

Differential Roles of Ag and ZnO in Bactericidal Activity of Ag/ZnO-Hydroxyapatite Nanocomposites against Multidrug-Resistant Clinical Isolates of *Pseudomonas aeruginosa*

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Abstract

Background: *Pseudomonas aeruginosa* is a Gram-negative bacterium that thrives in diverse environments, including the human body and on inert surfaces such as medical devices. It is notorious for resistance to nearly all conventional antimicrobial agents, driven by intrinsic and acquired mechanisms, posing significant therapeutic challenges. Hydroxyapatite (HAp) nanocomposites are widely studied for antimicrobial properties, but their biomedical applications remain in early stages. **Aim:** This study characterized four Ag/ZnO-HAp nanocomposites with Ag:ZnO mole ratios of 0.005/0.005 (nanocomposite A), 0.01/0.005 (nanocomposite B), 0.005/0.01 (nanocomposite C), and 0.01/0.01 (nanocomposite D) to advance therapeutic strategies. **Methods:** We performed agar diffusion, bactericidal, and time-kill assays against *P. aeruginosa* PAO1. We also determined minimum bactericidal concentrations (MBCs) for eight multidrug-resistant *P. aeruginosa* clinical isolates. **Results:** Agar diffusion assays showed inhibition zones of 8, 11, 11, and 12 mm for nanocomposites A, B, C, and D, respectively, while the control HAp showed no inhibition zone. The bactericidal assay showed log reductions of 1.34, 5.07, 1.67, and 5.69 for nanocomposites A, B, C, and D, respectively, and time-kill kinetics showed specific death rates (min^{-1}) of 0.034, 0.07, 0.054, and 0.082 for the same nanocomposites, respectively. MBCs for clinical isolates ranged from <0.06 to >4 mg/mL, independent of their antibiotic susceptibility profiles. **Conclusions:** Overall, the results suggest that bactericidal efficacy follows the order D > B > C > A. Ag content primarily determines bactericidal activity, with ZnO contributing secondarily. However, higher ZnO levels inhibit Ag

activity. Therefore, optimizing the Ag:ZnO ratio is crucial for maximizing bactericidal effects, and customizing the nanocomposites for specific bacterial strains is essential for effective biomedical applications.

Keywords

Hydroxyapatites (HAp), Silver/Zinc Oxide/HAp Nanocomposites, Bactericidal Activity, *Pseudomonas aeruginosa*

1. Introduction

Pseudomonas aeruginosa (*P. aeruginosa*) is a Gram-negative bacterium known for its exceptional metabolic versatility, enabling it to flourish in diverse environmental niches. As a result, *P. aeruginosa* can infect virtually any part of the human body and cause diseases such as lung, bloodstream, and urinary tract infections. Additionally, *P. aeruginosa* can form biofilms on indwelling medical devices, including urinary and central venous catheters, as well as on implanted surgical devices [1] [2]. Notably, this organism employs intrinsic, acquired, and adaptive resistance mechanisms, becoming multidrug-resistant to almost all traditional antibiotics [3]. *P. aeruginosa* is categorized by the World Health Organization as a priority “ESKAPE” organism due to its high adaptability and formidable antibiotic resistance [2]. Given the severity of this threat, new drugs or alternative therapies beyond traditional antibiotics are urgently needed to combat multidrug-resistant *P. aeruginosa*.

Hydroxyapatite (HAp), a bio-ceramic material similar to bone’s main inorganic component, has been widely studied for bone regeneration. Notably, its antibacterial effect increases when it is embedded with silver (Ag) or metal oxides. Ag and zinc oxide (ZnO) nanoparticles have been broadly studied for their antibacterial properties [4]–[6]. In Ag/ZnO-HAp nanocomposites, Ag within the hydroxyapatite (HAp) matrix releases Ag⁺ ions. These ions damage bacterial membranes, inactivate proteins, and impair DNA, resulting in metabolic dysfunction and increased reactive oxygen species (ROS). Meanwhile, ZnO releases Zn²⁺ ions and promotes additional ROS production, further damaging bacterial membranes and impairing cellular metabolism; thus, combining Ag and ZnO in HAp enhances antibacterial effects [4] [7]. HAp nanocomposites are primarily used in bone tissue engineering and in orthopedic or dental biomaterials. Research has focused on physicochemical properties, osteogenic capacity, biocompatibility, and antibacterial effects [5] [8] [9]. However, most studies on antibacterial effects remain limited to *in vitro* and preclinical phases [9] [10].

We previously reported the antibacterial effects of Ag/ZnO-HAp nanocomposites in four mole ratios against *E. coli* and *Aggregatibacter actinomycetemcomitans* [11]. Here, we confirmed a similar antibacterial effect against *P. aeruginosa* PAO1 using agar diffusion tests. We further assessed bactericidal activity using

bactericidal and time-kill assays. Overall, the results showed that Ag content primarily determined antibacterial activity, with ZnO contributing secondarily, and higher ZnO levels may inhibit Ag's bactericidal activity. We also tested potential biomedical applications by determining MBCs for eight multidrug-resistant clinical isolates of *P. aeruginosa*, ranging from <0.06 to >4 mg/mL, regardless of antibiotic susceptibility or resistance.

2. Materials and Methods

2.1. Bacterial Strains, Culture Conditions, and Chemicals

P. aeruginosa PAO1 and clinical isolates of *P. aeruginosa* were used from the previous study [12], with additional clinical isolates obtained from the State University of New York (SUNY) Downstate Medical Center (Brooklyn, NY). To prepare cultures, bacterial strains were grown aerobically on Luria-Bertani (LB; Becton, Dickinson and Company, Sparks, MD) agar plates or in LB broth at 37°C. All chemical reagents, antibiotics, and bacteriological media were purchased from Sigma-Aldrich (St. Louis, MO) or Fisher Scientific (Hampton, NH).

Four Ag/ZnO-HAp nanocomposites from the previous study [11] were used, which were prepared with the following Ag:ZnO mole ratios: nanocomposite A (0.005:0.005), nanocomposite B (0.01:0.005), nanocomposite C (0.005:0.01), and nanocomposite D (0.01:0.01). Each Ag/ZnO-HAp nanocomposite was dissolved in sterile distilled water to form a colloidal dispersion as previously described [13].

2.2. Agar Diffusion Test

Agar diffusion tests of Ag/ZnO nanocomposites were performed against *P. aeruginosa* as described previously [14]. Briefly, a bacterial lawn ($\sim 5 \times 10^9$ cells per milliliter) was prepared on the agar surface, and each Ag/ZnO-HAp nanocomposite (0.1 mg; 5 μ L) was applied to the lawn. Each nanocomposite had a specific Ag-to-ZnO mole ratio of A (nanocomposite-A, 0.005:0.005), B (nanocomposite-B, 0.010:0.005), C (nanocomposite-C, 0.005:0.010), D (nanocomposite-D, 0.010:0.010), and Cot (control; unmodified HAp). The agar plates were then incubated overnight (14 to 18 hours), and the results were assessed by measuring the diameter (mm) of the inhibition zones.

2.3. Bactericidal Assay and Log Reductions

A bactericidal assay of *P. aeruginosa* PAO1 was performed as previously described [14] with a minor modification. Briefly, Ag/ZnO nanocomposites were diluted (3, 2, 1, 0.5, 0.25, and 0.125 mg/mL) in LB broth. Fresh overnight cell cultures were inoculated into the nanocomposite dilutions (3×10^9 cells/mL). The inoculum was then incubated at 37°C for 18 hours without shaking. After incubation, an aliquot from each dilution was plated on LB agar plates and incubated for 24 hours to count colony-forming units (CFU) per mL. Standard deviations were calculated from three independent experiments. Bactericidal efficacy was quantified as log reductions ($\log_{10}[N_o] - \log_{10}[N_t]$), where N_o is the initial inoculum, and N_t is the

cell count at different nanocomposite concentrations after 18 hours.

2.4. Time-Killing Assay and Time-Kill Kinetics

A time-killing assay of *P. aeruginosa* PAO1 was performed as previously described [15] with a minor modification. Briefly, bacterial cells were diluted in LB broth (5×10^6 cells/mL) with 0.5 mg/mL of each nanocomposite and incubated at 37°C without shaking. At 30-minute intervals, a 100 µL aliquot was spread on an LB agar plate, and CFU per mL were counted. Experiments were performed in triplicate to determine standard deviations. Survival data at each time point were analyzed by time-kill kinetics to determine the specific death rate (k , min⁻¹) and decimal reduction time (D-value) as previously described [16]. The specific death rate (k) was calculated as $k = \ln(N_0/N_t)/t$, where N_0 is the initial cell count per mL, N_t is the cell count at time t , and t is the elapsed time in minutes. The D-value was calculated as $D = \ln(10)/k$.

2.5. Minimum Inhibitory Concentration (MIC) Determination

MIC levels of clinical isolates of *P. aeruginosa* were determined by the broth dilution method as described previously [14].

2.6. Minimum Bactericidal Concentration (MBC) Determination

MBC levels of Ag/ZnO-HAp nanocomposites were determined as described previously [14]. Briefly, serial two-fold dilutions of each nanocomposite were prepared in LB broth (4, 2, 1, 0.5, 0.25, 0.125, and 0.0625 mg/mL). Fresh overnight cultures of clinical isolates of *P. aeruginosa* were diluted in sterile saline, and a portion of the cells (5 µL; 5×10^9 cells/mL) was inoculated into each serial two-fold dilution. The cells were incubated overnight (16 to 18 hours) at 37°C and then spread on plain LB agar plates. The agar plates were incubated at 37°C for 24 hours to determine the MBC. MBC values were defined as the lowest nanocomposite concentration required to completely inhibit bacterial growth.

3. Results and Discussion

3.1. Antibacterial Activity of Ag/ZnO-HAp Nanocomposites against *P. aeruginosa*

An agar diffusion test was performed to assess the antibacterial activity of four Ag/ZnO-HAp nanocomposites (A, B, C, and D) and their parental HAp (control: Cot) against *P. aeruginosa* PAO1. As shown in **Figure 1**, the inhibition zone (diameter) of nanocomposite D was the largest (12 mm), nanocomposite A was the smallest (8 mm), and nanocomposites B and C were similar (11 mm). In contrast, the parental HAp showed no inhibition zone. These results demonstrate that all four Ag/ZnO-HAp nanocomposites exhibit antibacterial activity against *P. aeruginosa* PAO1, whereas the parental HAp does not. This finding is consistent with the previous report describing antibacterial effects in *E. coli* and *Aggregatibacter actinomycetemcomitans* [11]. Although the nanocomposites show clear inhibi-

tion zones, the variation in zone size may not be sufficient to differentiate their antibacterial efficacy.

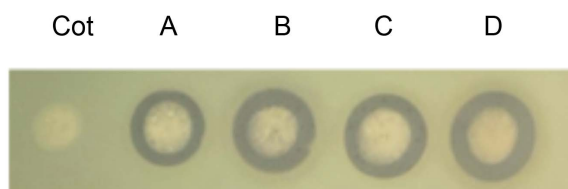


Figure 1. Agar diffusion test of Ag/ZnO-HAp nanocomposites against *P. aeruginosa* PAO1. A bacterial lawn was prepared on the agar surface, and each Ag/ZnO-HAp nanocomposite was applied to the lawn. Each nanocomposite had a specific mole ratio (Ag:ZnO): A (nanocomposite A, 0.005:0.005), B (nanocomposite B, 0.010:0.005), C (nanocomposite C, 0.005:0.010), D (nanocomposite D, 0.010:0.010), and Cot (control), as described in the Materials and Methods.

The agar (or disk) diffusion test is the most common method used in clinical microbiology for initial antimicrobial susceptibility screening because of its cost-effectiveness, simplicity, and high versatility [17]. Despite these advantages, the test method has notable limitations in precisely quantifying antimicrobial susceptibility or resistance. For example, high-molecular-weight or poorly soluble antimicrobial agents are unsuitable because their diffusion in agar matrices may be impeded, causing inaccurate inhibition zones [17] [18]. The Ag/ZnO-HAp nanocomposites used in this study have relatively high molecular weights (>1000 g/mol) and poor solubility in pure water. Consequently, the antibacterial activity of the nanocomposites, as measured by the size of the inhibition zones, may be underestimated.

3.2. Bactericidal Activity at Different Concentrations of Ag/ZnO-HAp Nanocomposites

Bactericidal activity was evaluated by measuring bacterial killing patterns at different concentrations of Ag/ZnO-HAp nanocomposites. Results showed that nanocomposite D killed cells 100% at 0.5 mg/mL over 18 hours, whereas nanocomposites A, B, and C killed cells 100% at 1 or 2 mg/mL over the same period (Figure 2). However, at 0.125 mg/mL, the initial inoculum (3×10^9 cells/mL) decreased to $\sim 2 \times 10^4$ cells/mL for nanocomposites B and D, while to $\sim 1.5 \times 10^8$ cells/mL for nanocomposites A and C. In parallel, bacterial colonies in the nanocomposite-untreated growth and in HAp-only controls were uncountable. This corresponds to log reductions of 1.34, 5.07, 1.67, and 5.69 for nanocomposites A, B, C, and D, respectively. Notably, the Ag content in nanocomposites B and D (0.01 mole) is twice that in nanocomposites A and C (0.005 mole) but does not correlate with the ZnO content. Thus, these results suggest that the enhanced bactericidal activity of nanocomposites B and D may be associated with a 2-fold increase in Ag. However, although Ag content is the primary factor in bactericidal activity rather than ZnO content, the bactericidal assay conditions, such as bacte-

rial culture conditions, media, and cell concentrations, may alter the relative roles of Ag and ZnO in bactericidal activity.

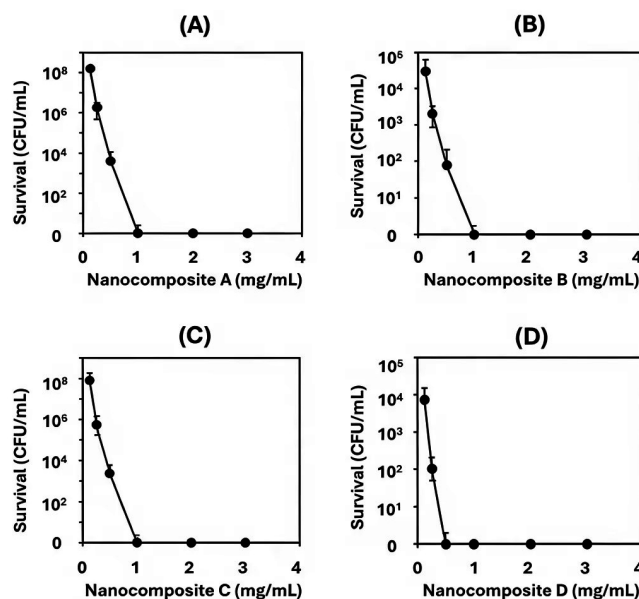


Figure 2. Bactericidal patterns of Ag/ZnO-HAP nanocomposites at different concentrations. Bactericidal patterns were determined by bacterial killing at varying concentrations (0.125, 0.25, 0.5, 1, 2, 3 mg/mL) of Ag/ZnO-HAP nanocomposites against *P. aeruginosa* PAO1, as described in the Materials and Methods. Standard deviations represent the results of three independent experiments.

3.3. Time-Killing in the Presence of Ag/ZnO-HAP Nanocomposites

A time-killing assay of *P. aeruginosa* PAO1 was performed in the presence of 0.5 mg/mL of each nanocomposite, including the parental HAP (control). Results showed that the initial inoculum (5×10^6 cells/mL) decreased to $\sim 3 \times 10^4$ cells/mL over the first 30 minutes for all nanocomposites as well as the control (**Figure 3(A)**). Over the next 120 minutes, cell concentrations for nanocomposites A, B, C, and D decreased to an average of 253, 4, 57, and 0 cells/mL, respectively, while cell concentrations in the control increased (**Figure 3(A)**). These results indicate that nanocomposites B and D have significantly higher killing rates than A and C, and that C and D have slightly higher killing rates than A and B. The differences in killing rate correspond to the amounts of Ag and ZnO in the nanocomposites. A 2-fold increase in Ag (from 0.005 to 0.01 mole) leads to significantly higher killing rates (A to B and C to D), and a 2-fold increase in ZnO (from 0.005 to 0.01 mole) results in slightly higher killing rates (A to C and B to D) (**Figure 3(A)**). Therefore, both Ag and ZnO increase bactericidal activity. However, Ag likely plays a primary role, with ZnO as a secondary contributor.

To assess bactericidal efficacy, we determined the time-kill kinetics of each nanocomposite by calculating the specific death rate (k) and decimal reduction time (D-value), as described in Materials and Methods, over a 120-minute period

starting at 30 minutes (cell concentration was 5.5×10^4 per mL), with measurements taken every 30 minutes. As shown in **Figure 3(B)**, nanocomposite D had the most potent bactericidal activity, with the highest specific death rate (0.082 min^{-1}) and the lowest D-value (28.08 minutes), implying that approximately 8.2% of surviving cells are killed each minute and that it takes 28.08 minutes to kill 90% of the cells. Conversely, nanocomposite A was the least potent, with the lowest specific death rate (0.034 min^{-1}) and the highest D-value (67.72 min). However, the control HAp showed a negative specific death rate and negative D-values, indicating that it promoted cell proliferation rather than inducing cell death (**Figure 3(A)** and **Figure 3(B)**). Overall, the results suggest that bactericidal efficacy follows the order $D > B > C > A$, as also supported by the log reductions in bacterial killing at 0.125 mg/mL for each nanocomposite, as shown in **Figure 2**.

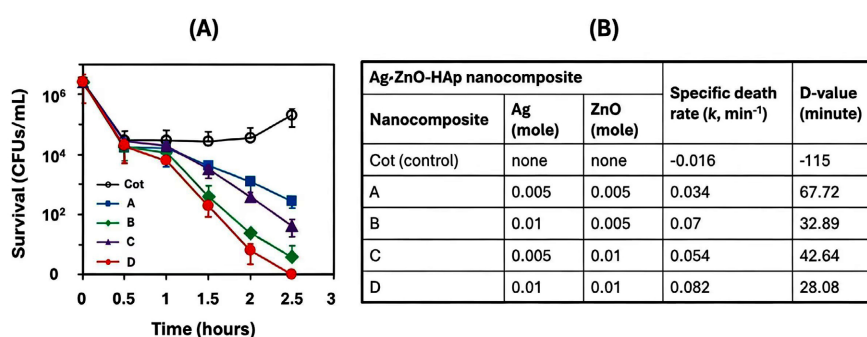


Figure 3. Time-killing and time-kill kinetics of *P. aeruginosa* PAO1. (A) Time-killing in the presence of Ag/ZnO-HAp nanocomposites (0.5 mg/mL), performed as described in Materials and Methods. Nanocomposite A: square; Nanocomposite B: diamond; Nanocomposite C: triangle; Nanocomposite D: closed circle; and Cot (control): open circle. Three independent experiments were used for standard deviation. (B) Time-kill kinetics were calculated using the specific death rate (k) and D-value for each nanocomposite, as described in the Materials and Methods.

The time-kill kinetics also demonstrated that doubling the Ag concentration from 0.005 to 0.01 mole (A to B) led to more than a two-fold increase in the specific death rate ($0.07/0.034 = 2.1$) when combined with 0.005 mole ZnO. In contrast, doubling Ag (C to D) resulted in less than a two-fold increase ($0.082/0.054 = 1.5$) when combined with 0.01 mole ZnO. The same D-value trends were also seen from A to B and C to D (**Figure 3(B)**). Likewise, doubling the ZnO concentration from 0.005 to 0.01 mole produced less than a two-fold increase in specific death rate, both A to C ($0.054/0.034 = 1.6$) and B to D ($0.082/0.07 = 1.2$) with the same as D-values when combined with either 0.005 or 0.01 mole Ag. These results suggest that Ag's bactericidal activity may be higher at lower ZnO concentrations, but that ZnO's bactericidal activity may not be significantly affected by Ag. Taken together, high ZnO levels may hinder Ag's bactericidal activity, and ZnO's effect may be insignificant on overall bactericidal activity. Thus, optimizing the Ag:ZnO ratio in HAp nanocomposites may be crucial for maximizing bactericidal activity.

3.4. Bactericidal Activity of Ag/ZnO-HAp Nanocomposites against Clinical Isolates of *P. aeruginosa*

HAp-based nanocomposites have been proposed for various biomedical applications, including bone tissue engineering, orthopedic implant coatings, and dental applications. These HAp-based nanocomposites are expected to exhibit high antimicrobial activity while maintaining biocompatibility and material stability [4] [8] [19]. To evaluate the Ag/ZnO-HAp nanocomposites in biomedical applications, eight multidrug-resistant clinical isolates of *P. aeruginosa* were used to determine MBC. Results showed that MBC levels for the four Ag/ZnO-HAp nanocomposites varied widely, ranging from ≤ 0.06 to >4 mg/mL, regardless of susceptibility or resistance to traditional antibiotics (Table 1). Although clinical isolates showed different MBC levels, nanocomposite D consistently had the lowest MBCs. Nanocomposite B had lower MBCs than the other two nanocomposites; nanocomposite C likely had lower MBCs than nanocomposite A, but, exceptionally, in isolate NY215, nanocomposite A showed lower MBCs than nanocomposites B and C (Table 1). These results indicate that the bactericidal activity of Ag/ZnO-HAp nanocomposites may be strain-specific, reflecting heterogeneity in cellular targets among isolates. Clinical isolates of *P. aeruginosa* have marked heterogeneity in both core genome sequences and accessory gene profiles [20]. Therefore, biomedical applications of Ag/ZnO-HAp nanocomposites may need to be customized to each clinical isolate.

Table 1. Bactericidal effect of Ag/ZnO-HAp nanocomposites against clinical isolates of *P. aeruginosa*.

Clinical isolates of <i>P. aeruginosa</i>	MBC (mg/mL) to Ag/ZnO-HAp nanocomposites				Antibiotic susceptibility*				
	A	B	C	D	CAR	CHL	CIP	KAN	TET
NY98	2	≤ 0.063	0.5	≤ 0.063	R	R	S	R	S
NY214	4	1	2	0.5	R	S	R	R	R
NY215	0.063	0.25	0.5	≤ 0.063	R	S	R	R	S
NY219	4	1	1	0.5	R	R	S	R	R
NY220	2	0.125	2	0.063	R	S	R	R	S
NY268	>4	2	>4	0.25	R	R	S	S	R
NY269	>4	2	>4	1	R	R	S	R	S
NY273-	>4	2	>4	1	R	R	S	S	R

MBC: minimum bactericidal concentration; MIC: minimum inhibitory concentration; *CAR: Carbenicillin (R: MIC: ≥ 128 $\mu\text{g/mL}$, S: MIC: <64 $\mu\text{g/mL}$); CHL: Chloramphenicol (R: MIC: ≥ 128 $\mu\text{g/mL}$, S: MIC: <64 $\mu\text{g/mL}$); CIP Ciprofloxacin (R: MIC: ≥ 2 $\mu\text{g/mL}$, S: MIC: <1 $\mu\text{g/mL}$); KAN: Kanamycin (R: MIC: ≥ 50 $\mu\text{g/mL}$, S: MIC: <25 $\mu\text{g/mL}$); TET: Tetracycline (R: MIC: ≥ 8 $\mu\text{g/mL}$, S: MIC: <4 $\mu\text{g/mL}$).

4. Conclusion

In this study, we confirmed the antibacterial activity of four previously reported Ag/ZnO-HAp nanocomposites (A, B, C, and D) [11] using the agar diffusion test against *P. aeruginosa* PAO1. We further analyzed patterns of bacterial death at

various concentrations to assess bactericidal effectiveness. We also performed time-killing assays against *P. aeruginosa* PAO1. The overall results showed that i) nanocomposite D exhibited the highest bactericidal activity, followed by B, C, and A; ii) Ag was a primary factor, and ZnO was a secondary contributor to bactericidal activity; and iii) increased ZnO inhibited the effect of Ag. Additionally, we determined the MBC levels of the nanocomposites against eight multidrug-resistant clinical isolates of *P. aeruginosa*. The MBC levels varied widely among clinical isolates, regardless of their susceptibility or resistance to traditional antibiotics. Taken together, these findings suggest that the nanocomposites are broadly effective against *P. aeruginosa*. However, to maximize bactericidal activity, it is essential to optimize the Ag:ZnO ratio and customize the nanocomposite for the targeted bacterial strain to ensure effective medical applications.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

- [1] Sachdeva, C., Satyamoorthy, K. and Murali, T.S. (2024) *Pseudomonas aeruginosa*: Metabolic Allies and Adversaries in the World of Polymicrobial Infections. *Critical Reviews in Microbiology*, **51**, 619-638. <https://doi.org/10.1080/1040841x.2024.2397359>
- [2] Tuon, F.F., Dantas, L.R., Suss, P.H. and Tasca Ribeiro, V.S. (2022) Pathogenesis of the *Pseudomonas Aeruginosa* Biofilm: A Review. *Pathogens*, **11**, Article 300. <https://doi.org/10.3390/pathogens11030300>
- [3] Horcajada, J.P., Montero, M., Oliver, A., Sorlí, L., Luque, S., Gómez-Zorrilla, S., *et al.* (2019) Epidemiology and Treatment of Multidrug-Resistant and Extensively Drug-Resistant *Pseudomonas Aeruginosa* Infections. *Clinical Microbiology Reviews*, **32**, e00031-19. <https://doi.org/10.1128/cmr.00031-19>
- [4] Hassanain, M., Abdel-Ghafar, H.M., Hamouda, H.I., El-Hosiny, F.I. and Ewais, E.M.M. (2024) Enhanced Antimicrobial Efficacy of Hydroxyapatite-Based Composites for Healthcare Applications. *Scientific Reports*, **14**, Article No. 26426. <https://doi.org/10.1038/s41598-024-76088-4>
- [5] Gebretsadik, A., Reddy, S.G., Gonfa, B.A. and Abebe, B. (2026) Ag-Decorated Cu-Doped ZnO Nanomaterial for Enhanced Antibacterial Application. *Scientific Reports*, **16**, Article No. 5552. <https://doi.org/10.1038/s41598-026-35838-2>
- [6] Zhang, Y., Liu, X., Li, Z., Zhu, S., Yuan, X., Cui, Z., *et al.* (2018) Nano Ag/ZnO-Incorporated Hydroxyapatite Composite Coatings: Highly Effective Infection Prevention and Excellent Osteointegration. *ACS Applied Materials & Interfaces*, **10**, 1266-1277. <https://doi.org/10.1021/acsami.7b17351>
- [7] Rai, M., Yadav, A. and Gade, A. (2009) Silver Nanoparticles as a New Generation of Antimicrobials. *Biotechnology Advances*, **27**, 76-83. <https://doi.org/10.1016/j.biotechadv.2008.09.002>
- [8] Liu, W., Cheong, N., He, Z. and Zhang, T. (2025) Application of Hydroxyapatite Composites in Bone Tissue Engineering: A Review. *Journal of Functional Biomaterials*, **16**, Article 127. <https://doi.org/10.3390/jfb16040127>
- [9] Omran, B.A., Tseng, B.S. and Baek, K. (2024) Nanocomposites against *Pseudomonas*

- Aeruginosa Biofilms: Recent Advances, Challenges, and Future Prospects. *Microbiological Research*, **282**, Article 127656. <https://doi.org/10.1016/j.micres.2024.127656>
- [10] Kucukosman, O.K., Pourmostafa, A., Dogan, E. and Bensalem, A. (2025) Balancing Antibacterial Activity and Toxicity in Silver-Loaded Hydroxyapatite: The Impact of the Silver Nanoparticle Incorporation Method. *Journal of Materials Chemistry B*, **13**, 9954-9967. <https://doi.org/10.1039/d5tb01113b>
- [11] Bensalem, A., Kucukosman, O.K., Raszkiewicz, J. and Topkaya, F. (2021) Synthesis, Characterization, Bactericidal Activity, and Mechanical Properties of Hydroxyapatite Nano Powders Impregnated with Silver and Zinc Oxide Nanoparticles (Ag-ZnO-Hap). *Ceramics International*, **47**, 21319-21324. <https://doi.org/10.1016/j.ceramint.2021.04.139>
- [12] Barrow, K. and Kwon, D.H. (2009) Alterations in Two-Component Regulatory Systems of *phoPQ* and *pmrAB* Are Associated with Polymyxin B Resistance in Clinical Isolates of *pseudomonas Aeruginosa*. *Antimicrobial Agents and Chemotherapy*, **53**, 5150-5154. <https://doi.org/10.1128/aac.00893-09>
- [13] Predoi, D., Iconaru, S.L., Predoi, M.V., Motelica-Heino, M., Guegan, R. and Buton, N. (2019) Evaluation of Antibacterial Activity of Zinc-Doped Hydroxyapatite Colloids and Dispersion Stability Using Ultrasounds. *Nanomaterials*, **9**, Article 515. <https://doi.org/10.3390/nano9040515>
- [14] Kwon, D., Vasanthan, N., Ibrahim, N. and Yahra, N. (2025) Antibacterial Properties of Quaternized Cellulose Nanocrystals in Clinical Isolates of Multidrug-Resistant Gram-Negative Bacteria. *The Journal of Antibiotics*, **78**, 442-448. <https://doi.org/10.1038/s41429-025-00829-4>
- [15] Kwon, D.H., Vasoya, M. and Sankaranarayanan, D. (2025) The Effect of Exogenous Glutathione on Meropenem Susceptibility in Klebsiella Pneumoniae-Carbapenemases (KPC)-Producing Bacteria. *The Journal of Antibiotics*, **78**, 633-637. <https://doi.org/10.1038/s41429-025-00850-7>
- [16] Donkor, E.S., Sosah, F.K., Odoom, A., Odai, B.T. and Kunadu, A.P. (2025) How Long Do Microorganisms Survive and Persist in Food? A Systematic Review. *Microorganisms*, **13**, Article 901. <https://doi.org/10.3390/microorganisms13040901>
- [17] Balouiri, M., Sadiki, M. and Ibnsouda, S.K. (2016) Methods for *in Vitro* Evaluating Antimicrobial Activity: A Review. *Journal of Pharmaceutical Analysis*, **6**, 71-79. <https://doi.org/10.1016/j.jpha.2015.11.005>
- [18] Hossain, T.J. (2024) Methods for Screening and Evaluation of Antimicrobial Activity: A Review of Protocols, Advantages, and Limitations. *European Journal of Microbiology and Immunology*, **14**, 97-115. <https://doi.org/10.1556/1886.2024.00035>
- [19] Kauke-Navarro, M., Knoedler, L., Knoedler, S. and Safi, A.F. (2024) Advancements in Facial Implantology: A Review of Hydroxyapatite Applications and Outcomes. *Frontiers in Surgery*, **11**, Article ID: 1409733. <https://doi.org/10.3389/fsurg.2024.1409733>
- [20] Mishra, P., Sahoo, D. and Sahu, M.C. (2026) Genetic Diversity of Pseudomonas Aeruginosa Isolated from Clinical Samples with ISSR Molecular Marker in a Tertiary Care Teaching Hospital. *Scientific Reports*, **16**, Article ID: 5315. <https://doi.org/10.1038/s41598-026-35090-8>