Original Research Article

Evaluation of the bactericidal effect of silver nanoparticles against Methicillin Resistant *Staphylococcus aureus* (MRSA) and Methicillin Sensitive *Staphylococcus aureus* (MSSA) strains isolated from mastitic milk of small ruminants and their surrounding environment in Aswan, Egypt

Randa Salah¹, Mohamed Karmi², Aml M. Abdel-Ra'ouf³*, Saber Kotb⁴
¹Department of Animal and Environmental hygiene, Faculty of Veterinary Medicine, Aswan University, Egypt.
²Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Aswan University, Egypt.
³Department of Animal Medicine, (Infectious diseases), Faculty of Veterinary Medicine, Aswan University, Egypt.
⁴Department of Animal and Poultry Hygiene and Environmental Sanitation, Faculty of Veterinary Medicine, Assiut University, Egypt.

ABSTRACT
In the recent years, emergence of infectious diseases caused by drug resistant pathogens had been increased; therefore there is an urgent need to search for new alternative and effective antimicrobial agents to overcome the drug resistance. In the present investigation, the study group consisted of 90 sheep and 90 goats with clinical evidences of mastitis in 17 (18.89%) goats and 5 sheep (5.56%) that manifested swollen udder with or without systemic signs of illness related to mastitis. Standard bacteriology was performed on pretreatment milk samples from the 17 goats and 5 mastitic ewes as well as 60 soil samples and 60 pail water samples. The bacteria isolated were identified as *Staphylococcus aureus* 12 (70.6%) from goats and 5 (100%) from sheep. In addition, *S. aureus* could also be identified in 41 (68.3%) soil samples and 42 (70%) water samples. The current study aimed to explore the bactericidal effect of silver nanoparticles (AgNPs) on methicillin- resistant *Staphylococcus aureus* (MRSA) and methicillin-sensitive *Staphylococcus aureus* (MSSA) strains isolated from mastitic milk and their surrounding environment (water and soil) in Aswan, Egypt. AgNPs solution was synthesized by typical one-step synthesis protocol using soluble starch and was characterized using transmission electron microscopy and atomic absorption spectrophotometer. The minimum inhibitory concentration (MIC) and minimum
bactericidal concentration (MBC) of AgNPs were examined to evaluate the bactericidal efficiency. In the present study, no significant discrepancy was observed between the influence of AgNPs on MRSA and MSSA (P > 0.05). It can be concluded that Ag-NPs have strong bactericidal effect against both MRSA and MSSA strains.

*Corresponding author: Aml M. Abdel-Ra'ouf, Department of Animal Medicine, (Infectious diseases), Faculty of Veterinary Medicine, Aswan University, Egypt. Email: aml.raouf@vet.aswu.edu.eg

1. Introduction

*Staphylococcus aureus* is one of the most pathogenic microorganisms for humans and animals since it causes severe infections, such as, wound infection, endocarditis, osteomyelitis, pneumonia, abscesses and food poisoning ([Tauxe, 2002; Deurenberg and Stobberingh, 2008; Chambers and DeLeo, 2009; Sowash et al., 2014]). *Staphylococcus aureus* is capable of acquiring mecA; a gene responsible for the production of penicillin binding protein 2a. This protein offers lower binding affinity for methicillin and other similar β-lactam antibiotics ([Katayama et al., 2005; Bustos-Martinez et al., 2006; Kinnevey et al., 2013]). The emergence of Methicillin Resistant *Staphylococcus aureus* (MRSA) becomes a worldwide problem ([David et al., 2008]). MRSA was first isolated in human in the United Kingdom in 1961 ([Barber, 1961]), while MRSA was first isolated from mastitic cow milk by [Devriese et al., (1972) in 1972. In the last few years, (MRSA) has been considered as a critical public health problem threatening humans and animals ([Petinaki and Spiliopoulou, 2012]). According to the aforementioned, researchers commence to search for new alternative antimicrobial agents for treatment of MRSA.

Recently, Nanoparticles (NPs) have been used as antimicrobial agent against drug resistant bacterial strains ([Koo et al., 2005; Gong et al., 2007; Lopez Goerne et al., 2012; Mahmoudi and Serpooshan, 2012; Rai et al., 2012; Franci et al., 2015; Kim et al., 2017; Wang et al., 2017; Yuan et al., 2017; Kumar et al., 2018]). Because of their unusual chemical and physical properties such as small size with large surface area and high reactivity, nanoparticles easily penetrate microorganisms and render their antimicrobial activity ([Morones et al., 2004]). Due to their high antimicrobial activity against viruses, bacteria and other eukaryotic microorganisms, safety and cost effectiveness, silver nanoparticles have been recognized to be the most effective nanoparticles ([Gong et al., 2007; Szmacinski et al., 2008; Lazar, 2011; Taraszkiewicz et al., 2013; Salomoni et al., 2015; Patra and Baek, 2017]). Roy et al., (2007) reported that the antimicrobial action of Ag-NPs is more effective than that of broad-spectrum antibiotics used worldwide. Therefore, AgNPs have been commonly used as antimicrobial agent against drug resistant bacterial strains ([Kvitek et al., 2008; Jones and Hoek, 2010; Lara et al., 2010; Ansari et al., 2011]). AgNPs can also be applied in cases of burn, traumatic wound dressings, diabetic ulcers, coating of catheters, dental works, medical devices and water filtration ([Jain and Pradeep, 2005; Silver et al., 2006; Gong et al., 2007; Kim et al., 2007; Thomas et al., 2007; Rai et al., 2009; Martinez-Gutierrez et al., 2010; Dosoky et al., 2015; Ibrahim et al., 2020]). In the present study, the bactericidal effect of silver nanoparticles against MRSA and MSSA strains isolated from mastitic milk of small ruminants and their surrounding environment in Aswan was investigated.

2. Materials and methods

2.1. Animals and samples

**Animals:**

A total of 90 sheep and 90 goats were subjected for clinical examination where 17 (18.89%) goats and 5 (5.56%) sheep showed obvious signs of clinical mastitis including swollen udder with or without systemic signs of illness related to mastitis based on the criteria described by [Dirksen et al. (1993)].

**Samples:**

**a. Milk**

Milk samples were collected from 17 goats and 5 sheep mastitic milk samples out of 90 sheep and 90 goats. About 10 ml of milk were hygienically drawn from each quarter into a sterile 50 ml falcon tubes. Milk sampling was done according to the recommendation of [National Mastitis Council (1999)], immediately transported to the laboratory in ice-cooled containers and analyzed within 24 to 48 h after collection.
b. Soil
Sixty soil samples were collected according to Clegg et al. (1983).

c. Water
Sixty pail water samples were taken from pail used for watering of animals in clean sterile transparent 50 ml falcon tubes.

2.2. Silver nitrate (Ag NO3) crystal, soluble starch

2.3. Cultures
MRSA and MSSA strains were isolated from milk of mastitic sheep and goats and their surrounding environment.

2.4. Isolation and identification of S. aureus
It was done according to Melter et al. (1999) and Lee (2003). The samples were immediately suspended in Tryptone Soya Broth (TSB, Oxoid) containing 10% NaCl and then incubated at 37°C for 24 h for selective enrichment of Staphylococci. Enrichment cultures were then streaked out on mannitol salt agar (MSA, Oxoid) and incubated at 37°C for 24 h. The presumptive isolates were phenotypically characterized by yellow fermentation of mannitol.

2.5. Phenotypic identification of Methicillin Resistant S. aureus (MRSA)
The positive S. aureus samples were re-streaked on mannitol salt agar (MSA, Oxoid) supplemented with 6 mg/L of oxacillin (Sigma, St. Louis, Mo.) for selective isolation of MRSA. A sample was positive for MRSA if one or more colonies were identified and one representative colony was selected from each sample for further testing. The isolates were further confirmed as MRSA by using DrySpot Staphytect Plus latex agglutination test kit (Oxoid, United Kingdom).

2.6. Inoculum preparation for minimal inhibitory concentrations (MIC):
Preparation of bacterial suspension: Colonies from MRSA and MSSA isolates were transferred to 5 ml nutrient broth and incubated at 37°C for 17 h. Tenfold serial dilution were prepared using sterile nutrient broth as a diluent, by mixing 9 ml nutrient broth and 1 ml bacterial suspension and 1ml was transferred from the first tube to the second tube till 10⁷ concentrations and 1 ml quantity from each dilution was spread on plate count agar and incubated at 37°C for 24 h. About 4-5 colonies from plate count agar contain 20-300 colonies were transferred to 5 ml nutrient broth then the broth culture was incubated at 35-37°C, until it achieved turbidity of the 0.5 McFarland standards. The turbidity of broth culture was adjusted with sterile broth to obtain turbidity comparable to that of the 0.5 McFarland standards. This suspension was containing about 1-2x10⁶ CFU / ml.

2.7. Synthesis of silver nanoparticles (Ag-NPs):
Stable AgNPs less than 100 nm were synthesized according to (Vigneshwaran et al., 2006). Briefly, 1gm of soluble starch was added to 100 ml of deionized water and heated till complete dissolution and cooling and 0.08 gm silver nitrate (AgNO₃) was added to 1ml of deionized water in dark glass bottle. Soluble starch solution was mixed with silver nitrate solution. This mixture was put in dark glass bottle, wrapped in aluminum foil and kept in autoclave at 121°C for 5 minutes. Clear yellow color solution indicated to the formation of AgNPs. Graphite Furnace Atomic Absorption (Model 210VGP) was used for determining the concentration of AgNPs stock solution. The size of AgNPs was measured by transmission electron microscopy (TEM) (JEOL-JEM-100CX) at Electron Microscopy Unit, Assiut University, Egypt.

2.8. Determination of minimum inhibitory concentration (MIC)
MIC technique was performed according to CLSI (2009) using successive serial 2 fold dilution technique. The original concentration of stock AgNPs solution was 100 µg / ml. The MIC technique was done in triplicate to confirm the MIC value for the tested organism.

2.9. MIC technique
Sterile capped test tubes were numbered from 1 to 6, one positive (MHB+ bacterial inoculation) and one negative (MHB+ AgNPs) tube. Two ml Muller Hinton Broth (MHB, Oxiod) were added to each tube, then 2 ml from AgNPs stock was added to the first tube and to the negative tube. The first tube was shacked and 2ml were transferred to the second tube. The contents of the second tube were mixed and then transferred 2ml to the third tube. Dilutions were continued in this manner till tube 6 and finally 2 ml were discarded. Finally, 0.1 ml of bacterial inoculum was added to all 6 tubes and to positive tube. All tubes were incubated at 37°C for 24 h.

2.10. Determination of minimum bactericidal concentration (MBC)
Five grams / L of sodium thiosulfate (Na₂S₂O₃) were added to Ag-NPs to halt the antimicrobial reaction between Ag-NPs and bacteria as described in the European quality standards (Nen, 1997). MBC was determined by subculturing the content of all
positive MIC tubes on mannitol salt agar (MSA) plates and incubated at 37°C for 24h. The MBC was determined as the lowest concentrations of silver nanoparticles that killed 100% of bacteria. Disinfection activity of AgNPs was measured by MBC/MIC ratio.

2.11. Statistical analysis:
MIC and MBC tests were performed in triplicate, and the results were expressed as the mean ± the standard errors of the mean. SPSS was used to compare these results.

3. Results
Table (1): Prevalence of isolated MRSA and MSSA from mastitic milk of goats and sheep, soil and water

| Sample       | Sample s | S. aureus | MRSA  | MSSA  |
|--------------|----------|-----------|-------|-------|
| Goats milk   | 17       | 12 (70.6%)| 5 (29.4%) | 7 (41.2%) |
| Sheep milk   | 5        | 5 (100%)  | 3 (60%)  | 2 (40%)  |
| Soil         | 60       | 41 (68.3%)| 21 (35%) | 20 (33.3%) |
| Water        | 60       | 42 (70%)  | 21 (35%) | 21 (35%) |
| Total        | 142      | 100 (70.4%)| 50 (35.2%) | 50 (35.2%) |

Figure (1): Negative and positive DrySpot Staphytect plus Latex agglutination test-kit

Figure (2): Transmission Electron Microscopy (TEM) image of Ag-NPs with spherical shapes (sizes ranged from 8.55 to 20.3nm).

Figure (3): Percentages of MRSA isolates showing values of MIC and MBC

Figure (4): Percentages of MSSA isolates showing values of MIC and MBC

Figure (5) Results of MIC test: Concentration of AgNPs (tube1=100 µg/ml; tube2=50 µg/ml; tube3=25 µg/ml; tube4=12.5 µg/ml; tube5=6.25 µg/ml; tube6= 3.125 µg/ml; P=positive tube and N=negative tube). Tube 4 is considered as MIC tube as it is the lowest concentration inhibits MRSA.
4. Discussion

In this study, clinical examination of a total of 90 goats and 90 sheep revealed the presence of clinical signs suggesting mastitis in 17 (18.89%) goats and 5 (5.56%) sheep. Results illustrated in table (1) revealed isolation of Staph. aureus from 12/17 (70.6%) of goat milk and in 5/5 (100%) of sheep milk, which is considered the most pathogenic microorganism for humans and animals. In this study, S. aureus was also present in 41/60 (68.3%) soil samples and in 42/60 (70%) water samples. Interestingly, such findings refer to the importance of soil and water as potential sources of infection to susceptible goats and sheep. MRSA was isolated from 5/17 (29.4%), 3/5 (60%), 21/60 (35%) and 21/60 (35%) of goat milk, sheep milk, soil and water samples respectively. Data presented in table (2) showed that the mean values of MIC and MBC of Ag-NPs against MRSA were 8.125±0.19 and 13.125±0.78 µg/ml, respectively. While, the mean values of MIC and MBC of Ag-NPs against MSSA were 7.5±0.73 and 9.375±0.41 µg/ml, respectively. The bactericidal effect of Ag-NPs against MRSA was higher than data of commercial antibiotic. For example, the MIC of gentamicin antibiotic against MRSA was 64 µg/ml (Ayala-Nunez et al., 2009). The significant difference in MIC and MBC values might be attributed to the size and shape of nanoparticles, the methods of preparation, type of reducing and stabilizing agent and the genetic variation of the isolated organisms (Ansari et al., 2011; Wady et al., 2014).

The MBC/MIC value is a parameter referring to the bactericidal efficiency of Ag-NPs. The obtained results in table (2) showed that Ag-NPs inhibited the growth of both of MRSA and MSSA in a bactericidal effect rather than a bacteriostatic effect (MBC/MIC ratio <4). The bactericidal activity agents are preferable than bacteriostatic as if the

Table (2): MIC and MBC and MBC/MIC ratio of Ag-NPs tested against MRSA and MSSA.

| Isolate Numbers (%) | Isolate Numbers (%) | MBC/MIC Ratio | MBC/MIC Ratio | MBC/MIC Ratio |
|---------------------|---------------------|----------------|----------------|----------------|
| MRSA (no=50)        | MSSA (no=50)        |                |                |                |
| MIC value (µg/ml)   | MIC value (µg/ml)   | MBC value (µg/ml) | MBC value (µg/ml) | MBC value (µg/ml) | MBC value (µg/ml) | MBC value (µg/ml) | MBC value (µg/ml) |
| 10                  | 10                  | 1              | 1              | 1              | 1              | 1              | 1              |
| 10                  | 10                  | 2              | 2              | 2              | 2              | 2              | 2              |
| 10                  | 10                  | 2              | 2              | 2              | 2              | 2              | 2              |
| 10                  | 10                  | 1              | 1              | 1              | 1              | 1              | 1              |
| Mean                | Mean                | 1.6            | 1.6            | 1.6            | 1.6            | 1.6            | 1.6            |
| 8.125±0.19          | 13.125±0.78         | 1.6            | 1.6            | 1.6            | 1.6            | 1.6            | 1.6            |

*: non-significant correlation (p > 0.05)
bacteria are killed rather than inhibited, this will lead to rapid combating of infection and reducing of bacterial resistance (French, 2006).

The mode of bactericidal action of AgNPs is still not clear. Several researchers have suggested that Ag-NPs bind to the surface of the cell membrane and changed their cellular permeability and the respiration (Lok et al., 2006; Panacek et al. 2006; Choi et al., 2008; Li et al., 2013). It is also proposed that Ag-NPs penetrate inside the bacteria and inactivate DNA replication, producing reactive oxygen species (ROS) causing death of the cell (Park et al., 2011; Gurunathan, 2015; Wang et al., 2017; Yuan et al., 2017). It is also obvious that there was no significant difference between the effect of Ag-NPs on MRSA and MSSA (p> 0.05). This result proved that the impact of AgNPs on both MRSA and MSSA was similar; explaining that resistant proteins that make bacteria resist antibiotics not influenced by nanoparticles and AgNPs not only act on PBP2a but act on a broad range of targets, such as cell membrane and cytoplasmic proteins and genomic DNA. This results agreed with (Kong and Jang 2008; Petica et al., 2008; Ayala-Nunez et al., 2009; Lara et al., 2010; Ansari et al., 2011; Cavassin et al., 2015; Kotb and Sayed 2015; Rangari et al., 2015).

5. Conclusion
Results of the current study revealed that the effect of AgNPs on MRSA and MSSA did not vary substantially. The bactericidal effect of Ag-NPs against MRSA was higher than that of the commercial antibiotics.

6. Conflict of interest statement
No conflicts of interest.

7. References
Ansari, M.A. and Alzohairy, M.A. (2018): One-Pot Facile Green Synthesis of Silver Nanoparticles Using Seed Extract of Phoenix dactylifera and Their Bactericidal Potential against MRSA. Evidence-Based Complementary and Alternative Medicine. 2018:1-9.
Ansari, M.A.; Khan, H.M.; Khan, A.A.; Sultan, A.; Azam, A.; Shahid, M. and Shujatullah, F. (2011): Antibacterial activity of silver nanoparticles dispersion against MSSA and MRSA isolated from wounds in a tertiary care hospital of North India. Inter. J. App. Biol. Pharm. Tech. 2(4):34-42.
Asghar, M.A.; Zahir, E.; Shahid, S.M.; Khan, M.N.; Asghar, M.A.; Iqbal, J. and Walker, G. (2018): Iron, copper and silver nanoparticles: Green synthesis using green and black tea leaves extracts and evaluation of antibacterial, antifungal and aflatoxin B1 adsorption activity. LWT. Food. Sci. Technol. 90: 98–107.
Ayala-Nunez, N.V.; Lara, H.H.; Turrent Liliana del, C.I. and Padilla, C.R. (2009): Silver nanoparticles toxicity and bactericidal effect against Methicillin-Resistant staphylococcus aureus: Nanoscale does matter. Nanobiotechnol.5:2-9.
Barber, M. (1961): Methicillin-resistant staphyloccoci. J. Clin. Path. 14: 385–393.
Bustos-Martinez, J.A.; Hamdan-Partida, A. and Gutierrez-Cardenas, M. (2006): Staphylococcus aureus: la reemergencia de un patógeno en la comunidad. Rev Biomed. 17:287–305.
Cavassin, E.D.; de Figueiredo, L.F.; Otoch, J.P.; Seckler, M.M. and de Oliveira, R.A. (2015): Comparison of methods to detect the in vitro activity of silver nanoparticles (AgNP) against multidrug resistant bacteria. J. Nanobiotechnol.13:1-16.
Chambers, H.F. and Deleo, F.R. (2009): Waves of resistance: Staphylococcus aureus in the antibioticera. Nat. Rev. Microbiol. 7: 629-641.
Choi, O.; Deng, K.; Kim, N.; Ross, L.; Surampalli, R. and Hu, Z. (2008): The inhibitory effects of silver nanoparticles, silver ions, and silver chloride colloids on microbial growth. J. Water Res.42:3066-3074.
Clegg, F.G.; Chiejina, S.N.; Duncan, A.L.; Kay, R.N. and Wray, C. (1983): Outbreaks of Salmonella newport infection in dairy herds and their relationship to management and contamination of the environment. Veterinary Record. 112: 580-584.
CLSI (2009): Methods for dilution of antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standard 5th ed. CLSI document; M07-A8 (ISBN 1-56238-689-1).
Das, B.; Dasha, S.K.; Mandala, D.; Ghoshb, T.; Chattopadhyaya, S.; Tripathya, S.; Das, S.; Deyc, S.K.; Dash, D. and Roy, S. (2015): Green synthesized silver nanoparticles destroy
multidrug resistant bacteria via reactive oxygen species mediated membrane damage. Arab. J. Chem. 10:862-876.

David, M.Z.; Glikman, D.; Crawford, S.E.; Peng, J.; King, K.J.; Hostetler, M.A.; Boyle-Vavra, S. and Daum, R.S. (2008): What is community associated methicillin-resistant <em>Staphylococcus aureus</em>? J. Infect. Dis. 197:1235-1243.

Deurenberg, R.H. and Stobberingh, E.E. (2008): The evolution of <em>Staphylococcus aureus</em>. Infect.Genet.Evol.8:747-763.

Devriese, L.A.; Van Damme, L.R. and Fameree, L. (1972): Methicillin (cloxacillin) resistant <em>Staphylococcus aureus</em> strains isolated from bovine mastitis cases. J. Vet. Med. B Infectious Diseases and Veterinary Public Health. 19:598–605.

Dirksen, G., Gründer, H-D. and Stöber, M.(1993). Rosenberg, Die Klinische Untersuchung des Rindes, (third ed., Verlagbuchhandlung Paul Parey, Berlin and Hamburg).

Dosoky, R.; Koth, S. and Farghali, M. (2015): Efficiency of silver nanoparticles against bacterial contaminants isolated from surface and ground water in Egypt. J. Adv. Vet. Anim. Res. 2(2): 175-184.

Franci, G.; Falanga, A.; Galdiero, S.; Palomba, L.; Rai, M.; Morelli, G. and Galdiero, M. (2015): Silver nanoparticles as potential antibacterial agents. Molecules. 20: 8856–8874.

French, G.L. (2006): Bactericidal agents in the treatment of MRSA infections- the potential role of dopamycin. J. Antimicro. Chemoth.37: 245-248.

Gong, P.; Li, H.M.; He, X.X.; Wang, K.M.; Hu, J.B.; Tan, W.H.; Zhang, S.C. and Yang ,X.H. (2007): Preparation and antibacterial activity of Fe3O4@Ag nanoparticles. Nanotechnology 18: 604–611.

Gurunathan, S. (2015): Biologically synthesized silver nanoparticles enhances antibiotic activity against Gram-negative bacteria. J. Ind. Eng. Chem. 29: 217–226.

Ibrahim, A.; Soliman, M.; Kothb, S. and Ali, M.M. (2020): Evaluation of fish skin as a biological dressing for metacarpal wounds in donkeys. BMC. Vet. Res. 16: 472.

Jain, P. and Pradeep, T. (2005): Potential of silver nanoparticle-coated polyurethane foam as an antibacterial water filter. Biotechnol Bioeng .90(1):59–63.

Jones, C.M. and Hoek, E.M.V. (2010): A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment. J. Nanopart. Res. 12:1531-1551.

Katayama, Y.; Robinson, D.A.; Enright, M.C. and H.F. Chambers, H.F. (2005): Genetic background affects stability of mecA in <em>Staphylococcus aureus</em>. J. Clin. Microbiol. 43: 2380-2383.

Kim, D.H.; Park, J.C.; Jeon, G.E.; Kim, C.S. and Seo, J.H. (2017): Effect of the Size and Shape of Silver Nanoparticles on Bacterial Growth and Metabolism by Monitoring Optical Density and Fluorescence Intensity. Biotechnol. Biopro. Eng. 22: 210-217.

Kim, J.S.; Kuk, E.; Yu, K.N.; Kim, J.H.; Park, S.J.; Lee, H.J.; Kim, S.H ; Park, Y.K.; Hwang, C.; Kim, Y.; Lee, Y.; Jeong, D. and Cho, M. (2007): Antimicrobial effects of silver nanoparticles. J. Nanomed. Nanotechnol, Biology and Medicine. 3(1): 95–101.

Kinnevey, P. M.; Shore, A.C.; Brennan, G.I.; Sullivan, D.J.; Ehricht, R.; Monecke, S.; Slickers, P. and Coleman, D.C. (2013): Emergence of Sequence Type 779 Methicillin-Resistant <em>Staphylococcus aureus</em> Harboring a Novel Pseudo Staphylococcal Cassette Chromosome mec (SCCmec)-SCCSCCRRISPR Composite Element in Irish Hospitals. Antimicrob. Agents. Chemother. 57 (1): 524–531.

Kong, H. and Jang, J. (2008): Antibacterial properties of novel poly (methyl methacrylate) nanofiber containing silver nanoparticles. Langmuir. 24: 2051–2056.

Koo, O. M.; Rubinstein, I. and Onyuksel, H. (2005): Role of nanotechnology in targeted drug delivery and imaging: A concise review. J. Toxicol. Environ. Health, Part A. 1:193–212.

Kotb, S. and Sayed, M. (2015): Bactericidal efficiency of silver nanoparticles against methicillin resistance (MRSA) and methicillin susceptible <em>Staphylococcus aureus</em> (MSSA) strains isolated from milk and its surrounding milking environment. Assiut Veterinary Medicine Journal. 61(144): 194-200.

Kulkarni, P.; Rathod, V.; Jyoti, H.; Patil, S.; Dattu, S. and Krishnaveni, R. (2014): Production of silver nanoparticles using...
*Aspergillus terreus* and its antibacterial activity against methicillin resistant *Staphylococcus aureus* (MRSA). Int. J. Latest Res. Sci. Technol. 3(4): 144-148.

Kumar, M.; Curtis, A. and Hoskins, C. (2018): Application of nanoparticle technologies in the combat against anti-microbial resistance. Pharmaceutics. 10: 11–27.

Kvitek, L.; Panacek, A.; Soukupova, J.; Kolar, M.; Vecerova, R.; Pucrek, R.; Holecova, M. and Zboril, R. (2008): Effect of surfactants and polymers on stability and antibacterial activity of silver nanoparticles (NPs). J. Phys. Chem. C. 112:5825–5834.

Lara, H.; Ayala-N’unez, N.; Ixtepan-turrent, L. and Rodriguez-padilla, C. (2010): Bactericidal effect of silver nanoparticles against multidrug-resistant bacteria. World. J. Microbiol. Biotechnol. 26(4): 615-621.

Lazar, V. (2011): Quorum sensing in Biofilms-How to destroy the bacterial citadels or their cohesion/power. Anaerobe. 17: 280–285.

Lee, J.H. (2003): Methicillin (Oxacillin)-Resistant *Staphylococcus aureus* Strains Isolated from Major Food Animals and Their Potential Transmission to Humans. Appl. Environ. Microbiol. 69(11): 6489–6494.

Li, C.; Fu, R.; Yu, C.; Li, Z.; Guan, H.; Hu, D.; Zhao, D. and Lu, L. (2013): Silver nanoparticle/chitosan oligosaccharide/poly(vinylalcohol) nanofibers as wound dressings: A preclinical study. Int. J. Nanomed. 8: 4131–4145.

Lok, C.N.; Ho, C.M.; Chen, R.; He, Q.Y.; Yu, W.Y.; Sun, H.; Tam, P.K.; Chiu, J.F. and Chen, C.M. (2006): Proteomic analysis of the mode of antibacterial action of silver nanoparticles. J. Proteome. Res. 5(4):916–924.

Lopez Goerne, T.M.; Alvarez Lemus, M.A.; Morales, V.A.; López, E.G. and Ocampo, P.C. (2012): Study of bacterial sensitivity to Ag–TiO2 nanoparticles. J. Nanomed. Nanotechnol. 5: 1–7.

Mahmoudi, M. and Serpooshan, V. (2012): Silver-coated engineered magnetic nanoparticles are promising for the success in the fight against antibacterial resistance threat. ACS Nano. 6: 2656–2664.

Martinez-Gutierrez, F.; Olive, P.L.; Banuelos, A.; Orrantia, E.; Nino, N.; Sanchez, E.M.; Ruiz, F.; Bach, H.; Av-Gay, Y. (2010): Synthesis, characterization, and evaluation of antimicrobial and cytotoxic effect of silver and titanium nanoparticles. Nanomedicine: Nanotechnology, Biology, and Medicine. 6: 681–688.

Melter, O.; Santos Sanches, I.; Schindler, J.; Aires de Sousa, M.; Mato, R.; Kovarova, V.; Zemlickova, H. and de Lencastre, H. (1999): Methicillin-resistant *Staphylococcus aureus* clonal types in the Czech Republic. Journal of Clinical Microbiology. 37: 2798–2803.

Morones, J.R.; Elechiguerra, J.L.; Camacho, A.; Holt, K.; Kouri, J.B.; Ramirez, J.T. and Yacaman, M.J. (2005): The bactericidal effect of silver nanoparticles. Journal of Nanotechnology.16: 2346-2353.

National Mastitis Council (1999): Laboratory handbook on bovine mastitis. National Mastitis Council, Madison, WI.

Nen, E.N. (1997): Chemical disinfectants and antiseptics-quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic, and institutional areas-test method and requirements (phase 2, step1). European committee for standardization, Brussels. 1276.

Panacek, A.; Kvitek, L.; Pucrek, R.; Kolar, M.; Vecerova, R.; Pizurova, N.; Sharma, V. K.; Neveca, T. and Zboril, R. (2006): Silver colloid nanoparticles: synthesis, characterization, and their antibacterial activity. J. Phys. Chem. B. 110(33): 16248-16253.

Park, M.V.; Neigh, A.M.; Vermeulen, J.P.; de la Fonteyne, L.J.; Verharen, H.W.; Briede, J.J.; Van Loveren, H. and De Jong, W.H. (2011): The effect of particle size on the cytotoxicity, inflammation, developmental toxicity and genotoxicity of silver nanoparticles. J. Biomaterials. 32(36): 9810-9817.

Patra, J. K. and Baek, K. H. (2017): Antibacterial activity and synergistic antibacterial potential of biosynthesized silver nanoparticles against foodborne pathogenic bacteria along with its anticandidal and antioxidant effects. Frontiers in Microbiology. 8: 167–180.

Petica, A.; Gavriliu, S.; Lungu, M.; Buruntea, N. and Panzarau, C. (2008): Colloidal silver solutions with antimicrobial properties. Mater. Sci. Eng. 152: 22–27.

Petinaki, E. and Spiliopoulou, I. (2012): Methicillin-resistant *Staphylococcus aureus* among companion and food-chain animals:
Rai, M. K.; Deshmukh, S. D.; Ingle, A. P. and Gade, A. K. (2012): Silver nanoparticles, the powerful Nano weapon against multidrug-resistant bacteria. J. Appl. Microbiol. 112: 841–852.
Rai, M.; Yadav, A. and Gade, A. (2009): Silver nanoparticles as a new generation of antimicrobials. Biotechnol. Adv. 27:76–83.
Rangari, A.A.; Sharma, N.K.; Goyal, M.R.; Thakur, R. and Singh, P. (2015): Evaluation and Efficacy of In-Vitro Antibacterial Activity of Silver Nano Particles Against Multidrug Resistant Bacterial Isolates from Skin Infections of Patients at a Tertiary Care Hospital in Western Uttar Pradesh of India. Int. J. Curr. Microbiol. App. Sci. 4(10): 764-773.
Rai, M. K.; Deshmukh, S. D.; Ingle, A. P. and Gade, A. K. (2012): Silver nanoparticles, the powerful Nano weapon against multidrug-resistant bacteria. J. Appl. Microbiol. 112: 841–852.
Rai, M.; Yadav, A. and Gade, A. (2009): Silver nanoparticles as a new generation of antimicrobials. Biotechnol. Adv. 27:76–83.
Roy, R.; Hoover, M.R.; Bhalla, A.S.; Slawecki, T.; Dey, S.; Cao, W.; Li. J. and Bhaskar, S. (2007): Ultra dilute Ag-aquasols with extraordinary bactericidal properties: role of the system Ag–O–H2O. Mater. Res. Innov. 11(1): 3–18.
Salomoni, R.; Léo, P. and Rodrigues, M. F. A. (2015): Antibacterial activity of silver nanoparticles (AgNPs) in Staphylococcus aureus and cytotoxicity effect in mammalian cells. The Battle Against Microbial Pathogens: Basic science, technological advances and educational programs. 851-857.
Silver, S.; Phung, L.T. and Silver, G. (2006): Silver as biocides in burn and wound dressings and bacterial resistance to silver compounds. J. Indust. Microbiol. Biotechnol. 33:627–634.
Sowash., Madeleine., G. and Uhlemann, AC. (2014): Community-Associated Methicillin-Resistant Staphylococcus aureus Case Studies. Methods. Mol. Biol. 1085: 25–69.
Surwade, P.; Ghildyal, C.; Weikel, C.; Luxton, T.; Peloquin, D.; Fan, X. and Shah, V. (2019): Augmented antibacterial activity of ampicillin with silver nanoparticles against methicillin-resistant Staphylococcus aureus (MRSA). J. Antibiotics.72:50–53.
Szymanski, H.; Lakowicz, J.R.; Catchmark, J. M.; Eid, K.; Anderson, J. P. and Middendorf, L. (2008): Correlation between scattering properties of silver particle arrays and fluorescence enhancement. Applied Spectroscopy. 62: 733–738.
Taraszkiewicz, A.; Fila, G.; Grinholc, M. and Nakonieczna, J. (2013): Innovative strategies to overcome biofilm resistance. BioMed. Res. Inter. 2013.
Tauxe, R.V. (2002): Emerging foodborne pathogens. Int. J. Food Microbiol.78:31-41.
Thomas, V.; Yallapu, M.M.; Sreedhar, B. and Bajpai, S.K. (2007): A versatile strategy to fabricate hydrogel-silver nanocomposites and investigation of their antimicrobial activity. J. Colloid Interface Sci. 315:389–395.
Vigneshwaran, N.; Nachane, R.P.; Balasubramanya, R.H. and Varadarajan, P.V. (2006): A novel one-pot —green synthesis of stable silver nanoparticles using soluble starch. Carbohydrate Research. 341: 2012-2018.
Wady, A. F.; Machado, A. L.; Foggi, C.C.; Zamperini, C. A.; Zucolotto, V.; Moffa, E.B., and Vergani, C.E. (2014): Effect of a silver nanoparticles solution on Staphylococcus aureus and Candida spp. Journal of Nanomaterials. 1–7.
Wang, L.; Hu, C. and Shao, L. (2017): The antimicrobial activity of nanoparticles: Present situation and prospects for the future. Inter. J. Nanomedicine. 12: 1227–1249.
Yuan, Y. G.; Peng, Q. L. and Gurunathan, S. (2017): Effects of Silver Nanoparticles on Multiple Drug-Resistant Strains of Staphylococcus aureus and Pseudomonas aeruginosa from Mastitis-Infected Goats: An Alternative Approach for Antimicrobial Therapy. Int. J.Mol. Sci. 18(3): 569.