# Antibacterial effects of Ceftriaxone/Zinc Oxide Nanoparticles Combination Against Ceftriaxone resistant *Escherichia coli* isolated from Urinary Tract Infections

**Sarah F. Al-Taie[1], Dhafar N. Al-Ugaili[2], Khawla A. Kasar[3], Laith A. Yaaqoob[4]**

*[1]Researcher, [2]Assistant Professor, College of Applied Biotechnology, [3]Lecturer, Department of Chemistry, College of Science, Al-Nahrain University, Baghdad, Iraq, [4]Assistant Professor, Department of Biotechnology, College of Science, University of Baghdad, Baghdad, Iraq*

## Abstract

This study was designed to shed light on the issue of pathogenic bacteria resistance to antibiotic, and to overcome it by taking the advantages of nanotechnology. A hundred and twenty urine samples were collected from patients of different ages and of both genders who showed symptoms of urinary tract infections, from three hospitals in Baghdad. Ceftriaxone *E. coli* was detected and isolated from these samples. More recently, nanotechnology application has grown in importance in this problem. In this study, we used Zinc oxide nanoparticle to enhance the activity of ceftriaxone. Zinc oxide nanoparticle (ZnO NP) was prepared by biological method of processing of a fresh leaf aloe vera plants. Further characterization methods, which are Fourier, transform infra-red Spectroscopy (FTIR), and Atomic Force Microscopy (AFM) (which showed that the average diameter of the newly created ZnO NP is 45.55 nm) were applied for checking the created nanoparticle. Antimicrobial ability of ZnO NP showed an inhibition zone with a 13 mm in diameter. The isolates of *E. coli* showed a resistance to ceftriaxone at a concentration of 100 µg/ml. The results of inhibition activity of ceftriaxone antibiotic against *Escherichia coli* isolates of the current study showed a remarkable change when mixed with ZnO NP, in which ceftriaxone became affective in inhibiting *Escherichia coli* growth.

***Keywords:*** *Urinary tract infections, Zinc oxide nanoparticles, b-Lactamase*

## Introduction

Antibiotics modification is a frequently used strategy for rendering an antibiotic ineffectiveness and a large number of modified antibiotics such as aminoglycoside antibiotics (e.g. kanamycin, streptomycin, and gentamycin), β-lactams, chloramphenicol and others are known to exist in producer bacteria[1,2]. Nanoparticles are new strategy in drug modification. Nanoparticles (NPs) in general have many properties that are different from those of traditionally used materials[3,4,5]. They have dimensions typically below 100 nm, which allows them to reach specific sites inside the body and even to be permeable to tissues and cells. Therefore, they can deliver the drugs in active forms at sites that conventional drugs may not reach by themselves and thus minimize the undesirable side effects[6].

In the past two decades, ZnO NPs have become one of the most common metal nanoparticles in biological applications because of their excellent biocompatibility, with high economic and low toxicity[7]. ZnO NPs have showed promising candidates in biomedicine, especially in anticancer and antibacterial fields, which are involved with their ability to trigger an excess reactive oxygen species (ROS) production, release zinc ions, then induce

cell apoptosis[8]. Zinc oxide (ZnO) nanoparticles (NPs) have been implicated in the studies of next-generation nanoantibiotics development against pathogenic microorganisms such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and others[9,10,11,12], since they have a unique physicochemical properties[10]. ZnO NPs can be combined with antibiotic and anti-inflammatory drugs to enhance antimicrobial activity against pathogenic microorganisms[13,14]. Their application has been extended to antibiotic drugs or medical devices, theragnostic, implants, and other devices used in clinics[15]. Their particle size and particle size distribution levels are the key parameters that determine NP uptake into biomembranes and thereby influence antimicrobial activity against pathogenic microorganisms[16]. Furthermore, large specific surface area levels of ZnO NPs/MPs facilitate membrane adsorption for antimicrobial actions[17].

Up till now few reports are available on synergistic bactericidal activity of inorganic nanomaterials in combination with b-lactam antibiotics. Combinations of ZnO NPs/MPs with other antibiotic drugs, metal oxide NPs/MPs, and devices have been used to enhance antimicrobial activity against pathogenic microorganisms[18,19]. They have synergistic or improved antimicrobial activity against *E. coli, S. aureus, Aeromonas veronii, P. aeruginosa, B. subtilis*, and *Klebsiella pneumoniae*. This work aimed to explore the antibacterial activity of Ceftriaxone/Zinc Oxide Nanoparticles hybrid against Ceftriaxone resist *E. coli* isolated from urinary tract infections.

## Material and Methods

### Synthesis of Zinc Oxide Nanoparticle (ZnO)

Fresh leaf aloe vera plants were collected, weighed (25 g) and washed with tap water and then with distilled water to remove the impurities[20]. The washed leaves were crushed and boiled for about 15 min in 100 ml distilled water. The boiled extract was cooled and filtered through Whatman filter paper. The filtered plant extract solution was used for the synthesis of ZnO NPs. The plant extract was stored in a refrigerator for further use.

### Preparation of ZnO Nanoparticle

Aqueous solutions of zinc acetate di-hydrate (5, 10 and 50 mmol kg−1) were prepared using distilled water. The above-prepared aloe vera plant extract was added drop wise to these solutions (~5ml min−1) in a round bottom flask under constant stirring and heating at 70◦C. About 15 min after the addition, freshly prepared 0.2 mol kg−1 KOH solution was added dropwise to the reaction mixture to maintain the pH of 11.5. After constant stirring and heating for 4 hrs., the ZnO NPs were collected and washed 3– 4 times with distilled water and then with ethanol. The NPs were dried in a hot air oven at 55−60˚C before further use. The formation of ZnO NPs was confirmed by the appearance of yellowish white color precipitates in the solution mixture

### Antimicrobial Activity of Zinc Oxide Nanoparticles

*E. coli* was cultured in the Muller – Hinton dishes from the broth by streaking methods by using sterilized loop. After that, 1 well was made in the agar. 50 µl of each dilution of ZnO nanoparticle was added to the well. The dish was sealed and left in an incubator at 37 ºC overnight, to be read in the next day.

### Ceftriaxone Dilution

By using micropipette, 100 µl of the stock solution (10 ml of distilled water was add to 1 gm of the antibiotic) was added to 900 µl of DW in an eppendorf to make dilution #1. Serial dilution method was done to prepare 4 dilutions.

### Ceftriaxone Resistance

*E coli* were cultured in the Muller – Hinton media and a ceftriaxone disk (35 µg) was put on the agar. Bacteria were allowed to grow overnight.

**Detecting the MIC**

*E. coli* was cultured in the Muller – Hinton dishes from the broth by streaking methods. Two wells per each dish were made. Fifty µl of each dilution of ceftriaxone was added to the wells. Dishes were sealed and left in an incubator at 37 ºC overnight, to be read in the next day.

Ceftriaxone – Nanoparticles Combination

Fifty µl of each dilution was put in 5 eppendorfs separately. By end of this step, 5 eppendorfs; each having 50 µl of a specific dilution of ZnO nanoparticles, was prepared. Fifty microliters of the targeted dilution/concentration of ceftriaxone were added to each eppendorf to have 100 µl of the solution in each.

Ceftriaxone/Zinc Oxide Nanoparticles Sonication

All eppendorfs were then mixed by using ultrasonic bath. The eppendorfs were sonicated in the device for 15 minutes and at 37 ºC.

Ceftriaxone/Zinc Oxide Nanoparticles Antimicrobial Activity

*E. coli* was cultured in the Muller – Hinton dishes from the broth by streaking methods. After finishing the culturing, 2 wells per each dish were made. 100 µl of each solution was added to the wells. Dishes were sealed and left in an incubator at 37 ºC overnight, to be read in the next day.

Atomic Force Microscopy (AFM)

The surface morphology of the ZnO nanoparticles was visualized by AFM under normal atmospheric conditions. ZnO NPs powder sample is fitted atop the scanner tube with less than 8mm thickness. The sample is placed on a 15 mm diameter steel disk.

Fourier Transform Infra-Red Spectroscopy (FTIR)

Fourier Transform Infrared (FTIR) spectroscopy analysis was carried out using FTIR spectrometer (8400S, Shimadzu, Japan) in attenuated total reflection mode and spectral range of 4000–400 cm -1 with a resolution of 4 cm. The antibiotic suspension was dropped onto the glass slides with the help of a pipette and allowed to dry at 30◦C in incubator.

## Results and Discussion

Production of ZnO Nanoparticles

The FTIR result of ZnO NPs exhibited many characteristic bands, which includes the bands at 3425.34 and 3434.98 $cm^{-1}$, which were due to the stretching vibration of O-H bond; and the bands at 420.45 and 567.03 $cm^{-1}$, which was due to due to the vibration of Zn-O.

The AFM appeared the surface roughness of the ZnO NPs[21]**.** The result showed that the 2D and 3D images of the sample have uniform height distribution around 45.55 nm as an average, as shown in figure-1 and figure-2.

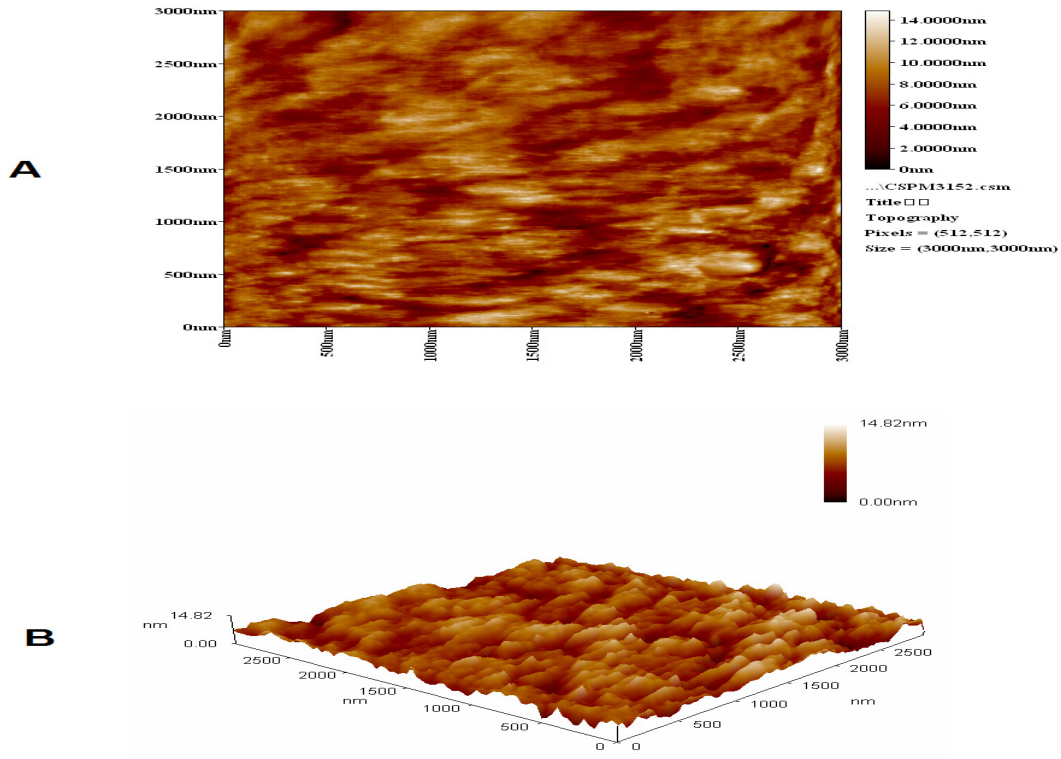


**Figure-1: AFM results for mixing of ZnO nanoparticle. A: The two-dimensional image of ZnO nanoparticle. B: The three-dimensional image of ZnO nanoparticle**

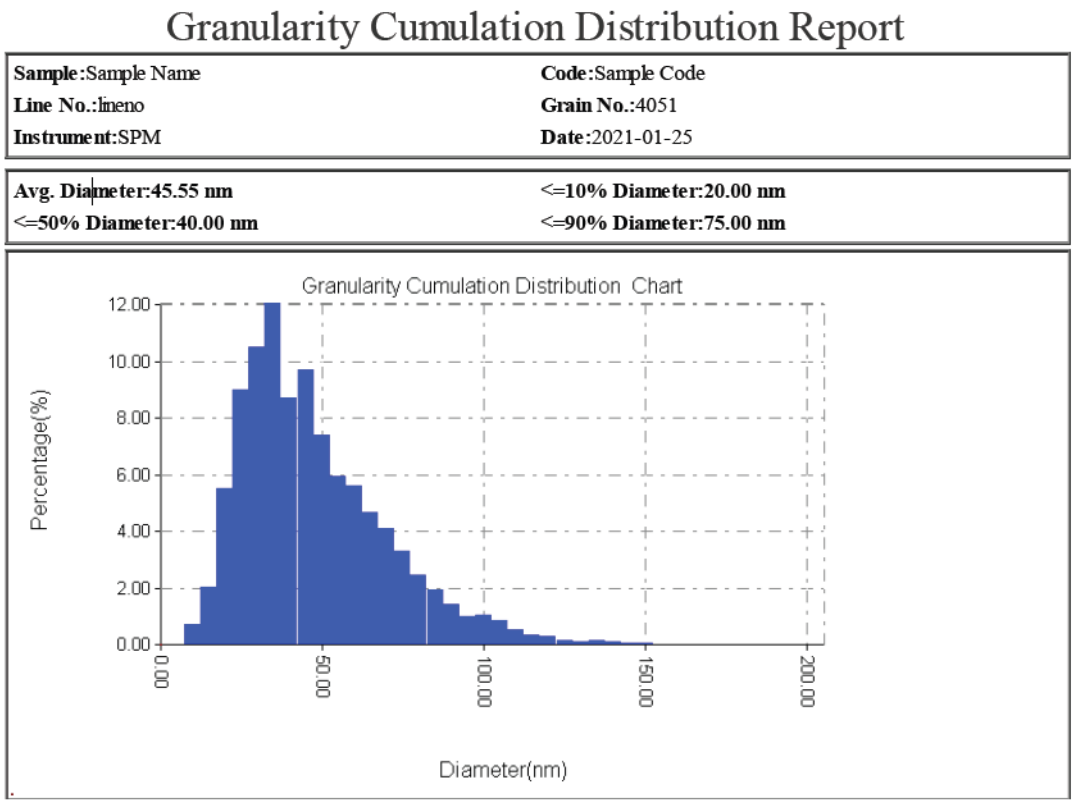


**Figure-2: AFM result report of ZnO nanoparticle**

ZnO Nanoparticle Sensitivity Test

On a Muller – Hinton agar, ZnO nanoparticles (with a concentration of 0.03 µg/µl) were put in wells and then the plates were inoculated with overnight bacterial culture. After examining the results on the next day, antimicrobial activity of ZnO NPs against *E. coli* isolates was tested with an inhibition zone of 13 mm. Dens Check machine were used for standardization the first Kahn tube to McFarland standard (1.5 X 108 CFU/ml).

**Ceftriaxone Sensitivity Test to *E. coli***

On Muller – Hinton agar, *E. coli* was allowed to grow overnight alongside a disk of ceftriaxone (35 µg. The results on the next day, it was found that *E. coli* is resistant to the ceftriaxone.

**Ceftriaxone MIC Test**

The use of the ceftriaxone antibiotic at a concentration of 100 µg/µl produce an obvious inhibitory effect against the tested isolates depending on the diameter of the inhibition zone compared with that of the antibiotic disc, while no antibacterial effect was detected for the antibiotic at the tested concentration of (10 µg/µl, 1 µg/µl, and 0.1 µg/µl as described in figure-3 which shows dilutions effect on *E. coli*.

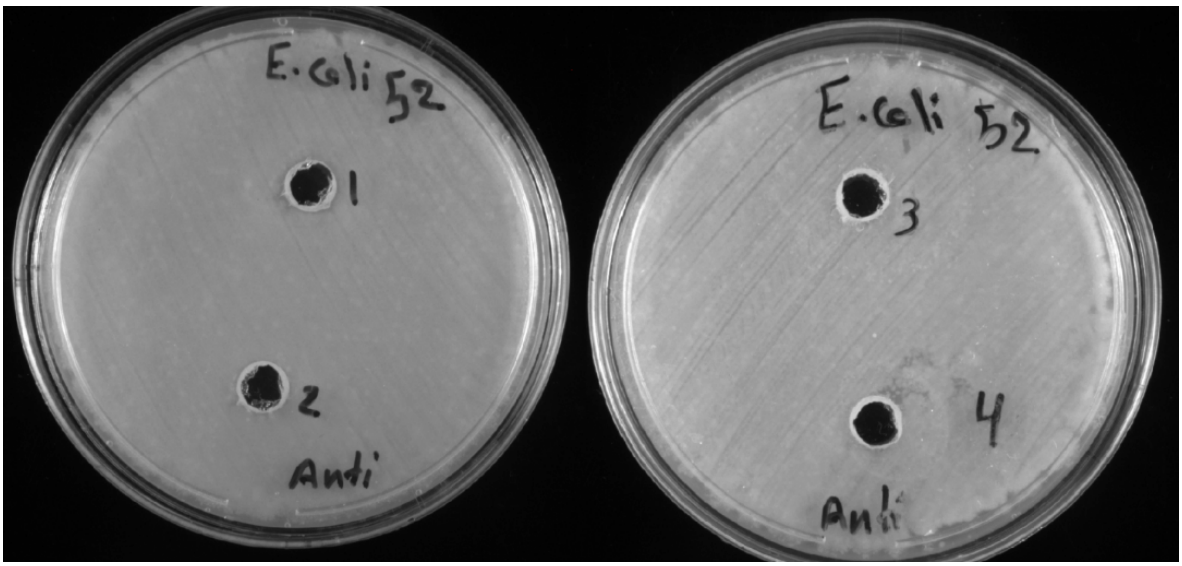


**Figure-3: Serial dilution of ceftriaxone. Dilution number 1 is showing no inhibition zone.**

Ceftriaxone Fourier transform infra-red Spectroscopy (FTIR) Test

The FTIR result of ceftriaxone carrier showed many characteristic bands at specific frequencies, which includes the bands at 3446.56 and 3259.47 $cm^{-1}$, which were caused by stretching vibration of O-H bond; the band at 1649.02 and 1502.44 $cm^{-1}$, which were as a result of the N-H bond; the bands at 1741.60 $cm^{-1}$, which was due to the C=O stretch; the two bands at 1398.30 and 1367.44 $cm^{-1,}$ which were due to C-H rock; and the band at 1033.77 $cm^{-1}$, which was due to the C-N stretch of aliphatic amines.

Hybrid Nanoceftriaxone Fourier transform infra-red Spectroscopy (FTIR) Test

The FTIR result of hybrid nanoceftriaxone showed the appearance of some characteristic bands as shown in figure-4, which includes the band at 3440.77 $cm^{-1}$, which was due the O-H alcoholic stretching; the bands at 1643.24 and 1627.81 $cm^{-1}$, which were caused by the aminic N-H bonds; and lastly the band at 513.03 and 441.67 $cm^{-1}$, which were due to the Zn=O group.

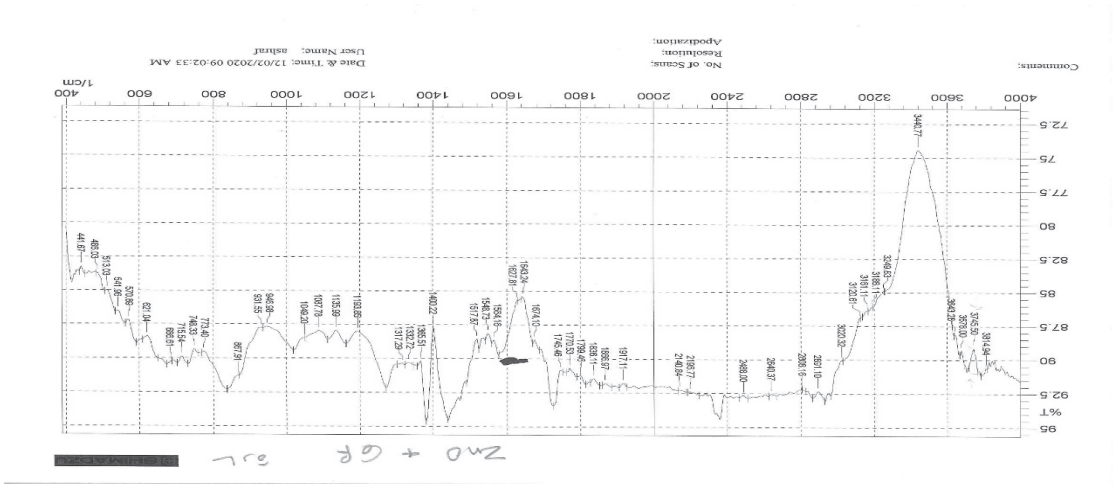

**Figure-4: FTIR result of hybrid nanoceftriaxone.**

The antimicrobial efficiency of ceftriaxone/ZnO NPs showed a remarkable inhibition activity with an inhibition zone of 19 mm compared with 13 mm for ZnO NPs when used alone. Figure-5 shows the inhibition zones resulted from the ceftriaxone-nanoparticles combinations.

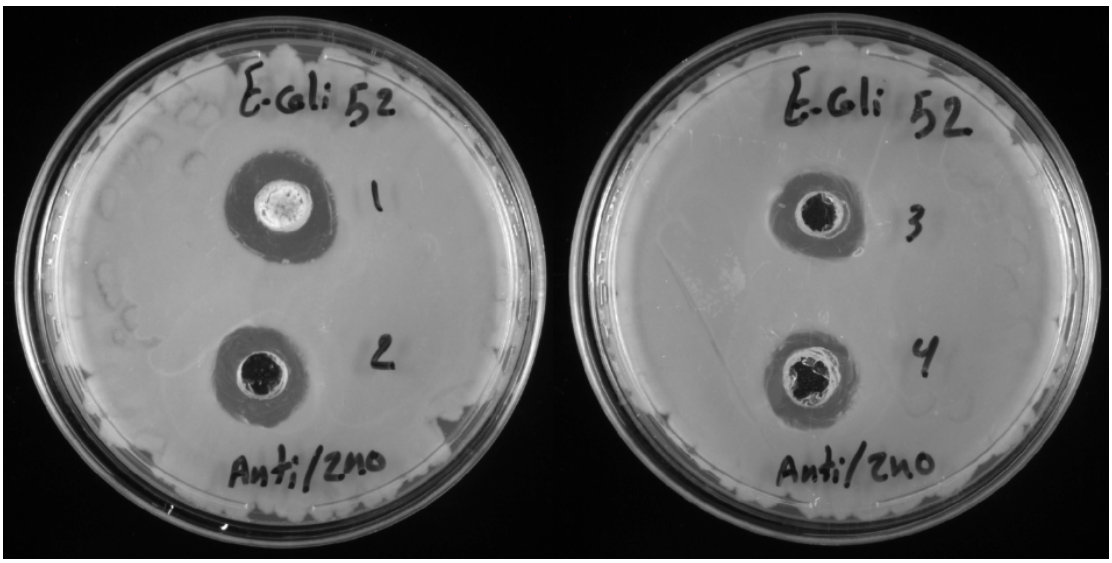


**Figure-5: Hybrid nanoceftriaxone concentrations on Muller – Hinton agar.**

According the results there is a greater capability of the hybrid nanoceftriaxone to inhibit *E. coli* growth on Muller – Hinton agar. As mentioned before, *E. coli* was able to resist free ceftriaxone concentration without any inhibition zone. The present study also showed that free ZnO nanoparticle had the ability to inhibit *E. coli* growth around the ZnO NP well, with an inhibition zone of 13 mm in diameter.

Creating the hybrid solution of ZnO NP with ceftriaxone has led to an increased killing ability of the ceftriaxone and ZnO NP to be 19 mm for the stock concertation of ZnO NP with dilution number 1 of ceftriaxone. Considering this area, the inhibition zone was increased by around 46% from what used in the stock concentration of free ZnO NP, and to activate dilution number 1 of ceftriaxone to able to act according to its mechanism of action. Table-1 shows each concentration of the Nanoceftriaxone with its killing zone diameter and the percentage of each increase.

**Table-1: Inhibition zones difference**

| | Free ZnO NP Diameter (mm) | Hybrid Nanoceftriaxone Diameter (mm) | Killing Elevation Percentage |
|---|---|---|---|
| Comparison #1 | 13 | 19 | 46.2 % |
| Comparison #2 | 13 | 17 | 30.8 % |
| Comparison #3 | 13 | 16 | 20.1 % |
| Comparison #4 | 13 | 14 | 7.7 % |

Antibiotic-tagged nanoparticles have been shown to increase antibiotic concentrations at the site of bacterium–antibiotic contact and promote antibiotic binding to microorganisms[22].

Luo *et al.,* (2013) showed ZnO nanorods could obviously achieve synergistic antibacterial effects with ceftriaxone against *Escherichia coli* (*E. coli*)[23], which agreed with the present result. Also, Cephalexin, other type of cephalosporins, also revealed an enhanced antibacterial activity against *E. coli* when mixed with ZnO NP according to Namasivayam *et al*., (2015)[24].

Our observations are in line with those of Banoee *et al.,* (2010), who observed that antibiotics (ciprofloxacin) have greater efficacy when combined with nanoparticles than when mixed with ampicillin. As ZnO nanoparticles were paired with ciprofloxacin, they reported a 27 percent and 22 percent rise in inhibition zone areas against *S. aureus* and *E. coli*, respectively[25].

The presence of an inhibition zone indicates that ZnO NPs biocidal action involves disrupting the membrane. It's likely that ZnO NPs antimicrobial effects are due to their tiny size, which is 250 times smaller than a bacterium cell. This makes it possible for them to bind to the microorganisms' cell walls, causing their degradation and, as a result, the cell death. Furthermore, the high rate of production of surface oxygen species from ZnO allows the bacteria to be killed[26]. As a result of the improved impact of nano-ZnO on ceftriaxone's antibacterial activity, the diameter of the inhibition region around the wells has increased significantly. This is thought to be due to the antibiotic-zinc nanoparticles combination's synergistic effect. Zinc nanoparticles greatly increased antibiotic effectiveness against *E. coli* at the concentrations tested. Ansari *et al*., (2012) published a paper on the antibacterial properties of ZnO nanoparticles as a potential new unconventional antibacterial agent that could be useful in fighting methicillin-resistant *S. aureus* and other drug-resistant bacteria[27], and our findings are considered to be in agreement.

Also, the current study's results were compared to those of Moodley, (2014), who paired gold nanoparticles GNPs with a particular antibiotic, ciprofloxacin, and investigated the antibacterial function of the ciprofloxacin-conjugated gold nanoparticles by exposing them to pathogenic bacteria such as *Staphylococcus aureus, E. coli, Klebsiella pneumoniae, Enterococococcus spp*., and discovered that conjugate nanoparticles improve antibiotic concentrations at the site of bacterium-antibiotic contact, facilitating antibiotic binding and entry into bacteria, which could have significant consequences for infection treatment[28].

## Conclusion

Zinc oxide nanoparticle (ZnO NP) prepared from fresh leaf aloe vera plants showed a small antibacterial ability and can act as drug carrier to overcome

emerging antibiotic resistance. When mixing with ceftriaxone, ZnO nanoparticles showed a synergistic effect leading to a greater inhibition zone. In fucture, more elaborate experimental have been needed to elucidate the mechanism of synergistic antibacterial impact.

**Conflict of Interest:** Nil/None to declare.

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**Ethical Clearance:** Samples were taken under the scientific ethics committee of the Iraqi Ministry of Health and the College of Applied Biotechnology, Al-Nahrain University, Baghdad, Iraq.

## References

1. Walker MS, Walker JB. Streptomycin biosynthesis and metabolism. Enzymatic phosphorylation of dihydrostreptobiosamine moieties of dihydro-streptomycin-(streptidino) phosphate and dihydrostreptomycin by *Streptomyces* extracts. J. Biol. Chem. 1970;245:6683-6689.
2. Benveniste R, Davies J. Aminoglycoside antibiotic-inactivating enzymes in actinomycetes similar to those present in clinical isolates of antibiotic-resistant bacteria. Proc. Natl. Acad. Sci. USA. 1973;70:2276-2280.
3. Chifiriuc C, Grumezescu A, Saviuc C, Croitoru C, Mihaiescu D, Lazar V. Improved antibacterial activity of cephalosporins loaded in magnetic chitosan microspheres. Int. J. Pharm. 2012;436:201-205.
4. Filippousi M, Papadimitriou S, Bikiaris D, Pavlidou E, Angelakeris M, Zamboulis D. Novel core-shell magnetic nanoparticles for Taxol encapsulation in biodegradable and biocompatible block copolymers: Preparation, characterization and release properties. Int. J. Pharm. 2013;448:221-230.
5. Liakos I, Rizzello L, Scurr D, Pompa P, Bayer I, Athanassiou A. All-natural composite wound dressing films of essential oils encapsulated in sodium alginate with antimicrobial properties. Int. J. Pharm. 2014;463:137-145.
6. Wilczewska A, Niemirowicz K, Markiewicz K, Car H. Nanoparticles as drug delivery systems. Pharmacol. Rep. 2012;64:1020-1037.
7. Sabir S, Arshad M, Chaudhari SK. Sci. World J. 2014;(14):1.
8. Mishra V, Sharma R, Jasuja ND, Gupta DK. A review on green synthesis of nanoparticles and evaluation of antimicrobial activity. International Journal of Green and Herbal Chemistry. 2014;3:81-94.
9. Makabenta JMV, Nabawy A, Li C-H, Schmidt-Malan S, Patel R, Rotello VM. Nanomaterial-based therapeutics for antibiotic-resistant bacterial infections. Nat. Rev. Microbiol. 2020.
10. Muzammil S, Hayat S, Fakhar EAM, Aslam B, Siddique MH, Nisar MA, Saqalein M, Atif M, Sarwar A, Khurshid A. Nanoantibiotics: Future nanotechnologies to combat antibiotic resistance. Front. Biosci. 2018;10:352-374.
11. da Silva BL, Abuçafy MP, Berbel Manaia E, Oshiro Junior JA, Chiari-Andréo BG, Pietro RCR, Chiavacci LA. Relationship Between Structure And Antimicrobial Activity Of Zinc Oxide Nanoparticles: An Overview. Int. J. Nanomed. 2019;14:9395-9410.
12. Jin S-E, Jin H-E. Synthesis, Characterization, and Three Dimensional Structure Generation of Zinc Oxide-Based Nanomedicine for Biomedical Applications. Pharmaceutics. 2019;11:575.
13. Sánchez-López E, Gomes D, Esteruelas G, Bonilla L, Lopez-Machado AL, Galindo R, Cano A, Espina M, Ettcheto M, Camins A. Metal-Based Nanoparticles as Antimicrobial Agents: An Overview. Nanomaterials. 2020;10:292.
14. Van Giau V, An SSA, Hulme J. Recent advances in the treatment of pathogenic infections using antibiotics and nano-drug delivery vehicles. Drug Des. Dev. Ther. 2019;13:327-343.
15. Bajwa N, Mehra NK, Jain K, Jain NK.

Pharmaceutical and biomedical applications of quantum dots. Artif. CellsNanomed. Biotechnol. 2016;44:758-768.

16. Sharma S, Kumar K, Thakur N, Chauhan S, Chauhan MS. The effect of shape and size of ZnO nanoparticles on their antimicrobial and photocatalytic activities: A green approach. Bull. Mater. Sci. 2019;43:20.
17. de Lucas-Gil E, Leret P, Monte-Serrano M, Reinosa JJ, Enríquez E, Del Campo A, Cañete M, Menéndez J, Fernández JF, Rubio-Marcos F. ZnO Nanoporous Spheres with Broad-Spectrum Antimicrobial Activity by Physicochemical Interactions. ACS Appl. Nano Mater. 2018;1:3214-3225.
18. Abo-Shama UH, El-Gendy H, Mousa WS, Hamouda RA, Yousuf WE, Hetta HF, Abdeen EE. Synergistic and Antagonistic Effects of Metal Nanoparticles in Combination with Antibiotics Against Some Reference Strains of Pathogenic Microorganisms.Infect. Drug Resist. 2020;13:351-362.
19. Shanmugam NR, Muthukumar S, Prasad S. A review on ZnO-based electrical biosensors for cardiac biomarker detection.Future Sci. OA. 2017;3:FSO196.
20. Supraja N, Prasad TNVKV, Krishna TG, David E. Appl. Nanosci. 2016;6:581.
21. Femi V, Prabha PH, Sudha P, Devibala B, Jerald AL. Anti-bacterial effect of ZnO-Au nanocomposites. Int. J. Biotechnol. Eng. 2011;1(1).
22. Allahverdiyev AM, Kon KV, Abamor ES, Bagirova M, Rafailo vich M. Coping with antibiotic resistance: combining nanoparticles with antibiotics and other antimicrobial agents. Expert Rev Anti Infect Ther. 2011;9(11):1035-1052.
23. Luo Z, Wu Q, Xue J, Ding Y. Selectively Enhanced Antibacterial Effects and Ultraviolet Activation of Antibiotics with ZnO Nanorods Against Escherichia Coli. Journal of Biomedical Nanotechnology. 2013;9(1):69-76.
24. Namasivayam KR, Prasanna M, Subathra M. Synergistic antibacterial activity of zinc oxide nanoparticles with antibiotics against the human pathogenic bacteria. Journal of Chemical and Pharmaceutical Research. 2015;7(3):133-138.
25. Banoee M, Seif S, Jafa ri-Fesharaki P, Shahverdi HR, Moballegh A, Moghaddam KM, Shahverdi AR. ZnO nanoparticles enhanced antibacterial activity of ciprofloxacin against *Staphylococcus aureus* and *Escherichia coli*. J Biomed Mater Res B Appl Biomater. 2010;93(2):557-561.
26. Al-Ugaili D, Fadhil A, Wohaieb S. Potential Activity of Zinc Oxide Nanoparticles and Ethanolic Olive Leaf Extract Against Oxacillin Resistant *Staphylococcus aureusin* vitro. Journal of Al-Nahrain University Science. 2017;17:162-169.
27. Ansari MA, Khan HM, Khan AA, Sultan A, Azam A. Characterization of clinical strains of MSSA, MRSA and MRSE isolated from skin and soft tissue infections and the antibacterial activity of ZnO nanoparticles. World J. Microbiol. Biotechnol. 2012;28:1605-1613.
28. Moodley N. Antimicrobial activity of ciprofloxacin-coated gold nanoparticles on selected pathogens. 2014.