PURE SCIENCES International Journal of Kerbala

Pure Sciences International Journal of Kerbala

Characterization of Cold Plasma Jet and Its Synergistic Effects with Antibiotics Against Staphylococcus Aureus

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PAPER INFO

Received: 27 .02.2026
Accepted: 29.03.2026
Published: 30.06.2026

Keywords:
Cold plasma, Staphylococcus aureus, OES, antibiotics.

Abstract

In this paper, a cold atmospheric-pressure argon (Ar) plasma jet system was constructed and used to inhibit *Staphylococcus aureus* bacteria. The system was used to generate a cold plasma using a homemade AC power supply that provided a variable voltage range (up to 15 kV_{p-p}) and a frequency range (up to 14 kHz). In this study, the operating parameters were fixed at 6 kV_{p-p} and 14 kHz to ensure stable discharge conditions. Initially, the general characteristics of the plasma jet, such as length, temperature, and optical emission spectroscopy (OES), were investigated. The results showed that the length of the plasma column is closely related to the plasma flow rate (laminated or turbulent). The gas temperature effect of the plasma flame was also confirmed, as the results demonstrated that the increase of the gas flow significantly reduced gas temperature. The OES of the Ar plasma was measured to confirm the presence of reactive oxygen and nitrogen species (RONS), as well as active free radicals, which are crucial for successful inhibition. *Staphylococcus aureus* bacteria were exposed to an Ar plasma jet for various time periods. Following exposure, the outcomes suggested that the inhibition regions were pronouncedly increased by the cold plasma jet. It is concluded that RONS was the main contributor to bacterial inactivation. Not only are its effects forceful but there are also other harmful constituents for this environment such as free radicals brought in from surrounding air particles. Finally, Ar cold plasma jet apparatuses were pitted against several standard antimicrobial test discs and *Staphylococcus aureus.*

DOI: 10.53851/psijk.v3.i10. 64-69

1. INTRODUCTION

Cold plasma jets at atmospheric pressure have attracted much attention because of their wide application prospects in the field of, biomedical (Asghar et al., 2021; Baniya et al., 2021; Pedroni et al., 2017), material surface treatment (Kostov et al., 2013) and other fields (Jiang et al., 2022). Traditional sterilization methods, including ultraviolet (UV) radiation, high temperatures (>120 °C), ethanol, and strong chemical agents, are effective for microbial inactivation(Hijnen et al., 2006; Holyoak et al., 1996). Nevertheless, concerns regarding their environmental and health impacts have prompted the search for alternative sterilization approaches that are safer, more convenient, and free from residual toxicity (Yang et al., 2009). Cold atmospheric pressure plasma jet is a promising alternative to traditional sterilization methods due to its distinct advantages, which include the ability to accurately target bacteria, allowing for flexible operation, providing definite curative effects, causing less toxic side effects, being safe, and convenient, so they are likely to find use in new treatment methods in the future (Homma et al., 2013). A cold plasma jet's physical characteristics are greatly influenced by the applied voltage, the type of working gas, and the distribution of the gas flow rate. Numerous studies (KC et al., 2025; Ogawa et al., 2018; Omran et al., 2020), have shown that the active species, particularly reactive oxygen and nitrogen species (RONS), play a crucial role in inhibiting and killing bacterial growth (Shrestha et al., 2025). Increasing the voltage and Ar flow rate will most likely lead to the

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generation of more active components, such as OH radicals and O atoms. Voltage parameters, inert gas types, and gas flow are important factors affecting the properties of cold plasma (Khlyustova et al., 2019; Kim and Chung, 2016; Shrestha et al., 2025). Omran, A.V., et al., (Omran et al., 2020) created a plasma jet source operated at atmospheric pressure for bacterial disinfection and it was compared its effectiveness with antibiotics. They investigated plasma characteristics, including electrical and thermal properties. They observed significant increases in bacterial inhibition zones by exposure to the plasma and compared them with inhibitions by several standard antimicrobial test discs. S. Das et al., (Lim et al., 2025) established an AC plasma jet device using an inert gas (argon) to estimate its antimicrobial efficiency against microorganisms isolated from clinical samples. They had changed parameters, like microbial concentration, cold plasma time exposure to bacteria, and distance from nozzle tube to the sample, to compare how well E. coli bacteria and S. aureus bacteria were killed off. They mentioned that oxidative stress induced by RONS, electrostatic stress caused by ions, might be crucial for bacterial inactivation.

The paper discussed important contributions to understanding the properties and mechanisms of a cold plasma jet by studying how these factors affect the plasma. Also, the importance of this research lies in conducting a comparative study between the effect of cold plasma and the traditional effectiveness of antibiotics against *Staphylococcus aureus* bacteria, which is among the most prominent pathogens known for their strong resistance to traditional treatments.

2 .EXPERIMENTAL WORK

2.1 Experimental facility

Figure 1 illustrates the structure of the atmospheric-pressure cold plasma jet device used in this paper. The plasma jet device consists of a Pyrex tube with an inner diameter of 2.5 mm and an outer diameter of 4 mm. Two aluminum ring electrodes with a thickness of 0.1 mm and 10 mm wide are attached to a Pyrex tube, separated by a distance of 10 mm. One electrode is connected to a homemade AC power supply, capable of delivering an adjustable voltage of up to 15 kV_{p-p} and a frequency range up to 14 kHz, while the counter-electrode is effectively grounded. The top side of Ar gas with purity 99.9998% is introduced to the Pyrex tube, and the gas flow rate is controlled by a flow meter (11420, USA). The applied voltage and discharge current waveforms were measured with a Digital Oscilloscope UTD2102CEX (UNI-T, China). The temperature of the gas plasma was measured by an alcohol thermometer at a distance from the nozzle of the Pyrex tube.

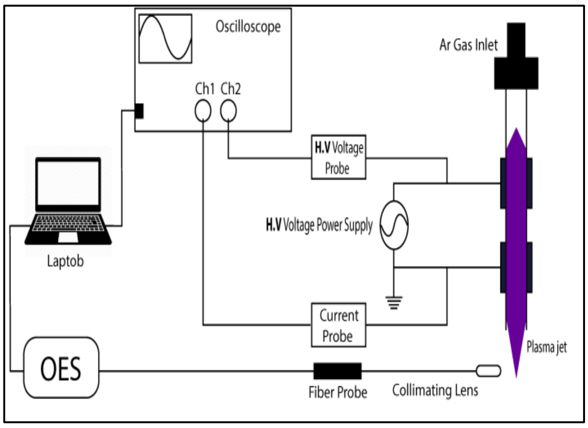


Figure 1. Schematic illustration of a cold plasma jet system.

2.2 Sample preparation

In this work, the *bacterium Staphylococcus aureus* was used, obtained from a laboratory stock culture at the Authority of Scientific Research, Ministry of Higher Education and Scientific Research/ Iraq. Bacterial concentration was measured using a spectrophotometer SP-3000 Plus (Optima) at 625 nm, as this apparatus has a linear range of 0.402 nm, corresponding to 6.5 × 10^8 bacterial cell-forming units per milliliter (CFU/ml). After preparing the suspension, 0.1 ml of it (containing 6.5 x 10^8 CFU/ml bacteria) was spread onto agar medium by means of a sterile swab in a typical Petri dish. After drying in an aseptic location for 10 min, the dishes were placed under Ar plasma jet treatments at different times and then incubated at 37 °C for 18 h.

The Ar cold plasma jet operated at a fixed frequency of 14 kHz with an applied voltage of 6 kV_{p-p} and an argon flow rate of 2. 5 slm was used for all treatments. All plasma treatments and biological assays were performed under stabilized environmental conditions, specifically at a room temperature of 25 ± 2 °C and a relative humidity (RH) of 45 ± 5%. The distance from the nozzle tube to the sample (bacteria) was fixed at 21 mm. The diameters of the inhibition zones that were formed on agar were measured to assess the influence of the Ar cold plasma jet treatment on the bacterial samples. To determine these areas, AutoCAD software was used to compute the diameter of the inhibition regions.

3. RESULTS AND DISCUSSIONS

To initiate the electrical discharge, a high-voltage fixed at 6 kV_{p-p} and 14 kHz frequency was applied to the upstream electrode with an AR gas flow rate of 1 SLM, as presented in Figure 2. The observed expansion of the plasma jet area upon exiting the nozzle is primarily attributed to the fluid-dynamic interaction between the Ar gas flow and the stationary ambient air, coupled with the electrostatic repulsion among the charged species. Furthermore, previous studies (Al-Rawaf et al., 2023; Liang et al., 2025) have demonstrated that the tube diameter plays a crucial role in shaping the plasma plume; a smaller diameter typically increases the gas velocity and restricts the radial expansion, whereas the specific diameter used in this study (2.5 mm) allows for an optimal expansion that enhances the treatment coverage. While this expansion effectively increases the treatment surface area, it consequently leads to a distributed concentration of reactive species per unit area, and it is a critical factor in determining the uniform inactivation of S. *aureus* across the treated surface.

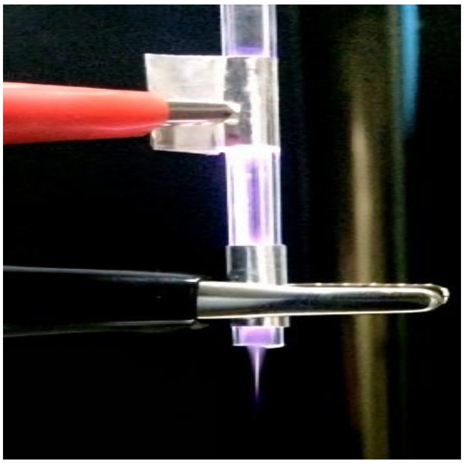


Figure 2. Ar cold plasma jet at 1 SLM.

The length of the Ar cold plasma jet plume was calculated as a function of the gas flow, as shown in Figure 3. Here, the growth of the AR plasma jet column growths as the gas flow rate increases, as it was observed that the maximum length of the argon plasma column (21 mm) appeared at a flow rate of 2.5 SLM.

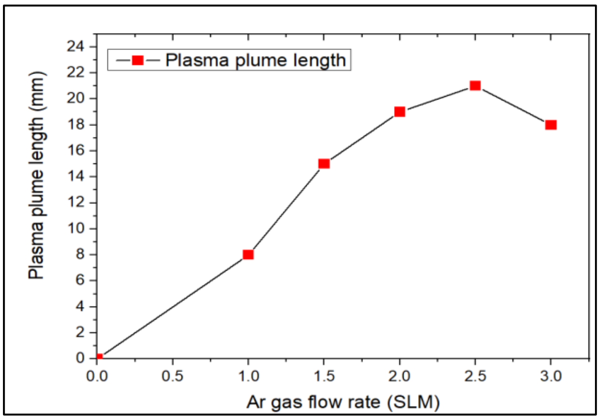


Figure 3. The length of the plasma plume as a function of Ar gas flow rate.

In addition, it is noted in Figure 3, that the growth of the AR plasma column increases proportionally with the gas flow in certain ranges from 1 to 2.5 SLM, which is called laminar flow. However, as soon as the flow moves to 3, it can be noticed that there is a drop in the length of the plasma plume, and it decreases to an amount of 18 mm. This means that the gas flow here has moved from laminar flow to turbulent flow that results in instabilities and deformations of the AR gas flow rate (Mericam-Bourdet et al., 2009).

An important characteristic of the AR plasma jet is low gas temperature, which gives the plasma plume significant applications in the medical and industrial fields. Figure 4 illustrates the time the plasma remains outside the nozzle tube and its relationship to the gas temperature at different gas flow rates. As shown, the highest gas temperature (42°C) was observed at a 1 SLM for 3 minutes. Conversely, the lowest gas temperature (33°C) was observed at a 2.5 SLM for 1.5 minutes. From the results above, an expulsive relationship between the decrease in temperature and the time and gas flow rate can be observed. In addition, it is noted that a thermal equilibrium appeared at all argon gas flow rates after three minutes of exposure to the plasma column.

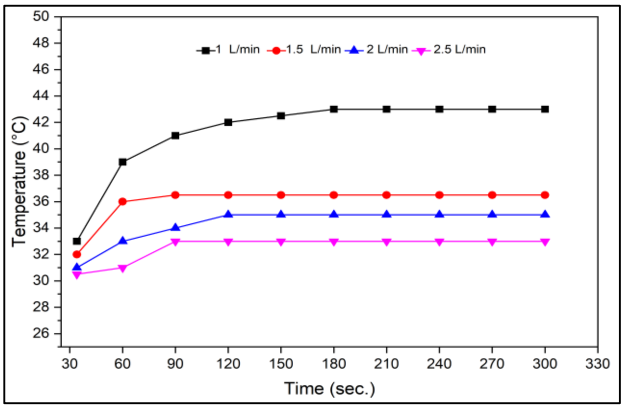


Figure 4. Influence of exposure time and Ar flow rate on gas temperature.

OES analysis is essential for understanding the nature of Ar cold plasma. This analysis allows for the identification of RONS and radicals. The presence of RONS is due to the generation of this Ar plasma jet in open air, where its energetic electrons and Ar metastable ions ionize and excite air molecules. Moreover, OH radicals are produced by the collision between water vapor molecules in the ambient air with electrons in the Ar plasma jet (Lamichhane et al., 2022; Roomy and Murbat, 2023). These species and radicals play a significant role in the medical field, especially in interpreting biological effects (Wang and Srivastava, 2010)

Figure 5 shows a typical spectrum of Ar plasma emitted in the ultraviolet-visible-infrared range. This spectrum was obtained at system conditions of 2.5 SLM and 6 kV_{p-p} with a frequency of 14 kHz. The wavelengths of the lines intensity in the Ar plasma jet are obtained from NIST (Ralchenko, 2005). The plasma plume length was 21 mm at these conditions.

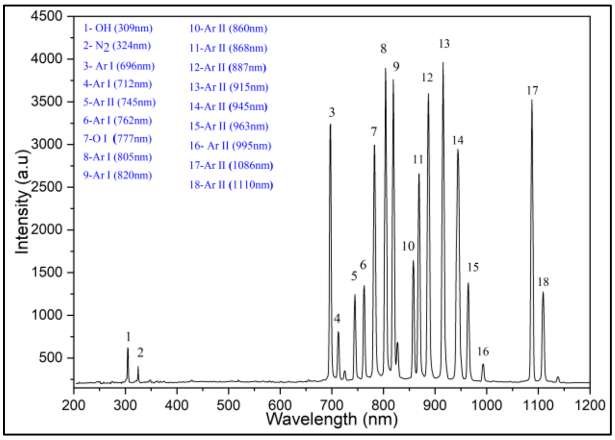
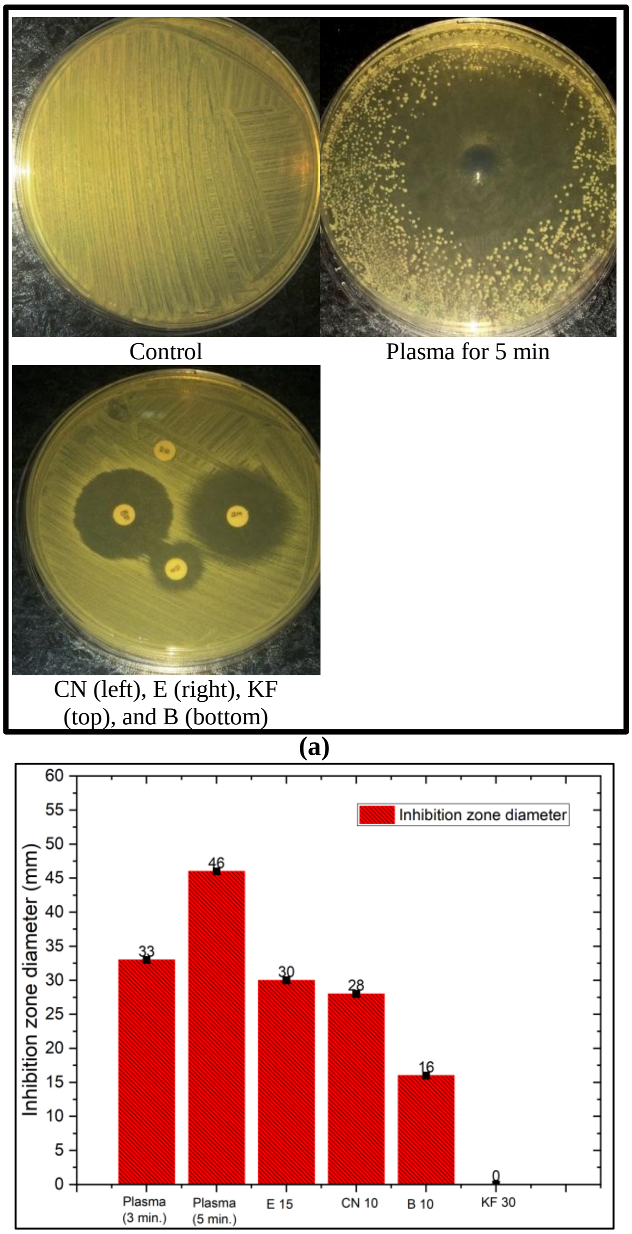


Figure 5. Typical OES spectra of the Ar plasma jet.

As illustrated in Figure 5, the OES spectra confirm that the cold Ar plasma jet generates a diverse array of RONS. Key emission lines were identified, including hydroxyl radicals (OH) at 309 nm and atomic oxygen (O I) at 777 nm, which are recognized as potent antibacterial agents. In this study, OES analysis was specifically employed as a qualitative diagnostic tool to establish the chemical fingerprint of the discharge. This approach prioritizes the identification of the biocidal species responsible for the observed synergistic effect with antibiotics, as their presence is the primary driver of oxidative stress and cell wall permeabilization in S. aureus. (Lim et al., 2025; Nie et al., 2018). Recent studies have demonstrated the effectiveness of cold argon plasma in inactivating a wide range of microorganisms, such as S-aureus and P-aeruginosa, as well as its role in promoting wound healing and reducing contamination of medical surfaces without causing significant damage to healthy tissue (Kondeti et al., 2018; Xu et al., 2015).

The *Staphylococcus aureus* bacteria were exposed to the Ar cold plasma jet for different times. After the exposure, round transparent areas marked the growth inactivation zones and were compared with the antibiogram disc diffusion method. To assess the effect of different bactericidal antibiotics, GENTAMICIN (CN), Erythromycin (E), Loloatin (B), and Cephalothin (KF) antibiotic discs at concentrations of 10, 15, 10, and 30 micrograms per disc, respectively, were placed on the surface of petri dishes containing bacteria. After 18 hours of incubation at 37°C, the areas of inhibition zones were measured using AutoCAD software. Figure 6 (a) shows a photograph comparison between the areas of bacterial growth inhibition of the Ar plasma jet and a group of antibiotics, (b) inhibition zone diameter of *Staphylococcus aureus* bacteria after Ar plasma jet exposure and antibiogram.



Control

Plasma for 5 min

CN (left), E (right), KF (top), and B (bottom)

(a)

(b)

Figure 6. (a) Photograph of the zones of *Staphylococcus aureus* bacteria growth inactivation following exposure to an Ar plasma jet and antibiotics. (b) Inhibition zone diameter of *Staphylococcus aureus* bacteria after Ar plasma jet exposure and antibiogram.

As shown in Figure 6, areas of inhibition appeared on bacteria exposed to plasma and most antibiotics. As is clear and expected, the plasma jet had a significant effect on expanding the area of inhibition, and this expansion varied according to the treatment duration, with the diameter of the inhibition area increasing as the time increased from 3 to 5 minutes. In contrast, when compared to the antibiotics, we observe that the areas of inhibition were prominent with CN 10 and E 15, while they were minimal with B 10 and nonexistent with KF 30. This comparison clearly demonstrates the role of plasma in expanding the inhibition zone compared to antibodies.

From these results, it is observed that the area of bacterial inactivation by plasma is large compared to the cold plasma column. It can be concluded that RONS from the surrounding air, in addition to free radicals generated within the ultraviolet zone, are key factors in the bacterial inactivation process. As demonstrated by Lo (2008) (Lu et al., 2008), reactive oxygen species (ROS) generated by plasma jets can diffuse radially outwards, thus inactivating cells.

4. CONCLUSION

In this work, the cold Ar plasma jet system was well constructed and developed to be an effective method for killing microorganisms when assayed against S. aureus. The results showed that the length of the plasma column grows as the gas flow rate increased. In addition, the results demonstrated that increasing the gas flow significantly reduced the gas temperature. OES confirmed the presence of RONS and radicals that have an effective impact on the process of inhibiting bacteria. The bacteria treated with Ar plasma jet exhibited an enlarged diameter of the inhibition zone as a function of irradiation time. Furthermore, the diameter of the zone of inhibition was greater than that of the plasma plume, indicating RONS contributed significantly to inactivation. It was also confirmed that S. aureus activity after treatment in plasma for 3 and 5 min decontamination showed an increase in the fixation zone compared to its activity after treatment with different antibiotics.

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