DOI: [10.18502/jfrh.v19i3.20054](https://doi.org/10.18502/jfrh.v19i3.20054)

Original Article

Evaluating the Efficacy of Plasma-Activated Water in the Elimination of Pathogenic Bacteria: An Experimental Study on Pseudomonas, Salmonella, Escherichia Coli, and Staphylococcus Aureus

Abolfazl Mazandarani; Ph.D.[1], Mojtaba Jabbari; B.A.[2], Shervin Goudarzi; Ph.D.[1], Mohammad Javad Khodashenas; M.Sc.[3]

1 Nuclear Science and Technology Research Institute, Plasma and Nuclear Fusion Research School, Atomic Energy Organization of Iran, Tehran, Iran
2 National Nuclear Industry Educational and Cultural Institute, Bandar Abbas, Iran
3 Department of Nuclear Engineering, Faculty of Physics and Energy Engineering, Amirkabir University of Technology, Tehran, Iran

Received August 2024; Revised and accepted September 2025

Abstract

Objective: This study aimed to evaluate the effectiveness of PAW in inhibiting and eliminating four major pathogenic bacterial species: Pseudomonas, Salmonella, Escherichia coli, and Staphylococcus aureus.
Materials and methods: Plasma-activated water (PAW) was generated using a dielectric barrier discharge (DBD) cold plasma device (10 kV, 20 kHz, 4.5 L/min airflow). Two-pipette electrodes generated plasma columns with reactive species. Water hardness, pH, and ozone were measured in triplicate. Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Salmonella spp. were cultured, suspended to 0.5 McFarland, diluted serially, and cultured using the pour plate method. Plasma-generating electrodes were immersed in bacterial suspensions and treated for 2.5, 5, 10, and 15 minutes. Samples were cultured in triplicate using the pour plate method and colony counts were analyzed using t-tests and ANOVA.
Results: Plasma-activated water (PAW) significantly altered pH and hardness and exhibited high bactericidal activity. Hardness increased dramatically post-plasma, while pH decreased. Ozone levels increased with plasma exposure. Duncan's test ($p < 0.05$) confirmed significant bacterial reduction. PAW completely eliminated some strains within 2.5-5 minutes. PAW eliminated Pseudomonas aeruginosa at all time points. S. aureus was reduced to 78 ± 9 CFU/mL at 2.5 minutes and eliminated thereafter. E. coli was eliminated at 5-15 minutes, with 53 ± 7 CFU/mL remaining at 2.5 minutes. Salmonella spp. was reduced to 66 ± 8 CFU/mL at 2.5 minutes and eliminated thereafter.
Conclusion: Increased ozone concentration along with ROS and RNS enhances disinfection, inactivating Pseudomonas aeruginosa, Salmonella spp., Escherichia coli, and Staphylococcus aureus within 5 minutes. Reactive species disrupt bacterial cell walls and membranes, providing antimicrobial effects. Plasma-activated water offers a portable, user-friendly, and eco-friendly alternative to chemical disinfectants for microbial decontamination in food, medical, sanitation, and hospital settings, while conserving water.

Keywords: Plasma-Activated Water; Pseudomonas; Salmonella; Escherichia Coli; Staphylococcus Aureus; Disinfection

Introduction

Plasma, often referred to as the fourth state of matter, is an ionized, quasi-neutral gas composed of free electrons, ions, neutral particles, reactive radicals, and electromagnetic radiation. These unique features

Correspondence:
Dr. Abolfazl Mazandarani
Email: amazandarani@aeoi.org.ir

differentiate it from the other three states of matter (solid, liquid, and gas) (1-3).

Atmospheric cold plasma (ACP) is a type of low-temperature plasma generated at atmospheric pressure. Due to the presence of reactive species and its non-thermal nature, it has found wide applications in medicine and biotechnology without causing thermal damage to biological tissues. Recent studies suggest ACP is effective in surface sterilization, wound healing acceleration, treatment of skin infections, and even combating cancer cells, highlighting its potential in managing inflammatory and infectious skin diseases (4).

Various methods exist for generating cold plasma, with dielectric barrier discharge (DBD) being one of the most common. This technique involves applying high-frequency alternating voltage across two conductive electrodes separated by a dielectric barrier, creating microdischarges that produce diverse reactive chemical species. These species play a significant role in enhancing chemical processes and controlling reactions (5, 6).

Plasma-activated water (PAW) refers to water that has been exposed to a plasma field, leading to modifications in its chemical properties. This process generates reactive oxygen and nitrogen species (RONS), electrons, ions, and ultraviolet radiation (7). These components contribute to PAW's disinfectant properties by lowering pH, increasing oxidation-reduction potential (ORP), and disrupting microbial cell structures. PAW has demonstrated strong antimicrobial activity against pathogens such as *E. coli* and *S. aureus*. Owing to its high biocompatibility and safety, PAW is increasingly applied in the food industry, agriculture, and medical fields (8-10).

The bacterial species investigated in this study are highly pathogenic and represent serious public health threats. *Escherichia coli* is one of the most significant human pathogens transmitted via contaminated water, food, or contact with animals. While it naturally resides in the intestines of humans and animals, pathogenic strains can cause severe diarrhea, urinary tract infections, and, in extreme cases, kidney failure, especially in children and the elderly (11, 12). *Staphylococcus aureus* is a major cause of hospital-acquired infections and antibiotic resistance. Commonly found on the skin, in the nose, throat, and gastrointestinal tract, it is responsible for conditions ranging from skin infections like impetigo and boils to more serious diseases such as pneumonia (13, 14). *Pseudomonas* species are frequently found in hospital environments, soil, and water, and can cause severe infections in immunocompromised individuals (15). *Salmonella* is a leading cause of foodborne illnesses and gastrointestinal infections, often transmitted through the ingestion of food or water contaminated with animal or human feces. Infections may result in diarrhea, fever, and abdominal pain, with increased severity in children, the elderly, and immunocompromised individuals (16, 17).

In this study, the effects of DBD-generated plasma-activated water were evaluated against four common pathogenic bacteria: *Pseudomonas*, *Salmonella*, *E. coli*, and *S. aureus*. These bacteria are among the primary causative agents of gastrointestinal and systemic infections in humans, commonly transmitted through ingestion of contaminated food, particularly raw vegetables. Each bacterium was initially cultured on an appropriate growth medium and subsequently treated with PAW. The effectiveness of PAW in microbial inactivation was systematically assessed.

Materials and methods

In this study, plasma-activated water (PAW) was generated using a dielectric barrier discharge (DBD) cold plasma device powered by an alternating high-voltage source (10 kV, 20 kHz), coupled with an aquarium air pump operating at a flow rate of 4.5 L/min. The system comprised two pipettes, each containing an electrode connected to the high-voltage power supply via conductive wires. Each pipette was independently connected to an air inlet to allow for consistent gas flow. Upon activation of the power supply, plasma columns were formed inside the pipettes, initiating microdischarges that led to the formation of reactive species and free radicals in the water. Figure 1 illustrates a schematic representation of the plasma generation system and the formation of plasma columns within the pipettes.

To monitor the physicochemical changes in water following plasma treatment, total water hardness, pH, and ozone concentration were measured using the TDS-3 SMART handheld hardness meter, NP-345 digital pH meter, and Vaheb ozone test kit, respectively. Each measurement was conducted in triplicate to reduce variability and enhance the reliability of results, with the mean values reported.

Following water parameter assessments, the standard strains of *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and clinical isolates of *Salmonella* spp. were cultured on nutrient agar and incubated at 37°C for 24 hours.

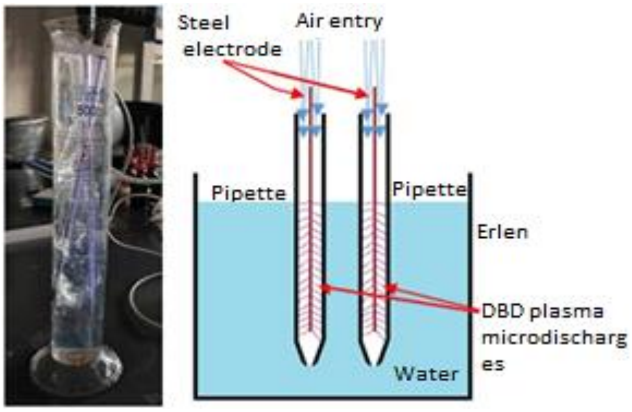

Figure 1: Schematic diagram of the experimental setup (right): plasma column formation within pipettes connected to the air pump (left)

Post-incubation, bacterial colonies were suspended in 5 mL of sterile physiological saline to achieve turbidity equivalent to 0.5 McFarland standard. Figure 2 presents an example of the bacterial dilution process.

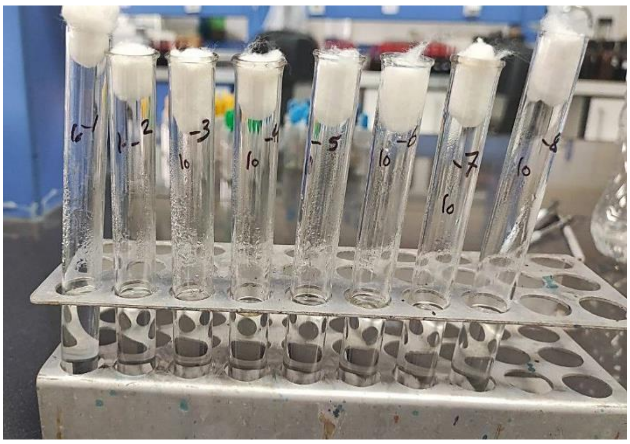

Figure 2: Example of bacterial suspension and dilution preparation

Next, 500 mL of physiological saline was poured into a graduated cylinder, and 100 µL of the prepared bacterial suspension (0.5 McFarland) was added. To determine the initial bacterial load, serial ten-fold dilutions (1:10) were prepared. From each dilution, 1 mL was mixed with 9 mL of molten nutrient agar (approximately 45°C) and cultured using the pour plate method. The inoculated plates were allowed to solidify and then incubated at 37°C for 24 hours. Figure 3 shows the sampling and culturing process.

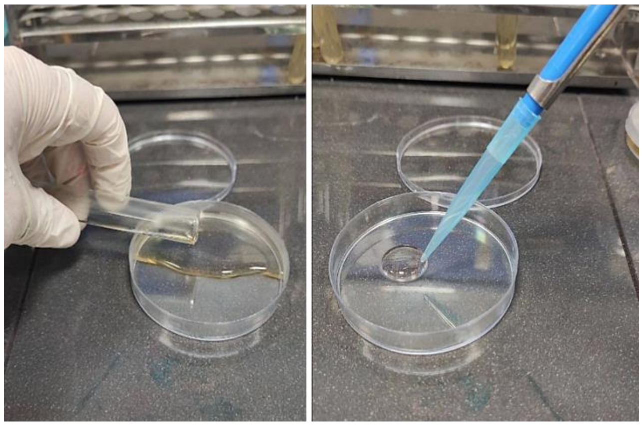

Figure 3: Sampling of bacterial suspensions (right) and inoculation of culture media using the pour plate technique (left)

Following sample preparation, the plasma-generating electrodes were immersed directly into the bacterial suspension inside the graduated cylinder, and the suspension was treated for 2.5, 5, 10, and 15 minutes. At each time point, samples were withdrawn and cultured separately using the pour plate method. Each time point was tested in triplicate to ensure the accuracy and reproducibility of the data. Figure 4 displays the bacterial dilution steps. All colony counts were conducted via the pour plate technique and statistically analyzed using t-tests and one-way ANOVA.

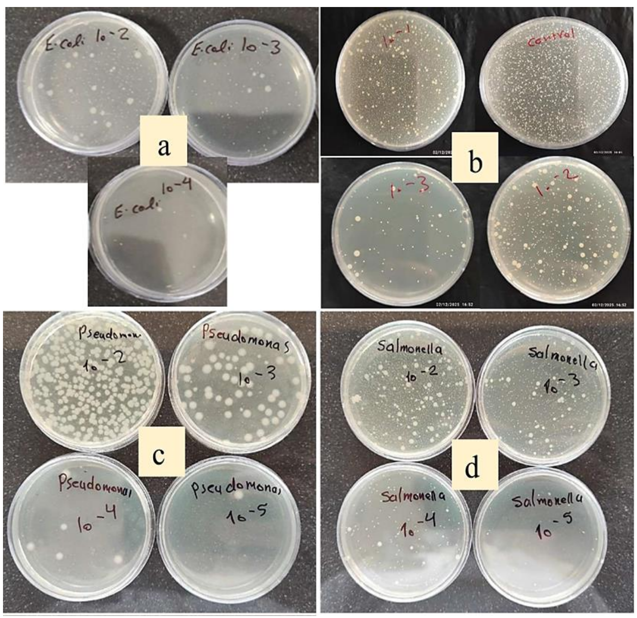

Figure 4: Serial dilution of bacterial suspensions: a) Escherichia coli, b) Staphylococcus aureus, c) Pseudomonas aeruginosa, d) Salmonella spp

Results

Water Parameters: Initially, the total hardness and pH of the water were measured for both the control sample and plasma-treated water. To reduce measurement error, all readings were repeated three times, and the mean values were recorded. According to Figure 5 and Table 1, the total hardness of the control sample (after addition of physiological saline) was 347 ppm. After plasma treatment for 2.5, 5, 10, and 15 minutes, the average total hardness increased to 4545.3 ppm, 5911 ppm, 7159.6 ppm, and 7934 ppm, respectively.

The pH of the control sample was measured at 7.15, which decreased following plasma treatment to average values of 5.3, 4.21, 3.28, and 2.48 for the respective time points. Ozone concentration in the untreated control was 0 mg/L. After plasma exposure for 2.5, 5, 10, and 15 minutes, the average ozone levels increased to 0.53 mg/L, 0.93 mg/L, 1.17 mg/L, and 1.23 mg/L, respectively. These observations are consistent with findings from previous studies (18).

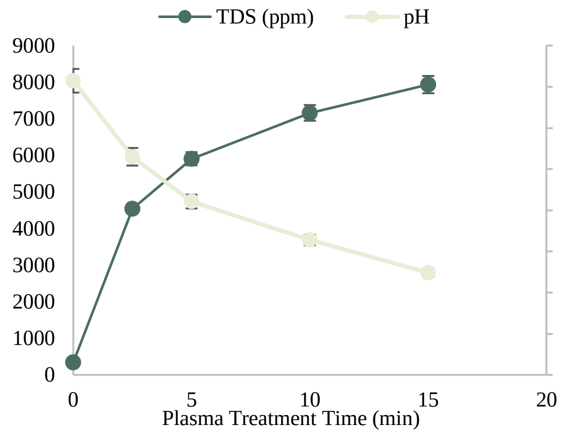

Figure 5: Changes in pH and total hardness of water as a function of plasma treatment time

Bacterial Enumeration: Following the culturing of both control and treated samples, no atypical microbial colonies were observed, and further identification tests were deemed unnecessary. Statistical comparison using Duncan's test at a 95% confidence level ($p < 0.05$) revealed a significant reduction in bacterial colony counts in plasma-treated samples compared to controls, indicating the antimicrobial efficacy of plasma treatment. In the control group, colony counts for all four bacterial species at a 10^{-3} dilution exceeded 100 CFU, the acceptable limit. In contrast, colony counts in plasma-treated samples showed a dramatic reduction across all time intervals. Table 2 presents the mean reduction in microbial load after 2.5, 5, 10, and 15 minutes of plasma-activated water treatment. Figures 6 and 7 illustrate the effects of plasma-activated water treatment on Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, and Salmonella spp.

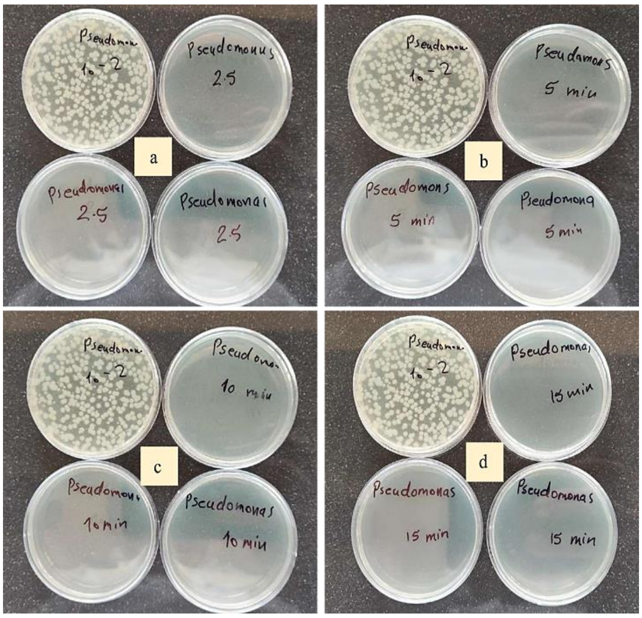

Figure 6: Effect of plasma-activated water treatment on Pseudomonas aeruginosa: a) Treatment duration: 2.5 minutes, b) 5 minutes, c) 10 minutes, d) 15 minutes

Discussion

Air-based dielectric barrier discharge plasma treatment of water significantly altered its properties. Hardness, acidity, and ozone levels are likely time-dependent. Specifically, plasma treatment increased total hardness to 4545.3 ppm, 5911 ppm, 7159.6 ppm, and 7934 ppm after 2.5, 5, 10, and 15 minutes, respectively (Figure 5, Table 1).

Table 1: Measurements of pH, total hardness, and ozone concentration in plasma-activated water at different treatment times

Plasma Treatment Time (min)	0	2.5	5	10	15
pH	7.15±0.04	5.3±0.06	4.21±0.05	3.28±0.03	2.48±0.02
TDS (ppm)	347±6.8	4545.3±11.2	5911±3.9	7159.7±6.3	7934±14.8
Ozon (mg)	0	0.53±0.05	0.93±0.05	1.17±0.05	1.23±0.05

Table 2: Comparison of mean microbial load reduction (CFU/mL) in plasma-activated water treatments at different exposure times

Bacteria (CFU/mL) Treatment	Control	2.5 min	5 min	10 min	15 min
Pseudomonas aeruginosa	1.05×10^6	0	0	0	0
Staphylococcus aureus	1.01×10^6	78 ± 9	0	0	0
Escherichia coli	9.3×10^5	53 ± 7	0	0	0
Salmonella spp.	1.47×10^6	66 ± 8	0	0	0

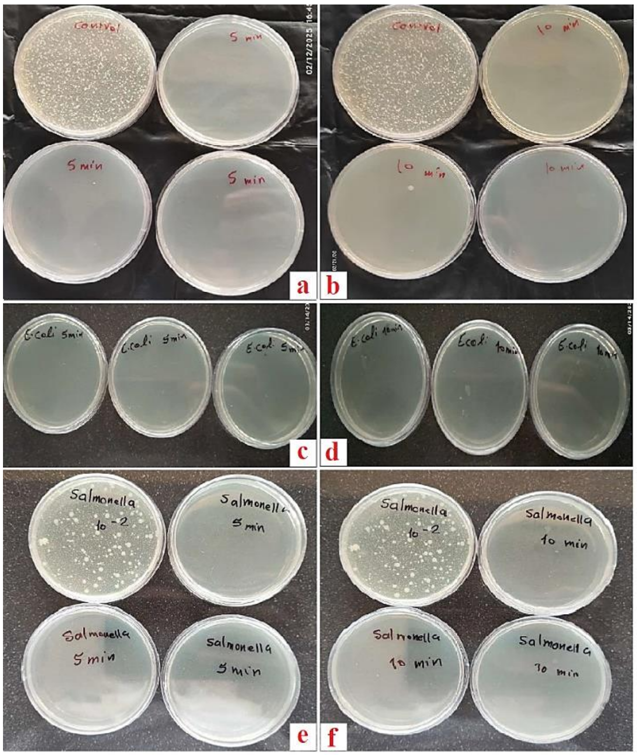

Figure 7: Control and plasma-treated culture plates for a–b) Staphylococcus aureus, c–d) Escherichia coli, e–f) Salmonella spp

Simultaneously, pH decreased to 5.3, 4.21, 3.28, and 2.48, and ozone concentration increased to 0.53 mg/L, 0.93 mg/L, 1.17 mg/L, and 1.23 mg/L for the same intervals. This increased hardness may benefit agriculture, while the reduced pH enhances antimicrobial activity and solid solubility, benefiting pharmaceutical and agricultural applications. The elevated ozone concentration improves disinfection, expanding the water's utility. These findings are consistent with prior research (18-20).

According to the results shown in Table 2 and Figure 6, the colony count of *Pseudomonas aeruginosa* in the control group was 1.05×10^6 CFU/mL. After plasma-activated water treatment for 2.5, 5, 10, and 15 minutes, complete elimination of bacterial colonies was observed at all time intervals. These findings indicate that *Pseudomonas aeruginosa* is highly susceptible to plasma treatment, showing no resistance under the tested conditions. The observed reduction in pH, increase in ozone concentration, and generation of various reactive free radicals in the plasma-treated water environment collectively contribute to creating unfavorable conditions for the survival of *Pseudomonas aeruginosa*. Hence, plasma-activated water can be considered a highly effective medium for inactivating this pathogen.

As shown in Figures 7a and 7b, colony-forming units (CFUs) of *Staphylococcus aureus* were enumerated using plates containing 30–300 colonies. In this experiment, dilutions of 10^{-1} and 10^{-2} produced more than 300 colonies and were thus uncountable. The 10^{-4} dilution yielded fewer than 30 colonies, and therefore, the 10^{-3} dilution plate—containing an average of 101 colonies—was selected for analysis.

In the control group, the bacterial load of *S. aureus* was calculated at 1.01×10^6 CFU/mL. Following plasma-activated water treatment, a substantial reduction in CFUs (78 ± 9 CFU/mL) was observed at the 2.5-minute mark. Complete elimination was achieved from the 5-minute treatment onwards. In a few plates, a single colony was noted, likely due to environmental contamination or experimental error, as no colonies were present in the other replicates.

Figures 7c and 7d show the control and treated cultures (5 and 10 minutes) for *Escherichia coli*. According to Table 2, the colony count for *E. coli* in the 10^{-3} dilution was 93 colonies. Plasma-activated water completely eliminated *E. coli* from the suspension at 5, 10, and 15-minute treatments. However, a remaining count of 53 ± 7 CFU/mL was recorded after 2.5 minutes. It is worth noting that spots seen in image d are due to plate error.

Figures 7e and 7f present the culture plates of control and treated samples (5 and 10 minutes) for *Salmonella* spp. As shown in Table 2, the 10^{-4} dilution plate was selected, containing 147 colonies. The initial bacterial count in the control was measured at 1.47×10^6 CFU/mL. Plasma-activated water reduced the microbial load to 66 ± 8 CFU/mL after 2.5 minutes and completely eliminated *Salmonella* in the 5, 10, and 15-minute treatments.

Salmonella, *E. coli*, and *S. aureus* exhibited relatively higher resistance to plasma treatment than *Pseudomonas aeruginosa*. These bacteria showed reduced counts at 2.5 minutes but were not completely eliminated. However, from 5 minutes onward, no viable colonies of any of the tested bacteria remained, indicating complete inactivation.

Conclusion

In conclusion, plasma-activated water represents an innovative, effective, safe, and environmentally friendly method for microbial decontamination, particularly in the food industry, medical environments, sanitation practices, and hospital settings. This technology could function as an alternative or adjunct to traditional chemical disinfectants, with the added benefit of substantial water conservation. Further studies are necessary to investigate the long-term stability of reactive compounds, potential effects on human health upon contact, and optimization of production methods.

Conflict of Interests

Authors declare no conflict of interests.

Acknowledgments

The authors extend their utmost gratitude and thanks to all the personnel of the National Nuclear Industry Educational and Cultural Institute (Bandar Abbas Branch) for their assistance in the implementation of this project.

References

1. Barjasteh A, Kaushik N, Choi EH, Kaushik NK. Cold atmospheric pressure plasma: a growing paradigm in diabetic wound healing—mechanism and clinical significance. International Journal of Molecular Sciences. 2023;24(23):16657.
2. Li HP, Ostrikov KK, Sun W. The energy tree: Non-equilibrium energy transfer in collision-dominated plasmas. Physics Reports. 2018;770:1-45.
3. Lu X, Naidis GV, Laroussi M, Reuter S, Graves DB, Ostrikov K. Reactive species in non-equilibrium atmospheric-pressure plasmas: Generation, transport, and biological effects. Physics Reports. 2016;630:1-84.
4. Zablah AC, Griffin T, Lio P. Cold Atmospheric Plasma in Inflammatory and Infectious Dermatology: A Scoping Review. Journal of Integrative Dermatology. 2025.
5. Mazandarani A, Goudarzi S, Ghafoorifard H, Eskandari A, Shahshenas S. Calculation of temperature and density for dielectric-barrier discharge (DBD) plasma using COMSOL. Journal of Nuclear Science, Engineering and Technology (JONSAT). 2020;40(4):99-108.
6. Khoja AH, Mazhar A, Saleem F, Mehran MT, Naqvi SR, Anwar M, et al. Recent developments in catalyst synthesis using DBD plasma for reforming applications. International Journal of Hydrogen Energy. 2021;46(29):15367-88.
7. Zhao YM, Ojha S, Burgess CM, Sun DW, Tiwari BK. Inactivation efficacy and mechanisms of plasma activated water on bacteria in planktonic state. J Appl Microbiol. 2020;129(5):1248-1260.
8. Zhao YM, Patange A, Sun DW, Tiwari B. Plasma-activated water: Physicochemical properties, microbial inactivation mechanisms, factors influencing antimicrobial effectiveness, and applications in the food industry. Compr Rev Food Sci Food Saf. 2020;19(6):3951-3979.
9. Zou F, Yang M, Wu J, Wang L, Wang H. The potential of plasma-activated water in safe and sustainable food production: a comprehensive review of recent advances and future trends. Crit Rev Food Sci Nutr. 2025:1-25.
10. Thirumdas R, Kothakota A, Annapure U, Siliveru K, Blundell R, Gatt R, et al. Plasma activated water (PAW): Chemistry, physico-chemical properties, applications in food and agriculture. Trends in food science & technology. 2018;77:21-31.
11. Li Y, Frey E, Mackenzie AM, Finlay BB. Human response to Escherichia coli O157:H7 infection: antibodies to secreted virulence factors. Infect Immun. 2000;68(9):5090-5.
12. Ekici G, Dümen E. Escherichia coli and food safety. InThe universe of Escherichia coli. IntechOpen. 2019.
13. Touaitia R, Mairi A, Ibrahim NA, Basher NS, Idres T, Touati A. Staphylococcus aureus: A Review of the Pathogenesis and Virulence Mechanisms. Antibiotics (Basel). 2025;14(5):470.
14. Mazandarani A, Goudarzi S, Jafarabadi M, Azimi Nekoo E. Effects of Cold Plasma on Staphylococcus Aureus. J Family Reprod Health. 2022;16(3):212-216.
15. Zhao L, Pu J, Liu Y, Cai H, Han M, Yu Y, et al. High prevalence of carbapenem-resistant Pseudomonas aeruginosa and identification of a novel VIM-type metallo-β-lactamase, VIM-92, in clinical isolates from northern China. Frontiers in Microbiology. 2025;16:1543509.
16. Coburn B, Grassl GA, Finlay BB. Salmonella, the host and disease: a brief review. Immunol Cell Biol.

2007;85(2):112-8.
17. Kahsay AG, Dejene TA, Kassaye E. A Systematic review on Prevalence, Serotypes and Antibiotic resistance of Salmonella in Ethiopia, 2010-2022. Infect Drug Resist. 2023;16:6703-6715.
18. Rathore V, Nema SK. Optimization of process parameters to generate plasma activated water and study of physicochemical properties of plasma activated solutions at optimum condition. Journal of Applied Physics. 2021;129(8).
19. Miranda FS, Tavares VK, Gomes MP, Neto NF, Chiappim W, Petraconi G, et al. Physicochemical characteristics and antimicrobial efficacy of plasma-activated water produced by an air-operated coaxial dielectric barrier discharge plasma. Water. 2023;15(23):4045.
20. Pal P, Pal UN, Singh V, Sharma NK, Singh M, Mishra A, et al. Experimental Investigation for the Generation and Characterization of Plasma-Activated Water. IEEE Transactions on Plasma Science. 2024.

Citation: Mazandarani A, Jabbari M, Goudarzi S, Khodashenas MJ. **Evaluating the Efficacy of Plasma-Activated Water in the Elimination of Pathogenic Bacteria: An Experimental Study on Pseudomonas, Salmonella, Escherichia Coli, and Staphylococcus Aureus.** J Family Reprod Health 2025; 19(3): 167-73.