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Original Research Paper

Pathology

ANTI BACTERIAL EFFECT OF SILVER NANOPARTICLE ON MRSA ISOLATES FROM ORAL INFECTIONS

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ABSTRACT

Aim of our study is to evaluate the antibacterial activity of silver Nano particle(Ag-NPs) against MRSA strains recovered from patients with oral infections

Material and Methods:- Samples were collected from Patients attending department of oral and maxillofacial surgery, Jaipur dental college who were diagnosed with oral infections. The samples were subjected to culture and sensitivity and MRSA (Methicillin resistant Staphylococcus aureus) screening. MRSA strains were treated with Ag-Nanoparticles to know the antibacterial affect

Results:- Out of 146 samples, 26 specimens showed Methicillin-resistant S aureus (MRSA) The MIC and MBC values of Ag-NPs against MRSA strains were observed to be very low (i.e. in the range of 12.5-100 µg/ml), indicating very well bacteriostatic (represented by the MIC) and bactericidal activity (represented by MBC).

Conclusion:- In recent years, there is increase in MRSA in oral and perioral samples with emergence of new resistant strains of MRSA. In our study we have seen Ag-NPs size 5-10nm has inhibited growth of Methicillin Resistant Staphylococcus aureus in vitro at nontoxic concentration, which makes it to use as anti-bacterial agent in oral infections

KEYWORDS : Oral infections, MRSA, MIC, MBC. Ag-NPs

Introduction:

Staphylococci is one of the constituents of the oral flora; however, their role in oral health and disease remains debatable[1]. Reported percentage of isolation rates for Staphylococcus aureus in healthy dentulous patient is 24-84% [2,3]. In addition, there are number of other oral infections (eg, angular cheilitis,[4] parotitis,[5] staphylococcal mucositis)[6] caused by this microorganism. Recent data suggested that it has a role in dental implant failure.[7,8].In certain studies S. epidermidis and S. aureus is also seen as the predominant microbe from the samples of oral infections and periodontists[9,10,11,12,13]

S. aureus is a member of the Micrococcaceae family, which appears as Gram-positive cocci in clusters. *S. aureus* is distinguished from other staphylococcal species on the basis of the gold pigmentation of colonies and positive results of coagulase, mannitol fermentation, and deoxyribonuclease tests[14]. Staphylococcus epidermidis is a gram-positive, coagulase negative cocci that is a part of the normal flora. Consequently, it is a true opportunistic pathogen, as it requires a major breach in the host's innate defenses. It is one of the leading pathogens of nosocomial infections[15]. Although these microbes of the skin and the nasopharynx in healthy individuals are harm less, but on some occasions S. aureus is known to cause severe infections[16]. One of the main concerns with S. aureus is the prevalence of methicillin resistant Staphylococcus aureus (MRSA) which was initially isolated 50 years ago, only after two years of introduction of methicillin in clinical practice and has developed into a major global health issue due to its pathogenic potential to cause bloodstream infections, pneumonia as well as surgical site infections[17]. Resistance of S. aureus to b-lactam antibiotics is acquired by the exogenous mecA gene, which encodes a modified form of Penicillin Binding Protein (PBP2a), that does not allow proper binding and thus prevents the inhibition of cell wall synthesis that this class of antibiotics cause[18,19]. MRSA strains are also resistant to a vast number of commonly available antibiotics[20]. They are very commonly involved in formation of biofilms making it all the more difficult to treat with antibiotics.[21]

Silver has been used for thousands of years as a precious metal by humans in different applications. Medicinal and preservative properties of silver have been known for over 2,000 years. Since the nineteenth century, silver based compounds have been widely used in bactericidal applications, in burns and wounds therapy, etc[22]. Over the last decades silver has been engineered into nanoparticles, structures from 1 to 100 nm in size. The antimicrobial activity of silver nanoparticles (Ag-NPs) appears significantly high than silver ions and other silver salts[23,24]. The antimicrobial activity of Ag-NPs is comparable or better than the broad spectrum of most prominent antibiotics used worldwide[25]. Silver has the highest bactericidal activity and biocompatibility amongst all the known antibacterial nanoparticles[26,27].

Microbes are unlikely to develop resistance against silver as they do against conventional and narrow target antibiotics, because the metal attacks a broad range of targets in the organisms, which means that they need to develop a range of mutations simultaneously to protect themselves. As a result, Ag-NPs have been applied to a wide range of products, the most important current use is as antimicrobial agents to prevent infection, such as in burn and traumatic wound dressings, diabetic ulcers, coating of catheters, dental works, scaffold, and medical devices[28,29].

Hence, the aim of aim of our study is to evaluate the antibacterial activity of Ag-NPs against MRSA strains recovered from patients with oral infections.

Materials And Methods

Patients attending department of oral and maxillofacial surgery, Jaipur dental college who were diagnosed with oral infections were included in the study. Informed consent was taken from all the patients. The sample collected from patients were Pus swabs, Oral Aspirates, Oral scrapings, Necrotic tissue ,Extracted tooth etc (Details of specimen types shown in table 1) and they were sent to microbiology department for bacterial identification and culture sensitivity testing.

TABLE I

SPECIMEN	MSSA	MRSA
Oral rinse	10	3
Angle of mouth swab	7	2
Tongue swab	8	2
Hard palate swab	4	4

Nares swab	**7**	
Upper denture swab	**16**	**6**
Lip swab	**6**	**0**
Face swab	**13**	**0**
Dentoalveolar abscess aspirate	**21**	**4**
Dental implant swab	**5**	**0**
Periapical tissue	**15**	**5**
Other	**8**	**0**
Specimens	**120**	**26**

Bacterial identification was done by Direct microscopy and culture on Blood agar and mannitol salt agar, further biochemical tests were done by the standard method to identify the isolates as Staphylococcus aureus. Those isolates identified as Staphylococcus aureus were subjected to antimicrobial susceptibility testing with known antimicrobial drugs by different methods of antimicrobial susceptibility testing. MRSA strains were identified on the basis of *Cefoxitin disc diffusion test*. MRSA isolates were used as the test organisms to evaluate the antimicrobial effects of Ag-NPs.

Silver nanoparticles dispersion and bacterial strains

Commercially prepared nanoparticles (5-10Nm) were obtained from Sigma-Aldrich. The dilutions were made in autoclaved Milli Q water.

Bacterial strains, medium, and cultivation

The clinical isolates which were identified as MRSA were used as the test organism to evaluate the antimicrobial effects of Ag-NPs. The reference strain of Staphylococcus aureus used in the study was S. aureus ATCC25923. The MRSA strains were aerobically cultured at 37C on Mueller-Hinton Agar (MHA) plates.

Minimal inhibitory concentration (MIC):

Bacterial strains grown overnight on MHA plates at 37^{0}C were used. The antimicrobial activity of Ag-NPs was examined using the standard broth dilution method (CLSI M07-A8). Luria-Bertani (LB) broth was used to determine the MIC using serial two-fold dilutions of Ag-NPs in concentrations ranging from 200 to 1.5625 µg/ml, initial bacterial inoculums of 2×10^{8} CFU/ml for 24 h incubation at 37^{0}C. The MIC is defined as the lowest concentration of antimicrobial agents that completely visually inhibits 99% growth of the microorganisms.

Minimal bactericidal concentration (MBC): After the MIC determination of the Ag-NPs was done, 50 µl aliquots from all tubes with no visible bacterial growth was looked for and were cultured on MHA plates not supplemented with Ag-NPs and were incubated for 24 h at 37^{0}C. The MBC endpoint is defined as the lowest concentration of antimicrobial agent that kills 100% of the initial bacterial population The MBC is the lowest broth dilution of antimicrobial that prevents growth of the organism on the agar plate. Failure of the organism to grow on the plate implies that only nonviable organisms are present

Bacterial viability and growth inhibition testing on bacterial growth curve: From fresh colonies on MHA plates, inoculations were done into 100 ml of Luria-Bertani (LB) broth (Hi-Media Mumbai, India). Growth was allowed until the optical density (OD) reached 0.1 at 600 nm (OD of 0.1 corresponds to 108 CFU/ml of medium). Further, 2×108 CFU/ml of above was added to 100 ml of liquid LB media supplemented with 5, 10, 15, 20 and 25 µg/ml of Ag-NPs. All the flasks were be put on rotator shaker (150 rpm) and incubated at 37°C. Control broths were used without nano particles. Optical density was measured after every 2 hour (up to 20 h) at 600 nm using Int.J.Curr.Microbiol.App.Sci (2015) 4(10): 764-773 767 spectrophotometer and the bacterial growth was determined. The growth inhibition percentage was obtained with respect to the positive control.

Result:

146 samples were collected from the patient over a two years period from 2015 to 2017 . In the laboratory, out of these, 120 specimens showed methicillin-sensitive S aureus (MSSA), and 26 specimen showed methicillin-resistant S aureus (MRSA). These MRSA strains were treated with Ag-Nanoparticles to know the antibacterial affect.

The MIC and MBC values of Ag-NPs against MRSA strains were observed to be very low (i.e. in the range of 12.5-100 µg/ml), indicating very well bacteriostatic (represented by the MIC) and bactericidal activity (represented by MBC) of the antibacterial agents (Table 1 & Fig. 1).The MIC and MBC value of Ag-NPs for reference strain *S. aureus* ATCC25923 was found very low i.e. 12.5 µg/ml and 25 µg/ml, respectively in comparison to MRSA,; also indicating very good bacteriostatic and bactericidal activity of the antibacterial agents (Table 2), (Fig.1).

TABLE II

MRSA ISOLATES 26		
NUMBER OF ISOLATES	**MIC (µg/ml)**	**MBC (µg/ml)**
4	**12.5**	**25**
5	**12.5**	**25**
3	**25**	**25**
8	**25**	**50**
4	**50**	**50**
2	**50**	**100**
S. aureus ATCC25923	**12.5**	**25**

Figure 1

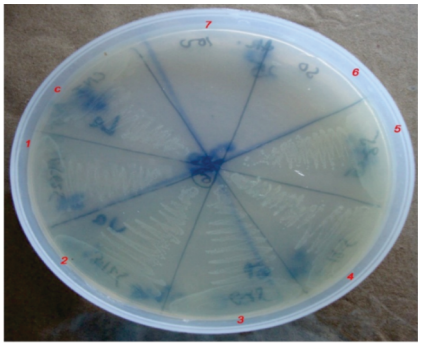

Fig 1. Bactericidal effect (MBC) of Ag-NPs dispersion against *S. aureus* ATCC25923 and MRSA C):control, 1): 1.562 µg/ml, 2): 3.12 µg/ml, 3): 6.24 µg/ml, 4): 12.5 µg/ml, 5): 25µg/ml, 6):50µg/ml, 7):100µg/ml

Effects of Ag-NPs dispersion on bacterial growth

The bacterial growth was monitored in liquid LB medium; with Ag-NPs in it. At all the concentrations (5, 10, 15, 20 and 25 µg/ml) of Ag-NPs, the nanoparticles caused a growth delay of the bacterial cells; slope of the bacterial growth curve continuously decreased with increasing nanoparticles concentration and at concentration of 20 µg/ml and 35µg/ml; Ag-NPs were found as an effective bactericidal agent

Discussion:-

Staphylococcus aureus accounts for 24%-84% in healthy adult dentate oral cavities [2,3] and 48% among the denture-wearing population.[4]. Confounding factors also include variations in referral patterns because S. aureus recovery rates increase in denture-wearing patients and patients with reduced salivary secretion. Centuries old treatment for wounds are based on Silver and silver-based compounds (Castellano *et al*.,2007)[30].

The aim of the current study was to determine the anti bacterial property of Ag-NPs against drug resistant MRSA isolates from oral infections. In order to do it, the oral isolates which were resistant to one or more antibiotics (methicillin/oxacillin/cefoxitin) were treated with different concentrations of a nanosilver suspension and described the effect on bacterial cell viability and growth rate. In our study, we have found 20 specimens to be MRSA.

The mechanism of the bactericidal effect of Ag-NPs needs to be

explained more. It has been proposed that Ag-NPs bind to the surface of the cell membrane, disrupting cellular permeability and the respiration functions of the cell. Smaller Ag-NPs having a large surface area available for interaction have a greater bactericidal effect than larger silver nanoparticles (Kvitek et al., 2008)[31]. It is also possible that Ag-NPs not only interact with the surface of the membrane, but also penetrate inside the bacteria and inactivate DNA replicating ability (Morones et al., 2005)[32] causing the devastation of the cell.

The reason why these bacteria were more susceptible to silver nanoparticles could be because the silver nanoparticles target protein synthesis, nucleic acid synthesis, and Gram positive cell wall synthesis. Dental caries and periodontal disease, the most widespread diseases affecting mankind, involve the adherence of bacteria and development of biofilms on both the natural and restored tooth surface. The mechanism of silver nanoparticles to attach to the surface of the cell membrane and disturb its function, penetrate bacteria, and release silver ions makes it a good treatment option for many oral infections; found that silver nanoparticles target the bacterial membrane, leading to a dissipation of the proton motive force (Sondi et al., 2004; Lok et al., 2006).[33,34]

In our study, the MIC and MBC values of Ag-NPs against MRSA strains were observed to be very low (i.e. in the range of 12.5-100 µg/ml), which is in accordance with Ansari et al. (2011) who found, the values of MIC and MBC of Ag-NPs against all clinical isolates of MSSA, MRSA and single strain of S.aureus ATCC25923 in the range of 12.5-50 µg/ml and 12.5-100 µg/ml, respectively (Ansari et al., 2011)[35]. Martinez-Castanon et al. (2008)[36] reported that Ag-NPs were inhibitory at concentration of 16.67 µg/ml against S. aureus ATCC 25923, but they used the Ag-NPs of size 29nm which was larger than the size used by us (10nm). This could be the reason of higher MIC in their study. The same reason could be applied for Shrivastava et al. (2007)[37] because the size of nanoparticles in their study was also larger than in our study The current study also showed better antibacterial activity as compared to earlier work of (Ayala-Nunez et al., 2009)[38] who reported MIC and MBC values of Ag-NPs 1800 µg/ml and 2700 µg/ml, respectively.

The growth curve of standard strain of S. aureus ATCC 25923 and MRSA were plotted in the presence of 0, 5, 10, 15, 20, and 25 µg/ml concentration of Ag-NPs. It is shown clearly in Figure 2 that as the concentration of Ag-NPs increases, reduction in bacterial growth was observed and this was even continued for 16 hrs. There was clear inhibitory action of Ag-NPs on S. aureus ATCC 25923 and MRSA at all concentrations.. The finding of Li et al. (2010) showed a complete growth inhibition for S. aureus ATCC 6538P at 20 µg/ml, while in case of our study no growth was observed up to 16 hrs at 25 µg/ml of Ag-NPs (Fig. 2). Thus, our result shows that there was very little difference between antibacterial activities of Ag-NPs against standard strain and methicillin-resistant strain, i.e. both were equally sensitive. The fact that the drug resistant and drug-susceptible strains were affected by Ag-NPs in the same manner indicates that the drug-resistant proteins that give bacteria the capacity to avoid antibiotics do not affect the efficacy of nanosilver.
Our study showed low MIC and MBC. Although clinicians prefer a bactericidal agent because bacterial killing should produce a faster resolution of the infection, improve clinical outcome, and reduce the likelihood of the emergence of resistance and the spread of infection. If oral pathogens are killed rather than inhibited, resistance mutations that might otherwise emerge as the result of antibiotic pressure are eliminated (French *et al.,* 2006)[39]. Being a good bacterisostatic and bcateriocidal, this noble metal tends to induce low bacterial resistance (Ip *et al.,* 2006)[40] and has low toxicity and minimal side effects when ingested since at most 2–4% is retained in tissues after absorption by the body. A notable health effect has been argyria, an irreversible pigmentation of the skin that is mostly an aesthetic concern (Drake *et al.,* 2005)[41]. Nanosilver size mediates MRSA inhibition and the cytotoxicity to human cells being smaller, nanoparticles the ones with a better antibacterial activity and nontoxic effect can be used.

The survival of micro-organisms within the oral cavity is dependent on their ability to adhere to surfaces and subsequently develop into a biofilm, a process influenced by the physico-chemical properties of the underlying surface (Al-Ahmad ,Hannig and Hannig,et.al 2009)[42]. On the tooth surface, the initial colonizers adhere to the acquired pellicle, a salivary-/dietary-derived proteinaceous layer, which can then influence the subsequent sequence of microbial colonization (Marsh and Bradshaw, 1995)[43]. The pellicle mediates the interactions among the solid tooth surface, oral fluids, and micro-organisms (Al-Ahmad ,Hannig and Hannig, 2009)[42]. In such circumstances , Ag-NPs have a promising role ot play.

The data presented here are novel in that they prove that Ag-NPs are effective bactericidal agents regardless of the drug-resistance mechanisms that exist in multidrug-resistant bacteria and show the importance of Ag-NPs in the nosocomial and community environment.

Conclusion

In recent years, there is increase in MRSA in oral and perioral samples with emergence of new resistant strains of MRS. In our study we have seen Ag-NPs (size 5-10nm) has inhibited growth of Methicillin Resistant Staphylococcus aureus in vitro at nontoxic concentration, which makes it to use as anti-bacterial agent in oral infections. In addition, it can be used in the manufacturing of pharmaceutical products to be used in dentistry.

Hence, finally, we conclude that Nano-biotechnology is an important area of research that needs attention for the application against multidrug- resistant microbes.Therefore, further studies must be done to assess the genotoxic and cytotoxic effects in human cells in order to evaluate the applications of Ag-NPs as a bactericidal agent in oral infections

References:

1. Singer AJ. Salivary bacteria. III. The pathogenicity of oral Staphylococci. J DentRes 1952;31:591-7.
2. Jackson MS, Bagg J, Gupta MN, Sturrock RD. Oral carriage of Staphylococci in patients with rheumatoid arthritis. Rheumatology (Oxford) 1999;38:572-5.
3. Ohara-Nemoto Y, Haraga H, Kimura S, Nemoto TK. Occurrence of staphylococci in the oral cavities of healthy adults and nasaleoral trafficking of the bacteria. J Med Microbiol 2008;57:95-9.
4. MacFarlane TW, Helnarska S. The microbiology of angular cheilitis. Br Dent J 1976;140:403-6.
5. Goldberg MH. Infections of the salivary glands. In: Topazian RG, Goldberg MH, editors. Management of infections in the oral and maxillofacial regions. Philadelphia [PA]: Saunders; 1981. p. 293-311.
6. Bagg J, Sweeney MP, Harvey-Wood K, Wiggins A. Possible role of Staphylococcus aureus in severe oral mucositis among elderly dehydrated patients. Microbiol Ecol Health Dis 1995;8:51-6.
7. Kronström M, Svenson B, Hellman M, Persson GR. Early implant failures in patients treated with Brånemark System titanium dental implants: a retrospective study. Int J Oral Maxillofac Implants 2001;16:201-7.
8. Rokadiya S, Malden NJ. An implant periapical lesion leading to acute osteomyelitis with isolation of Staphylococcus aureus. Br Dent J 2008;205:489-91.
9. Dahle´n G, Wikstro¨m M. Occurrence of enteric rods, staphylococci and Candida in subgingival samples. Oral Microbiol Immunol 1995;10(1):42–6.
10. Rams TE, Feik D, Slots J. Staphylococci in human periodontal diseases. Oral Microbiol Immunol 1990;5(1):29–32.
11. Smith AJ, Robertson D, Tang MK, Jackson MS, MacKenzie D, Bagg J. Staphylococcus aureus in the oral cavity: a three-year retrospective analysis of clinical laboratory data. Br Dent J 2003;195(12):701–3.
12. Loberto JCS, Martins CA, de P, Santos SSFD, Cortelli JR, Jorge AOC. Staphylococcus spp. in the oral cavity and periodontal pockets of chronic periodontitis patients. Braz J Microbiol 2004;35(1/2):64–8.
13. Slots J, Feik D, Rams TE. Age and sex relationships of superinfecting microorganisms in periodontitis patients. Oral Microbiol Immunol 1990;5(6):305–8.
14. Wilkinson BJ, Biology. In: Crossley KB, Archer GL, eds. The staphylococci in human disease. New York: Churchill Livingstone, 1997:1-38.
15. Nilsson M, Frykberg L, Flock J-I, Pei L, Lindberg M, Guss B. A Fibrinogen-Binding Protein of *Staphylococcus epidermidis*. *Infection and Immunity*. 1998;66(6):2666-2673.
16. Van Belkum A, Melles DC, Nouwen J, van Leeuwen WB, van Wamel W, Vos MC, et al. Co-evolutionary aspects of human colonisation and infection by Staphylococcus aureus. Infect Genet Evol 2009;9(1):32–47.
17. Stefani S, Chung DR, Lindsay JA, Friedrich AW, Kearns AM, Westh H, et al. Meticillin-resistant Staphylococcus aureus (MRSA): global epidemiology and harmonisation of typing methods. Int J Antimicrob Agents 2012;39(4):273–82.
18. Wielders CLC, Fluit AC, Brisse S, Verhoef J, Schmitz FJ. mecA gene is widely disseminated in Staphylococcus aureus population. J Clin Microbiol 2002;40(11):3970–5.
19. Fluit AC, Visser MR, Schmitz FJ. Molecular detection of antimicrobial resistance. Clin Microbiol Rev 2001;14(4):836–71.
20. Anupurba S, Sen M R, Nath G, Sharma B M, Gulati A K, Mohapatra T M. Prevalence of methicillin resistant staphylococcus aureus in a tertiary referral hospital in eastern

Uttar Pradesh. Indian J Med Microbiol 2003;21:49-51
21. Saginur R, StDenis M, Ferris W, et al. Multiple Combination Bactericidal Testing of Staphylococcal Biofilms from Implant-Associated Infections. *Antimicrobial Agents and Chemotherapy*. 2006;50(1):55-61. doi:10.1128/AAC.50.1.55-61.2006.
22. Klasen HJ, 2000. A historical review of the use of silver in the treatment of burns. II. Renewed interest for silver. Burns, 26:117-138.
23. Lok CN, Ho CM, Chen R, He QY, Yu WY, Sun H, Tam PK, Chiu JF, Che CM, 2006. Proteomic analysis of the mode of antibacterial action of silver nanoparticles. Journal of Proteome Research, 5:916–924.
24. Rai M, Yadav A, Gade A, 2009. Silver nanoparticles as a new generation of antimicrobials. Biotechnology Advances, 27:76–83.
25. Shrivastava, S., Bera, T., Roy, A., Singh, G., Ramachandrarao, P. and Dash, D. (2008) Characterization of enhanced antibacterial effects of novel silver nanoparticles. *Nanotechnol.* **18**, 103–112.
26. Cubillo A, Pecharroman C, Aguilar E, Santaren J, Moya J. Antibacterial activity of copper monodispersed nanoparticles into sepiolite. J Mater Sci . 2006; 41:5208–5212
27. Desai V, Kowshik M. Antimicrobial activity of titanium dioxide nanoparticles synthesized by sol-geltechnique. Res J Microbiol. 2009; 4:97–103.
28. Kim S, Kim HJ. Anti-bacterial performance of colloidal silver treated laminate wood flooring. Int Biodeterioration Biodegradation. 2006; 57:155–162.
29. Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, et al. Antimicrobial effects of silver nanoparticles.Nanomedicine: Nanotechnol Biol Med. 2007; 3(1):95–101
30. Castellano JJ, Shafii SM, Ko F, Donate G, Wright TE, Mannari RJ, Payne WG, Smith DJ, Robson MC 2007. Comparative evaluation of silver-containing antimicrobial dressing and drugs. International Wound Journal, 4(2):144-22.
31. Kvitek, L., Panacek, A., Soukupova, J., *et al.* 2008. Effect of surfactants and polymers on stability and antibacterial activity of silver nanoparticles (NPs). *J. Phys. Chem.,* 112: 5825–5834.
32. Morones, J.R., Elechiguerra, J.L., Camacho, A. 2005. The bactericidal effect of silver nanoparticles. *J. Nanotechnol.,* 16: 2346–2353.
33. Sondi, I., Salopek-Sondi, B. 2004. Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for gram-negative bacteria. *J. Colloid Interface Sci.,* 275: 177–182.
34. Lok, C.N., Ho, C.M., Chen, R., He, Q.Y., Yu, W.Y., Sun, H., Tam, P.K., Chiu, J.F., Che, C.M. 2007. Silver nanoparticles: partial oxidation and antibacterial activities. *J. Biol. Inorg. Chem.,* 12: 527–534.
35. Ansari, M.A., *et al.* 2011. Evaluation of antibacterial activity of silver nanoparticles against MSSA and MSRA on isolates from skin infections. *Biol. Med.,* 3(2): 141–146.
36. Martinez-Castanon, G.A., Nino-Martinez, N., Martinez-Gutierrez, F., Martinez-Mendoza, J.R., Ruiz, F. 2008. Synthesis and antibacterial activity of silver nanoparticles with different sizes. *J. Nanoparticles Res.,* 10: 1343–1348.
37. Shrivastava, S., Bera, T., Roy, A., Singh, G., Ramachandrarao, P., Dash, D. 2007. Characterization of enhanced antibacterial effects of novel silver nanoparticles. *Nanotechnology,* 18: 1–9.
38. Ayala-Nunez, N.V., Lara, H.H., Turrent Liliana del, C.I., Padilla, C.R. 2009. Silver nanoparticles toxicity and bactericidal effect against Methicillin-resistant *Staphylococcus aureus*: Nanoscale does matter. *Nanobiotechnology.* doi: 10:1007/s12030-009-9029-1.
39. French, G.L. 2006. Bactericidal agents in the treatment of MRSA infections—the potential role of daptomycin. *J. Antimicrob. Chemother.,* 58: 1107–1117.
40. Ip, M., Lui, S.L., Poon, V.K., Lung, I., Burd, A. 2006. Antimicrobial activities of silver dressings: an *in vitro* comparison. *J. Med. Microbiol.,* 55: 59–63.
41. Drake, P.L., Hazelwood, K.J. 2005. Exposure-related health effects of silver and silver compounds: a review. *Ann. Occup. Hyg.,* 49: 575–585.
42. Al-Ahmad, A., Follo, M., Selzer, A. C., Hellwig, E., Hannig, M., and Hannig, C. (2009). Bacterial colonization of enamel in situ investigated using fluorescence in situ hybridization. *J. Med. Microbiol.* 58, 1359–1366. doi: 10.1099/jmm.0.011213-0
43. Marsh, P.D. & Bradshaw, D.J. Journal of Industrial Microbiology (1995) 15: 169. https://doi.org/10.1007/BF01569822