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SYNTHESIS OF SILVER NANOPARTICLES AND VALUATION OF THEIR ANTIBACTERIAL ACTIVITY AGAINST GRAM POSITIVE & GRAM-NEGATIVE BACTERIUM

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Abstract

Background: Nanoparticles are involved in multiple applications of our everyday lives as well as a myriad of diagnostic and therapeutic uses. Nanoparticles have greater surface area to volume ratio, hence greater penetrability. This allows for both their efficacy and toxicity. From drug delivery systems to sun screens to imaging techniques, nanoparticles are part of various aspects of modern life. On the basis of the properties of silver, nanotechnology is now using silver in the form of silver nanoparticles. Silver nanoparticles hold great importance because several pathogenic bacteria have developed resistance against the antibiotics which were used for treating bacterial infections. Objective: The aim of this study was the synthesis of silver Nanoparticles and valuation of their antibacterial activity against Gram positive & Gram-negative bacteria. Methodology: Silver nanoparticles were prepared from E coli then were coated on the different antibiotics to enhance their antimicrobial activity against Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumonia, Pseudomonas aeruginosa. The respective zones of inhibitions formed were analyzed as increase in zone of inhibitions indicated the enhanced antimicrobial activity. Results: After coating the antibiotics with silver nanoparticles, the antibacterial activity of antibiotics against their respective bacterium increases. Conclusion: Moreover, comparison between gram positive & gram-negative bacteria against nanoparticle coated antibiotics was also studied which shows that effect of silver nanoparticles is more enhanced in case of gram-positive bacteria as compared to that of gram-negative bacteria.

Keywords: *Silver nanoparticles, antimicrobial activity, E. coli, Staphylococcus aureus, Bacillus subtilis, Klebsiellapneumonia, Pseudomonas aeruginosa.*

INTRODUCTION

Nanotechnology is the emerging field of science involved in the synthesis and application of materials and devices whose smallest functional organization, in at least one dimension is one billionth of a meter, i.e. nanometer (1). Nanoparticles are involved in multiple applications of our everyday lives as well as a myriad of diagnostic and therapeutic uses. Nanoparticles have greater surface area to volume ratio, hence greater penetrability. This allows for both their efficacy and toxicity (2).

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From drug delivery systems to sunscreens to imaging techniques, nanoparticles are part of various aspects of modern life Metallic nanoparticles such as silver and gold are getting attention due to their applications in different areas such as electronics, medicine, coatings cosmetics and biotechnology. Synthesis of these nanoparticles is an important research in nanotechnology (3).

There are many methods for the preparation of nanoparticles, but among them biological method is the most economical and environment friendly. As nanoparticles have specific characteristics, it has many applications in the field of medicine, biotechnology, food science, industries etc. A complete list of the possible applications of nanotechnology is too vast to discuss in detail, but their greatest application is observed in the development of new and efficient medical treatments by producing nano-medicine (4). Among these nanoparticles, Silver nanoparticles with distinctive characteristics of high antimicrobial activity have fascinated the scientists and researchers to develop nano silver-based medicine (5). Silver has been used in past in the form of silver nitrate and metallic silver for the treatment of burns, wounds and various bacterial infections. So, silver nanoparticles having metallic silver has made a significant comeback as a potential antimicrobial agent (6).

METHODOLOGY

SYNTHESIS OF SILVER NANOPARTICLES USING *ESCHERICHIA COLI*

Silver nanoparticles were prepared using *E. coli.* Lysogeny broth (LB broth) medium, a nutritionally rich medium primarily used for the growth of bacteria was used for the culturing of bacterial strain *Escherichia coli* provided by CASVAB, which in turn was used for the production of biomass for biosynthesis. The incubation of culture flask was done on an orbital shaker at 37°C& than agitated at 200 rpm. After 24 hours of growth, biomass was harvested and centrifuged at 10000 rpm for 10 minutes followed by the collection of supernatants for more reaction. For the biosynthesis of silver nanoparticles, different volumes of AgNO3 (0.002 M) was mixed with different volumes of culture supernatant in order to optimize the volume of the AgNO3 and supernatant (Sample1 was dilution of 2ml supernatant & 8ml silver nitrate, Sample 2 was dilution of 2ml supernatant & 5ml silver nitrate) (Fig. 1). It was then exposed to sunlight for 24 hours. Silver nanoparticles were also synthesized in the absence of light (7).

CHARACTERIZATION OF NANOPARTICLES

The presence of silver nanoparticles in the reaction mixture was shown by the appearance of brown color. This change in color shows that the Ag ions are efficiently reduced (Figure 2). The synthesis was further validated using Analytic jena Specord 200 UV Vis Spectrophotometer (7).

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ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES COATED ON ANTIBIOTICS

The sterile molten Mueller Hinton agar was poured into petri plates and then it was allowed to solidify. Then different cultures, *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumonia, Pseudomonas aeruginosa* provided by CASVAB were cultured on different plates and allowed to be absorbed in media. After 10 minutes different antibiotic discs recommended for each organism were administered on these cultures following CLSI guidelines. Cin (Clindamycin) antibiotic was used against gram positive bacteria while Cip (ciprofloxacin) antibiotic was used against gram negative bacteria. Four discs of each antibiotic were used against each organism. Out of these four, two were silver nanoparticle coated and two remain uncoated. These discs were placed on the plates containing organism and were incubated for 24 hours at 37°C. Zones of inhibition were observed after incubation.

COMPARISON OF RESPONSE OF GRAM POSITIVE &GRAM-NEGATIVE BACTERIA TO NANOPARTICLE COATED ANTIBIOTICS

Results of antibiotics shown against gram positive & gram-negative bacteria were compared. It was done by comparing the zones of inhibition formed in the case of gram-positive bacterium i.e. *Staphylococcus aureus* and *Bacillus subtilis* with that of formed in the case of gram-negative bacterium i.e. *Klebsiella pneumonia* and *Pseudomonas aeruginosa*.

Fig. 1. Preparation of nano particles at different concentrations

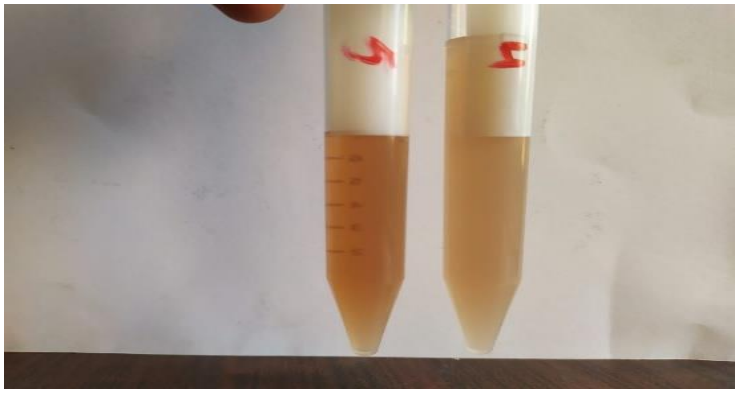

Fig. 2. Color of prepared nanoparticles

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RESULTS AND DISCUSSION

CHARACTERIZATION OF SILVER NANO PARTICLES

Silver nanoparticles were characterized by the following two ways.

1) THROUGH COLOR

The change of yellow color to brown confirms the presence of silver nanoparticles in the solution. From all the dilutions, the 1st sample which contains 2ml supernatant & 8ml silver nitrate showed the clear brown color so it was selected for further work.

2) THROUGH SPECTROPHOTOMETER

It is a very useful and reliable technique for the primary characterization of synthesized nanoparticles. It was used to monitor the synthesis of AgNPs (8).

Fig. 3. Absorbance Values

Sample 1 was dilution of 2ml supernatant & 8ml silver nitrate, Sample 2 was dilution of 2ml supernatant & 5ml silver nitrate, Sample 3 was distilled water, Sample 4 was E. coli culture (Fig. 3). The maximum absorbance value. i.e. 1.1112 was shown in case of 1st sample with dilution of 2m
l supernatant & 8ml silver nitrate indicates the formation of maximum silver nanoparticles (Fig. 3).

DETERMINATION OF ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES COATED ON ANTIBIOTICS

The biologically synthesized silver nanoparticles inhibited the tested microorganisms. The formation of zones of inhibition was observed as a result of the destabilization of the outer membrane, breakdown of the plasma membrane and reduction of intracellular ATP by the silver nanoparticles (7). There was a change found in the zones of inhibition after coating the antibiotics with AgNPs which is described below:

Panel (a) the zone of inhibition against the Staphylococcus aureus increases from 10mm to 20mm when the antibiotic is coated with silver nanoparticles. Panel (b) shows the zone of inhibition against the Bacillus subtilis increases from 11mm to 19mm when the antibiotic is coated with silver nanoparticles. Panel (c) shows the zone of inhibition against the Klebsiella pneumonia increases from 12mm to 15mm when the antibiotic is coated with silver nanoparticles. Panel (d) shows the zone of inhibition against Pseudomonas aeruginosa increases from 17mm to 20mm when the antibiotic is coated with silver nanoparticles (Fig. 4).

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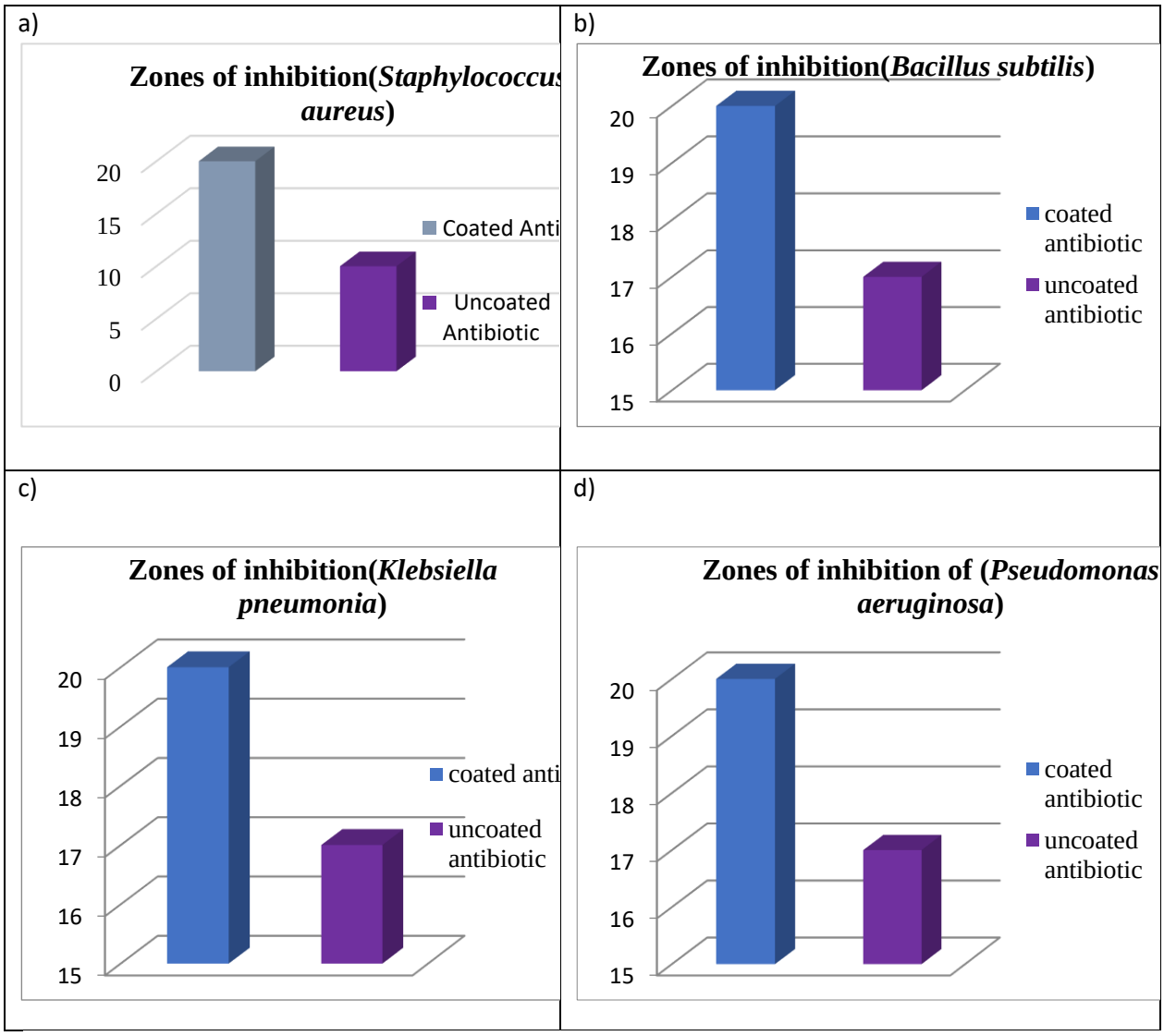

Fig. 4. Panel (a) demonstrates that zone of inhibition against the Staphylococcus aureus. Panel (b) demonstrates that zone of inhibition against the Bacillus subtilis Panel (c) demonstrates that zone of inhibition against the Klebsiella pneumonia Panel (d) demonstrates that zone of inhibition against Pseudomonas aeruginosa increases

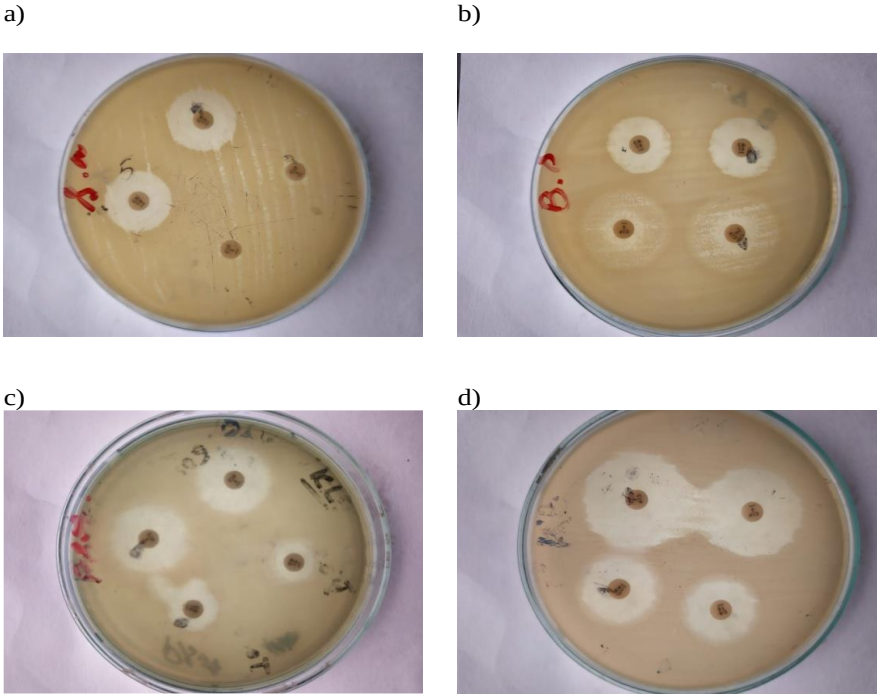

Fig. 4. The respective zone of inhibition against the *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumonia, Pseudomonas aeruginosa* when the antibiotic is coated with silver nanoparticles. Panel a and b shows the clindamycin antibiotic discs while panel c and d show the ciprofloxacin antibiotic disc.

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The overall change which occurs in the zones of inhibition around bacteria after coating the antibiotic with silver nanoparticles is shown below:

Table I: change in zone of inhibitions around bacterium due to silver nanoparticle coated antibiotics

Name of organisms	Antibiotics used	Zones of inhibition in mm	
		Control	Test
Staphylococcus aureus	Clindamycin	10	20
Bacillus subtilis	Clindamycin	11	19
Klebsiella pneumonia	Ciprofloxacin	12	15
Pseudomonas aeruginosa	Ciprofloxacin	17	20

It is inferred from above results that after coating the antibiotics with silver nanoparticles, the antibacterial activity of antibiotics against their respective bacterium increases.

COMPARISON BETWEEN GRAM POSITIVE & GRAM-NEGATIVE BACTERIA AGAINST NANOPARTICLE COATED ANTIBIOTICS

In case of *Staphylococcus aureus* and *Bacillus subtilis*, which are gram positive bacterium, zones of inhibition increase from 10mm to 20mm & 11mm to 19mm respectively while in case of *Klebsiella pneumonia and Pseudomonas aeruginosa*, which are gram negative bacterium, zones of inhibition increase from 12mm to 15mm & 17mm to 20mm respectively. It shows that the effect of silver nanoparticles is more evident and enhanced in case of gram-positive bacterium as compared to that of gram-negative bacterium.

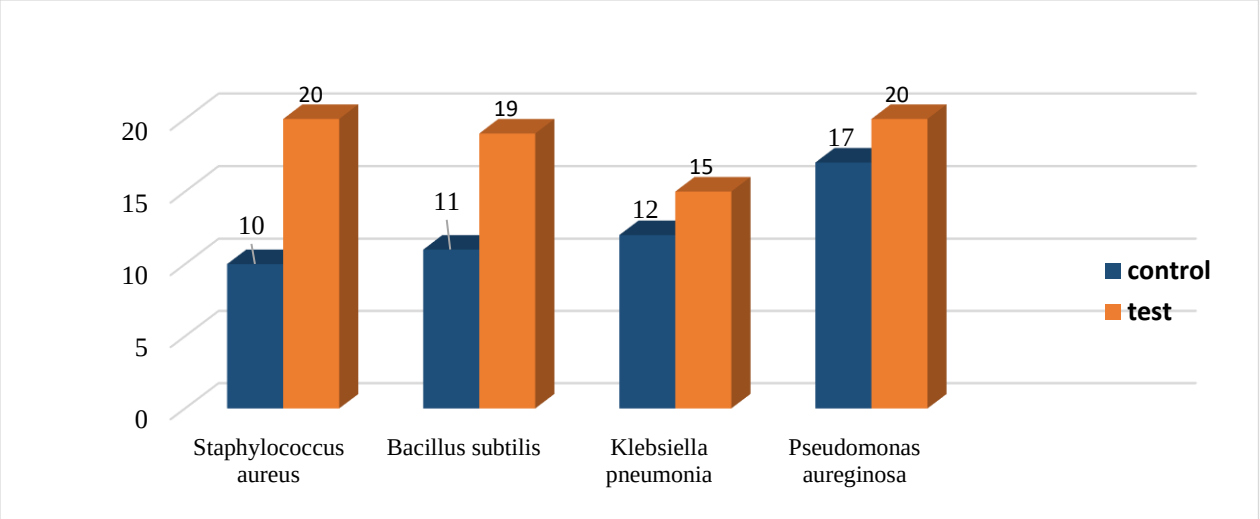

Fig. 6. Comparison between gram positive &gram-negative bacteria against nanoparticle coated antibiotics

Silver nanoparticles exhibits great antibacterial activity. Silver nanoparticles have been proved as an effective antibacterial material previously (9). In this study Silver nano-particle synthesis was confirmed through color change from yellow to brown which indicates the formation of silver nanoparticles as reported earlier (10). Furthermore, the increase in the respective zone of inhibitions of bacterium against different silver nanoparticle coated antibiotics showed the antibacterial activity of silver nanoparticle which is also proved by a study which includes the enhancement in antibacterial activities of different antibiotics in the presence of Ag-NPs (11, 12).

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While comparing the response of gram positive and gram-negative bacteria against nanoparticle coated antibiotics, it was observed that effect of silver nanoparticles is more prominent in case of gram-positive bacterium as compared to that of gram-negative bacterium which can be due to different reasons like. Interaction between silver nanoparticles and bacteria is related to the concentration of nanoparticles, strain, nature and incubation time etc. (13). It may be due to increased concentration of silver nanoparticles as it was proved in a study that increase in concentration of silver nano-particles increases its effect against gram positive bacterium (14). Antibiotic resistance towards bacteria also plays an important role to understand this aspect. In this case *Staphylococcus aureus*, a gram positive bacteria undergoes maximum effect from silver nanoparticle coated antibiotic Clindamycin because it was once reported that clindamycin had a resistance rate of 28% to *S. aureus* (15). While the sensitivity of Bacillus subtilis to clindamycin, was species specific (1). *Klebsiella pneumoniae* showed a resistance rate of 32%to Ciprofloxacin (3) and *P. aeruginosa* shows 35.2% resistance to ciprofloxacin (16, 17) therefore staphylococcus exhibits maximum effect against silver nanoparticle coated antibiotic due to low resistance rate against it.

CONCLUSION AND FUTURE PERSPECTIVES

Nanotechnology is contributing a lot for the production of many useful products, among which the nano medicines are most notable. Silver nanoparticles are the very effective candidate for enhancing the antibacterial activity of various antibiotics. Moreover, comparison between gram positive &gram-negative bacteria against nanoparticle coated antibiotics shows that effect of silver nanoparticles is more enhanced in case of gram-positive bacterium as compared to that of gram-negative bacterium.

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