International Food Research Journal 23(3): 1248-1254 (2016)
Journal homepage: *http://www.ifrj.upm.edu.my*

# Antibiotic resistance evolution of Methicillin Resistant *Staphylococcus aureus* (MRSA) and colloidal silver as the nanoweapon

*New, C.Y., Amalia, A.R., Ramzi, O.S.B. and Son, R.

*Food Safety and Research Center (FOSREC), Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang Malaysia*

**Article history**

*Received: 15 December 2015*
*Received in revised form: 2 January 2016*
*Accepted: 5 January 2016*

**Keywords**

*MRSA*
*Resistance*
*Antibiotics*
*Pathogen*
*Colloidal silver*

**Abstract**

As the society begin to realize the importance of combating antimicrobial resistance, going back to silver might be the solution. Silver has been known for its potential antimicrobial activity since ancient times and, the development of nanoparticles has increased its potential into becoming an antimicrobial agent that can be applied in broad-spectrum. Antimicrobial resistance has spread into an irrepressible manner which requires drastic action plan as a number of pathogenic bacteria began to acquire resistance genes. Methicillin Resistant *Staphylococcus aureus* (MRSA) is one of the earliest reported resistant clones which is the center of this study. This study focused on the dissemination and evolution of MRSA on its resistance towards antibiotics. Disc Diffusion Test was employed to create the antibiograms of MRSA isolates. All isolates showed resistance towards amoxicillin, ampicillin, cefazolin, oxacillin and penicillin. In contrast, all isolates were susceptible towards erythromycin. The findings also discovered isolates that were vancomycin-resistant (66.7%) and vancomycin-intermediate (33.3%). As the efficacy of antibiotic treatment is at a question, we also investigated on the antimicrobial activity of colloidal silver in the hope as an alternative treatment. Shiga Toxin producing *Escherichia coli* (STEC) and MRSA (ATCC 33591) was tested using modified Quantitative suspension test for the evaluation of bactericidal activity for chemical disinfectants and antiseptics based on BS EN 1276:2009. The outcome of this study indicated that the colloidal silver is working effectively against STEC and MRSA (ATCC 33591), showing killing percentages well above 99.0% at 4 minutes and 8 minutes of contact. Vancomycin-resistant *S. aureus* (VRSA) and Vancomycin-intermediate *S. aureus* (VISA) were also tested and the results indicated that VISA had higher killing percentages at 4 minutes (99.83%) and 8 minutes (99.85%) compared to VRSA at 4 minutes (96.72%) and 8 minutes (98.35%). This opens a solution to the rising problem of antimicrobial resistance.



## Introduction

Silver, a precious metal that was known long for its shining properties alongside gold was reported to have antimicrobial properties. From the ancient times, silver vessels were used as a disinfectant to store and purify water. The theory is also now currently used by the National Aeronautics Space Administration (NASA) for water storage and purification in its space shuttle. The mechanism action of silver's antimicrobial properties was extensively studied by many researchers to date. The metal in pure form is inert and does not react with human tissue or kill microorganisms until it is ionized (Melaiye and Youngs, 2005). Silver ions were reported to inhibit bacterial growth through protein inactivation where by it forms stable S-Ag bond with thiol-containing compounds in the cell membrane that are involved in transmembrane energy generation and ion transport (Klueh *et al*., 2000). Subsequently, this lead to membrane damages and cause apoptosis through oxidative damages as reported by Cheng *et al.* (2013). Silver ions can also act as a catalyst in oxidation reactions that results in the formation of disulfide bonds, which could possibly change the cellular enzymes and subsequently affect their function (Davies and Etris, 1997). This is to say that silver ions have the potential to inactivate essential enzymes, particularly cellular enzymes. This will disrupt the organisms' respiratory path by obstructing metabolic activity, and lead to death. On the other hand, silver was proposed to cause RNA or DNA alteration. Klueh *et al.* (2000) explained that it was possible that silver ions enters the cell and intercalates between the purine and pyrimidine base pairs, disrupting the hydrogen bonding between the two anti-parallel strands. This denatures and inhibits bacterial replication.

The antimicrobial properties of silver are further enhanced when nanotechnology surfaced. Through

*Corresponding author.
Email: *viviannew90@hotmail.com*

simple electrochemistry and irradiation under short wavelengths of light, nanoparticles silver also known as colloidal silver can be produced. Colloids are clusters of silver atoms that carry the same electrical charge, and thus mutually repel each other (Laroo, 2013). Clusters of silver atoms can be from a single monomer to millions of atoms forming many sizes in one dimension. Clusters larger than 100 nm in one dimension are no longer considered to fall in the category of nano clusters (Laroo, 2013). Nano particles size of silver increase the surface area to volume ratio, allowing the silver particles to penetrate through easily into the organism and cause destruction (Martinez-Castanon *et al.*, 2008). The smaller the size, the better. Colloidal silver is usually suspended in a solution, usually water, to prevent agglomeration and maintain effectiveness. The suspension is well-maintained through the presence of highly negative Zeta-potential which causes the mutual repelling action (Laroo, 2013). A slight disruption or contamination on the negatively Zeta-potential will cause agglomeration. Other than that, nano silver is coated with coating agents that are non-covalently attached to maintain an equilibrium state such as citric acid, various amino acids, cetyl trimethylammonium bromide (CTAB), and sodium dodecyl sulfate (SDS) (McShan *et al*., 2014).

The present study investigates on the antimicrobial activity of colloidal silver against Shiga Toxin producing *Escherichia coli* (STEC) and Methicillin Resistant *Staphylococcus aureus* (MRSA); the two pathogens that most feared of having the capabilities to be increasingly resistant and endangering the public's health. As the antimicrobial resistance becomes an alarming matter, many researchers are looking for alternative ways and treatments. Before that, we created an antibiogram profile of MRSA to study on its antibiotic resistance evolution.

## Materials and Method

*Bacterial Strains*

*Staphylococcus aureus* (ATCC 33591) defined as Methicillin resistant was used in this study. On the other hand, wild-type *Staphylococcus aureus* was also isolated from poultry sampled from Serdang, Malaysia through conventional method as stated in Bacteriological Analytical Method (BAM) by Bennett and Lancette (2001) with modification using selective media Mannitol Salt Agar (MSA) [Merck, Germany]. A total of 30 isolates were collected and all of the isolates were streaked onto Oxoid™ Brilliance™ MRSA 2 Agar [Thermo Oxoid, United Kingdom] for confirmation purpose. Out of 30 isolates, 9 isolates produced presumptive colonies of MRSA that produced denim blue colonies. The isolates were picked and kept on nutrient agar slants until further used. All presumptive colonies of MRSA were subjected to Antibiotic Susceptibility Testing according to Bauer *et al.* (1966) for antibiogram profiling.

*Escherichia coli* was isolated from ready-to-eat food through conventional methods as stated in Bacteriological Analytical Method (BAM) by Feng *et al.* (2002). It was then subjected to Polymerase Chain Reaction (PCR) for Shiga Toxin identification using targeted genes of stx 1 (Shiga toxin 1) and stx 2 (Shiga toxin 2) (Gannon *et al*., 2012). PCR conditions used were referred to Bai *et al*. (2010). The PCR products were subjected to gel electrophoresis using 1.25% of Agarose at 80 V for an hour. It was then viewed under UV light using GeneSnap [Syngene, United Kingdom]. Identified Shiga Toxin producing *E. coli* (STEC) were then grown on nutrient agar slants until further used.

*Antibiotic susceptibility testing*

The antibiotic susceptibility testing was performed according to Bauer *et al*. (1966) with modifications. Briefly, isolates were inoculated into Luria-Bertani (LB) Broth [Nacalai Tesque, Japan] and incubated for 24 hours at 37°C. The bacteria suspension was then spread using sterile cotton swab onto Mueller-Hinton Agar [Difco, USA] plates and allowed to dry. Antibiotic discs were arranged neatly using sterile forceps onto the swabbed plates before they are inverted and incubated for 24 hours at 37°C. The antibiotics used in this test were amoxicillin (25 µg), ampicillin (25 µg), cefazolin (30 µg), ciprofloxacin (10 µg), erythromycin (15 µg), kanamycin (30 µg), norfloxacin (10 µg), oxacillin (1 µg), penicillin (10 µg), tetracycline (10 µg), trimethoprim (5 µg) and vancomycin (30 µg); all purchased from Oxoid. After incubation, the diameters of the zone inhibition were measured and tabulated as according to Clinical and Laboratory Standards Institute (CLSI) for categorizing.

*Colloidal silver denotation*

The initial count of the bacteria was prior performed through plate counting method. Briefly, the bacteria was inoculated into nutrient broth and allowed to grow for 3 hours at 37°C. Prior to the experiment, a growth curve of each bacteria was plotted using plate counting method and, it was observed that a 3 hour growth was sufficient to have a count of well above 5 log CFU/ml (Data not shown). It was then subjected to 10 times of ten-fold serial

dilutions. For each dilution, 1 ml of the diluent was pipetted into duplicate sterile petri dish and added with sufficient amount of Plate Count Agar (PCA). It was then incubated at 37°C for 18 to 24 hours. The colonies formed were identified and enumerated.

Colloidal silver was obtained commercially. 9 ml of colloidal silver was transferred into a sterile test tube. The colloidal silver suspension was placed into the water bath at 20°C. 1 ml of 3 hour growth of bacterial suspension, initial count ranging from 5 log CFU/ml to 7 log CFU/ml in nutrient broth [Merck, Germany] was added into the colloidal silver suspension and the start watch was started immediately. The suspensions were allowed to mix according to the chosen contact times which were 4 minutes and 8 minutes. Immediately, after the chosen contact time, 1 ml of the mixed suspensions was transferred into another tube containing 1 ml of sterile distilled water and 8 ml of neutralizer (0.1% sodium sulphate, $Na_2SO_4$ [Merck, Germany]). It was allowed to stand for 5 minutes before performing serial dilutions where by 1 ml of the solution was pipetted and, serially diluted for ten-folds for six times in 0.85% saline, NaCl, solution. 1 ml of each dilution was pipetted out into duplicates empty petri dishes and added with standard method agar (SMA). Once the agar had been solidified, the plates were incubated at 37°C for 18 to 24 hours. The colonies formed were identified, enumerated and tabulated. The killing percentage was calculated following the equation [final count (CFU/ml) – initial count (CFU/ml)/initial count (CFU/ml)].

## Results and Discussion

*Staphylococcus aureus* was reported as the first pathogen that acquired resistivity against antibiotic treatment in 1960s, namely methicillin after a year it was introduced. Strains that showed resistivity towards beta-lactam antibiotics are classified as Methicillin resistant S. aureus (MRSA). The pathogen is constantly evolving with higher resistivity and the efficacy of antibiotic treatment might be non-relevant in the future. In this study, out of 30 isolates collected from poultry, 9 (30.00%) of the isolates were identified as positive wild-type MRSA. This signified the fast spreading of the pathogen and it is not only an issue anymore in the hospitals that is one of the important reservoirs of MRSA. Rajaduraipandi *et al.* (2006) indicated that infected or colonized patients and transient hand carriage on the health care workers is the predominant mode for patient to patient transmission. Subsequently, when the infected or colonized patients are discharged,

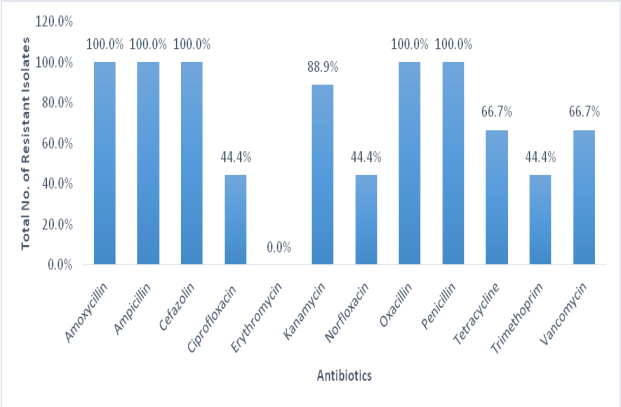


Figure 1. Antibiogram profile of wild-type MRSA

it widens the possible route of transmission of the pathogen and exacerbated the problem. In addition, the emergence of MRSA could happen simply through the horizontal gene transfer or transduction of mobile drug resistance genes from important reservoirs such as the wastewater and hospitals. It is no wonder the prevalence of MRSA leaps from year to year as reported by the National Nosocomial Infection Surveillance (NNIS) System data that demonstrated a steady increase in the incidence of nosocomial infections (NNIS, 2004). In 2010, a survey was conducted on the MRSA prevalence by Jarvis *et al.* (2012) and, they concluded that MRSA colonization has increased.

The 9 positive wild-type MRSA isolates were tested with 12 different antibiotic discs of different groups that have reported to be used against staphylococcal infection and, the antibiogram profile was as shown in Figure 1. Based on Figure 1, it was evaluated that all wild-type MRSA isolates were 100% resistant against amoxicillin, ampicillin, cefazolin, oxacillin, and penicillin. This further confirms the isolates were indeed MRSA based on the resistance towards amoxicillin, ampicillin, oxacillin and penicillin; all of those mentioned are beta-lactam antibiotics. The results also substantially prove that cefazolin, a suitable first-generation cephalosporins will not be effective against staphylococcal infection. On the other hand, erythromycin remained bactericidal against the wild-type MRSA isolates as shown in Figure 1. According to Harbarth *et al.* (1999), hospital acquired MRSA are resistant to erythromycin while community acquired MRSA are susceptible, although it may range up to 25% of resistance. Hence, the isolates were classified as community-acquired MRSA, a pathogen well-described in the general population. Community-acquired MRSA typically affects community that lacks of hospital association and, it can cause serious and fatal infections in otherwise healthy hosts (Naimi, 2001). None of the isolates were resistant indicating

that it can be used as antibiotic treatment suitable for minor skin infections through oral route. Despite that, the wild-type MRSA isolates began to show high resistance against Kanamycin, an antibiotic used to treat a wide variety of infections. About 88.9% of the isolates were resistance and it is likely that in the later years, it will be fully resistant to Kanamycin, rendering it ineffective. 66.7% of the isolates were resistant towards tetracycline in support of a recent finding reported by Turnidge *et al*. (2008) of MRSA's resistivity towards tetracycline while 44.4% of the isolates were resistant to trimethoprim, ciprofloxacin and norfloxacin.

Hiramatsu (2001) reported a new strain of *S. aureus* that was resistant towards vancomycin and this strain was termed as vancomycin-resistant *S. aureus* (VRSA). Similar strains have been detected in this study in which about 6 out of 9 isolates (66.7%) were resistant. Vancomycin use to be the drug of choice for serious infections caused by *S. aureus* strains. However, the reported results indicated otherwise and the occurrence of the strain was explained by Finks *et al.* (2009) in which they concluded that patients were co-infected or co-colonized with vancomycin resistant enterococci (VRE) and MRSA, which enables transfer of vanA gene from VRE to MRSA in a biofilm environment leading to a VRSA strain. Daum *et al.* (1992) reported that VRSA strains were found to have thicker peptidoglycan wall which may reduce the efficacy of antibiotics targeting cell walls. The remaining three isolates (44.4%) were reported as vancomycin-intermediate *S. aureus* (VISA). The disc diffusion test conducted can be considered as the acceptable primary test methods as mentioned by the Centers for Disease Control (CDC) and it is suggested for re-testing for further confirmation.

Multidrug resistant bacteria have created therapeutic problems especially to healthcare providers and, its consistent emergence will only cause hazard to the public health. The antibiogram profile created for all 9 isolates indicated different types of MRSA, primarily community-acquired MRSA as mentioned earlier. All isolates (100.0%) had a high MAR index of more than 0.2 with a range from 0.50 to 0.92. Two of the isolates (22.2%) were resistant to eleven types of antibiotics with a MAR index of 0.92. In contrast, only one isolate (11.1%) had a different antibiotic pattern and was resistant to six types of antibiotics. This signified that the bacterial isolates have been exposed to several antibiotics and the selective pressure of the antibiotics used in the management of bacterial infections could be the main reason of resistance (Poonia *et al*., 2014) apart from the bacteria acquiring the resistance gene via mutation or interspecies gene transmission. Laximanarayan *et al*. (2013) noted that resistance genes of pathogens were borne on chromosomal and increasingly, on transmissible extrachromosomal elements.

The wild-type MRSA isolates were obtained from poultry. The most likely source of resistant bacteria in food of animal origin is contamination from the animals' intestines during slaughter as stated by Laximanarayan *et al.* (2013). Contamination can possibly occur at every stage when there is insufficient food hygiene and sanitation. The spread of MRSA from people to animals either directly or indirectly has been well-reported (Catry *et al*., 2010). Most importantly, the fact that resistance genes can be transferred between different commensal bacterial species and from those to pathogens adds on to the complexity of numerous possible pathways of transmission of resistant bacteria.

The efficacy of antibiotics have decreased in recent years and the cause of it is complex. It is possible that it is due to many factors that lead in a stepwise fashion and as a result, the community is facing the emergence of resistant clones such as MRSA, *Escherichia coli* ST131 and *Klebsiella* ST258. The resistance is spreading worldwide and it has become a global epidemiology as those most vulnerable are at risk. For high-income countries, antibiotic resistance is treated with high rates and prolonged antibiotic use and if it is not effective, more expensive and broad-spectrum antibiotics will be used. As for low-income and middle-income countries, the aftermath of resistant which leads to high mortality rate, high hospitalization rate and high prevalence of hospital infections. Nevertheless, the burden of resistance is felt across the globe: longer duration of illness, higher rates of mortality in patients with resistant infection, increasing costs of treatment for resistant infections, and inability to do procedures that rely on effective antibiotics to prevent infection (Laximanarayan *et al*., 2013).

Tackling antibiotic resistance is not a simple task. It requires good operational excellence with detailed planning, execution and monitoring. Researchers are currently putting their heads together to intervene the spread of antibiotic resistance. Besides focusing on creating a stronger and effective antibiotic, research in molecular biology had yet to come out with solutions such as stopping plasmid replication (DeNap and Hergenrother, 2005) or resistance mechanisms such as efflux pump inhibitors (Kurincic *et al*., 2012) as well as bacteriophage treatment. Colloidal silver, on the other hand, has high antimicrobial properties as reported by many researchers (Kvitek *et al*., 2011; Rai *et al*., 2012; Laroo, 2013). When tested against

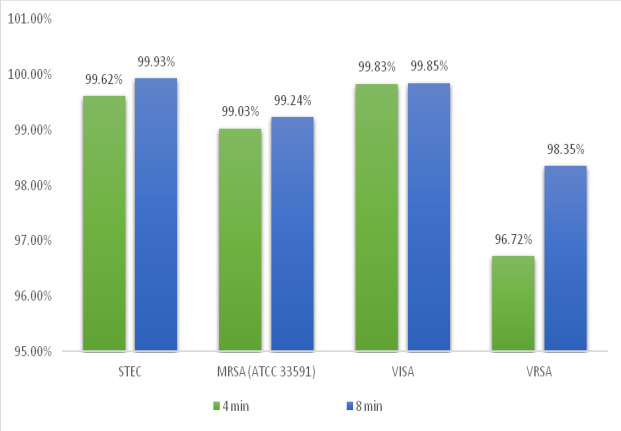


Figure 2. The killing percentages of different contact times according to the tested pathogens. STEC: Shiga toxin producing *Escherichia coli*; MRSA: Methicillin Resistant *Staphylococcus aureus*; VISA: Vancomycin-intermediate *Staphylococcus aureus*; VRSA: Vancomycin-resistant *Staphylococcus aureus*

MRSA either ATCC or wild-type, the results (Figure 2) were substantial to prove its efficacy and can be recommended as a possible intervention solution of antibiotic resistance.

The killing percentage of MRSA (ATCC 33591) recorded was 99.03% (2.01 log reduction) and 99.24% (2.12 log reduction) for 4 minutes and 8 minutes respectively. Two types of wild-type MRSA with Vancomycin-resistant and Vancomycin-intermediate were selected and labelled as VRSA and VISA. The results as shown in Figure 2 did not differ as much from MRSA (ATCC 33591). The killing percentage of VISA was 99.83% (2.78 log reduction) and 99.85% (2.81 log reduction) for 4 minutes and 8 minutes respectively. In contrast, VRSA recorded slight difference with a drop in killing percentage at 4 minutes (96.72%, 1.48 log reduction) and also at 8 minutes (98.35%, 1.78 log reduction). The most probable reason of VRSA to have a lower killing percentage could be due to the thick peptidoglycan layer of the bacteria. Aforementioned, VRSA has the ability to produce thicker cell walls (Daum *et al*., 1992). Consequently, the thick peptidoglycan layer minimized the ability of colloidal silver to penetrate into the bacterial cell. Kawahara *et al*. (2000) added that the peptidoglycan layer is negatively charged and the colloidal silver might get trapped by the peptidoglycan due to charges attraction as the charge surrounding the clusters of silver atoms is positively charged.

We tested the colloidal silver with another pathogen, Shiga Toxin producing *E. coli* (STEC) and the results were as satisfactory as MRSA (Figure 2). The killing percentage of STEC at 4 minutes and 8 minutes were 99.62% (2.42 log reduction) and 99.93% (3.15 log reduction) respectively. It was observed that Gram-negative bacteria were more susceptible to colloidal silver due to the absence of the peptidoglycan layer which supports the higher killing percentage when compared to Gram-positive bacteria. Based on the results, colloidal silver had indeed shine itself as an alternative to antibiotic resistance and could possibly be the next antibiotic.

The incorporation of colloidal silver into medicine had begun since 1990s but there were insufficient extensive studies on its toxicity as it is a metal. Examples of application include wound dressing, endotracheal tubes, surgical masks, and cotton fibers. The concept of application generally only involves coating of the colloidal silver onto the items. As such, this prevents the spread of infectious bacteria and subsequently reduces the rate of infection. On the other hand, consumption of colloidal silver as a dietary supplement, however, was not widely recommended due to side effects of long-term ingestion of gram quantities of silver (Silvestry-Rodriguez *et al*., 2007) where one will be suffering from argyria. The colloidal silver product used as a tested sample was found to be effective against MRSA and STEC at low concentration of colloidal silver (10 ppm). This is supported by Laroo (2013) by stating that the minimum inhibitory concentration of colloidal silver to exhibit its antimicrobial properties can be as low as 0.6 ppm compared to silver ions at 72 ppm or more. Note that, the minimum NOAEL (No Observable Adverse Effect Level) of silver is 10 g established by the World Health Organization (WHO) in 1996 in which the ingestion of the colloidal silver product should not cause toxicity. In a study of comparison between nano silver and ionic silver, conducted by Kvitek *et al.* (2011), it was stated that colloidal nano silver displayed more significant antimicrobial activities at low concentrations that are not toxic against human cells and does not cause harm to the environment. It can be suggested as an alternative antibiotic for consumption to reduce colonization of MRSA in patients but the intake should be kept to a standard regulatory and the patients are fully consulted by a physician.

## Conclusion

The wide spread of MRSA into the food commodities at present, presents a risk to the public health. The hazard of the pathogen exacerbates as its increasing resistance towards antibiotic was observed via the antibiogram profile and the MAR index. To combat antibiotic resistance, colloidal silver was tested to be suitable as an alternative strategy for treatment due to its efficient antimicrobial

properties as shown in the killing percentages of the pathogen MRSA (ATCC 33591 and wild-type) and STEC recorded well above 96% at 4 minutes and 8 minutes. The complexity of antimicrobial resistance and the solution to solve it is never an easy task. As the consequences affect everyone in the world, alternative treatment and strategies holds a key in combating antimicrobial resistance should be considered in safeguarding the future.

## References


Bai, J., Shi, X. and Nagaraja, T.G. 2010. A multiplex PCR procedure for the detection of six major virulence genes in *Escherichia coli* O157:H7. Journal of Microbiological Methods 82: 85-89.

Bauer, A.W., Kirby, W.M., Sherris, J.C. and Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology 45(4): 493-496.

Bennett, R.W. and Lancette, G.A. (January 2001). Bacteriological Analytical Manual Chapter 12: Staphylococcus aureus. Retrieved on December 23, 2015 from U.S. Food and Drug Administration website: *http://www.fda.gov/food/foodscienceresearch/laboratorymethods/ucm071429.htm*

Catry, B., van Duijkeren, E., Pomba, M.C., Greko, C., Moreno, M.A., Pyorala, S., Ruzauskas, M., Sanders, P., Threlfall, E.J., Ungemach, F., Torneke, K., Munoz-Madero, C. and Torren-Edo, J. 2010. Reflection paper on MRSA in food-producing and companion animals: epidemiology and controls options for human and animal health. Epidemiology and Infection 138: 626-644.

Cheng, Y.W., Yin, L.Y., Lin, S.H., Wiesner, M., Bernhardt, E. and Liu, J. 2011. Toxicity Reduction of Polymer-Stabilized Silver Nanoparticles by Sunlight. Journal of Physical Chemistry 115: 4425-4432.

Daum R.S., Gupta, S., Sabbagh, R. and Milewski W.M. 1992. Characterization of *Staphylococcus aureus* isolates with decreased susceptibility to vancomycin and teicoplanin: Isolation and purification of a constitutively produced protein associated with decreased susceptibility. Journal of Infectious Disease 166: 1066-1072.

Davies, R.L. and Etris, S.F. 1997. The Development and Functions of Silver in Water Purification and Disease Control. Catalysis Today 36: 107-114.

DeNap, J.C. and Hergenrother, P.J. 2005. Bacterial death comes full circle: targeting plasmid replication in drug-resistant bacteria. Organic and Biomolecular Chemistry 3: 959-966.

Finks, J., Well, E., Dyke T.L., Hussain, N., Plizga, L., Heddurshetti R., Wilkins, M., Rudrik, J., Hageman, J., Patel, J. and Miller, C. 2009. Vancomycin-resistant *Staphylococcus aureus*, Michigan, USA, 2007. Emergence of Infectious Disease 15: 943-945.

Feng, P., Weagent, S.D., Grant, M.A. and Burkhardt, W. (September 2002). Bacteriological Analytical Manual (BAM) Chapter 4: Enumeration of *Escherichia coli* and the Coliform Bacteria. Retrieved on December 23, 2015 from U.S. Food and Drug Administration website: *http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm064948.htm*

Gannon, V.P., King, R.K., Kim, J.Y. and Thomas E.J. 1992. Rapid and sensitive method for detection of Shiga-like toxin-producing *Escherichia coli* in ground beef using the polymerase chain reaction. Applied and Environment Microbiology 58: 3809-3815.

Harbarth, S., Dharan, S., LIassine, N., Herrault, P., Auckenthaler, R. and Pittet D. 1999. Randomized, placebo-controlled, double-blind trial to evaluate the efficacy of mupirocin for eradicating carriage of methicillin-resistant *Staphylococcus aureus.* Antimicrobial agents and Chemotherapy 43(6): 1412-1416.

Hiramatsu, K. 2001. Vancomycin-resistant *Staphylococcus aureus*: a new model of antibiotic resistance. The Lancet Infectious Diseases Commission 1(3): 147-155.

Jarvis, W.R., Jarvis, A.A. and Chinn, R.Y. 2012. National prevalence of methicillin-resistant *Staphylococcus aureus* in inpatients at United States health care facilities, 2010. American Journal of Infection Control 40(3): 194-200.

Kawahara, K., Tsuruda, K., Morishita, M. and Uchida, M. 2000. Antibacterial effect of silver-zeolite on oral bacteria under anaerobic conditions. Dental Materials 16(6): 452-455.

Klueh, U., Wagner, V., Kelly, S., Johnson, A. and Bryers, J.D. 2000. Efficacy of Silver-Coated Fabric to prevent bacterial colonization and subsequent device-based biofilm formation. Journal of Biomedical Materials Research Part B: Applied Biomaterials 53: 621-623.

Kurincic, M., Klancnik, A. and Smole Mozina, S. 2012. Epigallocatechin gallate as a modulato of *Campylobacter* resistance to macrolide antibiotics. International Journal of Antimicrobial Agents 40: 467-471.

Kvitek, L., Panacek, A., Prucek, R., Soukupova, J., Vanickova, M., Kolar, M. and Zobril, R. 2011. Antibacterial activity and toxicity of silver – nanosilver versus ionic silver. Journal of Physics Conference Series 304(1).

Laroo, H. 2013. Colloidal Nano Silver-Its production method, properties, standards and its bio-efficacy as an inorganic antibiotic. Journal Physical Chemistry and Biophysics 3:130

Laximinarayan, R., Duse, A., Wattal, C., Zaidi, A.K.M., Wertheim, H.F.L., Sumpradit, N., Vlieghe, E., Hara, G.L., Gould, I.M., Goossens, H., Greko, C., D So, A., Bigdeli, M., Tomson, G., Woodhouse, W., Ombaka, E., Peralta, A.Q., Qamar, F.N., Mir, F., Kariuki, S., Bhutta, Z.A., Coates, A., Bergstrom, R., Wright, G.D., Brown. E.D. and Cars, O. 2013. Antibiotic resistance – the need for global solutions. The Lancet Infectious Diseases Commission 13: 1057-1098.

Martinez-Castanon, G.A., Nino-Martinez, N., Martinez-Gutierrez, F., Martinez-Mendoza, J.R. and Ruiz,

F. 2008. Synthesis and antibacterial activity of silver nanoparticles with different sizes. Journal of Nanoparticle Research 10(8): 1343-1348.

McShan, D., Ray, C.P. and Yu, H. 2014. Molecular Toxicity Mechanism of Nanosilver. Journal of Food Drug Analysis 22(1): 116-127.

Melaiye, A. and Youngs, W.J. 2005. Silver and its application as an antimicrobial agent. Expert Opinion on Therapeutic Patients 15(2): 125-130.

Naimi, T.S., LeDell, K.H., Boxrud, D.J., Groom, A.V., Steward, C.D., Johnson, S.K., Besser, J.M., O'Boyle, C., Danila, R.N., Cheek, J.E., Osterholm, M.T., Moore, K.A. and Smith, K.E. 2001. Epidemiology and clonality of community-acquired methicillin-resistant *Staphylococcus aureus* in Minnesota, 1996-1998. Clinical infectious disease: an official publication of the Infectious Diseases Society of America 33(7): 990-996.

Poonia, S., Singh, T.S. and Tsering, D.C. 2014. Antibiotic susceptibility profile of bacteria isolated from natural sources of water from rural areas of East Sikkim. Indian Journal of Community Medicine: Official Publication of Indian Association of Preventive and Social Medicine 39(3): 156-160.

Rai, M.K., Deshmukh, S.D., Ingle, A.P. and Gade, A.K. 2012. Silver nanoparticles: the powerful nanoweapon against multidrug-resistant bacteria. Journal of Applied Microbiology 112: 841-852.

Rajaduraipandi, K., Mani, K.R., Pannerselvam, K., Mani, M., Bhaskar, M. and Manikandan, P. 2006. Prevalence and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus*: a multicenter study. Indian Journal of Medical Microbiology 24(1): 34-38.

Silvestry-Rodriguez, N., Sicairos-Ruelas, E.E., Gerba, C.P. and Bright, K.R. 2007. Silver as a Disinfectant. Reviews of Environmental Contamination and Toxicology 191: 23-45.

Turnidge, J., Rao, N., Chang, F-Y., Fowler, V.G.Jr., Kellie, S.M., Arnold, S., Lee, B.Y. and Tristan, A. 2008. Staphylococcus aureus. Retrieved on December 31, 2015 from Antimicrobe website: *http://www.antimicrobe.org/sample_staphylococcus.asp*