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Research Article

Comparision of the Bactericidal Effect of the UV and Blue-Light Regions on Selected *Escherichia Coli* and *Staphylococcus Aureus* Strains

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ABSTRACT

Some Bacteria are important microorganisms that threaten human health. Especially Escherichia coli and Staphylococcus aureus can cause serious diseases in humans. Antibiotics are used to stop these infections and prevent bacteria from multiplying. However, it has been observed that these antibiotics have side effects as well as their benefits. Therefore, the resistance of these bacteria can be reduced with rays of different wavelengths. In our study, the effects of three types of light with wavelengths of 254 nm, 365 nm and 460 nm on Escherichia coli and Staphylococcus aureus bacteria were investigated. The reason we chose these rays is that two of them have short wavelengths and are harmful rays, while the other one is in the visible region and is harmless. While a significant decrease in the number of colonies was observed under the operating conditions of 254 nm and 365 nm wavelength lights falling in the UV region, no colonies were observed in the 460 nm wavelength light. In the second trial results, 44% growth was achieved at 365 nm and 56% growth was inhibited. S. aureus growth stopped completely at 460 nm. At 254 nm, 14% growth was achieved and 86% growth was inhibited. It was observed that there was 3% growth and 97% growth inhibition at 365 nm. As can be clearly seen from the results, no significant bacterial growth was observed at 460 nm. In our literature studies, it can be seen that no study has been done on this wavelength before.

1. Introduction

Many bacteria that can adapt to adverse conditions in nature can cause diseases in living things. Because most of the boundaries within which pathogens can multiply are in harmony with the boundaries of the living things on or within them. When maximum or minimum temperature limits are approached or exceeded, death or weakening of the effectiveness of some factors occurs, even if microorganisms grow. Virus, bacteria and fungal spores are highly resistant to environmental conditions and can survive for long periods (years) and retain their infectious abilities. With this contagious feature, they spread among living things and cause diseases. People have been searching for cures and treatments for disease-causing bacteria for years. Many studies have been done on this subject [1-3]. The most important of these treatment methods is antibiotic treatments. However, antibiotics also have negative side effects. For this reason, different methods have been put forward to break antibiotic resistance. Some wavelengths of light can prevent bacteria from growing and multiplying. In this way, reproduction is prevented from reaching a level that could stop and cause infection.

1.1. The effects of light on bacteria

Lights of different wavelengths have long been used in microbial control because they do not require heat to kill the microorganism. The main areas of use of these microbial control lights are disinfection of air and surfaces of operating

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rooms, laboratories and biological safety cabinets. In addition, they can be used in closed places such as cafeterias and hospital rooms where people are crowded together, to prevent the spread of airborne diseases by reducing the number of pathogenic microorganisms in the air [4].

This microbial property of light disrupts the structure of the bacteria and causes some changes in the bacteria. These changes can be explained as follows.

Looking at the bacterial structure, monomers (simple molecular compounds with unsaturated or double bonds) are linked to each other by phosphodiester bonds formed between 5'-phosphate groups and 3'-hydroxyl groups, forming long polymers consisting of units up to several hundred million in number. These are the bonds made by the phosphates in DNA and RNA with deoxyribose and ribose. The double bond on thymine and cytosine bases in DNA absorbs ultraviolet light. This opens the added energy bond and allows it to react with the neighbouring base.

Reactive oxygen species (ROS) include oxygen radicals (free radicals released due to single-electron reduction from oxygen), single oxygen and peroxidases. These are usually tiny molecules and highly reactive due to the presence of unpaired valence shell electrons. High amounts of ROS are known to have a lethal effect on the cell. In some diseases, tissue destruction occurs as a result of the formation of free radicals. ROS in bacteria means light induced by sensitivity to endogenous light [5].

1.2. Phototoxic effects in microorganisms are as follows:

According to the wavelength used, the light causes the ROS to be induced when it is high, while it encourages the increase of ROS in a low amount. Intense blue light (preferably around 415 nm) is better than red light to kill bacteria. High-intensity visible light kills bacteria, while light in the visible and near-infrared region enhances bacterial growth.

In a 2013 article, blue light was used for CA-MRSA (methicillin-resistant *S.aureus*) infections from mouse skin abrasions. The wavelength has been tested in the range of 405-425 nm. Trials were performed on *S.aureus* and HaCat (human keratinocytes). When 170 J/cm^2 blue light was given, 4,75-log10 bacterial inactivation was observed in CA-MRSA and 0,29-log10 bacterial inactivation was observed in HaCaT. The given light was observed in two ways, for 30 minutes and for 24 hours. Bacterial proliferation was again observed in mouse wounds receiving daylight 24 hours after blue light therapy [5].

A study on bacteria with a light source in the visible region, it was observed that blue light was more effective on bacteria than red light [6].

One study mentioned that ultraviolet rays have lethal effects on bacteria, fungi, viruses, spores and cells. It has been emphasized that UV-rays, other than lethal effects, also mutagenically acted on microbes while proteins, especially nucleic acids, easily absorbed these rays and as a result, created the formation phenomenon of bonds between thymines located side by side in DNA strands. It is stated that this situation causes protein synthesis and other mechanisms in the bacteria to be disrupted by the structure of DNA and deaths [7].

In another study, eleven types of herbal bacteria, bacterial spores and mold spores were observed to be irradiated with different UV radiation doses of a 222 nm crypton-chloride excimer lamp and a 254 nm mercury lamp under laboratory conditions. Especially, it has been determined that microbes with high UV resistance and those with more effective repair mechanisms are more ineffective with 222 nm excimer lamp. The reason for this may be that low UV fluency mainly affects radiation-sensitive microorganisms with DNA damage; however, it has been stated that protein damage mechanisms at high UV fluency (various) can be held responsible for inactivation [8]. Another study investigated the wavelength and methicillin resistance effect of *Staphylococcus aureus* (MRSA) intensity on the bactericidal effect of 405 and 470 nm light. Irradiation at both wavelengths has been reported to reduce bacterial colonies at any intensity [9].

The reason why we chose *Escherichia coli* and *Staphylococcus aureus* microorganisms in our study is that they cause many respiratory, intestinal and abdominal diseases and can be found in many environments [10-15].

The most important species in the genus *Escherichia* is *Escherichia coli*. The width of *E.coli* in the form of bacillus is higher than its length. *E. coli* was first discovered in 1885 by The odor Escherich. Previously, these microorganisms were known as the *Bacterium coli* commune then *Escherichia coli* was named. These bacteria are the most common species that cause disease in humans and cause serious intestinal infections in humans. *E. coli* is an opportunistic pathogen for humans. *E. coli* has become the most studied model organism for understanding bacterial biology in general. *E. coli* is the most common species found in the large intestinal flora. It is also responsible for many bacterial infections. It forms urinary tract infections, intestinal infections and parenteral infections (pneumonia, meningitis, bacteremia) [16-19].

Staphylococcus aureus, one of the subjects of the study, is a bacterium that causes many infections, especially in humans. They are very common in nature because they are resistant to environmental conditions. The source of pathogenic staphylococci that infect humans is humans. These bacteria are mostly found in the nasal and throat cavities, human and animal feces, abscessed wounds and acne on the skin. They are also commonly found in food for hospital personnel and hospital settings, and in food facilities. Nasal staphylococci become dangerous by spreading around with carriers. Especially those who are carriers in the food industry and prepare food with their own hands are important sources of staphylococcal food poisoning [20, 21].

The way to destroy such bacteria is light sources. 254 nm and 365 nm light sources are light sources close to the UV region. When you look at it with the naked eye, especially when you look at 254 nm light, it may pose a health risk. However, the 460 nm visible light source does not pose a health risk. In this case, by applying it to local infection areas in the human body, the reproductive or colony-forming effects of bacteria can be reduced.

This study aims to prevent the growth and proliferation of two different bacterial species (Escherichia coli and Staphylococcus aureus) by weakening their resistance with rays of different wavelengths. Therefore, it is thought that by reducing the risk of infection with these two bacteria that cause respiratory tract infections, people's use of medication will be minimized and the treatment process will be shortened.

2. Material and Method

2.1. Material

In this study, microorganisms of *Staphylococcus aureus and Escherichia coli* were used. Nutrient Broth was used as the liquid medium (growth medium). TSA (Tripton Soy Agar) was used as a solid medium. FTS (physiological saline, 0.9 g NaCl) was used for dilutions. The light was given separately at wavelengths of 254-365-460 nm. Growth and results of microorganisms exposed to the light of different wavelengths were examined. The microorganisms we used in our project and 254-365 nm wavelength light sources were obtained from the Ege University biochemistry department and the 460 nm light source was obtained from the Nuclear Sciences Institute Thermoluminescence Laboratory at the same university. All experiments were done and evaluated in Biochemistry laboratories.

2.2.Method (preliminary trial)

S. aureus and E. coli strains were each inoculated into 25 mL of Nutrient Medium in 100 mL volume bottles and incubated for 24 hours at 37°C with a stirring speed of 150 rpm. After the incubation, 1000 µL of the cells produced was taken for the control group and inoculated into the empty petri dish. Then, TSA was poured on it (pour plate method) and left to freeze, stirring gently for homogeneous distribution. Frozen agar petri dishes were allowed to incubate at room temperature (25°C) for 24 h. For the sample group, 500 µL of cells were inoculated separately from each of the growth media into the tube containing 4.5 mL of FTS (physiological saline).

They formed a vortex and became homogeneous. The resulting cells were considered diluted to 10^{-1}. Then, 500 µL of these cells was taken and inoculated into the tube again with 4.5 mL of *FTS*. Vortex *was* performed. 500 µL of the resulting 10^{-2} diluted cells was taken and re-inoculated into the tube containing 4.5 mL FTS. Vortex was performed. This procedure was repeated until a 10^{-6} dilution was obtained. Then, the cast plate method was applied 3 times for 3 types of wavelengths from cells diluted 10^{-5} and 10^{-6}. As applied to the control group, 1000 µL of diluted cells were inoculated into empty petri dishes, TSA was poured on them, and the petri dishes were shaken gently to ensure homogeneous distribution of the cells in the agar. The planted agars were stored for freezing. The petri dishes were then left closed for 24 hours under light set at the specified wavelengths (Figure 1 and Figure 2).

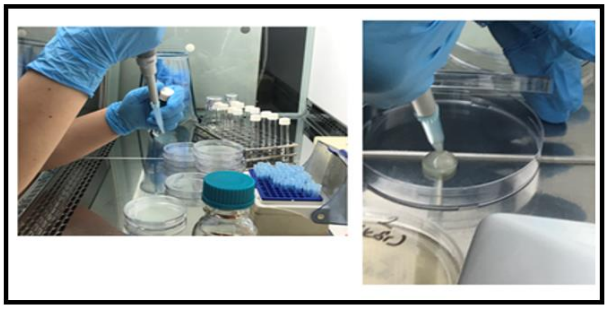

Figure 1. Taking bacteria into petri dishes

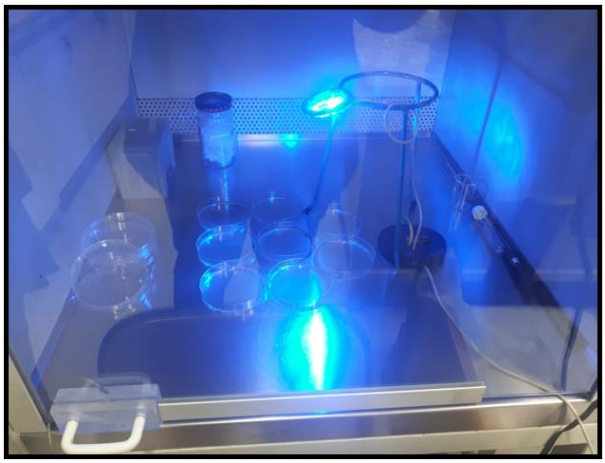

Figure 2. Blue light source with 460 nm wavelength used in experiments (pedri and light distance:20cm)

S. aureus and *E. coli* strains were inoculated in 25 mL Nutrient Broth in 100 mL volumes of flasks and incubated for 24 hours at 37°C 150 rpm mixing speed. After incubation, 500 µL of cells were inoculated into each tube containing 4.5 mL FTS (physiological saline), separately from each of the reproductive environments. By making a vortex, they became homogeneous. The diluted cells obtained continued to be diluted until their optical density (cell density-OD) was 0.5. Cells with an OD of 0.5 were considered the original cell. Then, 500 µL of these cells were taken and again inoculated into the tube with 4.5 mL FTS. Vortex was performed. The cells obtained were considered as 10^{-1}. 500 µL was taken from these cells again and again inoculated into a tube containing 4.5 mL of FTS. Vortex was performed. The cells obtained were considered as 10^{-2}. This procedure was repeated in the same manner until 10^{-6} dilution was achieved. Then, spread plate method was applied 3 times for 3 kinds of wavelengths from 10^{-4}, 10^{-5} and 10^{-6} diluted cells. 100 µL of diluted cells were previously inoculated into frozen petri dishes by pouring TSA and the cells were spread on the surface with a drigalski spatula. Petri dishes with cell transplantation were left open for 24 hours under the light set at the specified wavelengths. Without any dilution (10^{-4}, 10^{-5}, 10^{-6}) for the control group, sowing was done by spread plate method and left for 24 hours incubation at room temperature (25°C).

3. Results

When the microorganisms were exposed UV and blue lights for 24 hours, the number of colonies decreased. However, the number of colonies was never seen in both microorganisms exposed to 460 nm light in the visible region.

3.1. Result (preliminary trial)

Decrease in the number of colonies was observed as also seen in Table 1. Colony numbers were calculated and evaluated from the number of white dots in Petri dishes (Figure3). But the effect of 460 nm wavelength blue light gave very good results in both bacteria. It completely prevented the formation of colonies and was observed to disappear.

Table 1. Colony numbers of microorganisms exposed to different wavelengths of light for 24 hours

	Colony Number		
Microorganism	**254 nm**	**365 nm**	**460 nm**
S.aureus (10^{-5})	13	3	0
S.aureus (10^{-6})	0	0	0
E.coli (10^{-5})	~210	~2560	0
E.coli (10^{-6})	~1840	~432	0

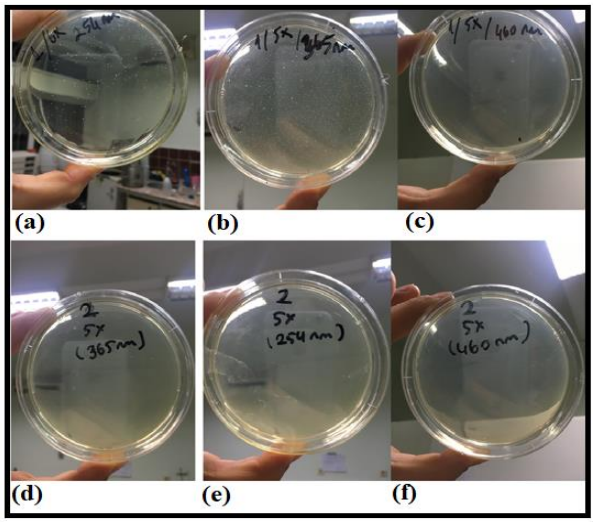

Figure 3. Bacterial colonies (a,b,c: E.coli; d,e,f : S.Aureus) seen under the influence of three different wavelengths

3.2. Results (2nd trial)

Reproduction of E. coli stopped completely at 254 nm (Table 2). *E. coli* generation time is 20 minutes.

Table 2. *E. coli* colonies exposed to three different wavelengths of light for 24 hours

Wavelength (*E.coli*)	**10^{-4}**	**10^{-5}**	**10^{-6}**
254 nm	None	None	None
365 nm	pireferasyon	60	6
460 nm	pireferasyon	117	12
Control	pireferasyon	136	14

If there were 60 growths for 136 control at 365 nm, according to the results, the number of bacteria growing was found to be 44%. The same calculation was done for 460 nm. There was 86% growth, 14% growth was prevented. Calculations were made for *S. aureus* based on the results of the first trial. *S. aureus* generation time is 30 minutes.

Table 3. *S. aureus* colonies exposed to three different wavelengths of light for 24 hours

Wavelength (*S. aureus*)	**10^{-5}**	**10^{-6}**
254	13	None
365	3	None
460	None	None

S. aureus reproduction completely stopped at 460 nm. At 254 nm, there was a 14% growth, 86% growth was prevented. There was 3% growth in 365nm, 97% growth was prevented (Table 3).

4. Conclusion and Discussion

When we look at the first trial results of the study in general, light sources with 3 different wavelengths (254 nm, 365 nm and 460 nm) were used. While a significant decrease in colony numbers was observed under the operating condition of 254 nm and 365 nm wavelength lights falling in the UV region, no colonies were observed in the 460 nm wavelength light (Table 1 and Figure 3). In the second trial results, 44% growth was achieved at 365 nm and 56% growth was inhibited. S. aureus growth stopped completely at 460 nm. At 254 nm, 14% growth was achieved, 86% growth was inhibited. At 365 nm there was 3% growth, 97% growth was inhibited.

As seen from the results, no significant bacterial growth was observed at 460 nm. In our literature studies, it can be seen that no study has been done at this wavelength before. In other studies, some results were obtained with visible light sources, but a decrease in colony reproduction was observed without destruction. A study stated that the effect of blue light is better than other lights [5].

Another study observed that wavelength of Staphylococcus aureus (MRSA) intensity and methicillin resistance irradiation at 405 and 470 nm reduced bacterial colonies at any intensity [9].

In one study, it was stated that a pressurized mercury vapor lamp was used in the treatment of the disease by destroying the DNA component of bacteria and viruses at a wavelength of 254 nm [22].

In our study, the effect of 460 nm light source on these bacteria is relatively high. In addition, the light source in the visible region does not cause much damage to normal human cells. In some cases, high-intensity visible light kills bacteria, while visible and near-infrared light increases bacterial growth.

As a result, it is important to evaluate the use of the 460 nm light source in visible areas where this type of bacteria is found, both in sterilization and in the treatment of local infections, and also in the examination and treatment of the new type of coronavirus. and the precautions to be taken.

4.1. Suggestions

For the purpose of our study, the effects of visible wavelength light on bacteria, especially E. coli and S. aureus, were examined and compared with other light sources close to UV regions. The 460 nm wavelength light source has been shown to be more effective on two types of bacteria. These two types of bacteria are extremely risky bacteria that threaten human health. Antibiotic treatment against them also negatively affects other beneficial bacteria in the body. It especially causes local throat infection. Therefore, it increases the possibility of using blue light with a wavelength of 460 nm against S. aureus. In the first stage of the treatment, thanks to the blue light source (460 nm) device, the reproduction of this bacterium, which first settles in the throat and mixes with the blood and causes infection, can be stopped. If the initial infection that occurs at the beginning of the human upper respiratory tract is eliminated, the treatment process will be shorter and the patient will recover more quickly. Additionally, such bacteria can cause skin infection. Especially the 460 nm wavelength light source we apply to these places can help us minimize infection. Infections in the body can then be eliminated by using low doses of antibiotics. In addition, the environments in the hospital environment can be purified from these bacteria and viruses by creating a sterile environment by using lights at these wavelengths. It is important to clean environments where the risk of infection may occur and to prevent the spread. Evaluating and applying such alternative methods in the fight against viruses in future studies will benefit new treatment methods.

Article Information Form

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The Declaration of Ethics Committee Approval
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The authors of the paper declare that they comply with the scientific, ethical and quotation rules of SAUJS in all processes of the paper and that they do not make any falsification on the data collected. In addition, they declare that Sakarya University Journal of Science and its editorial board have no responsibility for any ethical violations that may be encountered, and that this study has not been evaluated in any academic publication environment other than Sakarya University Journal of Science.

References

[1] S. Singha, R. Thomas,J. N. Viswakarma, V. K. Gupta, “Foodborne illnesses of *Escherichia coli* O157origin and its control

measures" Journal of Food Science and Technology, Vol. 60, no. 4), pp. 1274-1283, 2023.

[2] Y. Wang, K. Fu, "Genotoxins: The Mechanistic Links between Escherichia coli and Colorectal Cancer" Cancers, vol. 15(, no. 4, p. 1152, 2023.

[3] C. dos Anjos, L. G. Leanse, M. S. Ribeiro, F. P. Sellera, M. Dropa, V. E. Arana-Chavez, N. Lincopan, M. S. Baptista, F. C. Pogliani, T. Dai, C. P. Sabino, "New Insights into the Bacterial Targets of Antimicrobial Blue Light" Microbiology Spectrum, Vol.11, no. 2, 2023.

[4] N. Özkütük, Use of Ultraviolet Lamps, 5th National Sterilization Disinfection Congress, Antalya, Turkey, 2007.

[5] T. Dai, A. Grupta, Y. Huang, M.E. Sherwood, C.K. Murray, T. Kiellan, and M. R. Hamblin, ''Blue Light Eliminates Community-Acquired Methicillin-Resistant *Staphylococcus aureus* in Infected Mouse Skin Abrasions'', Photomedicine and Laser Surgery, Vol.31, p.11, 2013.

[6] R. Lubart, A. Lipovski, Y. Nitzan, And H. A. Friedmann, ''Possible Mechanism for The Bactericidal Effect of Visible Light'', Laser Therapy, vol. 20, no. 1, pp. 17-22, 2011.

[7] M. Arda, Fundamentals of Microbiology, Second Expanded Edition. Medisan Publication Series No 46, Ankara, 2000.

[8] M. Clauß, ''Higher effectiveness of photoinactivation of bacterial spores, UV resistant vegetative bacteria and mold spores with 222 nm compared to 254 nm wavelength'' Acta Hydrochimica et Hydrobiologica, vol. 34, pp. 525-532, 2006.

[9] V.V. Bumah, D.S. Masson-Meyers, S.E. Cashin, C.S. Enwemeka, ''Wavelength and bacterial density influence the bactericidal effect of blue light on methicillin-resistant *Staphylococcus aureus* (MRSA)'', Photomedicine and Laser Surgery, vol. 31, no. 11, pp. 547-553, 2013.

[10] YQ. Li, Q. Han, JL. Feng, WL. Tian, HZ. Mo,"Antibacterial characteristics and mechanisms of 3-poly-lysine against *Escherichia coli* and *Staphylococcus aureus*", Food Control, vol. 43, pp. 22-27, 2014.

[11] Y. Zhang, X. Liu, Y. Wang, P. Jiang, SY. Quek, "Antibacterial activity and mechanism of cinnamon essential oil against *Escherichia coli* and *Staphylococcus aureus*", Food Control, vol. 59, pp. 282-289, 2016.

[12] D.A. Phoenix, Z. Sayed, S. Hussain, F. Harris, M. Wainwright, "The phototoxicity of phenothiazinium derivatives against *Escherichia coli* and *Staphylococcus aureus*", FEMS Immunology and Medical Microbiology, vol. 39, pp. 17-22, 2003.

[13] C.L. Fischer, K.S. Walters, D.R. Drake, D.R. Blanchette, D.V. Dawson, K.A. Brogden, P.W. Wertz, "Sphingoid Bases Are Taken Up by *Escherichia coli* and *Staphylococcus aureus* and Induce Ultrastructural Damage", Skin Pharmacol Physiol, vol. 26, pp. 36–44, 2013.

[14] K.J. Williams, L. J. Piddock, "Accumulation of rifampicin by *Escherichia coli* and *Staphylococcus aureus*", Journal of Antimicrobial Chemotherapy, vol. 42, pp. 597-603, 1998.

[15] J. C. Lopez-Romero, H. González-Ríos, A. Borges, M. Simões, "Antibacterial Effects and Mode of Action of Selected Essential Oils Components against *Escherichia coli* and *Staphylococcus aureus*", Hindawi Publishing Corporation Evidence-Based Complementary and Alternative Medicine, vol. 2015, pp. 9, 2015.

[16] N. Turgut, "Investigation of the Presence of *E.Coli* O157-H7 in Lettuce Served for

Consumption in Aydın Province, Adnan Menderes University Health Sciences Institute, Department of Microbiology, Doctoral Thesis, 2015.

[17] D. Douglas Bannerman, Max J. Paape, Jai-Wei Lee, Xin Zhao, Jayne C. Hope, Pascal Rainard, "*Escherichia coli* and *Staphylococcus aureus* Elicit Differential Innate Immune Responses following Intramammary Infection", Clinical and Diagnostic Laboratory Immunology, vol. 11, no. 3, pp. 463-472, 2004

[18] F. Blanche, B. Cameron, F. X. Bernard, L. Maton, B. Manse, L. Ferrero, N. Ratet, C. Lecqo, A. Goniot, D. Bisch, J. Crouzet, "Differential Behaviors of *Staphylococcus aureus* and *Escherichia coli* Type II DNA Topoisomerases", Antimicrobial Agents and Chemotherapy, vol. 40, no. 12, 2714-2720 pp. 2714–2720, 1996.

[19] Q. L. Feng, J. Wu, G. Q. Chen, F. Z. Cui, T. N. Kim, J. O. Kim, "A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*", Journal of biomedical materials research, vol. 52, no. 4, pp. 662-668. 2000.

[20] U. R. Pothakamury, A. Monsalve-GonzBlez, G. V. Barbosa-Cbovas and B. G. Swansod, Inactivation of *Escherichia coli* and *Staphylococcus aureus* in model foods by pulsed electric field technology, Food Research International, vol. 28, no. 2, pp. 167-171, 1995.

[21] A. Gulbandılar, 'Investigation of *Staphylococcus Aureus* Carriage and Antibiotic Susceptibility in Nasal Mucosa in Kütahya Region', Dumlupınar University Journal of Science, no.18, pp. 1-6, 2009.

[22] P. Boyce, Controlling Tuberculosis Transmission with Ultraviolet Irradiation, Lighting Research Center Publications, 2007.