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Synergistic Effect of Zinc Oxide Nanoparticles with Antibiotics Against Resistant Staphylococcus aureus Isolated from SSTIs: A Cross-Sectional In Vitro Study

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Abstract

Background: Skin and soft tissue infections (SSTIs) are frequently encountered clinical conditions, most often caused by Staphylococcus aureus. The increasing prevalence of antibiotic-resistant strains, particularly methicillin-resistant S. aureus (MRSA), has made treatment more challenging. Nanotechnology, specifically zinc oxide nanoparticles (ZnO NPs), offers a promising approach to augment the efficacy of existing antibiotics. augment the efficacy of existing antibiotics.

Objective: This study aimed to evaluate the synergistic effect of ZnO nanoparticles combined with commonly used antibiotics against Staphylococcus aureus (S. aureus) isolated from patients with SSTIs.

Methods: We collected 100 S. aureus isolates from patients with SSTIs and identified them using standard microbiological methods. using standard microbiological methods. Antibiotic susceptibility was determined by the disc diffusion method on Mueller-Hinton agar. The antibiotics tested included ampicillin, cefoxitin, tetracycline, erythromycin, clindamycin, fusidic acid, levofloxacin, and co-trimoxazole. We supplemented each antibiotic disc with 100 µg of ZnO NP solution to assess changes in the zone of inhibition. inhibition.

Results: A notable increase in antibiotic sensitivity was observed following ZnO NP supplementation. The most significant enhancements in mean zone diameters were seen with erythromycin (+ 8.04 mm), levofloxacin (+ 7.75 mm), fusidic acid (+ 5.06 mm), and tetracycline (+ 4.44 mm). In contrast, ampicillin showed a marked decrease in efficacy (-9.68 mm), while cefoxitin exhibited a minimal change (-0.29 mm).

Conclusion: ZnO nanoparticles enhanced the antimicrobial activity of several antibiotics against resistant S. aureus from SSTIs. These findings support the use of ZnO NPs as potential adjuvants for the treatment of resistant infections, especially when options are limited. These findings support the potential use of ZnO NPs as a cost-effective adjuvant in managing antibiotic-resistant infections. This is particularly relevant in settings with limited treatment options. in settings with limited treatment options.

Introduction

Staphylococcus aureus is a leading cause of skin and soft tissue infections (SSTIs) worldwide and has become a significant burden on healthcare systems due to its capacity to develop resistance against multiple classes of antibiotics. [1] Every year, countless patients suffer severe complications from antibiotic-resistant Staphylococcus aureus infections, leading to prolonged hospital stays, increased healthcare costs, and death. To combat these diseases, we must develop new antimicrobials or enhance the effectiveness of existing ones. [2] Gaining Food and Drug Administration (FDA) approval for a new drug can take nearly ten years. This is largely due to the research required to ensure safety for human consumption. A time-saving option is to enhance the effectiveness of already approved drugs. [3, 4]

Nanotechnology can be employed to increase an antibiotic's antimicrobial effect and help overcome this challenge. [5]

Nanotechnology is the design and synthesis of nanoparticles using principles from physics, chemistry, engineering, and biology. [6] A nanoparticle is an ultra-fine particle with a size ranging from 1 to 100 nanometres in diameter. [7] Its small size results in a significant increase in the surface area-to-volume ratio, which is crucial for enhancing its antibacterial activity. [8] This increased surface area facilitates the generation of reactive oxygen species (ROS), which are key to the breakdown of bacterial cells. The nanoscale size also enhances the physical, chemical, and reactive properties of nanoparticles compared to their bulk counterparts, making them more potent and useful.[9] Engineered nanoparticles (NPs) are classified into three types: organic, inorganic, and carbon-based. [10] Inorganic NPs are either metal or metal oxides. Their qualities depend on the type of metal used. [11,12] Metallic NPs include aluminium, cadmium, cobalt, copper, gold, iron, lead, silver and zinc. Metal oxide NPs are modified forms of these metals. [13] Metal oxide NPs are more reactive than metals. Examples are cerium oxide, iron oxide, magnetite, silicon dioxide, titanium oxide, and zinc oxide. [14] Among these NPs, ZnO offers the greatest advantages for our needs. It has the highest photocatalytic efficiency and superior biocompatibility among NPs. [15] Zinc oxide also exhibits selectivity, durability, and heat resistance. These qualities make it effective against many microorganisms, including Staphylococcus aureus, a common cause of SSTIs. [16]

Zinc oxide nanoparticles (ZnO NP) function by producing reactive oxygen species (ROS), which induce oxidative stress and eventually destroy bacterial cells. [17]These ROS can damage bacterial DNA, proteins, and lipids, effectively circumventing common resistance mechanisms, such as beta-lactamases production in Methicillin-Resistant Staphylococcus aureus (MRSA), which normally deactivates antibiotics by breaking their beta-lactam ring. In addition, ZnO NPs also exhibit an inhibitory effect on biofilm production by Staphylococcus aureus. ZnO NPs can combat new resistance mechanisms in bacteria, especially Staphylococcus aureus.[18] Augmenting antibiotics with ZnO NPs increases their effectiveness and reduces the required dose, thereby reducing their side effects. [19] ZnO NP is listed as a GRAS (generally regarded as safe) substance for use in drugs by the Food and Drug Administration (FDA) in the US. [20] The compound in question is utilised in a multitude of products and systems, including sunscreens, nappy rash cream, biosensors, food additives, pigments, rubber manufacturing, and electronic materials. [21,22]

We hypothesise that augmenting antibiotics with ZnO NPs will significantly enhance their efficacy against Staphylococcus aureus by lowering the minimum inhibitory concentration (MIC) values by 2-fold or more. This study explores the synergistic effect of ZnO NPs in combination with diverse groups of antibiotics on a panel of clinically isolated Staphylococcus aureus strains.

Materials and Methods

Study Design and Setting

This cross-sectional comparative study was conducted in the Microbiology Laboratory at Jinnah Sindh Medical University (JSMU), Karachi, over a six-month period (May to November 2022).

Sample Collection and Bacterial Isolation

A total of 100 clinical samples originally collected for diagnostic purposes from patients with SSTIs were used in this study. These samples included wound swabs, pus aspirates, and tissue samples. Verbal informed consent was obtained from all patients.

The samples were cultured on blood and chocolate agar and incubated at 37°C for 24 hours. Staphylococcus aureus was identified using Gram staining, catalase test, and coagulase test.

Preparation of Zinc Oxide Nanoparticle Solution

ZnO NPs (Sigma-Aldrich) solution for antibiotics augmentation was prepared by dissolving 500 mg of ZnO NPs powder in 50 mL of distilled water and autoclaving at 121°C for 20 minutes. The resulting suspension had a concentration of 10 µg/µl. Antibiotic discs were supplemented with 10 µL of ZnO NP suspension (100 µg/disc) and then dried at 80°C.

Antibiotic Susceptibility Testing

Antibiotics used for testing:

- Beta-lactams: Ampicillin, Cefoxitin
- Macrolides: Erythromycin
- Lincosamides: Clindamycin
- Fluoroquinolones: Levofloxacin
- Sulfonamides: Co-trimoxazole
- Tetracyclines: Tetracycline
- Fusidane: Fusidic acid

Disc diffusion testing was performed on Mueller-Hinton agar plates according to standard microbiological protocols. For each Staphylococcus aureus isolate, antibiotic susceptibility was assessed using antibiotic-impregnated discs, with and without supplementation with zinc oxide nanoparticles (ZnO NPs). Specifically, 100 µg of ZnO NP solution was applied to each antibiotic disc before placement on the agar surface. After incubation at 37°C for 18–24 hours, the zones of inhibition around each disc were measured in millimetres using a calibrated ruler. The diameters of inhibition zones from ZnO NP-augmented discs were then compared to those from standard antibiotic discs to evaluate any enhancement in antibacterial activity.

Statistical Analysis

Mean zone diameters before and after ZnO NP augmentation were compared using paired t-tests. A p-value < 0.05 was considered statistically significant.

Results

Demographics

Among the 100 patients from whom Staphylococcus aureus isolates were collected, 57% were male and 43% female. The majority of patients fell within the range of 20 to 40 years. Regarding the types of skin and soft tissue infections, abscesses were the most common, accounting for 42% of cases, followed by cellulitis at 28%, surgical site infections at 18%, and other infections accounted for the remaining 12%.

Antibiotic Susceptibility Before ZnO Nanoparticle Augmentation

The antibiotic susceptibility testing revealed the highest resistance rates to ampicillin and erythromycin among the isolates. Testing with cefoxitin highlighted that 42% of the isolates were methicillin-resistant Staphylococcus aureus (MRSA), highlighting a considerable proportion of resistant strains in the study population.

A paired t-test was used to determine statistical significance, with p-values < 0.05 considered significant.

Discussion

This study demonstrates that zinc oxide nanoparticles significantly enhance the antimicrobial activity of several antibiotics against Staphylococcus aureus. Erythromycin, levofloxacin, fusidic acid, and tetracycline showed marked improvements in their zones of inhibition post ZnO NP augmentation. The Staphylococcus aureus isolates demonstrated notable differences in antibiotic sensitivity patterns before and after augmentation with ZnO nanoparticles (ZnO NPs). The mean increase in the diameter of the zone of inhibition following ZnO NP augmentation was 4.440 mm for tetracycline, 8.040 mm for erythromycin, 3.230 mm for clindamycin, 5.060 mm for fusidic acid, 7.750 mm for levofloxacin, and 5.030 mm for co-trimoxazole. Conversely, reductions were observed in cefoxitin (0.290 mm) and ampicillin (9.680 mm). Statistical evaluation revealed that the increases observed for erythromycin, fusidic acid, and levofloxacin were significant ($p < 0.05$, 95% CI within the positive range), confirming a true enhancement in antibacterial activity. In contrast, the minor reduction in cefoxitin and the decrease in ampicillin did not reach statistical significance ($p > 0.05$).

Similar results have been reported in international literature. After ZnO NP supplementation, an Egyptian study showed improved antimicrobial activity against Staphylococcus aureus. [23] Similarly, Pakistan found that levofloxacin's inhibitory zone increased by 5.01 mm after ZnO NPs were added. [24]

According to the proposed theory, ZnO NPs generate reactive oxygen species (ROS), disrupt vital cellular processes, and compromise the integrity of bacterial cell membranes, thereby increasing bacterial pathogenicity. Zinc oxide nanoparticles' intrinsic antibacterial activity likely acts in combination with antibiotics to enhance therapeutic outcomes.

The results of our study support previous literature indicating that ZnO NPs can improve the antibacterial activity of conventional medicines. In one experiment, a significant level of antibacterial activity was observed with ZnO NPs against various bacterial species, including methicillin-resistant Staphylococcus aureus (MRSA), when delivered in conjunction with conventional antibiotics. [25] Similarly, ZnO NPs were shown to provide synergistic effects against a variety of bacterial species when combined with erythromycin and amoxicillin. [26] Moreover, recent studies show that there is synergy in the use of ZnO NPs. In a study combining ZnO NPs with either ciprofloxacin or tetracycline, these researchers found that enhanced antibacterial efficacy against multidrug-resistant Staphylococcus aureus reduced bacterial viability at lower antibiotic doses. [27] Additionally, research showed that in 2023, ZnO NPs combined with antibiotics demonstrated better antimicrobial effects against MRSA than antibiotics alone. [28] This work illustrated how ZnO NPs can enhance antibiotic penetration while simultaneously reducing the risk of resistance by increasing membrane permeability and disrupting biofilm structure. These findings are more interesting in our studies, which notably demonstrated that ZnO NPs at sub-inhibitory concentrations increased the bactericidal efficacy of the drugs against Staphylococcus aureus isolated from SSTIs. Additional evidence even further supports our findings. One study examined the effects of ZnO NPs combined with doxycycline and found that they significantly reduced bacterial counts and biofilm formation in the chronic wound model, resulting in a 75% reduction in bacterial burden. [29] This suggests that ZnO NPs may be a useful adjuvant to enhance the effectiveness of antibiotics against infections caused by biofilm-forming bacteria.

Not all bacterial strains respond the same way to ZnO NPs' antimicrobial activity. One study found that observed bacterial susceptibility to ZnO NPs was robust against some Staphylococcus aureus strains, but ineffective against others. This variability insinuated that the type of infection causing the illness and the bacterium's genotype could affect findings. [30] In a more recent study with very similar findings, it was shown that environmental factors and biofilm formation could affect the intracellular antimicrobial activity of ZnO NPs, thereby considerably reducing efficacy in certain therapeutic settings. [31] The synergism between tetracycline and clindamycin, together with ZnO NPs, may result from their interference with bacterial protein synthesis and bile formation, thereby enhancing the antibiotics' activity. A study demonstrated that ZnO NPs increase bacterial membrane permeability, enabling greater intracellular antibiotic absorption. [32]

Similarly, the efficacy of levofloxacin and erythromycin in the presence of ZnO NPs could be atmospherically inhibited by blocking bacterial efflux pumps, thereby allowing higher intracellular concentrations of the respective antibiotics. The reduction in Staphylococcus aureus susceptibility to ampicillin and cefoxitin in the presence of ZnONPs was an intriguing discovery that raised concerns about the safety of ZnO NP–antibiotic combination therapy. While previous work has shown that zinc

oxide nanoparticles may enhance the therapeutic efficacy of some antibiotics against bacteria, it is plausible that they also contribute to reduced susceptibility to certain antibiotics by inhibiting bacterial cell wall synthesis or through another mechanism. This finding underscores the importance of careful evaluation and assessment of the mechanism prior to clinical translation.

Future research should be directed towards targeted clinical trials and the establishment of standardised guidelines for the safe and successful practice of combining ZnO NP–antibiotic pairings in line with these findings. While further research is required, ZnO NPs could play an important role in enhancing the efficacy of traditional antibiotics. Additionally, regulatory issues must be considered. Depending on their composition and purpose, ZnO NP–antibiotic combinations could be considered combination therapies or distinct pharmacological products. It will be imperative to ascertain their regulatory pathway as early as possible, as this will determine their safety testing, clinical trials, and approvals for clinical practice.

Conclusion

Zinc oxide nanoparticles demonstrated a statistically significant synergistic effect with several commonly used antibiotics against Staphylococcus aureus isolated from SSTIs. These findings support the potential for integrating ZnO NPs into clinical antimicrobial regimens, particularly for MRSA infections. Further in vivo studies and clinical trials are recommended to assess safety, dosing, and delivery mechanisms.

Recommendations
Larger multicenter studies across Pakistan
Evaluation of MICs and time-kill curves
Potential development of ZnO-based antibiotic dressings

Declarations

Conflict of Interest
The authors declare no conflict of interest.

Consent to Participate
Verbal informed consent was obtained from all patients from whom samples were collected for this study.

Ethics Approval and Standards
Ethical approval was obtained from the Institutional Review Board of JSMU with letter no JSMU/IRB/2022/-622. Patient confidentiality was maintained, and all procedures adhered to the principles outlined in the Declaration of Helsinki.

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Author Contribution

Mahnoor: Manuscript writing and analyzed the data Suresh: Analysis and Data compilation Hamza: Manuscript writing Kirsh: Tables and Review Maham: Review

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