Preprint Title Nanostructure Mediated Enhancement of Antibacterial Activity of Ampicillin Against *Staphylococcus aureus* and AFM Analysis of Morphological Changes Occuring Therein

Authors Mumtaz Ali, Shujat Ali, Abdul Latif, Samina Perveen, Shakil Ahmed, Manzoor Ahmad, Muhammad R. Shah, Ajmal Khan, Fazal Mabood, Ahmed Al-Rawahi and Ahmed Al-Harrasi

Publication Date 21 Okt 2019

Article Type Full Research Paper

ORCID® iDs Ajmal Khan - https://orcid.org/0000-0001-7851-6080

Nanostructure Mediated Enhancement of Antibacterial Activity of Ampicillin Against *Staphylococcus aureus* and AFM Analysis of Morphological Changes Occuring Therein

Mumtaz Ali[1], Shujat Ali[1], Abdul Latif[1], Samina Perveen[2], Shakil Ahmed[2], Manzoor Ahmad[1], Muhammad Raza Shah[2], Ajmal Khan[3,*], Fazal Mabood[4], Ahmed Al-Rawahi[3] and Ahmed Al-Harrasi[3,*]

[1]*Department of Chemistry, University of Malakand, Chakdara, Dir (L), Khyber Pakhtunkhwa, Pakistan*

[2]*H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan.*

[3]*UoN Chair of Oman's Medicinal Plants and Marine Natural Products, University of Nizwa, P.O. Box 33, Birkat Al Mauz, Nizwa 616, Sultanate of Oman*

[4]*Department of Biological Sciences & Chemistry, College of Arts and Sciences, University of Nizwa, Sultanate of Oma*

Email addresses of authors

Mumtaz Ali (mumtazphd@gmail.com)
Shujat Ali (shujatchem794@gmail.com)
Abdul Latif (dralatif2016@gmail.com)
Samina Perveen (samina_hej@yahoo.com)
Shakil Ahmed (shakilhej@yahoo.com)
Manzoor Ahmad (manzoorhej@yahoo.com)
Muhammad Raza Shah (raza_shahm@yahoo.com)
Ajmal Khan (ajmalkhan@ciit.net.pk)
Fazal Mabood (mehboob@unizwa.edu.om)
Ahmed Al-Rawahi (ahmed@unizwa.edu.om)
Ahmed Al-Harrasi (aharrasi@unizwa.edu.om)

*Corresponding authors
Dr. Ajmal Khan
E-mails: ajmalchemist@yahoo.com;
Prof. Ahmed Al-Harrasi
E-mails: aharrasi@unizwa.edu.om

Abstract:

Staphylococcus aureus is deliberated as one of the most challenging bacteria owing to its ability to develop resistance against antibacterial drugs. In an attempt to explore new approaches for enhancing the activity of antibiotics, here in this work, ampicillin is conjugated to Ag and Au nanoparticles (NPs) and its antibacterial potential was investigated against *S. aureus*. The antibacterial activity was assessed and the associated changes in the bacterial cell morphology were analyzed using atomic force microscopy (AFM) as well as other characterization techniques. Results showed that the antibacterial activity of ampicillin conjugated to gold and silver NPs was enhanced up to 10 and 5 times respectively, when compared with the non-conjugated antibiotic. The kinetics of the conjugated ampicillin were improved. Bacterial membrane destruction was scarcely evident after treating a cell culture with pure ampicillin for four hours. However, Ag conjugates have severely disrupted the cell membranes and Au conjugates have completely destroyed the cell morphology. The study gave an insight of the enhanced antimicrobial action of ampicillin and can be exploited for the devising nanoparticle's based antimicrobial agents. More sophisticated approaches such as faster and more efficient diagnostics, non-antimicrobial methodologies to prevent and treat infections and a better understanding of staphylococcal pathogenesis will also be required to forestall the future of the bacterial resistance.

Keywords: Bacterial resistance, ampicillin, antibacterial activity, Ag and Au nanoconjugates, AFM, cell morphology

Introduction

Nanotechnology has attracted significant attention because of the unique characteristics and increasing importance of nanomaterials in various fields especially in nanomedicine [1]. Their uniqueness is due to high surface area and more atoms at the particle boundaries. Among the different metallic NPs, silver and gold NPs have comprehensive range of uses in nano-scale strategies and tools due to their chemical inertness [2-5]. The worldwide increase in bacterial resistance to existing medicines is a long-standing problem for human health. Bacterial resistance to antimicrobial drugs has increased due to the irrational use of antibiotics, thus creating problems in the treatment of bacterial infections. The development and spread of resistance to antibiotics has compromised the clinical efficacy of currently existing antibiotics and highlighted the need for new antibacterial compounds [6]. β-Lactam antibiotics are the most widely used antibiotics for their effectiveness and safety profile, however occurrence of new, more antagonistic β-lactamases has reached the point where several marketed β-lactams are no longer clinically effective [7]. Therefore, immediate approaches are needed to develop new antimicrobial drugs to handle this problem. This has evoked a solid reaction from health consultants, who have implemented initiatives to inspire the discovery of new antibiotics. One of the capable approaches for restricting bacterial resistance is the application of metallic NPs as a powerful nano-weapon against multidrug resistant bacteria [8-10], because metallic NPs has the ability to target several bacterial structures [11]. There is a mounting evidence that the synergistic effect of antibiotics and NPs resulted in an increase in antibacterial activity of antibiotics [12-16] and gold and silver allay NPs, bound to antibiotics displayed enhanced antibacterial potential [17]. The Ag NPs of antibiotics including penicillin, vancomycin and amoxicillin, exhibited increased antibacterial activity against *S. aureus* and *E. coli* [18]. Our previous work has shown that the antibacterial effect of ceftriaxone against *E. coli* can be enhanced up to six times through conjugation with silver and gold NPs [19]. These findings are very important because such potent antibiotics can be made active in comparatively a small amount to treat infections, thereby decreasing side effects and minimizing the problem of drug resistance. In this paper, we present the enhancement of the antibacterial potential of ampicillin via conjugation to Au and Ag NPs. We have also explored the antibacterial action of

these nanoconjugates against *S. aureus* bacteria under atomic force microscope (AFM), which enabled us to obtain detailed and exciting close-up images of the nanoconjugates involved in various stages of antimicrobial actions. AFM is an appropriate tool for the study of living samples and a distinct vantage is that samples can be analyzed without fixation, vacuum and conductive coating. This technique is extremely efficient in getting images of tiny, highly fragile structures of bacteria, morphological changes suggestive of antibacterial activity [20-24] and a further detailed perception in the structure and mechanics of living specimens [25-26].

Results and discussion

The morphological analysis and mechanism of action of the antimicrobial activity of ampicillin conjugated with AgNPs (Mpn-AgNPs) and AuNPs (Mpn-AuNPs) on *staphylococcus aureus* using AFM was studied for the first time. *S. aureus* is a sensitive strain of bacteria that infect humans and can cause respiratory diseases, food poisoning and skin infections [27]. *S. aureus* is notorious for its capability to develop *resistance* to antibiotics and has created a worldwide problem in clinical treatment [28]. Ampicillin was capped with Ag and Au NPs by mixing its aqueous solution with ionic solutions of Ag and Au in the presence of triethylamine as a reducing agent. UV-visible spectroscopy was used to monitor the conjugation of ampicillin with Ag and Au NPs. The UV-visible spectra of the Mpn-AgNPs and Mpn-AuNPs exhibited surface plasmon bands (SPB) at 396 nm and 540 nm, respectively (**Fig. 4**), which can be correlated with the typical plasmonic absorption of Ag and Au NPs [29-30]. The conjugation of ampicillin with Ag and Au NPs was further confirmed by FT-IR spectroscopy (**Fig. 5**). The FTIR spectrum of ampicillin exhibited absorption bands in region 3512 cm^{-1} and 3205 cm^{-1} which could be associated with stretching vibrations of O–H and N–H groups, respectively. The band at 2968 cm^{-1} can be assigned to the stretching vibrations of C–H groups, carbonyl group of the lactame ring showed the stretching vibration at 1774 cm^{-1} and the amide carbonyl group exhibited band at 1688 cm^{-1}. The band at 1372 cm^{-1} could be assigned to the stretching vibrations of C–N of the lactame and thiazole.

The conjugation of ampicillin with Au and Ag NPs result in the decrease in absorbance intensities and merging of bands of O–H (3512 cm^{-1}), N–H (3205 cm^{-1}) and C=O (1774 and 1688 cm^{-1}) stretching [31]. Ag and Au NPs were then characterized by AFM and their size were found to be around 15-50 nm (**Fig. 6**).

The aim of this study was to examine the boosted antibacterial action and kinetics of the ampicillin Ag and Au NPs through AFM against *S. aureus*, which has not yet been explored. The membranolytic properties in the mechanisms of action of the antibiotics ampicillin, magainin and human platelets extract have been studied by using *Bacillus cereus* and *Escherichia coli* as the bacterial targets [32]. Similarly chitosan NPs of ampicillin trihydrate were synthesized and claimed that they would be capable of sustained delivery of ampicillin [33]. Another study is based on functionalized ampicillin with Ag and Au NPs and their antimicrobial activity against different bacterial strains by determining their minimum bactericidal concentration (MBC) [34]. This paper is offering the first description on visualizing the effect of ampicillin and its Ag and Au NPs on *S. aureus* by AFM. The minimum inhibitory concentrations (MICs) of ampicillin and its Au and Ag NPs were determined through a zone of inhibition [35]. The MICs of pure ampicillin and conjugated ampicillin were found to be 50 ± 0.1, 60 ± 0.3 µg mL^{-1} (which corresponds to a 10.8 µg ampicillin) and 75 ± 0.3 µg mL^{-1} (which correspond to a 4.52 µg ampicillin), respectively. While the MICs of bare Ag and Au NPs were calculated to be 85 ± 0.3 and 100 ± 0.2 µg mL^{-1}, respectively (**Fig. 7**).

The MIC for unconjugated ampicillin is in agreement with the literature value [36]. Although the MICs of Ag and Au conjugates were more than pure ampicillin, the conjugates contain only a small weight fraction of the ampicillin (18 % for Mpn-AgNPs and 6.03% for Mpn-AuNPs), which specifies that ampicillin conjugated to Ag and Au NPs is about 5 and 10 times more active than pure ampicillin, respectively. Further confirmation was carried by AFM which explored the more persuasive and rapid action of the conjugates. Morphological characterization of the control *S. aureus* samples showed typically round cells with normal shapes and flat membranes with a mean length of 1.052 µm, mean width of 1.082 µm and mean height of 0.104 µm and with a maximum height of 0.719 µm, as shown in **Fig. 8**. Bacterial cultures were then treated with pure ampicillin, its Ag and Au conjugates and bare Ag and Au NPs to study the comparative action and kinetics under AFM. Bacteria treated with MIC dose of unconjugated ampicillin for 1 hour showed slight effect and only small lesions were seen on bacterial cell surface **(Fig. 9a).** Cell Morphological degradation increased with time as a 2 hours treatment have further affected bacterial cells and after 4 hours considerable damages of cell bodies were observed (**Fig. 10a, 11a**). After 8 hours time period the cell morphologies were completely degraded and distorted (**Fig. 12a**). On the other

hand bacterial cultures treated with MIC dose of Mpn-AgNPs for 1 hour and 2 hours were found to affect the cells more than pure ampicillin (**Fig. 9b, 10b**) with complete destruction of bacterial cells after 4 hours treatment (**Fig. 11b**). A relatively stronger effect was observed in case of Mpn-AuNPs of MIC dose on the bacterial cells in 1 hour and 2 hours treatment (**Fig. 9c, 10c**), and a complete rupture of bacterial cells in 4 hours (**Fig. 11c**). Unconjugated Ag and Au NPs of MIC doses did not show any observable effect but only minimal morphological changes and only a very slight influence was observed even after treatment for 8 hours (**Fig. 12b, c**).

The interaction of NPs with a bacterial cell still needs further exploration, however many studies have shown that at first metal NPs adsorb to surface of a microorganism due to resultant electrostatic pressure and high affinity of metals towards Sulphur in the proteins [37]. After that, NPs get inside into the cell causing perforations and lead to the release of the cellular matrix [38-40]. Here in this case ampicillin reacts with the outer peptidoglycan layer of *S. aureus* thereby enhancing the membrane's permeability. Subsequently the NPs get into the cells through membranes and may be attached to the bacterial DNA and protein; thus, causing death of the cells by disturbing metabolism and vital functions [41-43]. Consequently, the mutual action of ampicillin and Ag and Au NPs lead to enhanced antibacterial potential [44] Transmission Electron Microscopy was used for studying the antibacterial potential of silver NPs against *E. coli* [38], but it represented *E. coli* when they were lifeless. Here in this study AFM explored noticeable investigation of *S. aureus* by providing a thorough topographic demonstration of shape, surface and phase imaging morphology that allowed analyses of height, width, length and boundary stiffness.

Conclusion

Ampicillin was conjugated with Ag and Au NPs and were characterized by UV-visible, FT-IR and AFM. The NPs were found to be very stable. The antibacterial potential of the synthesized NPs was studied against *S. aureus* and it was found that conjugated ampicillin exhibited antibacterial activity 5-10 times higher than the free drug. The kinetics and morphological changes in the bacterial cell were studied under AFM. The study gave an insight of the enhanced antimicrobial action of ampicillin and can be exploited for the devising nanoparticle's based antimicrobial agents. More sophisticated approaches such as faster and more

efficient diagnostics, non-antimicrobial methodologies to prevent and treat infections and a better understanding of staphylococcal pathogenesis will also be required to forestall the future of the bacterial resistance.

Experimental section

Materials

Silver nitrate ($AgNO_3$) and Tetrachloroauric acid trihydrate ($HAuCl_4.3H_2O$) was purchased from Merck, triethylamine (TEA) from Scharlau and ampicillin (Mpn) were supplied by Pharmagen Limited, Lahore, Pakistan. *Staphylococcus aureus ATCC 11632* (provided by H.E.J. Research institute of Chemistry (ICCBS), University of Karachi, Karachi Pakistan was used to evaluate the antibacterial activity of ampicillin and its silver and gold nano-conjugates. We used deionized water throughout experiment for the synthesis of NPs and further analysis.

Synthesis of silver NPs stabilized with Ampicillin (Mpn-AgNPs)

Solution of ampicillin (1 mM) and $AgNO_3$ (1 mM) were prepared in deionized water. These two solutions were mixed using optimized ratio (9:1 Ag:ampicillin mole ratio). The reaction mixture was stirred for 30 minutes and then 0.1 mL of triethylamine was added to it. The color of the reaction mixture turned to yellowish red; the reaction was carefully monitored through UV-visible spectroscopy. The reaction mixture was stirred for 2 hours then the suspensions were centrifuged to collect NPs. Unreacted precursors and reaction by-products were removed by washing the NPs repeatedly.

Synthesis of gold NPs stabilized with Ampicillin (Mpn-AuNPs)

1 mM solution of $HAuCl_4.3H_2O$ and a 1 mM solution of ampicillin were prepared in deionized water. These two solutions were mixed using optimized ratio (12:1 Au:ampicillin mole ratio). The reaction mixture was stirred for 30 minutes and then 0.1 mL of triethylamine was added to it. The reaction start immediately and colorless reaction mixture turned to purple red; the reaction was monitored by UV-visible spectroscopy. The reaction mixture was stirred for 2 hours

and the suspensions were centrifuged to collect NPs. Unreacted precursors and reaction by-products were removed by washing the NPs repeatedly.

Characterization

The synthesized ampicillin Ag and Au conjugates were characterized by UV-vis spectroscopy; the spectra were collected by a Thermo Scientific Evolution 300 spectrophotometer. FT-IR spectra were acquired with a Bruker Victor 22 spectrophotometer. Finally the shape and size of NPs were determined by AFM (AFM, Agilent Technologies 5500, USA). The instrument was used in ACAFM mode. The samples were dried on freshly cleaved mica surface for analysis at ambient temperature. Si cantilever of force constant 42 N/m, length 125 µm and resonance frequency 330 KHz was maintained throughout the analysis.

Quantification of the weight of ampicillin in the conjugates.

A known volume of suspension was centrifuged and the precipitated NPs were collected. The supernatant was repeatedly centrifuged to remove the synthesized NPs. The supernatant was then freeze-dried, and the residues weighed. Using this method the ampicillin was estimated as 18 wt% for Ag NPs and 6.03 wt% for Au NPs conjugates.

Stability of the NPs

UV-visible spectroscopy was used to describe temperature, salinity and pH stability of the suspensions. Coagulation is usually accompanied by color change and shift of the surface plasmon towards longer wavelengths [45]. The Ag and Au conjugates of ampicillin were found to be stable at 100^{o}C and 50^{o}C temperature, respectively (**Fig. 1**), in a 3-12 pH range (**Fig. 2**) and salt concentration up to 50 mM (**Fig. 3**).

Minimum Inhibitory Concentration (MIC) by Agar well diffusion method.

To calculate MICs, the agar-well diffusion method was employed [46]. MICs for ampicillin were measured with or without silver and gold NPs. In brief, nutrient agar was used as a medium to grow a lawn of *S. aureus ATCC 11632* at a concentration of 10^6 cells in one mL and duplicate dilutions were used to calculate minimum inhibition zones. The 60 mm well was made by using a

borer. The 500 µg ml^{-1} stock solution of ampicillin and its Ag & Au NPs were used to avoid nonspecific merged zones of inhibition. In each well different amounts of various concentrations ranging from 500-5 µg ml^{-1} were added. The plates were incubated at room temperature for 2 hours to allow the diffusion process to take place before it was incubated for 24-48 hours at 37 ºC ± 1. The zones of inhibition were measured by using a millimeter scale.

Antibacterial activity and Morphological changes of Staphylococcus aureus under AFM

S. aureus ATCC 11632 were grown on Tryptic soya agar (Oxoid UK) at 37 ±0.5 ºC for 24 hours in static condition and marked as stock *S. aureus* culture. On freshly cleaved mica slide, 10 µL drop(s) of polylysine was added and left to dry. Then, freshly incubated culture of *S. aureus* on tryptic soya agar (Oxoid UK) inoculated in sterilized distilled water to make 10^6 cfu of S. *aureus* and 5-10 µL droplets of this solution were transferred onto a freshly cleaved mica surface. The sample was characterized by atomic force microscopy to check its morphology of bacterial cells. MIC (50 µg) dose of ampicillin were added into test tubes of nutrient broth containing 10^6 cfu of *S. aureus* bacteria and incubated it for 1-8 hours respectively at 37 ±0.5 ºC after incubation 5-10 µL drops of each dose transferred on freshly cleaved mica coated with polylysine separately and left it for dry and was characterized by AFM. The same procedure was applied for Ampicillin conjugated with AgNPs, MIC (60 µg) dose was treated with 10^6 cfu of *S. aureus* for 1, 2, and 4 hours respectively and were characterized by AFM to check the cell changes and noted the effects of these conjugates. On the other hand Mpn-AuNPs (75 µg) were treated with 10^6 cfu of *S. aureus,* and incubated at 37±0.5 ºC. 5-10 µL of this suspension was transferred on freshly cleaved mica coated with polylysine and left it for dry and then was characterized by atomic force microscopy. On this way we recorded, control, treated with ampicillin, ampicillin conjugated with Ag and Au NPs and bare Ag & Au NPs images of *S. aureus* in similar condition using AFM (AFM, Agilent Technologies 5500, USA) in the ACAFM mode. We used high frequency Si cantilever having length of 125 µm, force constant 42 N/m and resonance frequency 330 KHz. All samples were prepared and analyzed in a same condition.

Abbreviations

Nanoparticles (NPs)

Atomic force microscopy (AFM)

Silver nitrate ($AgNO_3$)

Tetrachloroauric acid trihydrate ($HAuCl_4.3H_2O$)

Triethylamine (TEA)

Ampicillin (Mpn)

NPs stabilized with Ampicillin (Mpn-AgNPs)

Fourier-transform infrared (FTIR)

Silver nanoparticles (Ag NPs)

Gold nanoparticles (Au NPs)

Minimum inhibitory concentration (MIC)

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

All datasets on which the conclusions of the manuscript rely are presented in the paper.

Authors' contributions

MA, AK and AA supervised and designed the study. SA and SP performed all experiments. SA and MRS were analyzed data. AL and MA were involved in writing, editing of manuscript. All authors have read and approved the final version of the manuscript.

Conflicts of interest:

The authors declared that there is no conflict of interest.

Acknowledgments

The authors are thankful to ICCBS, HEJ Research Institute of Chemistry, University of Karachi for instrumental availability.

References

1. Doane, T. L.; Burda, C. The unique role of nanoparticles in nanomedicine: imaging, drug delivery and therapy. *Chemical Society Reviews* **2012,** *41* (7), 2885-2911.
2. Durán, N.; Marcato, P. D.; Conti, R. D.; Alves, O. L.; Costa, F.; Brocchi, M. Potential use of silver nanoparticles on pathogenic bacteria, their toxicity and possible mechanisms of action. *Journal of the Brazilian Chemical Society* **2010,** *21* (6), 949-959.
3. Alshehri, A. H.; Jakubowska, M.; Młożniak, A.; Horaczek, M.; Rudka, D.; Free, C.; Carey, J. D. Enhanced electrical conductivity of silver nanoparticles for high frequency electronic applications. *ACS applied materials & interfaces* **2012,** *4* (12), 7007-7010.
4. Tiwari, P. M.; Vig, K.; Dennis, V. A.; Singh, S. R. Functionalized gold nanoparticles and their biomedical applications. *Nanomaterials* **2011,** *1* (1), 31-63.
5. Li, N.; Zhao, P.; Astruc, D. Anisotropic gold nanoparticles: synthesis, properties, applications, and toxicity. *Angewandte Chemie International Edition* **2014,** *53* (7), 1756-1789.
6. Fair, R. J.; Tor, Y. Antibiotics and bacterial resistance in the 21st century. *Perspectives in medicinal chemistry* **2014,** *6*, 25.
7. Drawz, S. M.; Bonomo, R. A. Three decades of β-lactamase inhibitors. *Clinical microbiology reviews* **2010,** *23* (1), 160-201.
8. Rai, M.; Deshmukh, S.; Ingle, A.; Gade, A. Silver nanoparticles: the powerful nanoweapon against multidrug-resistant bacteria. *Journal of applied microbiology* **2012,** *112* (5), 841-852.
9. Lara, H. H.; Ayala-Núñez, N. V.; Turrent, L. d. C. I.; Padilla, C. R. Bactericidal effect of silver nanoparticles against multidrug-resistant bacteria. *World Journal of Microbiology and Biotechnology* **2010,** *26* (4), 615-621.
10. Li, X.; Robinson, S. M.; Gupta, A.; Saha, K.; Jiang, Z.; Moyano, D. F.; Sahar, A.; Riley, M. A.; Rotello, V. M. Functional gold nanoparticles as potent antimicrobial agents against multi-drug-resistant bacteria. *ACS nano* **2014,** *8* (10), 10682-10686.
11. Gordon, O.; Slenters, T. V.; Brunetto, P. S.; Villaruz, A. E.; Sturdevant, D. E.; Otto, M.; Landmann, R.; Fromm, K. M. Silver coordination polymers for prevention of implant infection: thiol interaction, impact on respiratory chain enzymes, and hydroxyl radical induction. *Antimicrobial agents and chemotherapy* **2010,** *54* (10), 4208-4218.
12. Naqvi, S.; Kiran, U.; Ali, M. I.; Jamal, A.; Hameed, A.; Ahmed, S.; Ali, N. Combined efficacy of biologically synthesized silver nanoparticles and different antibiotics against multidrug-resistant bacteria. *International journal of nanomedicine* **2013,** *8*, 3187-3195.
13. Allahverdiyev, A. M.; Kon, K. V.; Abamor, E. S.; Bagirova, M.; Rafailovich, M. Coping with antibiotic resistance: combining nanoparticles with antibiotics and other antimicrobial agents. *Expert review of anti-infective therapy* **2011,** *9* (11), 1035-1052.
14. Li, P.; Li, J.; Wu, C.; Wu, Q.; Li, J. Synergistic antibacterial effects of β-lactam antibiotic combined with silver nanoparticles. *Nanotechnology* **2005,** *16* (9), 1912.
15. Smekalova, M.; Aragon, V.; Panacek, A.; Prucek, R.; Zboril, R.; Kvitek, L. Enhanced antibacterial effect of antibiotics in combination with silver nanoparticles against animal pathogens. *The Veterinary Journal* **2015**.

16. Gurunathan, S. Rapid biological synthesis of silver nanoparticles and their enhanced antibacterial effects against Escherichia fergusonii and Streptococcus mutans. *Arabian Journal of Chemistry* **2014**.
17. Dos Santos, M. M.; Queiroz, M. J.; Baptista, P. V. Enhancement of antibiotic effect via gold: silver-alloy nanoparticles. *Journal of Nanoparticle Research* **2012,** *14* (5), 859.
18. Shahverdi, A. R.; Fakhimi, A.; Shahverdi, H. R.; Minaian, S. Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against Staphylococcus aureus and Escherichia coli. *Nanomedicine: Nanotechnology, Biology and Medicine* **2007,** *3* (2), 168-171.
19. Shah, M. R.; Ali, S.; Ateeq, M.; Perveen, S.; Ahmed, S.; Bertino, M. F.; Ali, M. Morphological analysis of the antimicrobial action of silver and gold nanoparticles stabilized with ceftriaxone on Escherichia coli using atomic force microscopy. *New Journal of Chemistry* **2014,** *38* (11), 5633-5640.
20. Niemirowicz, K.; Surel, U.; Wilczewska, A. Z.; Mystkowska, J.; Piktel, E.; Gu, X.; Namiot, Z.; Kułakowska, A.; Savage, P. B.; Bucki, R. Bactericidal activity and biocompatibility of ceragenin-coated magnetic nanoparticles. *Journal of nanobiotechnology* **2015,** *13* (1), 32.
21. de Araujo, A. R.; Quelemes, P. V.; Perfeito, M. L. G.; de Lima, L. I.; Sá, M. C.; Nunes, P. H. M.; Joanitti, G. A.; Eaton, P.; Soares, M. J. d. S.; de Souza de Almeida Leite, J. R. Antibacterial, antibiofilm and cytotoxic activities of Terminalia fagifolia Mart. extract and fractions. *Annals of Clinical Microbiology and Antimicrobials* **2015,** *14* (1), 25.
22. Neethirajan, S.; DiCicco, M. Atomic force microscopy study of the antibacterial effect of fosfomycin on methicillin-resistant Staphylococcus pseudintermedius. *Applied Nanoscience* **2014,** *4* (6), 703-709.
23. Marcio N, G.; Rafael RG, M.; Tarquin F, T.; Filipe DS, G.; Graciela C, P.; Kennedy CS, C.; Marcio CR, S.; Josmary R, S. Evaluation of the Antimicrobial Activity of Stryphnodendron barbatiman against Citrobacter freundii. *Materials Sciences and Applications* **2013,** *2013*.
24. Li, H.; Chen, Q.; Zhao, J.; Urmila, K. Enhancing the antimicrobial activity of natural extraction using the synthetic ultrasmall metal nanoparticles. *Scientific reports* **2015,** *5*.
25. Longo, G.; Kasas, S. Effects of antibacterial agents and drugs monitored by atomic force microscopy. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology* **2014,** *6* (3), 230-244.
26. Dufrêne, Y. F. Atomic force microscopy in microbiology: new structural and functional insights into the microbial cell surface. *MBio* **2014,** *5* (4), e01363-14.
27. McCaig, L. F.; McDonald, L. C.; Mandal, S.; Jernigan, D. B. Staphylococcus aureus-associated skin and soft tissue infections in ambulatory care. *Emerging infectious diseases* **2006,** *12* (11), 1715.
28. Costa, A. R.; Batistão, D. W.; Ribas, R. M.; Sousa, A. M.; Pereira, M. O.; Botelho, C. M. Staphylococcus aureus virulence factors and disease. **2013**.
29. Šileikaitė, A.; Prosyčevas, I.; Puišo, J.; Juraitis, A.; Guobienė, A. Analysis of silver nanoparticles produced by chemical reduction of silver salt solution. *Mater. Sci.-Medzg* **2006,** *12*, 287-291.
30. Pavlov, V.; Xiao, Y.; Shlyahovsky, B.; Willner, I. Aptamer-Functionalized Au Nanoparticles for the Amplified Optical Detection of Thrombin. *Journal of the American Chemical Society* **2004,** *126* (38), 11768-11769.

31. Kasthuri, J.; Veerapandian, S.; Rajendiran, N. Biological synthesis of silver and gold nanoparticles using apiin as reducing agent. *Colloids and Surfaces B: Biointerfaces* **2009,** *68* (1), 55-60.
32. Nikiyan, H.; Vasilchenko, A.; Deryabin, D. AFM investigations of various disturbing factors on bacterial cells. *Microscopy: Science, Technology, Applications and Education. A. Méndez-Vilas and J. Díaz (Eds.)* **2010**.
33. Saha, P.; Goyal, A. K.; Rath, G. Formulation and evaluation of chitosan-based ampicillin trihydrate nanoparticles. *Tropical journal of pharmaceutical research* **2010,** *9* (5).
34. Brown, A. N.; Smith, K.; Samuels, T. A.; Lu, J.; Obare, S. O.; Scott, M. E. Nanoparticles functionalized with ampicillin destroy multiple-antibiotic-resistant isolates of Pseudomonas aeruginosa and Enterobacter aerogenes and methicillin-resistant Staphylococcus aureus. *Applied and environmental microbiology* **2012,** *78* (8), 2768-2774.
35. Sultanova, N.; Makhmoor, T.; Abilov, Z.; Parween, Z.; Omurkamzinova, V.; Choudhary, M. I. Antioxidant and antimicrobial activities of Tamarixramosissima. *Journal of ethnopharmacology* **2001,** *78* (2), 201-205.
36. Chamundeeswari, M.; Sobhana, S.; Jacob, J. P.; Kumar, M. G.; Devi, M. P.; Sastry, T. P.; Mandal, A. B. Preparation, characterization and evaluation of a biopolymeric gold nanocomposite with antimicrobial activity. *Biotechnology and applied biochemistry* **2010,** *55* (1), 29-35.
37. Diaz-Visurraga, J.; Garcia, A.; Cardenas, G. Morphological changes induced in bacteria as evaluated by electron microscopy. *Microscopy: science, technology, applications and education. Formatex, Badajoz* **2010**, 307-315.
38. Morones, J. R.; Elechiguerra, J. L.; Camacho, A.; Holt, K.; Kouri, J. B.; Ramírez, J. T.; Yacaman, M. J. The bactericidal effect of silver nanoparticles. *Nanotechnology* **2005,** *16* (10), 2346.
39. Chakravarty, R.; Banerjee, P. C. Morphological changes in an acidophilic bacterium induced by heavy metals. *Extremophiles* **2008,** *12* (2), 279-284.
40. Nair, S.; Sasidharan, A.; Rani, V. D.; Menon, D.; Nair, S.; Manzoor, K.; Raina, S. Role of size scale of ZnO nanoparticles and microparticles on toxicity toward bacteria and osteoblast cancer cells. *Journal of Materials Science: Materials in Medicine* **2009,** *20* (1), 235-241.
41. Rai, M.; Yadav, A.; Gade, A. Silver nanoparticles as a new generation of antimicrobials. *Biotechnology advances* **2009,** *27* (1), 76-83.
42. Song, H.; Ko, K.; Oh, I.; Lee, B. Fabrication of silver nanoparticles and their antimicrobial mechanisms. *Eur Cells Mater* **2006,** *11* (Suppl 1), 58.
43. Sondi, I.; Salopek-Sondi, B. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. *Journal of colloid and interface science* **2004,** *275* (1), 177-182.
44. Rai, A.; Prabhune, A.; Perry, C. C. Antibiotic mediated synthesis of gold nanoparticles with potent antimicrobial activity and their application in antimicrobial coatings. *Journal of Materials Chemistry* **2010,** *20* (32), 6789-6798.
45. Kreibig, U.; Vollmer, M., *Optical properties of metal clusters*. Springer Science & Business Media: 2013; Vol. 25.
46. Carson, C.; Riley, T. Antimicrobial activity of the major components of the essential oil of Melaleuca alternifolia. *Journal of applied bacteriology* **1995,** *78* (3), 264-269.

Figures caption

Fig. 1: Heat stability of Mpn-AgNPs (A) and Mpn-AuNPs (B)

Fig. 2: PH stability of Mpn-AgNPs (A) and Mpn-AuNPs (B)

Fig. 3: Salt stability of Mpn-AgNPs (A) and Mpn-AuNPs (B)

Fig. 4: UV-visible spectrum of Mpn-AgNPs (A) and Mpn-AuNPs (B)

Fig. 5: FT-IR spectra of Mpn-AgNPs (A) and Mpn-AuNPs (B)

Fig. 6: AFM images of Mpn-AgNPs (A) and Mpn-AuNPs (B)

Fig. 7: Minimum inhibitory concentration of Ampicillin (1), Mpn-AgNPs (2) Mpn-AuNPs (3) bare AgNPs (4) and bare Au NPs (5)

Fig. 8: AFM images of *S. aureus* before treatment (control), Tophography (A), 3D (B)

Fig. 9: AFM images of *S. aureus* treated for 1h with (A) ampicillin (B) Mpn-AgNPs (C) Mpn-AuNPs

Fig. 10: AFM images of *S. aureus* treated for 2h with (A) Ampicillin (B) Mpn-AgNPs and (C) Mpn-AuNPs

Fig. 11: AFM images of *S. aureus* treated for 4h with (A) ampicillin (B) Mpn-AgNPs and (C) Mpn-AuNPs

Fig. 12: AFM images of *S. aureus* treated for 8h with (A) ampicillin (B) bare AgNPs and (C) bare AuNPs

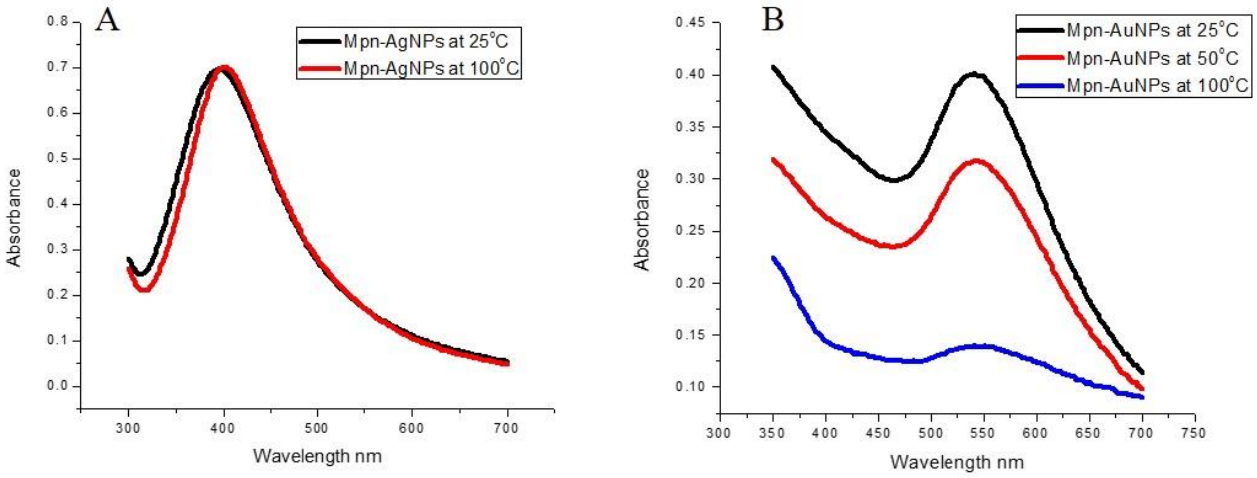
A
Mpn-AgNPs at 25°C
Mpn-AgNPs at 100°C
Absorbance
Wavelength nm
B
Mpn-AuNPs at 25°C
Mpn-AuNPs at 50°C
Mpn-AuNPs at 100°C
Absorbance
Wavelength nm

Figure-1

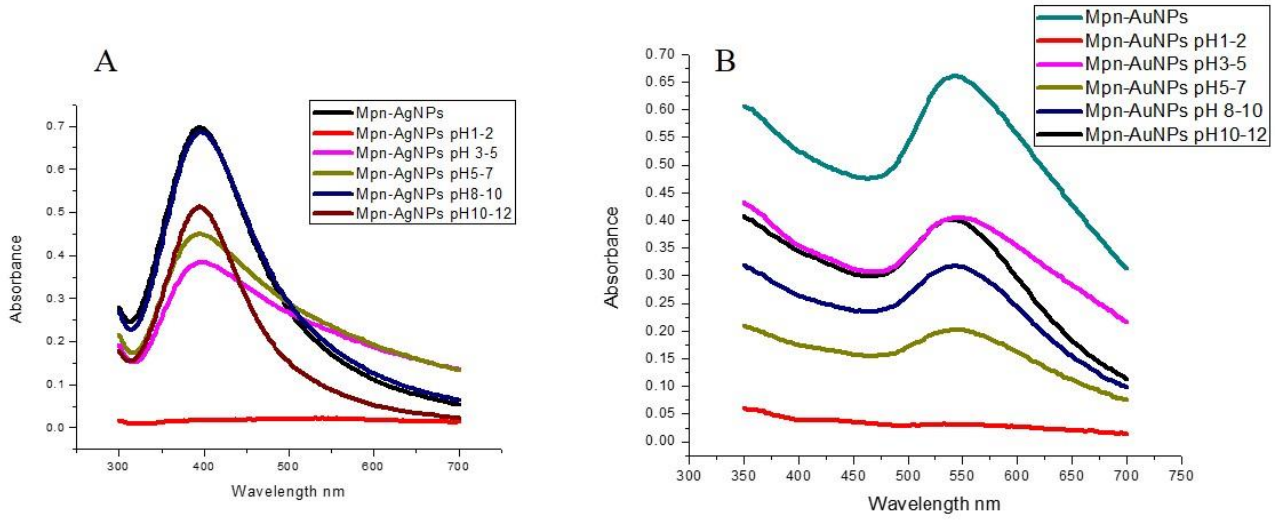
A
Absorbance
Wavelength nm
Mpn-AgNPs
Mpn-AgNPs pH1-2
Mpn-AgNPs pH 3-5
Mpn-AgNPs pH5-7
Mpn-AgNPs pH8-10
Mpn-AgNPs pH10-12
B
Absorbance
Wavelength nm
Mpn-AuNPs
Mpn-AuNPs pH1-2
Mpn-AuNPs pH3-5
Mpn-AuNPs pH5-7
Mpn-AuNPs pH 8-10
Mpn-AuNPs pH10-12

Figure-2

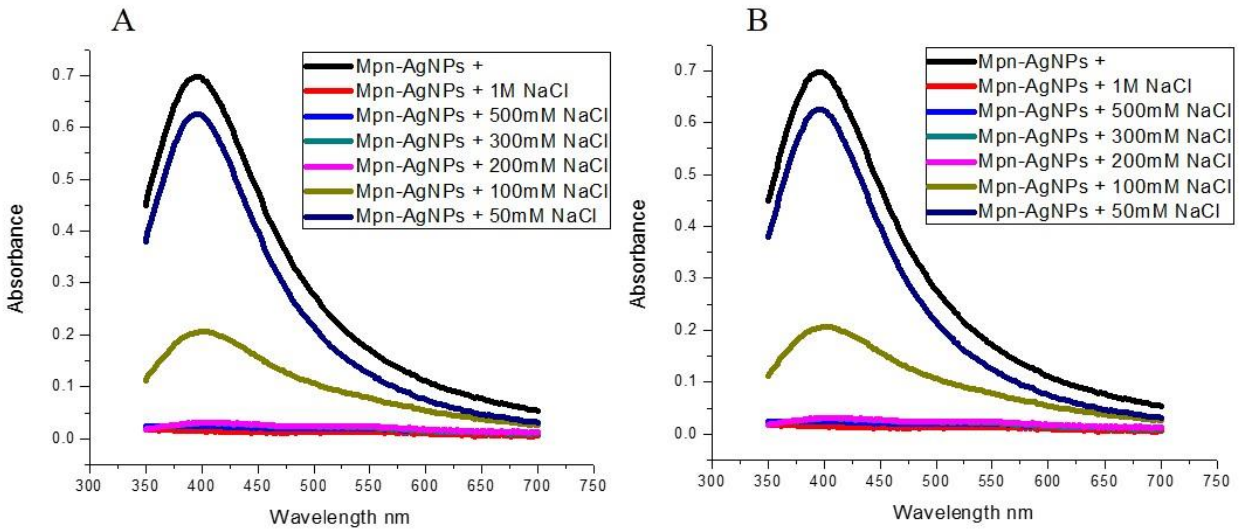
A
B
Absorbance
Wavelength nm
Mpn-AgNPs +
Mpn-AgNPs + 1M NaCl
Mpn-AgNPs + 500mM NaCl
Mpn-AgNPs + 300mM NaCl
Mpn-AgNPs + 200mM NaCl
Mpn-AgNPs + 100mM NaCl
Mpn-AgNPs + 50mM NaCl

Figure-3

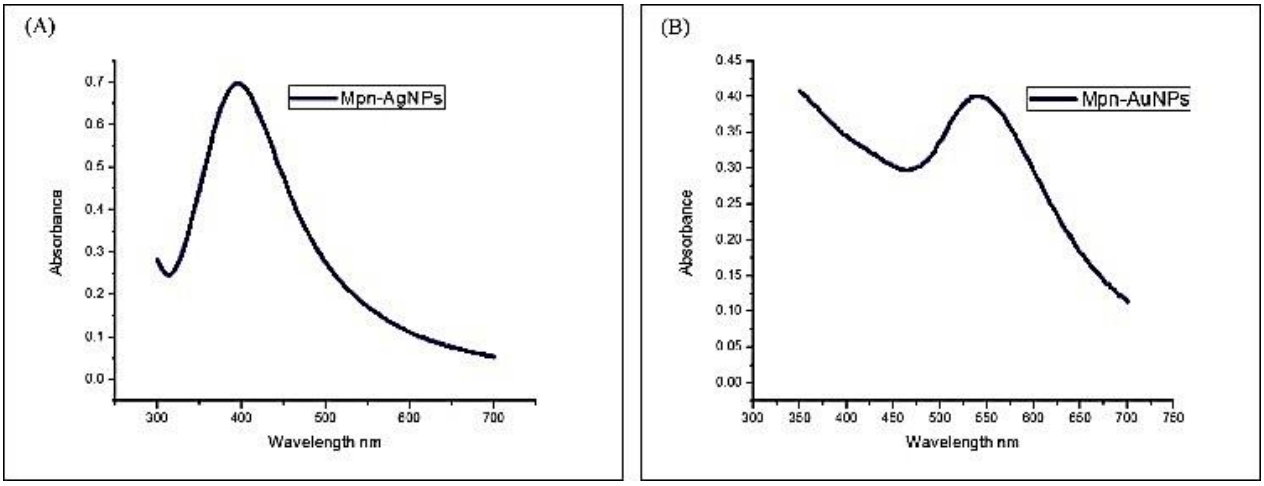
(A)
Mpn-AgNPs
Absorbance
Wavelength nm
(B)
Mpn-AuNPs
Absorbance
Wavelength nm

Figure-4

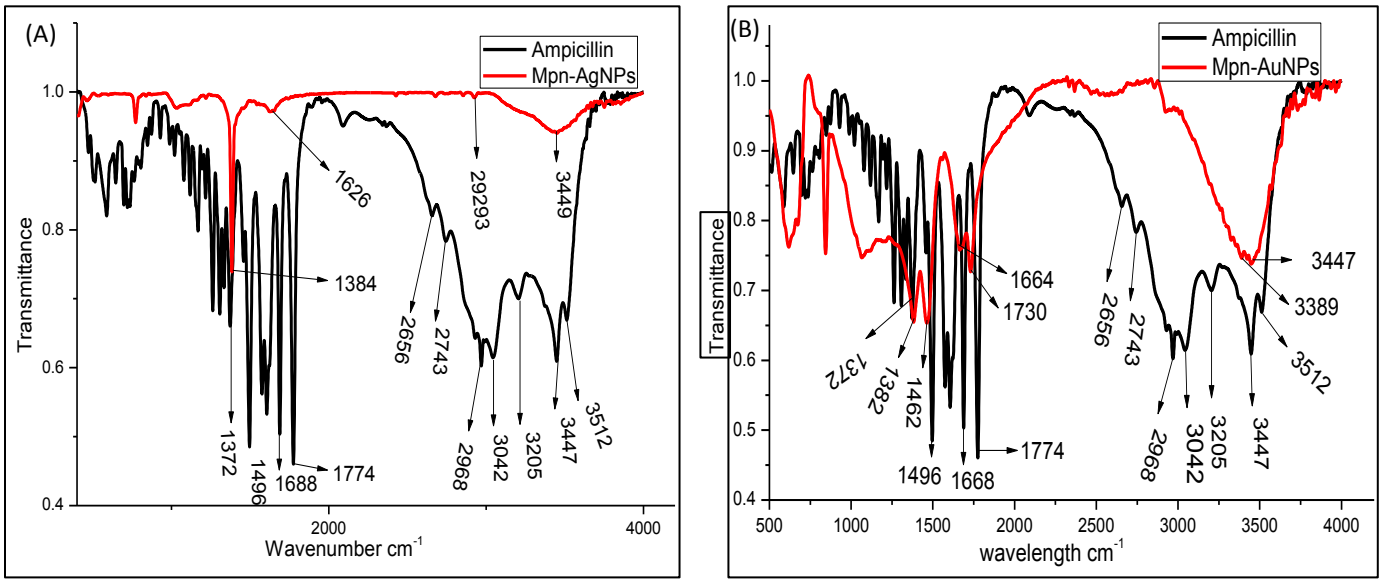
(A)
Ampicillin
Mpn-AgNPs
Transmittance
Wavenumber cm-1
1626
29293
3449
1384
2656
2743
1372
1496
1688
1774
2968
3042
3205
3447
3512
(B)
Ampicillin
Mpn-AuNPs
Transmittance
wavelength cm-1
1664
1730
3447
3389
2656
2743
1372
1382
1462
3512
1774
1496
1668
2968
3042
3205
3447

Figure-5

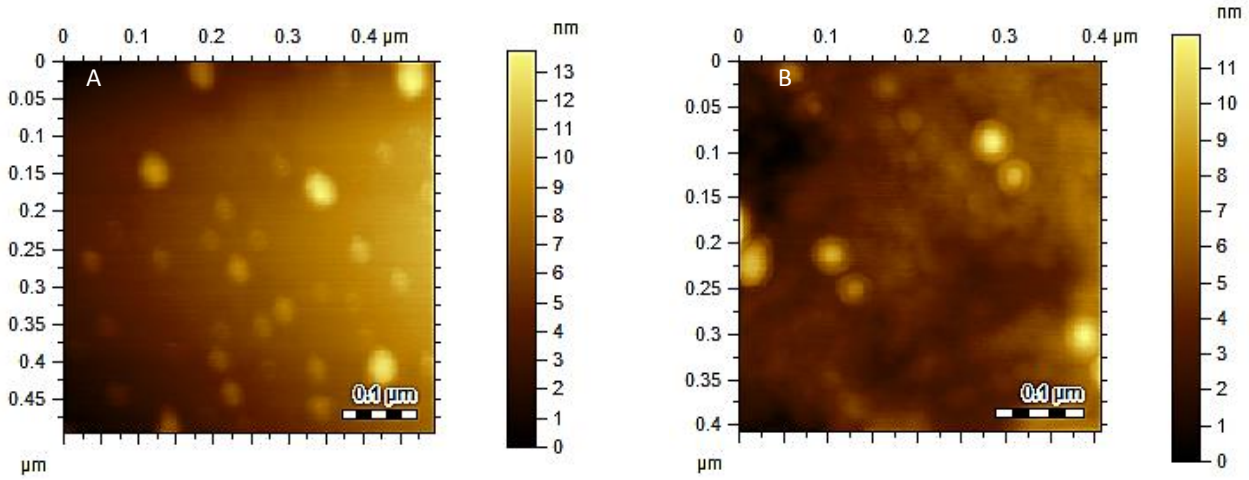

Figure-6

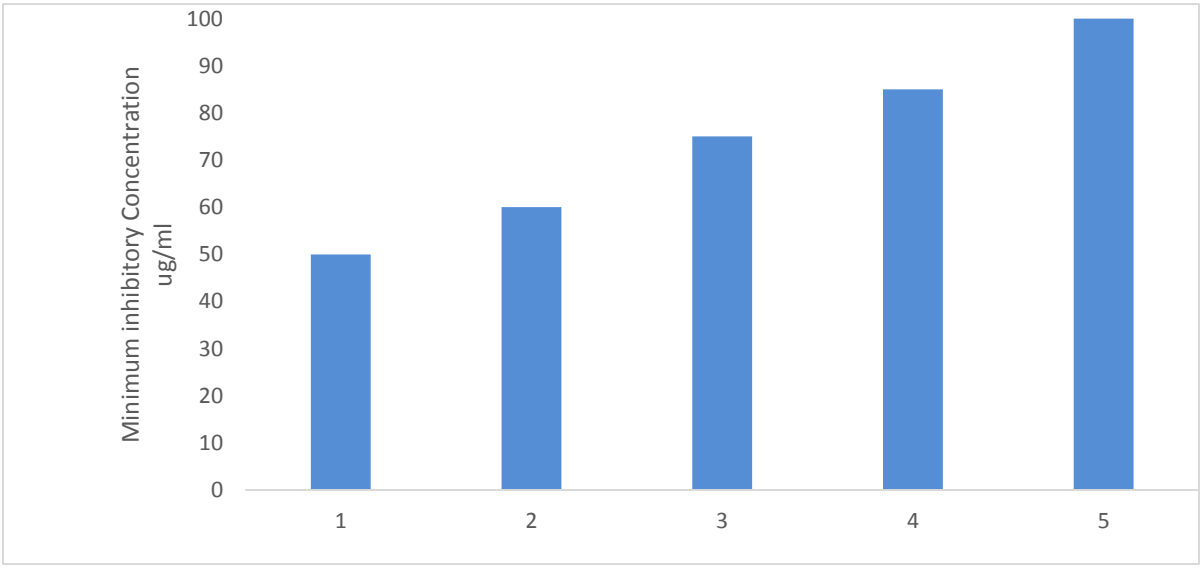
Minimum inhibitory Concentration ug/ml
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5

Figure-7

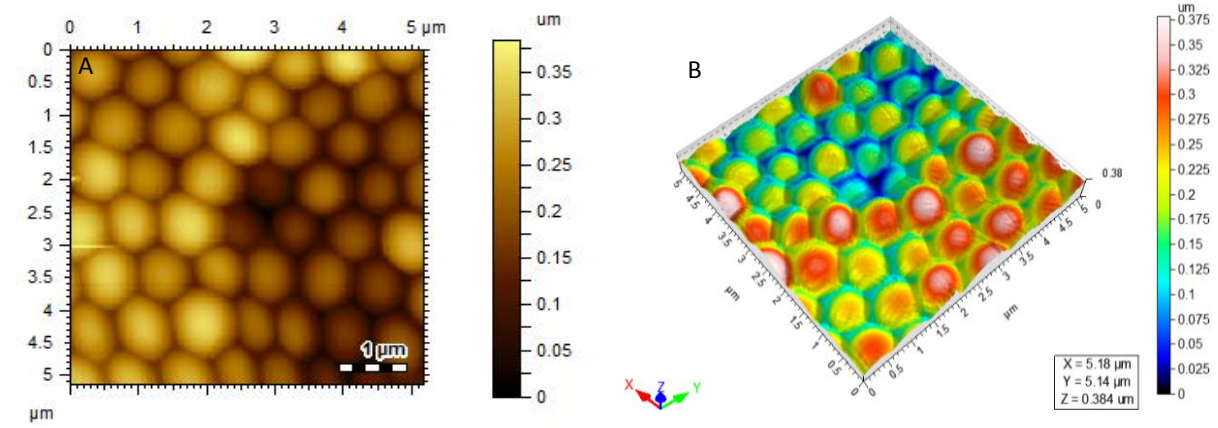

Figure-8

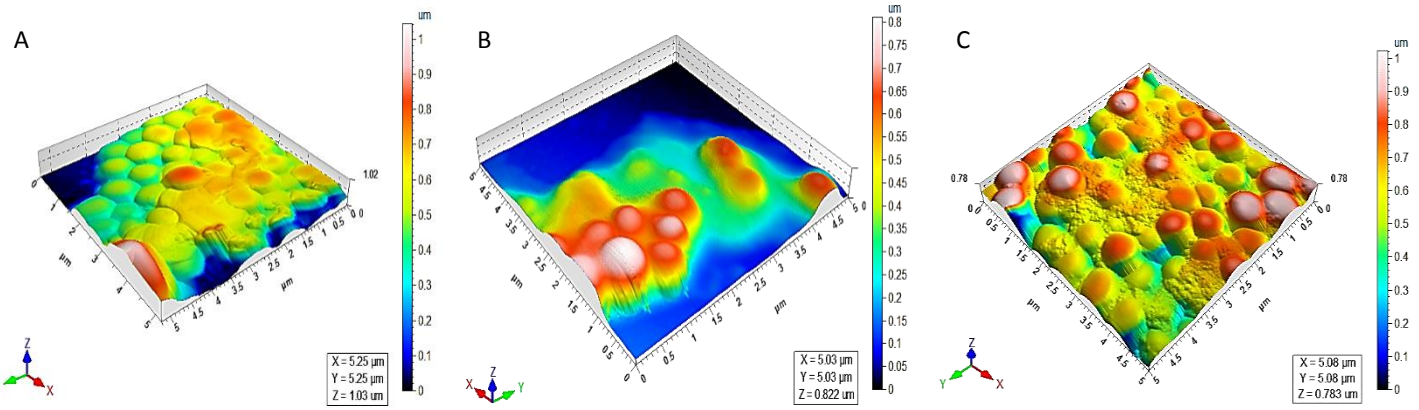
A
B
C
X = 5.25 µm
Y = 5.25 µm
Z = 1.03 um
X = 5.03 µm
Y = 5.03 µm
Z = 0.822 um
X = 5.08 µm
Y = 5.08 µm
Z = 0.783 um

Figure-9

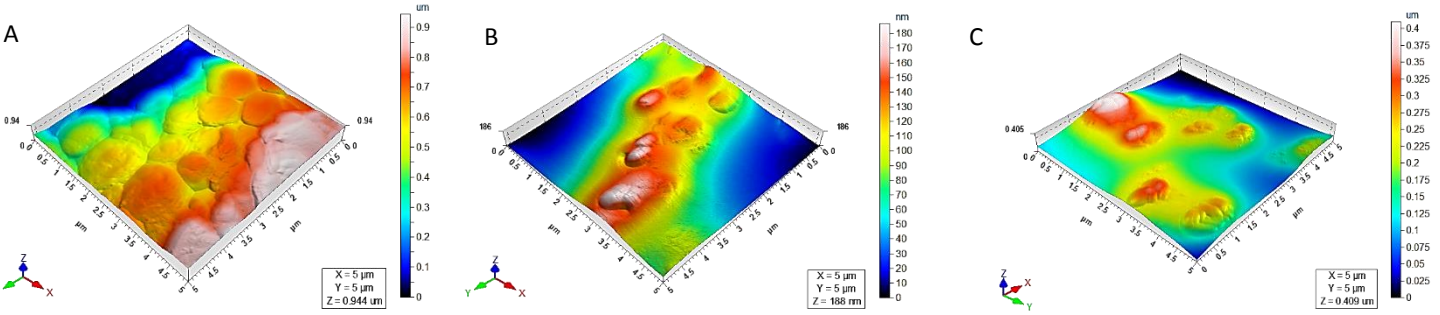

Figure-10

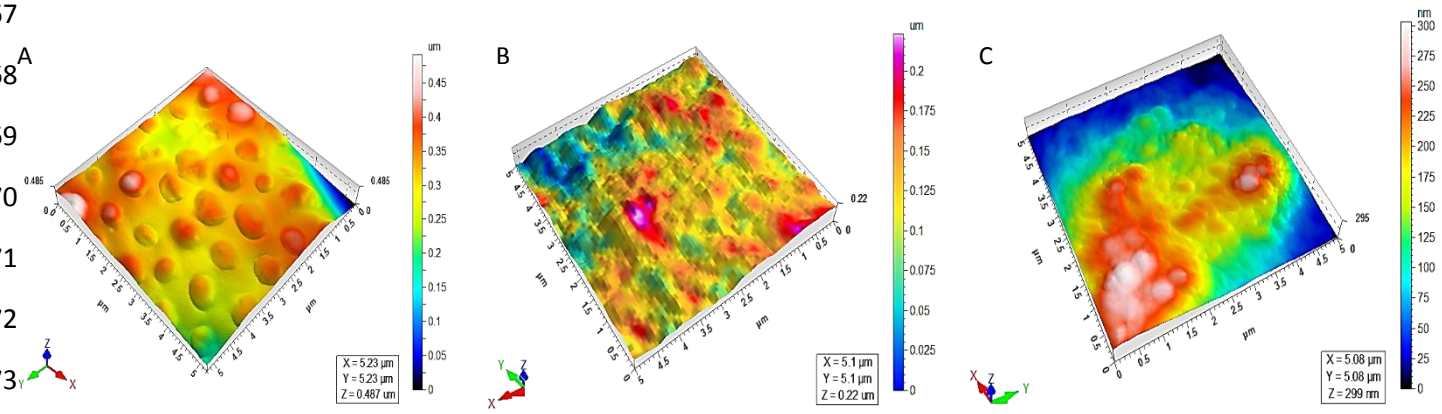
A
B
C
X = 5.23 µm
Y = 5.23 µm
Z = 0.487 µm
X = 5.1 µm
Y = 5.1 µm
Z = 0.22 µm
X = 5.08 µm
Y = 5.08 µm
Z = 299 nm

Figure-11

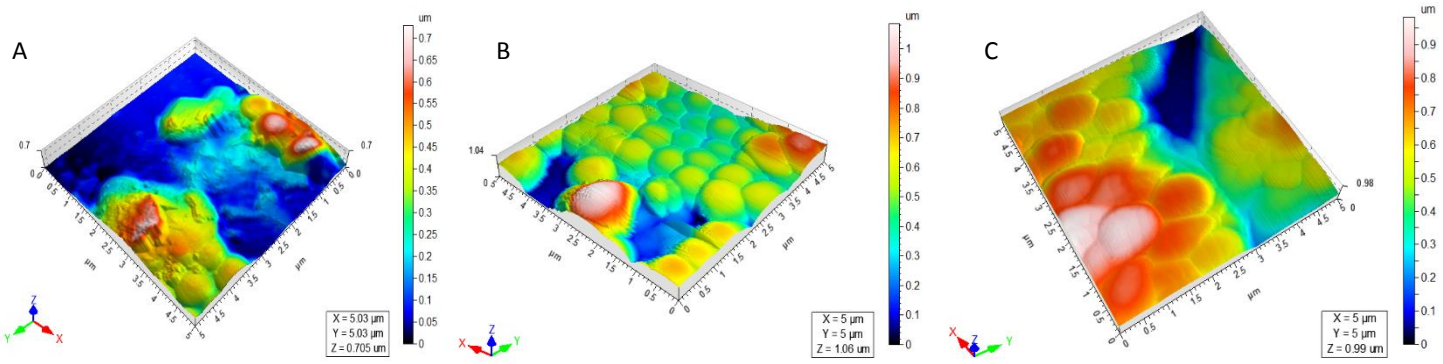
A
B
C
X = 5.03 µm
Y = 5.03 µm
Z = 0.705 um
X = 5 µm
Y = 5 µm
Z = 1.06 um
X = 5 µm
Y = 5 µm
Z = 0.99 um

Figure-12