Chapter 5
Antibacterial activity and mechanism of action of silver and gold nanoparticles
5.1. Introduction

Antimicrobial agents are vital to fight infectious diseases. However, emergence of bacterial resistance to antibacterial drugs has become a major problem because of their broad use and abuse. Bacteria developed resistance against many common antibacterial agents and thus infectious diseases continue to be one of the greatest health challenges worldwide. Bacterial drug resistance mainly includes mutation. Once resistance is acquired by bacteria, it can share, exchange and transfer its property vertically (to its progeny) or horizontally (to neighbouring bacteria) by transduction, transformation or by conjugation (Slonczewski and Foster, 2009). Moreover, drawbacks for conventional antimicrobial agents are not only the development of multiple drug resistance, but also adverse side effects. Drug resistance also enforces high-dose administration of antibiotics, often generating intolerable toxicity. This has prompted the development of alternative strategies to treat bacterial diseases (Baker-Austin et al., 2006). Among them, nanoscale materials have emerged as novel antimicrobial agents.

Metal nanoparticles possess unique properties which make it to have wide-range of applications in variety of areas including cell labeling and imaging (Parak et al., 2005), biosensing (Niemeyer, 2001), drug delivery (Langer, 2003), optoelectronics (Jackson et al., 2003), non-linear optical devices (Maier et al., 2001) surface enhanced Raman spectroscopy, (Li et al., 2004) therapeutics, catalysis and also as antimicrobial agents (Christopher et al., 2011; Ashokkumar et al., 2014). Among various metals, silver has a long history of use as an effective antimicrobial agent for the treatment of diseases, food preservation, and water purification (Jain et al. 2008). But with the advent of wide use of antibiotics, the medical application of silver as an antimicrobial agent was declined (Castellano et al., 2007). However with the emergence of nanotechnology, high surface to volume ratio of silver nanoparticles (AgNPs)
has been made them to have excellent antimicrobial activities (Song et al., 2009). In addition, gold nanoparticles (AuNPs) have long been considered as possible drug delivery vehicles applicable for therapy of a range of conditions. The excellent surface functionalization chemistry, optical and electronic properties and non toxic nature of gold nanoparticles has led to its various biomedical applications (Qian and Nie, 2008; Xia et al., 2011).

Microbially generated metal nanoparticles have been studied extensively for their wide antimicrobial applications. The small size and large surface area of nanoparticles is important in its antimicrobial activity (Guzmán et al., 2009; Arunachalam et al., 2012). Silver nanoparticles can have strong antibacterial activity towards both gram positive and gram negative bacteria (Nanda and Saravanan, 2009; Sadhasivam et al., 2010). Silver nanoparticles have been reported to attack gram negative bacteria by anchoring and penetrating the cell wall, leading to structural changes in the cell membrane and increase in cell permeability. This facilitates the uncontrolled transport through cytoplasmic membrane followed by cell death. (Morones et al., 2005; Sondi and Salopek-Sondi, 2004).

Several studies proposed the antimicrobial activity of AgNPs as due to the slow release of silver ions which react with thiol groups of proteins or interfere with DNA replication (Liau et al., 1999; Feng et al., 2000). Also silver nanoparticles may get attached to the cell membrane surface which in turn can damage or disturb the functions of the cell leading to bacterial death (Kim et al., 2007b). The ability of AgNPs to reach bacterial proximity and its high surface area produce high concentration of Ag$^+$ in cell surroundings, causing promising antibacterial effects (Wigginton et al., 2010). Silver nanoparticles were also reported to dephosphorylate the tyrosine residues, leading to signal transduction inhibition and bacterial growth inhibition (Shrivastava et al., 2008; Prabhu and Pouluse, 2012).
Electron spin resonance spectroscopy studies have suggested the effect of contact between silver nanoparticles and bacteria to result in the formation of free radicals, which damage the cell membrane and make it porous and ultimately lead to cell death (Danilcauk et al., 2006; Kim et al., 2007b). Thus the multiple antimicrobial mechanisms of AgNPs involve disruption of integrity of bacterial membrane by its attachment, prevention of cell division and DNA replication by its interaction with thiol groups of enzymes and phosphorus-containing bases and potential to induce oxidative stress by free radical formation (Morones et al., 2005). Therefore AgNPs are expected to offer new directions to tackle multidrug-resistant bacteria due to its multitargeted cellular effects (Leid et al., 2005). Moreover, AgNPs can be considered as an environment friendly antimicrobial agent because of its non toxic nature to human cells in low concentrations and weak ability of bacteria to develop resistance toward silver ions (Sukdeb et al., 2007; Shaverdi et al., 2007b). In addition, mammalian cells are known to phagocytose these nanoparticles and thereby reducing toxicity and free-radical damage (Arbab et al., 2005).

One of the major limitations of advanced medical device related diagnostics and therapeutic procedures are the device related infections. The major groups of organisms that cause device-related infections are Coagulase-Negative Staphylococci (CoNS) which are notorious for its biofilm formation and multidrug resistance. They are the part of normal flora of human skin and have relatively low virulence but are increasingly recognized as agents of clinically significant infection through the bloodstream and other sites. Although it is considered as commensal, risk factors for CoNS infection include the presence of foreign devices such as catheters and immune compromise. Moreover, CoNS have been reported to protect themselves against antibiotics and the host immune system via production of a matrix of exopolymeric substances. Once attached to medical devices, they secrete slimy
exopolysaccharide matrix which encapsulate developing colonies and protects from biocides and neutralizing antibiotics (Branda et al., 2005; Nichols et al., 1988). These processes ultimately result in CoNS mediated infection and sepsis.

Thus in the present study, the antibacterial activity of biosynthesized silver and gold nanoparticles were tested against multi drug resistant biofilm forming coagulase negative Staphylococcus epidermidis and Staphylococcus haemolyticus isolated from clinical samples such as pus, catheter tip, blood etc. In addition, MTCC cultures of Escherichia coli, Klebsiella pneumoniae, Salmonella enterica typhimurium, Staphylococcus aureus and Bacillus subtilis were also used to confirm the broad antimicrobial efficacy of biosynthesized nanoparticles. The antibacterial mechanisms of AgNPs were further analysed by AFM, HR-TEM.

5.2. Materials and Methods

5.2.1. Antibacterial activity of AgNPs and AuNPs by Well diffusion Assay

Biosynthesized AgNPs and AuNPs were evaluated for their antibacterial potential against selected bacterial pathogens by standard well diffusion method in Mueller Hinton Agar (MHA) plates as per previous reports (Dar et al., 2013). Three multi drug resistant and biofilm forming coagulase negative S. epidermidis (sample id-73,152, 78) and one S.hemolyticus (sample id-41) which were proved to have the presence of biofilm associated genes icaAB, aap, bhp, atle, fbe and embp isolated from clinical samples obtained from MOSC medical college, Kolencherry, Kerala, India were selected for the study. The broad antibacterial activity of biosynthesized AgNPs and AuNPs was also evaluated against other gram positive and gram negative bacterial pathogens like Staphylococcus aureus (MTCC 96), Bacillus subtilis (MTCC 121), Escherichia coli (MTCC 723), Klebsiella pneumoniae (MTCC 109) and Salmonella enterica typhimurium (MTCC 1251).
Pure cultures of CoNS strains were grown in Trypticase Soy broth (TSB) at 37°C for 18-24 hours. Pure cultures of MTCC cultures were grown in nutrient broth at 37°C for 18-24 hours. Wells of 6-mm were made on the Muller Hinton agar plates using a gel puncture and the plates were inoculated by swabbing the bacterial pathogens when the turbidity has equivalent related to that of a 0.5 McFarland Standard to create a confluent lawn of bacterial growth. Using a micropipette, 20 μl of biosynthesized AgNPs/AuNPs solution (20 μg/mL) was poured on to corresponding well. Also, 20 μL of 1 mM AgNO3/1 mM HAuCl4 solution and bacterial supernatant obtained after ultrasonication of biomass were poured on to respective wells as control. The diameter of zone of inhibition in millimeter around each well was measured after incubation at 37°C for 24 hours. The experiment was performed in triplicate and the data are presented as mean ± standard error.

5.2.2. **Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of AgNPs**

To determine the minimum inhibitory concentration (MIC) of silver nanoparticles, micro-broth dilution method was used according to the guidelines of the Clinical Laboratory Standards Institute (CLSI) using 96-well microtiter plates. The bacterial pathogens (CoNS) were grown overnight in TSB at 37°C. The overnight cultures of CoNS were diluted by Trypticase Soy broth to a 0.5 McFarland standard to obtain bacterial cell density around $10^8$ CFU/mL.

The Minimum inhibitory concentration of silver nanoparticles was determined in Trypticase Soy broth using serial two-fold dilutions of AgNPs in concentrations ranging from 200 to 0.391 μg/mL. Growth and sterility control wells were maintained in each micro titer plate and the plates were incubated at 37°C for 24 hours. The assays were performed in triplicate to confirm the value of MIC of AgNPs for each tested bacteria. The MIC results were noted by
checking the turbidity of the bacterial growth and the MBC were determined by spread plating bacterial culture on the Mueller Hinton Agar (MHA) plates, incubated at 37°C for 24 h. The MBC is the concentration at which the bacteria are completely killed (Quelemes et al., 2013; Zhang et al., 2014).

5.2.3. **Fluorescent Microscopy analysis of AgNPs treated bacterial cells**

The live or dead bacterial viability assay was determined using acridine orange (AO) fluorescent dye. The fluorescent dye was prepared by mixing of 0.05 mL of stock solution of 1% Acridine orange (HiMedia, India) and 5 mL of acetate buffer 0.2 M (pH 4.0). Silver nanoparticle treated (20 µg/mL) and untreated bacterial isolates were placed on a glass slide and dried at 50°C, fixed with absolute methanol for 2 min and air dried. The slides were then stained with acridine orange (AO) staining reagent for 1 min, washed with tap water and air dried. The samples were then observed under fluorescence microscope (OLYMPUS BX43F Fluorescent Microscope) (Dhas et al., 2014).

5.2.4. **Antibacterial mechanism of AgNPs**

5.2.4.1. **Atomic Force Microscopic (AFM) analysis of AgNPs treated bacterial cells**

The topological changes on the bacterial membrane after treatment with AgNPs (20 µg/mL) were analyzed using AFM. The treated and untreated CoNS samples were placed on glass cover slip and air-dried. AFM imaging was performed using an AFM (APER-A-100 SPM) operated in a tapping mode with Si cantilevers having a spring constant of 21 N/m operating at a resonance frequency of 160 kHz. The images were acquired at a scan field of 5 µm × 5 µm.
5.2.4.2. Transmission Electron Microscopic (TEM) analysis of AgNPs treated bacterial cells

The bactericidal action of biosynthesized silver nanoparticles and morphological changes of bacterial cells were analysed by TEM images at different magnifications. *Staphylococcus epidermidis* strain 152 and *Staphylococcus haemolyticus* 41 treated with AgNPs (both minimum inhibitory and minimum bactericidal concentration) and were incubated overnight at 37°C. The untreated cells were maintained as control. The bacteria were collected by centrifugation, washed, and a thin film of the sample was coated on to carbon coated-copper grid (JEOL, JEM-2100).

5.2.4.3. Bacterial genomic DNA degradation in the presence of AgNPs

The mechanism of antibacterial activity of biosynthesized AgNPs was also studied by DNA degradation experiment. Bacterial genomic DNA isolated from CoNS strains were treated with 3 doses of AgNPs such as sub inhibitory concentration (SIC), minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). This includes 1.56, 3.12 and 25 µg/mL of AgNPs against *S.epidermidis* strains and 3.12, 6.24 and 25 µg/mL of AgNPs against *S.haemolyticus* strain. The treated DNA in sterile phosphate buffer saline (PBS) was incubated at 37°C for 6 h. The untreated DNA suspended in sterile PBS was also maintained as control. Both the treated and the control DNA samples were electrophoresed using agarose gel electrophoresis, stained with ethidium bromide and visualized under UV light (Sen et al., 2013).

5.3. Results

5.3.1. Antibacterial activity of AgNPs and AuNPs

The antibacterial effect of AgNPs was investigated against clinical isolates of CoNS and MTCC bacterial pathogens like *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella*
*enterica* typhimurium by well diffusion assay and it showed activity against all the tested strains (Fig. 5.1 and 5.2). In comparison to AgNPs, there was no significant zone of inhibition by AgNO$_3$ and the bacterial supernatant, which confirmed the activity as due to the synthesized AgNPs only.

In the study, antibacterial activity of biosynthesized AuNPs was also performed and there was no zone of inhibition for biosynthesized AuNPs, which confirmed the gold nanoparticles as with no adverse effect against the test strains (Fig. 5.3 and 5.4). As AgNPs exhibited good antibacterial property, whereas the AuNPs did not show any inhibitory activity, AgNPs were selected further studies.

5.3.2. MIC and MBC of AgNPs

The antibacterial effect of biosynthesized AgNPs and antibiotics on the Coagulase- Negative Staphylococcal strains *S. epidermidis* and *S. haemolyticus* were quantitatively determined by the MIC. The MIC and MBC values of biogenic AgNPs for four CoNS strains were shown in Table 5.1. For *S. epidermidis* strains, the MIC was same at 3.125 µg/mL and for *S. haemolyticus* strain it was 6.25 µg/mL.

5.3.3. Fluorescent Microscopy analysis

The viability of bacterial cells after AgNPs treatment were analysed using acridine orange (AO) fluorescent dye. Interestingly the fluorescence image of bacterial cells (*S. epidermidis* 152 and *S. haemolyticus* 41) without AgNPs treatment appeared green indicating the cells as viable (Fig. 5.5 a, c). However nanoparticle treated bacterial cells were observed to be red (Fig. 5.5 b, d), due to the bactericidal action of AgNPs.
5.3.4. Antibacterial mechanism of AgNPs

5.3.4.1. Atomic Force Microscopic (AFM) analysis

The interaction of AgNPs with CoNS strains and the subsequent changes of cell morphology were studied by using AFM. Fig. 5.6 shows the representative AFM images of AgNPs treated and untreated \textit{Staphylococcus epidermidis} strain 152 and \textit{Staphylococcus haemolyticus} 41. It was found that untreated bacteria appeared spherical with no evidence of membrane rupture and collapse (Fig. 5.6 a, c). However, upon treatment with AgNPs, the bacteria demonstrated strong evidence of membrane disorganization compared to untreated CoNS (Fig. 5.6 b, d).

5.3.4.2. HR-TEM analysis of CoNS treated with AgNPs

High Resolution Transmission Electron Microscopic analysis of AgNPs treated and untreated bacterial cells revealed remarkable distribution of silver nanoparticles on the surface of bacterial cells with assisted detachment of cell membrane and leakage of cytoplasmic contents (Fig. 5.7 d-f, j-l). But the cell surface of \textit{Staphylococcus epidermidis} and \textit{Staphylococcus haemolyticus} without silver nanoparticle treatment observed under the same condition appeared smooth and found to have regular spherical morphology as expected (Fig. 5.7 a-c, g-i). AgNPs that accumulated in the cell membrane as well as some penetrating the cells can also be observed in the HR-TEM micrograph.

Untreated CoNS appeared as intact spherical cells with no evidence of membrane rupture and collapse. However, upon treatment with minimum inhibitory concentration of AgNPs, the bacteria demonstrated strong evidence of membrane disorganization with greater roughness as compared to the smooth surface of untreated bacteria (Fig. 5.7. d and j). In addition, \textit{S.epidermidis} and \textit{S.haemolyticus} treated with MBC of AgNPs showed extensive membrane
damage and leak out of the intracellular components causing shrinkage of cell and finally cell lysis (Fig. 5.7. f and l).

### 5.3.4.3. Bacterial genomic DNA degradation analysis

Bacterial DNA was treated with 3 different concentrations of AgNPs such as sub inhibitory concentration, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). No laddering patterns were observed in the gel in control DNA and those treated with sub inhibitory concentration of AgNPs, whereas a higher dose of AgNPs (MIC and MBC) showed a distinct ladder pattern after 6 hours of treatment (Fig. 5.8). Thus interaction of biosynthesized AgNPs with bacterial genomic DNA isolated from the CoNS strains revealed direct evidence of dose-dependent DNA damage.

**Fig. 5.1.** Antibacterial activity of biosynthesized AgNPs against (A) *S.epidermidis* 73(B) *S.epidermidis* 152(C) *S.epidermidis* 78 and (D) *S.haemolyticus* 41: (a) bacterial supernatant (b) AgNO₃
**Fig. 5.2.** Antibacterial activity of AgNPs against (A) *Staphylococcus aureus* (B) *Bacillus subtilis* (C) *Escherichia coli* (D) *Klebsiella pneumoniae* and (E) *Salmonella enterica typhimurium*: (a) AgNO$_3$ (b) bacterial supernatant
Fig. 5.3. Biosynthesized AuNPs with no toxicity towards (A) *S.epidermidis* 73 (B) *S.epidermidis* 152 (C) *S.epidermidis* 78 and (D) *S.haemolyticus* 41 : (a) bacterial supernatant (b) H\textsubscript{AuCl}_4
Fig. 5.4. Biosynthesized AuNPs with no toxicity towards (A) *Staphylococcus aureus* (B) *Bacillus subtilis* (C) *Escherichia coli* (D) *Klebsiella pneumoniae* and (E) *Salmonella enterica typhimurium* : (a) bacterial supernatant (b) HAuCl$_4$
**Table 5.1.** Minimum inhibitory concentration of biosynthesized AgNPs (µg/mL) against Cogulase Negative Staphylococci

<table>
<thead>
<tr>
<th>CoNS isolates</th>
<th>MIC (µg/ml)</th>
<th>MBC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. epidermidis</em> 73</td>
<td>3.125</td>
<td>25</td>
</tr>
<tr>
<td><em>S. epidermidis</em> 152</td>
<td>3.125</td>
<td>25</td>
</tr>
<tr>
<td><em>S. epidermidis</em> 78</td>
<td>3.125</td>
<td>25</td>
</tr>
<tr>
<td><em>S. haemolyticus</em> 41</td>
<td>6.25</td>
<td>25</td>
</tr>
</tbody>
</table>

**Fig. 5.5.** Fluorescent Microscopic images of (a) untreated *S. epidermidis* 152 (b) AgNPs treated *S. epidermidis* 152 (c) untreated *S. haemolyticus* 41 and (d) AgNPs treated *S. haemolyticus* 41
Fig. 5.6. AFM images of (a) untreated *S. epidermidis* 152 (b) AgNPs treated *S. epidermidis* 152 (c) untreated *S. haemolyticus* 41 and (d) AgNPs treated *S. haemolyticus* 41.
Fig. 5.7. Morphological changes of CoNS before and after treatment with AgNPs. (a-c) untreated *S. epidermidis* 152 (d) *S. epidermidis* 152 treated with MIC of AgNPs (e,f) *S. epidermidis* 152 treated with MBC of AgNPs (g-i) untreated *S. haemolyticus* 41 (j) *S. haemolyticus* 41 treated with MIC of AgNPs (k,l) *S. haemolyticus* 41 treated with MBC of AgNPs
Fig. 5.8. DNA degradation study of (a) *S. epidermidis* 73 (b) *S. epidermidis* 152 (c) *S. epidermidis* 78 and (d) *S. haemolyticus* 41 by AgNPs: lane 1 (control), lane 2 (treated with sub inhibitory concentration of AgNPs), lane 3 (treated with minimum inhibitory concentration of AgNPs), lane 4 (treated with minimum bactericidal concentration of AgNPs)
5.4. Discussion

AgNPs synthesized by diverse microorganisms show variable antimicrobial properties because of their difference in shape, size, and surface properties. Therefore, screening novel microbial sources for the identification of AgNPs with enhanced activity and better stability is highly interesting. The antibacterial effect of biologically synthesized nanoparticles was tested against a panel of human pathogens comprising both gram-positive and gram-negative bacteria by well diffusion assay. Among them, coagulase-negative staphylococci, in particular, *Staphylococcus epidermidis* is the most important pathogen involved in medical device related infections. Formation of biofilms and the high frequency of antibiotic-resistance observed in them have made it a clinically significant organism.

Silver nanoparticles showed excellent antibacterial activity against all tested bacteria compared to controls and the diameters of zone of inhibition varied for all the bacteria. The exact antibacterial action of AgNPs is not completely understood. However, the bactericidal efficiency of AgNPs is considered to be depends on different parameters including size, shape, and surface charge. Smaller particles are suggested to have greater surface area and are considered to reach the nuclear content of gram-negative bacteria very easily (Thiel et al., 2007). Size dependent mechanistic basis of antimicrobial activity of AgNPs has already been suggested. As per this, AgNPs with size greater than 10 nm are considered to interfere with cellular permeability as it is accumulated in cell surface. But those with less than 10 nm size have been shown to interfere with DNA and enzyme leading to cell death (Xiu et al., 2012). Moreover, the antibacterial activity of AgNPs is depends on the morphology of the particles. AgNPs with different shapes have also been described to have dissimilar microbicidal activity due to the difference in their effective surface areas and number of active facets which indicate importance of
shape of nanoparticles for its activity (Pal et al., 2007). The antimicrobial activity of the AgNPs is also considered to be due to the electrostatic attraction between positively charged nanoparticles and negatively charged bacterial cells (Sondi and Salopek-Sondi, 2004).

Eventhough gram-positive and gram-negative bacteria have differences in their membrane structure, most of them have a negative charge due to the presence of carboxyl, phosphate, and amino groups in the cell membrane (van der Wal et al., 1997; Abbaszadegan et al., 2015). Eventhough, there was no zone of inhibition for biosynthesized AuNPs, the strong inhibitory effect of AgNPs on the growth of clinical isolates of CoNS is highly supportive to the application of AgNPs for the surface modification of medical devices to minimize the CoNS infection. AuNPs by itself have already been reported to be non toxic to *Escherichia coli*, *Vibrio cholerae*, drug resistant isolates of MRSA, *Enterobacter aerogenes* and *Pseudomonas aeruginosa*, but have shown to enhance the bactericidal action of antibiotics (Brown et al., 2012). Antibacterial activity analysis of gold nanoparticles assessed against *Escherichia coli*, *Shewanella oneidensis* and *Bacillus subtilis* also showed its nontoxic effect towards bacteria (Suresh et al., 2011).

Fluorescent Microscopy image analysis of selected CoNS cell after treatment with AgNPs were also confirmed the antibacterial action of biosynthesized AgNPs. The live/dead cells can be easily differentiated based on the differential fluorescence exhibited when intercalated with Acridine orange (Manikandan and Wu, 2013). The live cells fluoresce green and dead cells orange. Previous studies on viability of bacteria in dental calculus described appearance of viable bacteria as apple green colour after staining with Acridine orange fluorescent stain. They also reported acridine orange as a fluorochrome strain with marked affinity for nucleic acids and stains both live and dead bacteria (Moolya et al., 2010; Mei et al., 2013).
Silver nanoparticles have been reported to have the ability to penetrate bacterial cell wall with the resulting structural changes leading to cell death (Prabhu and Poulose, 2012). AFM and TEM analysis of morphological changes of bacterial cell wall after treatment with silver nanoparticles as conducted in the study confirmed biosynthesized nanoparticles to have the ability to disorganize bacterial cell membrane resulting in the release of their cellular contents into the surrounding environment, through direct contact mechanisms. It was known that DNA can accomplish replication effectively when they are in a relaxed state only. Thus degradation study revealed effect of AgNPs on DNA and this may be due to the action of silver nanoparticles with major components of DNA like sulfur and phosphorus (Hatchett and Henry, 1996).

Various mechanisms have been suggested for the mode of action of silver nanoparticles and it is likely that more mechanisms may act together for the synergistic antibacterial properties of AgNP itself. But the chemical features of bacterial cell wall and biofilm may have determining effect on involved antibacterial mechanisms of AgNP against CoNS. The major mechanisms suggested for AgNPs include: interaction of NPs with cell components such as proteins, lipids and DNA (Wigginton et al., 2010; Chen et al. 2012; Basu et al., 2008). The interaction of AgNPs with bacterial cells has shown to induce formation of free radicals and thereby damaging to the cell membrane (Kim et al., 2007b). The silver ions released from silver nanoparticles have also been suggested to interact with thiol groups of many enzymes and also cause depletion of cellular antioxidant effect (Nagy et al., 2011).

The antibacterial mechanism of AgNPs studied using Surface Enhanced Raman Spectroscopy (SERS) has also demonstrated the influence of AgNPs on the metabolic processes of purines, possible inhibition of protein synthesis or complexation of Ag$^+$ ions with protein (Cui et al., 2013). All these justify the effect of AgNPs on multiple molecular targets and hence the observed results
suggest biosynthesized AgNPs as potential broad-spectrum agents against a variety of multidrug resistant bacteria and it have direct applications as surface modification agents on medical devices or pharmaceutical products. This may help to prevent transmission of drug-resistant pathogenic bacteria like CoNS, which cause device-related infections.