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RESEARCH ARTICLE

Preparation of the Hybrid Nano-ceftriaxone and Study of its Inhibitory Activity against Resistant Bacteria Isolated from Children with Nephrotic Syndrome

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ABSTRACT

Objective: Our research aims to train and utilize nano method by two ways to improve antibiotic use in treating blood infections in children with nephrotic syndrome.

Method: The research was done in date between October 2021 and May 2022. In 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 in age (months to 15 years). The blood culturing procedures were followed, and several analyses were performed, including biochemical parameters and Microbial examination (Bacterial Identification and Antibiotic Susceptibility Test). Preparation of nanohybrid ZnO-ceftriaxone (drug conjugate with ZnO by sol-gel method), nanohybrid LDH-ceftriaxone (drug conjugate with layers of Mg-AL-No3), were tested then by (FT-IR) Fourier-transform infrared spectroscopy, scanning electron microscope (SEM), X-ray powder diffraction (XRD), Atomic force microscopy (AFM) analysis. The effect of these nano drugs was examined in isolated bacteria.

Results: The nanohybrid ceftriaxone prepared with ZnO was not effective .while nano hybrid ceftriaxone with layers of Mg-Al-No3 was effective and its conjugation was successful.

INTRODUCTION

Nephrotic syndrome remains a common chronic illness marked by changes in perm selectivity on the glomerular capillary wall, resulting in an incapacity to control protein loss in the urine. Proteinuria in the nephrotic range stays definite as more than 1000 mg/m^2 per daytime or a random urine protein-to-creatinine ratio of more than 2 mg/mg.[1] 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 and acute renal failure are disorder complications. In nephrotic children, long-term therapy frequently includes corticosteroid, alkylating drugs, Calcineurin inhibitors, and mycophenolate mofetil (MMF).[2]

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Increased rates of systemic infections such peritonitis, pneumonia, cellulitis, and meningitis, and others, are a major cause of demise.[3]

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]).[4] 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 to combat pathogenic bacteria' resistance to most commonly used antibiotics. Several classes, including antimicrobial nanoparticles (NPs) and nanosized carriers for antibiotic delivery, have shown their efficacy in treating infectious diseases.[5]

Bacterial Infections Associated with Nephrotic Syndrome (NS)

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, and 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.[6]

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 %).[7] The pathogens that causing septicemia in study, MRSA and CONS, were not the most frequently reported pathogens of nephrotic syndrome.[7]

Ceftriaxone is among the most widely used antibiotics due to the strong antibacterial power, broad spectrum of action, and slight risk for toxicity.[8]

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.[9]

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.[10]

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.[11] 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.[12]

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,[13] 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.[14]

In specialized hospital's medical and emergency wards, the unsuitable use of ceftriaxone was so common. This may result in resistant infections, leading to treatment failure and increased therapy cost.[8] The proposed research aims to improve the antibiotic like ceftriaxone that resist these bacteria, which nosocomial, by nano-methods.

MATERIALS AND METHODS

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 (15), of both sexes, with NS.

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

Nutusi *et al.* performed an important blood culturing procedure which should be followed[15] 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 .Write it on the wall above its bed, or even in the patient notes, to confirm Identification.

2- The children patient was informed about the surgery and 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 and water). It is must then cleansed or rubbed till they were completely dry. It was agreed 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.

6- A 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. The puncture site was cleaned with a dry swab and pressure was applied. 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, there's a lot to do.

8- The blood culture container was gently rotated to combine 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 (2 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 (1-mL) was put in an EDTA tube and agitated for at minimum 15 minutes.

Nanohybrid LDH- Ceftriaxone Preparation

The nanohybrid antibiotic was made using the layered double hydroxide method.[16]

1 Magnesium nitrates solution $Mg(NO_3)2.6H_2O$: This solution was made by dissolving 32.05 gram of Mg(No3)2 in 250 mL of unionized distilled water.

2 Aluminum nitrates solution $Al(No_3)3.9H_2O$: This solution was made by dissolving 11.72 gm of aluminum nitrates $Al(No_3)3$ 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,[16] with some modulation; by mixed Magnesium nitrates solution with Aluminum nitrates solution, the precipitation was did in 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 hours . 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 and then kept.

B. Ceftriaxone solution:

This solution was made by melting 1 g of 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 eighteen 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 and kept.[17]

Preparation of Nanohybrid ZnO-Ceftriaxone

Nanohybrid Ceftriaxone The process was used to create the nanohybrid antibiotic. well-defined by Kolekar *et al*.,with some modification.

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. Ceftriaxone Solution: A solution was also prepared by dissolving 1 gm of ceftriaxone in 50% ethanol and adding it to 100 mL of ethanol.

C. Ion exchange method using Ceftriaxone Gel Sol for training nanohybrids made from zinc oxide layers: The methods used formerly published by[17] 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.

Concentrate and Petri Dish Preparation

- Forty-two from petri dishes were equipped, 14 of them are nano ceftriaxone (ZnO), 14 for nano (LDH), and 14 of them were free ceftriaxone (for each type of our bacteria).
- Any of the free and nano(ZnO,LDH) dishes were assigned a number based on the concentrations (0,1.25, 2.5, 5, 10, 20, 40)mg/mL with a duplicate for each

Table 1: Antibiotic concentration training

No. of tube	*Distilled water (µL)*	*Stock Solution (µL)*	*End volume (µL)*	*End concentration (mg/mL)*
1	1000	0	1000	0
2	968.75	31.25	1000	1.25
3	927.5	62.5	1000	2.5
4	875	125	1000	5
5	750	250	1000	10
6	500	500	1000	20

concentration.

- For each petri dish, two wells with a diameter of 5 mm were drilled into the media.
- A18 tube were prepared, 6 of which were Free-ceftriaxone, 6 of which were Nano-ceftriaxone(ZnO), and 6 of which were for Nano ceftriaxone (LDH).

Stock Solution Training

Free-Ceftriaxone 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.

Antibiotic Concentration Training:

The concentrations used in this test were made separately using the methods listed in Table 1 for free-Ceftriaxone and nano-Ceftriaxone.

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-Ano 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 characterized by AFM to determine the nanoparticles' diameters, sizes, and aggregation.

4.Detailed analysis of elements C, H and **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.

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.[18]

to see if Free-Ceftriaxone and Nano-Ceftriaxone have antibacterial activity, as:

The Media

Media for Nutrient Broth was made rendering 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.

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.

Activation of Bacteria Before 1 hour of culturing, *A bumanii* complex*, Staphylococcus warnie, and B cerus* were activated on nutrient broth.

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 100 of antibiotic concentration was added to each well, and 50 mL of active bacteria solution was placed on each petri plate and incubated for one day at 370°C. A diameter of the inhibitory zone surrounding the well was measured using a ruler, and growth was observed.

RESULTS AND DISCUSSION

Nanotechnology Study

Due to their unique physical and biological characteristics and their capacity for controlled drug release, nanoparticles have gained worldwide acceptance in various fields of medicine. Many inorganic nanoparticles with antibacterial properties, including gold, silver, zinc oxide, and titanium dioxide nanoparticles, have been developed in recent years. These inorganic nanoparticles can stop bacteria from multiplying by interference with replication and transcription, causing DNA damage by direct interaction, generating reactive oxygen species, and destroying the cell wall, among other things. They were also shown to be effective against resistant bacterial strains.[19]

Infrared spectrum (FT-IR)

FT-IR of Zinc Oxide (ZnO) FT-IR spectrum of free-ceftriaxone antibiotic (Figures 1 and 2): 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 around 1735 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

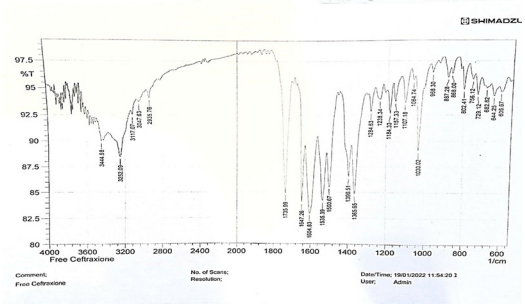

Figure 1: FT-IR for free-ceftriaxone

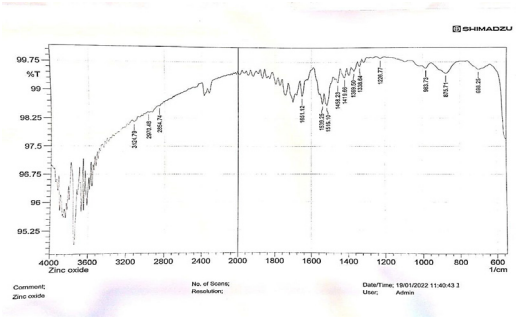

Figure 2: FT-IR for free-ZnO

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 is around 1107 for (C-OCH_3) stretching. The band at 802 due to aromatic (C-H) bending.

FT-IR spectrum of nano Nano-ZnO-Ceftriaxone (Figure 3). The doublet band around 3440 is attributed to (NH_2) stretching. The absorption band at 3279 for (O-H) groups stretching which was shifted to a 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 a lower frequency. The bands at 2947 were due to aliphatic (C-H) stretching which was shifted to a higher frequency. The strong band around 1739 due to carboxylic (C=O) stretching which was a little shifted to a higher frequency. The band at 1651 for amidic (C=O) stretching was also slightly shifted to a higher frequency. The bands at 1604, 1543 and 1516 were assigned to (C=N) groups stretching, which were shifted to a higher frequency. The bands around 1454 and 1419 are 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).

FT-IR for LDH

FT-IR spectrum of free LDH (Figure 8): 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.[20]

FT-IR spectrum of Nano-LDH-Ceftriaxone antibiotic (Figure 5): The band at 3460 attributed to (NH_2) stretching which was shifted to a higher frequency. The absorption

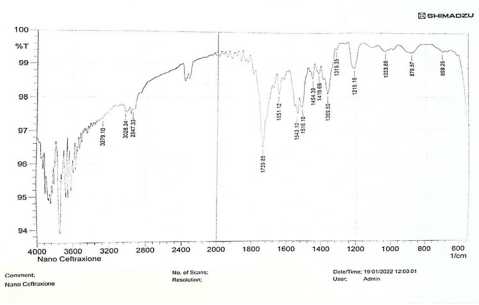

Figure 3: FT-IR for Nano-ZnO-Ceftriaxone

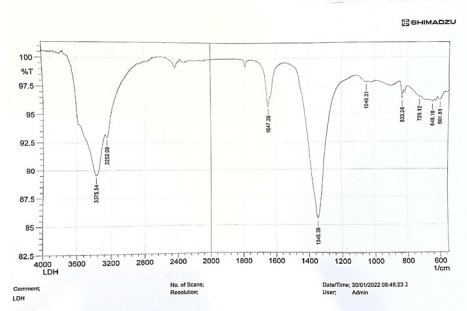

Figure 4: FT-IR for free LDH

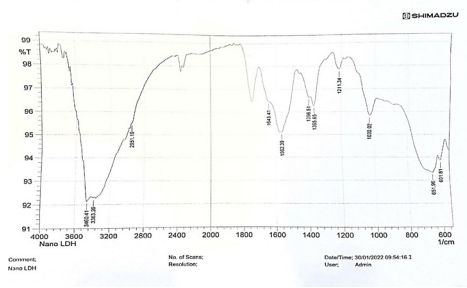

Figure 5: FT-IR for Nano-LDH-Ceftriaxone

band at 3383 for (O-H) groups stretching and amidic (N-H) stretching (overlapped) was also shifted to a higher frequency. The band at 3050 is assigned to the aromatic (C-H) stretching of thiazole ring. The bands at 2935 due to aliphatic (C-H) stretching. The strong band around1735 is due to carboxylic (C=O) stretching. The band at 1643 for amidic (C=O) stretching was a little shifted to lower frequency. The broad band at 1562 was assigned to (C=N) groups stretching (overlapped). The band around 1396 is 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 is near 840 due to aromatic (C-H) bending.

XRD

Figures 6 and 7 show 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 10.47° with a crystal distance of 0.84 nm as for the level (006), it appears at the angle of 22.03° with a crystal distance of 0.41 nm while the plane (009) appears at an angle of 34.44° and a crystalline distance equal 0.26 nm. And by observing the X-ray diffraction spectrum of anti-hybrid nanoparticles Mg/Al-Clo-LDH It shows diffraction of the

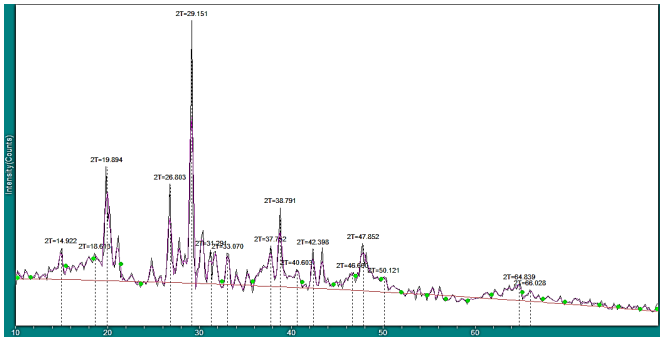

Figure 6: XRD for free LDH (A)

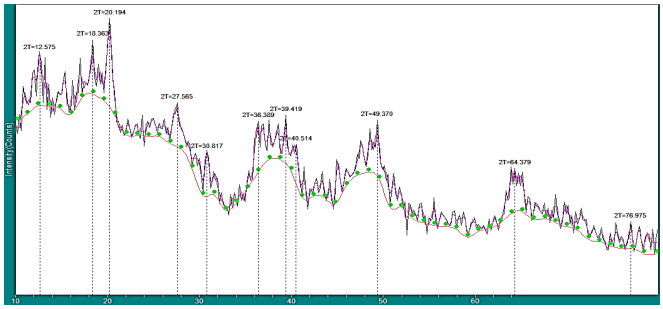

Figure7: XRD for Nano LDH (B)

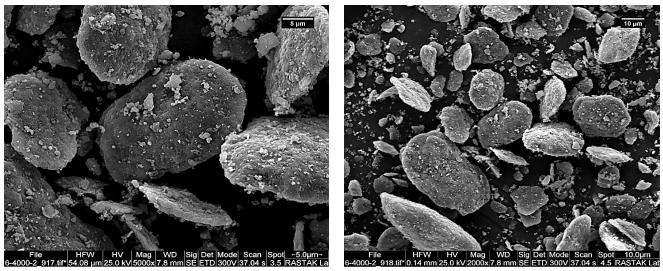

Figure 8: SEM for free ceftriaxone 10µm,5µm

(003) plane with a crystal distance 2.5 nm. It also shows the diffraction of the plane (006) with a crystal distance 0.94 nm in addition to the appearance of the plane (009) with a crystal distance of 0.7 nm.

Scanning Electronic Microscope Characterization (SEM)

Figures 8,9 and 10 show an image of scanning electron microscope of ceftriaxone layers, where it is observed the rough and irregular surfaces appeared in Ceftriaxone powder.[21]

As in Figure 8, a scanning electron microscope image of the dihydroxide layers is observed, in which plate-like structures with few pores and irregular shapes and sizes are observed.[22]

Atomic Force Microscope Characterization (AFM)

The exterior surface of the nanohybrid-ceftriaxone was studied by AFM. Figure 11 depicts semispherical shapes of Nano LDH ceftriaxone in two dimensions. The Figure depicted a three-dimensional picture of the surface section of the nanohybrid antibiotic, indicating that the preparation of the nanohybrid antibiotic was effective, with the elevation of molecular assemblies reaching 145.7 nm.

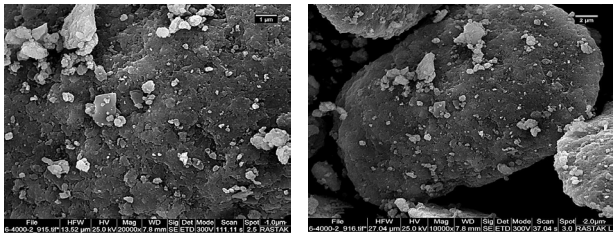

Figure 9: SEM for free ceftriaxone 2µm,1µm.

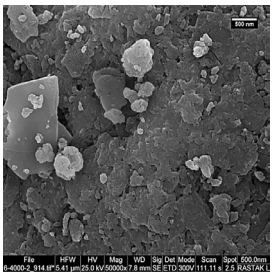

Figure 10: SEM for free ceftriaxone 500 nm

Table 2: 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 C: 10.51 S: 4.294 H: 1.997	11432 18279 3275 11364
2	Free Ceftriaxone	9.1460	Index 1	2.000	N: 12.09 C: 24.19 S: 10.02 H: 1.740	35042 49923 9177 11793

CHNS Elemental Analysis

The results in this analysis showed that the 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 the same elements were (24.19, 12.09, 1.74, 10.02) respectively as in Table 2 and 3.

These results appeared the percentage of ceftriaxone conjugated with LDH layers was 43.447. This result explained if use of nano LDH ceftriaxone was successful or useful as a treatment, which showed from carbon elements content that 43% of ceftriaxone found in nano drug (Mg/Al-NO3-cefetriaxone) and in this percentage, it remained high activity on tested bacteria in this study also nano LDH ceftriaxone can cause less side effects by increasing intracellular delivery similar.[20]

Bacterial Identification

Antimicrobial Activity of Ceftriaxone

A. Activity of Ceftriaxone for A. bumanii sp

Table 4 demonstrated that the widths of the inhibition zone of free ceftriaxone vary significantly (p 0.001) versus *A. bumanii* sp at all doses used when compared to the control. Furthermore, as concentration was raised, there was an increase in the

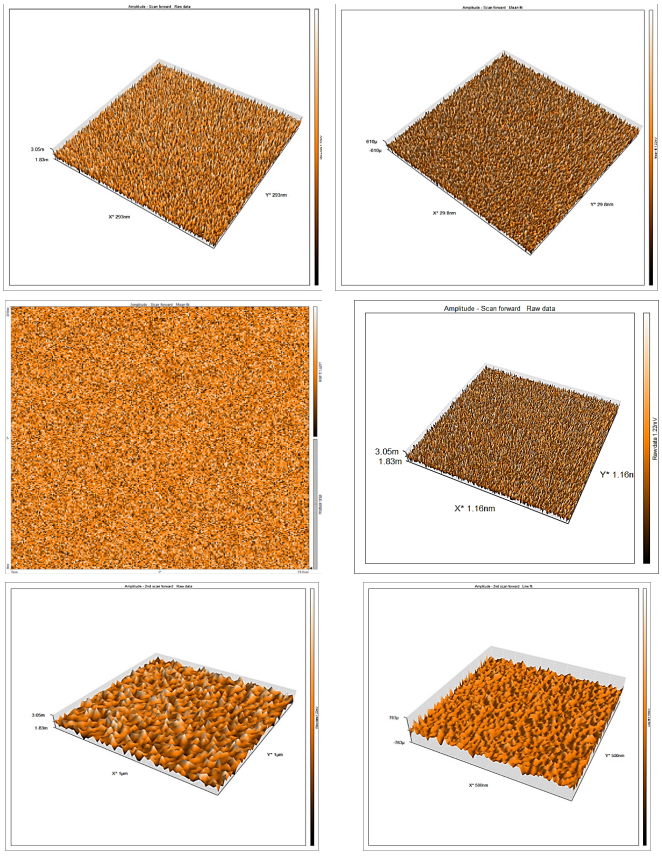

Figure 11: AFM for nano LDH-ceftriaxone

Table 3: Bacteria isolated from Nephrotic Syndrome patient children

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

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 *A. 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 sol-gel method, there are less significant differences in the diameters of inhibition zone of the nano ceftriaxone ZnO against *A. bumanii* 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.

In recent years, ceftriaxone has been a component of therapeutic regimens used to treat some of the most virulent

Table 4: The inhibition zone (mm) of free ceftriaxone and their nano-hybrid composites against *A. 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	
8.00	60.00 ± 0.00	41.25 ± 7.21	29.37 ± 0.75	0.000**

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

bacterial infections. However, rising bacterial resistance to third generation cephalosporin antibiotics like ceftriaxone and others has created severe clinical concerns. As a result of advancements in nanotechnology, better targeting and lower drug consumption, the concept of nanotherapeutics is becoming a tenable reality.[19] 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 and 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, and cefotaxime), the concentration of ceftriaxone was (30 g), and the number of isolates resistant to tested drug was 113.[23] *A. bumanii* was also shown in the case report study by Lahmidi et al. to be resistant to numerous antibiotics, notwithstanding ceftriaxone's conclusion that native valve infective endocarditis caused by *A. baumannii* was uncommon. Their case was significant because it highlighted the need for nosocomial infection control and preventive measures since infective endocarditis caused by the dreaded *A. baumannii* is a rare but potentially fatal cause of death in patients who had spent time in the intensive care unit.[24]

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, and 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.[23]

Layered double hydroxides (LDHs), which are based on nanohybrids, are now often employed in the medical profession as drug delivery systems because they may prevent the breakdown of active biomolecules, making them beneficial for sustained drug delivery. LDHs, also known as anionic clays, attracted interest because of their numerous features and significant applications, particularly in the field of medicine.[25] Third-generation cephalosporin ceftriaxone is a time-dependent killer used to treat life-threatening infections such

Table 5: The inhibition zone(mm) of free ceftriaxone and their nano-hybrid composites against *S. warnei*

Concentration µg/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**

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

gonorrhea, meningitis, and community-acquired pneumonia. This antibiotic's integration into the LDH structure enhances antimicrobial therapy by increasing intracellular delivery and minimizing harmful side effects.[20] 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.[20] 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.[26] 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 and Gram-negative bacteria. Previous research has shown that ZnO-NPs had broad spectrum antibacterial activity against clinical isolates examined in their study.[27] Notably, Ceftriaxone and 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, and 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 and 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 the 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.[26]

Antimicrobial Activity of Ceftriaxone for *S. warnerii*

Table 5 represents that there are substantial significant differences ($p \leq 0.001$) in the diameters of the inhibition zone of free ceftriaxone versus *S. 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 ($p \leq 0.01$) in the diameters of the zones of inhibition of the nano ceftriaxone LDH versus *S. 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 *S. 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.

Ceftriaxone kills the bacteria by preventing peptidoglycan cross-linking, which ultimately ends cell wall synthesis.[28]

S. 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 and 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.[29] 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.[29] *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, *S. 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.[30]

As a result, the use of ceftriaxone-conjugated metallic

Table 6: The inhibition zone(mm) of free ceftriaxone and their nano-hybrid composites against *B. cereus*

Concentration µg/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**

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

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.[31]

Antimicrobial Activity of Ceftriaxone for *Bacillus cereus*

The statistical analysis results in Table 6 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 ($p \leq 0.01$) in the diameters of the inhibition zone of the nano ceftriaxone LDH versus *B 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.

One of the microorganisms that causes nosocomial bloodstream infections is *B. cereus* (BSIs). Few papers, however, have described the clinical features and antibiotic susceptibility of *B. cereus* BSI and the significance of empirical therapy. In study[32] all isolates demonstrated gentamicin, imipenem, and vancomycin sensitivity. However, cephalosporin resistance was present in 48.3–100% of isolates.[32]

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 *B. subtilis*, *S. aureus*, *E. coli, Proteus vulgaris*.[31] 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.[20] 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.[31]

CONCLUSIONS

- Most bacterial species that causes blood infections to nephrotic syndrome children of Karbala were nosocomial bacterial infection.
- Some bacterial isolated was resistant to ceftriaxone
- 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.

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