American Journal of Microbiological Research, 2016, Vol. 4, No. 5, 132-137
Available online at http://pubs.sciepub.com/ajmr/4/5/1

DOI:10.12691/ajmr-4-5-1

Synergistic Effect of Biogenic Silver-nanoparticles with β. lactam Cefotaxime against Resistant *Staphylococcus arlettae* AUMC b-163 Isolated from T3A Pharmaceutical Cleanroom, Assiut, Egypt

M. H. A. Hassan, M.A. Ismail, A.M. Moharram, A. Shoreit*

Department of Botany and Microbiology, Faculty of Science, Assiut University, Egypt
*Corresponding author: ahmedshoreit@yahoo.com

Abstract The aim of this study was to biosynthesis silver nanoparticles (AgNPs) from *Staphylococcus arlettae* AUMC b-163 isolated from T3A pharmaceutical company cleanroom, its antimicrobial activity, and the synergistic effect of AgNPs in combination with commonly used antibiotic Cefotaxime sodium against resistant bacteria. The synthesized AgNPs from bacterial were characterized by using UV-VS spectrophotometer analysis, Fourier Transform Infrared Spectroscopy (FTIR), X-ray diffraction (XRD) and Transmission Electron Microscopy (TEM).UV-VS spectrophotometer analysis showed a peak at 420 nm corresponding to the Plasmon absorbance of silver nanoparticles and FTIR analysis showed the potential biomolecule responsible for the reduction of silver. The structural properties of silver nanoparticles were confirmed using XRD technique, while TEM micrographs revealed that the silver nanoparticles are dispersed and aggregated, and mostly having spherical shape within the size range between 8 and 35 nm. The synthesized silver nanoparticles exhibited a varied growth inhibition activity against the tested pathogenic bacteria. A significant increase in area of growth inhibition was observed when a combination of silver nanoparticles and Cefotaxime antibiotics was applied. The current results revealed that the synthesized silver nanoparticles produced by the bacterial strain *Staphylococcus arlettae* AUMC b-163 is a promising to be used in medical therapy due to their broad spectrum against some pathogenic bacteria, fungi and resistant tested bacteria.

Keywords: *antibacterial activity, β. lactam antibiotics, nanoparticles, Synergistic effect*

Cite This Article: M. H. A. Hassan, M.A. Ismail, A.M. Moharram, and A. Shoreit, "Synergistic Effect of Biogenic Silver-nanoparticles with β. lactam Cefotaxime against Resistant *Staphylococcus arlettae* AUMC b-163 Isolated from T3A Pharmaceutical Cleanroom, Assiut, Egypt." *American Journal of Microbiological Research*, vol. 4, no. 5 (2016): 132-137. doi: 10.12691/ajmr-4-5-1.

1. Introduction

Cleanrooms, where pharmaceutical medicaments were manufactured in pharmaceutical industries must be present in free of contamination by microorganisms, which may be inserted from exposure to air condition or contact with human body working there. Although the strict precautions some microorganisms allowed to be present in few amounts. During recent years resistance acquired by the pathogenic microorganisms to various available antibiotics has become a serious health issue [1]. Therefore, there is an urgent demand to find out new bactericidal materials, which counter act against bacterial resistance among them. Recent studies have shown that combining nanoparticles with antibiotics not only reduces the toxicity of both agents towards human cells by decreasing the requirement for high dosages but also enhances their bactericidal properties. Combining antibiotics with nanoparticles also restores their ability to destroy bacteria that have acquired resistance to them. Furthermore, nanoparticles tagged with antibiotics have been shown to increase the concentration of antibiotics at the site of bacterium–antibiotic interaction, and to facilitate binding of antibiotics to bacteria. Hence, nanoparticle-based antibacterial formulations could be effective bactericidal materials, as they will exhibit combined effects of silver and antibacterial agents. The enhanced activity of silver nanoparticles and antibiotics together has been reported earlier [2,3,4,5]. Green nanotechnology is to utilize microbial resources for the fabrication of nanoparticles. In the recent past, nanoparticles have been synthesized using various bacterialike *Bacillus licheniformis*, *Staphylococcus aureus*, and *Pseudomonas stutzeri* [6,7,8]. Compared with chemical and physical methods, biological synthesis of nanoparticles has proved to be free of any limitations associated with production of hazardous by products, and it is simple and cost effective in nature [9,10]. It can be expected that the high specific surface area and high fraction of surface atoms of silver nanoparticles will lead to high antimicrobial activity as compared with bulk silver metal. It was also interesting to note here that silver nanoparticles were able to exert inhibitory effect at a concentration that is below their cytotoxic limits,so they

were regarded as safe to be used as antimicrobials [11]. In this paper, a strain *Staphylococcus arlettae* AUMC b-163 was identified on Phenotypic and Phylogenetic characterization, studied the antibacterial activity of biogenic Silver-nanoparticles, characterized by UV-vis spectrophotometer, FTIR, XRD, TEM and itssynergistic effect with a β-lactam antibiotic in the third-generation class of cephalosporin's, (Cefotaxime sodium) against resistant strain S*taphylococcus arlettae* AUMC b-163 isolated fromT3A cleanroom, Assiut, Egypt.

2. Materials and Methods

2.1. Source of Microorganism

Air samples were collected from pharmaceutical cleanroom, at T3A pharmaceutical company Assiut, Egypt, using air sampler (Reuter Centrifugal Sampler), model RCS high flow (Biotest Hicon) with a flow rate of 100 L/min for 10 minutes, after sampling agar strip were incubated for 48 hours at 32° [12] the isolated colony were subcultured and obtained in pure culture, characterized henotypically & Phylogenetically identified and maintained at 4°C at Assiut University Mycological Center as (AUMC b-163) and selected for further study.

2.2. Phenotypic Characterization

Confirmation of the species, of the bacterial isolates was done by Gram staining and various biochemical tests.

2.3. Phylogenetic Characterization

The bacterial isolate of AUMC b-163was sent to Sol Gent Company (Daejeon, South Korea) for RNA gene sequencing. The sequence of the 16s rRNA gene has been widely used as a phylogenetic marker to study genetic relationships between different strains of bacteria. The analysis of this gene can therefore be considered as a standard method for the identification of bacteria at the family, genus and species levels, [13] bacterial DNA was extracted and isolated using SolGent purification bead. prior to sequencing, the ribosomal rRNA gene was amplified using the polymerase chain reaction (PCR) technique in which two universal bacterial primers 27F (forward) and 1492R (reverse) were incorporated in to the reaction mixture, primers used for gene amplification has the following composition: 27 F (5'- AGA GTT TGA TCM TGG CTC AG - 3'), and 1492R (5'- TAC GGY TAC CTT GTT ACG ACT T- 3').Sequence was further analyzed using BLAST from the national Center of biotechnology information (NCBI) website , phylogenetic analysis of sequences was done with the help of Meg Aligen (DNA star) software version 5.05.

2.4. Assay for the Synthesis of Nanoparticles

For silver nanoparticles biosynthesis studies, 250-ml Erlenmeyer conical flasks containing 100 ml nutrient broth were prepared, autoclaved and inoculated with 1 ml of the test strain. The inoculated flasks were incubated in a rotating shaker set at 200 rpm for 48 h at 35°C. After the end of incubation period, the cultures were centrifuged at 12,000 rpm for 10 min. Supernatant of the organism added to reaction vessels containing filter-sterilized AgNO3 solution at 0.1M final concentration. At the same time, experimental control containing supernatant without silver nitrate was prepared. All the reaction mixtures were incubated on rotating shaker (200 rpm) at 35°C for a period of 72 h in light.

2.5. Characterization of Silver Nanoparticles

2.5.1. Visual Observations:

The formation of AgNPs was followed by visual observation of color change into reddish color that indicated the reduction reaction [14].

2.5.2. UV-Vs Spectral Analysis

The reduction of pure Ag^+ was further confirmed by the sharp peak given by scanning the AgNPs of the reacting solution using Perkin-Elmer Lamda-45 spectrophotometer, in a 1cm path quartz cell at a resolution of 1 nm from 300 to 800 nm [15].

2.5.3. Fourier Transforms Infrared Spectroscopy (FTIR):

FTIR spectra of dried synthesized AgNPs were recorded using FTIR Nicolet Avatar 660 FTIR spectrometer [16].

2.5.4. X-ray Diffraction (XRD) Analysis

The synthesized AgNPs were dried and the powder form the sample was subjected for XRD analysis using X-ray diffractometer (Model PW 1710 control unit Philips Anode material Cu, 40 KV, 30 M.A, optics: Automatic divergence slit) with Cu Kα radiation λ=1.540562 °A [17].

2.5.5. Transmission Electron Microscopy (TEM) Analysis

The morphology of the AgNPs were investigated by TEM using JEOL- JEM-100 CXII instrument in electron microscope unit in Assiut University. By drying a drop of the washed colloidal dispersion onto a copper grid covered with a conductive polymer

2.6. Antimicrobial Activity of Bacterial Supernatant and AgNPs by Agar Well Diffusion Method

Silver nanoparticles (AgNPs) synthesized from test strain *Staphylococcus arlettae* AUMC b-163, and bacterial supernatant were tested for antimicrobial activity by agarwell - diffusion methods against various pathogenic organisms. The test organisms used were related to gram-negative bacteria (*Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027) and gram-positive bacteria (*Staphylococcus aureus* ATCC 6538P), and the fungal strains (*Candida albicans* ATCC 10231, and *Aspergillus niger* ATCC 16404). Each strain was swabbed on the surface of individual plates using sterile cotton swab, wells of size 6 mm diameter were made on nutrient agar plates, with the help of a sterilized corkborer. Different concentrations (100, 50, 25, 15 µL) of the AgNPs and bacterial supernatant was loaded separately into wells and all the plates were incubated at 35°C for 24h in case of bacterial strains and at 28°C for 72 h for fungal strains. The zones of inhibition were measured and

the lowest concentration of AgNPs and bacterial supernatant that inhibited the growth of the test organisms was recorded as the minimum inhibitory concentration (MIC) [16,18]

2.7. Evaluation of Synergistic Effects of AgNPs Combined with Cefotaxime Sodium Antibiotic

In this experiment, AgNPssynthesized from resistant strain *Staphylococcus arlettae* AUMC b-163 was used for studying the synergism between synthesized AgNPs andβ. lactam cefotaxime sodium antibiotic. Well-diffusion method on nutrient agar plates was used to assess the synergistic effect. Different concentrations of the tested antibacterial (200, 150, 100, 50, 25 and 12.5 µg /ml) were prepared to determine MIC. A 25 µL of freshly prepared AgNPs and 25 µL of each concentration of the antibacterial were loaded into each well as the final content of 50 µL of AgNPs and antibacterial per well. After incubation at 35°C for 24 hours, the zones of inhibition were measured, the assays were performed in triplicates [3]

2.8. Statistical Analysis

Results were compared statistically to understand the level of significance using Graphpad Prism v5.00 statistical software. The results are presented as mean ± standard deviation (S.D.). Statistical significance was set at $p < 0.05$.

3. Results

3.1. Phenotypic Characterization:

Staphylococcus arlettae AUMC b-163 is a Gram-positive coccus, 0.5–1.5 µm, non-motile, nonspore forming and aerobic growth. Colonies beige or yellow, opaque, with entire margins, 6–8 mm in diameter after 2 days' incubation at 37°C on brain heart infusion agar. Biochemical characterization,give positive results for catalase, alkaline phosphate, and acid production from L-arabinose, arbutin, beta-gentibiose, fructose, D-fucose (weak), glucose, lactose, trehalose, mannitol, raffinose, xylose, melezitose, turanose, ribose & sucrose. Negative results for nitrates reduction, oxidase, hyaluronidase, staphylokinase, Tween 80 hydrolysis, betaglucosidase, urease, acid production from: xylitol, cellobiose, rhamnose, sorbitol & salicin.

3.2. Phylogenetic Characterization:

The selected strain was isolated from pharmaceutical cleanroom was further subjected to molecular identification method. The sequence data of the bacterium was obtained and compared with the available gene sequences from organisms in the GenBank database. The AUMC b-163 strain was showed a very high percentage of similarity (99%) with the sequence of *staphylococcus* sp. mainly *Staphylococcus arlettae* (Figure 1). and it was maintained at Assiut University Mycological Center as *Staphylococcus arlettae* AUMC b-163. The GenBank accession numbers are represented in parentheses.

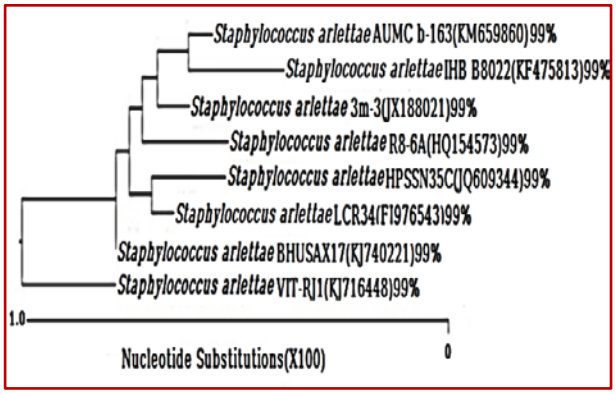

Figure 1. Phylogenetic analysis of 16S rRNA gene of isolates AUMC b-163 and other related *Staphylococcus arlettae*, GenBank accession number given in parentheses

3.3. Characterization of Silver Nanoparticles

3.3.1. Visual Observations

The color change from pale yellow to reddish after 24 h of incubation in the presence of light with *Staphylococcus arlettae* AUMC b-163 as shown in Figure 2. At the same time, experimental control containing supernatant without 0.1 M silver salt showed no color change when incubated at the same environmental condition (Figure 2).

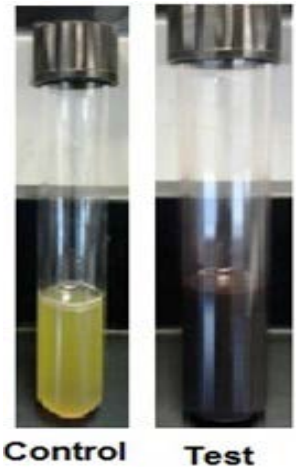

Figure 2. culture of *Staphylococcus arlettae* AUMC b-163 with and without silver salt (0.1M), Test: color change to reddish after 24 h of incubation in the presence of salver salt. Experimental control: showed no color change

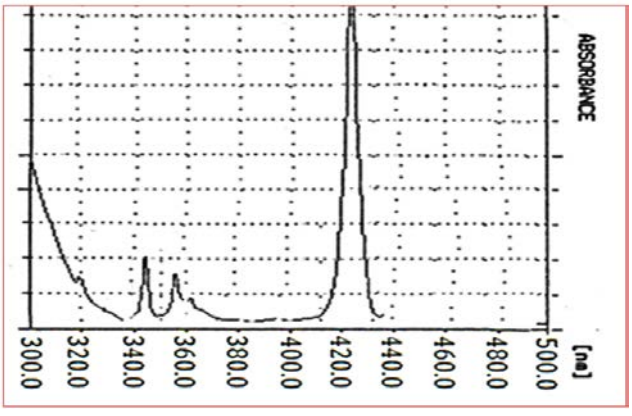

Figure 3. UV –Vis spectral analysis of AgNPs absorption band at 420 nm

3.3.2. UV –Vis Spectral Analysis

Bioreduction of silver nitrate ions was confirmed by the sharp peaks given by the AgNPs in the visible region from UV–VS spectrum of the reacting solution, (Figure 3) showed that,the UV–Vis absorption spectrum of silver nanoparticles exhibiting a strong broad peak at 420 nm. The spectrum showed a strong surface Plasmon absorption band indicating the presence of AgNPs.

3.3.3. FTIR analysis of Ag NPs:

The nature of the biomolecules involved in the reduction and formation of AgNPs was studied by FTIR (Figure 4). The FTIR signals of AgNPs were observed at 1078, 1290, 1648, 2140, 2410, 2963 and 3269 cm^{-1}. FTIR measurement was carried out to identify the potential biomolecule in enzyme filtrate responsible for the reduction of silver ions and capping agent responsible for the stability of the bioreduced silver nanoparticles. The FTIR spectra in 1400-1700 cm^{-1} region provides information about the presence of "C=O" and "N=H" groups which is responsible for the reduction of AgNO3 to Ag. The peaks in the region between 3269 to 2140 cm^{-1} were assigned to O-H stretching of alcohols and phenol compounds and aldehyde -C-H- stretching of alkanes. The peaks in the region 1078 and 1290 to 1648 cm-1 corresponds to N-H of primary and secondary amides and –C-N- stretching vibrations of amines or –C-O- stretching of alcohols, ethers, carboxylic acids and anhydrides.

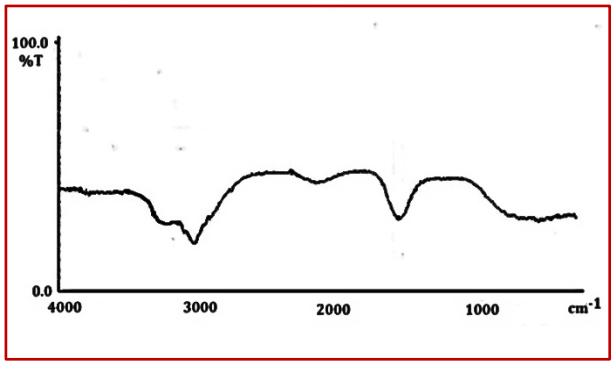

Figure 4. FTIR spectra of synthesized AgNPs of *Staphylococcus arlettae* AUMC b-163

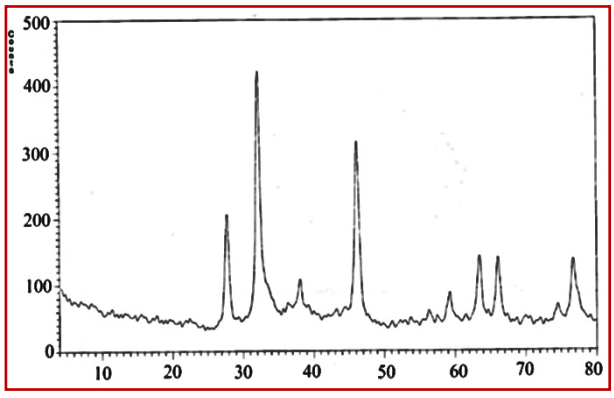

Figure 5. XRD pattern of synthesized AgNPs of *Staphylococcus arlettae* AUMC b-163

3.3.4. X-ray diffraction (XRD) Analysis

The crystalline nature of the particles was confirmed using XRD. Figure 5 shows X-ray powder diffraction patterns of the synthesized AgNPs at 80 °C. The peak positions are consistent with metallic silver. The peaks assigned in the AgNPs sample were 110, 200, 206, 222 and 310. A° number of Bragg reflections with 2θ values of 27.26°, 31.77°, 46.23°, 38.33°, 46.91°, 57.06°, 57.41°, 67.48°, 74.80° and 75.65°.

3.3.5. Transmission Electron Microscopy (TEM) Analysis

Transmission electron microscopy was carried out to know the morphology and size of the biosynthesized silver nanoparticles. The TEM image of AgNPs Figure 6 shown that the particles are spherical in shape and the size of the AgNPs was found to be in the range from 8 to 35 nm.

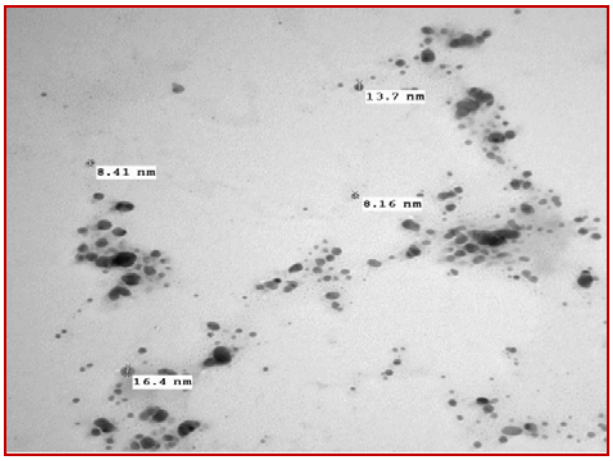

Figure 6. TEM micrograph of synthesized AgNPs showed that particles are spherical in shape and the size range from 8 to 35 nm.

3.4. Antimicrobial Activity and Synergistic Effect of AgNPs in Combination with Cefotaxime Sodium

The AgNPs exhibited good antibacterial activity against Gram-negative bacteria were *E. coli* ATCC 8739 7.66±0.58mm, *P. aeruginosa* ATCC 9027 8.33±1.53mm, gram-positive bacteria *S. aureus* ATCC 6538P 11.33±1.35 mm and resistant*S. arlettea* AUMC b-163 6.33±0.57. It also showed antifungal activity against*C. albicans* ATCC 10231 8.00±2.00 and *A. niger* ATCC 16404 10.00±2.00 mm Figure 7.

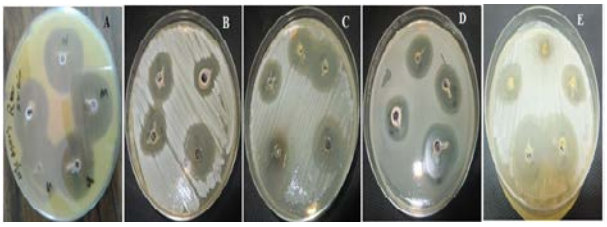

Figure 7. Inhibition zones of AgNPs against *S. aureus* ATCC 6538P (A), *S. arlettae* (R) AUMC b-163 (B), *E. Coli* ATCC 8739 (C), *C. Albicans* ATCC 10231(D) and *A. niger* ATCC 16404(E) culture plates were incubated at 35°C for 24 h in case of bacterial strains and at 28°C for 72 h for fungal strains

On the other hand, the combination of AgNPs with antibacterial agent's cefotaxime sodium was also investigated the inhibition activity of antibiotics increased in the presence of AgNPs against test strains as compared

to antibiotic alone. The maximum fold increase of antibacterial activity in combination with silver nanoparticles plus antibiotic was 85 % (Table 1).

Table 1. Antimicrobial activity of AgNPs synthesized from *S. arlettae* AUMC b-163 and its synergistic effect plus (Cefotaxime sodium antibiotic) expressed as (Zone of inhibition mm ±SD) against pathogenic microorganisms.

Tested microorganisms	Bacterial AgNPs only (50 µL)	Antibiotic only[a] MIC 200 µg/ml (50 µL)	Antibiotic (MIC) + AgNPs [b] (50 µL)	*Fold increase % = ((b−a)/a) × 100	P value
E. Coli ATCC 8739	7.66±0.58	9.66±2.08	11.33±2.08	17.27%	p < 0.05
P. aeruginosa ATCC 9027	8.33±1.53	0	9.33±0.57		
S. aureus ATCC 6538P	11.33±1.53	12.33±1.53	14.00±1.00	13.54%	
C. Albicans ATCC 10231	8.00±2.00	0	9.66±1.15		
A. niger ATCC 16404	10.00±2.00	0	11.66±0.58		
S. arlettae (R)AUMC b-163	6.33±0.57	6.66±0.57	12.33±2.89	85.14%	

* Percentage fold increases of individual antibiotics were calculated using the formula (b − a)/a) × 100 [3].

4. Discussion

The scope of the present study was based upon exploring the capabilities of bacterial strain *S. arlettae* AUMC b-163 to synthesize AgNPs along with their biomedical application in controlling infection. This research showed a great deal of capability towards synthesizing AgNPs. In addition, these nanoparticles alone and in conjugation with Cefotaxime antibiotic proved to be effective as antimicrobial agents.Change in color of the reaction mixture containing Ag salt and fungal cultural filtrate proved to be the first indication of AgNPs synthesis. When the reaction mixture was incubated under light conditions, the color of the liquid mixture changed to reddish after 24 hours of reaction (Figure 2). The change in color intensity (absorbance) was also monitored through ultraviolet-visible spectroscopy; peaks of the reaction mixture were obtained around 420 nm (Figure 3). It was reported that the reduction of Ag+ to atomic silver Ago corresponds to absorption at 420 nm [3,19,20]. Synthesis of AgNPs was previously linked with release of functional proteins in reaction mixture by bacterial supernatant, which might have helped reduction of the metal ions into nanoparticles. Various reports have provided evidence of extracellular generation of AgNPs by XRD and TEM images based upon these analytical [15,17,21]. In this study, *S. arlettae* AUMC b-163 was found to be an effective biological tool for the biosynthesis of stable AgNPs. Based on the results from this study, we can deduce that biologically synthesized AgNPs can be efficiently used as antimicrobial agents.AgNPs have been reported to be an effective as bactericidal agent [11,22,23,24]. Inpresent study the antimicrobial effect of AgNPs prepared from strains *S. arlettae* AUMC b-163 showed a great deal of capability towards synthesizing AgNPs. In addition, these nanoparticles alone and also in conjugation with antibiotics proved to be effective in controlling pathogenic organisms. The mechanism ofaction of AgNPs is still not well defined. Many proposed mechanisms about antibacterial effect of silver ions have been presented by researchers [3,19,20,24,25]. It was hypothesized that AgNPs have large surface area, which allows them to closely interact with antibiotic. The antibiotic molecules contain active groups like hydroxyl and amido groups, which can easily react with AgNPs and disrupt peptidoglycan in the cell wall [3]. Being positively charged, they attack negative charges of transmembrane proteins and can destroy the cell membrane and block the transport channels [20]. It might be possible that they penetrate inside the bacteria and disrupt cellular activities like transportation, protein synthesis, and nucleic acid functioning [26]. Likewise, cefotaxime in combination with positively charged AgNPs both inhibited and disrupted cell-wall synthesis. It has also been proposed that silver ions penetrate the cell intercalate themselves between pyrimidine and purine, and denature the DNA molecule [24].In synergism, the bactericidal effect is enhanced by interaction between antibiotics with AgNPs. As a result, antibiotic–AgNP conjugate is formed in which an AgNP core is surrounded by antibiotic molecules. Thus, the antimicrobial concentration is increased, which leads to increased destruction of bacteria [19]. More studies need to be conducted to find out the exact mechanism of action to develop a novel antimicrobial drug against multidrug-resistant bacteria

5. Conclusion

Antibiotic resistant bacteria have been continuously increasing over the past decade; hence, there is a need to detect another method to overcome this problem. In the present scenario, AgNPs have appeared as a promising antibacterial candidate in the medical field. Hence it could be potentially applied in the fabrication of silver impregnated antimicrobial materials for biomedical applications. We have demonstrated green process for the synthesis of silver nanoparticles from *S. arlettae* AUMC b-163 Within 24 hours of time, spherical shaped nanoparticles from 8 -35 nm size were formed. The method described is highly efficient and cost effective to produce silver nanoparticle. The antibacterial activity of antibacterial antibiotic cefotaxime against resistant *S. arlettae* AUMC b-163 was augmented when impregnated with AgNPs. Based on the results from this study, we can deduce that biologically synthesized AgNPs may function by binding to Thiol (SH) groups of membrane proteins, enzymes, and phosphate groups of DNA, and can be efficiently used as antimicrobial agents.

References

[1] Wright, GDs, “Bacterial resistance to antibiotics: enzymatic degradation and modification” *Advanced drug delivery reviews*, 57 (10), 1451-1470, 2005.

[2] Dar, MA.; Ingle A.; Rai Ms, “Enhanced antimicrobial activity of silver nanoparticles synthesized by *Cryphonectria* sp. evaluated

singly and in combination with antibiotics" *Nanomedicine: Nanotechnology, Biology and Medicine*, 9 (1), 105-110, 2013.

[3] Fayaz, AM.; Balaji K.; Girilal M.; Yadav R.; Kalaichelvan PT.; Venketesan Rs, "Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: a study against gram-positive and gram-negative bacteria" *Nanomedicine: Nanotechnology, Biology and Medicine*, 6 (1), 103-109, 2010.

[4] Geoprincy, G.; Saravanan P.; Gandhi NN.; Renganathan Ss, "A novel approach for studying the combined antimicrobial effects of silver nanoparticles and antibiotics through agar over layer method and disk diffusion method" *Digest Journal of Nanomaterials and Biostructures*, 6 (4), 1557-1565, 2011.

[5] Shahverdi, AR.; Fakhimi A.; Shahverdi HR.; Minaian Ss, "Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against*Staphylococcus aureus* and *Escherichia coli*" *Nanomedicine: Nanotechnology, Biology and Medicine*, 3 (2), 168-171, 2007.

[6] Kalimuthu, K.; Suresh Babu R.; Venkataraman D.; Bilal M.; Gurunathan Ss, "Biosynthesis of silver nanocrystals by *Bacillus licheniformis*" *Colloids and Surfaces B: Biointerfaces*, 65 (1), 150-153, 2008.

[7] Bhainsa, KC.; D'souza Ss, "Extracellular biosynthesis of silver nanoparticles using the fungus *Aspergillus fumigatus*" *Colloids and Surfaces B: Biointerfaces*, 47 (2), 160-164, 2006.

[8] Nanda, A.; Saravanan Ms, "Biosynthesis of silver nanoparticles from *Staphylococcus aureus* and its antimicrobial activity against MRSA and MRSE" *Nanomedicine: Nanotechnology, Biology and Medicine*, 5 (4), 452-456, 2009.

[9] Kim, JS.; Kuk E.; Yu KN.; Kim J-H.; Park SJ.; Lee HJ.; Kim SH.; Park YK.; Park YH.; Hwang C-Ys, "Antimicrobial effects of silver nanoparticles"*Nanomedicine: Nanotechnology, Biology and Medicine*, 3 (1), 95-101, 2007.

[10] Singh, M.; Manikandan S.; Kumaraguru As, "Nanoparticles: A new technology with wide applications" *Res J Nanosci Nanotechnol*, 1 (1), 1-11, 2011.

[11] Arya, V.; Komal R.; Kaur M.; Goyal A.; Town Ms, "Silver nanoparticles as a potent antimicrobial agent: a review" *Pharmacologyonline*, 3 118-124, 2011.

[12] Ljungqvist, B.; Reinmüller Bs, "Airborne viable particles and total number of airborne particles: comparative studies of active air sampling" *PDA Journal of Pharmaceutical Science and Technology*, 54 (2), 112-116, 2000.

[13] White, TJ.; Bruns T.; Lee S.; Taylor Js, "Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics" *PCR protocols: a guide to methods and applications*, 18 315-322, 1990.

[14] Gurunathan, S.; Kalishwaralal K.; Vaidyanathan R.; Venkataraman D.; Pandian SRK.; Muniyandi J.; Hariharan N.; Eom SHs, "Biosynthesis, purification and characterization of silver nanoparticles using *Escherichia coli*" *Colloids and Surfaces B: Biointerfaces*, 74 (1), 328-335, 2009.

[15] Saifuddin, N.; Wong C.; Yasumira As, "Rapid biosynthesis of silver nanoparticles using culture supernatant of bacteria with microwave irradiation" *Journal of Chemistry*, 6 (1), 61-70, 2009.

[16] Silambarasan, S.; Jayanthi As, "Biosynthesis of silver nanoparticles using *Pseudomonas fluorescens*" *Research Journal of Biotechnology Vol*, 8 3, 2013.

[17] Prema, P.; Raju Rs, "Fabrication and characterization of silver nanoparticle and its potential antibacterial activity" *Biotechnology and Bioprocess Engineering*, 14 (6), 842-847, 2009.

[18] Perez, C.; Pauli M.; Bazerque Ps, "An antibiotic assay by the agar well diffusion method" *Acta Biol Med Exp*, 15 113-115, 1990.

[19] Li, P.; Li J.; Wu C.; Wu Q.; Li Js, "Synergistic antibacterial effects of β-lactam antibiotic combined with silver nanoparticles" *Nanotechnology*, 16 (9), 1912, 2005.

[20] Davies, RL.; Etris SFs, "The development and functions of silver in water purification and disease control" *Catalysis Today*, 36 (1), 107-114, 1997.

[21] Narasimha, G.; Praveen B.; Mallikarjuna K.; Deva Prasad Raju Bs, "Mushrooms (Agaricus bisporus) mediated biosynthesis of sliver nanoparticles, characterization and their antimicrobial activity" *International Journal of Nano Dimension*, 2 29-36, 2011.

[22] Sondi, I.; Salopek-Sondi Bs, "Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria" *Journal of colloid and interface science*, 275 (1), 177-182, 2004.

[23] Lara, HH.; Ayala-Núnez NV.; Turrent LdCI.; Padilla CRs, "Bactericidal effect of silver nanoparticles against multidrug-resistant bacteria" *World Journal of Microbiology and Biotechnology*, 26 (4), 615-621, 2010.

[24] Rai, M.; Deshmukh S.; Ingle A.; Gade As, "Silver nanoparticles: the powerful nanoweapon against multidrug‐resistant bacteria" *Journal of applied microbiology*, 112 (5), 841-852, 2012.

[25] Kollef, MH.; Golan Y.; Micek ST.; Shorr AF.; Restrepo MIs, "Appraising contemporary strategies to combat multidrug resistant gram-negative bacterial infections–proceedings and data from the Gram-Negative Resistance Summit" *Clinical infectious diseases*, 53 (suppl 2), S33-S55, 2011.

[26] Senapati, S.; Ahmad A.; Khan MI.; Sastry M.; Kumar Rs, "Extracellular biosynthesis of bimetallic Au–Ag alloy nanoparticles" *Small*, 1 (5), 517-520, 2005.