4% ,and Colistin 0%**(Ioannou *et al.*, 2021).**

**Table (4-13) :Antibiotic susceptibility profile for Gram positive bacteria isolated from NS**

| Antibiotic | *Staphylococcus warnerii* (1) | *Staphylococcus warnerii* (2) | *Bacillus cerus*(1) | *Bacillus cerus* (2) | R [%] | I [%] | S [%] |
|---|---|---|---|---|---|---|---|
| Benzylpenicillin | R | R | | | 100 | 0 | 0 |
| Cefoxitin screen | + | + | | | | | |
| Clindamycin | R | R | | | 100 | 0 | 0 |
| Erythromycin | R | R | | | 100 | 0 | 0 |
| Fusidic Acid | R | R | | | 100 | 0 | 0 |
| Gentamicin | S | S | S | S | 0 | 0 | 100 |
| Inducible Clindamycin Resistance | _ | _ | | | | | |
| Levofloxacin | S | S | S | S | 0 | 0 | 100 |
| Moxifloxacin | I | I | S | S | 0 | 50 | 50 |
| Nitrofurantoin | S | S | | | 0 | 0 | 100 |
| Oxacillin | R | R | | | 100 | 0 | 0 |
| Rifampicin | R | R | | | 100 | 0 | 0 |
| Teicoplanin | R | R | | | 100 | 0 | 0 |
| Tetracycline | R | R | | | 100 | 0 | 0 |
| Tigecycline | S | S | | | 0 | 0 | 100 |
| Tobramycin | S | S | S | S | 0 | 0 | 100 |
| Trimethoprim/Sulfamethoxazole | R | R | S | S | 50 | 0 | 50 |
| Vancomycin | R | R | | | 100 | 0 | 0 |
| +Amoxicillin | R | R | | | 100 | 0 | 0 |
| +Amoxicillin/Clavulanic Acid | R | R | | | 100 | 0 | 0 |
| +Ampicillin | R | R | | | 100 | 0 | 0 |
| +Ampicillin/Sulbactam | R | R | | | 100 | 0 | 0 |
| +Carbenicillin | R | R | | | 100 | 0 | 0 |
| +Azithromycin | R | R | | | 100 | 0 | 0 |
| +Cefalotin | R | R | | | 100 | 0 | 0 |
| +Cefazolin | R | R | | | 100 | 0 | 0 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| +Cefalexin | R | R | | | 100 | 0 | 0 |
| +Cefepime | R | R | S | S | 50 | 0 | 50 |
| +Cefixime | R | R | S | S | 50 | 0 | 50 |
| +Cefotaxime | R | R | \|S | S | 50 | 0 | 50 |
| +Cefoxitin | R | R | | | 100 | 0 | 0 |
| +Cefotetan | R | R | | | 100 | 0 | 0 |
| +Cefpirome | R | R | | | 100 | 0 | 0 |
| +Ceftazidime | R | R | S | S | 50 | 0 | 50 |
| +Ceftriaxone | R | R | S | S | 50 | 0 | 50 |
| +Ciprofloxacin | S | S | | | 0 | 0 | 100 |
| +Clarithromycin | R | R | | | 100 | 0 | 0 |
| +Cloxacillin | R | R | | | 100 | 0 | 0 |
| +Daptomycin | S | S | | | 0 | 0 | 100 |
| +Dicloxacillin | R | R | | | 100 | 0 | 0 |
| +Doripenem | R | R | | | 100 | 0 | 0 |
| +Ertapenem | R | R | S | S | 50 | 0 | 50 |
| +Doxycycline | R | R | S | S | 50 | 0 | 50 |
| +Gemifloxacin | R | R | | | 100 | 0 | 0 |
| +Flucloxacillin | R | R | | | 100 | 0 | 0 |
| +Imipenem | R | R | S | S | 50 | 0 | 50 |
| +Meropenem | R | R | S | S | 50 | 0 | 50 |
| +Methicillin | R | R | | | 100 | 0 | 0 |
| +Norfloxacin | R | R | | | 100 | 0 | 0 |
| +Ofloxacin | S | S | S | S | 0 | 0 | 100 |
| +Oxacillin MIC | R | R | | | 100 | 0 | 0 |
| +Piperacillin | R | R | S | S | 50 | 0 | 50 |
| +Piperacillin/Tazobactam | R | R | S | | 50 | 0 | 50 |
| +Ticarcillin | R | R | S | S | 50 | 0 | 50 |
| +Ticarcillin/Clavulanicacid | R | R | S | S | 50 | 0 | 50 |
| +Trimethoprim | R | R | | | 100 | 0 | 0 |

( R=resistance ,I=intermediate ,S=sensitive )

Coagulase-negative *Staphylococcus warneri* is an opportunistic pathogen capable of causing several infections, especially in patients with indwelling medical devices **(Szczuka *et al.*, 2016).**Other studies found that this bacteria species was sensitive to oxacillin, penicillin G, clindamycin, erythromycin, gentamicin, tetracycline, ciprofloxacin, TMP/SMX, vancomycin, and linezolid. Novobiocin & polymyxin B were both toxic to the strain **(Ivić *et al.*, 2013).**While in study of Lourtet-Hascoet *et al.* ,showed it is resistant to Penicillin G 37.5%, Tetracycline 12.5%,Erythromycin 75%, Fosfomycin 75% , and sensitive to Methicillin ,Clindamycin ,Gentamicin ,SXT ,Vancomycin ,Teicoplanin ,Ofloxacin ,Rifampicin ,Linezolid , Daptomycin **(Lourtet-Hascoët *et al.*, 2018)** .

While *Bacillus cerus* Antibiotic susceptibility from blood isolates in study of Keda *et al*. also showed it is sensitive to Vancomycin 100%,Imipenem 100%,Gentamicin 100%,Amikacin 100%,Linezolid 100%,Chloramphinicol 100%,Rifampin 100% ,Levofloxacin 89.7%,Clindamycin 34.5%,Erythromycin 62.1%,Cefazolin 51.7%,Daptomycin 36.4%,Cefotaxime 81.8%,Ampicilin /sulbactam4%, Ampicillin 0%, Ceftazidime 0% **(Ikeda *et al.*, 2015)**.

### 4.8.2.Antimicrobial Activity of Ceftriaxone

#### 4.8.2.1. Antimicrobial Activity of Ceftriaxone for *Acinetobacter bumanii ssp*

Table (4-14) showed that there are significant differences *($P \leq 0.001$)* in the diameters of the inhibition zone of free ceftriaxone versus *Acinetobacter bumanii* ssp at all doses used when compared to the control Furthermore, as concentration was raised, there was an increase in the inhibitory zone. The diameters of the inhibition zones to Free-Ceftriaxone were (45.75, 45.62, 60, 60, 60, and 60) mm for the concentrations (0.25, 0.50, 1, 2, 4, and 8) mg/ml.

When we employed the Nano-Ceftriaxone that was made using the LDH layers method, there were significant differences ($P \leq 0.01$) in the diameters of the inhibition zone of a nano ceftriaxone LDH versus *Acinetobacter bumanii* ssp at all concentrations that were used compared to the control. The sizes of the inhibition zones to Nano-Ceftriaxone LDH were (27, 31.62, 42.5, 39.5, 42.5, and 41.5) mm at the following doses (0.25, 0.50, 1, 2, 4, and 8) mg/ml.

Noted when we used the Nano-Ceftriaxone which made with Zno soll gel method , there are less significant differences in the diameters of inhibition zone of the nano ceftriaxone Zno against *Acinetobacter bumanei* ssp at all concentrations that used compared with the control. The diameters of inhibition zone to Nano-Ceftriaxone Zno were (18.75, 22.62, 34.62, 18.75, 29.25, and 29.37) mm of the following concentrations (0.25, 0.50, 1, 2, 4 , and 8) mg/ml; respectively.

**Table (4-14): The inhibition zone (mm) of free Ceftriaxone and their nano-hybrid composites against *Acinetobacter buomani***

| **Concentration mg/ml** | **Free-Ceftriaxone** (Mean ± SD) | **LDH-Ceftriaxone** (Mean ± SD) | **ZnO-Ceftriaxone** (Mean ± SD) | ***P* value** |
|---|---|---|---|---|
| **0 (control)** | 0.0 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 1.000 |
| **0.25** | 45.75 ± 3.96 | 27 ± 1.41 | 18.75 ± 1.70 | 0.000** |
| **0.50** | 45.62 ± 11.06 | 31.62 ± 1.25 | 22.62 ± 4.32 | 0.0035* |
| **1.00** | 60.00 ± 0.00 | 42.50 ± 5.22 | 34.62 ± 3.90 | 0.000** |
| **2.00** | 60.00 ± 0.00 | 39.5 ± 4.84 | 18.75 ± 3.37 | 0.000** |
| **4.00** | 60.00 ± 0.00 | 42.5 ± 10.15 | 29.25 ± 2.59 | 0.0002** |
| **8.00** | 60.00 ± 0.00 | 41.25 ± 7.21 | 29.37 ± 0.75 | 0.000** |

* significance differences at $p < 0.05$ ,** high significance differences at $p < 0.001$

Ceftriaxone has been a part of therapeutic regime for combating some of the most aggressive bacterial infections in the last few decades. However, increasing bacterial resistance towards ceftriaxone and other third generation cephalosporin antibiotics has raised serious clinical. Advancement in nanotechnology has converted nano-therapeutic vision into a plausible reality with better targeting and reduced drug consumption**(Alshammari *et al.*, 2021)**.

Similar to pervious study Ziglam *et al.* in Libya ,during the two-year study, minimal inhibitory concentrations (MICs) were determined to use the agar dilution methodology & interpreted according the Clinical and Laboratory Standards Institute (CLSI) standards against all isolates to test sensitivity to such antibiotics, All *A. baumannii* isolates were resistant to third generation cephalosporins (ceftazidime, ceftriaxone, & cefotaxime), the concentration of ceftriaxone was (30 g), and the number of isolates resistant to tested drug was 113.**(Ziglam *et al*., 2012)** .

Also in the case report study of Lahmidi *et al.* showed *Acinetobacter bumanii* was resistant to many antibiotics as ceftriaxone concluded that native valve infective endocarditis caused by *A. baumannii* was rare. The relevance of their case was centered on the fact that infective endocarditis due to the dreaded *A. baumannii* is not a common cause but a possible cause of death in patients who have stayed in the intensive care unit; hence, the necessity of nosocomial infection control and prevention measures**(Lahmidi *et al.*, 2020).**

*A. baumannii* rapidly gained resistance to a wide range of antibiotics that arose in many regions of the world a few decades ago, mostly through the acquisition of gene clusters carried by plasmids, transposons, integrons, & resistant islands within the genome. This event resulted in an increase in multidrug resistance. To present,

certain strains of A. baumannii have developed resistance to almost every antibacterial agent now available, including Ceftriaxone**(Ziglam *et al.*, 2012).**

Nowadays, nanohybrids based layered double hydroxides (LDHs) are widely used in medical field as drug delivery systems because these nanomaterials are able to protect active biomolecules from decomposition thus making them useful in sustained drug delivery. LDHs also called anionic clays gained attention due to their diverse properties and important usage in many fields especially medicine**(EVA *et al.*,2020).**

Ceftriaxone, a third generation cephalosporin, is a time-dependent killer used to treat serious infections like community-acquired pneumonia, gonorrhea, and meningitis. The incorporation of this antibiotic into the structure of LDH improves antimicrobial therapy by raising intracellular delivery & reducing toxic side effects**(Duceac *et al.*, 2018).**

Because of its ability to modify surface and size distribution, layered double hydroxides (LDHs) nanoparticles can be utilized as drug carriers to reduce the risk of toxic side effects by targeting certain cells or tissues. These biocompatible inorganic compounds are more stable and less toxic than other drug delivery methods**(Duceac *et al.*, 2018).**

Several medicines have been successfully conjugated with zinc oxide nanoparticles. ZnO-NPs-conjugated drugs had potent side effects on MDR bacteria. ZnO-NPs and drug conjugated NPs had no impact on human cells**(Akbar *et al.*, 2021).**

Therapy with ZnO-NPs and drug conjugates revealed little cytotoxicity against human cell lines in the study of Akbar et al. ZnO-NPs were synthesized by direct precipitation and successfully coupled with β -cycldextrin. For the first time, ZnO-NPs were conjugated with these drugs and tested for antibacterial activities against

a panel of Gram-positive & Gram-negative bacteria. Previous research has shown that ZnO-NPs had broad spectrum antibacterial activity against clinical isolates examined in their study**(Jones *et al.*, 2008)** .

Notably, Ceftriaxone & Ampicillin are currently used to treat a variety of bacterial infections. Ceftriaxone is a wide cephalosporin antibiotic that is now used to treat bacterial infections in a variety of settings, such as the respiratory system, skin, soft tissue, & urinary tract. Inhibiting bacterial cell wall production is the mode of action. Similarly, Ampicillin is now used to treat a variety of bacterial infections, particularly respiratory & urinary tract infections. It causes cell lysis by reducing cell wall formation by targeting transpeptidase. The findings were positive in that coupling with ZnO NPs enhanced the effectiveness of both antibiotics. So because pharmacokinetic and pharmacodynamic characteristics of both antibiotics are well documented, testing these compounds to identify their translational value is sensible, and it is the subject of future research. Furthermore, future research will test if the aforementioned conjugate antibiotics can defeat increasingly resistant bacterial strains**(Akbar *et al.*, 2021).**

### 4.8.2.2. Antimicrobial Activity of Ceftriaxone for *Staphylococcus warnerii*

Table (4-15) represents that there are substantial significant differences ($P< 0.001$) in the diameters of the inhibition zone of free ceftriaxone versus *Staphylococcus warnei* at all concentrations used as compared to the control. Additionally, as concentration was raised, there was an increase in the inhibitory zone. The diameters of the inhibition zones to Free-Ceftriaxone were (39.87, 47.5, 46.87, 60, 60, and 60) mm at the following doses (0.25, 0.50, 1, 2, 4, and 8) mg/ml. When we used the Nano-Ceftriaxone that was made using LDH layers method, there were significant differences ($P< 0.001$) in the diameters of the zones of inhibition of the nano

ceftriaxone LDH versus *Staphylococcus warnei* at all concentrations that were used compared to the control. Furthermore, as concentration is increased, there is a rise in the inhibitory zone. The diameters of the inhibition zones to Nano-Ceftriaxone LDH were (25.25, 36.62, 38.5, 39.75, 42.375, and 54.37) mm at the following doses (0.25, 0.50, 1, 2, 4, and 8) mg/ml. There are very few (no effect) significant differences in the diameters of the inhibition zone of the nano ceftriaxone Zno versus *Staphylococcus warnei* at (0.25,0.50,)mg/ml concentrations when we used the Nano-Ceftriaxone made using Zno soll gel method. The diameters of the inhibition zones to Nano-Ceftriaxone Zno were (0, 0, 11.25, 11.00, 11.62, and 11.87) mm for the concentrations (0.25, 0.50, 1, 2, 4, and 8) mg/ml.

**Table(4-15):The inhibition zone(mm) of free Ceftriaxone and their nano-hybrid composites against *Staphylococcus warnei***

| **Concentration mg/ml** | **Free-Ceftriaxone** (Mean ± SD) | **LDH-Ceftriaxone** (Mean ± SD) | **ZnO-Ceftriaxone** (Mean ± SD) | ***P* value** |
|---|---|---|---|---|
| **0 (control)** | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 1.000 |
| **0.25** | 39.87 ± 2.05 | 25.25 ± 3.27 | 0.00 ± 0.00 | 0.000** |
| **0.50** | 47.5 ± 8.66 | 36.62 ± 3.22 | 0.00 ± 0.00 | 0.000** |
| **1.00** | 46.87 ± 5.54 | 38.50 ± 7.51 | 11.25 ± 1.04 | 0.000** |
| **2.00** | 60.00 ± 0.00 | 39.75 ± 5.61 | 11.00 ± 2.67 | 0.000** |
| **4.00** | 60.00 ± 0.00 | 42.375 ± 7.53 | 11.62 ± 1.25 | 0.000** |
| **8.00** | 60.00 ± 0.00 | 54.37 ± 6.10 | 11.87 ± 2.83 | 0.000** |

* significance differences at $p < 0.05$ ,** high significance differences at $p < 0.001$

Ceftriaxone kills the bacteria by preventing peptidoglycan cross-linking, which ultimately ends cell wall synthesis **(Hathout *et al.*, 2020).**

*Staphylococcus warneri* belongs to the coagulase-negative staphylococci (CNS). Because of their rare in clinical pathology, CNS are sometimes mislabeled as simple commensal bacteria, yet they are opportunistic pathogens which cause bacteremia & septicemia in immunocompromised patients. The CNS is the most frequent cause of bloodstream infection, particularly in catheter-related infections as well as skin and soft tissue infections**(Bai *et al.,* 2019)** .

In same study showed The results of the disk diffusion assays revealed that the three strains *S.warnei* which used were sensitive to antibiotic disks of ceftriaxone , and there were no obvious differences between the strains with inhibition zone diameter 25.9 ,26,and 26 **(Bai *et al.,* 2019).** *S. warneri* has the potential to cause severe infections in immunocompromised individuals, which is enhanced by presence of indwelling equipment and/or implants. The repair of the device is the primary treatment for such infections. In immunocompetent patients, *Staphylococcus warneri* may well be identified as the causal agent of an infection even in the lack of a foreign substance. In another study, Dimitriadi *et al*. reported a case of chronic uti caused by *S.warneri* in an adult healthy patient, its clinical significance, and resistance to commonly used beta-lactams***(Dimitriadi et al.,* 2014)**.

As a result, the use of ceftriaxone-conjugated metallic NPs has been suggested as an alternative option for inhibiting resistant pathogensAs the concentration of AgNPs was increased, the dose-dependent cytotoxic actions were observed. Ceftriaxone-conjugated AgNPs had more action than unconjugated AgNPs, indicating that they could be used to treat ceftriaxone-resistant bacteria **(Tewabe *et al.,* 2021).**

### 4.8.2.3. Antimicrobial Activity of Ceftriaxone for *Bacillus cereus*

The statistical analysis results in Table (4-16) show that there are significant differences ($P \leq 0.001$) in the diameters of the inhibition zones of free ceftriaxone

versus Bacillus cereus at all concentrations used as compared to the control. The diameters of the inhibition zones to Free-Ceftriaxone were (57.62, 60, 60, 60, 60, and 60) mm at the following concentrations (0.25, 0.50, 1, 2, 4, and 8) mg/ml. When we used the Nano-Ceftriaxone that was made using the LDH layers method, there were significant differences *(P ≤0.001*) in the diameters of the inhibition zone of the nano ceftriaxone LDH versus Bacillus cereus at all concentrations that were used when compared to the control. Furthermore, as concentration is increased, there is a rise in the inhibition zone .The diameters of the inhibition zones to Nano-Ceftriaxone LDH were (20.62, 25.87, 33.25, 39.75, 44.62, and 44.12) mm at the following concentrations (0.25, 0.50, 1, 2, 4, and 8) mg/ml. As we used the Nano-Ceftriaxone that was made with the Zno soll gel method, there was no inhibitory zone of the nano ceftriaxone Zno versus Bacillus cereus at all concentrations being used when compared to the control in all concentrations.

**Table(4-16):The inhibition zone(mm) of free Ceftriaxone and their nano-hybrid composites against *Bacillus cereus***

| **Concentration mg/ml** | **Free-Ceftriaxone** (Mean ± SD) | **LDH-Ceftriaxone** (Mean ± SD) | **ZnO-Ceftriaxone** (Mean ± SD) | ***P* value** |
|---|---|---|---|---|
| **0 (control)** | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 1.000 |
| **0.25** | 57.62 ± 4.47 | 20.62 ± 4.00 | 0.00 ± 0.00 | 0.000** |
| **0.50** | 60.00 ± 0.00 | 25.87 ± 0.75 | 0.00 ± 0.00 | 0.000** |
| **1.00** | 60.00 ± 0.00 | 33.25 ± 2.87 | 0.00 ± 0.00 | 0.000** |
| **2.00** | 60.00 ± 0.00 | 39.75 ± 1.65 | 0.00 ± 0.00 | 0.000** |
| **4.00** | 60.00 ± 0.00 | 44.62 ± 3.35 | 0.00 ± 0.00 | 0.000** |
| **8.00** | 60.00 ± 0.00 | 44.12 ± 4.21 | 0.00 ± 0.00 | 0.000** |

* significance differences at $p < 0.05$ ,** high significance differences at $p < 0.001$

*Bacillus cereus* is one of the pathogens causing nosocomial bloodstream infections (BSIs). However, few reports have documented the antimicrobial susceptibility and clinical characteristics of *Bacillus cereus* BSI and the importance of empirical therapy .In study**(Ikeda *et al.*, 2015)** all isolates showed sensitivity to vancomycin, gentamicin, and imipenem. However, 48.3–100 % of isolates were resistant to cephalosporins**(Ikeda *et al.*, 2015)**.

Metallic NPs functionalized using antibacterial medications can fight against bacterial threats passively by extending the drug's retention period at the target, or actively by conjugating to active molecules capable of binding to the target. In a study using biogenic AgNPs conjugated with ceftriaxone, greater anti-bacterial activities were seen as compared to both ceftriaxone and unconjugated AgNPs. The researchers discovered that ceftriaxone's antibacterial activity against test strains increased, while AuNPs produced by *Taramites* sp. improved the antibiotics' response rates in a synergistic way. These findings are consistent with previous research findings that showed increasing efficacies of ceftriaxone when coupled with AuNPs against *Bacillus subtilis, S. aureus, E. coli, & Proteus vulgaris***(Tewabe *et al.*, 2021)**.

Duceac et al. studied ceftriaxone intercalated nanostructures as controlled drug delivery systems. The results showed that including the active in the inorganic matrices provided advantages such as enhanced drug loading and prolonged release**(Duceac *et al.*, 2018).**

Furthermore, intercalation of ceftriaxone into layered structure for anionic clays improved antibiotic activity through controlled drug release. The authors projected that ceftriaxone-LDH nanohybrids might significantly improve antibiotic administration and medical treatment**(Tewabe *et al.*, 2021)**.

# Conclusions

# &

# Recommendations

## Conclusions

- Most bacterial species that causes blood infections to nephrotic syndrome children of Karbala were nosocomial bacterial infection.
- Most bacterial isolated was resistant to ceftriaxone.
- WBC had reversible correlation with Granulocytes and Monocytes in pediatric nephrotic syndrome patients.
- CRP had reversible correlation with WBC and serum Albumin.
- Reversible correlation between TG with serum cholesterol, TG with LDL and LDL with serum cholesterol .
- There are also reversible correlation in children nephrotic syndrome between GGT with LDH and ALP with Direct bilirubin .
- Nano-Ceftriaxone by Zinc oxide conjugate was neither successful nor effective.
- Nano-Ceftriaxone by LDH layers of $MgNo_3$ Al was successful and effective on bacteria isolated.
- β-Lactam resistance genes were found in all bacteria isolated from NS children have bacteremia.

## Recommendations

- Study family genes for patients of nephrotic syndrome who affected with this disease and appeared at over 4 years old .
- More studies on other drug showed be followed which used in children bacterial infection and use nano-method to improve it like vancomycin with conjugate with layers of (Mg-Al-$No_3$ )by precipitation method .
- Conducting study in relation to GGT and LDH .
- Conducting study about platelet in bacterial infection .
- AST test in Vitec system may give result not accurately so better to confirm it and if possible anglaises genetically .

# References

# References

ABASS, A. M., AHMED, M. E., IBRAHIM, I. G. & YAHIA, S. 2017. Bacterial resistance to antibiotics: current situation in Sudan. *Journal of Advances in Microbiology***,** 6(4),1-7.

ABBAS, A. O., HASSAN, F., ABDULLA, M. & ABBAS, Y. Y. 2019. The relationship between ABO blood group antigens and renal function test among chronic kidney disease patients in Khartoum state. *Saudi J Biomed Res,* 4**,** 33-36.

ABDEL-SALAM, M., ALLAM, H. A., KHOLOUSSI, S., IBRAHIM, A. A. E.-W. & ABD EL-HAKEIM, S. A. 2020. Study of Urinary Alpha Glutathione-S-Transferase (Alpha GST) in Children with Idiopathic Nephrotic Syndrome. *Journal of Renal and Hepatic Disorders,* 4**,** 34-40.

ABDU-ALLAH, S. N. & MUSSA, A. H. 2015. Study the inhibitory effects of Bacillus subtilis filtrates isolated from Iraqi soil in combination with MIC of ciprofloxacin and imipimen on Acinetobacter baumannii isolated from wound and burns. *World Journal of Pharmaceutical Research,* 4**,** 2041-2049.

ABRAR, S., AIN, N. U., LIAQAT, H., HUSSAIN, S., RASHEED, F. & RIAZ, S. 2019. Distribution of bla CTX− M, bla TEM, bla SHV and bla OXA genes in Extended-spectrum-β-lactamase-producing Clinical isolates: A three-year multi-center study from Lahore, Pakistan. *Antimicrobial Resistance & Infection Control,* 8**,** 1-10.

ABRAR, S., VAJEEHA, A., UL-AIN, N. & RIAZ, S. 2017. Distribution of CTX-M group I and group III β-lactamases produced by Escherichia coli and Klebsiella pneumoniae in Lahore, Pakistan. *Microbial pathogenesis,* 103**,** 8-12.

ADAMS, D. & OTÁROLA-CASTILLO, E. 20 .13Geomorph: an R package for the collection and analysis of geometric morphometric shape data. Methods Ecol Evol 4: 393–399. John Wiley & Sons, Ltd.

AHMAD, N., SINGH, A., GUPTA, A., PANT, P., SINGH, T. P., SHARMA, S. & SHARMA, P. 2022. Discovery of the Lead Molecules Targeting the First Step of the Histidine Biosynthesis Pathway of Acinetobacter baumannii. *Journal of Chemical Information and Modeling,* 7, 1744–1759.

AKBAR, N., ASLAM, Z., SIDDIQUI, R., SHAH, M. R. & KHAN, N. A. 2021. Zinc oxide nanoparticles conjugated with clinically-approved medicines as potential antibacterial molecules. *AMB Express,* 11**,** 1-16.

AKYOL, T., BULUCU, F., SENER, O., YAMANEL, L., AYDIN, A., INAL, V., BOZOGLU, E., DEMIRKAYA, E., EKEN, A. & MUSABAK, U. 2007. Functions and oxidative stress status of leukocytes in patients with nephrotic syndrome. *Biological trace element research,* 116**,** 237-247.

AL-BAHRANI, M. H. A. 2017. The Evolution of lipid Metabolism in Iraqi Children with Nephrotic Syndrome. *Biomedical and Pharmacology Journal,* 10**,** 1917-1924.

AL-JOBORY, L. S. A. & ABDULAAL, N. I. 2020. Checkerboard pattern of green tea and rosemary extracts on multi drugs resistant Bacillus cereus recovered from soft cheese in Kirkuk, Iraq. *Plant Archives,* 20**,** 6799-6805.

AL-MOSAWI, A. J. 2002. The etiology of chronic renal failure in 54 Iraqi children. *Pediatric Nephrology,* 17**,** 463-464.

ALHARES, F., ALBAKAA, A. & NASRAWI, A. 2020. Urinary Tract Infection in Children with Idiopathic Nephrotic Syndrome. *Prensa Med Argent,* 106**,** 6.

ALLEGRANZI, B., NEJAD, S. B., COMBESCURE, C., GRAAFMANS, W., ATTAR, H., DONALDSON, L. & PITTET, D. 2011. Burden of endemic health-care-associated infection in developing countries: systematic review and meta-analysis. *The Lancet,* 377**,** 228-241.

ALSAADI, Y. 2014. *Characterization of two component systems of Acinetobacter baumannii.*

AMIN, A. F., RABEEA, M. M. & ABD EL-RAHMAN, G. 2022. Audiometric Evaluation for children with idiopathic nephrotic syndrome . *Al-Azhar Med. J,* 51**,** 1133-1144.

ANDOLINO, T. P. & REID-ADAM, J. 2015. Nephrotic syndrome. *Pediatrics in review,* 36**,** 117-25; quiz 126, 129.

ANIGILAJE, E. A. & ADESINA, T. C. 2019. The pattern and outcomes of childhood renal diseases at University of Abuja Teaching Hospital, Abuja, Nigeria: A 4 year retrospective review. *Nigerian Postgraduate Medical Journal,* 26**,** 53.

ANOCHIE, I., EKE, F. & OKPERE, A. 2006. Childhood nephrotic syndrome: change in pattern and response to steroids. *Journal of the National Medical Association,* 98**,** 1977.

ASINOBI, A., ADEMOLA, A. & OGUNKUNLE, O. 2019. Steroid response in primary childhood nephrotic syndrome in a tropical African environment. *Nigerian Journal of Clinical Practice,* 22**,** 790-790.

ASLAM, M. & SHARMA, A. 2018. A Study of Lipid Profile and CRP in Children with Nephrotic Syndrome.

ASTUTI, K. D., MURYAWAN, M. H. & MELLYANA, O. 2015. Correlation between lipid profile and C-reactive protein in children with nephrotic syndrome. *Paediatrica Indonesiana,* 55**,** 1-6.

ATWAN, Q. & HAYDER, N. 2020. Eco-friendly synthesis of Silver nanoparticles by using green method: Improved interaction and application in vitro and in vivo. *The Iraqi Journal of Agricultural Science,* 51**,** 201-216.

AYINALEM, G. A., GELAW, B. K., BELAY, A. Z. & LINJESA, J. L. 2013. Drug use evaluation of ceftriaxone in medical ward of Dessie Referral Hospital, North East Ethiopia. *Int J Basic Clin Pharmacol,* 2**,** 711-717.

BAGGA, A. & MANTAN, M. 2005. Nephrotic syndrome in children. *Indian Journal of medical research,* 122**,** 13.

BAI, P., ZHANG, B., ZHAO, X., LI, D., YU, Y., ZHANG, X., HUANG, B. & LIU, C. 2019. Decreased metabolism and increased tolerance to extreme environments in Staphylococcus warneri during long-term spaceflight. *MicrobiologyOpen,* 8**,** e917.

BANERJEE, S., BASU, S., AKHTAR, S., SINHA, R., SEN, A. & SENGUPTA, J. 2020. Free vitamin D levels in steroid-sensitive nephrotic syndrome and healthy controls. *Pediatric Nephrology,* 35**,** 447-454.

BASU, B., BHATTACHARYYA, S., BARUA, S., NASKAR, A. & ROY, B. 2020. Efficacy of body weight vs body surface area-based prednisolone regimen in nephrotic syndrome. *Clinical and Experimental Nephrology,* 24**,** 622-629.

BEHERA, B., VISHNU, G. A., CHATTERJEE, S., SREEKUMAR, N., NAGABHUSHAN, A., RAJENDRAN, N., PRATHIK, B. & PANDYA, H. J. 2019. Emerging technologies for antibiotic susceptibility testing. *Biosensors and Bioelectronics,* 142**,** 111552.

BERKLEY, J. A. 2021. Bacterial Infections and Nutrition: A Primer. *Nutrition and Infectious Diseases.* Springer.

BIANCO, A., CAPOZZI, L., MONNO, M. R., DEL SAMBRO, L., MANZULLI, V., PESOLE, G., LOCONSOLE, D. & PARISI, A. 2021. Characterization of Bacillus cereus Group isolates from human bacteremia by whole-genome sequencing. *Frontiers in Microbiology***,** 3273.

BLUM, C. R. Index Number Lab-1535.

BOLOGNIA, J., JORIZZO, J. L., RAPINI, R. P., CALLEN, J., HORN, T., MANCINI, A., SALASCHE, S., SCHAFFER, J., SCHWARZ, T. & STINGL, G. 2008. Dermatology. St. Louis, MO: Mosby. Elsevier.

BRENCHLEY, P. E. 2003. Vascular permeability factors in steroid-sensitive nephrotic syndrome and focal segmental glomerulosclerosis. *Nephrology Dialysis Transplantation,* 18**,** vi21-vi25.

BUTLER, D. A., BIAGI, M., TAN, X., QASMIEH, S., BULMAN, Z. P. & WENZLER, E. 2019. Multidrug resistant Acinetobacter baumannii: resistance by any other name would still be hard to treat. *Current infectious disease reports,* 21**,** 1-17.

CADNAPAPHORNCHAI, M. A., TKACHENKO, O., SHCHEKOCHIKHIN, D. & SCHRIER, R. W. 2014. The nephrotic syndrome: pathogenesis and treatment

of edema formation and secondary complications. *Pediatric nephrology,* 29**,** 1159-1167.

CHEN, J.-R., LEE, S.-Y., YANG, B.-H. & LU, J.-J. 2008. Rapid identification and susceptibility testing using the VITEK 2 system using culture fluids from positive BacT/ALERT blood cultures. *J Microbiol Immunol Infect,* 41**,** 259-64.

CHEN, Y., TENOVER, F. C. & KOEHLER, T. M. 2004. β-Lactamase gene expression in a penicillin-resistant Bacillus anthracis strain. *Antimicrobial agents and chemotherapy,* 48**,** 4873-4877.

CHO, S.-W., LEE, J. S. & CHOI, S.-H. 2004. Enhanced oral bioavailability of poorly absorbed drugs. I. Screening of absorption carrier for the ceftriaxone complex. *Journal of pharmaceutical sciences,* 93**,** 612-620.

DIEN BARD, J. & MCELVANIA TEKIPPE, E. 2016. Diagnosis of bloodstream infections in children. *Journal of clinical microbiology,* 54**,** 1418-1424.

DIMITRIADI, D., CHARITIDOU, C. & CHARVALOS, E. 2014. Urinary tract infection due to beta-lactams-resistant Staphylococcus warneri: a case report. *The International Arabic Journal of Antimicrobial Agents,* 4.

DOUKAS, S. G., WU, Y.-C., HARA, K., ABDULLAH, R. & BRODER, A. Acute Liver Failure in a Patient with Cholestatic Liver Disease and Nephrotic Syndrome.

DOWNIE, M. L., GALLIBOIS, C., PAREKH, R. S. & NOONE, D. G. 2017. Nephrotic syndrome in infants and children: pathophysiology and management. *Paediatrics and international child health,* 37**,** 248-258.

DUCEAC, L. D., LUCA, A. C., MITREA, G., BANU, E. A., CIUHODARU, M. I., CIOMAGA, I., ICHIM, D. L. & BACIU, G. 2018. Ceftriaxone intercalated nanostructures used to improve medical treatment. *Mat. Plast,* 55**,** 613-615.

DURAN, N., OZER, B., DURAN, G. G., ONLEN, Y. & DEMIR, C. 2012. Antibiotic resistance genes & susceptibility patterns in staphylococci. *The Indian journal of medical research,* 135**,** 389.

EBRAHIMI, S., FARHADIAN, N., KARIMI, M. & EBRAHIMI, M. 2020. Enhanced bactericidal effect of ceftriaxone drug encapsulated in nanostructured lipid carrier against gram-negative Escherichia coli bacteria: drug formulation, optimization, and cell culture study. *Antimicrobial Resistance & Infection Control,* 9**,** 1-12.

EDDY, A. A. & SYMONS, J. M. 2003. Nephrotic syndrome in childhood. *The lancet,* 362**,** 629-639.

EL-HUSSEINI, A., EL-BASUONY, F., MAHMOUD, I., SHEASHAA, H., SABRY, A., HASSAN, R., TAHA, N., HASSAN, N., SAYED-AHMAD, N. & SOBH, M. 2005. Long-term effects of cyclosporine in children with

idiopathic nephrotic syndrome: a single-centre experience. *Nephrology Dialysis Transplantation,* 20**,** 2433-2438.

EL-RAB, S. M., HALAWANI, E. M. & HASSAN, A. M. 2018. Formulation of ceftriaxone conjugated gold nanoparticles and their medical applications against extended-spectrum β-lactamase producing bacteria and breast cancer. *Journal of microbiology and biotechnology,* 28**,** 1563-1572.

ELSHAMY, A. A. & ABOSHANAB, K. M. 2020. A review on bacterial resistance to carbapenems: epidemiology, detection and treatment options. *Future science OA,* 6**,** FSO438.

ESTEVEZ, M., VOYER, L., CRAVIOTTO, R., BALLART, I., GOICOA, M., PALACIOS, F., QUADRI, B., CORTI, S., WAINSTEIN, R. & SEN, L. 1989. Dysfunction of the Monocyte-Macrophage System in the Idiopathic Minimal Change Nephrotic Syndrome. *Acta Pædiatrica,* 78**,** 87-93.

EVA, L., DUCEAC, L. D., STAFIE, L., MARCU, C., MITREA, G., GOROFTEI, E. R. B., HANGANU, E., CIOMAGA, I. M., IORDACHE, A. C. & LUCA, A. C. Physical-Chemical Issues of Cephalosporin Intercalated Nanoparticles for Life-Threatening Infections Treatment. *drugs,* 5**,** 15.

FAIR, R. J. & TOR, Y. 2014. Antibiotics and bacterial resistance in the 21st century. *Perspectives in medicinal chemistry,* 6**,** PMC. S14459.

FAKHOURI, F., BOCQUET, N., TAUPIN, P., PRESNE, C., GAGNADOUX, M.-F., LANDAIS, P., LESAVRE, P., CHAUVEAU, D., KNEBELMANN, B. & BROYER, M. 2003. Steroid-sensitive nephrotic syndrome: from childhood to adulthood. *American journal of kidney diseases,* 41**,** 550-557.

FATAHI-BAFGHI, M. 2019. Antibiotic resistance genes in the Actinobacteria phylum. *European Journal of Clinical Microbiology & Infectious Diseases,* 38**,** 1599-1624.

FLOEGE, J. & FEEHALLY, J. 2018. Introduction to glomerular disease. *Comprehensive Clinical Nephrology E-Book***,** 182.

FOURNIER, P.-E., VALLENET, D., BARBE, V., AUDIC, S., OGATA, H., POIREL, L., RICHET, H., ROBERT, C., MANGENOT, S. & ABERGEL, C. 2006. Comparative genomics of multidrug resistance in Acinetobacter baumannii. *PLoS genetics,* 2**,** e7.

FRANKE, I., AYDIN, M., KURYLOWICZ, L., LOPEZ, C. E. L., GANSCHOW, R., LENTZE, M. J. & BORN, M. 2019. Clinical course & management of childhood nephrotic syndrome in Germany: a large epidemiological ESPED study. *BMC nephrology,* 20**,** 1-10.

FUCHSHUBER, A., GRIBOUVAL, O., RONNER, V., KROISS, S., KARLE, S., BRANDIS, M. & HILDEBRANDT, F. 2001. Clinical and genetic evaluation

of familial steroid-responsive nephrotic syndrome in childhood. *Journal of the American Society of Nephrology,* 12**,** 374-378.

GAJDÁCS, M. 2019. The concept of an ideal antibiotic: implications for drug design. *Molecules,* 24**,** 892.

GALES, A. C., SEIFERT, H., GUR, D., CASTANHEIRA, M., JONES, R. N. & SADER, H. S. Antimicrobial susceptibility of Acinetobacter calcoaceticus–Acinetobacter baumannii complex and Stenotrophomonas maltophilia clinical isolates: results from the SENTRY antimicrobial surveillance program (1997–2016). Open forum infectious diseases, 2019. Oxford University Press US, S34-S46.

GARIBYAN, L. & AVASHIA, N. 2013. Research techniques made simple: polymerase chain reaction (PCR). *The Journal of investigative dermatology,* 133**,** e6.

GEBREHIWOT, M., KASSA, M., GEBREHIWOT, H. & SIBHAT, M. 2020. Time to relapse and its predictors among children with nephrotic syndrome in comprehensive specialized hospitals, Tigray, Ethiopia, 2019. *International journal of pediatrics,* 2020.

GELLERMANN, J., WEBER, L., PAPE, L., TÖNSHOFF, B., HOYER, P. & QUERFELD, U. 2013. Mycophenolate mofetil versus cyclosporin A in children with frequently relapsing nephrotic syndrome. *Journal of the American Society of Nephrology,* 24**,** 1689-1697.

GHERARDI, G., ANGELETTI, S., PANITTI, M., POMPILIO, A., DI BONAVENTURA, G., CREA, F., AVOLA, A., FICO, L., PALAZZO, C. & SAPIA, G. F. 2012. Comparative evaluation of the Vitek-2 Compact and Phoenix systems for rapid identification and antibiotic susceptibility testing directly from blood cultures of Gram-negative and Gram-positive isolates. *Diagnostic microbiology and infectious disease,* 72**,** 20-31.

GIEDRAITIENĖ, A., VITKAUSKIENĖ, A., NAGINIENĖ, R. & PAVILONIS, A. 2011. Antibiotic resistance mechanisms of clinically important bacteria. *Medicina,* 47**,** 19.

GIULIANO, C., PATEL, C. R. & KALE-PRADHAN, P. B. 2019. A guide to bacterial culture identification and results interpretation. *Pharmacy and Therapeutics,* 44**,** 192.

GLASSET, B., HERBIN, S., GRANIER, S. A., CAVALIÉ, L., LAFEUILLE, E., GUÉRIN, C., RUIMY, R., CASAGRANDE-MAGNE, F., LEVAST, M. & CHAUTEMPS, N. 2018. Bacillus cereus, a serious cause of nosocomial infections: epidemiologic and genetic survey. *PloS one,* 13**,** e0194346.

GONZÁLEZ-BELLO, C. 2017. Antibiotic adjuvants–A strategy to unlock bacterial resistance to antibiotics. *Bioorganic & medicinal chemistry letters,* 27**,** 4221-4228.

GOODING, J. R., AGRAWAL, S., MCRITCHIE, S., ACUFF, Z., MERCHANT, M. L., KLEIN, J. B., SMOYER, W. E., SUMNER, S. J., MAHAN, J. & PATEL, H. 2020. Predicting and defining steroid resistance in pediatric nephrotic syndrome using plasma metabolomics. *Kidney international reports,* 5**,** 81-93.

GOTO, K., KONO, K., FUJII, H., GOTO, S. & NISHI, S. 2022. Clinical value of serum cholinesterase levels in Nephrotic syndrome: an observational study. *BMC nephrology,* 23**,** 1-7.

GREEN, M. R. & SAMBROOK, J. 2019. Analysis of DNA by agarose gel electrophoresis. *Cold Spring Harbor Protocols,* 2019**,** pdb. top100388.

GRIMBERT, P., AUDARD, V., REMY, P., LANG, P. & SAHALI, D. 2003. Recent approaches to the pathogenesis of minimal-change nephrotic syndrome. *Nephrology Dialysis Transplantation,* 18**,** 245-248.

GUHA, P., DE, A. & GHOSAL, M. 2009. Behavior profile of children with nephrotic syndrome. *Indian Journal of Psychiatry,* 51**,** 122.

GUO, J., LI, S., XU, S., JIANG, L., GAO, E. & LIU, Z. 2020. Nocardiosis in patients with nephrotic syndrome: a retrospective analysis of 11 cases and a literature review. *International Urology and Nephrology,* 52**,** 731-738.

GUO, R., LI, K., QIN, J., NIU, S. & HONG, W. 2020. Development of polycationic micelles as an efficient delivery system of antibiotics for overcoming the biological barriers to reverse multidrug resistance in Escherichia coli. *Nanoscale,* 12**,** 11251-11266.

GUPTA, N., RAI, D. B., JANGID, A. K. & KULHARI, H. J. M. I. M. 2019. Use of nanotechnology in antimicrobial therapy. 46**,** 143-172.

GURURAJA, M. 2013. Cephalosporin utilization evaluation in a University Teaching Hospital: a prospective study. *Journal of Drug Delivery and Therapeutics,* 3.

HA, J., HONG, S. K., HAN, G. H., KIM, M., YONG, D. & LEE, K. J. A. O. L. M. 2018. Same-day identification and antimicrobial susceptibility testing of bacteria in positive blood culture broths using short-term incubation on solid medium with the MicroFlex LT, Vitek-MS, and Vitek2 systems. 38**,** 235-241.

HAMIDIAN, M. & NIGRO, S. J. 2019. Emergence, molecular mechanisms and global spread of carbapenem-resistant Acinetobacter baumannii. *Microbial genomics,* 5.

HAMPSON, K. J., GAY, M. L. & BAND, M. E. 2021. Pediatric Nephrotic Syndrome: Pharmacologic and Nutrition Management. *Nutrition in Clinical Practice,* 36**,** 331-343.

HARI, P., KHANDELWAL, P. & SMOYER, W. E. 2020. Dyslipidemia and cardiovascular health in childhood nephrotic syndrome. *Pediatric Nephrology,* 35**,** 1601-1619.

HATHOUT, R. M., METWALLY, A. A., WOODMAN, T. J. & HARDY, J. G. 2020. Prediction of drug loading in the gelatin matrix using computational methods. *ACS omega,* 5**,** 1549-1556.

HINGORANI, S. R., WEISS, N. S. & WATKINS, S. L. 2002. Predictors of peritonitis in children with nephrotic syndrome. *Pediatric nephrology,* 17**,** 678-682.

HODSON, E. M. 2003. The management of idiopathic nephrotic syndrome in children. *Pediatric Drugs,* 5**,** 335-349.

HODSON, E. M., KNIGHT, J. F., WILLIS, N. S. & CRAIG, J. C. 2005. Corticosteroid therapy for nephrotic syndrome in children. *Cochrane database of systematic reviews*.

HOMBACH, M., MAURER, F. P., PFIFFNER, T., BÖTTGER, E. C. & FURRER, R. 2015. Standardization of operator-dependent variables affecting precision and accuracy of the disk diffusion method for antibiotic susceptibility testing. *Journal of clinical microbiology,* 53**,** 3864-3869.

HORII, T., NOTAKE, S., TAMAI, K. & YANAGISAWA, H. 2011. Bacillus cereus from blood cultures: virulence genes, antimicrobial susceptibility and risk factors for blood stream infection. *FEMS Immunology & Medical Microbiology,* 63**,** 202-209.

HORING, S., MASSARANI, A. S., LOFFLER, B. & RODEL, J. 2019. Rapid antibiotic susceptibility testing in blood culture diagnostics performed by direct inoculation using the VITEK®-2 and BD Phoenix™ platforms. *European Journal of Clinical Microbiology & Infectious Diseases,* 38**,** 471-478.

HOSEINI, R., OTUKESH, H., FERESHTEHNEJAD, S. M., TAHOORI, A., HOOMAN, N., RAHIMZADEH, N. & SADR, S. 2012. Prevalence and outcome of focal segmental glomerulosclerosis in Iranian children with nephrotic syndrome.*IJKD* 2012;6:18-24.

HUH, A. J. & KWON, Y. J. 2011. "Nanoantibiotics": a new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era. *Journal of controlled release,* 156**,** 128-145.

HUHAND,Y.&KWON,J.2011.Nanoantibiotics':anewparadigmfortreatinginfectiousdis-easesusingnanomaterialsintheantibioticsresistantera. JournalofControlledRelease.

HUMPHRIES, R. M., MAGNANO, P., BURNHAM, C.-A. D., DIEN BARD, J., DINGLE, T. C., CALLAN, K. & WESTBLADE, L. F. 2020. Evaluation of surrogate tests for the presence of mecA-mediated methicillin resistance in Staphylococcus capitis, Staphylococcus haemolyticus, Staphylococcus

hominis, and Staphylococcus warneri. *Journal of clinical microbiology,* 59**,** 2290-20.

IKEDA, M., YAGIHARA, Y., TATSUNO, K., OKAZAKI, M., OKUGAWA, S. & MORIYA, K. 2015. Clinical characteristics and antimicrobial susceptibility of Bacillus cereus blood stream infections. *Annals of Clinical Microbiology and Antimicrobials,* 14**,** 1-7.

ILYAS, M., ROY, S., ABBASI, S., LEGGIADRO, R. J., ENGLISH, B. K. & WYATT, R. J. 1996. Serious infections due to penicillin-resistant Streptococcus pneumoniae in two children with nephrotic syndrome. *Pediatric Nephrology,* 10**,** 639-641.

IOANNOU, P., MAVRIKAKI, V. & KOFTERIDIS, D. P. 2021. Infective endocarditis by Acinetobacter species: a systematic review. *Journal of Chemotherapy,* 33**,** 203-215.

IOREMBER, F. & AVILES, D. 2017. Anemia in nephrotic syndrome: approach to evaluation and treatment. *Pediatric Nephrology,* 32**,** 1323-1330.

ISAACMAN, D. J., KARASIC, R. B., REYNOLDS, E. A. & KOST, S. I. 1996. Effect of number of blood cultures and volume of blood on detection of bacteremia in children. *The Journal of pediatrics,* 128**,** 190-195.

ISAH, S. & ABDULAZEEZ, A. 2021. Evaluation of C-Reactive Protein and Interleukin-6 among Nephrotic Syndrome Patients in Kano Metropolis. *J Med Lab Sci,* 31**,** 1-11.

IVIĆ, I., KARANOVIĆ, J. & PAVIČIĆ-IVELJA, M. 2013. Sepsis with multiple abscesses caused by Staphylococcus warneri: a case report. *Central European Journal of Medicine,* 8**,** 45-47.

JEAN, S.-S. & HSUEH, P.-R. 2011. High burden of antimicrobial resistance in Asia. *International journal of antimicrobial agents,* 37**,** 291-295.

JESSIM, A. I., FAKHRY, S. S. & ALWASH, S. J. 2017. Detection and Determination of Bacillus cereusin Cooked Rice and Some Types of Spices with Ribosomal 16SrRNA gene Selected from Iraqi Public Restaurants. *International Journal of Bio-resource and Stress Management* , 8(3):382-387.

JONES, N., RAY, B., RANJIT, K. T. & MANNA, A. C. 2008. Antibacterial activity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms. *FEMS microbiology letters,* 279**,** 71-76.

JORGENSEN, J. H. & TURNIDGE, J. D. 2015. Susceptibility test methods: dilution and disk diffusion methods. *Manual of clinical microbiology***,**11, 1253-1273.

KADDAH, A., SABRY, S., EMIL, E. & EL-REFAEY, M. 2012. Epidemiology of primary nephrotic syndrome in Egyptian children. *J Nephrol,* 25**,** 732-737.

KALLASH, M. & MAHAN, J. D. 2021. Mechanisms and management of edema in pediatric nephrotic syndrome. *Pediatric Nephrology,* 36**,** 1719-1730.

KALRA, S., DARYANI, H., SAXENA, A., BHANDARI, S. & SHARMA, S. 2020. Infectious complications in children with nephrotic syndrome: Can they be prevented? *Medical Journal Armed Forces India*.

KANEKO, K., KIMATA, T., TSUJI, S., SHIMO, T., TAKAHASHI, M. & TANAKA, S. 2012. Serum albumin level accurately reflects antioxidant potentials in idiopathic nephrotic syndrome. *Clinical and experimental nephrology,* 16**,** 411-414.

KARMOSTAJI, A., NAJAR PEERAYEH, S. & HATEF SALMANIAN, A. 2013. Distribution of OXA-type class D β-lactamase genes among nosocomial multi drug resistant Acinetobacter baumannii isolated in Tehran hospitals. *Jundishapur Journal of Microbiology*. 2013 July; 6(5): e8219

KAYSEN, G. A. & AL BANDER, H. 1990. Metabolism of albumin and immunoglobulins in the nephrotic syndrome. *American journal of nephrology,* 10**,** 36-42.

KERLIN, B. A., BLATT, N. B., FUH, B., ZHAO, S., LEHMAN, A., BLANCHONG, C., MAHAN, J. D. & SMOYER, W. E. 2009. Epidemiology and risk factors for thromboembolic complications of childhood nephrotic syndrome: a Midwest Pediatric Nephrology Consortium (MWPNC) study. *The Journal of pediatrics,* 155**,** 105-110. e1.

KHATOON, U. T., RAO, G. N., MOHAN, K. M., RAMANAVICIENE, A. & RAMANAVICIUS, A. 2017. Antibacterial and antifungal activity of silver nanospheres synthesized by tri-sodium citrate assisted chemical approach. *Vacuum,* 146**,** 259-265.

KNIAZEWSKA, M. H., OBUCHOWICZ, A. K., WIELKOSZYNSKI, T., ZMUDZINSKA-KITCZAK, J., URBAN, K., MAREK, M., WITANOWSKA, J. & SIERON-STOŁTNY, K. 2009. Atherosclerosis risk factors in young patients formerly treated for idiopathic nephrotic syndrome. *Pediatric Nephrology*, 24**,** 549-554.

KONSTANTELOS, N., BANH, T., PATEL, V., VASILEVSKA-RISTOVSKA, J., BORGES, K., HUSSAIN-SHAMSY, N., NOONE, D., HEBERT, D., RADHAKRISHNAN, S. & LICHT, C. P. 2019. Association of low birth weight and prematurity with clinical outcomes of childhood nephrotic syndrome: a prospective cohort study. *Pediatric Nephrology,* 34**,** 1599-1605.

KRISHNAN, C., RAJESH, T., SHASHIDHARA, H., JAYAKRISHNAN, M. & GEETA, M. 2017. Major infections in children with nephrotic syndrome. *Int J Contemp Pediatr,* 4**,** 346-50.

KUMARASAMY, K. K., TOLEMAN, M. A., WALSH, T. R., BAGARIA, J., BUTT, F., BALAKRISHNAN, R., CHAUDHARY, U., DOUMITH, M., GISKE, C. G. & IRFAN, S. 2010. Emergence of a new antibiotic resistance

mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. *The Lancet infectious diseases,* 10**,** 597-602.

KWON, K. T., OH, W. S., SONG, J.-H., CHANG, H.-H., JUNG, S.-I., KIM, S.-W., RYU, S. Y., HEO, S. T., JUNG, D. S. & RHEE, J.-Y. 2007. Impact of imipenem resistance on mortality in patients with Acinetobacter bacteraemia. *Journal of Antimicrobial Chemotherapy,* 59**,** 525-530.

LAHMIDI, I., CHARMAKE III, D., ELOUAFI, N. & BAZID, Z. 2020. Acinetobacter baumannii native valve infective endocarditis: a case report. *Cureus* 12(11): e11527. doi:10.7759/cureus.11527

LEBEL, A., KROPACH, N., ASHKENAZI-HOFFNUNG, L., HUBER-YARON, A. & DAVIDOVITS, M. 2020. Infections in children with nephrotic syndrome: twenty years of experience. *Clinical pediatrics,* 59**,** 692-698.

LEE, D.-H. & JACOBS JR, D. R. 2005. Association between serum gamma-glutamyltransferase and C-reactive protein. *Atherosclerosis,* 178**,** 327-330.

LEE, H., JUNG, D., YEOM, J. S., SON, J. S., JUNG, S.-I., KIM, Y.-S., KIM, C. K., CHANG, H.-H., KIM, S.-W. & KI, H. K. 2009. Evaluation of ceftriaxone utilization at multicenter study. *The Korean journal of internal medicine,* 24**,** 374.

LI, B. & WEBSTER, T. J. 2018. Bacteria antibiotic resistance: New challenges and opportunities for implant-associated orthopedic infections. *Journal of Orthopaedic Research®,* 36**,** 22-32.

LI, H., LUO, Y.-F., WILLIAMS, B. J., BLACKWELL, T. S. & XIE, C.-M. 2012. Structure and function of OprD protein in Pseudomonas aeruginosa: from antibiotic resistance to novel therapies. *International Journal of Medical Microbiology,* 302**,** 63-68.

LI, T., LIU, C., LU, J., GAURAV, G. K. & CHEN, W. 2020. Determination of how tetracycline influences nitrogen removal performance, community structure, and functional genes of biofilm systems. *Journal of the Taiwan Institute of Chemical Engineers,* 106**,** 99-109.

LIM, C., TAKAHASHI, E., HONGSUWAN, M., WUTHIEKANUN, V., THAMLIKITKUL, V., HINJOY, S., DAY, N. P., PEACOCK, S. J. & LIMMATHUROTSAKUL, D. 2016. Epidemiology and burden of multidrug-resistant bacterial infection in a developing country. *elife,* 5**,** 1882.

LIN, A. & LORÉ, K. 2017. Granulocytes: new members of the antigen-presenting cell family. *Frontiers in immunology,* 8**,** 1781.

LIU, C., ZHAO, X., XIE, H., ZHANG, X., LI, K., MA, C. & FU, Q. 2020. Whole genome sequence and comparative genome analyses of multi-resistant

Staphylococcus warneri GD01 isolated from a diseased pig in China. *PloS one,* 15**,** 233363.

LOURTET-HASCOET, J., FELICE, M., BICART-SEE, A., BOUIGE, A., GIORDANO, G. & BONNET, E. 2018. Species and antimicrobial susceptibility testing of coagulase-negative staphylococci in periprosthetic joint infections. *Epidemiology & Infection,* 146**,** 1771-1776.

MAGHRABIA, A., BOUGHDADY, M. & MESHALI, M. 2021. Design and optimization of new enteric nanoparticles of ceftriaxone for oral delivery: in vitro and in vivo assessments. *International Journal of Nanomedicine,* 16**,** 5937.

MAHMOODI, B. K., TEN KATE, M. K., WAANDERS, F., VEEGER, N. J., BROUWER, J.-L. P., VOGT, L., NAVIS, G. & VAN DER MEER, J. 2008. High absolute risks and predictors of venous and arterial thromboembolic events in patients with nephrotic syndrome: results from a large retrospective cohort study. *Circulation,* 117**,** 224-230.

MALARKODI, C., RAJESHKUMAR, S., PAULKUMAR, K., VANAJA, M., GNANAJOBITHA, G., ANNADURAI, G. J. B. C. & APPLICATIONS 2014. Biosynthesis and antimicrobial activity of semiconductor nanoparticles against oral pathogens.

MARTINEAU, F., PICARD, F. J., GRENIER, L., ROY, P. H., OUELLETTE, M. & BERGERON, M. G. 2000. Multiplex PCR assays for the detection of clinically relevant antibiotic resistance genes in staphylococci isolated from patients infected after cardiac surgery. *Journal of Antimicrobial Chemotherapy,* 46**,** 527-534.

MAURER, F. P., CHRISTNER, M., HENTSCHKE, M. & ROHDE, H. 2017. Advances in rapid identification and susceptibility testing of bacteria in the clinical microbiology laboratory: implications for patient care and antimicrobial stewardship programs. *Infectious disease reports,* 9**,** 18-27.

MCKINNEY, P. A., FELTBOWER, R. G., BROCKLEBANK, J. T. & FITZPATRICK, M. M. 2001. Time trends and ethnic patterns of childhood nephrotic syndrome in Yorkshire, UK. *Pediatric nephrology,* 16**,** 1040-1044.

MEI, J., LI, Y.-T., ZHANG, H., XIAO, M.-M., NING, Y., ZHANG, Z.-Y. & ZHANG, G.-J. 2018. Molybdenum disulfide field-effect transistor biosensor for ultrasensitive detection of DNA by employing morpholino as probe. *Biosensors and Bioelectronics,* 110**,** 71-77.

MOHAMMED, S., AHMED, R. A. E. H. & SADEK, A. A. 2019. Serum Immunoglobulin G, IgM And IgG: IgM Ratio as Predictors for Outcome of Childhood Nephrotic Syndrome. *Sohag Medical Journal,* 23**,** 77-82.

MOHAMMED, T. F., SAHEB, O. A., NAIF, M. H. & YASEEN, Z. K. 2019. Congenital and Infantile Nephrotic Syndrome Single Center Study. *IRAQI POSTGRADUATE MEDICAL JOURNAL*. 18, 3.

MOHÁS, M., SZIGETI, N., MARKÓ, L., MOLNÁR, G. A., CSEH, J., LACZY, B., TAMASKÓ, M., BALLA, J., KAPPELMAYER, J. & WAGNER, L. 2008. Serum total LDH activity and LDH-2 isozyme in nephrotic syndrome. *Kidney and Blood Pressure Research,* 31**,** 47-54.

MONSERRAT-MARTINEZ, A., GAMBIN, Y. & SIERECKI, E. 2019. Thinking outside the bug: molecular targets and strategies to overcome antibiotic resistance. *International journal of molecular sciences,* 20**,** 1255.

MOORANI, K. N. 2011. Infections are common a cause of relapse in children with Nephrotic syndrome. *Pak Paed J,* 35**,** 213-9.

MORKA, K., BYSTROŃ, J., BANIA, J., KORZENIOWSKA-KOWAL, A., KORZEKWA, K., GUZ-REGNER, K. & BUGLA-PŁOSKOŃSKA, G. J. B. M. 2018. Identification of Yersinia enterocolitica isolates from humans, pigs and wild boars by MALDI TOF MS. 18**,** 1-10.

MUJAHID, A. & DICKERT, F. L. J. S. 2016. Blood group typing: from classical strategies to the application of synthetic antibodies generated by molecular imprinting. 16**,** 51.

MUKHERJEE, A., SADIQ, I. M., PRATHNA, T., CHANDRASEKARAN, N. J. S. A. M. P. C. C. R. & ADVANCES, T. 2011. Antimicrobial activity of aluminium oxide nanoparticles for potential clinical applications. 1**,** 245-251.

MURDOCK, C. B., BAKER, P. J., DELONG, E., ROE, C. R. & OSOFSKY, S. G. 1981. Urine and serum lactic dehydrogenase, lactic dehydrogenase isoenzymes, and alkaline phosphatase in the nephrotic syndrome. *Kidney International,* 19**,** 710-715.

MURRAY, P. & ZEITINGER, J. 1983. Evaluation of Mueller-Hinton agar for disk diffusion susceptibility tests. *Journal of clinical microbiology,* 18**,** 1269-1271.

MUZAMMIL, S., HAYAT, S., FAKHAR-E-ALAM, M., ASLAM, B., SIDDIQUE, M. H., NISAR, M. A., SAQALEIN, M., ATIF, M., SARWAR, A. & KHURSHID, A. J. F. B. 2018. Nanoantibiotics: Future nanotechnologies to combat antibiotic resistance. 10**,** 352-374.

NAIF, M. H. & MOHAMMED, T. F. 2019. Clinicopathological Features of Primary Nephrotic Syndrome in Iraqi Children. *Iraqi Medical Journal,* 65**,** 155-160.

NASROLLAHZADEH, M., SAJADI, S. M., SAJJADI, M. & ISSAABADI, Z. 2019. An introduction to nanotechnology. *Interface science and technology.* Elsevier.

NICHOLSON, D., EDWARDS, T. A., O'NEILL, A. J. & RANSON, N. A. 2020. Structure of the 70S ribosome from the human pathogen acinetobacter

baumannii in complex with clinically relevant antibiotics. *Structure,* 28**,** 1087-1100. e3.

NIEDERSTEBRUCH, N. & SIXT, D. 2013. Standard Nutrient Agar 1 as a substitute for blood-supplemented Müller–Hinton agar for antibiograms in developing countries. *European journal of clinical microbiology & infectious diseases,* 32**,** 237-241.

NINGSIH, F. F., SIREGAR, R. S. & TRISNAWATI, Y. 2021. COMPARISON OF HIGH SENSITIVITY C-REACTIVE PROTEIN VALUE FOR STEROID SENSITIVE NEPHROTIC SYNDROME PATIENTS WITH STEROID RESISTANT. *COMPARISON OF HIGH SENSITIVITY C-REACTIVE PROTEIN VALUE FOR STEROID SENSITIVE NEPHROTIC SYNDROME PATIENTS WITH STEROID RESISTANT,* 79**,** 8-8.

NOONE, D. G., IIJIMA, K. & PAREKH, R. 2018. Idiopathic nephrotic syndrome in children. *The Lancet,* 392**,** 61-74.

NTUSI, N., AUBIN, L., OLIVER, S., WHITELAW, A. & MENDELSON, M. 2010. Guideline for the optimal use of blood cultures: guideline. *South African Medical Journal,* 100**,** 839-843.

O'NEILL, J. 2016. Tackling drug-resistant infections globally: final report and recommendations.

OBAJULUWA, A., ONAOLAPO, J., OLAYINKA, B. & ADESHINA, G. 2017. Antibiotics susceptibility pattern of coagulase negative staphylococci isolates from orthopaedic patients. *International Educational Applied Scientific Research Journal* .1, 2456-5040.

ORTH, S. R. & RITZ, E. 1998. The nephrotic syndrome. *New England Journal of Medicine,* 338**,** 1202-1211.

ORUÇ, İ., SARı, H., ARAÇ, S., EYNEL, E., PEKKOLAY, Z., KADIROĞLU, A. K., YıLMAZ, Z., AYDıN, F. Y., AYDıN, E. & YıLDıRıM, Y. 2021. Frequency and relationship of ABO blood groups in patients with nephrotic syndrome. *Medical Research Journal,* 6**,** 301-304.

PADMAVATHY, N. & VIJAYARAGHAVAN, R. 2008. Enhanced bioactivity of ZnO nanoparticles—an antimicrobial study. *Science and technology of advanced materials*.

PAL, A. & KASKEL, F. 2016. History of nephrotic syndrome and evolution of its treatment. *Frontiers in pediatrics,* 4**,** 56.

PAL, S., TAK, Y. K., SONG, J. M. J. A. & MICROBIOLOGY, E. 2007. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium Escherichia coli. 73**,** 1712-1720.

PAPHITOU, N. I. 2013. Antimicrobial resistance: action to combat the rising microbial challenges. *International journal of antimicrobial agents,* 42**,** S25-S28.

PARK, C., KONG, M., LEE, J.-H., RYU, S. & PARK, S. 2018. Detection of bacillus cereus using bioluminescence assay with cell wall-binding domain conjugated magnetic nanoparticles. *BioChip Journal,* 12**,** 287-293.

PARK, S. J. & SHIN, J. I. 2011. Complications of nephrotic syndrome. *Korean journal of pediatrics,* 54**,** 322.

PEDROSO, S. H., SANDES, S. H., FILHO, R. A., NUNES, A. C., SERUFO, J. C., FARIAS, L. M., CARVALHO, M. A., BOMFIM, M. R. & SANTOS, S. G. 2018. Coagulase-negative staphylococci isolated from human bloodstream infections showed multidrug resistance profile. *Microbial Drug Resistance,* 24**,** 635-647.

PELEG, A. Y., SEIFERT, H. & PATERSON, D. L. 2008. Acinetobacter baumannii: emergence of a successful pathogen. *Clinical microbiology reviews,* 21**,** 538-582.

PELTOLA, V., MERTSOLA, J. & RUUSKANEN, O. 2006. Comparison of total white blood cell count and serum C-reactive protein levels in confirmed bacterial and viral infections. *The Journal of pediatrics,* 149**,** 721-724.

PEREZ-LLARENA, F. J. & BOU, G. 2016. Proteomics as a tool for studying bacterial virulence and antimicrobial resistance. *Frontiers in microbiology,* 7**,** 410.

PEREZ, F., HUJER, A. M., HUJER, K. M., DECKER, B. K., RATHER, P. N. & BONOMO, R. A. 2007. Global challenge of multidrug-resistant Acinetobacter baumannii. *Antimicrobial agents and chemotherapy,* 51**,** 3471-3484.

PETRI, W. 2011. Penicillins, cephalosporins, and other β-lactam antibiotics. *Goodman and Gilman's The Pharmacological Basis of Therapeutics 12th Ed McGraw-Hill, New York***,** 1477-1504.

PRASHANTH, K., VASANTH, T., SARANATHAN, R., MAKKI, A. R. & PAGAL, S. 2012. Antibiotic resistance, biofilms and quorum sensing in Acinetobacter species. *Antibiotic resistant bacteria: a continuous challenge in the new millennium***,** 179-212.

PRAVITSITTHIKUL, N., WILLIS, N. S., HODSON, E. M. & CRAIG, J. C. 2013. Non-corticosteroid immunosuppressive medications for steroid-sensitive nephrotic syndrome in children. *Cochrane database of systematic reviews*.

PRETE, G. D. 1992. Human Th1 and Th2 lymphocytes: their role in the pathophysiology of atopy. *Allergy,* 47**,** 450-455.

PRINSEN, B. H., DE SAIN-VAN DER, M. G., KAYSEN, G. A., STRAVER, H. W., VAN RIJN, H. J., STELLAARD, F., BERGER, R. & RABELINK, T. J.

2001. Transferrin synthesis is increased in nephrotic patients insufficiently to replace urinary losses. *Journal of the American Society of Nephrology,* 12**,** 1017-1025.

PRIYADHARSINI, J. V., GIRIJA, A. S. & PARAMASIVAM, A. 2018. In silico analysis of virulence genes in an emerging dental pathogen A. baumannii and related species. *Archives of oral biology,* 94**,** 93-98.

PULIDO, M. R., GARCÍA-QUINTANILLA, M., MARTIN-PENA, R., CISNEROS, J. M. & MCCONNELL, M. J. 2013. Progress on the development of rapid methods for antimicrobial susceptibility testing. *Journal of Antimicrobial Chemotherapy,* 68**,** 2710-2717.

QUAN, P.-L., SAUZADE, M. & BROUZES, E. 2018. dPCR: a technology review. *Sensors,* 18**,** 1271.

RACKI, S., ZAPUTOVIC, L., MAVRIC, Z., VUJICIC, B. & DVORNIK, S. 2006. C-reactive protein is a strong predictor of mortality in hemodialysis patients. *Renal failure,* 28**,** 427-433.

RAHUL, I., KRISHNAMURTHY, S., SATHEESH, S., BISWAL, N., BOBBY, Z. & LAKSHMINARAYANAN, S. 2015. Brachial artery flow-mediated dilatation and carotid intima medial thickness in pediatric nephrotic syndrome: a cross-sectional case–control study. *Clinical and experimental nephrology,* 19**,** 125-132.

RAI, V. K., AWASTHI, S. & VENKATESH, V. 2017. Prediction of early response to steroids in nephrotic syndrome patients aged between 2 and 10 years. *Clinical Epidemiology and Global Health,* 5**,** 203-208.

RANJBAR, R. & FARAHANI, A. 2019. Study of genetic diversity, biofilm formation, and detection of Carbapenemase, MBL, ESBL, and tetracycline resistance genes in multidrug-resistant Acinetobacter baumannii isolated from burn wound infections in Iran. *Antimicrobial Resistance & Infection Control,* 8**,** 1-11.

RAVANI, P., MAGNASCO, A., EDEFONTI, A., MURER, L., ROSSI, R., GHIO, L., BENETTI, E., SCOZZOLA, F., PASINI, A. & DALLERA, N. 2011. Short-term effects of rituximab in children with steroid-and calcineurin-dependent nephrotic syndrome: a randomized controlled trial. *Clinical Journal of the American Society of Nephrology,* 6**,** 1308-1315.

RAVANI, P., ROSSI, R., BONANNI, A., QUINN, R. R., SICA, F., BODRIA, M., PASINI, A., MONTINI, G., EDEFONTI, A. & BELINGHERI, M. 2015. Rituximab in children with steroid-dependent nephrotic syndrome: a

multicenter, open-label, noninferiority, randomized controlled trial. *Journal of the American Society of Nephrology,* 26**,** 2259-2266.

RELLER, L. B., WEINSTEIN, M., JORGENSEN, J. H. & FERRARO, M. J. 2009. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. *Clinical infectious diseases,* 49**,** 1749-1755.

REMUZZI, G. 2000. A unifying hypothesis for renal scarring linking protein trafficking to the different mediators of injury. *Nephrology Dialysis Transplantation,* 15**,** 58-60.

RESHI, A., BHAT, M., NAJAR, M., BANDAY, K., NAIK, M., SINGH, D. & WANI, F. 2008. Etiological profile of nephrotic syndrome in Kashmir. *Indian Journal of nephrology,* 18**,** 9.

RODER, C. & THOMSON, M. J. 2015. Auranofin: repurposing an old drug for a golden new age. *Drugs in R&D,* 15**,** 13-20.

RUF, R. G., LICHTENBERGER, A., KARLE, S. M., HAAS, J. P., ANACLETO, F. E., SCHULTHEISS, M., ZALEWSKI, I., IMM, A., RUF, E.-M. & MUCHA, B. 2004. Patients with mutations in NPHS2 (podocin) do not respond to standard steroid treatment of nephrotic syndrome. *Journal of the American Society of Nephrology,* 15**,** 722-732.

SAEED, B. M., ABBAS, B. A. & AL-JADAAN, S. A. Phylogenic tree of *Bacilus cereus* isolated from food sources and molecular detection of their virulence. *Bas.J.Vet.Res*.18, 2.

SANTAJIT, S. & INDRAWATTANA, N. 2016. Mechanisms of antimicrobial resistance in ESKAPE pathogens. *BioMed research international*. Article ID 2475067, 8 .

SARKER, M., ISLAM, M., SAAD, T., SHOMA, F., SHARMIN, L., KHAN, H., AFROOZ, F., FATMI, L., ALAM, A. & SALIMULLAH, A. 2012. Risk factor for relapse in childhood nephrotic syndrome-a hospital based retrospective study. *Faridpur Medical College Journal,* 7**,** 18-22.

SEIGEL, T. A., COCCHI, M. N., SALCICCIOLI, J., SHAPIRO, N. I., HOWELL, M., TANG, A. & DONNINO, M. W. 2012. Inadequacy of temperature and white blood cell count in predicting bacteremia in patients with suspected infection. *The Journal of emergency medicine,* 42**,** 254-259.

SHAIKH, S. A., JAIN, T., SANDHU, G., LATHA, N. & JAYARAM, B. 2007. From drug target to leads-sketching a physicochemical pathway for lead molecule design in silico. *Current pharmaceutical design,* 13**,** 3454-3470.

SHALLCROSS, L. J. & DAVIES, S. C. 2014. The World Health Assembly resolution on antimicrobial resistance. *Journal of antimicrobial chemotherapy,* 69**,** 2883-2885.

SHANTHA, G. P. S., KUMAR, A. A., BHISE, V., KHANNA, R., SIVAGNANAM, K. & SUBRAMANIAN, K. K. 2011. Prevalence of subclinical

hypothyroidism in patients with end-stage renal disease and the role of serum albumin: a cross-sectional study from South India. *Cardiorenal medicine,* 1**,** 255-260.

SHAREEF, L. G., SALMAN, A. A. & TAHER, M. A. 2021. Microorganisms Resistance Pattern and Antibiotic Prescriptions in Patients Admitted to the Intensive Care Unit in Ghazy AL Hariri Hospital. *Medico-legal Update,* 21.

SIEVERT, D. M., RICKS, P., EDWARDS, J. R., SCHNEIDER, A., PATEL, J., SRINIVASAN, A., KALLEN, A., LIMBAGO, B. & FRIDKIN, S. 2013. Antimicrobial-resistant pathogens associated with healthcare-associated infections summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2009–2010. *Infection Control & Hospital Epidemiology,* 34**,** 1-14.

SINGH, R., CAPALASH, N. & SHARMA, P. 2022. Vaccine development to control the rising scourge of antibiotic-resistant Acinetobacter baumannii: a systematic review. *3 Biotech,* 12**,** 1-14.

SINGHAL, R. & BRIMBLE, K. S. 2006. Thromboembolic complications in the nephrotic syndrome: pathophysiology and clinical management. *Thrombosis research,* 118**,** 397-407.

SINHA, A., BAGGA, A., GULATI, A. & HARI, P. 2012. Short-term efficacy of rituximab versus tacrolimus in steroid-dependent nephrotic syndrome. *Pediatric Nephrology,* 27**,** 235-241.

SINHA, A., SAHA, A., KUMAR, M., SHARMA, S., AFZAL, K., MEHTA, A., KALAIVANI, M., HARI, P. & BAGGA, A. 2015. Extending initial prednisolone treatment in a randomized control trial from 3 to 6 months did not significantly influence the course of illness in children with steroid-sensitive nephrotic syndrome. *Kidney international,* 87**,** 217-224.

SMILINE, A., VIJAYASHREE, J. & PARAMASIVAM, A. 2018. Molecular characterization of plasmid-encoded blaTEM, blaSHV and blaCTX-M among extended spectrum β-lactamases [ESBLs] producing Acinetobacter baumannii. *British journal of biomedical science,* 75**,** 200-202.

SMITH, D. A. & NEHRING, S. M. 2020. Bacteremia. *StatPearls [Internet]*.

SOUTHON, S. B., BERES, S. B., KACHROO, P., SAAVEDRA, M. O., ERLENDSDÓTTIR, H., HARALDSSON, G., YERRAMILLI, P., PRUITT, L., ZHU, L. & MUSSER, J. M. 2020. Population genomic molecular epidemiological study of macrolide-resistant Streptococcus pyogenes in Iceland, 1995 to 2016: identification of a large clonal population with a pbp2x mutation conferring reduced in vitro β-lactam susceptibility. *Journal of clinical microbiology,* 58**,** e00638-20.

SREEKANTH, S., BHATIA, P., MEENA, J., DAWMAN, L. & TIEWSOH, K. 2021. Iron deficiency in proteinuric children with nephrotic syndrome: A cross-sectional pilot study. *Archives de Pédiatrie,* 28**,** 485-487.

SRIVASTAVA, T., SIMON, S. D. & ALON, U. S. 1999. High incidence of focal segmental glomerulosclerosis in nephrotic syndrome of childhood. *Pediatric Nephrology,* 13**,** 13-18.

STÅHLBERG, A., THOMSEN, C., RUFF, D. & ÅMAN, P. 2012. Quantitative PCR analysis of DNA, RNAs, and proteins in the same single cell. *Clinical chemistry,* 58**,** 1682-1691.

SULEIMAN, S. A., ONAOLAPO, J. A. & OLAYINKA, B. O. 2020. Molecular detection of chloramphenicol-florfenicol resistance (cfr) genes among linezolid resistant MRSA isolates in Sokoto State, Nigeria. *Journal of Health & Biological Sciences,* 8**,** 1-6.

SYAL, K., MO, M., YU, H., IRIYA, R., JING, W., GUODONG, S., WANG, S., GRYS, T. E., HAYDEL, S. E. & TAO, N. 2017. Current and emerging techniques for antibiotic susceptibility tests. *Theranostics,* 7**,** 1795.

SZCZUKA, E., KRZYMIŃSKA, S. & KAZNOWSKI, A. 2016. Clonality, virulence and the occurrence of genes encoding antibiotic resistance among Staphylococcus warneri isolates from bloodstream infections. *Journal of Medical Microbiology,* 65**,** 828-836.

TAN, W., HAO, F., MCINTYRE, R. S., JIANG, L., JIANG, X., ZHANG, L., ZHAO, X., ZOU, Y., HU, Y. & LUO, X. 2020. Is returning to work during the COVID-19 pandemic stressful? A study on immediate mental health status and psychoneuroimmunity prevention measures of Chinese workforce. *Brain, behavior, and immunity,* 87**,** 84-92.

TEOH, C. W., ROBINSON, L. A. & NOONE, D. 2015. Perspectives on edema in childhood nephrotic syndrome. *American Journal of Physiology-Renal Physiology,* 309**,** F575-F582.

TEWABE, A., MAREW, T. & BIRHANU, G. 2021. The contribution of nano-based strategies in overcoming ceftriaxone resistance: a literature review. *Pharmacology Research & Perspectives,* 9**,** e00849.

TORRES, I., GIMENEZ, E., PASCUAL, T., BUENO, F., HUNTLEY, D., MARTÍNEZ, M. & NAVARRO, D. 2017. Short-term incubation of positive blood cultures in brain-heart infusion broth accelerates identification of bacteria by matrix-assisted laser desorption/ionization time-of-flight mass-spectrometry. *Journal of medical microbiology,* 66**,** 1752-1758.

TSUPRYKOV, O., CHEN, X., HOCHER, C.-F., SKOBLO, R., YIN, L. & HOCHER, B. 2018. Why should we measure free 25 (OH) vitamin D? *The Journal of steroid biochemistry and molecular biology,* 180**,** 87-104.

UNCU, N., BULBUL, M., YILDIZ, N., NOYAN, A., KOSAN, C., KAVUKCU, S., CALISKAN, S., GUNDUZ, Z., BESBAS, N. & GUVEN, A. G. 2010. Primary peritonitis in children with nephrotic syndrome: results of a 5-year multicenter study. *European journal of pediatrics,* 169**,** 73.

VALASEK, M. A. & REPA, J. J. 2005. The power of real-time PCR. *Advances in physiology education,* 29**,** 151-159.

VAN BELKUM, A., BACHMANN, T. T., LÜDKE, G., LISBY, J. G., KAHLMETER, G., MOHESS, A., BECKER, K., HAYS, J. P., WOODFORD, N. & MITSAKAKIS, K. 2019. Developmental roadmap for antimicrobial susceptibility testing systems. *Nature Reviews Microbiology,* 17**,** 51-62.

VAN DEN BERG, J. G., ATEN, J., CHAND, M. A., CLAESSEN, N., DIJKINK, L., WIJDENES, J., LAKKIS, F. G. & WEENING, J. J. 2000. Interleukin-4 and interleukin-13 act on glomerular visceral epithelial cells. *Journal of the American Society of Nephrology,* 11**,** 413-422.

VAZIRI, N. D. 2016. HDL abnormalities in nephrotic syndrome and chronic kidney disease. *Nature Reviews Nephrology,* 12**,** 37-47.

VICKERS, N. J. 2017. Animal communication: when i'm calling you, will you answer too? *Current biology,* 27**,** R713-R715.

WANG, Y., GUO, Y., ZHANG, L., YANG, Y., YANG, S., YANG, L., CHEN, H., LIU, C., LI, J. & XIE, G. 2021. Integration of multiplex PCR and CRISPR-Cas allows highly specific detection of multidrug-resistant Acinetobacter Baumannii. *Sensors and Actuators B: Chemical,* 334**,** 129600.

WANG, Y. & ZHANG, D. 2012. Synthesis, characterization, and controlled release antibacterial behavior of antibiotic intercalated Mg–Al layered double hydroxides. *Materials Research Bulletin,* 47**,** 3185-3194.

WANNAMETHEE, G., EBRAHIM, S. & GERALD SHAPER, A. 1995. Gamma-glutamyltransferase: determinants and association with mortality from ischemic heart disease and all causes. *American journal of epidemiology,* 142**,** 699-708.

WEI, T. Q., KRAMER, S., CHU, V. P., HUDSON, D., KILGORE, D., SALYER, S., PARKER, G., EYBERGER, A., ARENTZEN, R. & KOIV, H .2000 .An improved automated immunoassay for C-reactive protein on the Dimension® clinical chemistry system. *Journal of Automated Methods and Management in Chemistry,* 22**,** 125-131.

WEISSIG, V., PETTINGER, T. K. & MURDOCK, N. J. I. J. O. N. 2014. Nanopharmaceuticals (part 1): products on the market. 9**,** 4357.

WILSON, M. 2020. Critical factors in the recovery of pathogenic microorganisms in blood. *Clinical Microbiology and Infection,* 26**,** 174-179.

WONG, D., NIELSEN, T. B., BONOMO, R. A., PANTAPALANGKOOR, P., LUNA, B. & SPELLBERG, B. 2017. Clinical and pathophysiological overview of Acinetobacter infections: a century of challenges. *Clinical microbiology reviews,* 30**,** 409-447.

WOODFORD, N. & SUNDSFJORD, A. 2005. Molecular detection of antibiotic resistance: when and where? *Journal of Antimicrobial Chemotherapy,* 56**,** 259-261.

XU, A., ZHU, H., GAO, B., WENG, H., DING, Z., LI, M., WENG, X. & HE, G. 2020. Diagnosis of severe community-acquired pneumonia caused by Acinetobacter baumannii through next-generation sequencing: A case report. *BMC infectious diseases,* 20**,** 1-7.

XU, H., LI, W., MAO, J.-H. & PAN, Y.-X. 2017. Association between red blood cell distribution width and Henoch–Schonlein purpura nephritis. *Medicine,* 96,23.

YEH, E., PINSKY, B. A., BANAEI, N. & BARON, E. J. 2009. Hair sheep blood, citrated or defibrinated, fulfills all requirements of blood agar for diagnostic microbiology laboratory tests. *PloS one,* 4**,** 6141.

YOSHIOKA, K., OHASHI, Y., SAKAI, T., ITO, H., YOSHIKAWA, N., NAKAMURA, H., TANIZAWA, T., WADA, H. & MAKI, S. 2000. A multicenter trial of mizoribine compared with placebo in children with frequently relapsing nephrotic syndrome. *Kidney international,* 58**,** 317-324.

YOUNG, D. J. A. O. C. B. 1997. Effects of drugs on clinical laboratory tests. 34**,** 579-581.

YOUSEFICHAIJAN, P., REZAGHOLIZAMENJANY, M., RAFIEI, F., TAHERAHMADI, H. & RAFIEI, M. 2016. The relationship between blood biomarkers level and the prognosis of nephrotic syndrome in the children. *International Journal of Pediatrics,* 4**,** 3489-3497.

ZHANG, H., QIU, S., ZHONG, C., SHI, L., LI, J., ZHANG, T., ZHU, X. & WANG, M. 2021. Risk factors for poor prognosis of severe infection in children with idiopathic nephrotic syndrome: a double-center, retrospective study. *Frontiers in Pediatrics,* 9. Article 656215

ZHANG, L., PORNPATTANANANGKUL, D., HU, C.-M. & HUANG, C.-M. J. C. M. C. 2010. Development of nanoparticles for antimicrobial drug delivery. 17**,** 585-594.

ZHU, H., HAN, Q., ZHANG, D., WANG, Y., GAO, J., GENG, W., YANG, X. & CHEN, X. 2018. A diagnostic model for minimal change disease based on biological parameters. *PeerJ,* 6**,** e4237.

ZIGLAM, H., ELAHMER, O., AMRI, S., SHAREEF, F., GRERA, A., LABEEB, M. & ZORGANI, A. 2012. Antimicrobial resistance patterns among Acinetobacter baumannii isolated from burn intensive care unit in Tripoli, Libya. *The International Arabic Journal of Antimicrobial Agents*, 2.

ZORDAN, S., PRENGER-BERNINGHOFF, E., WEISS, R., VAN DER REIJDEN, T., VAN DEN BROEK, P., BALJER, G. & DIJKSHOORN, L. 2011. Multidrug-resistant Acinetobacter baumannii in veterinary clinics, Germany. *Emerging infectious diseases*, 17**,** 1751.

# الملخص

هدفت هذه الدراسة إلى استخدام طريقة النانو لتحسين استخدام المضادات الحيوية في معالجة ضمج الدم (التهاب الدم ) لدى الأطفال المصابين بالمتلازمة الكلوية (التناذر الكلوي)، وتحديد تجرثم الدم في هذه المجموعة من الأطفال ، ودراسة البكتيريا المعزولة لنمط الحساسية بالطريقة المظهرية وكذلك الكشف عن الجينات المقاومة للمضادات الحيوية وكذلك الكشف عن العلاقة بين بعض العوامل البيولوجية والمناعية لدى مرضى التناذر الكلوي من الأطفال مع اختلاف الأعمار.

اجرى البحث في الفترة ما بين تشرين الاول 2021 ومايو 2022 في مستشفى الاطفال التعليمي بمركز امراض الكلى / مديرية صحة كربلاء. تم جمع عينات من الدم الوريدي بحجم خمسة مليلتر من 116 طفل مصاب بالمتلازمة الكلوية و 42 طفل أصحاء. اجريت العديد من الاختبارات المهمة مثل زراعة عينات الدم ، اختبارات العوامل البيوكيميائية ؛ فيتامين د 3 ، تحليل الدهون الكامل ( TC، LDL، HDL و TG) ، اختبارات وظائف الكبد (الألبومين ، T. bilirubin، D. البيليروبين ، TP، ALT، AST، ALP، GGT، LDH) ، العوامل المناعية ( WBC، CRP، IgG ) ، العوامل الدموية CBC ، العوامل الفسيولوجية (الجلوكوز ، ABO) ، المعلومات العامة (العمر ، الوزن ، الجنس) ، والفحص الميكروبي (تحديد البكتيريا واختبار الحساسية للمضادات الحيوية).

تم تحضير Zno- ceftriaxone النانوي هجين (المضاد المقترن مع Zno بطريقة هلام السليكا) ، nanohybrid LDH-ceftriaxone (المضاد المقترن مع طبقات Mg-AL-No3) ، تم اختباره بعد ذلك بواسطة تحليل FTIR، SEM، XRD، AFM. كما تم فحص تأثير هذه الأدوية النانوية على البكتيريا المعزولة ، كما تم تحديد الجينات المقاومة للسيفترياكسون.

كانت النتائج للمعايير المقاسة في الأطفال المصابين بالتناذر الكلوي مقارنة بالأطفال الاصحاء كالتالي: وجد زيادة في مستوى الفيتامين د ،و زيادة في مستويات ال (الكوليسترول الكلي ، البروتينات الدهنية منخفضة الكثافة ، البروتينات الدهنية عالية الكثافة ،والدهون الثلاثية ).بينما وجد هناك نقصان كان في مستويات الألبومين ، والبروتين الكلي في مصل الدم، أيضا زيادة في مستويات البيليروبين الكلي و المباشر.

كان هناك نقصان في مستويات انزيمات الكبد : ALT، AST،و ALP )، ايضا وجد زيادة في مستويات الGGT و LDH، بالإضافة الى زيادة مستويات كريات الدم البيض وال CRP،بينما وجد هناك نقصان في مستوى الاجسام المضادة G .

وجد هناك ارتباط معنوي بين بعض العوامل لدى الأطفال الذين يعانون من التناذر الكلوي مثل بين ال CRP وكريات الدم البيض WBC (0.509) ، وكذلك بين CRP و الالبومين Albumin (0.515) ،

وكذلك WBC مع كل من Gran و Mid (0.727،0.856) على التوالي ، وكلها كانت ارتباط موجب كما وجدت علاقة ايجابية بين كل من: البيليروبين المباشر وال ALP بمقدار (0.566)، GGT وال LDH (0.640) ،الكوليسترول الكلي والبروتينات الدهنية منخفضة الكثافة بمقدار (0.808) وارتباط بين الدهون الثلاثية مع كلا من الكوليسترول والبروتينات الدهنية منخفضة الكثافة بمقدار (0.526,0.539) على التوالي.

في الأطفال المصابين بالمتلازمة الكلوية كانت فصيلة الدم B أكثر من الأصحاء. و كانت البكتيريا المعزولة عبارة عن *Acinetobacter bumanii* و *Staphylococcus warnerii* و *Bacillus cerus* كما وجد لكل منهم لديه جينات المقاومة لانزيم ال β-lactamase. لم يكن سيفترياكسون النانوي المحضر مع Zno فعالاً ، بينما كان سيفترياكسون النانوي الهجين مع طبقات Mg-Al-No3 فعالاً ضد البكتريا المعزولة ونجح اقترانه.

استنتجت الدراسة أن اكثر عدوى الدم في الاطفال المصابين بالتناذر الكلوي تأتي من بكتيريا ضمج المستشفيات. ان تقنية النانو بطريقة Zno soll gell لسيفترياكسون لم تنجح ولم تكن فعالة على البكتيريا. بينما كان ترسيب سيفترياكسون مع Mg-Al-No3 فعالًا وناجحًا حيث احتوت هذه الانواع البكتيرية المعزولة على جينات بيتا لاكتاماز.


**جامعة كربلاء**

# دراسة طبية حيوية لمتلازمة التناظر الكلوي مع تحسين المضاد الحيوي النانوي الهجين ضد المقاومة البكتيرية

**رسالة مقدمة**

إلى مجلس كلية العلوم الطبية التطبيقية - جامعة كربلاء

وهي جزء من متطلبات نيل شهادة الماجستير في التحليلات المرضية

**كتبت بواسطة**

**فاطمة عبدالحسن علي إبراهيم**

بكالوريوس تحليلات مرضية/ كلية العلوم الطبية التطبيقية – جامعة كربلاء/2016

**بإشراف**

**الأستاذ المساعد**

**د. علاء عبد الحسين كريم الدعمي**

**(ذوالقعدة)1444** **( حزيران) 2022**