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Antimicrobial Activity of ZnO Nanoparticles Against Gram Positive & Negative Bacterial Strains

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ABSTRACT
Zinc oxide nanoparticles (ZnO NPs), common antimicrobials with an unclear action mechanism, were studied for their concentration-dependent efficacy against Gram-negative *Pseudomonas aeruginosa* and Gram-positive *Enterococcus faecalis* . Pre-synthesized ZnO NPs of varying sizes were characterized using techniques like X-ray Diffraction, FT-IR Spectroscopy, and Transmission Electron Microscopy. The minimum inhibitory concentration (MIC) was determined via the standard micro-dilution method using Mueller Hinton broth cultures. Antimicrobial results confirmed that the tested ZnO NPs showed no antimicrobial activity against either *Pseudomonas aeruginosa* or *Enterococcus faecalis*.

KEYWORDS: ZnO nanoparticles, Antimicrobial efficacy, *Pseudomonas aeroginosa, Enterococcus faecalis*.

1. INTRODUCTION

ZnO, a wide band-gap semiconductor with unique properties, is gaining attention in electronics [1]. Its versatile nanostructures are ideal for various applications, including nanoscale optoelectronics [2], piezoelectric nanogenerators [3], and biotechnology [4]. ZnO also exhibits strong resistance to microorganisms [5]. The significant antibacterial activity of ZnO, alongside CaO and MgO, stems from the generation of reactive oxygen species (ROS) on their surfaces [6]. Inorganic oxides serve as essential mineral sources for humans and provide potent antimicrobial effects at low concentrations. Their activity is assessed by measuring changes in bacterial metabolic rates within a growth medium. Sawai et al. [7] employed conductometric methods to compare the minimal inhibitory concentration (MIC) of insoluble ceramic powders against common antibiotics. Their research revealed that the antibacterial efficacy of these powders is dependent on particle size, which is determined by specific processing parameters.
Researchers frequently correlate the antibacterial efficacy of inorganic agents with particle size [8, 9]. Nanocrystalline metal oxides are highly promising for biological applications due to their immense surface area. Inorganic materials offer advantages over organic ones, including enhanced durability, lower toxicity, better selectivity, and greater thermal stability. Commonly studied antimicrobial agents include semiconductor oxides like TiO2 and ZnO which exhibit photocatalytic action under UV exposure [10, 11]. The antimicrobial mechanism of these inorganic chemicals involves surface binding to microorganisms, followed by internalization and disruption of metabolic processes. Various oxides and metal ions have demonstrated therapeutic potential [12, 13]. While the antimicrobial properties of

ZnO are well-established, most investigations have focused on bulk material. Yamamoto's research [14] specifically examined the link between particle size (0.1–1 μm range) and antibacterial activity, quantifying the effect through electrical conductivity changes in powder slurries. That study determined that the antibacterial efficacy of ZnO increases with decreasing particle size.

2. MATERIAL AND METHOD

Materials

Zinc Nitrate Hydrated (Zn $(NO_3)_2.6H_2O$) and Gelatin powder, PEG 6000 were used.

Bacterial Strains

P. aeruginosa (Gram-negative) and *E. faecalis* (Gram-positive) strains were cultured in Mueller Hinton (MH) broth. Their respective Minimum Inhibitory Concentrations (MICs) for various ZnO nanoparticles were determined using the standard microdilution method.

Synthesis of ZnO Nanoparticles

ZnO nanopowder was synthesized via a sol-gel technique. The detailed procedure for producing varying nanoparticle sizes at different calcination temperatures is described in Ajay et al. [15].

Preparation of ZnO Suspension for Anti-bacterial Test

To prepare ZnO suspensions for antibacterial tests, a measured amount of dry ZnO nanoparticles was mixed in a glass beaker with distilled water and gently agitated. The mixture was then ultrasonicated for 30 minutes to disperse the particles and break down agglomerates, yielding a 1 mg/ml stock suspension [1]. This stock was subsequently diluted with distilled water to achieve the desired test concentrations [1]. To enhance suspension stability, PEG-600 dispersant was added at 10% by weight relative to the ZnO nanoparticles [1]. All prepared suspensions were then autoclaved at 121°C for 15 minutes before use in the antibacterial assays [1]. The concentrations used are detailed below. Different ZnO nanoparticle calcined at different temperatures having concentration of 1mg/ml were used with or without dispersant which are shown in Table 1.

Table 1: ZnO suspension concentration

Sample		Dispersant
A	ZnO@500°C	Yes
A_d	ZnO@500°C	No
B	ZnO@600°C	Yes
B_d	ZnO@600°C	No
C	ZnO@900°C	Yes
C_d	ZnO@900°C	No
D	ZnO@700°C	Yes
D_d	ZnO@700°C	No

Anti-bacterial Test using Microdilution Method

ZnO nanoparticle suspensions were prepared and tested for antibacterial efficacy using the micro-dilution method. To ensure monodispersity, dry ZnO powder was dispersed in water and sonicated for 30 minutes to break down agglomerates, forming a 1 mg/ml stock suspension. For stability, PEG-600 (at 10% w/w relative to ZnO) was added as a dispersant. The final suspensions were sterilized by autoclaving at 121°C for 15 minutes prior to use.

Serial dilutions ranging from 1000 µg/ml to1.5 µg/ml were prepared in microdilution wells. Each well contained Mueller-Hinton (MH) broth mixed with 10^5 colony forming units (CFU/ml) of log-phase bacterial cultures. The mixtures were incubated at 37°C with continuous shaking for 24 hours to prevent nanoparticle settling. The minimum inhibitory concentration (MIC) was defined as the lowest ZnO concentration that showed no visible bacterial growth.

Inoculum Preparation

A single colony was inoculated into 10mL of Mueller Hinton (MH) broth and incubated for 16–18 hours at 37°C, 200 rpm. A 100µL aliquot of this primary culture then initiated a secondary 10mL MH broth culture, grown under identical conditions until OD_{600} reached 1.0. This secondary culture was adjusted to 10^5 CFU/mL, and 100µL was plated per well in a 96-well microtiter plate.

Broth Microdilution Assay

To determine the minimum inhibitory concentration (MIC) of ZnO, a serial dilution was prepared across a 96-well microtiter plate. Wells 2-11 received 100 µL of sterile Mueller-Hinton Broth (MHB), and well 12 received 200 µL of unsterile MHB as a negative control. 200 µL of a ZnO suspension was added to well.

A 1:2 serial dilution was performed by transferring 100 µL sequentially from well 1 to well 10, discarding 100 µL from well 10 afterward. Next, 100 µL of bacterial culture (10^4 CFU) was added to wells 1-11. Well 11, lacking ZnO, served as the positive control. The plates were incubated at 37°C for 16-18 hours. The MIC (mg/L) was determined visually by observing the lowest concentration well without turbidity or visible bacterial growth.

3. CHARACTERIZAION

Materials were characterized in the previous study by Ajay K et al. [15] using specific instruments: X-ray diffraction patterns were obtained via a Panalytical X'Pert powder diffractometer, transmission electron microscopy (TEM) with a Hitachi H-7500, thermogravimetric analysis (TGA-DSC) on Universal V4.1D TA instruments, and the FT-IR spectrum of ZnO nanoparticles was measured using a Varian 670-IR spectrometer.

4. RESULTS AND DISCUSSIONS

Characterization results (XRD, FTIR, TGA and TEM) for the ZnO nanoparticles were previously reported by Ajay K. et al. (2025) [15]

Antibacterial effect of ZnO NPs

ZnO nanoparticles' antibacterial activity was assessed against *Pseudomonas aeruginosa* (Gram-negative) and *Enterococcus faecalis* (Gram-positive). The Minimum Inhibitory Concentration (MIC) was determined using a standard microdilution method. Serial dilutions of ZnO nanoparticles (1.5–1000 µg/ml) in MH broth were mixed with bacterial cultures (10^5 CFU/ml) and incubated for 24 hours at 37°C. The study investigated the effect of ZnO nanoparticle suspensions both with and without a dispersant. Size dependent Antibacterial activity of ZnO nanoparticle is shown in the Table 2a and Table 2b below.

ZnO nanoparticles affected bacterial strains variably. The use of dispersants did not alter the antibacterial effect of ZnO suspensions. Specifically, ZnO had a negative effect on the growth of *Enterococcus faecalis* (Figure 1a) and *Pseudomonas aeruginosa* (Figure 1b). Enterococcus faecalis

and Pseudomonas aeroginosa showed a positive growth of these bacterial strains indicating that the inefficiency of ZnO nanoparticle as an antimicrobial agent against these strains.

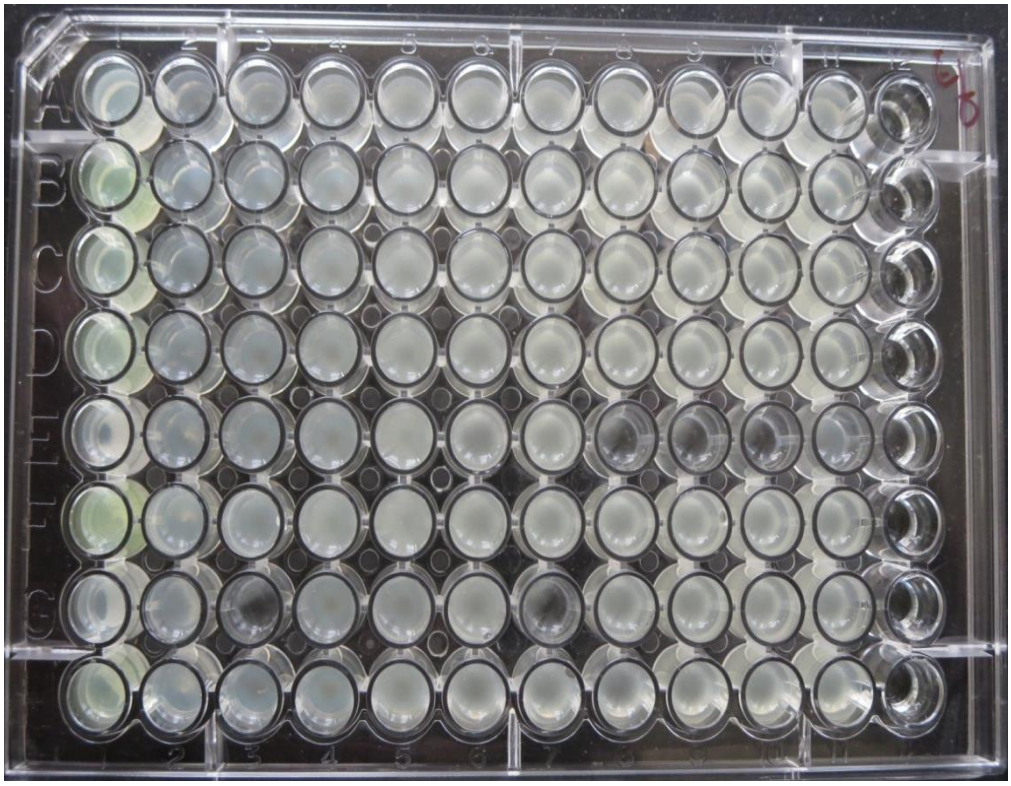

Figure 1a: Microtitre dilution results for a) Enterococcus faecalis

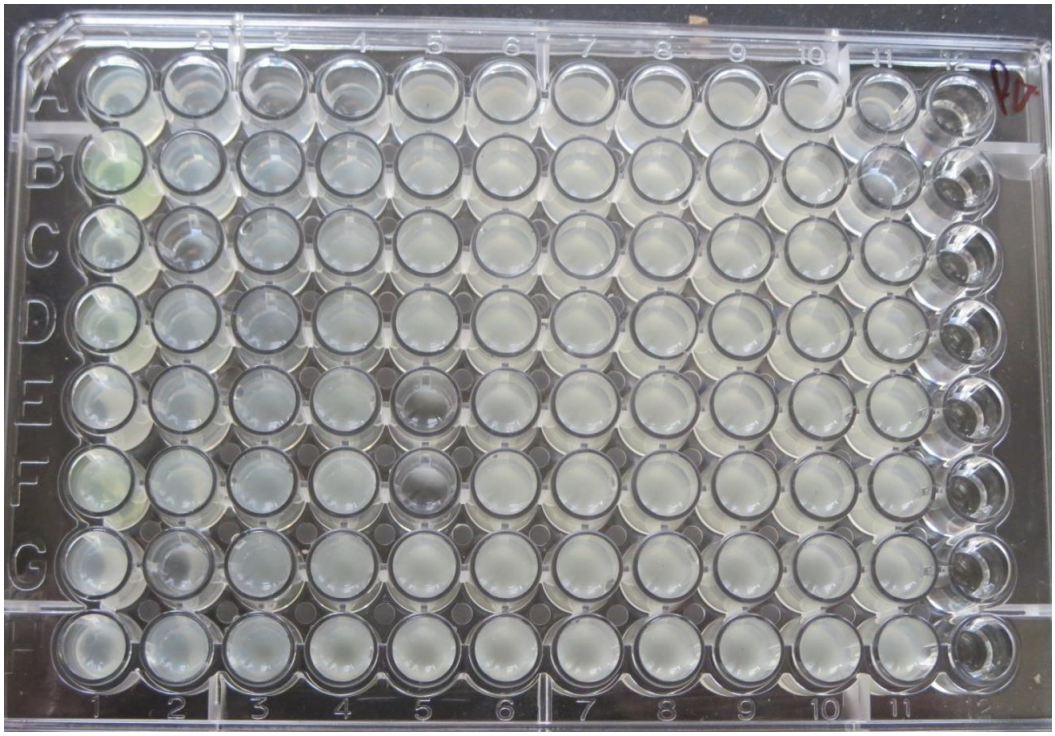

Figure 1b: Microtitre dilution results for Pseudomonas aeroginosa

Table 2a: Microtitre dilution results for Enterococcus faecalis

Well	1	2	3	4	5	6	7	8	9	10	11	12
µg/ml	**1000**	**500**	**250**	**125**	**62**	**31**	**15**	**7**	**3.9**	**1.95**	**No particles**	**No particles& inoculums**
Zno@500°C (A)	++	++	++	++	++	++	++	++	++	++	++	-
Zno@500°C (A_d)	++	++	++	++	++	++	++	++	++	++	++	-
Zno@600°C (B)	++	++	++	++	++	++	++	++	++	++	++	-
Zno@600°C (B_d)	++	++	++	++	++	++	++	++	++	++	++	-
Zno@900°C (C)	++	++	++	++	++	++	++	++	++	++	++	-
Zno@900°C (C_d)	++	++	++	++	++	++	++	++	++	++	++	-
Zno@700°C (D)	++	++	++	++	++	++	++	++	++	++	++	-
Zno@500°C (D_d)	++	++	++	++	++	++	++	++	++	++	++	-

Table 2b: Microtitre dilution results for Pseudomonas aeroginosa

Well	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
µg/ml	**1000**	**500**	**250**	**125**	**62**	**31**	**15**	**7**	**3.9**	**1.95**	**No particles**	**No particles& inoculums**
Zno@500°C (A)	++	++	++	++	++	++	++	++	++	++	++	-
Zno@500°C (A_d)	++	++	++	++	++	++	++	++	++	++	++	-
Zno@600°C (B)	++	++	++	++	++	++	++	++	++	++	++	-
Zno@600°C (B_d)	++	++	++	++	++	++	++	++	++	++	++	-
Zno@900°C (C)	++	++	++	++	++	++	++	++	++	++	++	-
Zno@900°C (C_d)	++	++	++	++	++	++	++	++	++	++	++	-
Zno@700°C (D)	++	++	++	++	++	++	++	++	++	++	++	-
Zno@700°C (D_d)	++	++	++	++	++	++	++	++	++	++	++	-

5. CONCLUSION

In case of Pseudomonas aeroginosa and Enterococcus faecalis did not showed a strong antibacterial activity. Where as in the previous study ZnO nanoparticles exhibit good antibacterial activity against food borne pathogen Staphylococcus aureus and E. coli, which showed that the antibacterial activity mechanism works differently against different pathogens.

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