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Investigation of the effects of atmospheric pressure cold plasma on bacterial isolates

Atmosferik basınç soğuk plazmanın bakteri izolatları üzerine etkilerinin araştırılması

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ABSTRACT

Background: Atmospheric pressure cold plasma is a type of non-thermal plasma used for antimicrobial activity in the health, food and agriculture sectors. This study was carried out to investigate the efficacy of atmospheric pressure cold plasma on different bacterial isolates.
Materials and Methods: The study was conducted on standard reference bacterial strains; *Escherichia coli* (O157:H7), *Bacillus subtilis* (ATCC 6633), *Enterobacter aerogenes* (ATCC 13048), *Rhodococcus equi* (ATCC 6939), *Salmonella typhimurium* (ATCC 14028), *Enterococcus feacalis* (ATCC 2912) and field isolates; 12 *Escherichia coli* isolates, 22 *Staphylococcus spp*, 18 *Klebsiella spp*., 2 *Enterococcus spp*., 3 *Acinetobacter spp*., 8 *Candida spp*., 1 *Morgarella mongarii*, 2 *Corynebacterium spp*., 1 *Streptococcus pyogenes*. Atmospheric pressure cold plasma jet and plasma activated medium (PAM) were applied to the isolates and their efficacy was investigated.
Results: As a result of the study, it was found that cold plasma was effective against *Escherichia coli* (O157:H7), *Enterobacter aerogenes* (ATCC 13048), *Salmonella typhimurium, Rhodococcus equi* (ATCC 6939) and *Enterococcus feacalis* (ATCC 2912) among standard bacterial strains, while it was effective against *Streptococcus pyogenes* (group A Bet) and *Staphylococcus epidermidis* among field isolates.
Conclusions: As a result of our study, the antimicrobial activity of atmospheric pressure cold plasma and PAM was demonstrated, and we believe that it will contribute to the health, food and agriculture sectors.
Keywords: Atmospheric pressure cold plasma, Plasma activated media, Bacterial isolate, Microbial activity

ÖZET

Amaç: Atmosferik basınç soğuk plazma sağlık, gıda ve tarım sektöründe antimikrobiyal etkinliği için kullanılan termal olmayan plazma türüdür. Bu çalışma farklı bakteri izolatları üzerine atmosferik basınç soğuk plazmanın etkinliğinin araştırılması amacıyla gerçekleştirildi.
Materyal ve Metot: Çalışma standart referans bakteri suşu olan; *Escherichia coli* (O157:H7), *Bacillus subtilis* (ATCC 6633), *Enterobacter aerogenes* (ATCC 13048), *Rhodococcus equi* (ATCC 6939), *Salmonella typhimurium* (ATCC 14028), *Enterococcus feacalis* (ATCC 2912) ve saha izolatı olan 12 adet *Escherichia coli* izolatı, 22 adet *Staphylococcus* spp., 18 adet *Klebsiella* spp., 2 adet *Enterococcus* spp., 3 adet *Acinetobacter* spp., 8 adet *Candida* spp., 1 adet *Morgarella mongarii*, 2 adet *Corynebacterium* spp., 1 adet *Streptococcus pyogenes* üzerine gerçekleştirildi. İzolatlara atmosferik basınç soğuk plazma jeti ve plazma ile aktive edilmiş ortam (PAM) uygulandı ve etkinliği araştırıldı.
Bulgular: Çalışma sonucunda standart bakteri suşlarında *Escherichia coli* (O157:H7), *Enterobacter aerogenes* (ATCC 13048), *Salmonella typhimurium, Rhodococcus equi* (ATCC 6939) ve *Enterococcus feacalis* (ATCC 2912)'e soğuk plazmanın etki ettiği, saha izolatlarından ise *Streptococcus pyogenes* (A grubuBet) ile *Staphylococcus epidermidis*'e etki gösterdiği bulundu.
Sonuç: Çalışmamız sonucunda atmosferik basınç soğuk plazmanın ve PAM'ın antimikrobiyal etkinliği ortaya konuldu ve bu yönüyle sağlık, gıda ve tarım sektörlerine katkı sağlayacağını düşünmekteyiz.
Anahtar Kelimeler: Atmosferik basınç soğuk plazma, Plazma ile aktive edilmiş ortam, Bakteri izolatı, Mikrobiyal etkinlik

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INTRODUCTION

The fourth state of matter, the plasma state, results from the ionization of atoms and/or molecules. This state contains neutral particles, ultraviolet photons, electrons, and positive/negative ions. In addition, different free radicals and reactive species are formed depending on the type of ionized gas. In the laboratory environment, non-thermal plasmas produced by passing different gases through direct or alternating current at low temperature (<1000K) and 1 atm pressure are called atmospheric pressure cold plasma. In the medical field, this plasma is used either as a jet or as a dielectric barrier discharge. Atmospheric pressure cold plasma jets are plasmas that have the ability to leave the environment in which they are produced (Brun et al., 2012; Cha et al., 2014; Heinlin et al., 2011; Hoffmann et al., 2013; Hosseini et al., 2020; Laroussi, 2002; Vecchio et al., 2013). Atmospheric pressure cold plasma jet can be applied directly to the application area or indirectly by penetrating distilled water, 0.9% NaCl solution, organic substances and phosphate buffered saline solutions. This newly formed medium is called plasma-activated medium (PAM) (Cheng et al., 2020; Oztan et al., 2022).

The antimicrobial activity of atmospheric pressure cold plasma has been investigated in the literature and reported to be effective against a variety of bacteria, spores, biofilms, fungi, viruses through reactive oxygen species (ROS), lipid peroxidation, DNA / cell membrane damage (Bernhardt et al., 2019; Brun et al., 2012; Ermolaeva et al., 2011; Hosseini et al., 2020; Isbary et al., 2013). The antimicrobial activity of plasma was first demonstrated by Laroussi et al. in 1996 (Hoffmann et al., 2013). In the following years, attempts have been made to explain the antimicrobial mechanism of plasma, and many authors have reported that the main factor of this mechanism is due to ROS products such as oxygen, nitrogen dioxide and hydroxyl radicals formed as a result of plasma (Hoffmann et al., 2013).

Escherichia coli O157 is a common strain of *Escherichia coli* that causes severe gastroenteritis in human. It was first identified in 1982 and was found in the feces of healthy cattle. Food contaminated with feces, dairy products, animal contact, water and environmental factors have all been implicated as sources of transmission. It causes symptoms such as severe abdominal pain and bloody diarrhea in human (Mead et al., 1998; Pennington, 2010). *Bacillus subtilis* is a gram-positive, aerobic soil bacterium found in the natural environment (Oggioni et al., 1998). *Enterobacter aerogenes* is a gram-negative, antibiotic-resistant bacterium that affects the respiratory tract (Malléa et al., 2002). *Rhodococcus equi* is a bacterium that causes pneumonia in horses and immunocompromised humans (Hondalus et al., 1994). *Salmonella* is a bacterium that causes enteric infections in humans and animals (Branchu et al., 2018). *Enterococcus feacalis* is among the main components of the gastrointestinal flora and is resistant to antibiotics. It is considered a major source of nosocomial infections (McBride et al., 2007). *Staphylococcus species* can cause many different infections such as skin infections, endocarditis, nasal infections and urinary tract infections (Foster, 1996). *Klebsiella* is the second species found in the human gastrointestinal tract (Ristuccia et al., 1984). *Acinetobacter* is a gram-negative and antibiotic-resistant coccobacillus that is more common in temperate climates (Munoz-Price et al., 2008).

The aim of this study was to investigate the efficacy of atmospheric pressure cold plasma jet and PAM on bacterial isolates.

MATERIALS AND METHODS

Bacteria sampling

The study was carried out using standard bacterial strains from the culture collection of Karabük University Medical Faculty Medical Microbiology Laboratory and other field bacterial isolates available in the collection. Standard reference bacterial strains were *Escherichia coli* (O157:H7), *Bacillus subtilis* (ATCC 6633), *Enterobacter aerogenes* (ATCC 13048), *Rhodococcus equi* (ATCC 6939), *Salmonella typhimurium* (ATCC 14028), *Enterococcus feacalis* (ATCC 2912), and field isolates were 12 *Escherichia coli* isolates, 22 *Staphylococcus spp*, 18 *Klebsiella spp*., 2 *Enterococcus spp*., 3 *Acinetobacter spp*., 8 *Candida spp*., 1 *Morgarella mongarii*, 2 *Corynebacterium spp*., 1 *Streptococcus pyogenes*. Bacteria were inoculated onto blood agar and Mueller Hinton agar (MHA) to grow single colonies, and these single colonies were inoculated into Mueller Hinton broth (MHB) and kept in an oven at 37ºC overnight. The cultures were adjusted to McFarland 0.5 density and 1 ml dilution of the cultures adjusted to McFarland 0.5 density was obtained.

Atmospheric cold pressure plasma jet and plasma activated medium (PAM) generation

The atmospheric cold plasma jet was obtained by a setup with a manually adjustable flow meter (0-5 L/min, 5 L), 99% Ar cylinder, voltmeter, ammeter, plasma pen (heat resistant made of Boron glass), high voltage power supply, high voltage electrode, ground electrode, optical emission spectroscopy and gas armature. Ar (99%) was used as the discharge gas to obtain atmospheric pressure cold plasma. A flow meter with 0-5 L/min, 5 L interval settings was used to adjust the flow rate of Ar gas delivered to the plasma pen. A capacitive electrode design was used to initiate the discharge in the plasma pen. Borosilicate glass with a diameter of 4 mm was used as the discharge tube. Voltage and frequency values were measured using a high voltage probe. The radicals formed in the plasma were detected by optical emission spectrometry with a range of 360-920 nm (Figure 1). To generate the plasma, a sine wave signal with a frequency of 10kHz at a potential of 6kV was applied to the high voltage electrode of the plasma pen with capacitive electrode design. The total power of the system was measured as 10W.

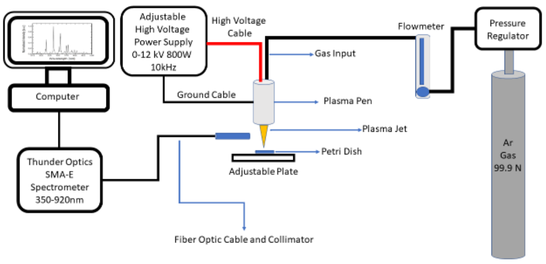

Figure 1. Atmospheric cold pressure plasma jet setup.

PAM was obtained by applying an atmospheric cold plasma jet to 5 ml of distilled water in a petri dish for 10 min and 30 min.

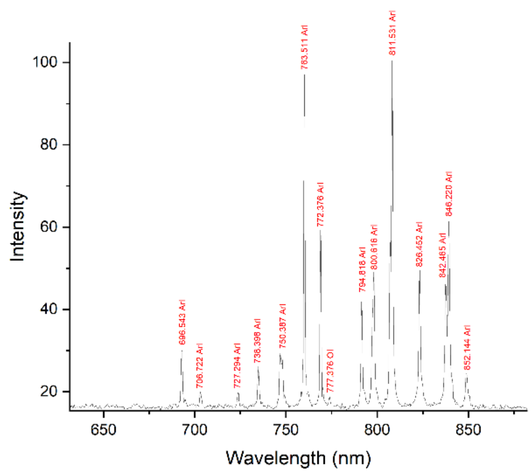

Figure 2. Optical emission spectrum of argon plasma.

Figure 2 shows the generation of spectral lines of argon plasma using an optical emission spectrometer. As a result of the plasma reaction, when ambient air was added to the discharge, Argon spectral lines were revealed due to O2 radicals and Ar gas.

Determination of antibacterial activity

Standard bacterial cultures inoculated in MHB and incubated overnight at 37ºC in an oven were adjusted by diluting the cultures to McFarland 0.5 density, and 1 ml dilution of the cultures adjusted to McFarland 0.5 was obtained. Atmospheric pressure cold plasma jet (10 min) was then applied directly to standard bacterial strains and field isolates. In addition, 200 µl of PAM was added to the standard strains for 10 min and 30 min separately for each reconstitution and incubated. After 3 hours, each tube was inoculated onto MHA plates and the plates were incubated in an oven. After overnight incubation, the plates were evaluated for the presence of growth.

RESULTS

The study was approved by the decision of the non-interventional local ethics committee of Karabük University, number 2022/1068, dated 29.09.2022. Atmospheric pressure cold plasma jet was applied to standard bacterial strains (*Escherichia coli*, *Bacillus subtilis*, *Enterobacter aerogenes*, *Rhodococcus equi*, *Salmonella typhimurium*, *Enterococcus feacalis*) for 10 min and the effect was obtained in *Escherichia coli* (O157:H7), *Enterobacter aerogenes* (ATCC 13048), *Salmonella typhimurium* (ATCC 14028) strains. The effect was obtained in *Rhodococcus equi* (ATCC 6939) as a result of 10 min PAM application to standard bacterial strains. The effect was obtained in *Rhodococcus equi* (ATCC 6939) and *Enterococcus feacalis* (ATCC 2912) as a result of 30 min PAM application to standard reference bacterial strains.

Atmospheric pressure cold plasma jet, which is most effective on standard bacterial strains, was also tested on field isolates for 10 min and was effective on *Streptococcus pyogenes* (group ABet) and *Staphylococcus epidermidis*. No effect was observed on other field isolates.

DISCUSSION

In this study investigating the efficacy of atmospheric pressure cold plasma jet and PAM on bacterial isolates, it was found that 10 min jet was effective against *Escherichia coli* (O157:H7), *Enterobacter aerogenes* (ATCC 13048) and *Salmonella typhimurium* in standard bacterial strains, while 10 min PAM was effective against *Rhodococcus equi* (ATCC 6939) and *Enterococcus feacalis* (ATCC 2912). In field isolates, 10 min jet was found to be effective against *Streptococcus pyogenes* (group ABet) and *Staphylococcus epidermidis*. We believe that the difference in efficacy between plasma jet and PAM is due to differences in bacterial resistance.

The antimicrobial efficacy of atmospheric pressure cold plasma has been the subject of research in dentistry, food industry, agriculture and medicine and positive effects have been observed (Balcı et al., 2021; Kandemir et al., 2021; Usta et al., 2017; Yüksel et al., 2017).

In dentistry, Li et al. used cold plasma against *Enterococcus faecalis* biofilms during root canal treatment and found that biofilms in the environment disappeared after 12 min (Li et al., 2015). Yang et al. reported that argon cold plasma inactivated *Streptococcus mutans* in the mouth in 15 s and *Lactobacillus acidophilus* in 5 min and can be used to deactivate oral bacteria (Yang et al., 2011).

Yong et al. examined the effectiveness of cold plasma against *Escherichia coli*, *Salmonella Typhimurium* and *Listeria monocytogenes* in the food industry and reported that the bacterial load decreased as a result of plasma application, and reported that this feature could be used to extend the shelf life of sliced cheese (Yong et al., 2015). Gurol et al. used cold plasma against *Escherichia coli* in raw milk and reported a 54% reduction in bacterial load after 3 min of application (Gurol et al., 2012).

It has been reported that cold plasma can be used in the agricultural sector for decontamination against *Escherichia coli, Staphylococcus aureus, Candida albicans* and *Bacillus subtilis* in seeds, fruits and vegetables (Kandemir et al., 2021).

Cold plasma has been reported to contribute to the reduction of the load of *Escherichia coli* and *Staphylococcus aureus* in the environment at different flow rates and durations in medical wound treatment (Usta et al.).

CONCLUSION

Our study shows that atmospheric pressure cold plasma jet and PAM affect different bacterial species. As such, it can be used effectively against common bacteria in the food, health, and agriculture sectors.

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