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ORIGINAL ARTICLE

Antibacterial Synergy of Silver Nanoparticles with Gentamicin and Chloramphenicol against *Enterococcus faecalis*

Sagar Katva†, Satyajeet Das†, Harpreet Singh Moti, Anupam Jyoti, Sanket Kaushik

Amity Institute of Biotechnology, Amity University, Jaipur, Rajasthan, India
†These two authors have contributed equally to this work.

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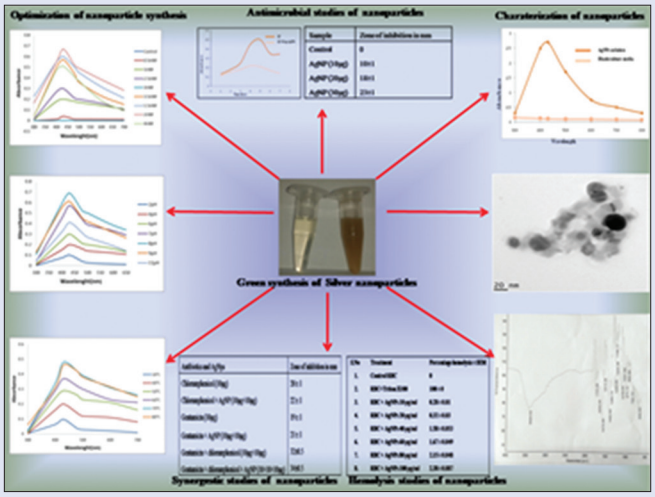
ABSTRACT

Background: *Enterococcus faecalis* (Ef) is a multidrug-resistant pathogenic bacteria associated with hospital-acquired infections. Ef is involved in a number of infectious diseases. It generally infects patients with the weekend immune system, i.e. a person mostly acquires Ef infections in the hospital, especially in intensive care units and thus, is more likely to be resistant to many antibiotics. Development of resistance against various antibiotics and emergence of drug-resistant strains is a growing global concern. **Objective:** Due to the unselective use of antibiotics for a long time multidrug resistant bacteria and extensively drug-resistant, which is now posing a new challenge to the medical community. To treat infections caused by Ef, the synergistic effect of different antibiotics with silver nanoparticles (AgNPs) was tested against Ef. **Materials and Methods:** In the present study, synthesis of AgNPs was carried out from the cell-free supernatant of *Klebsiella pneumoniae*. AgNPs were characterized using various techniques, namely, ultraviolet-visible spectrophotometry, transmission electron microscopy, and Fourier transform infrared spectroscopy. Moreover, process optimization was done for enhanced production of AgNPs. In addition, antimicrobial activity of the nanoparticles was also tested. Furthermore, the nanoparticles were evaluated for their antimicrobial activities in combination with gentamicin and chloramphenicol, against Ef. **Results:** The results showed that the combination of gentamicin and chloramphenicol with AgNPs has a better antibacterial effect. To add to this, hemolytic activity of AgNPs was evaluated against human red blood corpuscles (RBCs). AgNPs were found to be nontoxic to RBCs. **Conclusion:** The collective effect of AgNps with Gentamicin and Chloramphenicol was more as compared to AgNps alone which indicate the synergistic effect of these components. These observations show the potential of AgNPs in combination with above-stated antibiotics against Ef infections.

Keywords: Antibiotic synergy, green synthesis, silver nanoparticle

SUMMARY

- *Enterococcus faecalis* (Ef) is a multidrug-resistant bacteria with is resistant to wide range of antibiotics
- Due to this increasing resistance, there is a need to find a new approach to overcome the infections caused by Ef
- The combined effect of silver nanoparticles (AgNPs) with gentamicin and chloramphenicol was notably seen against Ef
- Furthermore, the AgNPs were nontoxic to the human red blood corpuscles which confirm its nontoxic nature.



Abbreviations used: Ef: *Enterococcus faecalis*, MDR: Multidrug resistance, AgNPs: Silver nanoparticles, Kp: *Klebsiella pneumoniae*, RBCs: Red blood corpuscles, ENPs: Engineered nanoparticles, FTIR: Fourier transform infrared spectroscopy, TEM: Transmission electron microscopy, $AgNO_3$: Silver nitrate, EDTA: Ethylenediaminetetraacetic acid, PBS: Phosphate-buffered saline.

Correspondence:
Dr. Sanket Kaushik,
Amity Institute of Biotechnology,
Amity University Jaipur,
Rajasthan, India.
E-mail: sanketkaushik@gmail.com

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INTRODUCTION

Nanotechnology deals with the synthesis and applications of nanostructures in various areas such as chemistry, physics, biology, and medicine.[1] Nanoparticles produced from metals are called metal nanoparticles which can also be synthesized using biological sources.[2-9] Due to strong antimicrobial activity[10] and use in water treatment,[11,12] silver nanoparticles (AgNPs) have received much more interest as compared to other metallic nanoparticles. Nanoparticles which are intentionally created are called engineered nanoparticles (ENPs).[13,14] Due to various applications of nanoparticles in numerous areas use of ENPs will result in accumulation of various toxic byproducts of ENPs in the ecosystem. In this regard looking at the toxic nature of ENPs people have started synthesizing nanoparticles from a biological source.[15] In the recent past, nanoparticles have been synthesized using bacteria, plants, and fungus. Biological methods were adopted by people as they were easy to synthesize,

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toxicity issues were less as compared to ENPs, and the conditions can be better optimized in case of biological synthesis as compared to ENPs. It has been reported[16] that the growing resistance of many bacterial strains to traditional antibiotics has led to the development of other more potent methods for controlling bacterial infections. The antibacterial nature of silver is enhanced in the form of nanoparticles as AgNPs has been reported to have the ability to penetrate the bacterial cell wall and damage the cell membrane that leads to the death of the cell.[12] AgNPs were reported to have antibacterial activity against a number of bacterial species.[14,17,18] The presence of protein caps in nanoparticles helps in stabilization and binding to cell surface receptor results in increased binding and uptake of drug or genetic material on human cells.[15,16] The present study deals with the extracellular biosynthesis of AgNPs, using cell filtrate of *Klebsiella pneumoniae* (Kp). Nanoparticle synthesis was also optimized with respect to temperature, pH and silver nitrate ($AgNO_3$) concentration. Furthermore, characterization of AgNPs was carried out using ultraviolet (UV)-visible spectrophotometry, Fourier transforms infrared spectroscopy (FTIR) and transmission electron microscopy (TEM). Moreover, antimicrobial studies have also been elucidated against *Enterococcus faecalis* (Ef) in different concentrations, and antimicrobial studies of AgNPs have also been done in combination with gentamicin and chloramphenicol. The collective effect of gentamicin and chloramphenicol conjugated with AgNP is observed to be more as compared to the antibiotics and AgNPs alone. Thus, this study suggests the synergistic usage of AgNP with gentamicin and chloramphenicol which will help combat the growing resistance against gentamicin and chloramphenicol.

MATERIALS AND METHODS

Chemicals, reagents, and culture strains

The chemicals and reagents used in the experiments were purchased from Hi-media, India. The bacterial strains used in the experiments were procured from National Centre for Cell Sciences (NCCS), Pune, India.

Synthesis of silver nanoparticles from *Klebsiella pneumoniae*

AgNPs were synthesized using primary broth culture of Kp was added in 200 ml of Nutrient Broth in test tubes with one control (without inoculation). After inoculation, the test tubes were incubated at 37°C for 24 h in a shaker incubator. After the 24 h of incubation, the culture was centrifuged at 8000 rpm for 10 min. After centrifugation, the supernatant was collected in other autoclaved test tubes, and the pellet was discarded. 2 mM $AgNO_3$ was added to the supernatant of Kp under sterilized conditions, and the tubes were monitored at the regular interval for color change.

Characterization of bacterial silver nanoparticles

The synthesized nanoparticles were characterized using different techniques.

Analysis by ultraviolet-Vis spectroscopy

After addition of $AgNO_3$ in cell-free supernatant of Kp, it was incubated for 1–2 days. UV-visible absorption measurements were carried out on SL 164 Double Beam UV-visible Spectrophotometer at 300–800 nm wavelength at the interval of 10 nm.

Fourier transform infrared spectroscopy analysis

Further FTIR measurements were made to locate the possible biomolecules, which are responsible for the reduction of silver ions to AgNPs and stabilization of AgNPs in colloidal solution. For FTIR analysis samples were mixed with potassium bromide in the ratio of 1:100. The recurrence extent is measured as wave numbers normally over the reach 4000–600 cm^{-1}.

Transmission electron microscopy analysis

To examine the size of synthesized AgNPs the transmission electron microscopic analysis was performed. For TEM measurements, a drop of solution containing synthesized AgNPs was placed on the carbon coated copper grids and kept in infrared light until sample gets dried before loading them onto a specimen holder. TEM micrographs were taken and size and shapes were analyzed.

Optimization of silver nanoparticles synthesis

Effect of silver nitrate concentration

The concentration of $AgNO_3$ was varied between 0.5 mM to 4 mM. Samples with different $AgNO_3$ concentrations were incubated for 2 days. UV-visible spectra were recorded at regular time interval from 24 to 72 h.

Effect of pH

Effect of change in pH was seen on the synthesis of AgNPs. pH value ranging from 2.0 to 11.0 were used to analyze the effect of change in pH on the rate of AgNPs synthesis. pH was adjusted using 0.1N HCl and 0.1N NaOH. Samples were incubated for 2 days and UV-visible spectra were recorded.

Effect of temperature

AgNPs synthesis was also optimized with respect to change in temperature. Temperature ranging from 30°C to 80°C was used for synthesis of nanoparticles. After sample incubation for 2 days, UV-visible spectra were recorded.

Antibacterial assay test by disc diffusion method

The antibacterial assays were performed using standard disc diffusion method (Kirby-Bauer method) in Nutrient agar (NA) media. The fresh overnight culture of inoculum (200 µl) of Ef was spread onto solidified NA plates. AgNPs was purified by centrifuging supernatant at 10,000 rpm followed up by washing it twice with sterile water. Sterile paper discs were made of Whatman filter paper (Grade-1) of 4 mm diameter, impregnated with standard antibiotic concentrations and combinations and nanoparticles solution and a control disc (sterile paper disc dipped in water) were placed on each plate. The cultured agar plates were incubated at 37°C for 24 h. After 24 h of incubation, inhibition zones were observed.

Antibacterial assay test in broth media

The antibacterial assays were also performed in suspension cultures of Ef. The test Ef species was inoculated in nutrient broth media in different culture tubes. One of the tubes was kept as control in which no AgNPs were added and other with 10 µg silver nanoparticle solution. UV-visible spectra were recorded at regular time interval from 24 to 72 h.

Synergistic activity test of chloramphenicol and gentamicin with nanoparticles

Synergistic activity of the AgNPs with chloramphenicol and gentamicin was studied in different combinations as AgNP+chloramphenicol, AgNP+gentamicin, chloramphenicol+gentamicin, and chloramphenicol+gentamicin+AgNP by disc diffusion method (Kirby-Bauer method). NA plates were prepared and streaked evenly with Ef culture. Antibiotic discs were prepared by impregnating sterile discs in sterile water (for control), AgNP, chloramphenicol and gentamicin, singly and in combinations, AgNP+chloramphenicol, AgNP+gentamicin, chloramphenicol+gentamicin and chloramphenicol+gentamicin+AgNP and in uniform concentration, i.e., 10 µg of each component. These discs were placed in the Ef streaked NA plates in quadratic

manner as control, AgNP, chloramphenicol + gentamicin and chloramphenicol + gentamicin + AgNP and incubated for 24 h at 37°C.

Hemolysis assay

The hemolytic assay was done to study the hemolytic activity of AgNPs with human erythrocytes. To perform hemolytic assay, about 10 ml of blood was taken from human volunteer. Blood sample was added in a tube containing ethylenediaminetetraacetic acid. After addition of the sample, it was centrifuged at 3000 rpm for 15 min. RBCs were isolated by discarding the supernatant and white blood cell. The RBC pellet was washed with phosphate-buffered saline (PBS). RBCs prediluted in PBS were added to AgNPs ranging from 10 µg/ml to 100 µg/ml. RBCs mixed with 1.5 ml triton X-100 was taken as positive control. All the samples were incubated at 37°C for 1 h, followed by centrifugation at 3000 rpm for 15 min and the supernatant was used for spectroscopic analysis at 540 nm. The percentage of hemolysis was calculated from the formula:

OD_{540} (sample) – OD_{540} (0% lysis)/OD_{540} (100% lysis) – OD_{540} (0% lysis) ×100%

RESULTS

Preparation of bacterial culture

A loop full of lyophilized Kp (procured from NCCS, Pune) culture was inoculated into 10 ml sterile nutrient broth and incubated overnight at 37°C in shaker incubator (primary culture). Then, from this primary culture, 1 ml was inoculated in 100 ml sterile nutrient broth and incubated overnight at 37°C in shaker incubator (secondary culture). This was further used for synthesis of AgNP.

Synthesis of silver nanoparticles

After 90 h incubation, the color of bacterial supernatant containing $AgNO_3$ was changed perfectly from yellow to brown because of reduction of Ag + to Ag^0 due to excitation of surface plasmon vibrations in the particles, and thus provide a convenient means of visually determining their presence in the samples and there is no change in color in uninoculated (control) sample [Figure 1]. It showed the successful synthesis of AgNPs by the bacteria.

Characterization of silver nanoparticles

The synthesis of AgNPs was confirmed by UV-visible spectrophotometer (Thermo Scientific). The change in color of $AgNO_3$ treated culture supernatant initially indicated the formation of AgNPs. The oscillation waves of electrons of AgNPs are in resonance with the light waves gives increase to a unique surface plasmon resonance (SPR) absorption band (400–440 nm), which is also the origin of the observed color. Thus, the extracellular synthesis of AgNPs using Ef was monitored in UV-visible spectroscopy. The UV-visible absorption spectra of the AgNPs were observed in a range of 300–800 nm. A strong peak specific for the synthesis of AgNPs was obtained at 420–430 nm. According to the observation UV-visible spectra recorded at different time intervals showed increased absorbance with increasing time of incubation. The absorbance spectra of reaction mixture containing aqueous solution of 2 mM silver nitrate and the pellet of bacteria after incubation was recorded [Figure 2]. In the recent years, various studies have characterized the size of AgNPs by TEM. TEM analysis was also done to further characterize the nanoparticles and it was found that synthesized AgNPs were nearly 20 nm sizes and found to be well dispersed in aqueous medium [Figure 3]. FTIR analysis was also done for the characterization of AgNPs. FTIR absorption spectrum of AgNP powder is shown in Figure 4. The absorbance bands analysis in bioreduction and absorbed in the regions is 3424.53, 1575.69, 1402.82, and 1076.05. The presence of peak at 3424.53 cm^{-1} corresponds to N–H stretch which belongs to primary and secondary amines. The peaks seen at 1575.69 cm^{-1} is assigned to stretching vibrations of–COO– groups present in amino acid residues in the protein. The peaks seen at 1402.82 and 1076.05 cm^{-1} correspond to–C–N stretching vibrations. In this regard, it can be stated that, FTIR spectrum analysis established the presence of capping protein around the AgNPs synthesized from culture supernatant of Kp.

Optimization of silver nanoparticles synthesis

Optimization with respect to silver nitrate concentration

$AgNO_3$ concentration ranging from 0.5 mM to 4 mM was used for synthesis of AgNPs. Different concentrations of $AgNO_3$ were added in the culture supernatant of Kp and absorbance was taken at regular intervals from 24 to 120 h. It was observed that maximum peak was obtained at 2 mM $AgNO_3$ concentration at 430 nm. Synthesis rate was less at concentration more or <2 mM $AgNO_3$ concentration. It was also observed that for all the concentrations of $AgNO_3$ used, the amount of synthesis of AgNPs was highest at 90 h of incubation [Figure 5].

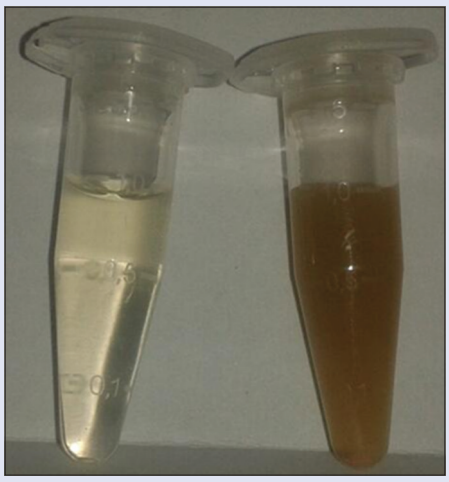

Figure 1: Uninoculated media and bacterial culture with 2 mM silver nitrate

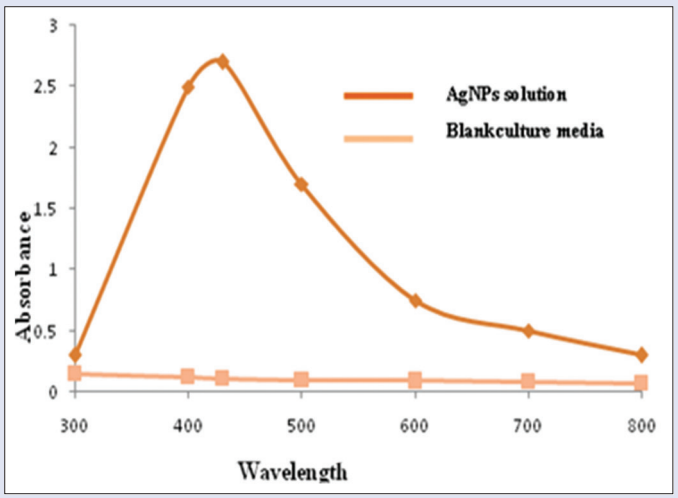

Figure 2: Optical Density (OD) scan at ultraviolet visible spectra

Optimization with respect to pH

pH value ranging from pH 2 to pH 11 were used for synthesis of AgNPs. $AgNO_3$ were added in culture supernatant of Kp with varying pH ranges. Absorbance at 430 nm was taken for all the samples at regular intervals from 24 to 120 h. It was observed that maximum value at 430 was observed at pH 8. It was also observed that for all the pH values used the amount of synthesis of AgNPs was highest at pH 8 at 90 h of incubation [Figure 6].

Optimization with respect to temperature

Temperatures ranging from 30°C to 90°C were used for synthesis of AgNPs. $AgNO_3$ were added in culture supernatant of Kp with varying temperature ranges. For all set of reactions pH was 8 and $AgNO_3$ concentration was 2 mM. Synthesis of AgNPs was monitored after every 8–10 h. Maximum value at 430 nm was obtained at 70°C and it remained stable for longer time period, indicated rapid and stabilized synthesis [Figure 7].

Antibacterial activity of silver nanoparticles

The antimicrobial activity of the synthesized AgNPs was tested against Ef. As indicated from the observations, the zone of inhibition increased with the increase in the concentration of AgNPs [Table 1]. The antibacterial activity against Ef was measured by measuring the diameter of the zone of inhibition. According to the observations, the reaction mixture containing AgNPs exhibited antimicrobial activity against the test bacteria.

Antibacterial assay test in broth media

The antibacterial activity of the synthesized AgNPs was also evaluated in broth media against Ef. In the control sample where no AgNPs were added in the media the growth was found to be normal. The absorbance was taken at regular intervals and it was observed that the value increased randomly indicating normal bacterial growth. However when AgNPs were added in the culture media, it was observed that the growth of the bacteria was reduced which was indicated by decrease in absorbance recorded after regular interval [Figure 8].

Synergistic effect of chloramphenicol and gentamicin with nanoparticles

When tested together the combination of antibiotic-antibiotic, antibiotic-nanoparticles, and antibiotic-antibiotic-nanoparticles worked in an effective manner and the combination showed synergistic effect against Ef. Chloramphenicol + gentamicin were added in combination with AgNPs separately and together as

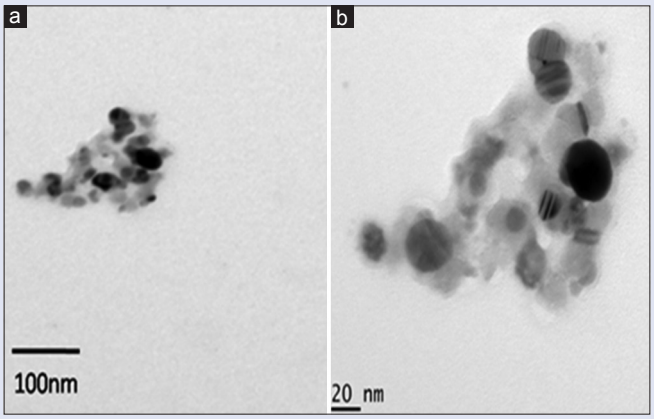

Figure 3: Transmission electron microscopy images of silver nanoparticles at (a) low and (b) high magnification

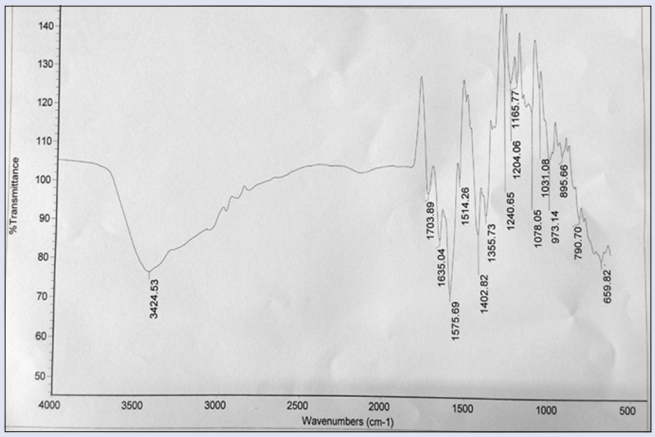

Figure 4: Fourier transform infrared spectroscopy spectra of synthesized silver nanoparticles

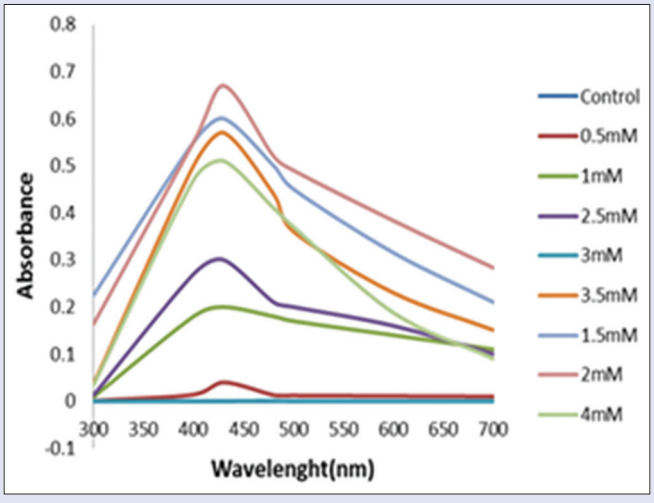

Figure 5: Optimization of silver nitrate concentration for silver nanoparticle synthesis

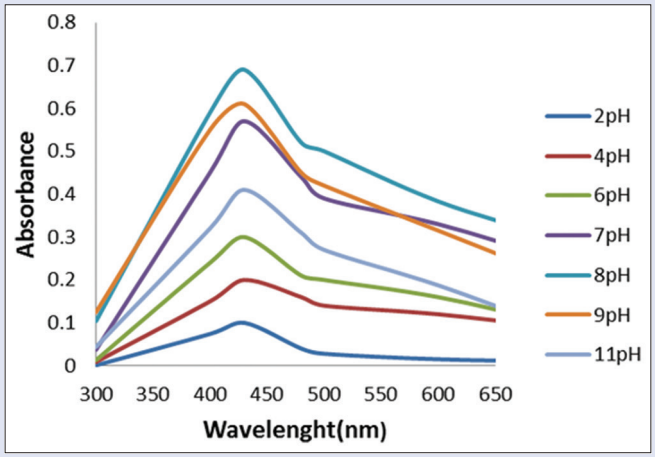

Figure 6: pH optimization of silver nanoparticle synthesis

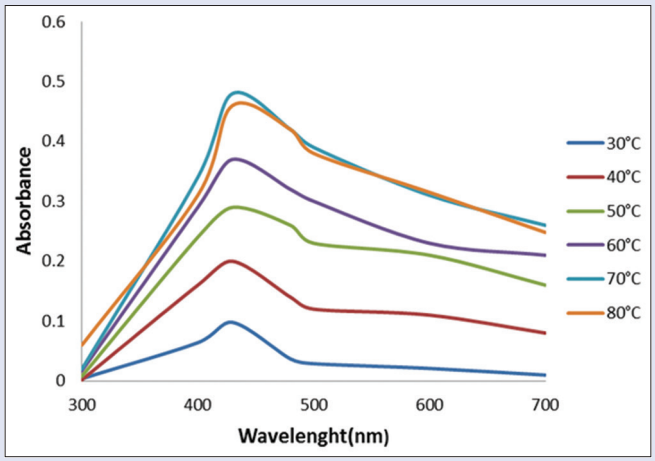

Figure 7: Temperature optimization of silver nanoparticle synthesis

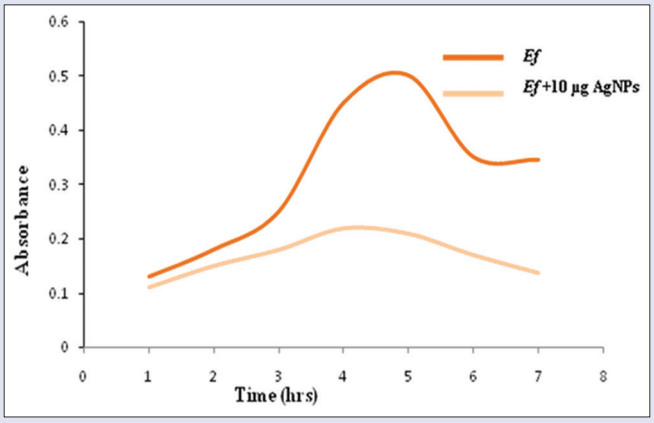

Figure 8: Antibacterial assay test in broth media

chloramphenicol + gentamicin + AgNp. Enhancement of antibacterial activities of Chloramphenicol and Gentamicin was observed by measuring the diameter of the zone of inhibition of the combined antibiotics with the nanoparticles. The overall result is shown in Table 1.

Hemolysis assay

The results of the hemolytic studies of AgNPs on diluted human RBC for 1 h of incubation are shown in Table 2. It was observed that incubation of AgNPs with RBCs did not cause any hemolysis when absorbance values were taken. The absorbance value of RBCs in PBS and in 1% triton X-100 was used along with the values of RBCs treated with AgNPs in six different concentrations on the formula.

DISCUSSION

Ef is a Gram-positive bacterial pathogen which causes a large number of infections. It is well documented that resistance of Ef for different antibiotics is increasing with time. This increase in the resistance to different antibiotics can be controlled using new approaches. In this regard, it is worth mentioning here that silver also possesses antimicrobial properties mostly in the form of nanoparticles. As the resistance to different antibiotic is increasing day by day silver has again gained importance as an antimicrobial agent.[19] It is already reported that the antimicrobial activity of AgNPs is more than the antimicrobial activity of silver metal alone.[20] We have previously reported synthesis of AgNPs from biological sources and its optimization as well as potential applications.[21-24] In this study, we have studied the combined effects of AgNPs with gentamicin and chloramphenicol against Ef bacteria using the disc-diffusion method. It was observed that the antimicrobial effect of AgNPs increased with increase in the AgNPs concentration. When AgNPs were used in combination with either of the antibiotics it showed increased antimicrobial effect. To add to this, we also studied the synergistic effect of the antibiotics alone and the antibiotics in combination with AgNPs. It was observed that the synergistic effect of the three showed the maximum antimicrobial effect. Gentamicin and chloramphenicol are both protein synthesis inhibitors. Gentamicin works by irreversibly binding the 30 s subunit of the bacterial ribosome, interrupting protein synthesis, whereas chloramphenicol prevents protein chain elongation by inhibiting the peptidyl transferase activity of the bacterial ribosome. The combined effect of AgNPs with gentamicin and chloramphenicol was notably seen against Ef. The exact mechanism of the action is still under investigation; however, several mechanisms have been proposed. It has been reported that as compared to AgNPs alone the combination of antibiotic + AgNPs complexes will release Ag + at a higher rate, moreover it has also been proposed that the combination of antibiotic with AgNPs through the active groups of antibiotics such as hydroxyl group and amine group will result in conjugation of both the molecules. This will result in the increase in the effective concentration of antibiotic at a specific site.[25-29] To add to this, we have also studied the toxicity properties of AgNPs. We have performed hemolysis assay of AgNPs and our results were in agreement with the previous studies.[23] It was observed that AgNPs did not cause any harm to blood cells, especially RBCs. This justifies the use of nanoparticles made from biological sources as compared the nanoparticles made from chemical and physical methods which are more toxic that the once made from biological sources.

Table 1: Antibacterial activity (Kirby-Bauer method) of nanoparticle and synergistic studies of nanoparticles along with antibiotics

Antibiotics and AgNps	Zone of inhibition in mm
Control	0
AgNPs (10 µg)	10±1
AgNPs (20 µg)	18±1
AgNPs (30 µg)	23±1
Chloramphenicol (10 µg)	20±1
Chloramphenicol + AgNPs (10 µg + 10 µg)	22±1
Gentamicin (10 µg)	19±1
Gentamicin + AgNPs (10 µg + 10 µg)	21±1
Gentamicin + chloramphenicol (10 µg + 10 µg)	32±0.5
Gentamicin + chloramphenicol + AgNPs (10 µg + 10 µg + 10 µg)	34±0.5

AgNPs: Silver nanoparticles

Table 2: Hemolysis study of silver nanoparticles on red blood corpuscles

Treatment	Percentage hemolysis±SEM
Control RBC	0
RBC + Triton X-100	100±0
RBC + AgNPs 10 µg/ml	0.28±0.01
RBC + AgNPs 20 µg/ml	0.32±0.03
RBC + AgNPs 40 µg/ml	1.58±0.053
RBC + AgNPs 60 µg/ml	1.67±0.049
RBC + AgNPs 80 µg/ml	2.15±0.048
RBC + AgNPs 100 µg/ml	2.38±0.087

SEM: Standard error of mean; RBC: Red blood corpuscle; AgNPs: Silver nanoparticles

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Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Kristich CJ, Rice LB, Arias CA. Enterococcal infection—treatment and antibiotic resistance. Enterococci: From Commensals to Leading Causes of Drug Resistant Infection. Boston: Massachusetts Eye and Ear Infirmary; 2014.
2. Albrecht MA, Evans CW, Raston CL. Green chemistry and the health implications of nanoparticles. Green Chem 2006;8:417-32.
3. Sinha SN, Paul D. Eco-friendly green synthesis and spectrophotometric characterization of silver nanoparticles synthesized using some common Indian spices. Int J Green Herbal Chem 2004;3:401-8.
4. Arunachalam K, Annamalai SK, Arunachalam AM, Raghavendra R, Kennedy S. One step green synthesis of phytochemicals mediated gold nanoparticles from *Aegle marmales* for the prevention of urinary catheter infection. Int J Pharm Pharm Sci 2014;6:700-6.
5. Lee HJ, Song JY, Kim BS. Biological synthesis of copper nanoparticles using *Magnolia kobus* leaf extract and their antibacterial activity. J Chem Technol Biotechnol 2013;88:1971-7.
6. Darroudi M, Sabouri Z, Kazemi Oskuee R, Khorsand Zak A, Kargar H, Hamid MH. Sol–gel synthesis, characterization, and neurotoxicity effect of zinc oxide nanoparticles using gum tragacanth. Ceramics Int 2013;39:9195-9.
7. Nadagouda MN, Castle AB, Murdock RC, Hussain SM, Varma RS. *In vitro* biocompatibility of nanoscale zerovalent iron particles (NZVI) synthesized using tea polyphenols. Green Chem 2010;12:114-22.
8. Khazaei A, Rahmati S, Hekmatian Z, Saeednia S. A green approach for the synthesis of palladium nanoparticles supported on pectin: Application as a catalyst for solvent-free Mizoroki–Heck reaction. J Mol Catal A Chem 2013;372:160-6.
9. Husen A, Siddiqi KS. Plants and microbes assisted selenium nanoparticles: Characterization and application. J Nanobiotechnology 2014;12:28.
10. Rajakumar G, Rahuman AA, Roopan SM, Khanna VG, Elango G, Kamaraj C, *et al*. Fungus-mediated biosynthesis and characterization of TiO2 nanoparticles and their activity against pathogenic bacteria. Spectrochim Acta A Mol Biomol Spectrosc 2012;91:23-9.
11. Agarwal R, Agrawal NK, Singh R. Cicer arietinum leaf extract mediated synthesis of silver nanoparticles and screening of their antimicrobial activity. Adv Sci Eng Med 2014;6:203-7.
12. Davies RL, Etris SF. The development and functions of silver in water purification and disease control. Catal Today 1997;36:107-14.
13. Con TH, Loan DK. Preparation of silver nano-particles and use as a material for water sterilization. Environ Asia 2011;4:62-6.
14. Arif N, Yadav V, Singh S, Singh S, Mishra RK, Sharma S, et al. Current Trends of Engineered Nanoparticles (ENPs) in Sustainable Agriculture: An Overview. J Environ Anal Toxicol 2016;6:1-5.
15. Iravani S, Korbekandi H, Mirmohammadi SV, Zolfaghari B. Synthesis of silver nanoparticles: chemical, physical and biological methods. Research in Pharmaceutical Sciences 2014;9:385-406.
16. Devendra J, Hemanth KD, Sumita K, Kothari SL. Synthesis of plant-mediated silver nanoparticles using papaya fruit extract and evaluation of their antimicrobial activities. Dig J Nanomater Biostruct 2009;4:557-63.
17. Sondi I, Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: A case study on *E. coli* as a model for Gram-negative bacteria. J Colloid Interface Sci 2004;275:177-82.
18. Li P, Li J, Wu C, Wu Q, Li J. Synergistic antibacterial effects of β-lactam antibiotic combined with silver nanoparticles. Nanotechnology 2005;16:1912-7.
19. Davies J, Davies D. Origins and Evolution of Antibiotic Resistance. Microbiology and Molecular Biology Reviews 2010;74:417-33.
20. Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, *et al*. Antimicrobial effects of silver nanoparticles. Nanomedicine 2007;3:95-101.
21. Hu CM, Zhang L, Aryal S, Cheung C, Fang RH, Zhang L. Erythrocyte membrane-camouflaged polymeric nanoparticles as a biomimetic delivery platform. Proc Natl Acad Sci U S A 2011;108:10980-5.
22. El-Nour KM, Eftaiha AA, Al-Warthan A, Ammar RA. Synthesis and applications of silver nanoparticles. Arabian J Chem 2010;3:135-40.
23. Balaji DS, Basavaraja S, Deshpande R, Mahesh DB, Prabhakar BK, Venkataraman A. Extracellular biosynthesis of functionalized silver nanoparticles by strains of *Cladosporium cladosporioides* fungus. Colloids Surf B Biointerfaces 2009;68:88-92.
24. Deshpande LM, Chopade BA. Plasmid mediated silver resistance in *Acinetobacter baumannii*. Biometals 1994;7:49-56.
25. Dixit A, Das S, Jyoti A, Kaushik S. Biogenic synthesis of silver nanoparticles and its potential application in prevention of acute ear infections. J Pharm Sci Res 2017;9:14-7.
26. Kumar N, Das S, Jyoti A, Kaushik S. Synergistic effect of silver nanoparticles with doxycycline againt *Klebsiella Pneumonia*. Int J Pharm Pharm Sci 2016;8:183-6.
27. Rajora N, Kaushik S, Jyoti A, Kothari SL. Rapid synthesis of silver nanoparticles by *Pseudomonas stutzeri* isolated from textile soil under optimised conditions and evaluation of their antimicrobial and cytotoxicity properties. IET Nanobiotechnol 2016;10:367-73.
28. Sharma K, Kaushik S, Jyoti A. Green synthesis of silver nanoparticles by using waste vegetable peel and its antibacterial activities. J Pharm Sci Res 2016;8:313-6.
29. Allahverdiyev AM, Kon KV, Abamor ES, Bagirova M, Rafailovich M. Coping with antibiotic resistance: Combining nanoparticles with antibiotics and other antimicrobial agents. Expert Rev Anti Infect Ther 2011;9:1035-52.