KJVR
Korean Journal of Veterinary Research

Original Article

Microbiology

pISSN 2466-1384 · eISSN 2466-1392
Korean J Vet Res 2024;64(3):e19
https://doi.org/10.14405/kjvr.20240032

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Conflict of interest:
The authors declare no conflict of interest.

Received: May 13, 2024
Revised: Jul 1, 2024
Accepted: Jul 2, 2024

Harnessing blue light for cost-effective and eco-friendly antimicrobial solutions in poultry farming

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Abstract

Blue light in the visible spectrum naturally fights bacteria and effectively deactivates various Gram-positive and Gram-negative bacteria and fungi using photodynamic processes. It works against drug-resistant strains within these species and is gentler on mammalian cells than ultraviolet rays or chemicals. This study evaluated blue light as an affordable way to sanitize surfaces in poultry settings, such as eggs and litter. The study used an light-emitting diode array to remove harmful *Escherichia coli* and *Staphylococcus aureus* from these items and observed how blue light affected their survival rates. Blue light (455 nm) at 30 mW/cm^2 for one hour decreased the survival of *S. aureus* and *E. coli* on solid agar plates to 29.88% and 21.04%, respectively, compared to non-irradiated cultures. Similarly, on untreated surfaces (such as plastic used for feeding and drinking), the survival rates dropped to 25.8% and 15.6%, respectively. The survival percentages on treated eggs were 50% (*S. aureus*) and 36.47% (*E. coli*) and 49% (*S. aureus*) and 48.2% (*E. coli*) on treated litter. Hence, blue light technology offers a promising alternative to traditional antimicrobial methods by leveraging specific wavelengths to target microbial cells. This approach can significantly reduce the microbial load in poultry environments, enhancing food safety and animal health. This paper reports the first use of blue light as an antibacterial within poultry research in Iraq, offering a fresh approach to disinfection in this field.

Keywords: blue light; antimicrobial stewardship; poultry; cost savings; anti-bacterial agents

Introduction

The poultry industry is one of the fastest growing and most important income sources for many countries. According to estimates, more than nine billion chickens were slaughtered in the United States in 2019 [1]. Global poultry meat production reached 133.3 million tons in 2020 [2]. This means there were 27.8 billion poultry birds as opposed to the 7.7 billion human population [3]. This overproduction of poultry products has adverse effects on humans and the environment. Individuals or corporations of varying capacities manage commercial poultry production [4]. Therefore, the types of farms, practices, and biological safety procedures vary from one producer to another. This causes variations in the quality

control of poultry products and could impose risks to public health when hygiene and biosafety measures are not controlled [5].

Throughout history, birds have been associated with pandemics, such as the avian influenza pandemic, serving as a significant reservoir for zoonotic pathogens [6,7]. Of these, the pathogenic bacterial and viral contamination of poultry products poses significant public health risks [8,9]. In addition, litter-based poultry projects require periodic decontamination of the litter and the equipment, which becomes an obligatory procedure in case of disease outbreaks. Current decontamination procedures include physical and chemical methods. Nevertheless, these procedures are environmentally challenging and expensive. Therefore, a low-cost sanitization method is needed that is easy to install at any poultry farm, has a minimal maintenance requirement, is environmentally safe, and would be of significant value to the production quality and consumers' health [10].

Researchers have described blue light, particularly wavelengths between 400 and 470 nm, as an intrinsically effective, low-cost antimicrobial capable of killing microorganisms and reducing biofilm [11,12]. A previous study [13] reported that a 405-nanometer light-emitting diode (LED) array could kill both Gram-positive bacteria (*Staphylococcus aureus*–methicillin-resistant *S. aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, and *Clostridium perfringens*) and Gram-negative bacteria (*Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris*, and *Klebsiella pneumoniae*). Blue light inactivation also affects airborne bacteria [14]. Furthermore, light in the blue spectrum effectively inactivates viruses, including coronaviruses [15,16] and several fungal species [17]. Despite having lower germicidal capacity than ultraviolet (UV) light, blue light benefits from its safety on mammalian cells, allowing its widespread use in various applications for extended exposure times without harming human cells [18]. In addition, blue light is equally effective against drug-sensitive and drug-resistant microorganisms [19,20].

The mode of action of blue light has been reported via the photoexcitation of intracellular porphyrins, which cause energy transfer and the generation of cytotoxic reactive oxygen species (ROS), predominantly singlet oxygen ($^1O_2$) [13,21,22]. A series of photophysical and photochemical processes trigger the effects of photodynamic inactivation, following the excitation of the photosensitizer by light. These steps produce ROS through 2 separate pathways: types I and II mechanisms of action, which break down biomolecules [23]. Both processes produce ROS. These ROS interact immediately with the biological parts of the cell wall, such as lipids, proteins, amino acid residues, nucleic acid bases, and pigments in some cells [24]. Overall, the antimicrobial potentials of blue light are diverse and can find applications in various biological and industrial settings, including the poultry industry. The practical effectiveness of the antimicrobial properties of blue light was assessed by conducting tests on the egg surfaces, litter, industry-related surfaces, and equipment.

This study chose a low-cost LED light source that can be easily purchased from markets and installed by anyone without prior training.

Materials and Methods

Ethical statement

The current study was conducted in accordance with the relevant guidelines and regulations and received approval from the College of Veterinary Medicine, University of Basrah (No. 34/2022).

Materials and equipment

The media and nutrient agar were obtained from Oxoid Co., USA. The strains used in this study included *S. aureus*, a community-associated *S. aureus* USA300_LAC strain, and antibiotic-resistant *E. coli* that was locally isolated from a poultry farm and previously verified using polymerase chain reaction, 16S ribosomal sequencing, and biochemical tests. Eggs of comparable sizes and shapes were purchased from a local market, while litter and poultry equipment were acquired from a poultry farm at College of Veterinary Medicine, University of Basrah. All materials used in each experiment were sterilized using an autoclave or UV light, except eggs, which were disinfected using 70% ethanol. All experiments were conducted under sterile conditions inside a UV-light-supported biological hood. The incubation conditions and colony-forming unit (CFU) counting were done by plating inocula on nutrient agar at 37°C for 24 hours.

Light source and irradiation

The white and blue light sources were low-cost commercial LED bulbs purchased from a local market (ROZHled Co. KRG, Iraq). Based on the manufacturer's description, the blue light LED was an indium–gallium–nitride LED array with a brightness of 900 lumens. The accuracy was confirmed by measuring the wavelength, wattage, and voltage using a spectrometer (SpectroVio; Lab Junior, Korea), a wattmeter, and a voltmeter. The irradiance was adjusted to the desired level and focused on the surfaces at the approximate level before beginning the experiments. Consequently, the irradiance of light on the target

https://doi.org/10.14405/kjvr.20240032

surface was adjusted by changing the distance between the LED array aperture and the target (cell culture or object surface). The light sensor measured the irradiance (mW/cm^2) for the fluence calculations (J/cm^2), and this value was multiplied by the irradiation time. (Thorlabs Inc., USA). For fluence calculations (J/cm^2), a light sensor was used to measure the irradiance (mW/cm^2) multiplied by the irradiation time. Furthermore, using the Lux meter (Testo 450, Germany), illuminances were measured prior to each experiment (data not shown).

Blue light treatment

Light dose and time are important factors that determine the efficiency of antimicrobial activation [13]. Therefore, an initial titration determined the best light irradiance and exposure time (Data not shown). For ease, in all the experiments, a 30 cm distance was maintained between the LED array light source and the targeted objects (eggs, surfaces, litter, and petri dishes), corresponding to 30 ± 2 mW/cm^2 irradiance.

Plates experiment

Overnight cultures of *E. coli* and *S. aureus* were grown in nutrient broth for 18 hours. Suspensions of 1 × 10^6 CFU/mL were prepared by diluting the overnight cultures in normal saline, and 50 µL from each suspension were pipetted and spread on 90 mm diameter nutrient agar plates using an L-shape glass spreader. After the blue light treatment, the plates were incubated at 37°C for 24 hours. The CFU was determined by counting the colonies on the tested plates. The results were compared with the untreated ones [11]. The temperature of the plates before, during, and after light exposure was monitored using an infrared thermometer.

Surfaces experiment

A wet cotton swap was used to spread bacterial suspensions (1 × 10^6 CFU/mL) across identical-sized confined areas (4 × 4 cm) of the bench areas and plastic materials. These contaminated spots were allowed to dry in the dark for 10 to 15 minutes before subjecting them to the blue light for 60 minutes (excluding the no-light treatment controls). After exposure, the bacteria were collected from each spot using wet cotton swaps and re-suspended in 10 mL of tubes containing normal saline. After vigorous shaking, 50 µL from each sample was plated on nutrient agar for CFU determination [25].

Eggs experiment

The eggs were prepared for the blue light exposure experiment by conducting contamination and decontamination processes using a slight modification of a methodology reported elsewhere [26]. Each egg was disinfected by immersion in containers with 70% ethanol. The disinfected eggs were transferred to UV-sterilized plastic egg trays using sterile large thump forceps, which were then closed with lids immediately to dry and prevent contamination. Sterile cups with caps (250 mL capacity) were filled with 40 mL of normal saline bacterial suspensions of 1 × 10^6 CFU/mL and closed tightly. A group of cups contained only 40 mL of sterile normal saline and were used to test the quality of the alcohol disinfection of the eggs. Sterility was ensured by conducting all procedures in a UV-equipped biological safety cabinet sterilized using 70% ethanol and UV irradiation. Each disinfected egg was contaminated separately by immersing it in a plastic cup that contained the bacterial suspensions or sterile normal saline. After shaking gently for 5 minutes, the eggs were transferred and placed with eggs in UV-sterilized plastic egg trays and covered immediately to dry and prevent contamination.

After opening the tray lids, the contaminated eggs were exposed to blue light inside a biosafety cabinet for one hour. The non-contaminated trays of eggs were left open inside the biosafety cabinet without light treatment in a dark, separated section. After the LED light treatment, the blue light-treated eggs were immersed individually in sterile cups containing 40 mL of sterile normal saline and shaken for 5 minutes. The killing efficiency was measured by plating 50 µL of these suspensions on nutrient agar plates and incubating them for 24 hours for CFU counting. These results were compared with the no-treatment control. Furthermore, 50 µL of the control samples of sterile normal saline (from eggs dipped in the sterile normal saline-containing cups) were plated on nutrient agar to verify the disinfection quality of the eggs [25].

Litter contamination

The litter was weighted and autoclaved in a thermal-resistant bag. Subsequently, 5 mL of *E. coli* bacteria suspension (1 × 10^{10}) was added frequently and mixed vigorously. Using sterile thump forceps, 10 g of the contaminated litter was distributed equally to sterile plastic petri dishes. Except for the control plates, the other plates were treated with blue light for one hour. Two grams from each petri dish were suspended in tubes containing 10 mL of sterile saline. The tubes were left to stand for 10 minutes and shaken vigorously before plating 50 µL of each suspension on nutrient agar [27].

Statistical analysis

Each data point in the figure represents the mean ± standard

deviation of 3 independent biological experiments, with at least 3 replicates collected for each data point. The data were analyzed using Student t-tests in IBM SPSS ver. 25.0 (IBM Corp., USA); *p*-values less than 0.05 versus the untreated control were considered significant.

Results

Light wavelength

The optical emission spectrum showed that the commercial blue LED light bulb had a single band 455 nm wavelength (Fig. 1).

Exposure to 455 nm blue light is toxic to *S. aureus* and *E. coli* grown on Petri dish plates

This study tested the hypothesis that exposure of solid cultures of *S. aureus* and *E. coli* to blue light for 60 minutes reduces their viability. Hence, solid cultures of *S. aureus* and *E. coli* were exposed to 60-minute cycles of 30 ± 2 mW/cm^2 of 455 nm blue light. A significant reduction of *S. aureus* and *E. coli* viability was observed. The survival rates of *S. aureus* and *E. coli* on solid agar plates exposed to 455 nm light decreased to 29.88% and 21.04%, respectively, compared to the untreated cultures ($p < 0.005$) (Fig. 2A and B). The antibacterial effect of the white light LED was compared with the blue light LED to contrast with other light color forms. The survival rates of *S. aureus* and *E. coli* on solid agar plates exposed to white LED light decreased to 76.4% and 74.3%, respectively, compared to the untreated cultures ($p < 0.005$) (Fig. 2C).

The surface temperature of the agar petri dish plates was pressured using an infrared thermometer to exclude the effect of temperature generated by the light source. Throughout the experiment, the temperature of the surface of the light-treated plates and other light-treated objects increased by less than 1°C (data not shown).

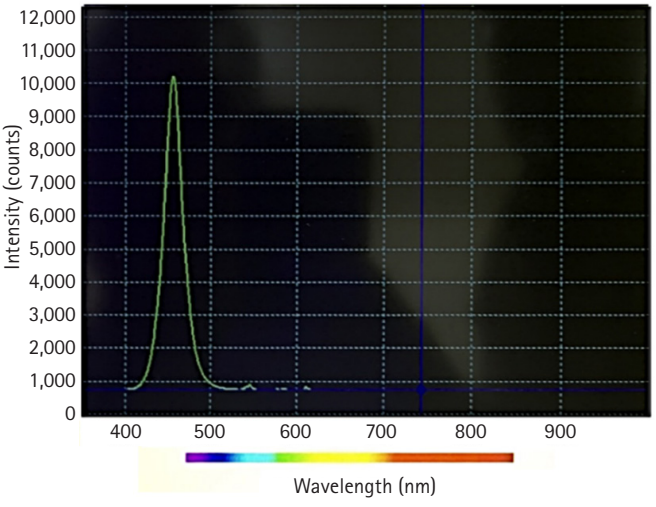

Fig. 1. Optical emission spectrum (SpectroVio spectrometer, Lab Junior) of the 455 nm light-emitting diode array.

Exposure to 455 nm blue light is toxic to *S. aureus* and *E. coli* on surfaces.

The blue light-treated surfaces (plastics used in feeders and waterers) had 25.8% and 15.6% fewer *S. aureus* and *E. coli* bacteria than on the untreated surfaces ($p < 0.05$) (Fig. 3).

Exposure to 455nm blue light is toxic to *S. aureus* and *E. coli* on Eggs and Litter.

S. aureus and *E. coli* were 50% and 36.47%, respectively, more likely to survive on blue light-treated eggs than on cultures not treated ($p < 0.005$) (Fig. 4A and B). The consistency, edibility, or color of the internal contents of the eggs were unaffected by the blue light. Furthermore, the sterilization controls did not show any colonies on the plates, indicating the efficacy of the egg disinfection process. The survival percentages of *S. aureus* and *E. coli* on the blue light-treated litter were 49% and 48.2%, respectively, lower than on the untreated litter (Fig. 4C).

Discussion

The poultry industry must always keep infections under control to prevent economic losses and, more importantly, prevent the spread of public health maladies. Antibacterial blue-light therapy is a promising technique that could combat pathogens and recurrent infections. Greater recognition of poultry production as a source of pathogens has led to demands for increased investment in more effective conventional sanitation, as well as the promotion of the development of an array of novel cleaning and decontamination technologies. This study used a low-cost commercial blue light to disinfect eggs, litter, and other plastic subjects. Endogenous photosensitizing chromophores in pathogenic microorganisms could be responsible for the antimicrobial efficacy of blue light within the 400 to 470 nm wavelength range. Several factors may influence the efficacy of blue light as an antimicrobial, including time of exposure, intensity, mode of exposure, and the presence of synergistic chemicals [20,28].

The optical emission spectrum of the commercial LED array used in this study confirmed a single band wavelength of 455 nm. The treatment effectively reduced the bacterial load of pathogenic bacterial isolates *S. aureus* and *E. coli* (Fig. 2). In ad-

https://doi.org/10.14405/kjvr.20240032

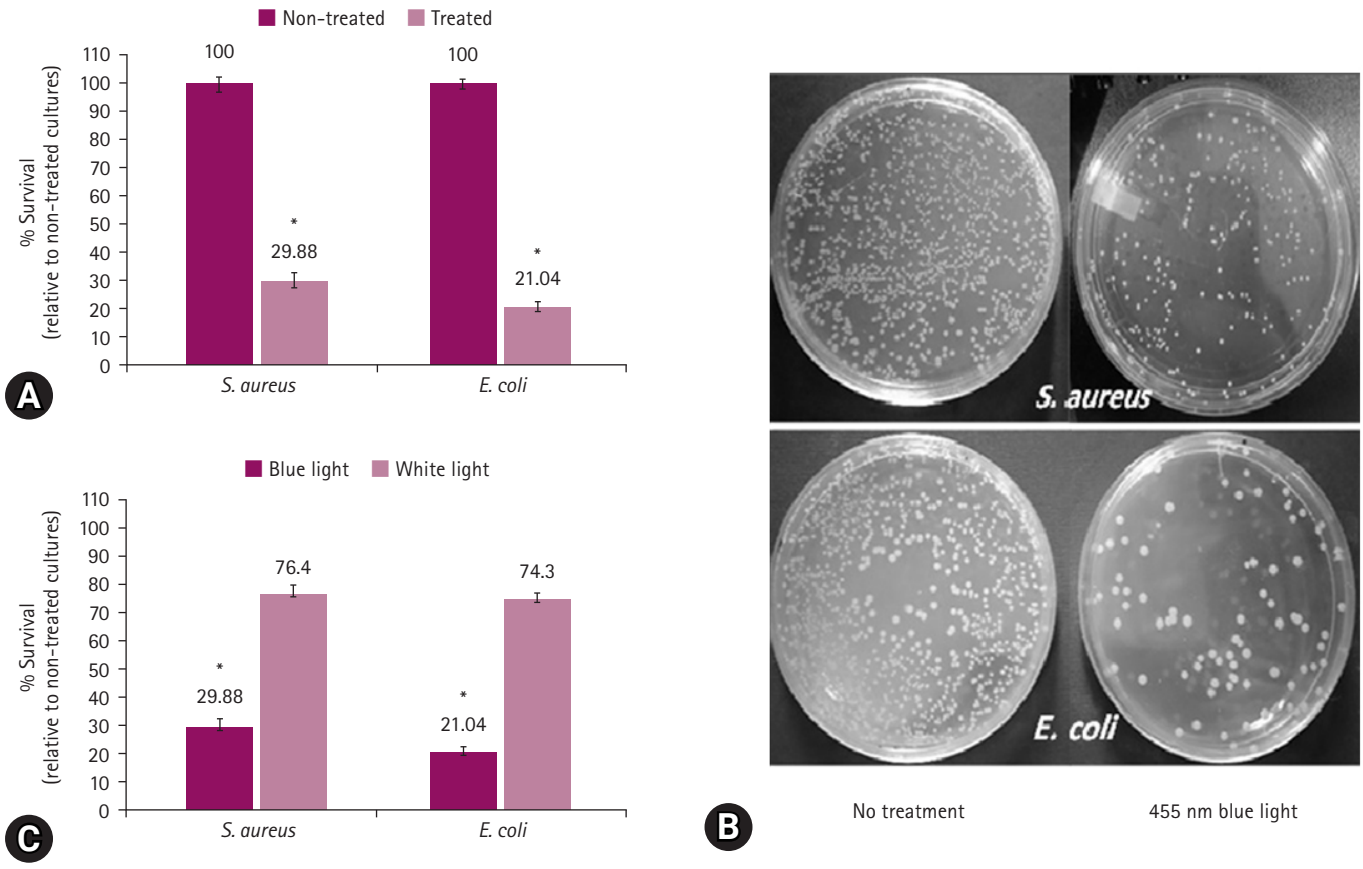

Fig. 2. *Staphylococcus aureus* and *Escherichia coli* viability on solid agar petri dish plates subjected to 455 nm blue light (A, B) or white light (C) for 60 minutes. *Significant differences between the groups at $p < 0.05$.

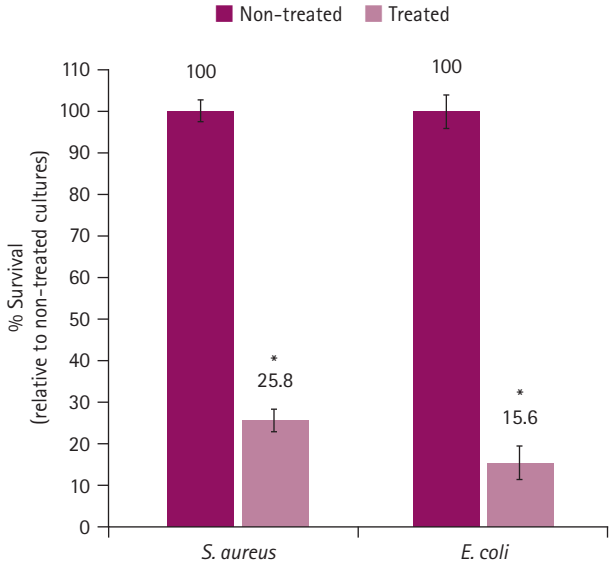

Fig. 3. *Staphylococcus aureus* and *Escherichia coli* viability on surfaces subjected to 455 nm blue light for 60 minutes. *Significant difference between the groups at $p < 0.05$.

dition, the white light also slightly reduced the viability of cells on plates (Fig. 2C), which is consistent with other studies that showed that visible light (500–800 nm) has an antimicrobial effect [29].

The effect of 455 nm blue light on the surface of petri dishes was significant for *S. aureus* and *E. coli*. Comparably, the 455 nm wavelength was effective in other studies [30]. ROS is believed to be the mechanism for the disinfecting effect of blue light. *Staphylococci* exposed to visible light may be photo-dynamically inactivated through the photoexcitation of intracellular porphyrins [13]. In all the experiments, *S. aureus* was less susceptible to the blue light treatment than *E. coli*. Variations in the *E. coli* and *S. aureus* background responses to blue light have been documented [19,31].

The shape of the exposed area limited the treatment ability of blue light to reduce bacterial viability in all the experiments in this study. The curvy shape of eggs limits the use of blue light for egg decontamination on curved surfaces. This was obvious by comparing Figs. 3, 4A, where *S. aureus* and *E. coli* showed

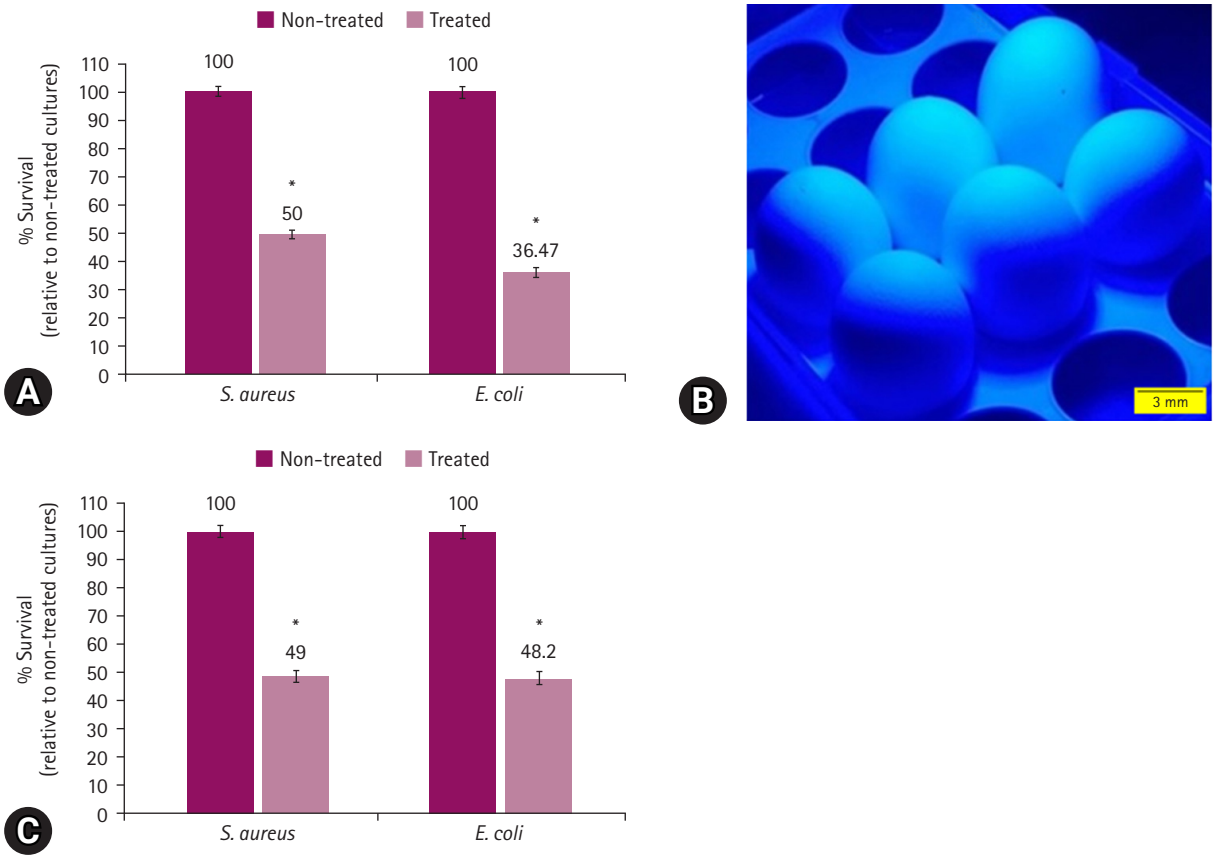

Fig. 4. *Staphylococcus aureus* and *Escherichia coli* viability on eggs (A, B) and litter (C) subjected to 455 nm blue light for 60 minutes. Scale bar = 3 mm. *Significant difference between the groups at $p < 0.05$.

higher egg survival rates than on flat surfaces. Therefore, manually or mechanically turning eggs could increase the efficacy of decontamination by increasing the surface area exposed to light [25]. This turning method could be similar to that used in egg incubators, where eggs are turned automatically.

In conclusion, integrating blue light into incubator systems shows promise for reducing infection-related losses in poultry farming. Although the treatment decreased survival rates on litter, it was not as drastic as on flat surfaces. Focusing on the exposed litter areas through regular turning might mitigate this effect. This study highlighted the effectiveness of 455 nm light against bacterial pathogens, offering safety benefits for human exposure and overcoming antibiotic resistance. Its simplicity and wavelength properties make implementing these findings broadly across poultry production settings feasible, enhancing environmental sanitation without demanding intricate technical expertise.

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https://doi.org/10.14405/kjvr.20240032

Funding

The grant was received in 2024. All expenses were covered by the authors. The the experiments were conducted at University of Basrah Laboratories, Iraq.

Acknowledgments

The staff of the University of Basra's College of Veterinary Medicine and Physics department at the College of Science are thanked by the authors for providing the necessary assistance and resources for the study.

Data Availability Statements

Data can be made available upon reasonable request to the corresponding author.

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