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Research Article

ANTIBIOTIC SENSITIVITY OF GOLD AND SILVER NANOPARTICLES AGAINST ENTEROCOCCAL PATHOGENS

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ABSTRACT

Although disinfection methods currently used in drinking water treatment can effectively control microbial pathogens, research in the past few decades have revealed a dilemma between effective disinfection and formation of harmful and to consider innovative approaches that enhance the effectiveness. With the rapid advancement of nanoscience and nanotechnology, detailed knowledge of interactions between engineered nanomaterials and cells, tissues and organisms have become increasingly important, especially in regard to possible hazards to human health. The study was mainly designed to study the effect of silver and gold nanoparticles against the enterococcal pathogens. The organisms were also tested against the antibiotics to prove of their susceptibility toward the drugs. The enterococcal pathogens studied here showed a positive response for all the drugs used. The silver and gold nanoparticles are studied in this context to prove of their effect on the bacterial pathogens. Gold and silver particles are studied separately and are found to show some significant effect on the growth of bacteria. The pathogens studied showed susceptibility toward both the particles (gold and silver). And the effect of the nanoparticles was completely dose dependent, i.e., the effect was found to be more at higher concentration. All effects were statistically significant at the 0.05 significance level. There was a significant effect of the nanoparticles (gold and silver) among the four different concentrations remembered at the $p<0.05$ level. Gold nanoparticles showed a significance to the concentration ($F [1,7]=13.36364$, $p=0.035353$).

Keywords: Gold nanoparticles, Silver nanoparticles, Enterococcus, Minimum inhibitory concentration.

INTRODUCTION

Pathogenic bacteria remain a major health concern, which are responsible for causing a large number of deaths and hospitalizations each year [1]. Although we have current treatments such as antibiotics, bacteria are gaining resistance to these therapeutics at an increased rate. That leads to the development and optimization of novel treatments against bacterial infections [1-3]. A range of potential solutions have been researched in hope that new treatments and diagnostic techniques will be developed. A large group of these studies includes the implementation of nanotechnologies and nanomaterials to create new antibacterial nanomedicines that increased effectiveness and efficiency [4,5]. Nanomedicine is defined as the monitoring, repair, construction, and control of human biological systems at the molecular level, using engineered nanodevices and nanostructures. This is the field of science and technology for diagnosing, treating, and preventing disease and traumatic injury, of relieving pain and of preserving and improving human health, using molecular tools and molecular knowledge of the human body [1,2,6].

Nanotechnologies are developing rapidly in numerous industries and in particular those related to the medicine field. Silver and gold has long been used in medicine, even in ancient times it was used as a preservative and also to reduce inflammation and prevent against infection of wounds [7]. It is due to this prior knowledge that further studies using these particles as antibacterial agents has been perused, and bulk silver has already been used in appliances that are prone to microbial contamination, such as a fridge with an internal silver lining [8,9].

Silver on scaling down to the nanoscale provides a higher specific surface area and a higher fraction of surface atoms that improves its antimicrobial activity compared with bulk silver [10]. A study already confirmed the effectiveness of silver nanoparticle (NP) as an antimicrobial agent against *Escherichia coli* [11]. In the study, 10^5 colony forming units of Gram-negative *E. coli* colonies were grown on Luria-Bertani agar plates with different concentrations of Ag-NPs. Ag-NPs at concentrations of 10 μg cm^{-3} showed inhibition of bacterial growth by up to 70%, and at concentrations of 50-60 μg cm^{-3} there was 100% inhibition of bacterial growth [12-14].

Silver ions antimicrobial activity is believed to work by impairing DNAs ability to replicate and through the inactivation of key proteins by denaturation when they are bound together [15]. Although it is still unknown what type of interaction takes place between the NPs and the constituents of the outer membrane [4,16]. Another study showed that with the use of silver NPs in conjunction with antibiotics such as penicillin G [17], amoxicillin [1], erythromycin, clindamycin, and vancomycin, provided an increase in the effectiveness of the antibiotics [18,19]. Silver NPs show great potential as antimicrobial

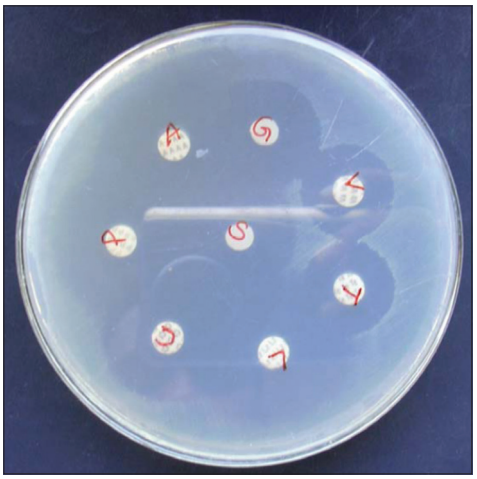

Fig. 1: The Zone of inhibition of the isolate toward the different antibiotics. A: Ampicillin; P: Penicillin; C: Ciprofloxacin; V: Vancomycin; T: Teicoplanin; L: Linezolid; G: Gentamycin; S: Streptomycin

agents in applications, as they are of low cost and easily synthesized, and could be used to treat material surfaces to provide highly effective antibacterial materials, medical equipment such as in devices and paints [20-22].

The antibacterial NPs fall into three general categories: Naturally occurring antibacterial substances, metals and metal oxides [23], and novel engineered nanomaterials [24]. These NPs interact with microbial cells through a variety of mechanisms [25]. The NPs can either directly interact with the microbial cells, e.g., interrupting transmembrane electron transfer, disrupting/penetrating the cell envelope, or oxidizing cell components, or produce secondary products (e.g., reactive oxygen species or dissolved heavy metal ions) that cause damage [26].

The study was mainly designed to study the effect of silver and gold NPs against the enterococcal pathogens. The organisms were also tested against the antibiotics to prove of their susceptibility toward the drugs. To make a positive statement, we made an attempt to find out the action of these nanoparticles against the bacterial pathogens using the minimum inhibitory concentration (MIC) inhibition assay.

METHODS

Silver NPs and gold NPs of approximately 100 nm were obtained from Sigma-Aldrich in powder presentation.

Antibiotic susceptibility testing

All the Enterococcal isolates were tested for antibiotic sensitivity pattern against ampicillin (l0 µg), penicillin (10 U), ciprofloxacin (5 µg), vancomycin (30 µg), linezolid (30 µg). High-level aminoglycoside resistance was tested against gentamicin (120 µg) and streptomycin (300 µg) by Kirby–Bauer disk diffusion method. Commercially available Hi-media disks were used. The strength of disks used and their zone size interpretative standards were according to guidelines of NCCLS standards 69. Antibiotic susceptibility testing had done with quality control strains by using *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212, for appropriate antimicrobials.

Disk diffusion method

The isolates were screened for their susceptibility using the Kirby–Bauer disk diffusion method. Mueller-Hinton agar medium was used for the growth and the inoculated samples were incubated for 16-18 hrs at 35°C in an ambient air incubator. With the help of straight wire 3-4 identical colonies were picked up and were inoculated into 5 ml of nutrient broth. The broth was incubated at 35°C for 3-4 hrs, so as to obtain moderate turbidity if necessary turbidity was adjusted to 0.5 McFarland standards. A streak was made on the medium using sterile cotton swab in all directions and rotating the plate every time.

The antibiotic disks were applied with aseptic precautions. Disks were deposited with centers at least 24 mm apart. The plates were incubated at 35°C in ambient air for 16-18 hrs. After incubation, the plates were observed for the zone of inhibition around the disks. Zone showing complete inhibition was measured to the nearest whole millimeter.

The serial dilution tubes were done in triplicates, where one tube serves as control (without treatment) and the other two with treatments (gold and silver). Both gold and silver NPs were tested for their efficacy at different concentrations like 0.75%, 0.50%, 0.25%, 0.01% g/mol. The inoculum was streaked unto the respective plates with and without treatment. The plates were further incubated at 37°C for 18-20 hrs.

MIC values

The lowest concentration of drug which could inhibit the growth of the strain is taken as MIC of the drug for the strain. The diameter of the ring of inhibition was measured with respect to the control.

RESULTS AND DISCUSSION

Antibiotic susceptibility testing

The isolates screened for showed positive results to all the drugs tested. The zone of inhibition was measured by the diameter of the inhibition circle. Both the species responded the same to the disks (Table 1 and Fig. 1).

MIC values under the treatment of NPs

The isolates showed sensitivity to both the silver and gold nanoparticles. Both the particles are dose dependent and the gold nanoparticles showed a higher activity levels when compared to the silver nanoparticles. A two-way ANOVA between the treatments and the concentrations was conducted to compare the inhibition efficacy in both silver and gold nanoparticles toward the enterococcal strains. All effects were statistically significant at the 0.05 significance level. There was a significant effect of the nanoparticles (gold and silver) among the four different concentrations remembered at the $p<0.05$ level. Gold nanoparticles showed a significance to the concentration ($F [1,7]=13.36364$, $p=0.035353$). A significant effect was also found among the four different concentrations remembered at the $p<0.05$

Table 1: The values of diameter of inhibition Zones. All the values are expressed in mm and are the average of triplicates

Antibiotic	Disk potency (µg)	Inhibition zone diameter (mm)		
		S	I	R
Ampicillin	10	≥17	14-16	13
Penicillin	10 U	29	-	28
Ciprofloxacin	5	≥21	16-20	≤15
Vancomycin	30	≥17	-	≤14
Teicoplanin	30	14	11	10
Linezolid	30	27-31	-	-
Gentamycin	120	≥10	7	6
Streptomycin	300	≥10	9	6

S: Sensitive; I: Intermediate response; R: Resistant

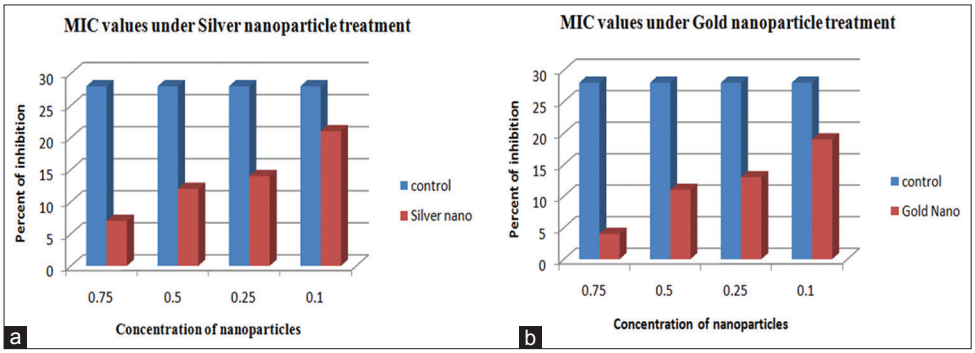

Fig. 2: Minimum inhibitory concentration (MIC) values of the silver and gold nanoparticles at different concentrations. All the values are average of triplicates and expressed in % of inhibition. (a) MIC values under silver nanoparticle treatment (b) MIC values under gold nanoparticle treatment

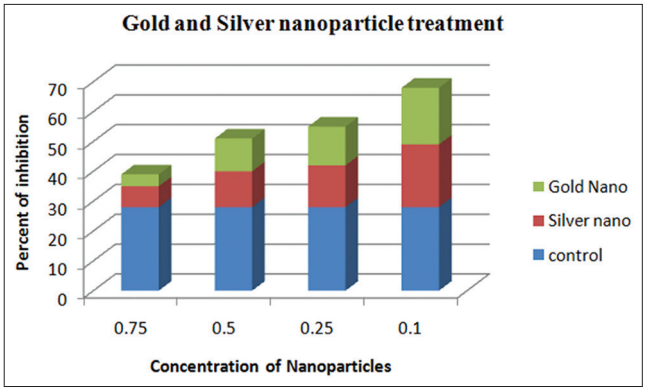

Fig. 3: Comparative analysis of the minimum inhibitory concentration values of the silver and gold nanoparticles at different concentrations. All the values are average of triplicates and expressed in % of inhibition

level. High concentration of 0.75% showed a significance in both the silver and gold treatments (F [3,3]=15.9091, p=0.000862) (Figs. 2 and 3).

CONCLUSION

Nanoparticles have been proved of its importance in the field of medicine. It is sued against many diseases which are caused by bacteria and virus. To date, there are a few studies done on the activity of the nanoparticles toward the bacterial pathogens. Many effective antibiotics are still available and are being used in the antibacterial therapy. However, keeping in view of their side effects and increases resistance to the drugs makes the field of science to look forward for the nanoparticles.

The enterococcal pathogens studied here showed a positive response for all the drugs used. The silver and gold nanoparticles are studied in this context to prove of their effect on the bacterial pathogens. Gold and silver particles are studied separately and are found to show some significant effect on the growth of bacteria. The pathogens studied showed susceptibility toward both the particles (gold and silver). And the effect of the nanoparticles was completely dose dependent, i.e., the effect was found to be more at higher concentration.

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