



## Egyptian Journal of Biophysics and Biomedical Engineering

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



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**T**HE 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].

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Received: 25/12/2024; Accepted: 4/1/2025

DOI: 10.21608/EJBBE.2024.336817.1078

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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.

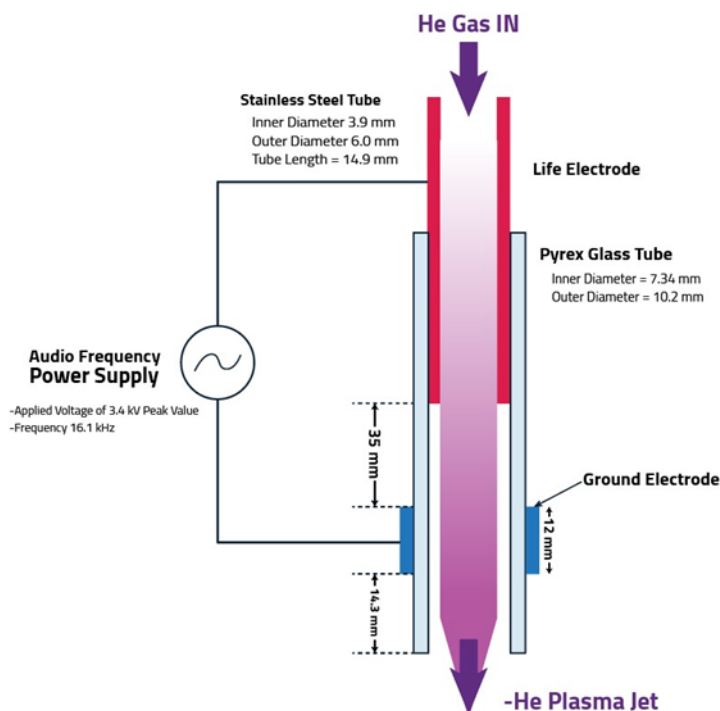


Fig. 1 diagram of cold atmospheric plasma (CAP) jet system.

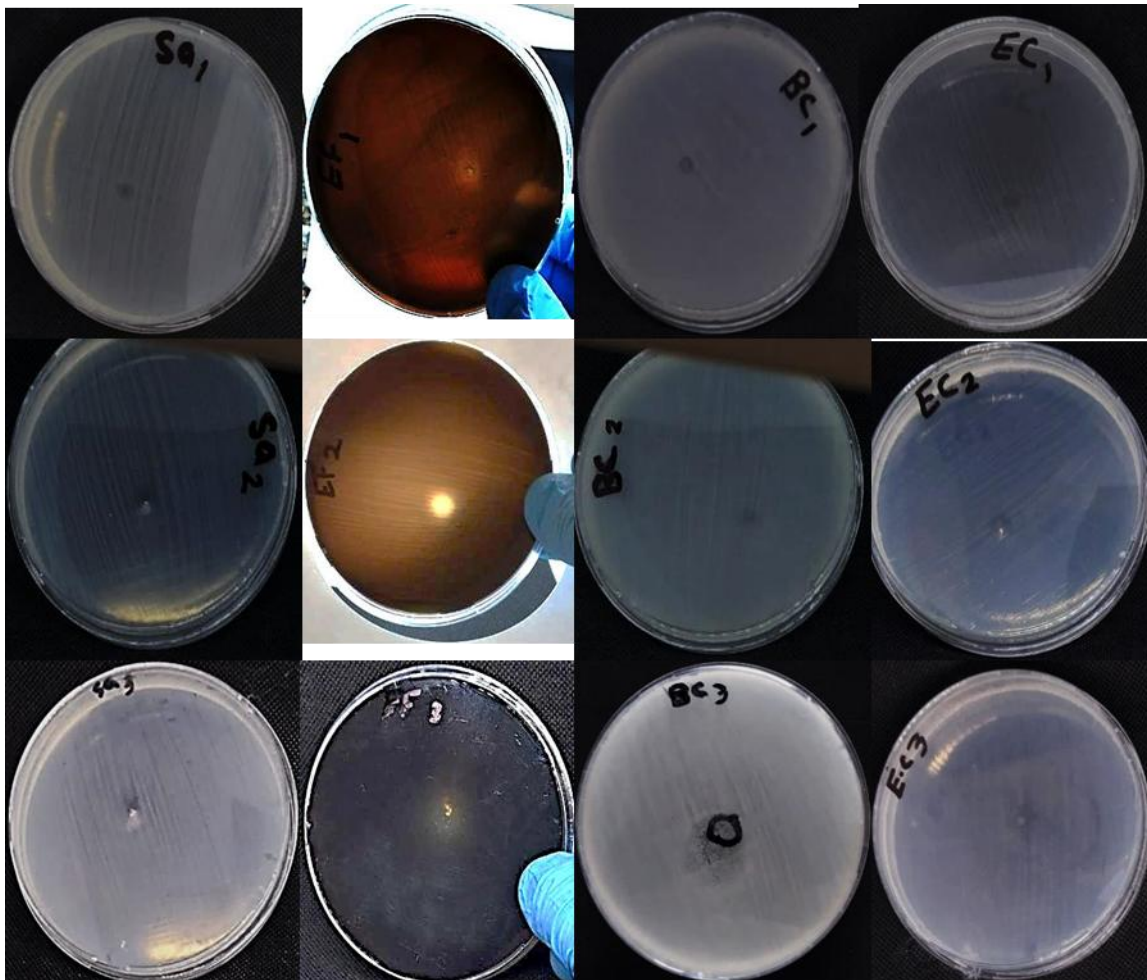
### Antibacterial studies

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°C. Afterwards, we measured the inhibition zones and recorded them in triplicate.

## Results

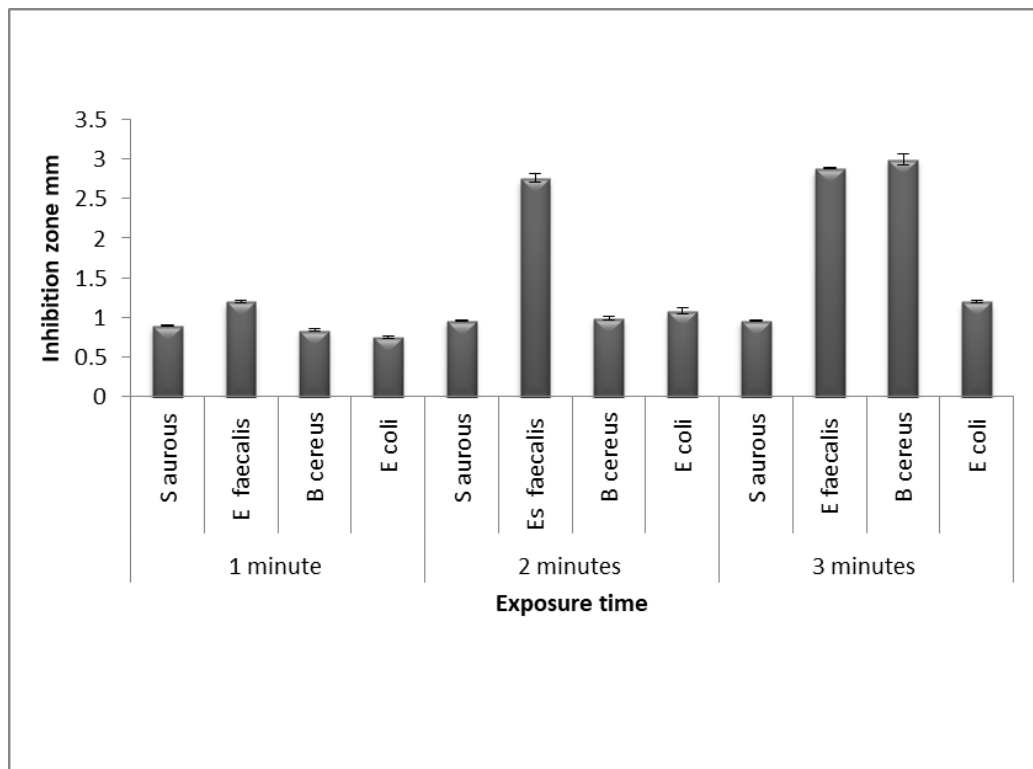
Figures 2 and 3 presented in this study demonstrate the efficacy of plasma jet in inhibiting the growth of various pathogenic bacteria, including Gram-positive strains such as *Enterococcus faecalis*, *Staphylococcus aureus*, and *Bacillus cereus*, as well as Gram-negative bacteria like *Escherichia 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  $1.2\pm 0.02$  mm,  $0.9\pm 0.01$  mm,  $0.84\pm 0.01$  mm, and  $0.85\pm 0.01$  mm for *Enterococcus faecalis*, *Staphylococcus aureus*, *Bacillus cereus*, and *Escherichia coli*, respectively.

Increasing the exposure time to two minutes resulted in higher inhibition values:  $2.76\pm 0.05$  mm,  $1.08\pm 0.04$  mm,  $0.99\pm 0.03$  mm, and  $0.96\pm 0.01$  mm for *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus cereus*, respectively. Finally, after three minutes of exposure, the inhibition values further increased to  $3.00\pm 0.07$  mm,  $2.88\pm 0.01$  mm,  $1.2\pm 0.02$  mm, and  $0.96\pm 0.01$  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  $0.84\pm 0.01$  mm to  $3.00\pm 0.07$  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.

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

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تمثل الهدف من هذه الدراسة الحالية قياس فاعلية وقدرة بلازما الضغط الجوي البارد (CAP) في مكافحة التلوث البكتيري. وقد تم تطبيق CAP كعلاج موضعي على أمل توفير طريقة أقل خطورة مقارنة بعمليات الإستئصال الجراحية. وقد تم اعداد نفائة الهيليوم باستخدام 3.4 كيلو فولت وتردد 16.1 كيلو هرتز. تم إجراء اختبار النشاط المضاد للبكتيريا 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 عن 1 و 2 و 3 دقائق) أن المعالجه البلازما ببلازما الضغط الجوي البارد يمكنها تثبيط والحد من نمو البكتيريا المررضة الموجبة لصبغة جرام (المكورات المعوية البرازية، والمكورات العنقودية الذهبية، والعصيات المخية) وكذلك البكتيريا السالبة لصبغة جرام (الإشريكية القولونية) و تتاثر كفاءتها بشكل مباشر بالسلاطات 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 البكتيريا العسوية 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 السالبة لصبغة جرام.