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Biogenic synthesis and effect of silver nanoparticles (AgNPs) to combat catheter-related urinary tract infections

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ABSTRACT

Silver nanoparticles (AgNPs) have been considered as antimicrobial agents for decades. Rather than chemical synthesis, biosynthesis of AgNPs is emerging as a significant and effective method. This study mainly focused on synthesizing eco-friendly AgNPs from coral-associated bacteria. A total of 57 coral bacterial isolates were screened and the isolate MGL- D10 was selected for synthesizing AgNPs. The isolate MGL- D10 was identified as *Alcaligenes* sp. using 16S rDNA sequence based phylogenetic analysis. The synthesized AgNPs MGL- D10 was then characterized using UV–Vis spectroscopy, FTIR, and X-ray diffraction (XRD) analysis. Further, the morphology and the size of the synthesized AgNPs was observed through Atomic Force Microscopy (AFM), Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). Microscopic analysis revealed the size of the synthesized AgNPs MGL- D10 was 30–50 nm. The synthesized AgNPs MGL- D10 showed antimicrobial activity against urinary tract infection (UTI) causing clinical isolates such as *Bacillus* sp., *E. coli*, *K. pneumonia, P. aeruginosa*, *S. aureus* and *C. albicans*. Antibiofilm effect of synthesized AgNPs MGL- D10 against *S. aureus* was assessed using confocal laser scanning microscopy. Catheter experiments also proved the antibiofilm and antimicrobial effect of synthesized AgNPs MGL- D10. The obtained results exhibit that the coating of synthesized AgNPs MGL- D10 on catheters effectively inhibited the growth and biofilm formation of UTI causing pathogens. The present study will pave a way for successful and eco-friendly methods of protection against urinary tract infection causing pathogens and nosocomial infections.

1. Introduction

Nanotechnology is considered as an emerging scientific field that has a great impact on human daily lives with diverse applications in many aspects (Nikalje, 2015). Current research on nanoscience significantly deals with synthesizing of metal nanoparticles. Metal nanoparticles have diverse applications and have been used extensively as biosensors, bio-labeling, cancer therapeutics, textiles, home and industrial appliances etc. Among the metal nanoparticles, AgNPs have been mostly used as antimicrobial and anti-inflammatory agents in wound dressings, eye treatment, dental hygiene, bone substitute biomaterials, antimicrobial filters, and disinfecting medical devices as well as in the coating of catheters (Salata, 2004). Silver is a non-hazardous and safe antimicrobial agent which has been used for centuries (Lansdown, 2006). Most of the antimicrobial agents have several drawbacks such as poor stability, toxicity to the environment, and non-specificity of targeting microbes (Butola and Mohammad, 2016). Other few antimicrobial agents are highly irritant and costly to formulate (Sondi and Salopek- Sondi, 2004). Silver has the unique property to bind cellular components which are considered as much more important than nucleic acids (Clement and Jarrett, 1994).

Synthesis of AgNPs can be performed using physical, chemical and biological methods. Physical and chemical methods to synthesize nanoparticles mostly end up with several drawbacks such as low-yield, harsh reducing agents, energy-intensive mechanisms, irregular particle size and instability in aggregation, production of hazardous waste, difficult to scale up, and may require costly organo metallic precursors (Iravani et al., 2014). Biological methods are considered as safer and effective methods to synthesize nanoparticles (Pantidos and Horsfall, 2014). Bacteria are the most potential bio-factories for several metal nanoparticles including gold, silver, platinum, palladium, titanium dioxide, magnetite, and cadmium sulfide. Bacteria-mediated synthesis of AgNPs is the preferable method when compared to other techniques. Moreover, bacteria mediated AgNPs are easier to develop and are eco-friendly. Bacterial synthesis of silver nanoparticles can be performed by both intracellular (biomass) and extracellular (cell extracts).

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Intracellular methods need ultrasonication and additional reactions with specific detergents to release the synthesized nanoparticles. Therefore, it is essential to rely on extracellular methods for synthesizing AgNPs due to its simple downstream processing which favors large-scale production (Iravani et al., 2014; Deepak et al., 2011; Das et al., 2014).

Nowadays there is a prevalence of multiple antibiotic resistance offered by several clinical pathogens including urinary tract pathogens which arise due to excess antibiotic usage and due to antibiotic accumulation in the environment. This kind of resistance is exhibited by *Staphylococcus* sp., *Streptococcus* sp., *Klebsiella* sp., *Enterococcus* sp., *Proteus* sp., *Pseudomonas* sp. and *E. coli* due to its biofilm forming potential (Flores-Mireles et al., 2015). Usage of silver nanoparticles as antimicrobial agent can overcome the multidrug resistance and it is a viable alternative when compared to antibiotics (Salomoni et al., 2017). In the field of medicine, biofilm formations have been considered as the worldwide obstacle in preventing catheter associated infections (Batoni et al., 2016). In this study, AgNPs were successfully synthesized by using coral-associated bacteria. Biosynthesized AgNPs were assessed for their anti-biofilm and antimicrobial activity. In addition, AgNPs were coated on urinary catheter and evaluated for antimicrobial and anti-biofilm potential.

2. Materials and methods

2.1. Isolation and screening of coral bacteria for the synthesis of AgNPs

Bacteria were isolated from the healthy coral samples as described by Koren and Rosenberg (2016). Briefly, coral (*Favites* sp) was collected from Palk Bay located near Mandapam, Gulf of Mannar, Marine National Park in the southeast coast of India, Latitude: 9° 16′ 32.56″ N Longitude: 79° 07′ 25.03″ E) fragments measuring 1 × 1 cm weighing approximately 2 g was collected and rinsed with sterile seawater and then homogenized with phosphate buffer (pH 7.0). The samples were serially diluted and then inoculated on ZoBell marine agar medium. After 48 h of incubation at 30 °C, the morphologically distinct colonies were picked and maintained as pure culture and stored in agar slants at 4 °C. All the isolated bacteria were screened for the synthesis of AgNPs as described by Yugandhar and Savithramma (2016) with necessary modifications. To screen for the synthesis of AgNPs, the bacterial cultures were inoculated in to 96- deep well plate containing 2 ml of nutrient broth (Himedia) and incubated at 37 °C for 48 h with an orbital shaking of 200 rpm. After incubation, cell free supernatant (CFS) was collected by centrifugation at 12000 rpm for 20 min. Then 1 mM Silver nitrate solution was prepared and mixed with the 100 µl of CFS and incubated under both light and dark conditions for 24–48 h and was observed for the visual color change.

2.2. DNA extraction and identification of bacteria

Genomic DNA was extracted and the identification of bacteria was performed using 16S rDNA gene sequence (Lane, 1991). The universal primers namely, forward primer 16S-27F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and reverse primer 16S-1492R (5′-ACG GCT ACC TTG TTA CGA CT-3′) were used for the 16S rDNA amplification. The amplified products were then visualized on a 1% agarose gel electrophoresis containing ethidium bromide. The amplified DNA was purified using DNA purification kit (Thermo Fischer) and then sequenced and the retrieved sequence was submitted to Genbank for accession number. The obtained 16S rDNA gene sequence was compared and a phylogenetic tree was constructed using the MEGA software.

2.3. Biosynthesis of AgNPs

The bacterial isolate MGL-D10 was inoculated into a 250 ml Erlenmeyer flask containing 100 ml of sterile nutrient broth and the culture flask was incubated at 200 rpm at 37°C for 48 h. AgNPs were extracted from the biosynthesized medium by centrifugation at 10000 rpm for 30 min and the pellet obtained was washed repeatedly with sterile distilled water to remove the impurities. The resulting pellet was air dried and used for characterization studies. Biosynthesized AgNPs were dissolved in sterile distilled water and the formation of AgNPs was analyzed using UV–Vis spectroscopy (Agilent) in the range of 200–800 nm. For the functional group determination of the synthesized AgNPs, FTIR analysis was performed using the spectrum in transmittance mode in the range of 400–4000 cm^{-1}. X-ray diffraction pattern of the AgNPs was recorded using a computer controlled XRD-system (JEOL) and the data was used for identifying the lattice parameter and the structure of the nanoparticles (Zaki et al., 2011; Zhang et al., 2016; Anandalakshmi et al., 2016).

2.4. Particle size determination using microscopic analysis

The size and morphology of the synthesized AgNPs was determined by microscopic image analysis. The size of the synthesized particles was evaluated using transmission electron microscopy (JEOL 3010 HR-TEM). Samples for TEM analysis were prepared by placing a drop of the synthesized AgNPs on a carbon coated copper drips and was allowed to air dry and then examined under TEM. Microstructure and the sample homogeneity were determined by SEM/EDS Hitachi (Japan) S3400-N Gold ion sputtering (E-1010) – Hitachi: 15 seconds EDS: Horiba – Emax – H7021. Atomic force microscope (AFM-SPA 400, Seiko instruments) was operated under tapping mode and was used to determine the morphology of the synthesized nanoparticle (Kiran et al., 2017).

2.5. Antimicrobial screening of AgNPs

Antimicrobial activity of AgNPs MGL-D10 against urinary tract infection (UTI) causing pathogens was assessed by using well diffusion method (Shanmughapriya et al., 2008; Kiran et al., 2014). The UTI causing pathogens such as *Bacillus* sp, *C. albicans, E. coli, K. pneumonia, P. aeruginosa*, and *S. aureus* were collected from clinical laboratories. The assay was performed using AgNPs MGL-D10 dissolved in sterile distilled water at a concentration of 50 µg/ml. Mueller-Hinton agar plates were prepared and wells were made using a sterile steel cork borer and 50 µl of stock AgNPsMGL-D10 solution was added into the wells and incubated at 37 °C for the formation of zone of inhibition.

2.6. Minimal inhibitory concentration (MIC) determination

To determine the MIC, AgNPs MGL-D10 of different concentrations of 5–80 µg/ml was prepared and added to a sterile 96 well plate, containing 2 ml of LB broth and inoculated with 100 µl of overnight grown cultures of the test pathogens and incubated at 37 °C for 16–18 h. After incubation, OD was measured at 620 nm in a UV–Vis spectrophotometer.

2.7. Antibiofilm effect of AgNPs

Potential biofilm forming clinical isolate *S. aureus* was identified using a Congo red assay and was used in the biofilm inhibition assay. Initial dose selection of AgNPs was performed based on microtitre plate assay as described in the literature (Kiran et al., 2017). Concentrations of AgNPs MGL-D10 includes 25, 50 and 100 µg/ml and the assays were carried out in 96-well plates. Confocal analysis was carried out based on the protocol mentioned in Kiran et al. (2017). Briefly, overnight grown culture of *S. aureus* was inoculated into sterile LB broth containing 50 µg/ml of AgNPs MGL-D10 and flasks with *S.aureus* culture was set as the control. Sterile glass cover slips measuring 1 × 1 cm was placed inside the LB broth using sterile forceps. After incubation at 37 °C for 48 h, the cover slips were rinsed with PBS to remove loosely attached planktonic cells and then stained with 0.1% acridine orange, air dried

and examined under the confocal microscope.

2.8. Effect on coating of AgNPs in catheter

Effect of AgNPs on catheter coating was verified using antimicrobial assay. Briefly, all the UTI causing pathogens were swabbed on to the surface of MHA plates. To this coated and uncoated catheter was placed and incubated at 37 °C for 24 h and observed for zone of inhibition. Among the pathogens tested for antimicrobial catheter assay, a representative strain of *S. aureus* was selected for catheter biofilm assay. Urinary catheters were cut into pieces of 1 × 1 cm and were immersed in the synthesized AgNPs MGL-D10 at a concentration of 50 µg/ml and incubated for 4 h with shaking for coating these evenly. Then the catheter pieces were washed with sterile distilled water to remove excess AgNPs MGL-D10 which may induce aggregation. The pieces were then air dried under sterile conditions in laminar air flow chamber. The AgNPs MGL-D10 coated catheter was used for the antimicrobial assay (Eloff, 1998) with suitable modifications. Briefly, MHA plates were prepared and swabbed with overnight grown culture of the UTI causing pathogens. Then the coated pieces of catheter were placed on the surface of MHA plates and incubated at 37 °C for 24 h and observed for the zone of inhibition. The effect of AgNPs MGL-D10 on biofilm formation in catheter was assessed using 2, 3, 5,-triphenyltetrazolium chloride (TTC) assay. In brief, 5 ml of LB broth *S. aureus* culture was allowed to form biofilm on the AgNPs coated catheter pieces during incubation at 37 °C for 48 h. The flasks containing uncoated catheter served as the control. After incubation, the catheter was washed with sterile PBS solution to detach loosely bound cells. Then the catheter was placed on 6-well plate containing LB broth and 100 µl of TTC solution (0.5% w/v) and observed for the color change.

3. Results

3.1. Screening of bacteria for the biosynthesis of AgNPs MGL-D10

Among the 54 coral bacterial isolates, the strain MGL-D10 was selected based on the change of $AgNO_3$ solution from colorless to brown color. This was due to the reduction of Ag + into AgO from $AgNO_3$. Visual color change of the silver nitrate solution after addition of cell-free supernatant is shown in Fig. 1A and B. For further confirmation of AgNPs MGL-D10 synthesis, a spectrum was recorded at a wavelength range of 200–800 nm. A single surface plasmon resonance peak at 418 nm was obtained within 24 h (Fig. 1C). The color change of yellow to brown and surface plasmon resonance peak at the wavelength of 410–450 nm indicates the synthesis of AgNPs and single peak indicates the synthesis of spherical AgNPs of size < 50 nm.

3.2. Identification of AgNPs-MGL-D10 bacteria

The evolutionary history was inferred by using Kimura 2-parameter model based maximum likelihood method. Neighbor-Joining and BioNJ algorithms were used for obtaining iThe ntial trees for the heuristic search. Maximum Composite Likelihood (MCL) approach was used to estimate a matrix of pairwise distances in the Neighbor Joining anf BioNJ algorithms. Superior log-likelihood value was used to select the topology.(Kimura, 1981). Evolutionary rate differences were modelled by using a discrete Gamma distribution among sites (5 categories (+G, parameter = 0.1000). All positions with less than 95% site coverage were eliminated. Evolutionary analysis was conducted using MEGA6 and the tree showed maximum representatives with *Alcaligenes faecalis* (Fig. 2). The sequence was deposited to Genbank with an accession number MF804858.

3.3. Characterization of AgNPs

3.3.1. FTIR and XRD analysis

The FTIR analysis (Fig. 3) was carried out to identify the major functional groups present in the synthesized AgNPs MGL-D10. The spectrum showed seven distinct peaks at 3439.70, 2923.56, 1639.23, 1370, 1231.16, 1028.71 and 544.78 cm^{-1}. The obtained peaks evidenced the presence of capping/stabilization agent within the nanoparticle. A broad peak at 3439.70 indicates O—H stretching vibration which may be due to the presence of alcohol and phenol. Peak at 2923.56 may be due to the presence of aromatic groups in the synthesized AgNPs. Peak at 1639.23 may be due to the presence of proteins. FTIR analysis confirms the presence of biomolecules causing the reduction of AgNPs. XRD diffractogram showed integrated intense peaks at (200), (220), (311), for cubic phase of silver. The peaks obtained were similar to the reported study on AgNPs. XRD pattern analysis showed that the obtained nanoparticles were crystalline in nature.

3.4. Microscopic analysis

Microscopic analysis revealed that the AgNPs synthesized were spherical in nature with almost even size distribution (Fig. 4). EDAX analysis also confirmed the presence of silver ions. TEM results showed the presence of pure silver and the size of the synthesized particles was in the range of 30–50 nm. TEM analysis further evidenced the shape of the synthesized nanoparticles as spherical. Further AFM analysis also proved the synthesized AgNPs MGL-D10 was uniform in shape with spherical morphology.

3.5. Antimicrobial activity against UTI pathogens

Antimicrobial activity of the synthesized AgNPs against urinary pathogens was assessed. The greater zone of inhibition was observed for *P. aeruginosa,* followed by *S. aureus, Bacillus Sp., C. albicans, E. coli,* and *K. pneumonia* (Fig. 5). On MIC determination 10 µg concentrations of AgNPs were found to be effective in inhibition of 50% of the UTI causing pathogens and 30–40 µg of AgNPs was found effective in

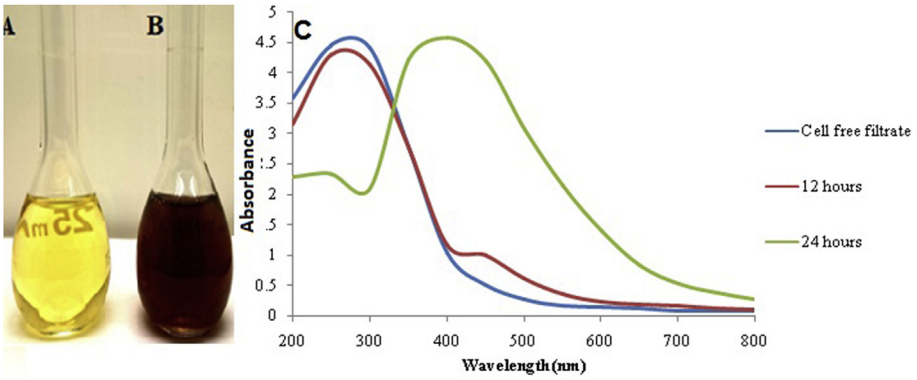

Fig. 1. A) Cell free supernatant of MGL-D10 B) color change of the supernatant after the addition of silver nitrate solution indicating the biosynthesis of AgNPs after 24 h C) UV- Vis spectrum analysis of MGL-D10 indicating peak at 418 nm. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

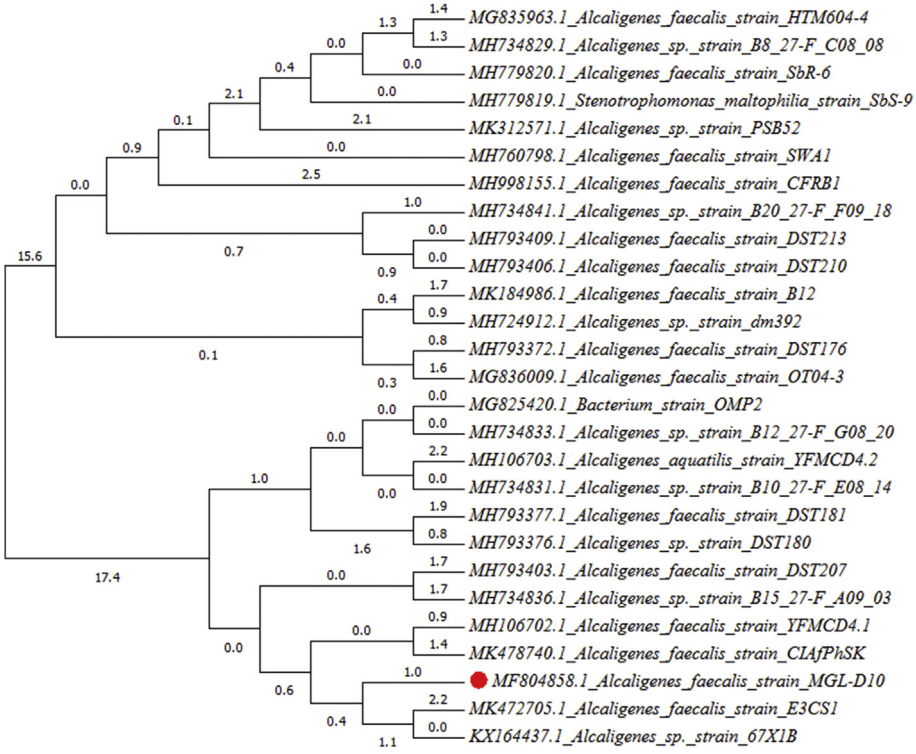

Fig. 2. Phylogenetic analysis of MGL-D10 by Maximum Likelihood method showed cluster with *Alcaligenesfaecalis.*

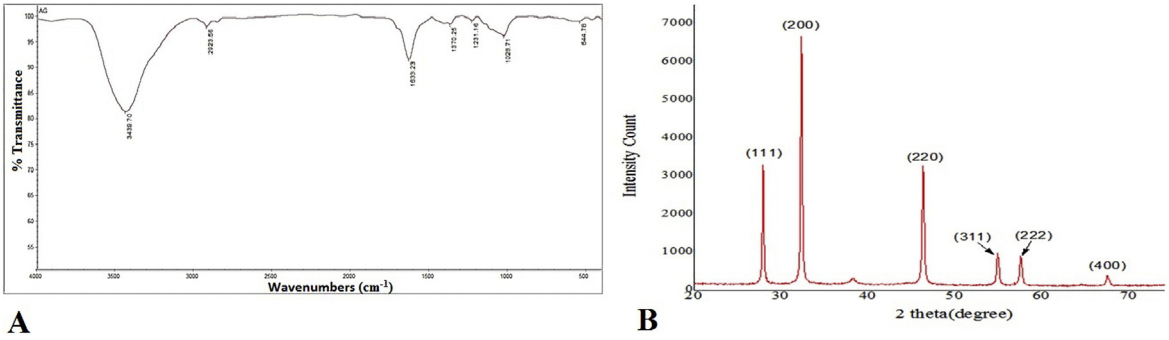

Fig. 3. A) FT-IR spectra and B) XRD analysis of synthesized silver nanoparticles MGL-D10.

inhibition of both Gram positive and Gram negative bacteria as well as *C. albicans* (Fig. 6).

3.6. Antibiofilm potential of AgNPs MGL-D10

Based on the preliminary microtitre growth inhibition assay AgNPs of 50 µg/ml was found effective in inhibition of *S. aureus.* In confocal microscopy image analysis, the control biofilm of *S. aureus* showed dense agglomeration of colonies (Fig. 7A) whereas AgNPs MGL-D10 treated showed protection against the biofilm formation (Fig. 7B). The concentration of 50 µg/ml was found to be effective in disrupting the biofilm. Bacteria within the biofilm are highly resistant to antibiotics and hence nanoparticle-based treatments are effective to prevent biofilm formation.

3.7. Effect of AgNPs on coating urinary catheter

On assessment of antimicrobial activity of AgNPs MGL-D10 on catheter, the coated catheter showed significant zone of inhibition whereas the uncoated catheter did not show any inhibition against the pathogens *Bacillus* sp, *C. albicans, E. coli, K. pneumonia, P. aeruginosa*, and *S. aureus*. The results obtained revealed that the silver coated catheter was found to be effective in controlling urinary tract infections causing pathogens (Fig. 8). Biofilm formation and its assessment using TTC assay revealed the uncoated catheter showed red formazan color indicating the viable cells and in the AgNPs MGL-D10 coated catheter no red color formation was observed as an evidence of inhibitory effect of the synthesized AgNPs MGL-D10 on biofilm formation (Fig. 9). The MIC dose of 30 µg/ml of synthesized AgNPs was used for the coating assays and it showed significant effect on antimicrobial and biofilm formation. Low concentrations of the nanoparticles are always recommended for medical applications and for coating of biomedical devices for better biocompatibility.

4. Discussion

Silver is known for its antimicrobial activity and in the form of nanoparticles they emphasize maximum potential to enter the bacterial cells and inhibit DNA synthesis by altering metabolic enzymes of the bacteria (Salomoni et al., 2017). AgNPs can release silver ions (Ag+) which can interfere with the enzymes and sulphydryl groups of proteins. Ag + causes oxidative stress to the bacteria and thus exhibits

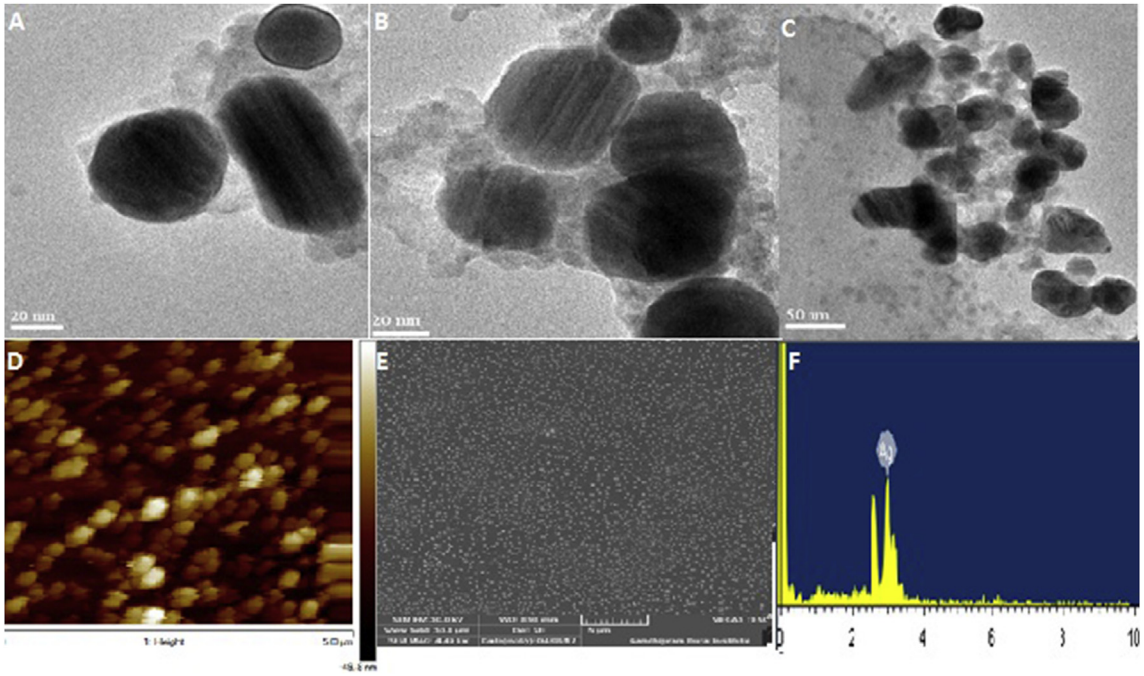

Fig. 4. Microscopic analysis of the nanoparticles showing spherical shape and evidences the size of the particles were in the range of 30–50 nm.

antimicrobial activity. Recent reports evidenced antibacterial resistance towards antibiotics, bacteria have started exhibiting resistance towards AgNPs on continuous exposure (Yun'an Qing et al., 2018). So, there is a need to find out potential and stable AgNPs and assess their stability on coated implants. AgNPs are widely used in numerous fields with diverse applications due to their antibacterial activity and other biocompatible properties (Chernousova and Epple, 2013). AgNPs can be synthesized through several techniques such as chemical, physical, photochemical, laser ablation, irradiation methods and through green synthesis or other biological methods. (Iravani, 2014). Biological methods include, utilizing bacteria for synthesizing AgNPs and bacterial synthesis of AgNPs and is preferred much because of their capping capability (Ahmed et al.,

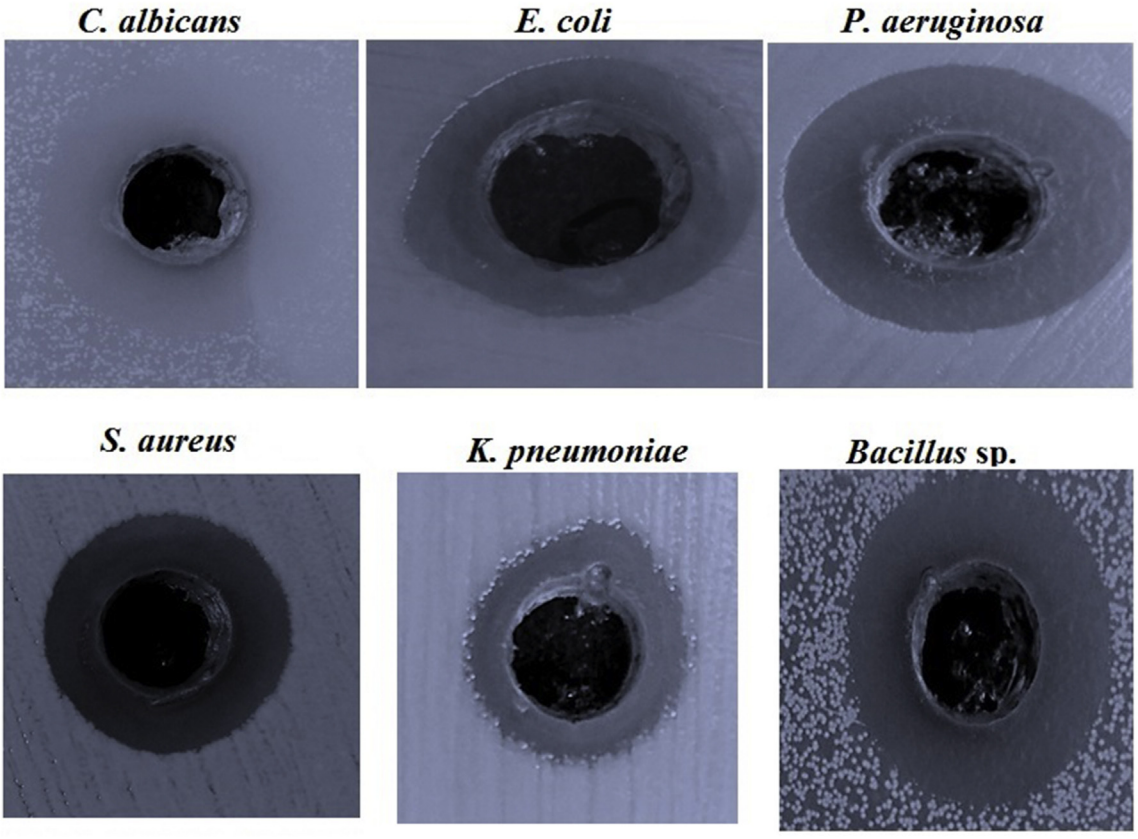

Fig. 5. Assessment of antimicrobial activity using well diffusion method for the pathogens *C. albicans*, *E.coli*, *P. aeruginosa*, *S.aureus*, *K.pneumoniae* and *Bacillus* sp.

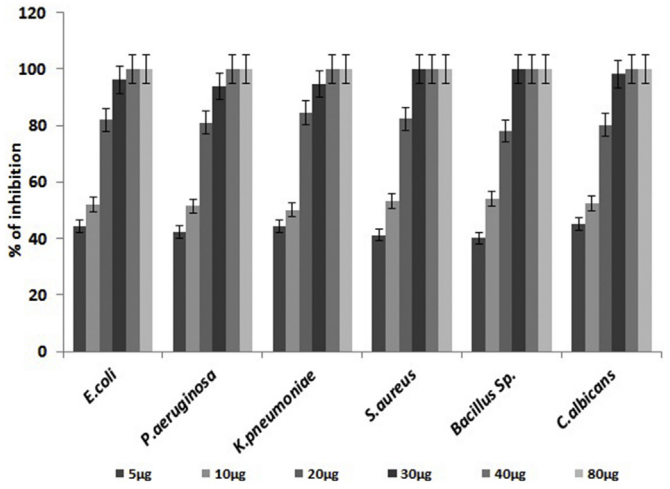

Fig. 6. MIC determination of AgNPs MGL-D10 against UTI pathogens and it was observed 30–40 µg/ml was effective in the inhbition of both Gram positive and Gram negative bacteria as well as *C. albicans*.

2016). Synthesis of AgNPs with aid of bacteria is emerging as an alternative source and a promising field for future therapeutics. The bacteria mediated synthesis of nanoparticles has been found to be more stable (Kalishwaralal et al., 2008), fast, non-toxic, possessing well-defined morphology and uniformity in sizes (Gurunathan et al., 2015). The literature emphasizes the shape and size of the AgNPs is important because antibacterial or inhibition activity against pathogens is shape dependent (Leuck et al., 2015). Coral-associated bacteria are known for the production of antimicrobial compounds and other significant enzymes which exhibit antimicrobial activity (Zaheer, 2012). This study attempts to identify the AgNP synthesizing bacteria and focuses on the assessment of characterization, antimicrobial activity and stability of AgNPs synthesized from coral associated bacteria. This study is the first report on coral-associated bacteria for AgNP synthesis. Healthy coral fragments were collected from southeastern coast of India which is home to many coral reefs. A total of 54 isolates were obtained and screened for the synthesis of AgNPs. The strain, MGL D10 was found to exhibit maximum AgNPs synthesis within 48 h. Based on 16S rDNA gene sequence, the AgNP synthesizing MGL-D10 was identified as *Alcaligenes faecalis* and it is well known for heavy metal resistance (Abo-Amer et al., 2015). Both cell pellets and cell free supernatant was screened for synthesis of AgNPs. The reduction was visualized in the cell free supernatant, this indicates biomolecules produced extracellular by the bacteria may be responsible for AgNPs synthesis. Within 24 h AgNps reduction was observed, generally biological methods are considered for slow production of AgNPs synthesis. Several studies suggest that a broad peak between 410–450 nm attributes to the spherical nature of the nanoparticles (Jyoti et al., 2016). Stability and soluble nature of the AgNPs are also considered as an important factor (Stevens et al., 2009) for the synthesized AgNPs. MGL-D10 was found to be stable, soluble in water and is crystalline in nature. The strain *Alcaligenes faecalis* is already known for activities like heavy metal resistance and AgNP synthesis (El-Deeb et al., 2013). FTIR analysis showed stretch bands and bonds of AgNPs, and the presence of biomolecules capped in the synthesized AgNPs was identified. Few reports suggest that higher antibacterial and antibiofilm activity of biologically synthesized AgNPs is due to the surface attached biomolecules (Singh et al., 2016). The biologically (bacterial) mediated synthesis of AgNPs is non-toxic to the environment, cheap and there is no need of any additional capping or stabilizing agents (Makarov et al., 2014). XRD analysis revealed the crystalline nature of AgNPs and the peak detection confirmed the presence of silver. The structure and size of AgNPs was detected through SEM, TEM and AFM as spherical and with uniformity in size. The AgNPs MGL-D10 exhibits antimicrobial activity against all the tested pathogens such as *Bacillus* sp, *C. albicans*, *E. coli*, *P. aeroginosa*, *K. pneumonia*, and *S. aureus* which are considered as common clinical pathogens causing UTI. These clinical pathogens are often reported for the nosocomial infection and secondary complications while using medical devices. Antibacterial activity assessment showed inhibition of all the test clinical pathogens. This may be due to the smaller size and ability to penetrate the cell wall. AgNPs disrupt the structure of the cell membrane of bacteria making the drug-resistant bacteria to susceptible (Li et al., 2010). In recent years, consequences and the adverse effects of catheter-associated urinary tract infections which may lead to mortality have been on the rise (Nicolle, 2012). Hence, medical devices coated with antimicrobial agents are important for avoiding bacterial growth and biofilm formation which is the primary cause of infections. AgNPs are known for their antimicrobial activities and they are the most excellent tools for avoiding catheter-associated microbial infections (Morones et al., 2005). Confocal analysis evidences the anti-biofilm effect of AgNPs MGL-D10 and the experiments using TTC proved the effectiveness of the coating of AgNPs MGL-D10 on the urinary catheter. This study reveals that AgNPs can be used as an effective tool to inhibit pathogens by coating medical devices with such nanoparticles.

5. Conclusion

Biogenic synthesis of AgNPs was achieved using coral-associated bacteria. The AgNPs showed strong antibiofilm and antimicrobial activity against clinical pathogens causing UTI infections. The CLSM images showed effective disruption of *S. aureus* biofilm in AgNPs MGL-D10 treatment. Catheter experiments showed antibiofilm and antimicrobial effect of synthesized AgNPs MGL- D10. Therefore, precoating of synthesized AgNPs MGL- D10 on catheters can act as a protective shield aganist UTI causing pathogens. This study demonstrates AgNPs based method to develop treatment approaches to check urinary tract infection causing pathogens and nosocomial infections.

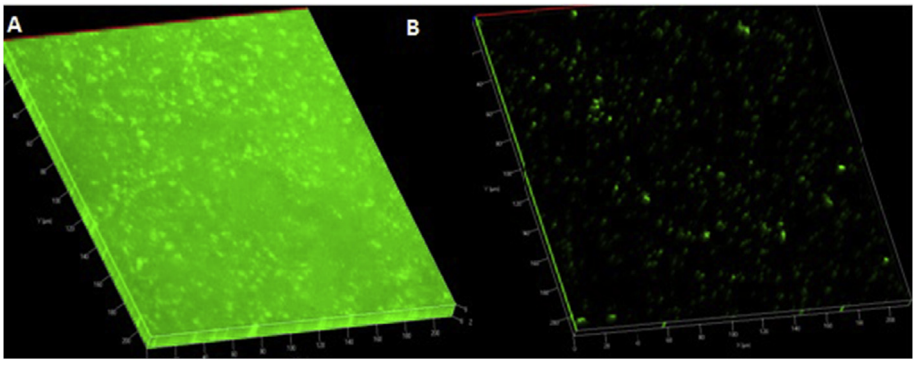

Fig. 7. Antibiofilm effect of AgNPsMGL-D10 as evident by confocal microscopy image analysis.

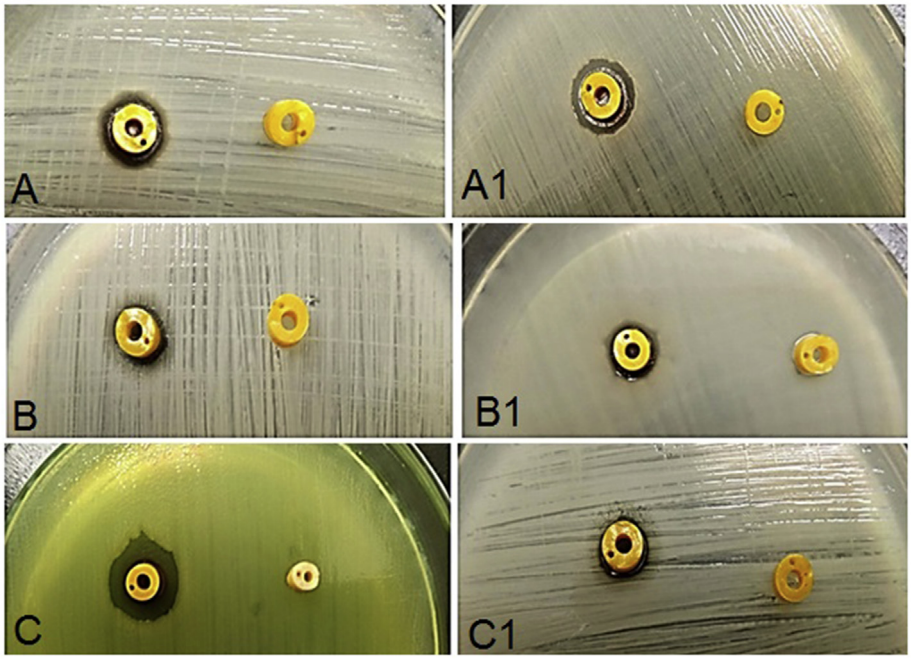

Fig. 8. Antimicrobial activity of AgNPsMGL-D10 coated on the catheter against UTI pathogens.

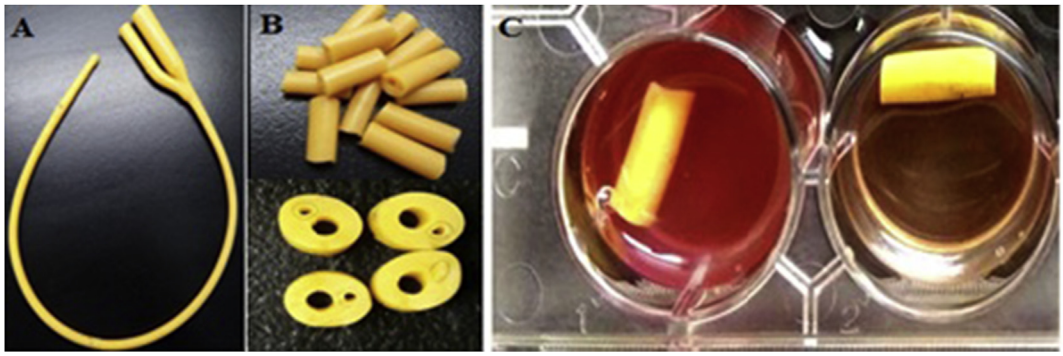

Fig. 9. Biofilm formation assessment using TTC assay evidences the AgNPsMGL-D10 coated catheter showing absence of red color whereas the uncoated catheter showed red color indicating the viable cells of *S. aureus.* (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

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