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ORIGINAL PAPER

Effect of biofabricated gold nanoparticle-based antibiotic conjugates on minimum inhibitory concentration of bacterial isolates of clinical origin

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Abstract Medical device related infections caused by coagulase-negative staphylococci (CoNS) are difficult to treat mainly because of the increased bacterial genetic tolerance to antibiotics and its notorious biofilm formation property which has been reported to be achieved through a wide range of mechanisms. Current study is the demonstration of persistent antibiotic delivering potential and broad spectrum of activity of nanoantibiotic combinations designed from gold nanoparticles in conjugation with known antimicrobial agents. Such formulations are of potential applications as surface engineering agents on medical devices to prevent device mediated infection caused by pathogens like CoNS. For this, highly stable gold nanoparticles fabricated by a *Bacillus* sp. were functionalized with ciprofloxacin, gentamycin, rifampicin, and vancomycin and these nanoparticle-antibiotic conjugates were studied for its effectiveness against selected CoNS like *Staphylococcus epidermidis* and *Staphylococcus haemolyticus*. Very interestingly, the designed conjugates were identified to have a profound antibacterial efficiency compared to pure antibiotic and AuNPs. Thus, the result of the study is with highly significant medical applications as the test organisms used are emerging opportunistic pathogens.

Keywords Biofabrication · Gold nanoparticles · Nanoantibiotics · CoNS

Introduction

Microbial development of resistance mechanisms and escalation of emerging and re-emerging infectious diseases associated with medical procedures have become a serious problem with great concern to public health [1]. This demands design of new antimicrobial drugs with highly efficient and improved delivery potential by utilization of knowledge from recent advances in nanomaterial research [2]. The unique physical properties of nanoscale materials have shown to have promises to develop wide array methods for cost-effective diagnosis, rapid determination of resistance of antibacterial drugs, and efficient delivery of antimicrobial agents [3]. Their application as drug delivery vehicles is due to its advantages such as controlled release, improved solubility, specific site-targeted delivery, minimized side effects, and enhanced cellular internalization. Additional advantages of nanoantimicrobial formulations including its protective effect toward antimicrobial drugs and ability to overcome resistance in conjugation with antibiotics [4–6]. This demands studies on exploration of antimicrobial nanomaterials and novel nanosized platforms for clinical applications.

Nanoparticles can act as mediators of drug release, and thus, they have been used to enhance the selectivity and efficiency of the drug delivery system. In addition, because of their extremely small size and high surface area, their surfaces can be modified with hydrophobic, hydrophilic, cationic, anionic, or any neutral moieties and can easily travel into the target cells and allow high drug load. Various nanoparticles have been investigated as efficient delivery vehicles for the effective administration of wide range of antimicrobial agents.

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They are also suggested to protect antimicrobial drugs from resistant mechanisms in target microbes. Also, many types of lipophilic and water-soluble antibiotics have been reported to be conjugated inside or on the surface of nanoparticles, or carried via encapsulation [7]. Among all metal nanoparticles, gold nanoparticles (AuNPs) have been considered to be highly useful platform for the efficient drug delivery/carrier system due to their facile and well-studied synthesis, easy surface functionalization and biocompatibility and less toxicity [8]. Also, AuNPs have shown to increase drug concentration at infected site with reduced toxicity of the drug [9]. This make gold nanoparticles to be used as therapeutic agents or vaccine carriers in to specific cells to increase the efficiency of antibiotics therapy in treating serious bacterial infections at a reduced antibiotics dosage with minimal adverse effects.

Gold nanoparticle conjugates were reported to have promising applications against both gram-positive and gram-negative bacteria and also against those where the biofilm formation forms the basis of infection. Vancomycin-bound biogenic gold nanoparticles (VBGNP) have shown to have excellent antibacterial activity against resistant *Staphylococcus aureus* and *Escherichia coli* compared to their free forms [10]. Also, vancomycin-capped AuNPs were demonstrated to have enhanced antibacterial activity against vancomycin-resistant enterococci (VRE) [11]. In addition to this, AuNPs functionalized with ampicillin were found to have effective action as broad spectrum bactericides against wide range of bacteria [12]. Gold nanoparticles have also been exploited for its function as carrier for antibiotics such as streptomycin, neomycin, gentamycin, kanamycin [13–16], and fluorouracil [17]. These studies indicate the promising applications of AuNPs, and hence, it is of particular interest to design AuNP-based conjugates to engineer surface of medical devices to efficiently prevent organisms like CoNS which cause infections associated with insertion of medical devices.

CoNS are highly specialized organisms for the diversity in mechanisms for biofilm formation which make them to escape easily from the action of antimicrobial drugs. As biofilm-forming microorganisms are notoriously resistant to antimicrobial agents, their treatment is very difficult and biofilm formation on surfaces of medical implants is very problematic. Thus, in the current work, biogenic AuNPs functionalized with antibiotics were studied for its effectiveness against multidrug-resistant and biofilm-forming coagulase-negative staphylococcal (CoNS) strains isolated from clinical samples. Gold nanoparticles fabricated by the selected *Bacillus* sp. were characterized by UV–Vis spectroscopy, FTIR, HR-TEM, and EDS. Very interestingly, biosynthesized AuNPs when conjugated with ciprofloxacin, gentamycin, rifampicin, and vancomycin were found to have reduced minimum inhibitory concentration (MIC) when compared to their unconjugated free forms against tested CoNS which adds novelty and significance of the study.

Experimental details

Biosynthesis and purification of gold nanoparticles

Bacillus sp. (SJ 14) isolated and characterized for silver nanoparticle (AgNPs) synthesizing property in our previous study [18] was also selected as source organism for the synthesis of gold nanoparticles using aqueous solution of gold(III) chloride hydrate ($HAuCl_4$; Sigma-Aldrich). Bacterial biomass was grown in a specially prepared liquid culture medium (0.5 % peptone, 0.15 % beef extract, 0.15 % yeast extract, and 1 % NaCl, pH 7). The inoculated broth was maintained at room temperature under constant agitation at 200 rpm in a rotating shaker for 24 h. After incubation, the biomass was collected by centrifugation at 10,000 rpm for 10 min and washed thoroughly with sterile deionized water to remove any adhering media components. About 2 g of bacterial wet biomass was resuspended in 100-mL aqueous solution of filter-sterilized 1 mM $HAuCl_4$ in a 250-mL Erlenmeyer flask for the synthesis of gold nanoparticles. The bacterial biomass incubated without chloroauric acid ($HAuCl_4$) was maintained as control. After synthesis, the whole bacterial mixture with AuNPs was centrifuged at 15, 000 rpm for 15 min, and the collected pellets were washed and resuspended in 50 mM Tris buffer (pH 7). The cells were disrupted by ultrasonication over three 15-s periods and with an interval of 45 s between periods (Sonics-Vibra Cell). The resulting solution was then filtered through a 0.22-μm filter (Millipore), and the purified AuNPs thus obtained was used for further characterization.

Characterization of gold nanoparticles

The formation of gold nanoparticles was indicated by a visual color change from light yellow to dark purple. The bioreduction of the $AuCl^{4-}$ ions in solution was monitored by measuring the UV–Vis spectra of the reaction mixture using Hitachi U5100 spectrophotometer in the range of 400–800 nm at a resolution of 1 nm. The purified AuNPs were also characterized by Fourier transform infrared (FTIR) spectrometer to identify the possible biomolecules at a resolution of 4 cm^{-1} in the range of 4000–450 cm^{-1} (Schimadzu IR Prestige 21). High-resolution transmission electron microscopy (HR-TEM) and selected area electron diffraction (SAED) studies were prepared by drop coating of Au nanoparticles onto carbon-coated copper grids (JEOL-TEM) to confirm the microstructure of the nanoparticles. The surface interatomic distribution of AuNPs was also analyzed using energy-dispersive spectroscopy (EDS).

Antibacterial activity of gold nanoparticles

Antibacterial activity of AuNPs was evaluated against multi-drug-resistant, biofilm-forming coagulase-negative *Staphylococcus epidermidis* (sample ID 73,152, 78) and *Staphylococcus haemolyticus* (sample ID 41) strains isolated from clinical samples obtained from the MOSC Medical College, Kolencherry, Kerala, India [18]. The inoculum of each bacterium was developed by growing the CoNS strains in trypticase soy broth (TSB) at 37 °C for 18–24 h. The turbidity of the culture was maintained by comparing with 0.5 McFarland Standard by diluting with 0.9 % NaCl solution. Wells of 6 mm were made on the Muller-Hinton agar (MHA) plates using a gel puncture, and the plates were inoculated by swabbing the bacterial pathogens to create a confluent lawn of bacterial growth. Wells were then filled with 20 μl of biosynthesized AuNPs (20 μg/ml). $HAuCl_4$ solution (1 mM) and bacterial supernatant obtained after ultrasonication of biomass were poured on to respective wells as negative control, and plates were incubated at 37 °C for 24 h. The formation of clear zone around the wells is taken as an indication of antibacterial activity. The experiment was performed in triplicate.

Disk diffusion assay to evaluate the combined effects of AuNPs and antibiotics

The antibacterial potential of antibiotics in the presence of biosynthesized AuNPs was assessed by standard disk diffusion method against CoNS strains on MHA plates [19]. The standard antibiotic disks (gentamycin 30 μg/disk, ciprofloxacin 30 μg/disk, nalidixic acid 30 μg/disk, methicillin 5 μg/disk, rifampicin 15 μg/disk, and vancomycin 30 μg/disk) were purchased from Himedia (Mumbai, India). The inocula were prepared by diluting the overnight cultures of bacterial pathogens in TSB with 0.9 % NaCl to a 0.5 McFarland standard and were swabbed uniformly onto the individual MHA plates using sterile cotton swabs. Then, AuNPs impregnated air-dried antibiotic disks (20 μl of freshly prepared AuNPs with the final content of 20 μg of AuNPs per disk) and antibiotic disk alone as control were placed aseptically on the surface of the plates. Plates were incubated at 37 °C for 24 h to check the antibacterial activity and were expressed as zone of inhibition in millimeter around each disk. The fold increase area of different antibiotics against CoNS strains was calculated by the equation $(b^2-a^2) / a^2$, where a and b are zone of inhibitions for antibiotic and antibiotic plus AgNPs, respectively. The assays were implemented in triplicate.

Functionalization biogenic AuNPs with antibiotics

For this, stock solution (1 mg/ml) of ciprofloxacin, gentamycin, rifampicin, and vancomycin hydrochloride (Himedia, Mumbai, India) were prepared. Further, antibiotics-bound biogenic gold nanoparticles were prepared by stirring 1 ml of antibiotic solution with equal volume of biosynthesized AuNPs (20 μg/ml) for 15 min, followed by incubation for overnight at room temperature. The resulting functionalized nanoparticles were centrifuged, and the pellet was resuspended in sterile deionized water and stored in the dark at 4 °C [10, 12]. The formations of antibiotic-AuNP conjugates were characterized using UV–Vis spectroscopy (Hitachi U5100 spectrophotometer) in the range of 300–800 nm and FTIR (Schimadzu IR Prestige 21) in the range of 4000–450 cm^{-1}.

Analysis of MIC of free and AuNP-conjugated antibiotics

The MIC of biogenic gold nanoparticles functionalized with antibiotics against CoNS strains (*S. epidermidis* 73,152, 78 and *S.haemolyticus* 41) were conducted by standard microbroth dilution method according to the guidelines of the Clinical Laboratory Standards Institute (CLSI) using 96-well microtiter plates (Thermo Scientific Varioskan Flash multimode reader).

The CoNS strains were inoculated into TSB and incubated at 37 °C. The overnight cultures of CoNS were diluted by TSB to a 0.5 McFarland standard with bacterial cell density around 10^8 CFU/ml. Subsequently, the cultures were added to two-fold dilutions of antibiotics and AuNP-conjugated antibiotics in concentrations ranging from 500 to 0.976 μg/ml. Growth and sterility control wells were maintained in each microtiter plate, and the plates were incubated at 37 °C for 24 h. The assays were performed in triplicate to confirm the value of MIC of both free and AuNP-bound antibiotics for each tested bacteria. The MIC endpoint was considered as the lowest concentration at which there is no visible growth in the wells [10].

Results and discussion

CoNS species including the well-documented *S. epidermidis* are the major cause of device-associated infections. Other CoNS species such as *S. haemolyticus* are also extensively associated with implantation failure. The success in the colonization and development of CoNS infection has been related to the capacity of these organisms to produce biofilm. Once biofilm formation occurs on medical devices, dissemination of cells from this to other parts of the body happens which result in CoNS infection. Added challenge to this is the emerging resistance among these microorganisms toward traditional antibacterial treatments. So, alternative strategies to control CoNS infections by exploring the unique features of nanoparticles are highly attractive for these biofilm-mediated, drug-resistant, and device-centered infections. By engineering

surface of medical devices with NP-antibiotic conjugates, the early steps in bacterial infection can be prevented which offer much clinical applications.

Synthesis and characterization of AuNPs

Bacillus sp. SJ 14 (KJ451478) isolated previously from soil of jewelry premises were used for the biosynthesis of gold nanoparticles. Biosynthesis of AuNPs was visually inspected by distinct color change of reaction mixture from pale yellow to dark purple within 6 h after treating bacterial biomass with 1 mM $AuCl_4$ (Fig. 1a). The appearance of dark purple color is the first evidence of the reduction of the gold ions to gold nanoparticles and is due to the excitation of surface plasmon resonance (SPR) of typical gold nanoparticles [20]. In addition, the intensity of color increased up to 24 h and maintained throughout the experiment. However, no color change was observed in the flasks containing bacterial biomass without 1 mM $AuCl_4$.

Further UV–Vis spectroscopy analysis was used to check the formation and stability of biosynthesized AuNPs in aqueous solution, because metallic nanoparticles display characteristic optical absorption spectra in the UV–Vis region. The spectra showed an intense peak at 540 nm (Fig. 2) corresponding to the surface plasmon band of gold nanoparticles [21]. The narrow peak also revealed the formed gold nanoparticles as monodispersed in solutions. In addition, the stability of the gold nanoparticles were analyzed by UV–vis spectroscopy after 1 year of storage at 4 °C. There was no change in color of the AuNP solution and absorption peaks even after this period, which might be taken as indication of stability of biosynthesized gold nanoparticles. This may be due to the capping of AuNPs with biomolecules during synthesis, which balances the electrostatic force around them and provides stability against aggregation [22]. The role of proteins involved in the reduction of Au ions and AuNPs formation was investigated using FTIR spectroscopy. The FTIR spectrum of the biosynthesized gold nanoparticles showed an intense

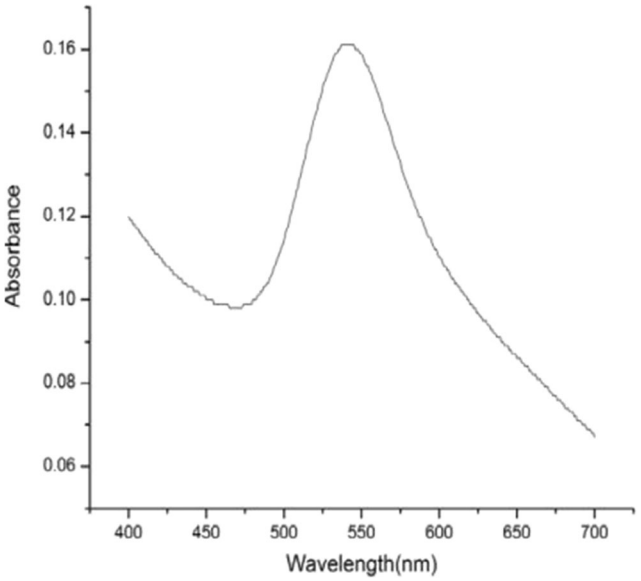

Fig. 2 UV–Vis spectra of AuNPs synthesized by *Bacillus* sp.

absorption bands located in the region of 3381–3259 cm^{-1} and also at 1643 and 1633 cm^{-1} (Fig. 3). This may be assigned to the amide bands of proteins and arises due to carbonyl stretch and free-N–H stretch vibrations in the amide linkages of the proteins. Thus, based on FTIR spectroscopic study, it was confirmed that the carbonyl group of amino acid residues and peptides of proteins have the stronger ability to bind metal. This evidence also suggests the possible role of proteins in the formation and also stabilization of AuNPs by covering around them to prevent agglomeration [23]. In addition, the capped proteins have previously been reported to retain their secondary structure on nanoparticle surface to make them biocompatible [24].

Further, the morphological analysis of the gold nanoparticles was done with HR-TEM. It was found that the synthesized nanoparticles were predominantly spherical in shape. In addition, hexagonal and triangular nanoparticles were also found and the sizes of formed AuNPs were in the range of 12–32 nm (Fig. 4a–g). The corresponding image of SAED

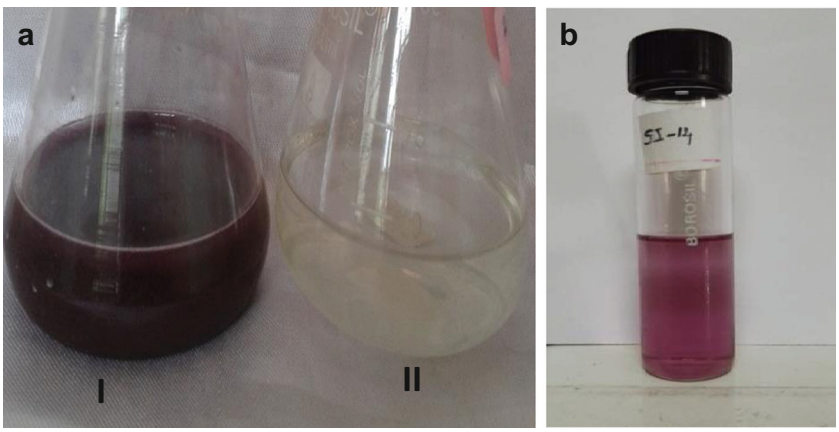

Fig. 1 **a** Visual observation of the biosynthesis of AuNPs by *Bacillus* sp. after 24 h. *I*, bacterial biomass with 1 mM $HAuCl_4$ solution (color change from pale yellow to dark purple); *II*, bacterial biomass without $HAuCl_4$ (**b**) purified AuNPs

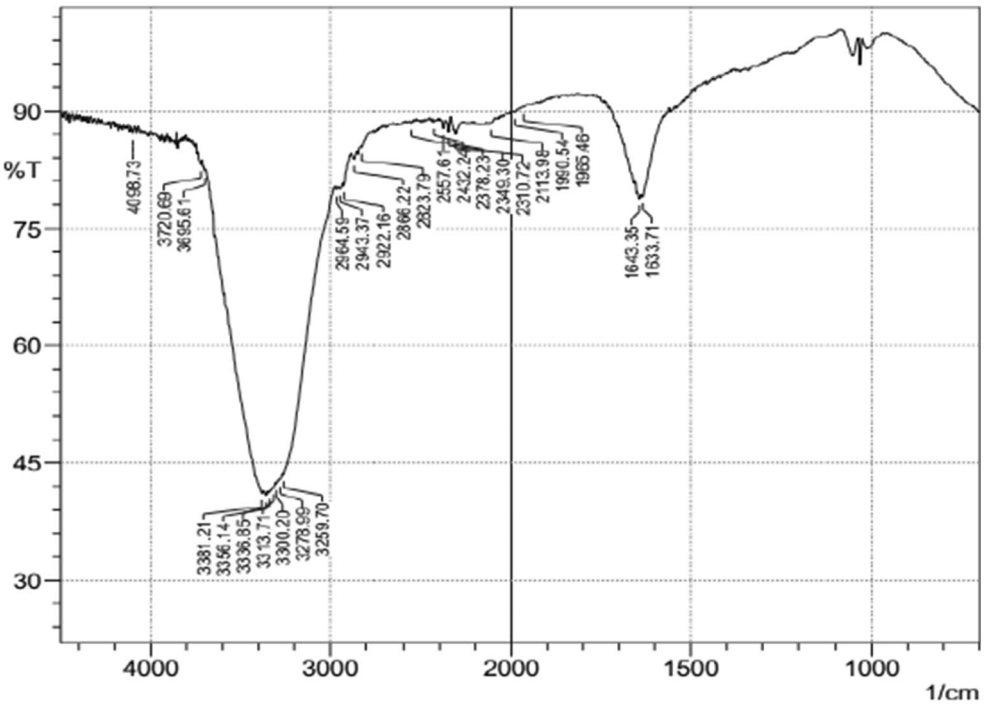

Fig. 3 FTIR spectra of gold nanoparticles synthesized using *Bacillus* sp.

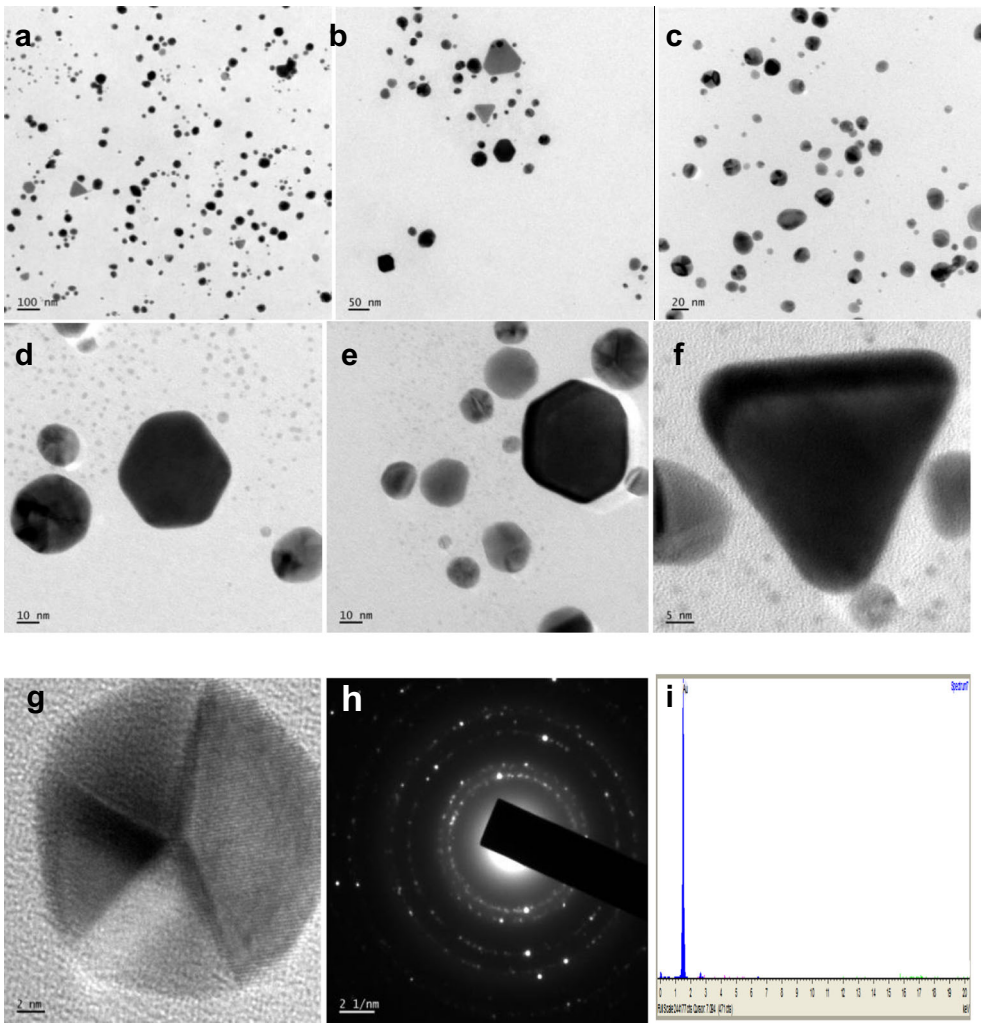

Fig. 4 HR-TEM images indicating the formation of spherical, triangular, and hexagonal gold nanoparticles at different magnifications (**a**–**g**) SAED pattern of AuNPs (**h**) EDS spectrum of silver nanoparticles (**i**)

patterns with bright circular spots revealed the biosynthesized gold nanoparticles as crystalline in nature (Fig. 4h). The EDS spectrum of AuNPs showed strong signals at 1.5 keV, which confirmed the presence of elemental gold in the sample (Fig. 4i).

Antibacterial activity of gold nanoparticles

In the study, antibacterial activity of biosynthesized AuNPs were performed against multidrug-resistant, biofilm-forming coagulase-negative *S. epidermidis* (sample ID 73,152, 78) and *S. haemolyticus* (sample ID 41) strains based on standard well diffusion assay. There was no zone of inhibition for biosynthesized AuNPs, which confirmed the gold nanoparticles as with no adverse effect against the test strains (Online Resource 1). AuNPs by itself have already been reported to be nontoxic to *E. coli*, *Vibrio cholerae*, drug-resistant isolates of MRSA, *Enterobacter aerogenes*, and *Pseudomonas aeruginosa*, but have shown to become bactericidal in the presence of antibiotic ampicillin [12]. Antibacterial activity analysis of gold nanoparticles assessed against *E. coli*, *Shewanella oneidensis*, and *Bacillus subtilis* also showed its nontoxic effect toward bacteria [25].

The antibacterial efficacy of biosynthesized gold nanoparticles in combination with various antibiotics was analyzed based on disk diffusion method. Table 1 shows the diameter of the zone of inhibition around antibiotics disk with and without gold nanoparticles against CoNS strains. The activity of all antibiotics except methicillin and nalidixic acid against all the test strains were increased and highest fold increase in area was observed in the case of vancomycin against *S. epidermidis strain* 73 (Online Resource 2). The prevalence of vancomycin resistance among clinical isolates of CoNS-like *S. epidermidis*, *S. haemolyticus*, *Staphylococcus warneri*, and *Staphylococcus hominis* has become a growing challenge as presented in many studies and hence the observed the result is highly significant [26, 27]. Because of the large surface area to volume ratio of AuNPs, more antibiotic molecules might have possibly adsorbed on the nanoparticle surface to act as a single group against microorganisms [15]. Thus, the observed

Table 1 Zone of inhibition of antibiotics in the presence and absence of gold nanoparticles against CoNS

CoNS	Antibiotics	Zone of inhibition (mm)		
		Antibiotics only (a)	Antibiotic + AuNPs (b)	Increase in fold area (b^2-a^2/a^2)
S.epidermidis 73	Gentamycin	26	28	0.16
	Ciprofloxacin	35	37	0.12
	Methicillin	No zone	No zone	0
	Nalidixic acid	19	19	0
	Rifampicin	36	40	0.23
	Vancomycin	14	16	0.31
S.epidermidis 152	Gentamycin	16	18	0.27
	Ciprofloxacin	25	27	0.17
	Methicillin	No zone	No zone	0
	Nalidixic acid	No zone	No zone	0
	Rifampicin	No zone	No zone	0
	Vancomycin	15	16	0.16
S.epidermidis 78	Gentamycin	29	30	0
	Ciprofloxacin	33	36	0.19
	Methicillin	No zone	No zone	0
	Nalidixic acid	18	18	0
	Rifampicin	39	42	0.16
	Vancomycin	20	22	0.21
S.haemolyticus 41	Gentamycin	24	26	0.17
	Ciprofloxacin	21	22	0.10
	Methicillin	No zone	No zone	0
	Nalidixic acid	No zone	No zone	0
	Rifampicin	35	37	0.12
	Vancomycin	20	20	0

In the absence of bacterial growth inhibition zones, the antibiotic disk diameter (6 mm) were used to calculate the increase in fold area

result provide an insight into the development of new antimicrobial formulations based on biosynthesized AuNPs with potential inhibitory effect toward multidrug-resistant isolates of coagulase-negative staphylococci.

Characterization of AuNPs functionalized with antibiotics

The formation of biosynthesized AuNPs functionalized with four antibiotics, ciprofloxacin, gentamycin, rifampicin, and vancomycin were monitored using UV–Vis spectroscopy (Hitachi U5100). The SPR band typical of the gold nanoparticles lies in the visible region of the electromagnetic spectrum, and it provides information regarding particle size distribution and aggregation [8]. The UV–Vis spectra of AuNPs conjugated with four antibiotics (Fig. 5) showed no significant change in the position of peak and color of the resulting solution even after conjugating with antibiotics. This indicates the nonaggregated nature of conjugates formed and is in accordance with previous report on functionalization of AuNPs with streptomycin, neomycin, gentamycin, and kanamycin [13].

FTIR (Schimadzu IR Prestige 21) was used to investigate the nature of binding of antibiotics to gold nanoparticles. The IR spectra of free antibiotics and antibiotic-coated gold nanoparticles are shown in Fig. 6. All the antibiotics except rifampicin showed characteristic absorption band at 16,300–1635 cm^{-1} corresponding to the N–H bending frequency [13]. In the case of antibiotic-coated gold nanoparticles, N–H bending peak was shifted to higher wavelength. Rifampicin showed an absorption band at 2922 and 2939 cm^{-1} due to symmetrical and asymmetrical stretching of methylene group [15], and this band is shifted to 2949 in rifampicin–AuNPs conjugate. In addition, N–H bending peak at 1645 cm^{-1} was also observed in the IR spectrum of rifampicin-coated gold nanoparticles. Based on the spectral data, binding of the amino groups of antibiotics to the surface of gold nanoparticles could be confirmed. Gold nanoparticles have strong affinity for amine groups and a previous report on vancomycin-bound biogenic AuNPs suggested the ability of gold nanoparticles to react with vancomycin by ionic interaction between amino group of antibiotic and negative surface charge of gold nanoparticles [10, 28].

Antibacterial activity of AuNPs functionalized antibiotics

The antibacterial activity of antibiotics and antibiotic-bound biogenic gold nanoparticles were tested against CoNS strains based on liquid broth dilution method, and MICs of antibiotics were analyzed (Table 2). It was found that MICs of all AuNP-conjugated antibiotics reduced significantly compared to their free forms except rifampicin-bound AuNPs against *S. epidermidis* 152, and vancomycin-bound AuNPs against *S.haemolyticus* 41. As CoNS have the ability to express resistance to multiple antibiotics, treatment of CoNS infections is complicated. Also, resistance to methicillin is almost universal among CoNS, and so, vancomycin is usually the antibiotic of choice for treatment. However, it has been reported that this

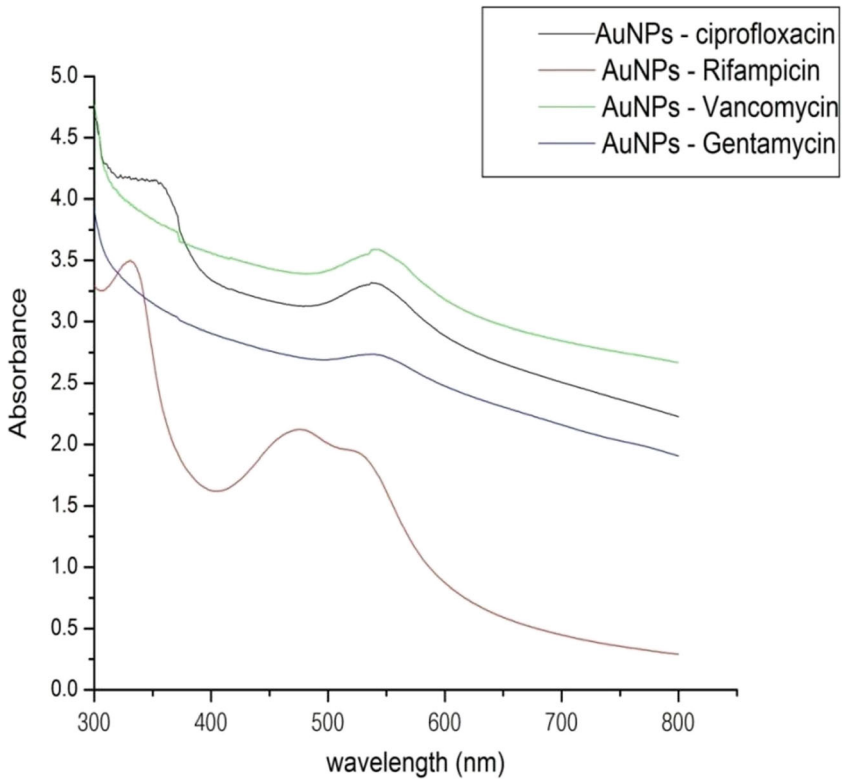

Fig. 5 The UV–Vis absorption spectra of biogenic gold nanoparticles functionalized with antibiotics

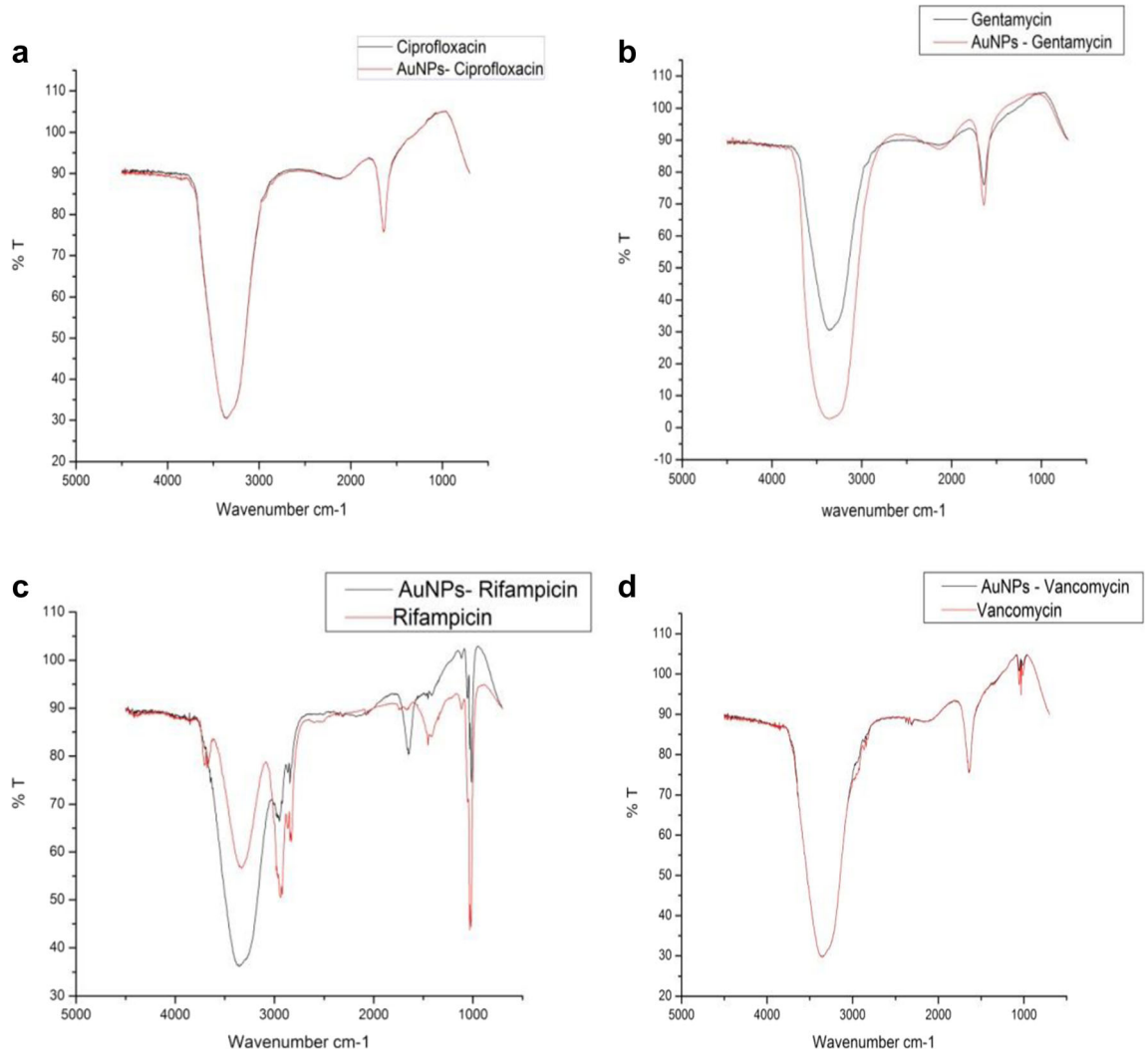

Fig. 6 FTIR spectra of gold nanoparticles-antibiotics conjugates **a** AuNPs-ciprofloxacin, **b** AuNPs-gentamycin, **c** AuNPs-rifampicin, and **d** AuNPs-vancomycin

glycopeptide susceptibilities of clinically significant staphylococci are also decreasing. However, AuNP-bound vancomycin was found to reduce the MIC of this antibiotic from 62.5 to 15.65 μg/ml against all tested *S. epidermidis* isolates. Similar result was also observed with ciprofloxacin against *S. epidermidis* 152 and *S. haemolyticus* 41. Both gentamycin and rifampicin were less effective against *S. epidermidis* 152 compared to other antibiotics. Also, the antibacterial efficacy of gentamycin is increased when bound with AuNPs and reduced its MIC from 125 to 31.25 μg/ml.

Table 2 Minimum inhibitory concentration (μg/ml) of antibiotics and antibiotics bound gold nanoparticles against CoNS strains

Antibiotics	CoNS isolates							
	73		152		78		41	
	A	B	A	B	A	B	A	B
Ciprofloxacin	31.25	7.81	62.5	15.62	31.25	3.9	62.5	15.62
Gentamycin	31.25	3.9	125	31.25	31.25	15.62	31.25	7.81
Rifampicin	31.25	7.81	125	125	31.25	15.62	62.5	15.62
Vancomycin	62.5	15.65	62.5	15.62	62.5	15.62	62.5	62.5

Columns A and B represent the MIC values in μg/ml of antibiotics and AuNPs functionalized antibiotics, respectively

Antibacterial efficiency of AuNP-bound antibiotics has already been reported and in the case of gentamycin, which contains active groups that react with nanogold by chelation. Gold nanoparticles possess a large surface to volume ratio and because of this more number of drug molecules gets adsorbed on its surface. When the antimicrobial groups come in close proximity with a nanogold core it has been suggested to act very effectively against the microorganisms [15]. Because of this, vancomycin-capped AuNPs (Au@Van NPs) has reported to have 64-fold improved efficacy against VRE strains and *E. coli* than vancomycin alone [11]. A similar result has also

been reported for ciprofloxacin-coated AuNPs [28]. In addition, the antibacterial mechanism of VBGNP against vancomycin-resistant *S. aureus* (VRSA) was reported to be due to the non specific binding of VBGNP to the transpeptidase instead to terminal peptides (D Lac) of VRSA strain [10]. Thus, it can be confirmed that gold nanoparticles act as a good anchor for carrying more amount of drugs effectively on its surface via electrostatic attraction and which signifies application of AuNP-based nanoconjugates for surface engineering applications.

The important advantages of nanoparticle-based antimicrobial drug delivery includes improved solubility of poorly water-soluble drugs, prolonged drug half-life and systemic circulation time, and sustained and stimuli-responsive drug release, which eventually lowers administration frequency and dose [6, 29]. In addition, minimized systemic side effects through targeted delivery of antimicrobial drugs as well as combined, synergistic, and resistance overcoming effects via co-delivery of multiple antimicrobial drugs can also be achieved using nanoparticle carriers [30]. Because of the enhanced antibacterial effect of AuNP-antibiotic conjugates, it can be used as potential template for the designing of new biocompatible antibacterial agents to engineer surface of medical devices to prevent bacterial colonization and to overcome drug resistance. Thus, the antibacterial effect of gold nanoparticles functionalized with antibiotics on the survival of CoNS investigated in this study is very innovative and promising and the conjugates developed in the study can be of immense application to modify the surface of medical device or targeted delivery of antibiotics to CoNS to limit its infection.

Conclusion

In the present study, biosynthesis of highly stable AuNPs by soil *Bacillus* sp. and its antibacterial activity functionalized with antibiotics were studied to combat multidrug-resistant bacteria. AuNPs were functionalized with antibiotics ciprofloxacin, gentamycin, rifampicin, and vancomycin and characterized using UV–Vis spectroscopy and FTIR. Even though AuNPs does not have any antibacterial activity, it was found to act as carrier for drugs and the antibiotic-bound AuNPs showed very effective activity against CoNS strains compared to pure drugs. Thus, antibacterial effects of AuNP-antibiotic conjugate developed in the study shows biosynthesized AuNPs as an effective drug delivery vehicle which is of much potential applications.

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