Study of Antibacterial Effect of Zinc Oxide Nanomaterials Was Chemically Synthesized Against *Staphylococcus Aureus* Isolates from Different Infection Sites

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Abstract. In this study, about 312 clinical samples were collected from different health institutions in Babylon governorate-Iraq, during the period from 1/10/2021 to 12/1/2022, by taking swabs from Burns (50 samples), wounds (55 samples), nose swabs (50 samples), abscesses (50 samples), diabetic foot (52 samples), and samples from the various inflammatory site such as urine (55 samples), various phenotypic, microscopic, and biochemical tests were conducted on it to detect about the presence of S. *aureus* among the isolates, these tests proved the return of (105 isolates out of 312 samples) isolated from different sites at clinical samples (Percentage of 33.65%) to the genus *S. aureus*, Zinc oxide nanoparticles were also chemically synthesized by the sol-gel method. The diagnosis of the nano-material was confirmed by conducting four specialized analyzes to reveal the properties of the synthesized material, by (XRD, AFM, FTIR, FESEM), the synthesized nanomaterial was also tested on *Staphylococcus aureus* bacteria to know its antibacterial activity and its effectiveness in inhibiting the growth of *Staphylococcus aureus* bacteria, used statistical program SPSS version 26 with biological part by calculating least significant differences with one way ANOVA.

Keywords: zinc oxide nanoparticles, antibacterial, *S. aureus*

INTRODUCTION

Staphylococci are a group of gram-positive bacteria, non-motile, not form spores, spherical in shape, size ranging between 0.5 – 1.5 µm in diameter, maybe occurring at many forms, single cocci, in pairs (tetrads) as short chains or random clusters **[3]**

The division of staphylococci may occur in more than one plane, and as the result of these divisions, irregular clusters (grape-like) appearance will be formed **[2]**.

With some exception of anaerobic species bacteria like *staphylococcus Sacchrolyticus* and *staphylococcus aureus* subspecies anaerobius, all others are facultative anaerobes **[4],** Staphylococci characteristically by catalase-positive, but rare catalase-negative strains reported by researches **[19],** Other traits to most staphylococcus are cytochrome oxidase negative, with some exceptions like (*S. Sciuri*, and *S. Vitulinus*) **[1].**

Staphylococci are susceptible to lysostaphin (is a zinc metalloenzyme which has specific lytic action to *staphylococcus aureus*, is composed of three enzymes Gly glycine endopeptidase, β-N-acetyl glucose amidase, and N- acetyl muramyl alanine amidase and work by glycine -glycine bonds in cell wall breakdown) **[1]**, because they contain the cell wall with peptidoglycan embedded on it teichoic acid **[6]**, staphylococci can grow in the high concentration of salts in the presence of 10% NaCl (halophiles) between (18 ºC - 40º C) **[7]**, Metabolism of staphylococci respiratory and fermentative **[8]**.

Objective of search: Study of the effect of zinc oxide nanoparticles were chemically synthesized on bacteria.

1

2

LITERATURE REVIEW

ANTIBACTERIAL SUSCEPTIBILITIES

Another name that is resistant to antimicrobial or antibiotics, *staphylococcus aureus* has many genes responsible for resistance to antibiotics, like methicillin, penicillin, piperacillin, …etc. **[10]**.

Resistant mediated by mecA gene, that was another new name called mecC gene, is the most resistant mechanism is by resistant to β lactam antibiotics family (penicillin, generation 1-4 cephalosporins, and carbapenem) [**9**].

The mecA gene and other homologous genes related with staphylococcus aureus on mobile DNA (SCC mec) staphylococcal cassette chromosome encode for penicillin-binding protein (PBP) that was responsible for decreased activities of β- lactam antibiotics, and mecA gene described by different forms with different species of staphylococcus (s. *scuiri* (mecA1), and *s. vitulinus* (mecA2)) [**10**].

Now appear new strain have resistant to newer antibiotics like tigecycline, daptomycin, as below:

TABLE 1. different types of *S. aureus* resistant or sensitive to antibiotics **[15]**

No.	Abbrev.	Full name
1	MRSA	methicillin-resistant Staphylococcus aureus
2	LRSA	linezolid resistant staphylococcus aureus
3	MSSA	methicillin-sensitive staphylococcus aureus
4	TRSA	tigecycline resistant staphylococcus aureus
5	VRSA	vancomycin-resistant staphylococcus aureus
6	VISA	vancomycin-intermediate Staphylococcus aureus
7	NRSA	nafcillin resistant staphylococcus aureus
8	ORSA	oxacillin resistant staphylococcus aureus
		And others

There is also another form of resistance to antibiotics behave by staphylococcus aureus and many other bacteria, is by tolerance mechanism, which is mean inhibited by antibiotics and not killed, and that can be used to determine MIC and MBC (MIC mean minimum inhibitory concentration, MBC mean minimum bactericidal concentration) **[12]**, Community-acquired methicillin-resistant antibiotics (CA-MRSA) strains almost sensitive to non-β-lactam antimicrobial, and make them contrast with the hospital-acquired methicillin-resistant *staphylococcus aureus* (HA-MRSA) **[17]**

ZINC OXIDE NANOPARTICLES

One of the materials that we have studied the most is zinc oxide (ZnO), which is a semiconductor oxide that presents particular characteristics, such as large bandwidth ~ 3.36 eV, modulable electrical conductivity, highly transparent when presented in the form of film, and crystallizes in a wurtzite-type hexagonal phase, these characteristics make possible its use in nanoelectronics, among its main applications we can mention. gas sensors, photocatalysts, solar cells, anti-reflective layers, transparent conductive electrodes, thermal mirrors, among others **[14]**

Research work carried out consisted in the manufacture of tablets based on nanometric ZnO powders, these powders were obtained through the homogeneous precipitation technique, which consists of the synthesis of the powder from a solution that contains the ions of the chemical element of interest. The precipitation of the compound is achieved by adding an agent called precipitant, subsequently, the solid-liquid phases are separated by centrifugation, and finally, the paste obtained is thermally dried to obtain powders. **[12]**

The homogeneous precipitation technique allows obtaining nanoparticles of the order of approximately 28 nm, with different geometries. Nano-sponges, nano bars, nanoflowers, and nanospheres, depending on the process and drying conditions. The size and geometry of the dust particles are known by scanning electron microscopy [**16]**

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METHODS AND MATERIALS

CHEMICALLY PREPARATION OF (ZnO NPs) BY SOL-GEL METHOD

1. Zinc Oxide Nanoparticles (ZnO NPs) can be prepared by a sol-gel method according to [**1]**, with some modifications, by the steps:
2. Mix 20 gm from zinc acetate dihydrate (Zn $(CH_3COO)_2 \cdot 2H_2O$) with 150 ml deionized water and the solution will be stirred at 40º C for 20 minutes to produce zinc acetate solution (first solution)
3. Mix 80 gm from sodium hydroxide (NaOH) with 80 ml deionized water and stirrer at 40º C for 20 minutes to produce sodium hydroxide solution (second solution)
4. Mixing both solutions (first and second solution) to form (third solution)
5. The third solution will titration by adding 100 ml of absolute ethanol (99.9% concentration) slowly (drop by drop) with a stirrer at 40º C for 90 minutes.
6. Wash the product with deionized water to remove any NaoH not react, and filtration with Whatman filter paper with pores diameter (2.5µm) three times, the production stays at (60º C) overnight.
7. Calcify the product in an oven at 400º C for 3 hours, or at 250º C for 4 hours
8. White powder will be produced at the end (zinc oxide nanoparticles formation)

$$Zn\,(CH_3COO)_2\,.2H_2O + 2\,NaOH \longrightarrow ZnO + 2CH_3COONa + 2H_2O$$

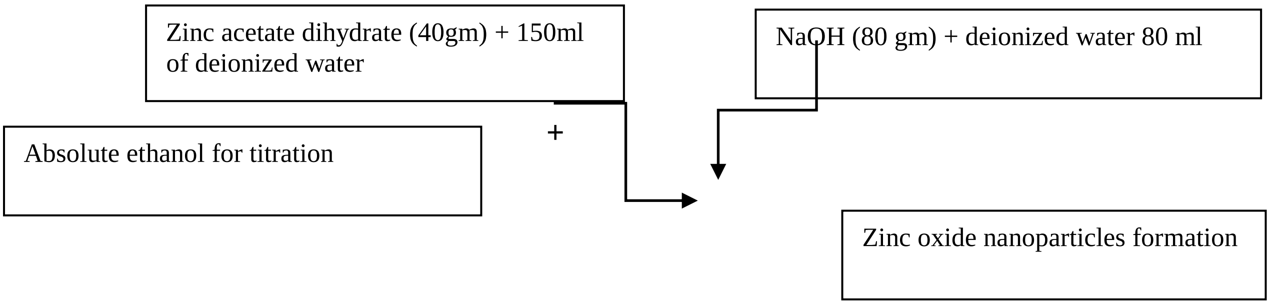

FIGURE 2. diagram of zinc oxide nanoparticles preparation

EFFECTIVE INHIBITORY (ZnO NPs) AGAINST *S. AUREUS* [13]

Prepared four concentrations from (ZnO NPS) to study inhibitory activity on bacteria (400 µg/ml, 200 µg/ml, 100 µg/ml, 50 µg/ml) by mixing nanoparticles with deionized water with magnetic stirrer and heat60º C four one hour and then under ultra-sonic for 15 minutes to make a colloidal solution, concentrations prepared according to the mathematical equation**.**

$$C = \frac{w}{v}\frac{w}{v}$$

Where C= wanted concentration, W= wanted the weight of (ZnO NPs), V= volume by ml
Then prepare other concentrations according to the previous equation or according to the below equation

$$C1V1 = C2V2$$

Where C_1 = Initial Concentration, V_1= Initial Volume, C_2 = Final Concentration, V_2 = Final Volume.

RESULTS AND DISCUSSION

CHARACTERIZATION OF NANOMATERIALS XRD ANALYSIS

X-ray diffraction examines crystallite structures, phases presence in ZnO NPs, atoms orientations, and determine crystals size by depending on peaks and diffraction of (Ɵ angle), that represent all diffractions zinc oxide nanoparticles synthesized chemically by using zinc acetate as a precursor, as the figure below showed presence zinc oxide nanoparticles (ZnO NPs) at diffraction angles between (20^o-80^o).

Sharp peaks on the figure below indicate crystalloid structure and formation of Zinc oxide nanoparticles:

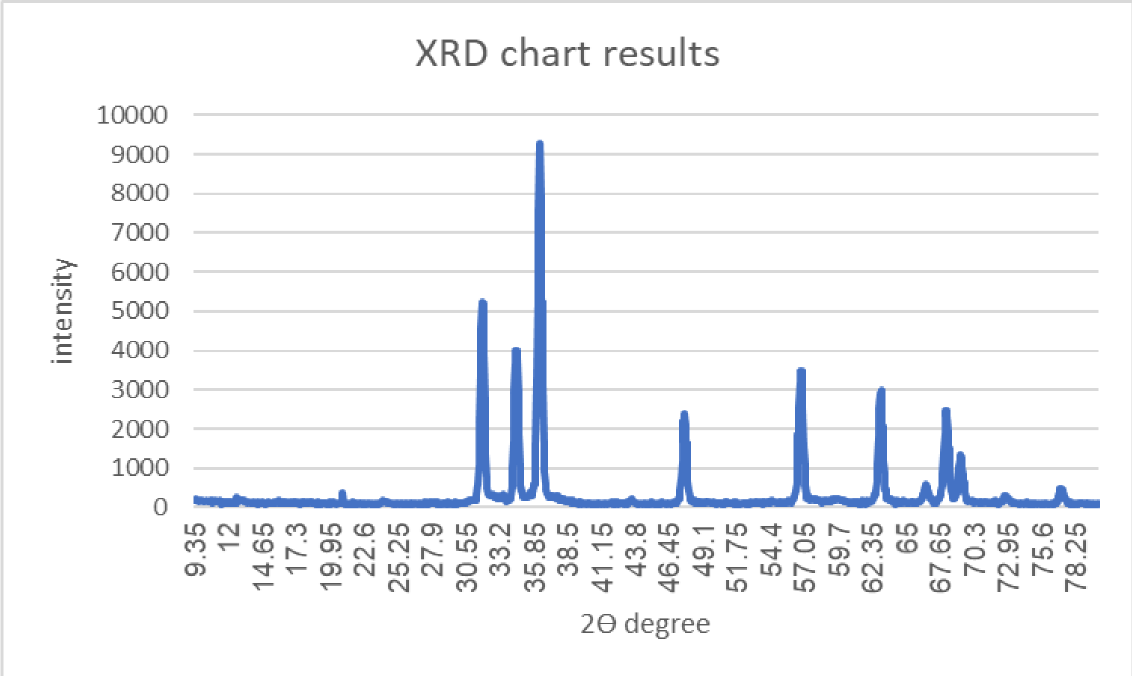

FIGURE 2. XRD results

TABLE 2. Brag`s law and Scherrer's law **[18]**

Law name	Law	Meanings
Brag`s law	$n\lambda = 2d \sin \Theta$	λ = x-ray wavelength (λ=0.15406 nm), d = spacing between atomic planes
Scherrer's law	D=0.98$\lambda\Delta(2\theta)$. cos (θ)	Δ (2θ) = full width at half maximum (in radians) of the peak (FWHM), Ɵ =Bragg`s angle

From Scherrer's equation in the table, we can calculate the size of nanoparticles synthesized and compare it with standard results (the nanoparticle average size was 75.83nm).

AFM ANALYSIS

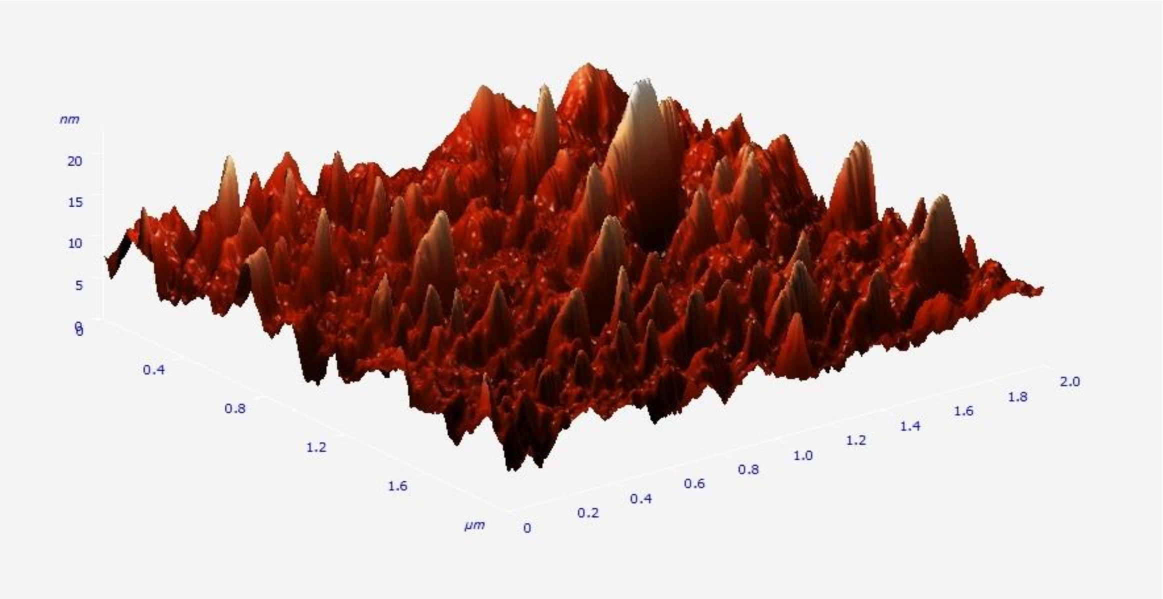

FIGURE 3. AFM 3D picture

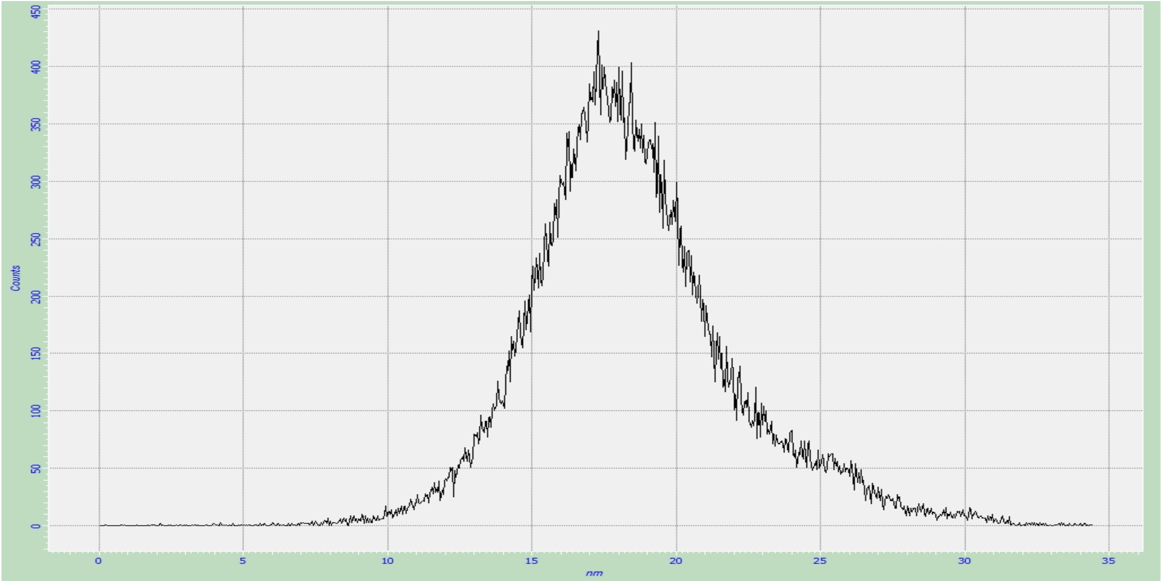

FIGURE 4. Histogram of AFM

Dimension of particles at AFM test was (amount of sampling max= 34.4035nm, min= 0, average of roughness= 2.68703nm, root mean square= 3.54966nm).

FTIR ANALYSIS

FTIR test showed functional groups related to chemo-synthesized zinc oxide nanoparticles, the FTIR range of the ZnO nanoparticles orchestrated by sol-gel method, which was acquired in the scope of 400-4000 cm^{-1} (wavenumber), The band between the 450-500 cm^{-1} correlated to metal oxide security (ZnO).

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From the Figure (4-8) we can see peaks at the points (3900- 3600 cm^{-1}, 3600-3300 cm^{-1}, 3000-2700 cm^{-1}, 2400-2100 cm^{-1}, 1500-1200 cm^{-1}, 1200-900 cm^{-1}, and under 450 cm^{-1}).

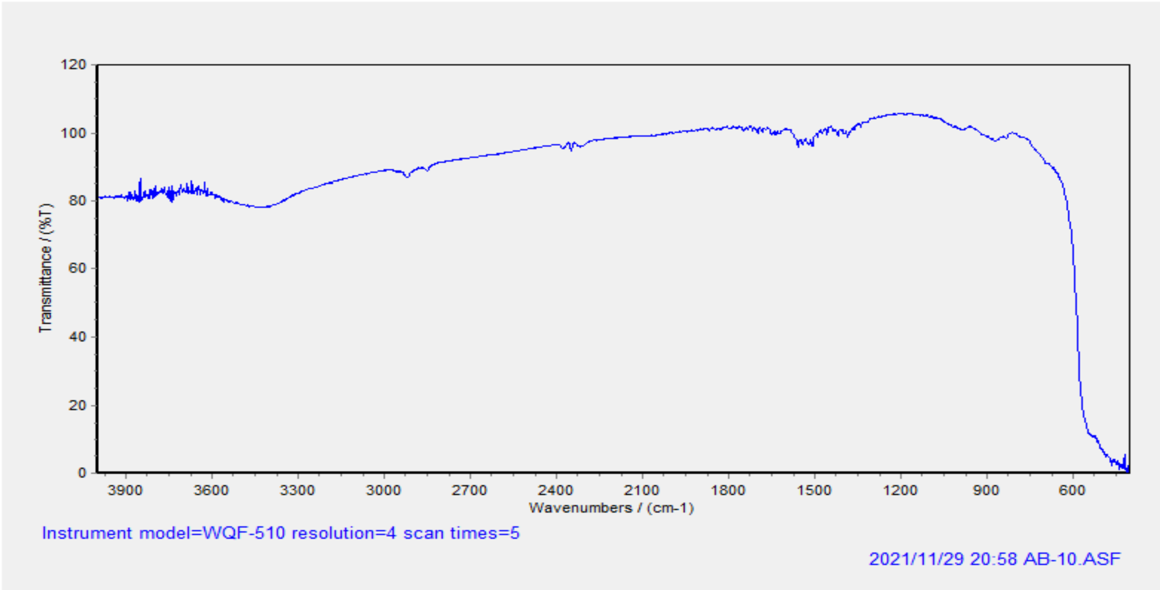

FIGURE 5. FTIR result

FESEM ANALYSIS

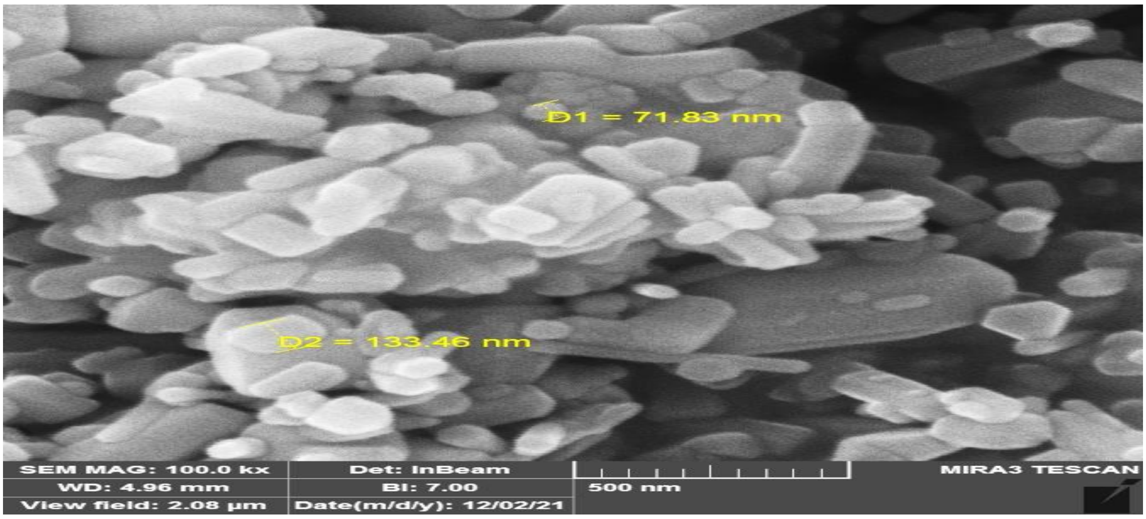

FIGURE 6. FESEM picture

ANTIBACTERIAL EFFECT OF ZnO NPs

TABLE 3. Antibacterial effect of ZnO NPs

No.	site of Sample	Diameter of inhibition zone (mm) / Con.			
		400 µg /ml	200 µg /ml	100 µg /ml	50 µg /ml
1	Burns*	12±2	10±2	7±1	6±1
2	Wounds*	14±1	11±2	9±1	8±1
3	Urine*	13±2	9±2	6±1	6±1
4	Nose swab*	15±1	12±2	10±2	8±1
5	Abscesses*	14±2	11±1	8±2	5±1
6	Diabetic foot*	16±1	13±1	9±2	6±2

There are significant differences between concentrations at $P \leq 0.05$ LSD=0.887
*meaning significance differences

When reading the graph of the results of this examination, it was found that the highest peak was at level 101 with an angle and at an intensity that reached where the peak was sharp, clear, and high. Standard values, by measuring the three highest peaks that appeared in our examination results and as mentioned by **[19]**, Also the results of the AFM examination in the current study gave results indicating the presence of 10 points with a height (17.347nm), which was consistent with the study **[2]** which also found the presence of (9 points) with a height of (19.352nm), Through the graph of FTIR analysis, we find that the highest peak was at the band between (450-500 cm^{-1}) and this site is related to nano-zinc as indicated by **[20]**.

When viewing the (500nm) image, the picture becomes clearer, as the sizes of the nanoparticles are clear and can be measured. It was noticed that there are different sizes in the picture, starting with sizes much smaller than (71 nm), as well as the presence of medium sizes up to (133 nm), and there are sizes that appear larger than this size, Through the results of the current study, it was found that there are significant differences at the level of significance ($P \leq 0.05$) between the different concentrations, where the (least significance differences LSD) between the different concentrations.

CONCLUSION

The nanomaterial synthesized in this study was effective in inhibiting the growth of bacteria at different concentrations.

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REFERENCES

[1] A. S. C. Thesis G, "Scholar commons cell division regulation in *staphylococcus aureus*," *medical microbiology*, vol. 2, no. 2, pp. 211-227, 2017.

[2] B. G. B. L. Timsit J. F., "Expert consensus based clinical practice gudilines managements of intravasculer

catheters in intensive care unit," *intensive care,* vol. 6, no. 2, pp. 118-125, 2020.

[3] C. D. J. S. B. &. M. O. Gorden Y. S., "pathogenicity and virulence of staphylococcus aureus," pp. 210-221, 25 June 2021.

[4] C. M. a. R. R. Fiedot Tibola M., "Deposition of zinc oxide on different polymer textile and their antibacterial properties," *material journal,* vol. 5, no. 2, pp. 178-185, 2018.

[5] G. S. C. J. Akpomie K., "One pot synthesis of zinc oxide nanoparticles via chemical precipitaion for bromophenol blue absortion and antifungal activity against filamentous fungi," *sci rep ,* vol. 7, no. 4, pp. 210-227, 2021.

[6] H. A.M., "Staphylococcus aureus infections in malaysia: a review of antimicrobial resistant and characteristsic of the clinical isolates," *biochemistry and medical biology,* vol. 7, no. 2, pp. 101-112, 2019.

[7] I. S., "Bacteria in nanoparticle synthesis current status and future prospects," *international scholaely research* , vol. 4, no. 3, pp. 1-18, 2014.

[8] K. C. C. J. S. S. A. Geo F. Brook, Medical microbiology, Adelberg: Mc GrawHill, 2019.

[9] K. E.F., "Synthesis and characterization of zinc oxide nanoparticles loaded chloramphincol for antibiotics applications," *annual of tropical medicine and public health,* vol. 1, no. 5, pp. 210-2018, 2019.

[10] Kumar M., "Multidrug resistant staphylococcus aureus," *emerging infectious diseases ,* vol. 9, no. 22, pp. 1666-1669, 2016.

[11] L. A., "Novel anticellule antibiotics delivery system against staphylococcus aureus cloxacillin loaded poly lactide glycolide," *nanobiomedicine,* vol. 4, no. 1, pp. 1189-1203, 2020.

[12] M. R. Jowad, "detection of virulence factors and antibiotic resistance gene in staphylococcus aureus isolated from clinical cases in AL-Diwaniya city," in *master thesis*, Iraq, 2013.

[13] Masri A.S., "The use of nanomedicine for targeted therapy against bacterial infections," *Antibiotics as nanoparticles*, pp. 1-12, 4 8 2019.

[14] N. S. D. S. a. A. R. Alsinjensipriya A., "synthesis and characterization of ZnO nanoparticles by novel sol-gel technique," in *AIP conferenc proceeding 2244*, Delhi, 2020.

[15] P. A. a. K. B. Laux C., "Staphylococcus aureus colonization of the human nose and interaction with other microbiome members," *gram positive pathogen,* vol. 3, no. 6, pp. 723-730, 2019.

[16] R. N., "Biofilm formation mechanisms and targets for developing antibiofilm agents," *future medicinal chemistry,* vol. 4, no. 8, pp. 493-512, 2015.

[17] Rodrigues S.A., "Comparative study of chemically synthesized, physically, biosynthesized zinc oxide nanoparticles at different characters and different activities," *Journal of biotechnology materials* , pp. 493-512, 18 3 2020.

[18] S. A., "Susciptibility profile for methiciliin resistant staphylococcus aureus to linzolid in clinical isolates," *International journal of health sciences* , vol. 5, no. 2, pp. 1-4, 2017.

[19] V.-. E. C., "study of susceptibility antibiotics and moleculaer characterization of high virulence staphylococcus aureus strains isolated from rural hospital in Ethiopia," *medicinal Ethipian Journal,* vol. 6, no. 2, pp. 112-119, 2020.

[20] W.-C. M., "Identification of *staphylococcus aureus* by polymerase chain reaction directly from positive blood culture and effect on patient care," *Candian journal of hospital pharmacy,* vol. 2, no. 5, pp. 43-49, 2021.