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RESEARCH ARTICLE

SILVER NANOPARTICLES: A GAME CHANGER IN DENTAL ANTIBACTERIAL THERAPY

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ABSTRACT

Introduction: Antimicrobial resistance (AMR) is projected to become a leading cause of death in the coming decades.Hence, new methods for development of further generation of antibiotics are urgently needed. Metalloantibiotics are aninfinite, wide and underexplored group of compounds.Inorganic compounds and metal ions have a history of antimicrobial activity and got attention in increase of antimicrobial resistance. **Aim**: To check the effectiveness of silver nanoparticles on *Staphylococcus aureus*when combined with conventional antibiotic used in dentistry. **Methodology**: Silver nanoparticles (AgNPs) with an average particle size of 5 nm were used in solution form for the study.silver nanoparticles (AgNPs) were utilized in solution. Brain Heart Infusion broth was used to cultivate a conventional strain of Staphylococcus aureus, which was then aerobically incubated at 37°C for a whole day. Following centrifugation, the culture was reconstituted in saline and adjusted to 0.5 McFarland standard (≈1 × 10^2 CFU/mL). A sterile swab was used to produce a bacterial lawn on Mueller-Hinton agar plates. AgNPs alone, antibiotics alone, and antibiotics with AgNPs were the three categories into which the discs were separated. Zones of inhibition were assessed to evaluate antibacterial activity after plates were incubated at 37°C for a whole day. **Result**: The combination of silver nanoparticles with different antibiotics showed varying inhibition zones. The highest inhibition zone was observed with silver nanoparticles and doxycycline (23.4 mm), while the lowest was with silver nanoparticles (Ag-NPs)and cephalosporin (15.2 mm). **Conclusion:** AgNPs may help combat emerging antimicrobial resistance and improve the effectiveness of traditional antimicrobial treatments.

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INTRODUCTION

Globally, antimicrobial resistance (AMR) is on the rise and has emerged as a significant global health issue. Antimicrobial resistance was linked to an estimated 4.95 million fatalities in 2019. [1] By 2050, this figure is expected to increase to 10–11 million deaths per year, in part because of the extensive and frequently improper use of antibiotics, especially their over prescription during the COVID-19 epidemic. Staphylococcus aureus is a significant bacterium that causes a variety of infections in humans and is linked to high rates of morbidity and mortality. Its capacity to build biofilms, which increases its resistance to antimicrobial agents, is one of its primary pathogenicity factors [2]. Inorganic and metal-based compounds have had a small but important role in medicine in the 20th century. However, in the last few years, a number of metals have gotten a lot of attention as possible antimicrobial agents because antimicrobial resistance (AMR) is growing quickly [3]. Recent studies emphasize the utilization of silver-based compounds as synergistic agents alongside conventional antibiotics, as well as the capacity of silver ions to directly inhibit or eradicate bacterial pathogens through diverse antimicrobial mechanisms. Also, metal ions have been known to kill bacteria for a long time, and they are getting more attention because more and more microorganisms are becoming resistant to drugs. As a result, researchers are now working on new antibacterial methods that use combinations of metal ions and nanoparticles to make them more effective against bacteria and to get around drug resistance [4,5]. The aim of the study was to assess the effectiveness of silver nanoparticles (Ag-NPs) when combined with a conventional antibiotic used in dentistry on *Staphylococcus aureus*.

MATERIAL AND METHODS

Silver nanoparticles formulation Silver nanoparticles of an average particle size of 5 nm used in a solution form. Purity > 99% Ag, Molar mass – 107.87,density 1-4g/cc

Microorganism: This study utilized a standardized strain of Staphylococcus aureus. We got the bacterial strain from a microbiology lab and kept it in normal culture conditions.

Preparation of Bacterial Culture

The revived culture of Staphylococcus aureus was inoculated into Brain Heart Infusion (BHI) broth and Incubated at 37°C for 24 hours in an aerobic environment to stimulate active bacterial growth. After being incubated, the bacterial

suspension was spun in a centrifuge. The bacterial pellet was put back into sterile normal saline to make a new culture suspension. Using a spectrophotometer, the turbidity of the bacterial suspension was set to the 0.5 McFarland standard, which is about 1×10^8 CFU/ml.

Preparation of Agar Plates: Lab made sterile Mueller–Hinton agar plates and let them harden in a clean place.

The best way to test how well antibiotics work is to use Mueller–Hinton agar because its composition never changes and antibiotics spread easily through it.

Inoculating a bacterial lawn: A clean cotton swab was dipped into the standardized bacterial suspension, and the extra liquid was taken off by pressing the swab against the wall of the tube. The swab was streaked evenly in three directions across the whole surface of the Mueller-Hinton agar plate to make sure that the bacteria were spread out evenly and a confluent bacterial lawn formed. To make sure that the bacteria were spread evenly and a confluent bacterial lawn formed, the swab was streaked evenly in three directions across the whole surface of the Mueller–Hinton agar plate.After inoculation, the plates were allowed to stand for 5–10 minutesto permit absorption of the inoculum into the agar surface.

Preparation of Test Discs

Sterile blank antimicrobial susceptibility discs used for the test. Four most commonly used antibiotics in dentistry were selected

- Doxycycline30 µg
- Amoxicillin30 µg
- Cephalosporin 30 µg
- Metronidazole 30 µg

The discs were divided into three groups:

Group I – Silver nanoparticles Alone

Group II – Antibiotics Alone

Discs were infused with the respective antibiotic solutions.

Group III – Antibiotics + Silver Nanoparticles: Discs were loaded with the selected antibiotics combined with 0.625 mg/ml **silver nanoparticles (AgNPs)**. The prepared discs were allowed to dry under sterile conditions to ensure proper absorption of the antimicrobial agents. Using sterile forceps, the antibiotic discs were carefully placed on the inoculated Mueller–Hinton agar plates. The discs were positioned at equal distances from each other to avoid overlapping of inhibition zones.

Each plate contained discs representing:

- Silver nanoparticles
- Antibiotics alone
- Antibiotics with silver nanoparticles

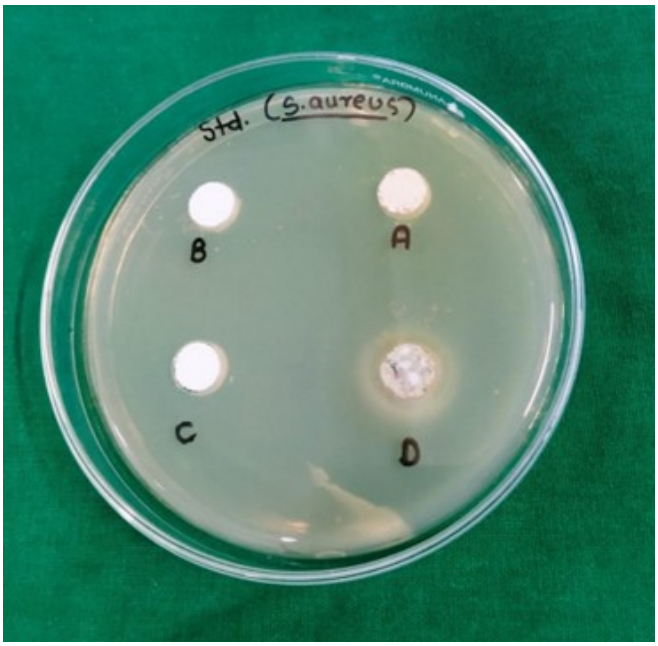
The discs were gently pressed to ensure complete contact with the agar surface.

Incubation: The inoculated plates with antimicrobial discs were incubated in an aerobic incubator at 37°C for 24 hours. This incubation period allows sufficient time for bacterial growth and diffusion of antimicrobial agents into the agar medium.[6]

Measurement of Zone of Inhibition: After 24 hours of incubation, the plates were examined for clear circular zones around the discs, indicating inhibition of bacterial growth. The diameter of the zone of inhibition was measured inmillimeters (mm) using a digital caliper or transparent ruler. The measurement was taken from edge to edge across the center of the disc, including the disc diameter. The size of the inhibition zone represents the antibacterial effectiveness of the tested agents against *Staphylococcus aureus*.

RESULTS

The zone of inhibition by Silver nanoparticles – 19.8mm (Figure -1). The zone of inhibition of plain Cephalosporin is 17.2 mm. The zone of inhibition of plain Amoxicillin is 19.9mm. The zone of inhibition of plain Metronidazole is 19.8mm. The zone of inhibition of plain Doxycycline is 18.5. (Figure -2). The combination of silver (Ag) nanoparticles 0.625 mg/ml with different antibiotics showed varying inhibition zones. The highest zone of inhibition was observed with silver nanoparticles and doxycycline (23.4 mm), while the lowest was with silver nanoparticles and cephalosporins (15.2 mm) Statistical analysis using the Kruskal-Wallis test showed a p-value of 0.429, indicating no statistically significant difference between the groups ($p \leq 0.05$).(Table 1,Figure-3)



(Figure -1) D. The zone of inhibition by Silver nanoparticles19.8 mm

Statistical analysis: Statistical Analysis: The data collected was entered in Microsoft Excel (2020) and analysed using SPSS® (IBM Corp. Released 2012 IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY, USA: IBM Corp.).

(Figure -2) Antibiotic sensitivity test A. Cephalosporin B. Amoxicillin C. Metronidazole D.Doxycycline

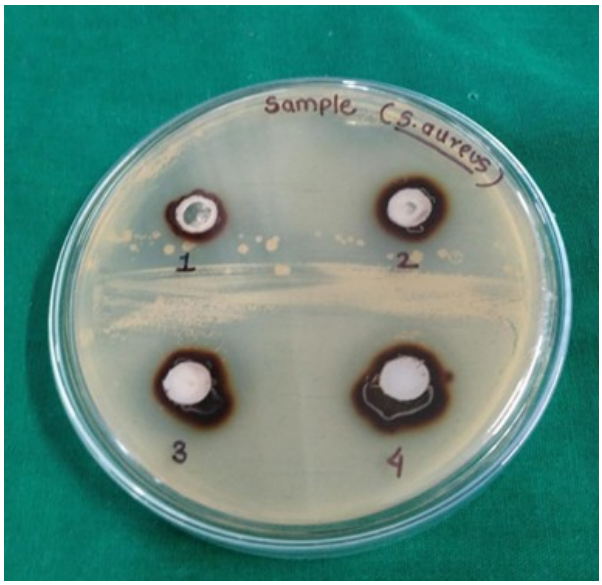


Figure 3. Antibiotic sensitivity test 1.Cephalosporin+ AgNP ,2.Amoxicillin +AgNP3.Metronidazole+ AgNP4. Doxycycline+ Ag NP

Test organism	Sample	Result (zone of inhibition in mm)
s.aureus	A.Silver Nano particles + Cephalosporin	15.2
	B. Silver Nano particles+ Amoxicillin	18.3
	C. Silver Nano particles+ Metronidazole	17.2
	D. Silver Nano particles +Doxycycline	23.4
	A .Cephalosporin	17.2
	B. Amoxicillin	19.9
	C.Metronidazole	19.8
	D.Doxycycline	18.5

Kruskal Wallis test was carried out to determine the differences between the groups. All statistical tests were performed at a significance level of 5% ($p < 0.05$).

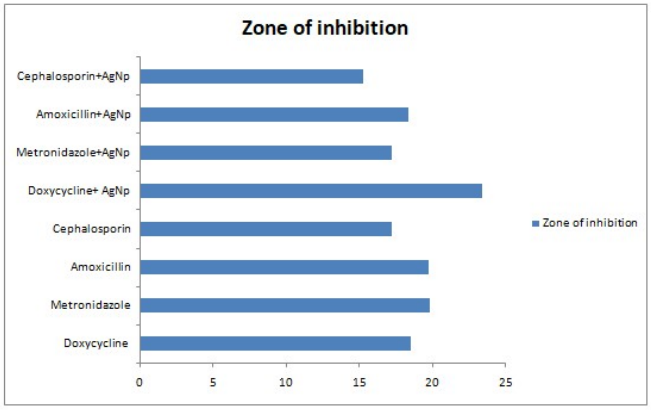


Figure 4.

DISCUSSION

Antimicrobial resistance (AMR) is widely recognized as a major global public health threat, yet its origins and implications extend beyond the simple overuse of antibiotics. AMR is a complex, multifactorial phenomenon shaped by biological, clinical, dental, environmental, and social dynamics, with particular attention to the emerging role of dentistry. A narrative literature review was performed, drawing from textbooks, peer-reviewed articles, and official World Health Organization (WHO) reports, with emphasis on recent findings on periodontal biofilms as reservoirs of resistance genes. The analysis shows that AMR develops through bacterial mutations, horizontal gene transfer, environmental contamination, healthcare-associated practices, and patient behaviors, all of which interact to sustain its spread [7]. Common oral pathogens, including Streptococci, staphylococci and anaerobes, have demonstrated some levels of resistance to frequently prescribed antibiotics like amoxicillin and metronidazole. The study outlines foundational principles for antibiotic usage in pediatric dentistry, emphasizing prevention, adjunctive therapy, proper selection, and dosing. Specific clinical scenarios, such as pulpal infections, facial swelling, dental trauma. Emerging trends in antibiotic management includes the potential of metal nanoparticles and artificial intelligence [8]. Selective toxicity refers to the ability of a drug to target and inhibit harmful microorganisms or abnormal cells (such as cancer cells) without significantly harming the host.This concept is especially important in the development of antibiotics, antivirals, antifungals, and anticancer agents, where the goal is to selectively target the pathogens while minimizing damage to normal, healthy cells. The basis for selective toxicity lies in differences in the structure and function in the pathogen or abnormal cells compared to the host cells. For eg:Antibiotics: Selective toxicity is often attained by targeting constituents or processes that are unique to bacteria, such as the bacterial cell wall. Penicillin, for example, targets the bacterial cell wall synthesis process without affecting human cells. The concept of selective toxicity is important in the development of effective, safe therapeutic agents. However, achieving seamless selectivity is often difficult, and side effects or resistance may develop over time [9-10]. The spectrum of a drug is the range of microorganisms or conditions that the drug can effectively treat. In the context of antimicrobial drugs, the spectrum refer to the variety of bacteria, fungi, viruses, or parasites that the drug can target. Drugs can have a narrow or broad spectrum activity depending on how many different pathogens they can act against. Drug resistance take place when microorganisms (such as bacteria, viruses, fungi) evolve mechanisms that

reduce the effectiveness of a drug or treatment designed to target them. This can make infections or diseases harder to treat and more dangerous. Humans are more often infected by microorganisms such as bacterium, virus, etc. in the living environment. Some metal ions have strong inhibitory and bactericidal effects. AgNPs have broad-spectrum antibacterial activity, effective against both Gram-positive, Gram-negative bacterias.AgNPs can effectively inhibit the formation of biofilm. Metal ions have aextensive history of antimicrobial activity and have received attention in antimicrobial resistance. Silver nanoparticles (Ag-NPs) represent a new generation of antimicrobials. Ag-NPs have very broad range of antimicrobial activity and kill both Gramnegative and Gram-positive bacteria, including *E.coli, Staphylococcus aureus, Bacillus subtilis, Streptococcus mutans, and Staphylococcus epidermis* [12][13]. According toZhao and Stevens the antimicrobial activity of Ag-NPs is significantly high. Silver is toxic element to microorganisms than other metals in the following sequence: Ag >Hg >Cu>Cd>Cr >Pb> Co>Au>Zn>Fe >Mn>Mo >Sn and Ag-NPs have more effect than other silver salts in facilitating their antimicrobial activity. Kim et al.in 2007 stated that silver has low toxicity to mammalian cells and silver has a less propensity to induce microbial resistance than so many other antimicrobial materials [13]. Study done by Kyung-Hwan Cho et. al 2005 showed the growth inhibition ring of *S. aureus* and *E. coli* treated by Ag-NPs [14]. A combination of silver nanoparticles (AgNPs) and an antibiotic can synergistically inhibit bacterial growth, especially against the drug-resistant bacteria. Mohammed A. Abd Ali in 2022 was studied that erythromycin E had the greatest synergistic impact with AgNPs (0.1 mg/ml), but Streptomycin and Tetracycline had only 6 mm inhibitory zones when paired with AgNPs (0.1 mg/ml) in comparison.[15]Study done by Hui Li et.al. 2023 stated that AgNPs andAg^{+} have excellent antibacterialeffects on *S.aureus* and methicillin-resistant *staphylococcus aureus.*(MRSA)[16] Wen-Ru Li et.al Used Ag-NPs on *S. aureus* cells did not show any growth over 7 days. Respiratory chain dehydrogenase was inhibited by Ag-NPs, and the higher of concentration of Ag-NPs, the lower of the activity of the enzyme. The same phenomenon occurred in the *E. coli* cells exposed to Ag-NPs (Li et al. 2010).[17-18]

CONCLUSION

Silver nanoparticles (AgNPs) showed strong antibacterial efficacy against Staphylococcus aureus within the constraints of this in vitro investigation. In comparison to antibiotics used alone, a discernible increase in the zone of inhibition was seen when combined with routinely used dental antibiotics, suggesting a synergistic impact. This implies that AgNPs may help combat emerging antimicrobial resistance and improve the effectiveness of traditional antimicrobial treatments. The combined therapy demonstrated excellent antibacterial activity among the studied groups, indicating its potential as an adjuvant in the treatment of dental infections. However, before widespread clinical application, more in vivo research and clinical trials are required to assess the safety, ideal dosage, and long-term effects of such combinations.

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