Egyptian Journal of Biophysics and Biomedical Engineering
https://ejbbe.journals.ekb.eg/

Antibacterial Effects of Helium Cold Atmospheric Plasma Jet against Gram-Positive and Gram-Negative Bacteria

Mahmoud M. Ismail[a*], Tamer M. Elsayed[a], Amir Eissa[a], Amer M Abdelaziz[b], Mohamed H. Sharaf[b]

[a] *Department of Physics, Faculty of Science, Al-Azhar University, Nasr City 11884, Cairo, Egypt*
[b]*Department of botany and microbiology, Faculty of Science, Al-Azhar University, Nasr City 11884, Cairo, Egypt*

THE GOAL of the present study was to investigate the potency and validity of cold atmospheric plasma (CAP) in combating bacterial contamination. CAP is applied as a topical treatment with the hope of providing a less invasive approach compared to surgical debridement and irrigation. The setup of helium CAP jet has been done with 3.4 KV and frequency 16.1 KHz. The antibacterial activity of the plasma jet was performed on Muller Hinton agar media (MHA) for *Staphylococcus aureus*, *Bacillus cereus,* and *Escherichia coli,* but selective *Enterococcus* agar media (Bile Esculin Agar Media) (BEA) was used for *Enterococcus faecalis*. CAP treatment (1, 2, and 3 min) showed that plasma jet can inhibit and limit the growth of pathogenic G +ve (*Enterococcus faecalis*, *Staphylococcus aureus*, and *Bacillus cereus*) as well as G –ve bacteria (*Escherichia coli*) depended directly on the strains and also depended greatly on the exposure. The plasma jet has antibacterial potency against Gram-Positive *Bacillus cereus*, *Enterococcus faecalis*, *Staphylococcus aureus*, and *Staphylococcus aureus* in addition to Gram-Negative bacteria *Escherichia coli*.

Keywords: Cold atmospheric plasma, Plasma jet, Antibacterial, Gram-Positive, Gram-Negative.

Introduction

Cold atmospheric plasma (CAP) is a type of gas in an ionized form that is acknowledged to be a fourth state of matter. Using lower temperatures, a non-thermal plasma is produced and comprises a range of different chemical and physical species; as primary excited atoms, secondary excited atoms, free radicals, molecules, as well as ions. The characteristics of any plasma source have a considerable influence on the chemical composition as well as the existence of both primary and secondary reactive species [1-3]. A significant number of research studies have demonstrated that CAP is capable of effectively neutralizing a diverse range of organisms, including bacteria, bacterial spores, yeast, fungi, and viruses [4-5]. CAP is extensively utilized in the field of biomedicine, undertaking various functions including the modification of hydrophilic and chemically reactive materials, the eradication of bacteria, skin disinfection, wound management, caries treatment, sterilization of implant surfaces, enhancement of blood coagulation, and the demonstration of anti-cancer effects [6-7].
Antibiotics made their clinical debut in the early 1940s as a treatment for serious bacterial infections. However, by the 1950s, notable instances of antibiotic resistance had already been observed [8]. The Centers for Disease Control and Prevention (CDC) previously reported that at least 2 million individuals in the United States are infected annually with antibiotic-resistant bacterial strains, leading to approximately 25,000 fatalities [9]. In addition to the medical challenges associated with treating patients suffering from bacterial contamination, the proliferation of resistance to antibiotics leads to considerable additional costs for the healthcare system and imposes significant economic burdens. Estimates suggest that these costs may reach as high as $1 billion annually, while global losses in gross domestic product could amount to $3 trillion.[10-11].
There has been a growing emphasis on the advancement and application of CAP sources that can fulfill the specifications necessary for medical instrumentation. [12]. The extensively documented application of different plasma types to sterilize non-biomedical materials has created a large room for improvement in the field of antiseptics where plasma medicine can offer a significant contribution [13-16].
For low-temperature (LTP) to act as a bactericide, it is essential that this plasma can produce reactive oxygen species (ROS) as well as reactive nitrogen species (RNS). It was also observed that LTP treatment can kill bacteria without the latter being able to acquire resistance [17].

*Corresponding author e-mail: m10m10e10@gmail.com
Received: 25/12/2024; Accepted: 4/1/2025
DOI: 10.21608/EJBBE.2025.346490.1082

The increasing consumption of antibiotics has caused the advent of bacteria resistant to traditional antibiotics, rendering them ineffective in treating these pathogens [18]. Antibiotic-resistant bacteria are one of the most important threats facing humanity, as they increase deaths and consume a lot of energy of health workers, in addition to the indirect impact on the global economy, especially in developing countries that suffer from a high rate of infection [19]. Consequently, scientists and researchers have sought effective and safe alternatives that are both beneficial for individual health and environmentally friendly. Some researchers have used natural materials, while others have employed physical methods [20-22]. Plasma jets are applied for sterilization and therapeutic treatments due to their unique properties [23]. Plasma jets have several mechanisms in inhibition of bacterial growth, from which : they produce a variety of ROS and RNS that can damage bacterial cell membranes, denaturation of proteins, and breakdown bacterial nucleic acids [24]. Reactive oxygen species cause lipid peroxidation, leading to structural leakage of cellular contents [25]. Another mechanism is that Plasma jets can lead to resistant bacterial cell lysis through the ionized particles that can penetrate bacterial biofilms [26]. The objective of the study is to evaluate the effectiveness and validity of cold atmospheric pressure plasma as a treatment for bacterial contamination. The study aims to determine cold atmospheric pressure plasma's antibacterial properties against various bacterial strains, including both Gram-positive and Gram-negative bacteria.

Material and methods

Cold Atmospheric Plasma (CAP) Device

A cold atmospheric plasma jet was developed independently utilizing a power supply capable of producing high-frequency output. As in Figure 1, the plasma jet consists of a life electrode which consists of a metal tube (stainless steel) with an inner diameter of 3.9 mm, outer diameter of 6 mm and, length of 14.9 cm which is enclosed in a Pyrex tube with an inner diameter of 7.34 mm, outer diameter 10.2 mm and the ground electrodes is formed from aluminum strip with length 12mm, the axial distance from the circular life electrode to the circular ground electrode is about 35 mm.

The plasma jet system utilizes a voltage of 3.4 kV at the frequency of 16.1 kHz with helium as a discharge gas.

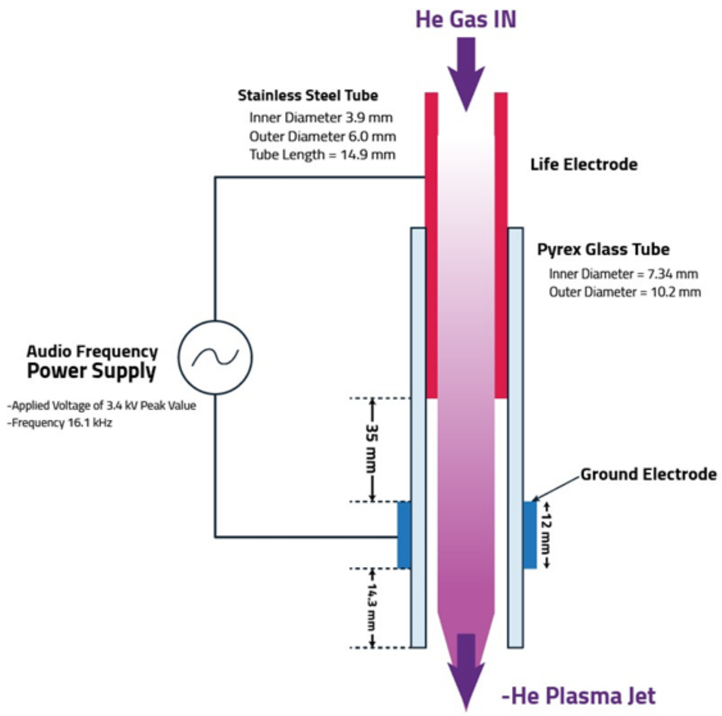

Fig. 1 diagram of cold atmospheric plasma (CAP) jet system.

Antibacterial studies

The antibacterial activity of plasma jet was performed on Muller Hinton agar media (MHA) for *Staphylococcus aureus*, Bacillus *cereus*, and *Escherichia coli* but Selective *Enterococcus* agar media (Bile Esculin Agar media) (BEA) was used for *Enterococcus faecalis*. The bacteria were cultured on MHA and BEA and then exposed to plasma jet for one, two, and three minutes and we incubated the plates for a duration of 24 h while maintaining the temperature at 37℃. Afterwards, we measured the inhibition zones and recorded them in triplicate.

Results

Figures 2 and 3 presented in this study demonstrate the efficacy of plasma jet in inhibiting the growth of various pathogenic bacteria, including Gram-positive strains such as *Enterococcus faecalis, Staphylococcus aureus*, and *Bacillus cereus*, as well as Gram-negative bacteria like *Escherichia coli.* The ability of the plasma jet to halt bacterial growth was found to be dependent on the specific strains tested and the duration of exposure.

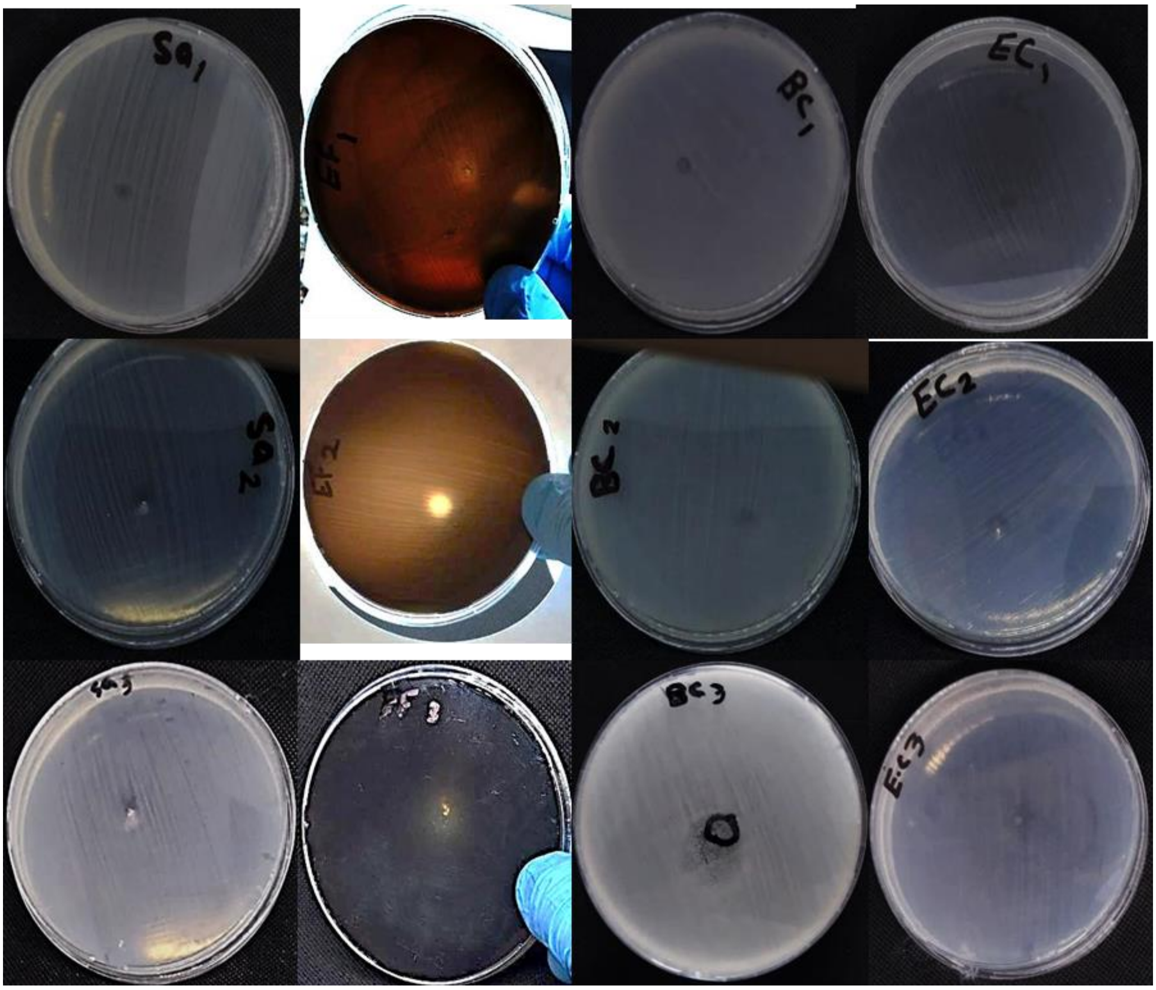

Fig. 2 Antibacterial activity of plasma jet under different exposure times. (Sa1: *Bacillus cereus* exposed to plasma jet for 1 minute , Sa2: *Bacillus cereus* wide-open to PJ for 2 minutes , Sa3: *Bacillus cereus* wide-open to PJ for 3 minutes ,EF1: *Enterococcus faecalis* wide-open to PJ for 1 minute, EF2: *Enterococcus faecalis* wide-open to PJ for 2 minutes, EF3: *Enterococcus faecalis* wide-open to PJ for 3 minutes , BC1: *Bacillus cereus* exposed to plasma jet for 1 minute , BC2: *Bacillus cereus* exposed to plasma jet for 2 minutes , BC3: *Bacillus cereus* wide-open to PJ for 3 minutes, EC1: *Escherichia coli* wide-open to PJ for 1 minute , EC2: *Escherichia coli* wide-open to PJ for 2 minutes, and EC3: *Escherichia coli* wide-open to PJ for 3 minutes).

These findings align with previous research conducted by Morabit et al. [27], who observed that plasma jet treatment led to damage to bacterial nucleic acids, resulting in genetic mutations and destruction of the bacterial cell wall. Consequently, this caused significant harm to the bacterial cells. Furthermore, other studies have also reported the effectiveness of plasma jet in combating both Gram-positive and Gram-negative bacteria [28]. Overall, these findings highlight the potential of plasma jet technology as a promising approach for bacterial control and treatment.

Exposure to a plasma jet for different time durations was found to have varying effects on the growth of bacteria [29-33].

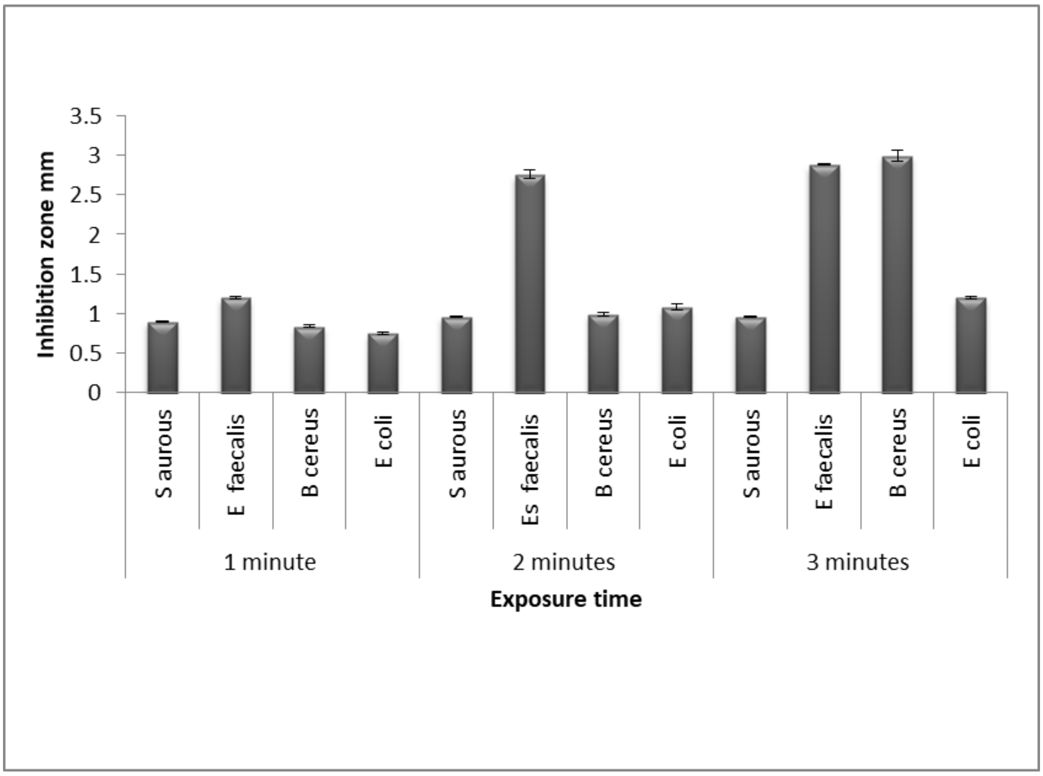

Fig. 3 Inhibition zone of bacteria growth after treatment by plasma jet with different exposure times.

In the present study, the plasma jet was applied for one minute, two minutes, and three minutes to test its inhibition potential against *Enterococcus faecalis, Staphylococcus aureus, Bacillus cereus*, and *Escherichia coli.* The results showed that after one minute of exposure, the inhibition values were recorded as 1.2±0.02 mm, 0.9±0.01 mm, 0.84±0.01 mm, and 0.85±0.01 mm for *Enterococcus faecalis, Staphylococcus aureus, Bacillus cereus, and Escherichia coli,* respectively.

Increasing the exposure time to two minutes resulted in higher inhibition values: 2.76±0.05 mm, 1.08±0.04 mm, 0.99±0.03 mm, and 0.96±0.01 mm for *Enterococcus faecalis, Staphylococcus aureus, Escherichia coli,* and *Bacillus cereus,* respectively. Finally, after three minutes of exposure, the inhibition values further increased to 3.00 ±0.07 mm, 2.88±0.01 mm, 1.2 ±0.02 mm, and 0.96±0.01 mm for *Bacillus cereus, Enterococcus faecalis, Staphylococcus aureus*, *Escherichia coli*, and *Staphylococcus aureus*, respectively. These findings clearly indicate that increasing the exposure time to the plasma jet resulted in enhanced inhibition of bacterial growth, except for *Staphylococcus aureus,* which remained unaffected by the increase in exposure time. Notably, the plasma jet's capacity to inhibit *Bacillus cereus showed* a significant increase, as the inhibition value increased from 0.84±0.01 mm to 3.00±0.07 mm when the exposure time was extended from one minute to three minutes. These results highlight the potential of plasma jet technology as a promising approach for bacterial growth control, particularly against *Bacillus cereus*. Further studies are warranted to explore the underlying mechanisms and optimize the application of plasma jet in various settings.

Conclusion

The helium CAP (cold atmospheric plasma) has shown significant antibacterial potency against Gram-positive bacteria including *Bacillus cereus (Bc), Enterococcus faecalis (Ef), and Staphylococcus aureus (Sa).* Furthermore, it has also demonstrated effectiveness against Gram-negative bacteria such as *Escherichia coli (Ec).* These findings have important implications for the potential use of plasma jet treatments in various medical and industrial applications. The results suggest that by optimizing the exposure duration of the plasma jet, the antibacterial effectiveness can be further enhanced, thereby improving the overall outcomes of such treatments. This research highlights the promising potential of helium CAP in combating bacterial infections and provides insights into its application in various fields.

Acknowledgement
I would like to express my gratitude to the group of plasma research in the faculty of science – Al Azhar University in Egypt.

Funding
This study did not receive or obtain any particular grant at all from any sources in the public, private, or non-profit sectors.

Conflict of interest
We have no conflict of interest to declare.

References

1. Tabares FL, Junkar I. (2021) Cold plasma systems and their application in surface treatments for medicine. Molecules. 26:1903. https://doi.org/10.3390/molecules26071903.
2. Veda Prakash G, Patel K, Behera N, Kumar A. (2020) Expansion dynamics of atmospheric pressure helium plasma jet in ambient air. Phys. Lett. 384:126497. https://doi.org/10.1016/j.physleta.2020.126497.
3. Veda Prakash G, Behera N, Patel K, Kumar A. Concise characterization of cold atmospheric pressure helium plasma jet. AIP Adv. 2021; 11:085239. https://doi.org/10.1063/5.0061857.
4. Laroussi M. (2008) Mechanisms of interaction of cold plasma with bacteria. AIP Conference Proceedings. 1041:87–90. https://doi.org/10.1063/1.2997283.
5. Mravlje J, Regvar M, Vogel-Mikus K. (2021) Development of cold plasma technologies for surface decontamination of seed fungal pathogens: Present status and perspectives. J. Fungi (Basel) 7:650. https://doi.org/10.3390/jof7080650.
6. Brany D, Dvorska D, Halasova E, Skovierova H. (2020) Cold atmospheric plasma: A powerful tool for modern medicine. Int. J. Mol. Sci. 21:2923. https://doi.org/10.3390/ijms21082932.
7. T VONW, Schmidt A, Bekeschus S, Wende K, Weltmann KD. (2019) Plasma medicine: A field of applied redox biology. In Vivo. 33:1011–1026. https://doi.org/10.21873/invivo.11570.
8. Ventola, C. L. (2015) The antibiotic resistance crisis: part 1: causes and threats. *P T* 40, 277–83.
9. Centers for Disease Control and Prevention. About Antimicrobial Resistance | Antibiotic/Antimicrobial Resistance | CDC. Available at, https://www.cdc.gov/drugresistance/about.html. (Accessed: 19th December 2018) (2018).
10. Naylor, N. R. *et al*. (2018)Estimating the burden of antimicrobial resistance: a systematic literature review. *Antimicrob. Resist. Infect. Control* 7:58. https://doi.org/10.1186/s13756-018-0336-y.
11. Shrestha, P. *et al*. (2018) Enumerating the economic cost of antimicrobial resistance per antibiotic consumed to inform the evaluation of interventions affecting their use. *Antimicrob. Resist. Infect. Control* 7:98. https://doi.org/10.1186/s13756-018-0384-3.
12. International Organization for Standardization. ISO 14937:2009 - Sterilization of health care products–General requirements for characterization of a sterilizing agent and the development, validation and routine control of a sterilization process for medical devices. Available at, https://www.iso.org/standard/44954.html. (Accessed: 3rd January 2019).
13. Weltmann, K. D. *et al*. (2010) Atmospheric-pressure plasma sources: Prospective tools for plasma medicine. *Pure Appl. Chem.* 82, 1223–1237. http://dx.doi.org/10.1351/PAC-CON-09-10-35.
14. Shintani, H., Sakudo, A., Burke, P. & McDonnell, G. (2010) Gas plasma sterilization of microorganisms and mechanisms of action. *Exp. Ther. Med.* 1, 731–738. https://doi.org/10.3892/etm.2010.136.
15. Ferreira, S. D. *et al*. (2001) Effect of gas-plasma sterilization on the osteoinductive capacity of demineralized bone matrix. *Clin. Orthop. Relat. Res*. 233–9. https://doi.org/10.1097/00003086-200107000-00032.
16. Shintani, H. *et al*. (2007) Inactivation of microorganisms and endotoxins by low temperature nitrogen gas plasma exposure. *Biocontrol Sci.* 12, 131–43. https://doi.org/10.4265/bio.12.131.
17. Nicol, McKayla J., Timothy R. Brubaker, Brian J. Honish II, Alyssa N. Simmons, Ali Kazemi, Madison A. Geissel, Connor T. Whalen, Christopher A. Siedlecki, Sven G. Bilén, Sean D. Knecht, and Girish S. Kirimanjeswara (2020) Antibacterial Effects of Low-Temperature Plasma Generated by Atmospheric-Pressure Plasma Jet Are Mediated by Reactive Oxygen Species." *Scientific Reports* 10 (3066). https://doi.org/10.1038/s41598-020-59652-6.
18. Abdelaziz AM, A. Abdel-Maksoud M, Fatima S, Almutairi S, Kiani BH, Hashem AH (2024) Anabasis setifera leaf extract from arid habitat: A treasure trove of bioactive phytochemicals with potent antimicrobial, anticancer, and antioxidant properties. PLoS One 19 (10):e0310298. https://doi.org/10.1371/journal.pone.0310298

19. Bashar MA, Hamed A, El-Tabakh MA, Hashem AH, Zaki AA, Al-Askar AA, Abou-Amra ES, El-Beeh ME, Mehany AB, Shaaban M (2024) Anticancer, antimicrobial, insecticidal and molecular docking of sarcotrocheliol and cholesterol from the marine soft coral Sarcophyton Trocheliophorum. Scientific Reports 14 (1):28028. https://doi.org/10.1038/s41598-024-75446-6.

20. Elkady FM, Badr BM, Hashem AH, Abdulrahman MS, Abdelaziz AM, Al-Askar AA, AbdElgayed G, Hashem HR (2024) Unveiling the Launaea nudicaulis (L.) Hook medicinal bioactivities: phytochemical analysis, antibacterial, antibiofilm, and anticancer activities. Frontiers in Microbiology 15:1454623. https://doi.org/10.3389/fmicb.2024.1454623.

21. Hashem AH, Al-Askar AA, Abd Elgawad H, Abdelaziz AM (2023) Bacterial endophytes from Moringa oleifera leaves as a promising source for bioactive compounds. Separations 10 (7):395. https://doi.org/10.3390/separations10070395.

22. Khattab AM, Abo-Taleb HA, Abdelaziz AM, El-Tabakh MA, El-Feky MM, Abu-Elghait M (2022) Daphnia magna and Gammarus pulex, novel promising agents for biomedical and agricultural applications. Scientific Reports 12 (1):13690. https://doi.org/10.1038/s41598-022-17790-z

23. Liu D, Huang Q, Gu W, Zeng X-A (2022) A review of bacterial biofilm control by physical strategies. Critical Reviews in Food Science and Nutrition 62 (13):3453-3470. https://doi.org/10.1080/10408398.2020.1865872

24. Lotfy K (2020) The impact of the carrier gas composition of non-thermal atmospheric pressure plasma jet for bacteria sterilization. AIP Advances 10 (1). https://doi.org/10.1063/1.5099923

25. Morabit Y, Hasan MI, Whalley RD, Robert E, Modic M, Walsh JL (2021) A review of the gas and liquid phase interactions in low-temperature plasma jets used for biomedical applications. The European Physical Journal D 75:1-26. https://doi.org/10.1140/epjd/s10053-020-00004-4

26. Shehabeldine AM, Abdelaziz AM, Abdel-Maksoud MA, El-Tayeb MA, Kiani BH, Hussein AS (2024) Antimicrobial characteristics of endophytic Aspergillus terreus and acute oral toxicity analysis. Electronic Journal of Biotechnology 72:1-11. https://doi.org/10.1016/j.ejbt.2024.07.003

27. Weltmann KD, Brandenburg R, von Woedtke T, Ehlbeck J, Foest R, Stieber M, Kindel E (2008) Antimicrobial treatment of heat sensitive products by miniaturized atmospheric pressure plasma jets (APPJs). Journal of Physics D: Applied Physics 41 (19):194008. https://doi.org/10.1088/0022-3727/41/19/194008

28. Zhu T, Fu S, Xie W, Li F, Liu Y (2024) Comparison of inactivation characteristics of Escherichia coli and Staphylococcus aureus in water by rotary plasma jet sterilization. Environmental Technology & Innovation 36:103746. https://doi.org/10.1016/j.eti.2024.103746

29. Shi X, Liu S, Jiang R, Chen J, Jin S, Mei D, Zhou R, Fang Z, Cullen P.J. (2024) Development and characterization of touchable air plasma jet device for inactivation of oral bacteria. Results in Physics 36:105405. https://doi.org/10.1016/j.rinp.2022.105405

30. Maho T, Binois R, Brulé-Morabito F, Demasure M, Douat C, Dozias S, Bocanegra P.E, Goard I, Hocqueloux L, Helloco C.L, Orel I, Pouvesle J, Prazuck T, Stancampiano A, Tocaben C, Robert E (2021) Anti-Bacterial Action of Plasma Multi-Jets in the Context of Chronic Wound Healing. Appl. Sci. 11:9598. https://doi.org/10.3390/app11209598.

31. Valentina Puca V, Beatrice Marinacci B, Pinti M, Cintio F.D, Sinjari B, Marcantonio M.C.D, Mincione G, Acharya T.R, Kaushik N.K, Choi E.H, Sallese M, Guarnieri S, Grande R, Perrotti V (2024) Antimicrobial efficacy of direct air gas soft jet plasma for the in vitro reduction of oral bacterial biofilms. Scientific Reports 14:10882. https://doi.org/10.1038/s41598-024-61438-z.

32. Jurov A, Škoro N, Spasić K, Modic M, Hojnik N, Vujošević D, Đurović M, Petrović Z.L, Cvelbar U (2022) Helium atmospheric pressure plasma jet parameters and their influence on bacteria deactivation in a medium. Eur. Phys. J. D 76:29. https://doi.org/10.1140/epjd/s10053-022-00357-y.

33. Konchekov E.M, Gudkova V.V, Burmistrov D.E, Konkova A.S, Zimina M.A, Khatueva M.D, Polyakova V.A, Stepanenko A.A, Pavlik T.I, Borzosekov V.D, Malakhov D.V, Kolik L.V, Gusein-zade N, Gudkov S.V (2024) Bacterial Decontamination of Water-Containing Objects Using Piezoelectric Direct Discharge Plasma and Plasma Jet. Biomolecules 14:181. https://doi.org/10.3390/biom14020181.

التأثيرات المضادة للبكتيريا لبلازما الهيليوم الباردة ضد البكتيريا الموجبة والسالبة لصبغة جرام

محمود محمد اسماعيل[1]، تامر محمود السيد[1] ، وأمير محمد عيسى[1]، عامر مرسى عبدالعزيز[2] ، محمد حامد شرف[2]

[1]قسم الفيزياء، كلية العلوم(بنين)، جامعة الأزهر؛ [2]قسم النبات والميكروبيولوجى، كلية العلوم(بنين)، جامعة الأزهر

تمثل الهدف من هذه الدراسة الحالية قياس فاعلية وقدرة بلازما الضغط الجوي البارد (CAP) في مكافحة التلوث البكتيري. وقد تم تطبيقCAP كعلاج موضعي على أمل توفير طريقة أقل خطورة مقارنة بعمليات الإستئصال الجراحية. وقد تم اعداد نفاثة الهيليوم باستخدام ٣.٤ كيلو فولت وتردد ١٦.١ كيلو هرتز. تم إجراء اختبار النشاط المضاد للبكتريا بعد التعرض للبلازما على وسائط أجار مولر هينتون (MHA) للمكورات العنقودية الذهبية Staphylococcus aureus والعصيات المخية Bacillus cereus والإشريكية القولونية Escherichia coli ، ولكن تم استخدام وسائط أجار المكورات المعوية الانتقائية (BEA) (Bile Esculin Agar Media) للمكورات المعوية البرازية. أظهرت المعالجة ببلازما الضغط الجوي البارد (لمدة من الزمن عبارة عن١ و٢ و٣ دقائق) أن المعالجه البلازما ببلازما الضغط الجوي البارد يمكنها تثبيط والحد من نمو البكتيريا الممرضة الموجبة لصبغة جرام (المكورات المعوية البرازية، والمكورات العنقودية الذهبية، والعصيات المخية) وكذلك البكتيريا الساالبة لصبغة جرام (الإشريكية القولونية) و تتاثر كفائتها بشكل مباشر بالسلالات البكتيرية و زمن التعرض.

و كخلاصه فاننا نستطيع القول ان بلازما الضغط الجوي البارد كان لها تأثير و فاعلية مضادة للنشاط البكتيري و خاصه ضد البكتريا العصوية موجبة الجرام، والمكورات المعوية ، والمكورات العنقودية الذهبية، والمكورات العنقودية الذهبية، بالإضافة إلى البكتيريا سالبة الجرام الإشريكية القولونية.

الكلمات الدالة : بلازما الغلاف الجوي الباردة – بلازما جت – مضاد للبكتيريا– البكتيريا الموجبة لصبغة جرام – البكتيريا السالبه لصبغة جرام.