Antibacterial effects of green laser in vicinity of silver nanoparticles on methicillin-resistant staphylococcus aureus (MRSA)

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ABSTRACT—Methicillin-resistant *Staphylococcus aureus* (MRSA) is a type of staph bacteria that is resistant to most of the available antibiotics. So different methods have been introduced to overcome this great threat to public health. Photoactivated metal nanoparticles have been presented as good alternatives to antibiotics. In this paper, S. aureus ATCC 33591 as reference strains and clinical MRSA bacteria, isolated from wounds of burn patients, was used. After identification of the bacteria and testing its antibiotic susceptibility, the minimum inhibitory concentration test was performed to study the antibacterial effect of Ag-NPs on MRSA. Our results showed that the bacterial colony population of both samples of *S. aureus* strains was reduced significantly in the presence of Ag-NPs. The antibacterial effect was enhanced by utilizing a green laser. Interestingly, the bacterial growth has completely inhibited after 2 minutes of laser irradiation in the BHI agar medium. Also, in the nutrient agar medium and Müller-Hinton agar medium, significant synergistic bacterial suppression has been observed by combination the antibacterial effects of the laser and the Ag-Nps.

KEYWORDS: Antibacterial activity, Green laser, Methicillin-resistant *S. aureus* (MRSA), Oxygen species, Silver nanoparticles.

I. INTRODUCTION

Antibiotic resistance is a serious public health threat worldwide which leads to many problems such as prolongation of hospital stay and increase in medical cost. One of the most common drug-resistant bacteria is *Staphylococcus aureus*, particularly methicillin-resistant *S. aureus (MRSA)* which is also resistant to most common β-lactam antibiotics like penicillins and cephalosporins [1,2]. *MRSA* is a Gram-positive and spherical (coccal) bacterium that causes different to cure infections like surgical and nonsurgical wound infections. As the resistance mechanism of MRSA prevents antibiotics from bacterial cell wall disruption, these infections can become serious and long-lasting [3]. Therefore, in recent years considerable attention has been paid to various methods that could kill or suppress the growth of bacteria [4].

Recent researches have shown that nanoparticles (NPs) are effective alternatives to antibiotics for eradicating resistant bacteria as several biomolecules are targeted by NPs at once. NPs are divided into four groups: organic, inorganic, carbon, and composite nanoparticles. The size of these tiny materials varies from 1 to 100 nanometers. At the nanoscale, the behavior of matter is unpredictable and surprising like high reactivity, adsorption capacity, and specific area. Furthermore, they have antibacterial and antioxidant properties due to their effective treatment strategies such as the production of reactive oxygen species (ROS), DNA degradation, membrane interaction, and reduction of ATP. There has been a lot of interesting research on the strong antibacterial activity of metal NPs. Metal NPs such as silver, gold, platinum, and copper-oxide NPs are used in many products and antimicrobial formulations because of their cytotoxicity and bioactivity interaction with microbes [5-10].

Furthermore, many studies have shown that the laser beam causes biochemical and physiological changes in the cellular components. The therapeutic effects of the laser depend on the wavelength, spectral profile, applied energy, energy density, type of emission (continuous or pulsed), temporal profile, and exposure time [11] Related to irradiance level, six classes of light-tissue interaction modes could be classified. The modes include 1. photobiomodulation, 2.photochemical reaction, 3. Thermal effects, 4. photoablation,5.plasma-induced photoablation, and 6. photodisruption. In photobiomodulation mode, also called biostimulation or low-level light therapy (LLLT), chromophore molecules absorb the light and their electrons excite to higher energy levels. The mechanism release reactive-oxygen species (ROS), nitric oxide (NO), and the synthesis of adenosine triphosphate (ATP). The cytotoxic effects of ROS species can cause cancer cell apoptosis. In the second mode, photochemical reaction (also called photodynamic therapy (PDT)), a photosensitive drug is used, additionally. The wavelength of the selected laser corresponds to the absorption peak of the photosensitizer. The light absorption cause formation of highly toxic singlet oxygen ($^1O_2$) and consequently the diseased cell death. The other modes are related to high-energy laser lights (upper than 10 W/cm^2) that thermal reactions cause the tissue to coagulate, vaporize, carbonize, or melt [12].

The LLLT and PDT modes have been investigated for inhibition of *Staphylococcus aureus* bacteria. In 2011, it was observed that a Helium-Neon laser has an inhibitory effect on the growth of *Staphylococcus aureus*. The longer the laser irradiation, the lower the growth rate of the bacteria. In addition, it has been shown that the use of caffeic acid as a photosensitizer in the PDT process reduces bacterial populations. [13]. Furthermore, different researches have been done on the toxicity of silver nanoparticles (Ag-NPs) and their antibacterial effect on gram-negative bacteria (such as *Escherichia. coli, Vibrio. cholera, Pseudomonas. aeruginosa, and Scrub. typhus*) [14,15], and also gram-positive bacteria (such as Methicillin-resistant *Staphylococcus aureus*) [16,17]. Of course, by decreasing the size and also related to particle shape, the antibacterial activity could be increased due to more contact with bacteria [18]. Moreover, the synergy of laser irradiation and Ag-NPs have shown the enhancement of antimicrobial activity. The enhancement is related to the creation of oxidative stress, production of reactive oxygen species (ROS), which damages the protein and DNA of the cell [17,19]. The optical properties of Ag-NPs, the localized surface plasmon resonance (LSPR) absorption, can be controlled by the particle shape, composition, size, and refractive index of their environment [20, 21]. As the LSPR band of Ag-NPs is in the UV-visible region of the spectrum, their antibacterial treatments have been studied using various laser wavelengths in this region [22]. In newer strategies, Ag-NPs, blue light, and antibiotics have combined to combat *MRSA* infections [17]. As the absorption peak of Ag nanospheres shifts toward a higher wavelength (redshift) by increasing the size [23], selection of higher wavelength lasers (such as green lasers) with higher penetration depth in the skin, could help heal deeper wounds infections [12].

In this study, we have sampled methicillin-resistant strains of *Staphylococcus aureus* (MRSA) bacteria from the burn patients and also used *S. aureus* ATCC 33591 as reference. Then we have used a gram staining method to identify the bacteria. Also, the bacterial resistance to antibiotic discs was examined. To extract bacterial DNA, a standard PCR protocol has been used. After that, the antibacterial effect of Ag-NPs was evaluated through the minimum inhibitory concentration/ minimum bactericidal concentration (MIC/MBC) test. Finally, the antibacterial effects of green laser were investigated by preparing sterile tubes including ATCC 33591 or a Clinical sample of S. aureus strains on Muller-Hinton agar, nutrient agar, and HBI agar mediums after addition of Ag-NPs and irradiating for 1 and 2 minutes.

II. EXPERIMENTAL PROCEDURE

A. *Collection of bacteria*

S. aureus ATCC 33591, used as reference strains, were collected from the laboratory of Azad University Central-Tehran branch. Also, methicillin-resistant strains of *Staphylococcus aureus (MRSA)* bacteria were sampled from the burn patient's wound of Shahid Motahari burns hospital by using sterile swabs. The samples were cultured in a nutrient agar medium.

B. *Identification of bacteria*

To identify the bacteria, a gram staining method was used. 10 BHI media plates were prepared. Then they were cultured in 4 steps and incubated for 24 hours. Moreover, a catalase test and also coagulase test were performed.

A catalase test is used to detect the difference between *Staphylococcus aureus* (catalase-positive) and *Streptococcus* (catalase-negative). And also, the aim of the coagulase test is the detection of differentiation between coagulase-positive Staphylococcus aureus and staphylococcus aureus (coagulase-negative)
Also, DNase test on DNase medium mannitol test on mannitol salt agar, and hemolysis test on blood agar medium were performed. All mediums belong to the Merck Company, Germany.

The purpose of using a DNase medium is to determine the activity of staphylococcal specific microorganisms.

The enriched medium, blood agar medium, and mannitol salt agar are used for the clinical samples to isolate pathogenic microorganisms.

C. *Determination of antibiotic susceptibility*

The goal of antibiotic susceptibility testing is to determine the bacterial resistance to antibiotic discs. To do this experiment, 10 Mueller Hinton Agar plates were prepared. Then the standard half-McFarland solution was made in 10 tubes with O.D 0.08-13. Finally, 9 discs of antibiotics (oxacillin, gentamicin, ciprofloxacin, vancomycin, erythromycin, tetracycline, penicillin, amikacin, and clindamycin) were used and 3 discs were placed in each plate containing bacteria. After that, those were incubated for 24 hours. Afterward, the zone of inhibition was observed.

D. *DNA extraction of resistant staph bacteria*

To extract bacterial DNA from the resistant staph bacteria, the Boiling method, a standard PCR protocol have been used. In this method, the bacteria were grown on agar plates by taking 1 to 3 colonies from each plate. After inoculation, it was added to 300 µl of sterile water in microtubes. Then they were put in a bain-marie (water bath) at 80 °C for 20 minutes and after that, at 96 °C for 20 mins again. After these times, microtubes were placed in a centrifuge at 14000 rpm for 15 min. After that, the clear (transparent) supernatant (liquid) was poured into other sterile microtubes.

To confirm DNA extraction quantitatively, a nanodrop machine (Thermo Fisher, Germany) and also electrophoresis (Bio-rad UV transilluminator, France) in 1% agar gel were used to confirm its quality.

E. *PCR (Polymerase chain reaction)*

PCR reaction, the chemical reaction that molecular biologists use to amplify pieces of DNA, was performed for all isolates and S. *aureus* ATCC 33591 sample (control), by using a temperature and time program of Thermocycler (SensoQuest, USA) that is shown in Table 1 and specific primers of the mecA gene in a volume of 25 µl as follows: Master mix 12.5 µl, forward primer 0.7 µl, reverse primer 0.7 µl, DDW (sterile double distilled water) 6.1 µl, and the extracted DNA 5 µl.

Table 1. A temperature and time program for Thermocycler in PCR

Thermocycler steps	Time (s)	Temperature (°C)
Step 1	300	94
Step 2	60	94
Step 3	60	50
Step 4	120	72
Step 5	600	72

F. *MIC/MBC test (minimum inhibitory concentration/ minimum bactericidal concentration)*

The minimum inhibitory concentration (MIC) test was carried out to evaluate the antibacterial

effect of Ag-NPs on *S. aureus* bacteria methicillin-resistant strains and *S. aureus* ATCC 33591 by the standard broth dilution method according to CLSI. 95 µl of Muller-Hinton broth and 5 µl of 0.5 McFarlands'standard suspensions of bacteria were provided. A serial dilution of the Ag-NPs was done in the amount of 100 µl in the first well of each row of a sterile microplate and diluted in the well onwards. After that, the microplate was placed in the incubator for 18-24 hours. Therefore, the MIC well is evaluated by the turbidity and transparency of each well in a row of the microplate. Also, the MBC test was performed by culturing the MIC well and one well before and after that on the divided Muller-Hinton agar, which was inoculated after 18-24 hours.

G. *Laser irradiation*

The colloidal silver in the concentration of 1000 ppm with a particle size of 50-100 nm was purchased. Then, the concentration of Ag-NPs based on the result of MIC was prepared.

The pulsed laser (Takfam Sazan Shafa, Iran) parameters include wavelength: 532 nm, pulse duration: 3.5 ns, repetition rate: 2.97 kHz, optical power: 77.7 mW, and energy: 26.16 µJ. Also, the time of irradiation includes 1 and 2 minutes. The Schematic diagram of our experimental setup is shown in Fig. 1.

12 sterile tubes were prepared with a concentration of MIC and were divided into 3 groups. Each group contains 4 tubes including Clinical sample of *S.* aureus strains + medium, Clinical sample of S. aureus strains + medium+ Ag-NPs, *S. aureus* ATCC 33591 sample + medium, S. aureus ATCC 33591 sample + medium+ Ag-NPs. 1 group was not irradiated, but the other 2 groups were exposed to laser radiation (4 tubes for 1 minute and 4 tubes for 2 minutes). After that these 12 tubes were cultured on three media, 100 µl of each tube was poured on muller-Hinton agar, nutrient, and BHI (brain heart infusion) by Pasteur pipette, and they were inoculated for 24 hours. Finally, colony counting was performed for all plates.

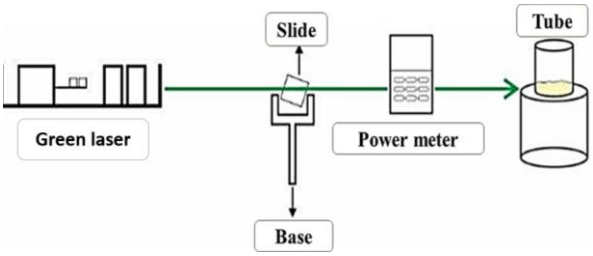


Fig. **1** Schematic diagram of the experimental setup.

III. RESULTS AND DISCUSSIONS

The Gram staining results (Fig. 2) showed that the identity of the bacterial strain was acceptable. Furthermore, the bacteria were spherical, clustered, and purple, indicating that it was a gram-positive bacterium. Moreover, the results of both catalase and coagulase tests were also positive. The enzyme catalase is present in resistant *Staph* bacteria.

Also, the bacterium is coagulase-positive means fibrinogen is altered and converted to fibrin, and cell accumulation has occurred in resistant Staph bacteria.

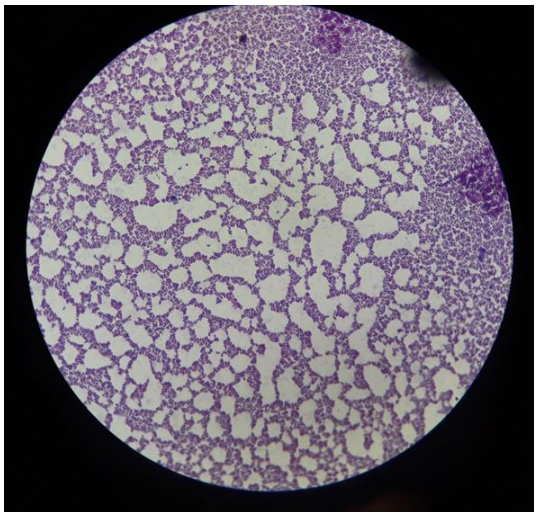


Fig. **2** A gram stain of Methicillin-resistant *Staphylococcus aureus.*

The bacteria were also grown in different mediums. After inoculating bacteria on Dnase medium, hydrochloric acid was poured on the plates. The halo formation was a sign of the presence of deoxyribonuclease in *S. aureus* bacteria methicillin-resistant strain (as shown in Fig. 3.a). Also, Fig. 3.b indicated that the plates containing bacteria and mannitol salt agar medium discolored from pink to yellow due to

the ability of bacteria to ferment mannitol, after inoculating. And about culturing bacteria on the last medium, blood agar medium, the transparency around the colonies indicated beta-hemolysis for bacteria (as shown in Fig. 3.c).

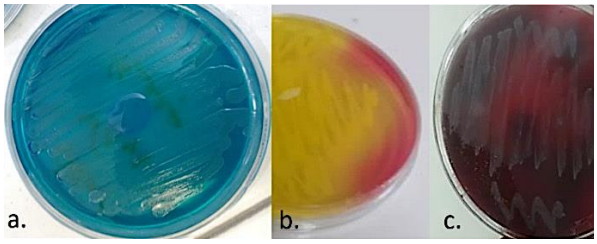


Fig. **3** a) halo formation after pouring the hydrochloric acid on the plates with Dnase medium, b) discolouring on mannitol salt agar medium, c) transparency around the colonies on blood agar medium.

The results of the antibiotic testing were illustrated in Table 2. In this table, the zone of inhibition of antibiotics such as tetracycline, oxacillin, amikacin, vancomycin, gentamicin, ciprofloxacin, erythromycin, penicillin, clindamycin on 6 clinical samples of *S. aureus* bacteria methicillin-resistant strain were shown. The difference in the zone of inhibition for each clinical strain is due to the fact that each strain was isolated from patients in different parts of the hospital.

Table **2** The Zone of Inhibition (mm) of Clinical samples of *S. aureus* strains

	The Zone of Inhibition (mm) of Clinical samples of *S. aureus* strains					
Antibiotics	**1**	**2**	**3**	**4**	**5**	**6**
Tetracycline	8	8	R	8	8	4
Oxacillin	19	R	R	R	19	R
Amikacin	10	R	R	R	10	R
Vancomycin	19	13	12	14	19	13
Gentamicin	13	7	8	9	13	6
Ciprofloxacin	R	R	7	R	R	R
Erythromycin	R	R	R	R	R	R
Clindamycin	R	R	16	16	R	7
Penicillin	R	R	R	R	R	R

The antibacterial effect of Ag-NPs on both Clinical samples of *S. aureus* strains and *S. aureus* ATCC 33591 samples were evaluated by MIC/MBC tests. The minimum inhibitory concentration (MIC) was the same for the two samples and it was observed at the sixth well (15.625 ppm).

Nanodrop spectrum verified the DNA extraction. Also, Gel electrophoresis results indicated that some of the samples are positive in terms of having this gene. As shown in Fig. 4. the band is created for 7 samples. The MecA gene has 310 bp, so this gene is well visible in extracted DNA with a 50 bp ladder.

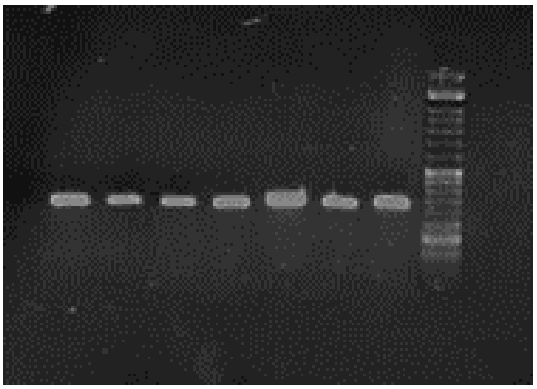


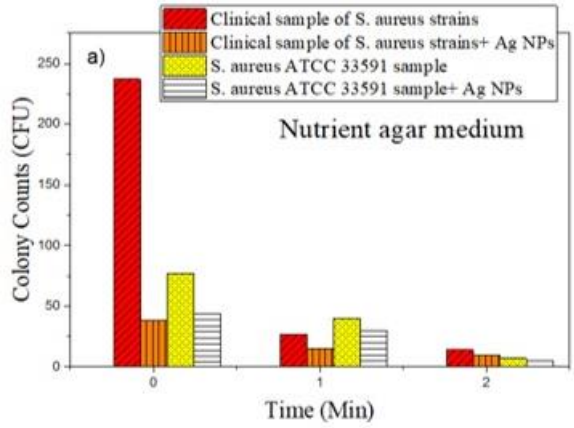
Fig. **4** With the presence of the mecA gene in isolates, the bands are created for 7 samples

Fig 5 shows the results of colony counting of 12 tubes before and after laser irradiation. Every 4 tubes include a clinical sample of *S. aureus* strains + medium, clinical sample of *S. aureus* strains + medium+ Ag-NPs, *S. aureus* ATCC 33591 sample + medium, *S. aureus* ATCC 33591 sample + medium+ Ag-NPs. They were cultured on three mediums: nutrient agar (Fig. 5. a), muller-Hinton agar (Fig. 5. b), and BHI agar (Fig. 5. c).

As can be seen in Fig. 5.a, both ATCC and clinical sample of *S. aureus* strains have grown on nutrient agar medium before laser irradiation (at t=0). However, after the addition of Ag-NPs, the population of those colonies was reduced significantly, especially in the case of the clinical sample. After laser irradiation (at t=1 min), the number of bacterial cells was greatly reduced and those containing Ag-NPs were further reduced. The increasing inhibition was seen at t=2 min. The interesting issue is that the inhibition for ATCC bacteria is more than clinical bacteria on nutrient agar medium at t =2 min. This phenomenon is due to the fact that clinical bacteria are more resistant than ATCC ones.

As presented in Fig. 5.b, both bacteria were grown on Muller-Hinton agar medium in the absence of laser irradiation (at t=0), and the Ag-NPs had a greater inhibitory effect on clinical bacteria than ATCC bacteria. Laser irradiation at both times of 1 and 2 minutes had a more excellent inhibitory effect on clinical bacteria and clinical bacteria+ Ag-NPs than ATCC bacteria and ATCC bacteria+ Ag-NPs; In addition, samples containing Ag-NPs have shown enhancement of inhibitory effect due to antibacterial activity of Ag-NPs.

On HBI agar medium, as shown in Fig. 5.c, in the absence of laser irradiation, both bacteria have grown and the samples containing Ag-NPs had less growth. With laser irradiation at two times, 1 and 2 minutes, the growth of the ATCC bacterial colony population has significantly reduced compared to clinical bacteria, to the extent that ATCC bacteria has completely destroyed and killed. Intensification of inhibition of bacteria growth by Ag-NPs in both samples is due to its antibacterial properties.



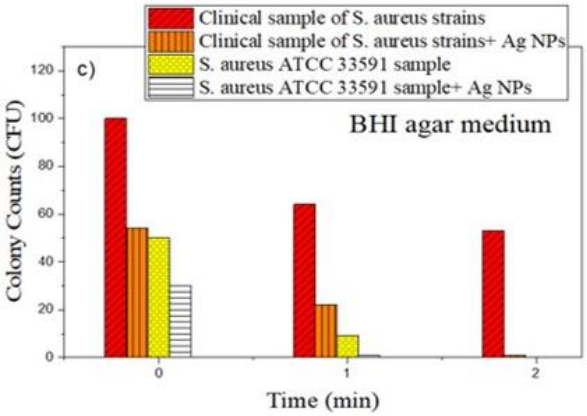


Fig. **5** Colony counting of 12 tubes before and after laser irradiation in the presence and absence of Ag-NPs.

Comparing the bacterial population growth of ATCC and clinical bacteria in Fig. 5. a-c, has shown that both bacteria have grown in the absence of laser irradiation. Also, the population of both bacteria was reduced significantly in the presence of Ag-NPs in all mediums. Interestingly, the growth of ATCC bacteria on BHI agar medium was less than other mediums and after 2 minutes of laser irradiation, the ATCC bacteria in the absence and presence of Ag-NPs has not grown completely. However, the clinical bacteria in the absence of Ag-NPs were highly resistant.

General study of the number of bacteria cells:

The number of clinical bacterial cells (in the absence of Ag-Nps) concerning broth medium (indicated as red columns with diagonal lines):

On nutrient agar medium:

The number of bacterial cells (indicated as red columns with diagonal lines) decreased by 88.60% after one minute of laser irradiation and about 94% after two minutes of irradiation.

On Müller-Hinton agar medium:

The bacterial count decreased by 87% after one minute of laser irradiation and 91.3% after two minutes of irradiation.

On BHI agar medium:

The number of bacteria cells decreased by 36% after one minute of irradiation and 47% after two minutes of irradiation.

The number of clinical bacterial cells concerning broth medium in the vicinity of Ag-Nps (indicated as orange vertical striped columns):

On Nutrient agar medium:

The bacterial count was reduced by 84% in the vicinity of Ag-Nps. The synergy of laser and Ag-Nps reduced the bacterial count to 93.7% after one minute of irradiation. The toxicity of Ag-NPs after photoactivation resulted in bacterial count reduction up to 95.8% after two minutes of irradiation.

On Müller-Hinton agar medium:

The bacterial count in the vicinity of Ag-Nps reduced by 84.8%. The count was reduced to 90% after one minute of laser irradiation and 92.6% after two minutes of irradiation.

On BHI agar medium:

The bacterial count decreased by 46% in the vicinity of Ag-Nps. The count was reduced to 78% after one minute of laser irradiation. The results showed significant bacterial reduction (up to 99%) after 2 minutes of irradiation.

The number of ATCC bacterial cells (in the absence of Ag-Nps) concerning broth medium (indicated as yellow dotted diamond columns):

On Nutrient agar medium:

The number of bacteria cells reduced by 48% after one minute of laser irradiation and 91% after two minutes of irradiation.

On Müller-Hinton agar medium:

The number of bacterial cells reduced by 60% after one minute of laser irradiation and 75.7% after two minutes of irradiation.

On BHI agar medium:

The number of bacteria cells reduced by 82% after one minute of laser irradiation and the bacterial growth was inhibited completely (100%) after two minutes of irradiation.

The number of ATCC bacterial cells concerning broth medium in the vicinity of Ag-Nps (indicated as white horizontal striped columns):

On Nutrient agar medium:

The number of bacteria cells reduced by about 42.8% in the vicinity of Ag-Nps. The bacterial count decreased by 61% after one minute of irradiation and 93.5% after two minutes of irradiation.

On Müller-Hinton agar medium:

In the vicinity of Ag-Nps, the number of bacteria cells reduced by 34.3%. It reduced to 68.6% after one minute of laser irradiation and to 78.6% after two minutes of irradiation.

On BHI agar medium:

The number of bacteria cells reduced by 40% in the vicinity of Ag-Nps. After one minute of laser irradiation, it reduced to 98%, and the bacterial growth was totally inhibited (100%) after two minutes of irradiation.

Besides, to compare the results of bacterial growth inhibition in the Nutrient agar medium, Müller-Hinton agar medium, and BHI agar medium, after two minutes of laser irradiation, in presence of Ag-Nps, and two minutes of laser irradiation in presence of Ag-Nps, table 3 have been depicted. As can be seen, the laser and Ag-Nps have antibacterial effects, independently. However, simultaneous application of them cause photoactivation of Ag-Nps which resulted in efficiency enhancement of the bacterial growth inhibition. The enhancement process is observed in the BHI agar medium, more obviously.

Table **3** Percentages of bacterial count reduction.

		Nutrient agar	**Müller-Hinton agar**	**BHI agar**
Clinical sample	Laser	94%	91.3%	47%
	Ag-Nps	84%	84.8%	46%
	Laser+Ag-Nps	95.8%	92.6%	99%
ATCC sample	Laser	91%	75.7%	100%
	Ag-Nps	42.8%	34.3%	40%
	Laser+Ag-Nps	93.5%	78.6%	100%

The antibacterial activity of Ag-Nps is due to the interactions that occur between the bacterial cell wall and the nanoparticles, which destroy the cell wall and suppress the bacterial growth and finally destroy it. Silver nanoparticles easily release ions throughout the membrane, and as the charges on the cell wall of the bacterium are negative, the nanoparticle adhesion and instability that occurs in the cell membrane causes the bacterial cell wall destruction [15, 16].

The antibacterial efficacy of Ag-NPs was enhanced after the exposure to green laser radiation. Resonant oscillation of conduction electrons at the Ag-NPs surface, called surface plasmon resonance (SPR), causes strong absorption of light at the resonance frequency. The SPR frequency is related to various factors such as size, shape, and the environment of Nps. For instance, the increment of Ag-Nps size results in frequency red-shift which lets us use a green laser for photoactivation of 50-100 nm NPs. As the penetration depth of light increases by increasing the wavelength, lasers with higher wavelengths could be used to treat the deeper infected wounds.

When the Ag-NPs absorb the laser light, the electrons are excited from the singlet state to the triplet state. In this long-lasting state, the Ag-NPs interact with ambient molecular oxygen and the electron transfer reactions yield to the production of highly toxic reactive oxygen species (ROS) such as $^{\cdot}OH$, $O2^{\cdot-}$ which cause the inactivation of bacterial biomolecules including DNA, proteins, and lipids [16-23].

IV. Conclusion

Methicillin-resistant *Staphylococcus aureus (MRSA)* causes difficulty to treat *staph* infections because of its high antibiotic resistance. So, considerable attention has been paid to alternative antimicrobial approaches. In this paper, we have introduced the combination of green laser and silver nanoparticles as a harmless method to kill or inhibit the growth of bacteria.

We have compared the bacterial population growth of ATCC 33591 and clinical samples of S. aureus strains in the vicinity of Ag-NPs, after 1 and 2 minutes of laser irradiation. Colony counting showed population reduction of both bacteria in the presence of Ag-NPs in all Muller-Hinton agar, nutrient agar, and HBI agar mediums. On the BHI agar medium, the ATCC bacterial growth was less than two other mediums and the bacterial growth was completely inhibited after 2 minutes of laser irradiation. Subsequently, one of the best alternatives to antibiotics is the photoactivation of silver nanoparticles. As they have the potential to target several biomolecules together, strong antibacterial activity is expected.

References

[1] B. Aslam, W. Wang, M.I. Arshad, M. Khurshid, S. Muzammil, M.H. Rasool, M.A. Nisar, R.F. Alvi, M.A. Aslam, M.U. Qamar, and M.K. Salamat, "Antibiotic resistance: a rundown of a global crisis, Infection and drug resistance," Infect Drug Resist, vol. 11, pp. 1645 (1-14), 2018

[2] R.M. Klevens, M.A. Morrison, J. Nadle, S. Petit, K. Gershman, S. Ray, L.H. Harrison, R. Lynfield, G. Dumyati, J.M. Townes, and A.S. Craig. "Invasive methicillin-resistant Staphylococcus aureus infections in the United States," Jama, vol. 298, no. 15, pp. 1763-1771, 2007.

[3] A. S. Haddadin, S. A. Fappiano, and P. A. Lipsett, "Methicillin-resistant Staphylococcus aureus (MRSA) in the intensive care unit," Postgraduate medical journal, vol. 78, pp. 385-392, 2002.

[4] P. Yang, N. Wang, C. Wang, Y. Yao, X. Fu, W. Yu, R. Cai, and M. Yao, "460 nm visible light irradiation eradicates MRSA via inducing prophage activation," Journal of Photochemistry and Photobiology B: Biology, vol. 166, pp. 311-22, 2017.

[5] Y.N. Slavin, J. Asnis, U.O. Häfeli, and H. Bach, "Metal nanoparticles: understanding the mechanisms behind antibacterial activity", Journal of nanobiotechnology, vol. 15, no. 1, pp. 1-20, 2017.

[6] R.S. Ghaderi, M. Kazemi, and S. Soleimanpour, "Nanoparticles are More Successful Competitor than Antibiotics in Treating Bacterial Infections: A Review of the Literature," Iranian Journal of Medical Microbiology, vol. 15, no. 1, pp. 18-45, 2021.

[7] A.P. Ingle, N. Duran, and M. Rai, "Bioactivity, mechanism of action, and cytotoxicity of copper-based nanoparticles: a review," Applied

microbiology and biotechnology, vol. 98, pp. 1001-1009, 2014.

[8] H. Itohiya, Y. Matsushima, S. Shirakawa, S. Kajiyama, A. Yashima, T. Nagano, and K. Gomi, "Organic resolution function and effects of platinum nanoparticles on bacteria and organic matter," PloS one, vol. 14, no. 9, pp. e0222634 (1-12), 2019.

[9] J.R. Morones, J.L. Elechiguerra, A. Camacho, K. Holt, J.B. Kouri, J.T. Ramírez, and M.J. Yacaman, "The bactericidal effect.t of silver nanoparticles," Nanotechnology, vol. 16, no. 10, pp. 2346 (1-8), 2005.

[10] A.P. Ingle, N. Duran, and M. Rai, "Bioactivity, mechanism of action, and cytotoxicity of copper-based nanoparticles: a review," Applied microbiology and biotechnology, vol. 98, no. 3, pp. 1001-1009, 2014.

[11] H. F. chambers, "The changing epidemiology of staphylococcus aureus," Special issue, vol. 7, pp. 178–182, 2001.

[12] G. Keiser, *Biophotonics*, Springer, chapter 6, 2016.

[13] S. E. McBirney, K. Trinh, A. Wong-Beringer, and A. M. Armani, "Wavelength- normalized spectroscopic analysis of staphylococcus aureus and pseudomonas aeruginosa growth rates," Biomedical optics express, vol. 7, pp. 4034-4042. 2016.

[14] I. Sondi and B. Salopek-Sondi, "Silver nanoparticles as antimicrobial agent: a case study on E-coli as a model for gram-negative bacteria," J. Colloid Interface Sci. vol. 275, pp. 177–182, 2004.

[15] J.R. Morones, J.L. Elechiguerra, A. Camacho, K. Holt, J.B. Kouri, J.T. Ramírez, and M.J. Yacaman, "The bactericidal effect of silver nanoparticles," Nanotechnology, vol. 16, pp. 2346-2353, 2005.

[16] A.F. Wady, A.L. Machado, C.C. Foggi, C.A. Zamperini, V. Zucolotto, E.B. Moffa, and C.E. Vergani, "Effect of a Silver Nanoparticles Solution on Staphylococcus aureus and Candida spp," Journal of Nanomaterials, vol. 2014, pp. 1-7, 2014.

[17] F.E. Akram, T. El-Tayeb, K. Abou-Aisha, and M. El-Azizi, "A combination of silver nanoparticles and visible blue light enhances the antibacterial efficacy of ineffective antibiotics against methicillin-resistant Staphylococcus aureus (MRSA)," Annals of clinical microbiology and antimicrobials, vol. 15, no. 1, pp. 1-3, 2016.

[18] S. Pal, Y. K. Tak, and J. M. Song, "Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium Escherichia coli," Appl. Environ. Microbiol, vol. 73, pp. 1712–1720, 2007.

[19] A. Al-Sharqi, K. Apun, M. Vincent, D. Kanakaraju, and L.M. Bilung, "Enhancement of the antibacterial efficiency of silver nanoparticles against gram-positive and gram-negative bacteria using blue laser light," International Journal of Photoenergy, vol. 2019, pp. 1-12, 2019.

[20] C. Noguez, "Surface plasmons on metal nanoparticles: the influence of shape and physical environment," J. Phys. Chem. C, vol. 111, no. 10, pp. 3806-3819, 2007.

[21] D. Paramelle, A. Sadovoy, S. Gorelik, P. Free, J. Hobley, and D.G. Fernig, "A rapid method to estimate the concentration of citrate capped silver nanoparticles from UV-visible light spectra," Analyst. vol. 139, no. 19, pp. 4855-4861, 2014.

[22] R.T. da Silva, M.V. Petri, E.Y. Valencia, P.H. Camargo, S.I. de Torresi, and B. Spira, "Visible light plasmon excitation of silver nanoparticles against antibiotic-resistant Pseudomonas aeruginosa," Photodiagnosis and Photodynamic Therapy, vol. 1, no. 31, pp. 101908,2020.

[23] D. Paramelle, A. Sadovoy, S. Gorelik, P. Free, J. Hobley, and D. G. Fernig, "A rapid method to estimate the concentration of citrate capped silver nanoparticles from UV-visible light spectra," Analyst, vol. 139, pp. 4855-4861, 2014.

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