Antibacterial Effects of ZnO Nanoparticles in Combination with Other antibiotics

Ruaa Maan[1],*

Alyaa Hussein Ashour[2],

Raneen Salam Al-Obaidi[3],

Dina Hameed Haider [4],

Dunya Abdullah Mohammed[5]

Omar A Mahmoud [5] ,

Wassan A. Hassan[6]

1.Department of Forensic Chemistry, Higher Institute of Forensic Sciences, Al-Nahrain University, Baghdad, Iraq

2. College of medicine, Al-Nahrain University, Baghdad, Iraq

3. Alnahrain renewable energy research Center, Al-Nahrain University, Baghdad, Iraq

4. Department of Forensic Chemistry, Higher Institute of Forensic Sciences, Al-Nahrain University, Baghdad, Iraq

5. Department of Forensic Biology, Higher Institute of Forensic Sciences, Al-Nahrain University, Baghdad, Iraq

6. Department of Forensic Engineering, Higher Institute of Forensic Sciences, Al-Nahrain University, Baghdad, Iraq

*****Corresponding author: Ruaa Maan Attallah

E-mail: **ruaamaan@gmail.com**

Antibacterial Effects of ZnO Nanoparticles in Combination with Other antibiotics

Ruaa Maan[1], Alyaa Hussein Ashour[2], Raneen Salam Al-Obaidi[3], Dina Hameed Haider [4], Dunya Abdullah Mohammed [5]Omar A Mahmoud [5] ,Wassan A. Hassan[6]*

1.Department of Forensic Chemistry, Higher Institute of Forensic Sciences, Al-Nahrain University, Baghdad, Iraq

2. College of medicine, Al-Nahrain University, Baghdad, Iraq

3. Alnahrain renewable energy research Center, Al-Nahrain University, Baghdad, Iraq

4. Department of Forensic Chemistry, Higher Institute of Forensic Sciences, Al-Nahrain University, Baghdad, Iraq

5. Department of Forensic Biology, Higher Institute of Forensic Sciences, Al-Nahrain University, Baghdad, Iraq

6. Department of Forensic Engineering, Higher Institute of Forensic Sciences, Al-Nahrain University, Baghdad, Iraq

*****Corresponding author: Ruaa Maan Attallah

E-mail: **ruaamaan@gmail.com**

Abstract

This study investigates the antibacterial activity of zinc oxide nanoparticles (ZnO-NPs) against *Acinetobacter baumannii* plus *Staphylococcus aureus*. The well diffusion method was used to estimate the inhibition zones of different concentrations of ZnO-NPs, as well as combined with ciprofloxacin and ceftriaxone. The results indicate that high concentrations of ZnO-NPs alone exhibit strong antibacterial activity and statistically, the p-value is non-significant, and their combination with antibiotics enhances bacterial inhibition. The mixture of ZnO-NPs with ciprofloxacin and ceftriaxone significantly enhanced the antibacterial effect against both Gram-positive and negative bacteria, suggesting a potential strategy for overcoming antibiotic resistance.

Key words: Antibacterial activity, ZnO Nanoparticles, antibiotic resistance, Gram-positive bacteria, Gram- negative bacteria

الخلاصة

تستقصي هذه الدراسة النشاط المضاد للبكتيريا لجسيمات أكسيد الزنك النانوية (ZnO-NPs) ضد Acinetobacter baumannii بكتيريا و Staphylococcus aureus. تم استخدام طريقة well diffusion method لتقدير مناطق التثبيط لتركيزات مختلفة من جسيمات أكسيد الزنك النانوية وحدها، وكذلك عند دمجها مع سيبروفلوكساسين (ciprofloxacin) وسيفتاكسون (ceftriaxone). تشير النتائج إلى أن التركيزات العالية

من جسيمات أكسيد الزنك النانوية وحدها تظهر نشاطًا قويًا مضادًا للبكتيريا، ومن الناحية الإحصائية، فإن قيمة p غير معنوية.كما عزز خليط جسيمات أكسيد الزنك النانوية مع سيبروفلوكساسين وسيفتاكسون بشكل ملحوظ التأثير كالمضاد للبكتيريا ضد كل من البكتيريا المستخدمة، مما يشير إلى استراتيجية محتملة للتغلب على مقاومة المضادات الحيوية.

Introduction

Nanotechnology plays a critical role in modern material science, with applications ranging from medicine to industry. Among the various nanoparticles, ZnO-NPs have received considerable attention due to their stability, low toxicity, and broad-spectrum antimicrobial properties [1, 2]. The development of antibiotic-resistant bacteria such as *Staphylococcus aureus* and *Acinetobacter baumannii* requires new therapeutic approaches [3]. This study aims to evaluate the antibacterial activity of ZnO-NPs alone and in a mixture with ciprofloxacin and ceftriaxone. ZnO-NPs have unique properties, including a high surface area-to-volume ratio, which improves their interaction with bacterial cells [4, 5]. Previous research has demonstrated that ZnO-NPs can disturb bacterial membranes, generate reactive oxygen species (ROS), and interfere with essential cellular processes [6]. The growing prevalence of multidrug-resistant (MDR) bacteria highlights the urgent need for novel antimicrobial strategies. In this paper, we will study the antibacterial activity of concentrations of zinc oxide nanoparticles against *Acinetobacter baumannii* and *Staphylococcus aureus* bacteria, also Combine the antibiotic with concentrations of zinc oxide nanoparticles and study antibacterial activity against *Acinetobacter baumannii* and *Staphylococcus aureus* bacteria.

Materials and Methods

Preparation of Culture Media: Muller-Hinton agar was used for bacterial culturing. The medium was prepared and sterilized using an autoclave at 121°C for 15 minutes. The bacteria used in this study were clinical isolates of *Acinetobacter baumannii* and *Staphylococcus aureus* obtained from hospital samples.

Well Diffusion Method: Sterile cotton swabs were used to inoculate bacterial cultures onto agar plates. Wells of 6-8 mm diameter were created and filled with different concentrations of ZnO-NPs [5, 2.5, 1.25, 0.625, 0.313, 0.156, 0.078, and 0.039 mg/ml]. Plates were incubated at 37°C for 24 hours, and inhibition zones were measured. The antibacterial effect of nanoparticles was achieved by well diffusion method. The steps of this work were achieved all inside the fume hood, dipping a sterile cotton swab into the standardized bacterial suspension and removing excess inoculum by lightly pressing the swab against the tube wall at a level above that of the liquid, then Muller-Hinton Agar plate surface is inoculated by streaking with the swab then a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork, containing the inoculum. A volume (75µL) of the ZnO solution at different concentrations, [5, 2.5, 1.25, 0.625, 0.313, 0.156, 0.078, 0.039 mg/ml] is introduced into the well, incubated for 18 to 24 hours at 37oC. The growth of bacteria was well observed and the nanoparticles effect on the growth of bacteria was tested by observing the inhibition zone, which means growth stops and is measured by the ruler.

Results and Discussion

Inhibition Zone of ZnO-NP against bacteria: According to Table 1 the comparison between the inhibition zone diameter of different concentrations of Zinc oxide against *Acinetobacter baumannii* and *Staphylococcus aureus*, the current result showed that the increasing concentrations of zinc oxide lead to an increase the inhibition zone for both types of bacteria, and statistically, the p-value is non-significant $p \geq 0.05$. Figure 1 shows a linear relationship between them.

Table 1: Comparison of zone diameter of different concentration of Zinc oxide against bacteria

Zinc Conc. (mg/ml)	***Acinetobacter baumannii*** **mean±SD**	***Staphylococcus aureus*** **mean±SD**
5.0	15.04+1.46	15.07+1.55
2.5	12.75+2.03	13.43+1.77
1.25	9.21+1.87	10.59+2.49
0.625	8.0+1.0	8.5+1.61
0.313	0.0+0.0	0.0+0.0
0.156	0.0+0.0	0.0+0.0
0.0781	0.0+0.0	0.0+0.0
0.039	0.0+0.0	0.0+0.0

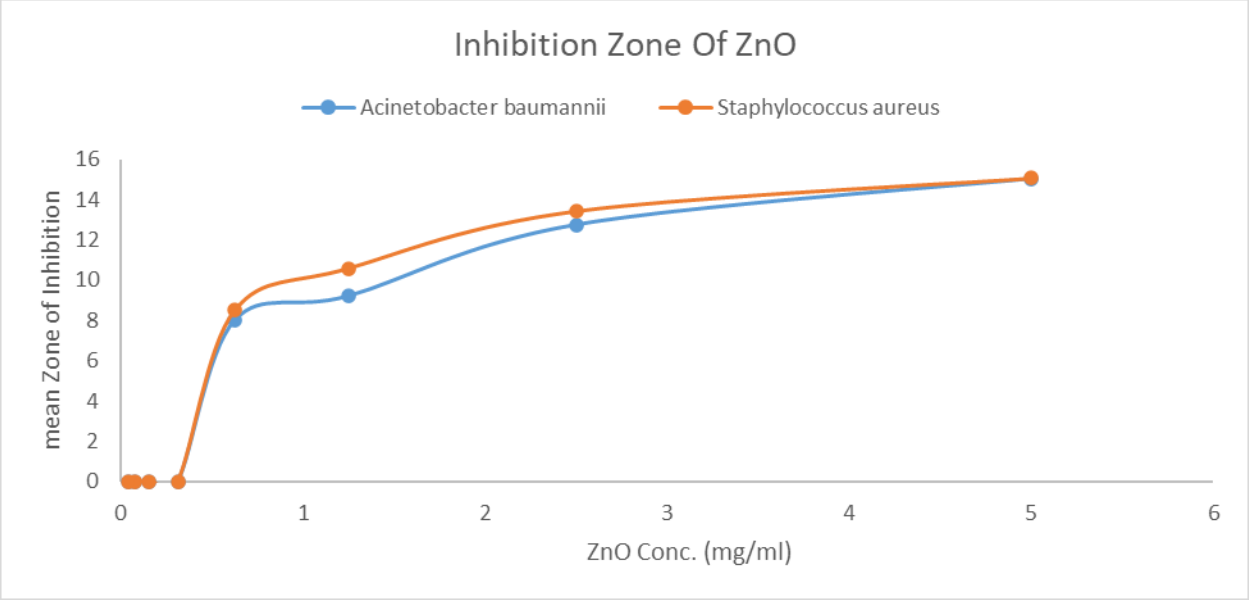

Figure 1: The linear relationship between zinc oxide and the zone of inhibition

The curve accurately represents the inhibition zones for *Acinetobacter baumannii* and *Staphylococcus aureus*, showing a plateau at higher concentrations.

By using well diffusion method, the present study suggested that ZnO-NPs had high antibacterial activity against both Gram-positive and Gram-negative bacteria, and this method is suitable for detecting the antibacterial activity of ZnO-NPs against *Acinetobacter baumannii* and *Staphylococcus aureus* bacteria. ZnO-NPs have a high activity against bacteria and this is found in many published researches [7, 8].

This study found that in the high concentrations of ZnO-NP, the inhibition zone is large, which mean that particle size and concentration of ZnO-NPs play an important role in the antibacterial activity [9]. ZnO-NPs antibacterial activity directly correlates with their concentration was reported by several studies, likewise, the activity is size dependent, however, this dependency is also influenced by the concentration of NPs. Larger surface area and higher concentration are accountable for ZnO-NP antibacterial activity[10].e It has been explaining that larger surface areas and higher concentrations of smaller particles may provide additional antibacterial activity and that is accordance with the results obtained[11]. It's also shown that the inhibition of Gram-negative bacteria requires higher concentrations of ZnO NPs [12]. This is likely because the peptidoglycan layer that surrounds Gram-positive bacteria can promote ZnO attack inside the cell, while the cell wall components of Gram-negative bacteria, such as lipopolysaccharides, can counter this attack. It's found that the concentration of zinc oxide has no effect in the inhibition region on bacteria, and this differs with the current results obtained [13]. Also, the small size (<100 nm) and the high surface-to-volume ratio of NPs facilitate the prerequisite interaction with the microorganisms. The antimicrobial activity of NPs such as ZnO is usually attributed to its crystal structure, size, shape, and surface area [14, 15, 16, 17].

Combination with Antibiotics: ZnO-NPs were combined with ciprofloxacin and ceftriaxone at 5 mg/ml and 2.5 mg/ml concentrations. The well diffusion method was applied to determine the enhancement in antibacterial activity.

Inhibition Zone of ZnO-NP mixed ciprofloxacin against bacteria: Tables (2) and (3) showed a comparison of the inhibition zone diameter of each of *Acinetobacter baumannii* and *Staphylococcus aureus* bacteria after treatment with different concentrations of mixture of Zinc oxide with ciprofloxacin [5.0 mg/ml and 2.5 mg/ml] respectively. Results are similar to sensitivity to pure ZnO as increasing concentrations of ZnO mixture with ciprofloxacin [5.0 mg/ml and 2.5 mg/ml] lead to an increase in the inhibition zone for both types of bacteria but with a significant increase in inhibition zone area compared to the pure ZnO.

Table 2 Comparison of zone diameter of different concentration of mixture of Zinc oxide in combination with ciprofloxacin 5.0 mg/ml between the bacteria.

Zinc Conc. (mg/ml)	*Acinetobacter baumannii* mean±SD	*Staphylococcus aureus* mean±SD
5.0	27.63+1.44	27.37+2.14
2.5	25.96+2.48	26.07+2.42
1.25	24.08+2.55	24.03+3.01
0.625	20.67+2.79	21.77+3.29
0.313	18.13+3.19	18.6+4.12
0.156	15.25+2.57	17.03+4.34
0.0781	13.92+2.96	15.07+4.61
0.039	12.71+3.24	13.53+4.63

Table 3: Comparison of zone diameter of different concentration of mixture of Zinc oxide in combination with ciprofloxacin 2.5 mg/ml between *the* bacteria.

Zinc Conc. (mg/ml)	*Acinetobacter baumannii* mean±SD	*Staphylococcus aureus* mean±SD
5.0	25.29+2.12	24.63+2.55
2.5	23.5+2.9	23.6+2.8
1.25	20.92+4.2	20.97+3.25
0.625	18.21+3.95	18.27+3.92
0.313	16.23+3.09	15.34+3.71
0.156	13.65+2.74	13.46+3.58
0.0781	10.69+3.25	11.55+2.84
0.039	10.0+1.6	10.44+2.5

Antibiotics were found to be a valuable weapon to combat bacterial infection, but their popularity had also become their undoing. Although the drugs crippled harmful microbes from within, bacteria that survived such sabotage developed resistance that made them even more dangerous [18].

Drug resistance developed in part because conventional antibiotics such as ciprofloxacin did not physically damage a microbe's cell wall. Instead, entered the target less disruptively and moved on to disrupt the DNA within block cell division or, trigger cellular self-destruction [19].

Strains that survived this assault, however, could evolve to defend themselves against future attacks, opening the door for deadlier versions of bacteria such as methicillin-resistant Staphylococcus aureus (MRSA) [19], to counter this, we have to develop to supplement pure antibiotics by destroying outer protective membranes of bacteria, ensuring that their

morphing days are through, this is done by combining nanoparticles with antibiotics as in ZnO NPs-CIP in the present study.

Many studies have shown that metal nanoparticles combined with antibiotics have better effects against both Gram- positive and Gram-negative bacteria [20, 21, 22]. So, combining ciprofloxacin with nanoparticles of zinc oxide will improve efficiency against bacteria, and this has been proven by the present study results that when combining nanoparticles of zinc oxide with ciprofloxacin, obtained a greater inhibition zone than zinc oxide or ciprofloxacin alone. Where it raised for ciprofloxacin (5 mg/ml) from 11.86 (mm) and 12.77 (mm) to 27.63(mm) and 27.37(mm) for *Acinetobacter baumannii* and *Staphylococcus aureus* respectively, and raised for ciprofloxacin (2.5 mg/ml) from 11.0(mm) and 12.3(mm) to 25.29(mm) and 24.63(mm) for *Acinetobacter baumannii* and *Staphylococcus aureus* respectively. These results are in line with previous research, where they confirmed that the efficiency of the antibacterial activity of ciprofloxacin increases in the presence of ZnO-NPs [23, 24, 25, 26, 27]. In a related development, previous research [28] has shown that adding nanoparticles can reduce the antibacterial activity of antibiotics.

The explanation was mentioned previously that increasing the concentration leads to increases the anti-bacterial activity and thus increases the inhibition zone and increases the sensitivity to the bacteria and it is agreed with another research [29].

A previous research, suggested the increased antibacterial activity of ciprofloxacin in the presence of ZnO-NPs may be due to membrane damage and accumulation of antibacterial agents in the cells [30].

The exact mechanism of antibacterial action of ZnO-NPs with antibiotics has not yet been elucidated. It has been suggested the synergistic activity between conventional antibiotics and NPs due to inhibition of the export of antibacterial agents by blocking efflux pumps or by enhancing the entrance of antibiotics into the cell by disrupting the bacterial membrane [31]. Previous studies concluded that ZnO-NPs may induce genotoxicity indirectly by promoting oxidative stress or by directly passing through the cellular membrane and interacting with the DNA to damage all four bases or producing thymine–tyrosine cross-linking [32].

Other authors [33] proposed an explanation for the increased activity of ciprofloxacin in the presence of ZnO nanoparticles against *S. aureus* that ZnO nanoparticles may interfere with the pumping activity of Nor A protein by inducing faster electron transfer kinetics in its active site. This protein mediates the active efflux of hydrophilic fluoroquinolones from the bacterial cell providing resistance to the bacterium and this led to the inhibition of the antibacterial activity of ciprofloxacin. Interference with NorA protein restores ciprofloxacin action.

Others explained that ZnO-NP damaged the cell membrane through reactive oxygen species (ROS) generation with free electrons and holes in the presence of light, which assisted ciprofloxacin to enter into the cell and, thereby, inhibit bacterial growth [23].

Inhibition Zone of ZnO-NP mixed ceftriaxone against bacteria: Tables (4) and (5) show a comparison of the diameter of the inhibition zone of *Acinetobacter baumannii* and *Staphylococcus aureus* after treatment with a different concentration of zinc oxide mixed with ceftriaxone (5.0 mg/ml and 2.5 mg/ml), respectively. The results are similar to pure ZnO and ZnO combined with ciprofloxacin. Increasing concentrations of ZnO mixed with

ceftriaxone (5.0 mg/ml and 2.5 mg/ml) increase the inhibition zone of both types of bacteria but with a remarkable increase in the area of the inhibition zone compared to the pure ZnO.

Table 4: Comparison of zone diameter of different concentration of mixture of Zinc oxide in combination with ceftriaxone 5.0 mg/ml between the Bacteria.

Zinc Conc. (mg/ml)	*Acinetobacter baumannii* mean±SD	*Staphylococcus aureus* mean±SD
5.0	27.87+1.87	27.77+1.66
2.5	26.26+2.61	26.42+2.04
1.25	24.52+3.07	24.54+2.58
0.625	22.04+4.13	21.77+3.0
0.313	18.96+4.47	19.5+3.5
0.156	16.74+4.29	17.77+4.03
0.0781	15.13+4.32	15.31+3.92
0.039	14.39+4.27	14.0+4.54

Table 5: Comparison of zone diameter of different concentration of mixture of Zinc oxide in combination with ceftriaxone 2.5 mg/ml between the Bacteria

Zinc Conc. (mg/ml)	*Acinetobacter baumannii* mean±SD	*Staphylococcus aureus* mean±SD
5.0	25.21+1.53	25.1+1.54
2.5	23.25+2.52	22.97+2.31
1.25	20.96+3.18	20.27+3.11
0.625	17.75+3.27	17.67+3.24
0.313	14.46+3.48	15.47+3.0
0.156	12.0+3.13	12.33+2.58
0.0781	11.2+3.03	10.17+2.04
0.039	9.73+1.56	8.85+1.5

Obtain a large inhibition zone of Ceftriaxone approximately or slightly higher than that obtained in combining nanoscale ZnO with ciprofloxacin as it reaches 27.87 (mm) and 27.77(mm) for *Acinetobacter baumannii* and *Staphylococcus aureus* at 5 mg/ml of Ceftriaxone combined with ZnO-NPs and reaches to 25.21 (mm) and 25.1(mm) for *Acinetobacter baumannii* and *Staphylococcus aureus* at 2.5 mg/ml of ceftriaxone combined with ZnO-NPs.

These results are compatible with previous results [34] showed that the antibacterial effect of ceftriaxone increased significantly when used in combination with ZnO nanoparticles. While other results showed that the combination of ZnO-NPs and ceftriaxone has no effect on gram-positive and explained the reason for this due to either to decrease in particle size or to low concentration of those compounds [35].

It demonstrated that ZnO with ceftriaxone achieves obvious synergistic antibacterial effects against E. coli. Meanwhile, ceftriaxone-ZnO has higher antibacterial activity under UV activation, compared to pure ceftriaxone or ZnO [36].

Previous studies have shown that combining nanoparticles with ceftriaxone improves antibacterial activity where [37] elucidated that ceftriaxone combined with AuNP was highly effective against bacterial strains compared to ceftriaxone and AuNP alone. [38] Illustrate, ceftriaxone–nanoparticle conjugate showed the greatest antimicrobial activity against both Gram-positive and Gram-negative bacteria compared to ceftriaxone alone and explained the reason for this due to the negatively charged cell wall of bacteria being ruptured by silver ions from silver nanoparticles and eventually resulting in cell death.

The results of Shanmuganathan [39] display the improvement of the antibacterial activity of Ag-NPs after conjugation with ceftriaxone and the application of ceftriaxone-conjugated nanoparticles as a choice for the inhibition of the pathogens. Nano-metal and even nano-polymer improve their antibacterial activity when combined with ceftriaxone as Chitosan reported in a previous study [40]. However, this did not happen with Geovana [41] as the area of the inhibition zone decreased when silver was combined with ceftriaxone antibiotic when compared with Ag-NPs alone against *P. aeruginosa.*

Briefly mentioned previously an explanation of the enhancement of ZnO nanoparticles with ciprofloxacin, this increased synergistic activity will depend on the assumption that ZnO NPs may interfere with the pumping activity of the NorA bacterial protein. The same thing happened with the synergistic activity between zinc oxide and ceftriaxone, and this was confirmed by Rashmi [42] in previous research.

Conclusion

This study confirms the significant antibacterial effects of ZnO-NPs, both alone and in combination with antibiotics. The synergistic action of ZnO-NPs with ciprofloxacin and ceftriaxone suggests a promising approach to improving antibiotic efficacy against resistant bacterial strains. Further studies should explore the clinical applications of ZnO-NPs and their potential use in developing advanced antimicrobial treatments.

Future Directions

Future research should focus on the in vivo efficacy of ZnO-NPs, assessing their pharmacokinetics, toxicity, and potential for clinical applications. Investigating the mechanisms of bacterial resistance to ZnO-NPs and optimizing nanoparticle formulations could further enhance their therapeutic potential. The combination of ZnO-NPs with other antimicrobial agents, such as natural compounds or peptides, may offer additional benefits in overcoming antibiotic resistance.

References

[1] Sahoo, S. (2010). Socio-ethical issues and nanotechnology development: Perspectives from India. 10th IEEE International Conference on Nanotechnology, 1205–1210.

[2] Yadav, V. (2013). Nanotechnology, big things from a tiny world: A review. AEEE, 3(6), 771–778.

[3] Raghunath, A., & Perumal, E. (2017). Metal oxide nanoparticles as antimicrobial agents: A promise for the future. International Journal of Antimicrobial Agents, 49(2), 137–152.

[4] Sun, Y., Mayers, B., Herricks, T., & Xia, Y. (2003). Polyol synthesis of uniform silver nanowires: A plausible growth mechanism and the supporting evidence. Nano Letters, 3(7), 955–960.

[5] Dhage, S. R., Pasricha, R., & Ravi, V. (2005). Synthesis of fine particles of ZnO at 100°C. Materials Letters, 59(7), 779–781.

[6] Liu, Y., He, L., Mustapha, A., Li, H., Hu, Z. Q., & Lin, M. (2009). Antibacterial activities of zinc oxide nanoparticles against Escherichia coli O157:H7. Journal of Applied Microbiology, 107(4), 1193–1201.

[7] 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(1), 71–76.

[8] Boucher, H. W., & Corey, G. R. (2008). Epidemiology of methicillin-resistant Staphylococcus aureus. Clinical Infectious Diseases, 46(Supplement_5), S344–S349.

[9] Peleg, A. Y., Seifert, H., & Paterson, D. L. (2008). Acinetobacter baumannii: Emergence of a successful pathogen. Clinical Microbiology Reviews, 21(3), 538–582.

[10] A. R. Shahverdi, A. Fakhimi, H. R. Shahverdi, and S. Minaian, “Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against Staphylococcus aureus and Escherichia coli,” Nanomedicine Nanotechnology, Biol. Med., vol. 3, no. 2, pp. 168–171, 2007

[11] F. Mirhosseini, M. Amiri, A. Daneshkazemi, H. Zandi, and Z. S. Javadi, “Antimicrobial Effect of Different Sizes of Nano Zinc Oxide on Oral Microorganisms,” Front. Dent., vol. 16, no. 2, p. 105, 2019.

[12] B. L. da Silva, M. P. Abuçafy, E. B. Manaia, J. A. O. Junior, B. G. Chiari-Andréo, R. C. L. R. Pietro, and L. A. Chiavacci, “Relationship between structure and antimicrobial activity of zinc oxide nanoparticles: An Overview,” Int. J. Nanomedicine, vol. 14, p. 9395, 2019.

[13] K. Fattah, A. Gamal, S. Ibrahim, E. Mohamed, and A. Saleh, “Investigation of the efficacy of synthesized silver and zinc oxide nanoparticles against multi-drug resistant gram negative bacterial clinical isolates,” Arch Clin Microbiol, vol. 8, no. 6, p. 67, 2017.

[14] M. Alizadeh-Sani, H. Hamishehkar, A. Khezerlou, M. Maleki, M. Azizi-Lalabadi, V. Bagheri, P. Safaei, T. Azimi, M. Hashemi, and A. Ehsani, “Kinetics Analysis and Susceptibility Coefficient of the Pathogenic Bacteria by Titanium Dioxide and Zinc Oxide Nanoparticles,” Adv. Pharm. Bull., vol. 10, no. 1, p. 56, 2020.

[15] P. A. Kareem, E. G. H. Alsammak, Y. J. Abdullah, and Q. M. Bdaiwi, “Estimation of antibacterial activity of zinc oxide, titanium dioxide, and silver nanoparticles against multidrug-resistant bacteria isolated from clinical cases in Amara City, Iraq.,” Drug Invent. Today, vol. 11, no. 11, 2019.

[16] A. Azam, A. S. Ahmed, M. Oves, M. S. Khan, S. S. Habib, and A. Memic, “Antimicrobial activity of metal oxide nanoparticles against Gram-positive and Gram-negative bacteria: a comparative study,” Int. J. Nanomedicine, vol. 7, p. 6003, 2012.

[17] M. J. Hajipour, K. M. Fromm, A. A. Ashkarran, D. J. de Aberasturi, I. R. de Larramendi, T. Rojo, V. Serpooshan, W. J. Parak, and M. Mahmoudi, “Antibacterial properties of nanoparticles,” Trends Biotechnol., vol. 30, no. 10, pp. 499–511, 2012.

[18] K. Hiramatsu, Y. Katayama, M. Matsuo, T. Sasaki, Y. Morimoto, A. Sekiguchi, and T. Baba, "Multi-drug-resistant Staphylococcus aureus and future chemotherapy," J. Infect. Chemother., vol. 20, no. 10, pp. 593–601, 2014.

[19] H. F. Chambers and F. R. DeLeo, "Waves of resistance: Staphylococcus aureus in the antibiotic era," Nat. Rev. Microbiol., vol. 7, no. 9, pp. 629–641, 2009.

[20] Jones, N., Ray, B., Ranjit, K. T., & Manna, A. C. (2008). Antibacterial activity of ZnO nanoparticle suspensions. FEMS Microbiol. Lett., 279(1), 71–76.

[21] A. S. H. Hameed, C. Karthikeyan, A. P. Ahamed, N. Thajuddin, N. S. Alharbi, S. A. Alharbi, and G. Ravi, "In vitro antibacterial activity of ZnO and Nd doped ZnO nanoparticles against ESBL producing Escherichia coli and Klebsiella pneumoniae," Sci. Rep., vol. 6, no. 1, pp. 1–11, 2016.

[22] Y. Xie, Y. He, P. L. Irwin, T. Jin, and X. Shi, "Antibacterial activity and mechanism of action of zinc oxide nanoparticles against Campylobacter jejuni," Appl. Environ. Microbiol., vol. 77, no. 7, pp. 2325–2331, 2011.

[23] P. Patra, S. Mitra, N. Debnath, P. Pramanik, and A. Goswami, "Ciprofloxacin conjugated zinc oxide nanoparticle: A camouflage towards multidrug resistant bacteria," Bull. Mater. Sci., vol. 37, no. 2, pp. 199–206, 2014.

[24] R. Farzana, P. Iqra, F. Shafaq, S. Sumaira, K. Zakia, T. Hunaiza, and M. Husna, "Antimicrobial behavior of Zinc oxide nanoparticles and β-lactam antibiotics against pathogenic Bacteria," Arch. Clin. Microbiol, vol. 8, p. 57, 2017.

[25] S. Sepideh, K. Z. Bahri, P. M. Reza, S. H. Reza, A. Massoud, B. Roya, N. Z. Esmail, and S. A. Reza, "Preparation of ciprofloxacin-coated zinc oxide nanoparticles and their antibacterial effects against clinical isolates of Staphylococcus aureus and Escherichia coli," Arzneimittelforschung, vol. 61, no. 08, pp. 472–476, 2011.

[26] A. Venubabu Thati, S. Roy, M. V. N. A. Prasad, C. T. Shivannavar, and S. M. Gaddad, "Nanostructured zinc oxide enhances the activity of antibiotics against Staphylococcus aureus," J. Biosci Tech, vol. 1, no. 2, pp. 64–69, 2010.

[27] S. Iram, J. A. Khan, N. Aman, A. Nadhman, Z. Zulfiqar, and M. A. Yameen, "Enhancing the anti-enterococci activity of different antibiotics by combining with metal oxide nanoparticles," Jundishapur J. Microbiol., vol. 9, no. 3, 2016.

[28] Z. E. Nazari, M. Banoee, A. A. Sepahi, F. Rafii, and A. R. Shahverdi, "The combination effects of trivalent gold ions and gold nanoparticles with different antibiotics against resistant Pseudomonas aeruginosa," Gold Bull., vol. 45, no. 2, pp. 53–59, 2012.

[29] E. Pazos-Ortiz, J. H. Roque-Ruiz, E. A. Hinojos-Márquez, J. López-Esparza, A. Donohué-Cornejo, J. C. Cuevas-González, L. F. Espinosa-Cristóbal, and S. Y. Reyes-López, "Dose-dependent antimicrobial activity of silver nanoparticles on polycaprolactone fibers against gram-positive and gram-negative bacteria," J. Nanomater., vol. 2017, 2017.

[30] F. Ghasemi and R. Jalal, "Antimicrobial action of zinc oxide nanoparticles in combination with ciprofloxacin and ceftazidime against multidrug-resistant Acinetobacter baumannii," J. Glob. Antimicrob. Resist., vol. 6, pp. 118–122, 2016.

[31] M. Banoee, S. Seif, Z. E. Nazari, P. Jafari-Fesharaki, H. R. Shahverdi, A. Moballegh, K. M. Moghaddam, and A. R. Shahverdi, "ZnO nanoparticles enhanced antibacterial activity of ciprofloxacin against Staphylococcus aureus and Escherichia coli," J. Biomed. Mater. Res. Part B Appl. Biomater., vol. 93, no. 2, pp. 557–561, 2010.

[32] V. Sharma, R. K. Shukla, N. Saxena, D. Parmar, M. Das, and A. Dhawan, “DNA damaging potential of zinc oxide nanoparticles in human epidermal cells,” Toxicol. Lett., vol. 185, no. 3, pp. 211–218, 2009.

[33] A. M. Allahverdiyev, K. V. Kon, E. S. Abamor, M. Bagirova, and M. Rafailovich, “Coping with antibiotic resistance: combining nanoparticles with antibiotics and other antimicrobial agents,” Expert Rev. Anti. Infect. Ther., vol. 9, no. 11, pp. 1035–1052, 2011.

[34] F. Rubab, M. F. Chaudhary, and N. M. Butt, “Comparative and Synergistic Studies of Antibacterial Effect of ZnO Nanoparticles and Antibiotics for Pathogens in Drinking Water.” TechConnect Briefs.,vol.3,pp.12-17,2016.

[35] A. A. Slman, “Antibacterial activity of ZnO nanoparticle on some gram-positive and gram-negative bacteria,” Iraqi J. Phys., vol. 10, no. 18, pp. 5–10, 2012.

[36] Z. Luo, Q. Wu, J. Xue, and Y. Ding, “Selectively enhanced antibacterial effects and ultraviolet activation of antibiotics with ZnO nanorods against Escherichia coli,” J. Biomed. Nanotechnol., vol. 9, no. 1, pp. 69–76, 2013.

[37] S. M. F. G. El-Rab, E. M. Halawani, and A. M. Hassan, “Formulation of ceftriaxone conjugated gold nanoparticles and their medical applications against extended-spectrum β-lactamase producing bacteria and breast cancer,” J. Microbiol. Biotechnol., vol. 28, no. 9, pp. 1563–1572, 2018.

[38] M. Harshiny, M. Matheswaran, G. Arthanareeswaran, S. Kumaran, and S. Rajasree, “Enhancement of antibacterial properties of silver nanoparticles–ceftriaxone conjugate through Mukia maderaspatana leaf extract mediated synthesis,” Ecotoxicol. Environ. Saf., vol. 121, pp. 135–141, 2015.

[39] R. Shanmuganathan, D. MubarakAli, D. Prabakar, H. Muthukumar, N. Thajuddin, S. S. Kumar, and A. Pugazhendhi, “An enhancement of antimicrobial efficacy of biogenic and ceftriaxone-conjugated silver nanoparticles: green approach,” Environ. Sci. Pollut. Res., vol. 25, no. 11, pp. 10362–10370, 2018.

[40] S. Mushtaq, J. A. Khan, F. Rabbani, U. Latif, M. Arfan, and M. A. Yameen, “Biocompatible biodegradable polymeric antibacterial nanoparticles for enhancing the effects of a third-generation cephalosporin against resistant bacteria,” J. Med. Microbiol., vol. 66, no. 3, pp. 318–327, 2017.

[41] G. D. Savi, A. C. Trombin, J. da Silva Generoso, T. Barichello, J. C. Possato, J. V. V. Ronconi, and M. M. da Silva Paula, “< b> Antibacterial Acivity of Gold and Silver Nanoparticles Impregnated With Antimicrobial Agents,” Saúde e Pesqui. ISSN 2176-9206, vol. 6, no. 2, 2013.

[42] R. M. Bhande, C. N. Khobragade, R. S. Mane, and S. Bhande, “Enhanced synergism of antibiotics with zinc oxide nanoparticles against extended spectrum β-lactamase producers implicated in urinary tract infections,” J. nanoparticle Res., vol. 15, no. 1, p. 1413, 2013.