INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

IN VITRO ANTIBACTERIAL ACTIVITY EXHIBITED BY SILVER, COPPER AND NICKEL NANOPARTICLES STABILIZED BY PEG AND PVP POLYMERS

S. Kanchana and J. Santhanalakshmi*

Department of Physical Chemistry, University of Madras,
Guindy campus, Chennai – 600 025, Tamil Nadu, India.

ABSTRACT

Infectious diseases caused by bacterial pathogens pose constant challenges to health care industries worldwide. Bacteria by being ubiquitous and capable of getting transmitted through various sources frequently develop antibiotic resistance, thus making their control ever unsolved. Nanoparticles of different metals have been proved to be highly specific and effective antimicrobial agents in several reports. Owing to the precise action, nanoparticles are considered as preferable agents for designing drugs in modern medicine for controlling bacterial pathogens. In this study using poly ethylene glycol (PEG) and poly vinyl pyrrolidone (PVP) polymers as stabilizing agents five different nanoparticles (NPs) of three metals namely, copper (Cu), nickel (Ni) and silver (Ag), were synthesized. Subsequent to their physico-chemical characterization, the antibacterial properties against three bacterial test organisms viz., *Staphylococcus aureus, Escherichia coli* and *Salmonella typhi* were studied. The minimum inhibitory concentration (MIC) of nanoparticles were determined using resazurin microtitre assay. Among the NPs synthesized with PEG, CuNPs showed substantial antibacterial activity on all the test bacteria with MIC of 0.075mg/ml while the NiNPs showed moderate activity especially with the lowest MIC against *S. typhi* (0.312 mg/ml). Regarding NPs synthesized with PVP CuNPs exhibited higher inhibitory activity with the lowest MIC of 0.078 mg/ml against *E. coli*. NiNPs demonstrated fair antibacterial activity with the lowest MIC of 0.625 against *S. typhi*. Surprisingly the AgNPs could inhibit the bacteria only at higher concentrations with MIC of 5.0 mg/ml. Overall, PEG based NPs were better in the antibacterial action than those of PVP stabilized NPs with the respective MIC ranges of 0.078 – 0.625 mg/ml and 0.078 – 5.0 mg/ml. Further studies for augmenting antibacterial activity of PEG stabilized NPs are under progress. Salient results are discussed.

Keywords: Antibacterial, Metal nanoparticles, Copper, Nickel, Silver, PEG, PVP.

1. INTRODUCTION

Infections due to bacterial pathogens constitute major cause of morbidity and mortality in developing countries. As the bacteria are ubiquitous in nature, it could be easily transmitted to human host wherever poor sanitation and ill hygiene prevail. Although the use of antibiotics are effective in controlling bacterial infections, the ever growing antibiotic resistant bacterial pathogens pose challenges to medical and hospital managements. Owing to the indiscriminate use of antibiotics, there has been alarming increase of antibiotic resistant bacteria in recent years. Antibiotic resistance has become more common among both gram positive and gram negative bacteria[1].

In recent years the emergence of multidrug resistant pathogens has become a major threat to human health[2]. Infections complicated by resistant forms of the bacteria *Staphylococcus aureus* and

Pseudomonas aeruginosa are given global concern[3]. Resistance to multiple drugs including ampicillin, streptomycin, tetracycline, rifampicin, etc. exhibited by certain stains of pathogenic bacteria such as *Escherichia coli* (O157:H7) and *Mycobacteium tuberculosis* are considered as major threats to the health care industry[4,5]. Based on global surveillance on antibiotic resistance in 2014 the World Health Organization has reported that deaths of 25,000 and 23,000 people each occur in Europe and United States annually due to infections by multi-drug resistant bacteria[6].

In order to overcome the challenge of antibiotic resistance, treatments of bacterial infections with many natural drugs sourced from plants, marine water, metals, etc. are attempted. In recent years there is a growing interest in the application of nanoparticles as antimicrobial agents. By virtue of their smaller size, unique surface property and precise activity, nanoparticles serve as potential candidates for combating bacterial infections complicated by antibiotic resistance. Unlike the common antibiotics the metal nanoparticles cause lethality to bacteria by targeting multiple structures[7]. The nanoparticles, by virtue of their specific and precise action, could be considered as suitable alternative to antibiotics thus to control the drug resisting bacteria[8].

Nanoparticles of different types have found potential application in the field of medicine over the years. Silver nanoparticles are extensively employed in topical ointments for the purpose of preventing infections of burn and open wounds[9]. In recent years many research studies have reported the antimicrobial properties of nanoparticles of various metals such as copper[10,11], Nickel, Zinc[12], Gold[13], etc. As the nanoparticles of metals have smaller size than the pores of bacterial cell membrane, there will be facile transmission across the cell wall and entry into the cytoplasm and eventual cause of cell lethality[10].

The bacteria *Staphylococcus aureus*, a gram positive aerobic bacteria, although recognized as a commensal living on skin and soft tissues of healthy human, when the immune system declines it can cause an array of infections ranging from skin, ear, urinary tract and wound infections to life threatening invasions, pneumonia, endocarditis, etc. Also there has occurred the emergence of methicillin resistant *S. aurues* (MRSA) strains and of late, drug resistance multiple drugs (MDR-MRSA) causing challenges in the treatment of infections[14]. The bacteria *Escherichia coli*, a gram negative aero anaerobic normal intestinal flora of human, could emerge as an opportunistic pathogen to cause mild intestinal infections (e.g., traveler's diarrhea) and serious extra-intestinal infections (e.g., meningitis)[15]. The rapid global raise of cephalosporin-resistant *E. coli* (ESCR-EC) infections during the recent decades is considered as the biggest threat to medical care[16]. The bacteria *Salmonella typhi*, a gram negative aero anaerobic bacteria, is common cause of blood stream infections, typhoid (enteric fever) and febrile illness in low and middle income countries. Reports in recent years address the serious emergence of Salmonella strains resistant to fluoroquinolone and extended spectrum cephalosporin antibiotics[17]. All these three bacteria are frequently encountered with antibiotic resistance in both social community and in hospital settings worldwide. The present study was carried out with the objectives of determining the antibacterial properties of PEG and PVP capped nanoparticles of copper, nickel and silver metals against three bacterial pathogens and to compare the efficiency for potential application in infection controls.

2. MATERIALS AND METHODS

2.1. Synthesis of metal nanoparticles, purification and characterization:

Nanoparticles of copper, nickel and silver were synthesized in the presence of poly ethylene glycol (PEG) and poly vinyl pyrrolidone (PVP) stabilizers. The polyol process prescribed by Sun and Xia[18] was adopted with modifications for the synthesis of nanoparticles. Using PEG two types of nanoparticles were synthesized namely copper (PEG-CuNP) and nickel (PEG-NiNP) and three types of nanoparticles were synthesized using PVP viz., copper (PVP-CuNP), nickel (PVP-NiNP) and silver (PVP-AgNP). Powder samples of each of the metal nanoparticle thus prepared were stored in an air tight container purged with N_2 gas. Characterization of each of the nanoparticles was initially done by visual monitoring of color change of the solution. Synthesized nanoparticles were subjected to powder X-ray diffraction study using X'pert PROPAN analytical instrument and the average size value was determined using Debye-Scherer's formula[19]. Subsequently the transmission electron microscopy (TEM) and scanning electron microscopy (SEM) were performed for copper and silver nanoparticles at an accelerating voltage of 5kV and magnification of (x10k) in order to record their surface morphology and size distributions.

2.2. Assay of antibacterial activity:

2.2.1. Test organisms

Cultures of three bacterial pathogens including a gram positive bacteria (*Staphylococcus aureus*) and two of the gram negative bacteria (*Escherichia coli* and *Salmonella typhi*) were

obtained from Microbial Type Culture Collection (MTCC), Chandigarh, India. Stock cultures were sub-cultured individually onto Nutrient Agar (NA) culture plates and preserved until further use in NA slants at 4^{o}C. For the purpose of performing antibacterial assay nutrient broth cultures of each bacterial strain were prepared by overnight incubation at 37^{o}C and the turbidity of the cultures were adjusted corresponding to 0.5 McFarland standard using sterile NA broth.

2.2.2. Determination of antibacterial activity

Antibacterial activity of each of the metal nanoparticles was determined in terms of minimum inhibitory concentration (MIC) using a microtitre plate based assay prescribed by Sarkar et al.[20] with slight modifications.

The assay initially involved the preparation of indicator solution by completely dissolving 270 mg of resazurin dye in 40 mL of distilled water using a vortex mixer. A sterile 96 well microtiter plate was taken and five rows of it, each for a particular nanoparticle, were labelled for independent assay of antibacterial activity of test materials (i.e., nanoparticles). First well of each of these rows were added independently with 100 µL of stock solution of each nanoparticle (100 mg/mL) prepared in distilled water. Remaining wells were added with 50 µL of sterile nutrient broth. Serial dilution of each of the test materials was carried out using a micropipette thus to obtain concentrations of 5, 2.5, 1.25, 0.625, 0.312, 0.156, 0.078 and 0.036 mg/mL. In parallel two more rows of wells each for a positive control (prepared with a broad spectrum antibiotic Streptomycin) and a negative control (prepared with only test material in nutrient broth) were also maintained. Finally a volume of 10 µL of overnight culture of test bacteria was added to all the wells independently except to those of the negative control. The plates were prepared in triplicates for each test bacteria and incubated at 37°C for 24 h. Positive result was visually recorded if the color of the well contents changed from purple to pink or colorless. The lowest concentration (highest dilution) of the test material which caused the color change was taken as minimal inhibitory concentration (MIC) value.

3. RESULTS

3.1. Synthesis and characterization of metal nanoparticles

The synthesis of different metal nanoparticles viz., PEG-CuNP, PEG-NiNP, PVP-CuNP, PVP-NiNP and PVP-AgNP were identified by visual inspection of change of colors correspondingly for each of them. The X-ray diffractogram indicated the size of these nanoparticles to be 21$\pm$1 nm (PEG-CuNP), 26$\pm$1 nm (PEG-NiNP), 22$\pm$1 (PVP-CuNP), 24$\pm$1 nm (PVP-NiNP) and 55$\pm$1 (PVP-AgNP) (fig 1). The TEM and SEM revealed the spherical nature of copper and silver nanoparticles. While the copper nanoparticles appeared discrete the silver nanoparticles were observed to be denser and aggregated flocs (fig 2).

3.2. Antibacterial activity

The antibacterial activities of five different nanoparticles against three bacterial pathogens at various concentrations are depicted in Tables 1 and 2. The PEG stabilized copper nanoparticles caused inhibition of growth of all the test bacteria even at higher dilutions. The PEG stabilized nickel nanoparticles inhibited bacterial growth at lower dilutions, but were less effective at higher dilutions (Table 1). The antibacterial activity of the two PEG stabilized nanoparticles was in the order of CuNP>NiNP.

On the other hand the PVP stabilized nanoparticles produced varying effects on the test organisms (Table 2). While the PVP stabilized copper nanoparticles could inhibit bacterial growth at higher dilutions, the nickel and silver nanoparticles were observed to be less effective. The PVP stabilized silver nanoparticles were observed to be least effective as they could exhibit their antibacterial activity only at lower dilution. The antibacterial activity of these three PVP stabilized nanoparticles was in the order of CuNP > NiNP > AgNP. As anticipated, there was inhibition and growth of bacteria respectively in the positive and negative controls.

The antibacterial activity in terms of minimum inhibitory concentration (MIC) determined for each nanoparticle is presented in Table 3. Among the different nanoparticles tested, the PEG stabilized copper nanoparticles (PEG-CuNP) demonstrated the best antibacterial activity as they inhibited the growth of all the tested bacteria with a MIC of 0.078 mg/mL. The PVP stabilized copper nanoparticles (PVP-CuNP) showed satisfactory antibacterial activities exhibiting a minimum of 0.078 mg/mL and a maximum of 0.156 mg/mL as their MICs against tested bacteria. While, the PEG stabilized nickel nanoparticles (PEG-NiNP) consumed moderate MIC values ranging from 0.312 to 0.625 mg/mL compared to its PVP counterpart (PVP-NiNP) which showed bacterial growth inhibition only at MIC values of >0.625 mg/mL. The PVP stabilized silver nanoparticles (PVP-AgNP) were comparatively lower in their efficiency as they were effective only at the concentrations of >5mg/mL (Table 3).

The individual analysis of responses of each test bacteria to different nanoparticles are presented in the Figures 1 – 3. The bacteria *S. aureus* was highly sensitive to PEG-CuNP followed by PVP-

CuNP, PEG-NiNP and PVP-NiNP (fig 3). The bacteria *E. coli* showed equally higher sensitivity to PEG-CuNP and PVP-CuNP followed by PEG-NiNP and PVP-NiNP (fig 4). The response of the bacteria *S. typhi* to the tested nanoparticles was in the order of PEG-CuNP > PVP-CuNP > PEG-NiNP > PVP-NiNP (fig 5). All of the tested bacteria were least sensitive to PVP-AgNP.

Comparative analysis of antibacterial efficiency of different nanoparticles revealed that the copper nanoparticles stabilized by both PEG and PVP (PEG-CuNP and PVP-CuNP) were better in the antibacterial action than the other nanoparticles as they exhibited their antibacterial activities with the MIC values as low as 0.078 mg/mL. From the view point of influence of polymeric stabilizers on the antibacterial activity of nanoparticles, it was observed that the PEG stabilized metal nanoparticles (PEG-CuNP and PEG-NiNP) were comparatively more efficient regarding the MIC values ranging from 0.078 to 0.625 mg/mL than those stabilized by PVP (PVP-CuNP, PVP-NiNP and PVP-AgNP) with the MIC values ranging from 0.078 to 5 mg/mL.

4. DISCUSSION

The present study evaluated the antibacterial activities of two of PEG stabilized metal nanoparticles namely copper (PEG-CuNP) and nickel (PEG-NiNP) and three PVP stabilized nanoparticles namely copper (PVP-CuNP), nickel (PVP-NiNP) and silver (PVP-AgNP) against three commonly known bacterial pathogens namely *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*.

The copper has been recognized as an antimicrobial agent since ancient days. Copper nanoparticles (CuNPs) exhibit their antimicrobial activity by virtue of their high surface-to-volume ratio and easy interaction with other particles[21]. The antibacterial assay carried out in the present study demonstrated the superior potential of copper nanoparticles stabilized by both PEG and PVP falling in line with the reported ones. The PEG-CuNP could inhibit the growth of all of the tested bacterial strains with MIC of 0.078 mg/mL. The PVP-CuNP utilized MICs of 0.078 and 0.156 mg/mL respectively to inhibit bacterial strains (Tables 1-3). Also it utilized a lowest MIC of 0.156 mg/mL against *S. typhi*. Even at higher dilutions it was found to be efficient in its action. Many earlier researchers have demonstrated the antimicrobial activity of copper nanoparticles against variety of pathogens including the gram positive and gram negative bacteria[22,23]. Both PEG-CuNP and PVP-CuNP synthesized in the present study displayed comparatively better efficiency on the test bacteria than that of the earlier research group reports and evident from figs. 3-5.

Nickel has been in frequent application in experimental environment as a model chemical owing to its moderate toxic property[12]. However, the reports explaining the antimicrobial activity of nickel nanoparticles (NiNPs) are scanty. The PEG-NiNP and PVP-NiNP employed in the present study exhibited moderate antibacterial activity with the former one showing better efficiency. The MIC ranges required for antibacterial activity by these two nanoparticles were 0.312-0.625 and 0.625-2.5 mg/mL respectively (Table 3). The lowest MICs utilized by PEG-NiNPs and PVP-NiNPs respectively were 0.312 and 0.156 mg/mL, each against *S. typhi*. Similar to our finding, Baek and An[12] have demonstrated the moderate antibacterial activity of NiO nanoparticles against *E. coli*, *B. subtilis* and *S. aureus* with respective median effective concentrations (EC50) of 115.7, 85.8 and 121.1 mg/L.

The silver nanoparticles (AgNPs) have been proved to possess antimicrobial activity against bacteria, viruses and other eukaryotic microorganisms[24]. Antimicrobial activity of AgNPs is attributed to the release of Ag+ and even low concentrations of Ag+ can contribute to the inhibitory activity[25]. Research studies conducted in recent years have documented the antibacterial activity of AgNPs against many bacterial pathogens including *S. aureus*[26-28], *E. coli*[25,29,30], *S. typhi*[31], *K. pneumoniae*[32], *P. aeruginosa*[33], *Proteus mirabilis*[28], etc. Surprisingly, the PVP-AgNPs synthesized in the present study could inhibit the growth of all the tested bacterial strains only at higher concentrations (Tables 2 & 3). Its requirement of MIC value of 5 mg/mL, being higher compared to other nanoparticles, revealed its poor bactericidal activity (figs. 3-5). Comparatively lesser efficiency of PVP-AgNPs could be attributed to its larger size. The antibacterial activity of silver nanoparticles is greatly influenced by the size, morphology and the concentration. Generally smaller particles have larger surface area to be in contact with the bacterial cells, showing a larger activity[34]. Proportionate with the concentration of silver ions the size of the silver nanoparticles would increase. Particles with larger size usually have smaller surface area and show reduced infiltration rate subsequently with a poor antibacterial effect[26]. Concomitant with our finding Agnihotri et al.[35], who studied the antibacterial effect of AgNPs with the sizes ranging 5-100 nm against *S. aureus* and *E. coli*, demonstrated the diminishing effect of bactericidal activity proportionated with the increase in size. Besides, the shape of the silver nanoparticles also influences the antibacterial activity[26]. The atomic density of polyhydron surface is directly proportional to the antibacterial

effect of the nanoparticle[36]. This concept could be agreeable as the PVP-AgNPs of the present study were found to be denser flocs, which might have negatively influenced the antibacterial activity.

The present study employed PEG and PVP polymers as chemical agents to bring about the stability of metal nanoparticles. Pure nanoparticles synthesized by chemical methods tend to agglomerate owing to the interparticle interaction (e.g., Vander Waals force) and magnetic interaction, which eventually reduce the specific surface area and interfacial free energy resulting in waning of reactivity of particles. This can be counteracted by the application of a stabilizer thereby the dispersion and steric hindrance can be enhanced[37]. Such effects would enable the particles to easily interact with outer membrane components of the bacterial cell causing significant changes like cell wall damage and death[38]. Research studies conducted on the augmenting effects of polymeric stabilizers such as starch[39] and chitosan[26,29] on bactericidal activity of AgNPs support this view.

Among the two stabilizers used in the present study significant difference in antibacterial activities between them were noted (Table 2; Fig 3-5). While the PEG stabilized NPs exhibited better antibacterial activities with the MIC values ranging 0.078 – 0.625 compared to PVP stabilized NPs showing MICs ranging mg/ml and 0.078 – 5.0 mg/mL. Our finding is in agreement with the studies of Dey et al.[40] which reported the floundering bactericidal activity of PVP stabilized AgNPs (MIC 269.5 µg/mL) compared to tri-sodium citrate AgNPs (MIC 26.75 µg/mL) against *S. aureus* and *E. coli*. It is generally understood that the antibacterial activity of metal nanoparticles depend on the adequate release of their ions, for example, Cu^{2+}, Ni^{2+}, Ag^{+}, etc., which is essential for exerting the toxicity. The comparatively inferior bactericidal activity of PVP-NiNPs and PVP-AgNPs in the present study could be attributed to inadequate of release of respective ions (i.e, Ni^{2+} and Ag^{+}) from the nanoparticles under the influence of PVP stabilizer. This speculation can be well explained by the findings of Xiu et al.[25], who experimented the antibacterial activities of PEG and PVP stabilized AgNPs against *E. coli*. This research group[25] reported the survival rate of the bacteria as 6%, 7% and 13% respectively when treated with PEG-AgNPs sized 3 nm, 5 nm and 11 nm. On the other hand the survival rates of the bacteria corresponding to different sized PVP-AgNPs (given in parenthesis) were 11% (20 nm) and 21% (40 nm).

5. CONCLUSION

The metal nanoparticles exhibit different degrees of antibacterial activities depending on the stabilizer used and their particle size. Among the three types of nanoparticles tested the copper nanoparticles display superior antibacterial activity followed by nickel and silver nanoparticles showing moderate and lower activities. Stabilization of nanoparticles with polymeric substances could result in the enhancement of the bactericidal activities. The two types of stabilizers viz., PEG and PVP vary in their influences on the antibacterial activities of nanoparticles. While the effect of PEG on antibacterial property of nanoparticles was encouraging, the PVP displayed poor performance particularly with nickel and silver nanoparticles, which could be due to its nature of causing larger size of particles and hindrance of release of ions. As the PEG stabilized copper nanoparticles (PEG-CuNPs) have been observed to possess promising antibacterial property, carrying out further in-depth studies to develop it as a potential agent could help combating drug resistant bacterial pathogens so as to ensure successful medical care.

Table 1: Antibacterial activity of PEG capped metal nanoparticles against three test bacteria

S. No	Test material & Bacteria tested	Concentration of drug (mg/mL) & response of test organism									
		5 mg	2.5 mg	1.25 mg	0.625 mg	0.312 mg	0.156 mg	0.078 mg	0.036 mg	P.C	N.C
	PEG-CuNP										
1	*Staphylococcus aureus*	-	-	-	-	-	-	-	+	-	+
2	*Escherichia coli*	-	-	-	-	-	-	-	+	-	+
3	*Salmonella typhi*	-	-	-	-	-	-	-	+	-	+
	PEG-NiNP										
1	*Staphylococcus aureus*	-	-	-	-	+	+	+	+	-	+
2	*Escherichia coli*	-	-	-	-	+	+	+	+	-	+
3	*Salmonella typhi*	-	-	-	-	-	+	+	+	-	+

"CuNP"- Copper nanoparticle; "NiNP"- Nickel nanoparticle; "+" – Growth of bacteria;
"-" – Inhibition of bacteria; "PC"- Positive control; "NC"- Negative control

Table 2: Antibacterial activity of PVP capped metal nanoparticles against three test bacteria

S. No	Test material & Bacteria tested	Concentration of drug (mg/mL) & response of test organism									
		5 mg	2.5 mg	1.25 mg	0.625 mg	0.312 mg	0.156 mg	0.078 mg	0.036 mg	P.C	N.C
	PVP-CuNP										
1	*Staphylococcus aureus*	-	-	-	-	-	-	+	+	-	+
2	*Escherichia coli*	-	-	-	-	-	-	-	+	-	+
3	*Salmonella typhi*	-	-	-	-	-	-	+	+	-	+
	PVP-NiNP										
1	*Staphylococcus aureus*	-	-	-	+	+	+	+	+	-	+
2	*Escherichia coli*	-	-	+	+	+	+	+	+	-	+
3	*Salmonella typhi*	-	-	-	-	+	+	+	+	-	+
	PVP-AgNP										
1	*Staphylococcus aureus*	-	+	+	+	+	+	+	+	-	+
2	*Escherichia coli*	-	+	+	+	+	+	+	+	-	+
3	*Salmonella typhi*	-	+	+	+	+	+	+	+	-	+

"CuNP"- Copper nanoparticle; "NiNP"- Nickel nanoparticle; "AgNP"- Silver nanoparticle;
"+" – Growth of bacteria; "-" – Inhibition of bacteria; "PC"- Positive control; "NC"- Negative control

Table 3: Dose dependent antibacterial activity of metal nanoparticles

Bacteria tested	Metal nanoparticle and MIC values (mg/ML)				
	PEG-CuNP	PEG-NiNP	PVP-CuNP	PVP-NiNP	PVP-AgNP
Staphylococcus aureus	0.078 mg	0.625 mg	0.156 mg	1.25 mg	5 mg
Escherichia coli	0.078 mg	0.625 mg	0.078 mg	2.5 mg	5 mg
Salmonella typhi	0.078 mg	0.312mg	0.156mg	0.625 mg	5 mg

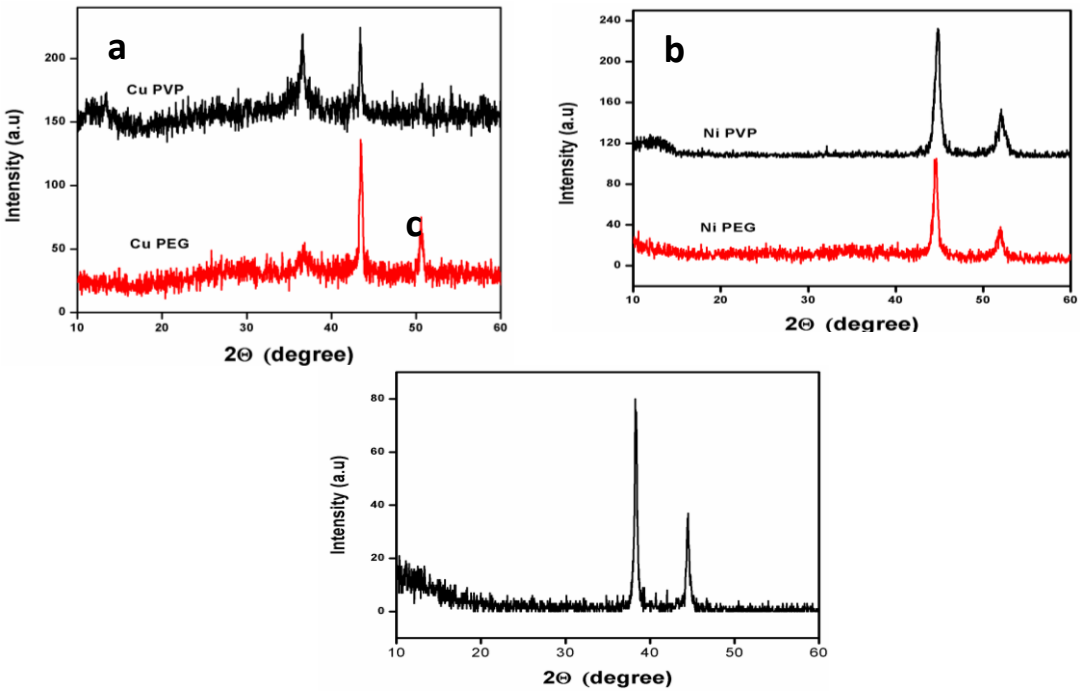

Fig. 1: XRD images of Nanoparticles a) CuNPs b) NiNPs c) AgNPs

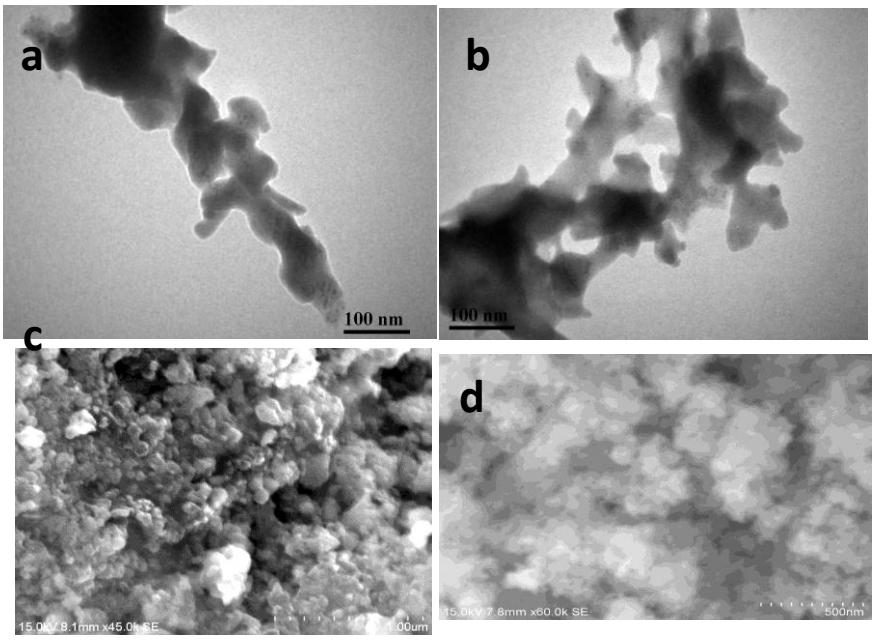

Fig. 2: TEM (a & b) and SEM (c & d) images of CuNPs and AgNPs

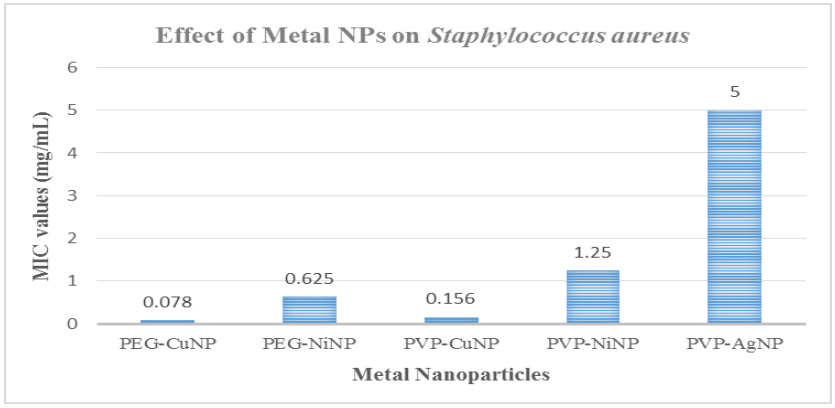

Fig. 3: MIC requirement by metal nanoparticles against *Staphylococcus aureus*

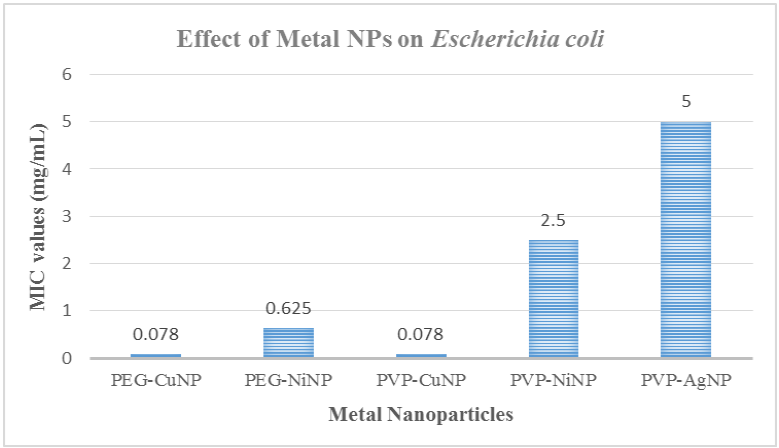

Fig. 4: MIC requirement by metal nanoparticles against *Escherichia coli*

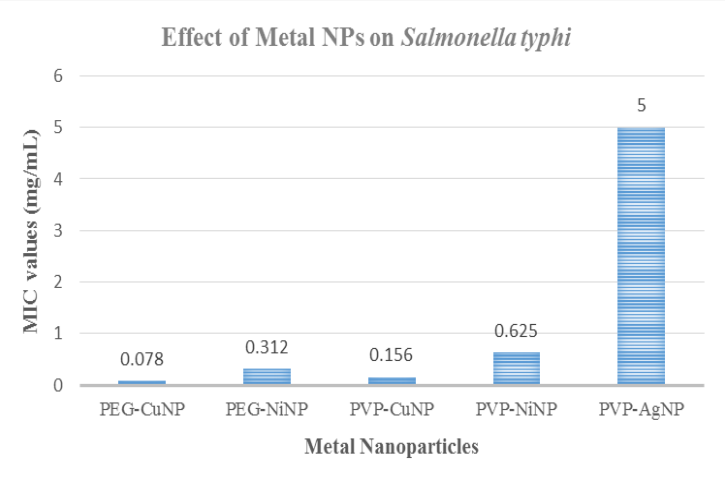

Fig. 5: MIC requirement by metal nanoparticles against *Salmonella typhi*

REFERENCES

1. Chuanchuen R, Karkhoff-Schweizer RR and Schweizer HP. High-level triclosan resistance in Pseudomonas aeruginosa is solely a result of efflux. Am J Infect Cont. 2003;31:124–27.
2. Franci G, Falanga A, Galdiero S, Palomba L, Rai M, Morelli G and Galdiero M. Silver nanoparticles as potential antibacterial agents. Molecules. 2015;20:8856-74.
3. Emily A, Morell BA and Balkin DM. Methicillin-resistant Staphylococcus aureus: a pervasive pathogen highlights the need for new antimicrobial development. Yale J Biol Med. 2010;84(4): 223–33.
4. Meng J, Zhao S, Doyle MP and Joseph SW. Antibiotic resistance of Escherichia coli O157:H7 and O157:NM isolated from animals, food, and humans. J Food Prot. 1998 61(11):1511–14.
5. Blair JMA, Webber MA, Baylay AJ, Ogbolu DO and Piddock LJV. Molecular mechanisms of antibiotic resistance. Nat Rev Microbiol. 2015;13:42-51.
6. World Health Organization. Antimicrobial Resistance: Global Report on Surveillance 2014 http://www.who.int/drugresistance/documents/surveillancereport/en/ (accessed Sep 1, 2016).
7. Gordon O, VigSlenters T and Brunetto PS. Silver coordination polymers for prevention of implant infection: thiol interaction, impact on respiratory chain enzyme and hydroxyl radical induction. Antimicrob Agents Chemother. 2010;54(10):4208-18.
8. Rai MK, Deshmukh SD, Ingle AP and Gade A.K. Silver nanoparticles: The powerful nanoweapon against multidrug-resistant bacteria. J App Microbiol. 2012;112:841–852.
9. Ip M, Lui SL, Poon VK, Lung I and Burd A. Antimicrobial activities of silver dressings: an in vitro comparison. J Med Microbiol. 2006;55:59-63.
10. Azam A, Ahmed AS, Oves M, Khan MS, Habib SS and Memic A. Antimicrobial activity of metal oxide nanoparticles against Gram-positive and Gram-negative bacteria: a comparative study. Int J Nanomed. 2012;7:6003–09.
11. Rani R, Kumar H, Salar RJ and Purewal SS. Antibacterial activity of copper oxide nanoparticles against gram-negative bacterial strain synthesized by reverse micelle technique. Int J Pharma Res Dev. 2014;6(1):72-78.
12. Baek YW and An YJ. Microbial toxicity of metal oxide nanoparticles (CuO, NiO, ZnO, and Sb2O3) to Escherichia coli, Bacillus subtilis, and Streptococcus aureus. Sci Tot Environ. 2011;409:1603-08.
13. Cui Y, ZhaoY , Tian Y, Zhang W, Lü X and Jiang X. The molecular mechanism of action of bactericidal gold nanoparticles on Escherichia coli. Biomaterials. 2012;33:2327-33.
14. Dubey D, Rath S, Sahu MC, Pattnaik L, Debata NK, Rabindra N and Padhy RN. Surveillance of infection status of drug resistant Staphylococcus aureus in an Indian teaching hospital. Asian Pac J Trop Dis. 2013; 3(2): 133–42.

15. Chassaing B and Darfeuille-Michaud A. The commensal microbiota and enteropathogens in the pathogenesis of inflammatory bowel diseases. Gastroenterol. 2011;140:1720–28.
16. Pitout JD. Infections with extended-spectrum beta-lactamase-produc- ing enterobacteriaceae: changing epidemiology and drug treatment choices. Drugs. 2010;70:313– 33.
17. Crump JA, Karlsson MS, Gordon MA and Parry CM. Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive salmonella infections. Clin Microbiol Rev. 2015;28(4):901-37.
18. Sun Y and Xia Y. Shape controlled synthesis of gold and silver nanoparticles. Science. 2002;298: 2176-79.
19. Huang W and Tang X. Preparation, structure and magnetic properties of mesoporous magnetite hollow spheres. J Colloid Interface Sci. 2005;28(2):432-36.
20. Sarkar SD, Nahar L and Kumarasamy Y. Microtitre plate–based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. Methods. 2007;42:321-24.
21. Tamilvanan A, Balamurugan K, Ponappa K and Kumar BM. Copper nanoparticles: synthetic strategies, properties and multifunctional application. Int J Nanosci. 2014;13(2):143001-22.
22. Hsiao MT, Chen SF, Shieh DB and Yeh CS. One pot synthesis of hollow Au3Cu1 spherical like and biomineral botallackite Cu-2(OH)(3)Cl flower like architectures exhibiting antimicrobial activity. J Phys Chem B. 2006;110:205-10.
23. Theivasanthi T and Alagar M. Studies of copper nanoparticles effect on microorganisms. Annals Bio Res. 2011;2(3):368-73.
24. Rai M, Yadav A and Gade A. Silver nanoparticles as a new generation of antimicrobials. Biotechnol Adv. 2009;27:76-83.
25. Xiu ZM, Zhang QB, Puppala HL, Colvin VL and Alvarez PJJ. Negligible particle-specific antibacterial activity of silver nanoparticles. Nano Lett. 2012;12:4271−75.
26. Cao XL, Cheng C, Ma YL and Zhao CS. Preparation of silver nanoparticles with antimicrobial activities and the researches of their biocompatibilities. J Mater Sci: Mater Med. 2010; 21: 2861-68.
27. Paredes D, Ortiz C and Torres R. Synthesis, characterization, and evaluation of antibacterial effect of Ag nanoparticles against Escherichia coli O157:H7 and methicillin-resistant Staphylococcus aureus (MRSA). Int J Nanomed. 2014;9:1717–29.
28. Singh K, Panghal M, Kadyan S and Yadav JP. Evaluation of antimicrobial activity of synthesized silver nanoparticles using Phyllanthus amarus and Tinospora cordifolia medicinal plants. J Nanomed Nanotechnol. 2014;5(6):1-5.
29. Namasivayam SKR and Roy EA. Enhanced antibiofilm activity of chitosan stabilized chromogenic silver nanoparticles against Escherichia coli. Int J Sci Res Pub. 2013;3(4):1-9.
30. Salem W, Leitner DR, Zingl FG, Schratter G, Prassl R, Goessler W, Reidl J and Schild S. Antibacterial activity of silver and zinc nanoparticles against Vibrio cholerae and enterotoxic Escherichia coli. Int J Med Microbiol. 2015;305:85–95.
31. Shameli K, Ahmad MB, Jazayeri SD, Shabanzadeh P, Sangpour P, Jahangirian H and Gharayebi Y. Investigation of antibacterial properties silver nanoparticles prepared via green method. Chem Cent J. 2012;6(73):1-10.
32. Naraginti S and Sivakumar A. Eco-friendly synthesis of silver and gold nanoparticles with enhanced bactericidal activity and study of silver catalyzed reduction of 4-nitrophenol. Spectrochim Acta A Mol Biomol Spectrosc. 2014;28:357–62.
33. Collins TL, Markus E.A, Hassett DJ and Robinson JB. The effect of a cationic porphyrin on Pseudomonas aeruginosa biofilms. Curr Microbiol. 2010;61:411–16.
34. Baker C, Pradhan A, Pakis L, Pochan DJ and Shah SI. Synthesis and antibacterial properties of silver nanoparticles. J Nanosci Nanotechnol. 2005; 5: 244-49.
35. Agnihotri S, Mukherji S and Mukherji S. Size-controlled silver nanoparticles synthesized over the range 5 100 nm using the same protocol and their antibacterial efficacy. RSC Adv. 2014;4:3974-83.
36. Sharma VK, Yngard RA and Lin Y. Silver nanoparticles: green synthesis and their antimicrobial activities. Adv Colloid interface Sci. 2009;145:83-96
37. He F, Zhao D and Liu J. Stabilization of Fe-Pd nanoparticles with sodium carboxymethyl cellulose for enhanced

transport and de-chlorination trichloroethylene in soil and groundwater. Ind Eng Chem Res. 2007;46:29-34.
38. Prema P and Thangapandiyan S. In vitro antibacterial activity of gold nanoparticles capped with polysaccharide stabilizing agents. Int J Pharm Pham Sci. 2013;5(1):310-14.
39. Mohanty S, Mishra S, Jena P, Jacob B, Sarkar B and Sonawane A. An investigation on the antibacterial, cytotoxic, and antibiofilm efficacy of starch-stabilized silver nanoparticles. Nanomed: Nanotechnol Biol Med. 2012;8(6):916-24.
40. Dey A, Dasgupta A, Kumar V, Tyagi A and Verma AK. Evaluation of the antibacterial efficacy of polyvinylpyrrolidone (PVP) and tri-sodium citrate (TSC) silver nanoparticles. Int Nano Lett. 2015; 5:223–30.