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Antibiotic Susceptibility Profile of Carbapenem-Resistant *Klebsiella pneumoniae* Isolates after Exposure to Non-Thermal Plasma

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ABSTRACT: ***Background:*** *Carbapenem-resistant Klebsiella pneumoniae (CRKP) should be considered an emerging clinical threat. Because these isolates are resistant to nearly all commonly used antibiotics, control of their spread is important.* ***Objective:*** *This study aimed to examine the impact of non-thermal plasma on the CRKP isolates from Iraqi patients.* ***Methods:*** *A total of 150 specimens were collected from different clinical sources. Thirty isolates of K. pneumoniae were obtained in this study. The antibiotic susceptibility testing was conducted using Kirby-Bauer's disk diffusion method before exposing the isolates to non-thermal plasma for 15, 30, 60, 90, and 120 seconds at a temperature of 37 °C for 24 hours.* ***Results:*** *The results of the study suggested that K. pneumoniae isolates exhibited differential antibiotic susceptibility following treatment with a nonthermal plasma. Both piperacillin, ceftazidime, and imipenem demonstrated increased efficacy 15 seconds after exposure with an inhibition rate of 100%, while ciprofloxacin and gentamicin showed optimal efficacy with inhibition rates of 97% and 90%, respectively. However, amikacin displayed resistant phenotypes at 15 seconds with an inhibition rate of 87%, and the inhibition rate with tetracycline and cefepime was the same at 83%; as for the time of 30 seconds and above, all nine tested antibiotics showed an inhibition rate of 100%.* ***Conclusions:*** *The results demonstrate the important impact of nonthermal plasma on the antibiotic susceptibility profiles of K. pneumoniae isolates. Understanding the role of nonthermal plasma in antibiotic resistance can help in developing more effective strategies for controlling infections caused by multi-resistant organisms. Further research is needed to clarify the underlying processes and their implications for treatment.*

KEYWORDS: *Nonthermal plasma; Carbapenem resistance; Klebsiella pneumoniae; Antibiotic; Bacteria; Antibacterial*

INTRODUCTION

Klebsiella pneumoniae is an ESKAPE pathogen that has a demonstrated high threat of causing life-threatening nosocomial contagion in critically ill and immune-compromised individuals [1]. The World Health Organization (WHO) stated that we are close to a post-antibiotic age when antibiotics cannot successfully deal with multidrug-resistant (MDR) bacterial disease [2]. The resistance mechanisms of beta-lactam include an efflux pump, reduced permeability, change transpeptidases, and inactivation by β-lactamases [3]. Antimicrobial resistance (AMR) is an inevitable evolutionary operation made by organisms out of genetic mutation to live in lethal selective pressure, especially antimicrobial agents [4].

Through the last few contracts, the global utilization of antibiotics has increased due to growing demand and population growth. The widespread use of antibiotics has led to the appearance of resistant bacteria (ARB). Antibiotic resistance indicates the capacity of bacteria post-sensitive to

antibiotics to resist their effects. Bacteria can become resistant to antibiotics by alternation in their chromosomal DNA, which changes the proteins they create, or via horizontal gene transfer (HGT), confer the transmission of new genetic material between independent bacteria [5], [6]. Many various mechanisms of intermediate MDR are involved in the evofelution of carbapenemases, AmpC enzymes, and ESβLs [1]. The occurrence of carbapenem-resistant *Enterobacteriaceae (CRE)* strains has been increasingly reported with extended clinical requests, and about 70%–90% of CRE strains are carbapenem-resistant *K. pneumoniae* (CRKp) in clinical practice [7]. Carbapenem-resistant *K. pneumoniae* (CRKP) infections demonstrate challenges in clinical administration as alternate treatment choices may be limited, leading to a high average of treatment failure and increased death [8]. An alternative technique to dealing with these resistant bacterium types is nonthermal plasma [9]. Plasma is considered the fourth case of substance; it has unique characteristics that recognize it from the following three essential states: solids, liquids, and gases. It is created through atoms that have undergone ionization, meaning that they have absent or obtained electrons, resulting in a mix of free electrons and ions [10]. Nonthermal plasma is a mixture of powerful local electric fields, short-lived and long-lived reactive oxygen, and nitrogen species (RONS), UV photons furthermore active electrons and ions at gas temperatures nigh to ambient temperature [11]. Among the most long-lived forms are (H_2O_2) and (HNO_2/NO_2^-), while ($^1O_2$) and (NO) belong to the second group. Their possible cellular goals are lipids, proteins, and DNA. Whole of these reactive forms can make, among alia, lipid peroxidation, protein destruction, and membrane disorder, finally resulting in cell death [12]. RONS contain powerful oxidizing agents (H_2O_2 and O_3) and reactive free radicals (NO, OH, and superoxide) that can harm different parts of bacterial organisms. An additional chain reaction in the cytoplasm oxidizes cellular proteins or microbe DNA, causing cell death [13], [14]. The mixture of those particles displays antimicrobial efficiency against a wide domain of microorganisms, such as bacteria, molds, yeasts, and even bacterial and fungal spores [15]. The current research was conducted to determine the effect of nonthermal plasma on the resistance rate of CRKP isolates periods of 15, 30, 60, 90, and 120 sec to several antimicrobial agents.

MATERIALS AND METHODS

Collection and Identification of *K. pneumoniae* Isolates

During a period between October to November 2023, 150 clinical specimens were collected from different clinical sources (urine, blood, burns, sputum, ear swabs, and stool) from Baghdad Teaching Hospital, Ghazi AL-Hariri Hospital for surgical specialties, Burns Hospital, and Al-Imamein Al-Kadhimaen Medical City. All clinical specimens were cultured primarily in enclosed containers containing nutrient agar then sub-cultured in brain heart infusion broth bottles and incubated at 37 °C for 24 hours to enrich the bacteria, then sub-cultured on MacConkey agar, Blood agar, and EMB agar. Phenotypic characteristics were determined by colony morphology on the culture media that different shapes, sizes, odors, colors, textures, opacity, and margins of colonies. Gram stain was used to detect and differentiate the form and arrangement of bacteria via microscopic examination. of biochemical examination were used for the identification *K. pneumoniae*. The identification of CRKP was confirmed by the VITEK-2 compact system.

Evaluation of the Effect of Nonthermal Plasma on *K. pneumoniae* Susceptibility to Antibiotics

The susceptibility of three *K. pneumonia* isolates (isolate from urine samples, isolate from wound swab, and isolate from blood) labeled 1, 2, and 3 respectively to different antibiotics at different times from exposure to non-thermal plasma (0, 15, 30, 60, 90, and 120) second were tested using Kirby Bauer's disk diffusion technique. Based on the standards set via the Clinical and Laboratory Standards Institute (CLSI) guidelines [16]. The bacterial isolates under examination were cultured on a brain heart agar medium and incubated for 24 h at 37 °C. Then standardized bacterial suspension from a few colonies of overnight culture, and the correction was set to 0.5 McFarland (1.5×10^8 CFU/ml). Then, the bacterial suspension was exposed to non-thermal plasma for 0.15, 30, 60, 90, and 120. The bacterial suspension was spread on Muller-Hinton agar plates using sterile cotton swabs in three directions. The sterile forceps placed antimicrobial discs on each Muller-Hinton agar plate; the plates were incubated at 37 °C for 24 hours. A metric ruler was used to measure the inhibitory zones that formed around the discs, and the measurements were taken in (mm).

Statistical Analysis

The study's data results were estimated to employ Excel Professional Plus 2021 (Microsoft Software, Inc.). All statistical analyses have significant $p<0.05$.

Source of Non-Thermal Plasma

The plasma jet device includes two copper metal electrodes. Figure 1 displays the experiential schematic of an atmospheric plasma jet device. The first electrode denoted the electrode with high voltage. An insulating layer surrounds a Pyrex glass tube that is 15 cm long and 1 mm thick. This tube comprises two nozzles: one for introducing the operator gas and another as an output nozzle with a diameter of 6 mm, which allows for the ejection of the plasma torch. The second electrode was positioned as a ground electrode on the outside surface of the nozzle. The high-voltage electrodes were connected to a high-power source with a direct current (DC) voltage of 5 KV. The gas discharge used argon gas with a purity of 99.99%. The gas flow was maintained at a constant rate of 3 liters per minute, regulated by a flow meter. The plasma jet propagated through the atmosphere, and the length of the plasma plume was about 20 mm.

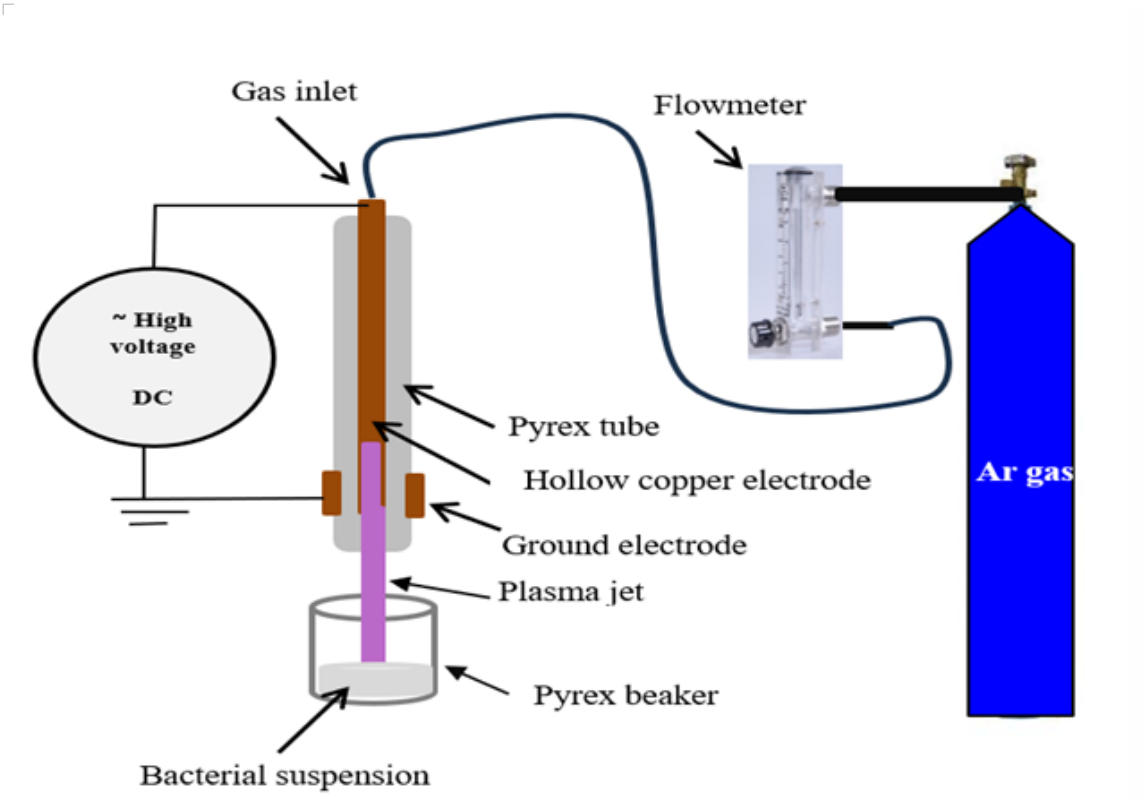

Figure 1. A schematic showing the setup of a non-thermal plasma jet device

RESULTS AND DISCUSSION

Source of the Clinical Isolates of *Klebsiella pneumoniae*

The clinical samples were obtained and collected from different sources, one hundred and fifty sources from Baghdad hospitals. Thirty *K. pneumoniae* isolates were recognized by employing colonies' appearance on culture media, microscopic examinations, biochemical examinations, and identification confirmed by the VITEK-2 compact system. According to the sources of infection, Table 1 describes the distribution of *K. pneumoniae* isolates used in this investigation.

Table 1. Distribution of *K. pneumoniae* isolates according to clinical source

Source of specimen	No. (%)
Urine	14 (46.6 %)
Blood	5 (16.6 %)
Burns	5 (16.6 %)
Sputum	3 (10 %)
Ear swabs	2 (6.6 %)
Stool	1 (3.3 %)

The high percentage of *K. pneumoniae* isolates from the urine samples and long-term use of catheters, particularly in hospitalized patients. This result is associated with an increased incidence of *K. pneumoniae* in urine samples, particularly in elderly women suffering from urinary tract infections [17].

The Effect of Non-Thermal Plasma and Antibiotics Against Bacterial Isolates

The current study investigated the effect of non-thermal plasma on three bacterial isolates' resistance, for bacterial isolates code No.1 and code No. 3 indicate that exposure to NTP for 15 seconds led to complete inhibition of the bacteria through the absence of bacterial growth as shown in Table 2.

Table 2. Antibiotic susceptibility of *K. pneumoniae* for isolate code No.1 and No. 3 to different antimicrobial agents utilizing Kirby Bauer's disk diffusion method after exposure to non-thermal plasma for 0 and 15 sec. The diameter ranges of the inhibition zone were calculated in millimeters via a ruler. According to the Clinical & Laboratory Standards Institute (CLSI) M100 33nd Edition 2023

Antimicrobial agent	Isolate code No. 1		Isolate code No. 3	
	Antibiotic	Antibiotic+ NTP	Antibiotic	Antibiotic+ NTP
Imipenem (IMI 10 μg)	R = 16	no growth	R = 18	no growth
Gentamicin (GN 10 μg)	R = 0	no growth	S = 21	no growth
Ciprofloxacin (CIP 5 μg)	R = 18	no growth	S = 24	no growth
Amikacin (AK 30 μg)	R = 0	no growth	S = 23	no growth
Tetracycline (TCG 15 μg)	S = 21	no growth	R = 20	no growth
Ceftazidime (CAZ 30 μg)	R = 0	no growth	R = 12	no growth
Pipracilline (PRL 100 μg)	R = 0	no growth	R = 0	no growth
Cefepime (FEM 30 μg)	R = 0	no growth	R = 0	no growth
Tigecycline (TE 30 μg)	S = 27	no growth	S = 23	no growth

As for bacterial isolation code B, the results shown in Table 3 and Figure 2 indicated that exposure to plasma for 15 seconds caused a clear increase in the inhibition rate of bacteria with different antibiotics. The inhibition zone of the piperacillin and ceftazidime antibiotics after exposure to non-thermal plasma NTP increases to 30 mm. Also, with Cefepime, Amikacin, and gentamicin antibiotics the inhibition increased from 0 mm to 25, 26, and 27 mm, respectively. Also, for the antibiotics of Imipenem and Ciprofloxacin the diameter of inhibition increased to 30 and 29 mm, respectively. The reason for the increased inhibition rate may be due to the combined action of the antibiotics in addition to the effect of the plasma, which led to an increased inhibition rate. No increase was recorded in the inhibitory effectiveness of the antibiotic's tetracycline and Tigecycline. As for the exposure times of 30, 60, 90, and 120 seconds. The growth of bacterial isolates has an inhibition rate of 100%. This indicates the complete and comprehensive elimination of bacteria at these times. Our results show a complete and total inhibition at 30 seconds, which is superior to the study [18], [19] which demonstrated the inhibition of bacteria with non-thermal plasma for a longer time.

Table 3. Antibiotic susceptibility profile of *K. pneumoniae* after exposure to non-thermal plasma for 0 and 15 sec

Antimicrobial agent	**Isolate code No. 2**	
	Antibiotic	**Antibiotic+NTP**
Imipenem (IMI 10μg)	R = 10	S = 30
Gentamicinμg (GN 10 μg)	R = 0	S = 27
Ciprofloxacin (CIP 5 μg)	R = 19	S = 29
Amikacin (AK 30 μg)	R = 0	S = 26
Tetracycline (TE 15 μg)	S = 25	S = 25
Ceftazidime (CAZ 30 μg)	R = 0	S = 30
Pipracilline (PRL 100 μg)	R = 0	S = 30
Cefepime (FEM 30 μg)	R = 0	S = 25
Tigecycline (TCG 30 μg)	S = 27	S = 27

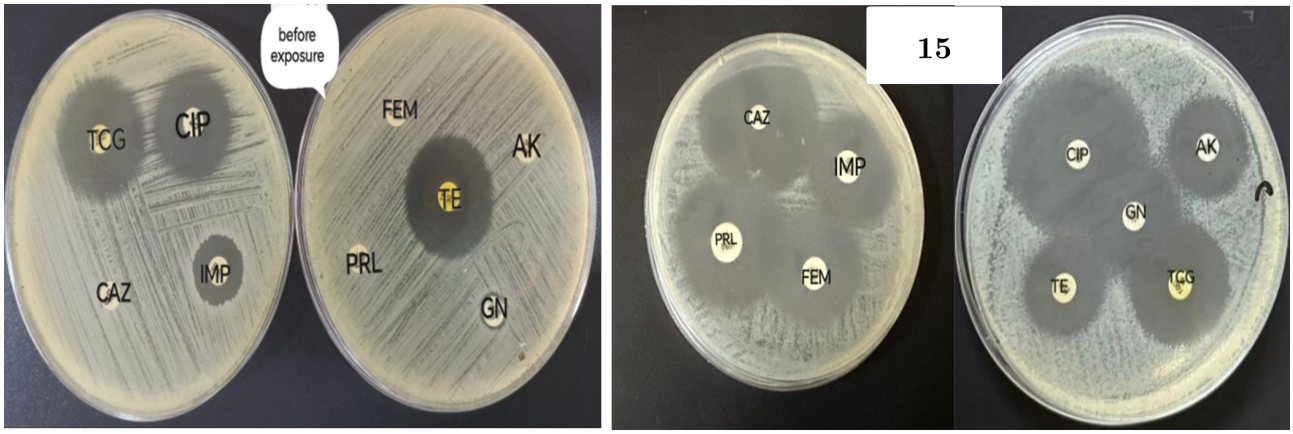

Figure 2. Inhibition zone of antibiotics before and after treated with non-thermal plasma at different treatment times

K. pneumoniae has developed resistance to carbapenem antibiotics, which has now spread globally. Unfortunately, over 50% of patients being treated for *K. pneumoniae* infections do not respond to carbapenem medications [20]. Carbapenems are a category of antibiotics frequently seen as the last option for treating severe bacterial infections, particularly those caused by germs resistant to several drugs [21]. One of the factors that affects its decontamination ability and mechanism is the duration of exposure to plasma.

This study was focused on the effect of nonthermal plasma exposure times of 15 seconds, exposure of more than 15 seconds led to the complete elimination of *K. pneumonia.* It would be impossible to detect the combined effect of nonthermal plasma and antibiotics if the exposure times result in a total decrease in the metabolic activity of bacterial cells. The efficacy of nonthermal plasma combined with antibiotics has been shown as a potential approach in the treatment of bacterial pathogens [22]. Plasma generates reactive oxygen and nitrogen radicals (RONS) such as OH, O, O_3, H_2O_2, NO, NO_2^-, NO_3^-, UV, ions, and charged particles [23]. The interactions between nonthermal plasma and live cells and tissues are mostly mediated by reactive oxygen and nitrogen species, as shown by previous studies [24]. These reactive radicals cause damage to the cellular composition, damage to proteins by oxidizing them, and also by damaging the DNA (deoxyribonucleic acid) of the cells thus leading to the denaturation of microbial cells [25]. RONS may play a significant role in bacterial inactivation by causing membrane damage to bacterial cells and interfering with lipid peroxidation events that disrupt the bonding of microbial cell structures [23]. According to previous studies, the antibacterial activities of nonthermal plasma are attributed to a synergistic interaction among ROS, RNS, and UV radiation [26], [27]. It is believed that free radicals (ROS or RNS) interact with DNA, proteins, and membrane lipids in several ways that lead to lipid peroxidation and disruption of enzyme activity [28], [29]. All these factors collectively result in the oxidation of several amino acids from proteins and alterations to the 3-dimensional structure of proteins. These variables appear in cells in a way that compromises their intracellular functioning. These cells can't keep their cytoplasmic

pH levels steady, have easily damaged membranes, and will ultimately become inactive because their intracellular contents leak out [26], [30]. Studying the impact of nonthermal plasma on sensitivity might provide valuable insights into the development of resistance in *K. pneumoniae*. It contributes to the formulation of strategies to avoid or overcome resistance. This investigation enhances our comprehension of the correlation between bacteria, antibiotics, and environmental variables in the treatment of diseases caused by pathogens.

CONCLUSION

K. pneumoniae susceptibility to antibiotics such as ciprofloxacin, imipenem, gentamicin, cefepime, ceftazidime, amikacin, tetracycline, and piperacillin may be significantly altered by non-thermal plasma. When selecting antibiotics to treat *K. pneumoniae* infections, it is helpful to be aware of the correlation between the action of non-thermal plasma and the efficacy of the drugs.

SUPPLEMENTARY MATERIAL

None.

AUTHOR CONTRIBUTIONS

Sanaa A. Hamza: Collected samples examined, and wrote the first draft of the manuscript. Raghad S. Mohammed and Mohammed F. Al Marjani: Conception and design of the study. Mounir M. Bekhit: Analyzed and interpreted the results.

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DATA AVAILABILITY STATEMENT

None.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

[1] M. Romance, M. U. Khan, M. S. Islam, M. F. Islam, and M. H. Haque, "Draft genome sequence of multidrug-resistant *Klebsiella pneumoniae* Hakim-RU strain isolated from a patient with urinary tract infections in Bangladesh," *Microbiology Resource Announcements*, vol. 13, no. 5, p. e0008924, 2024. doi: 10.1128/mra.00089-24.

[2] S. Yoo, K.-M. Lee, N. Kim, T. N. Vu, R. Abadie, and D. Yong, "Designing phage cocktails to combat the emergence of bacteriophage-resistant mutants in multidrug-resistant *Klebsiella pneumoniae*," *Microbiology Spectrum*, vol. 12, no. 1, p. e0125823, 2024. doi: 10.1128/spectrum.01258-23.

[3] T. Sawa, K. Kooguchi, and K. Moriyama, "Molecular diversity of extended-spectrum β-lactamases and carbapenemases, and antimicrobial resistance," *Journal of Intensive Care*, vol. 8, no. 1, p. 13, 2020. doi: 10.1186/s40560-020-0429-6.

[4] N. Medugu, I. A. Tickler, C. Duru, R. Egah, A. O. James, V. Odili, F. Hanga, E. K. Olateju, B. Jibir, B. E. Ebruke, G. Olanipekun, F. C. Tenover, and S. K. Obaro, "Phenotypic and molecular characterization of Beta-lactam resistant multidrug-resistant Enterobacterales isolated from patients attending six hospitals in Northern Nigeria," *Scientific Reports*, vol. 13, no. 1, p. 10 306, 2023. doi: 10.1038/s41598-023-37621-z.

[5] I. Courti, C. Muja, T. Maho, F. P. Sainct, and P. Guillot, "Degradation of bacterial antibiotic resistance genes during exposure to non-thermal atmospheric pressure plasma," *Antibiotics*, vol. 11, no. 6, p. 747, 2022. doi: 10.3390/antibiotics11060747.

[6] G. D. Wright, "Q&A: Antibiotic resistance: Where does it come from and what can we do about it?" *BMC Biology*, vol. 8, no. 1, p. 123, 2010. doi: 10.1186/1741-7007-8-123.

[7] Z. Xu, B. Li, Y. Jiang, J. Huang, L. Su, W. Wu, Q. Pang, Z. Li, J. Zhang, X. Li, J. Wang, F. Cen, L. Peng, J. Liang, F. Wang, C. Liu, C. Shen, Y. Liu, and Y. Yang, "Development of a quadruple qRT-PCR assay for simultaneous identification of hypervirulent and carbapenem-resistant *Klebsiella pneumoniae*," *Microbiology Spectrum*, vol. 12, no. 1, p. e0071923, 2024. doi: 10.1128/spectrum.00719-23.

[8] Q. Luo, L. Xu, Y. Wang, H. Fu, T. Xiao, W. Yu, W. Zhou, K. Zhang, J. Shen, J. Ji, C. Ying, and Y. Xiao, "Clinical relevance, mechanisms, and evolution of polymyxin B heteroresistance carbapenem-resistant *Klebsiella pneumoniae*: A genomic, retrospective cohort study," *Clinical Microbiology and Infection*, vol. 30, no. 4, pp. 507–514, 2024. doi: 10.1016/j.cmi.2024.01.014.

[9] B. Olayiwola, F. O'Neill, C. Frewen, D. F. Kavanagh, R. O'Hara, and L. O'Neill, "Cold plasma deposition of tobramycin as an approach to localized antibiotic delivery to combat biofilm formation," *Pathogens*, vol. 13, no. 4, p. 326, 2024. doi: 10.3390/pathogens13040326.

[10] M. I. Valdez-Narváez, M. T. Fernández-Felipe, A. Martinez, and D. Rodrigo, "Inactivation of Bacillus cereus spores and vegetative cells in inert matrix and rice grains using low-pressure cold plasma," *Foods*, vol. 13, no. 14, p. 2223, 2024. doi: 10.3390/foods13142223.

[11] A. Helmke, I. Curril, J. Mrotzek, J. Schulz, and W. Viöl, "Non-thermal plasma for decontamination of bacteria trapped in particulate matter filters: Plasma source characteristics and antibacterial potential," *Journal of Physics D: Applied Physics*, vol. 57, no. 26, p. 265 202, 2024. doi: 10.1088/1361-6463/ad35d3.

[12] J. Moszczyńska, K. Roszek, and M. Wiśniewski, "Non-thermal plasma application in medicine-focus on reactive species involvement," *International Journal of Molecular Sciences*, vol. 24, no. 16, p. 12 667, 2023. doi: 10.3390/ijms241612667.

[13] R. S. Mohammed and M. F. Al -Marjani, "DBD plasma as a practical approach to sterilization of dental instruments," *Physica Scripta*, vol. 99, no. 4, p. 045 601, 2024. doi: 10.1088/1402-4896/ad2e5a.

[14] U. Pundjung Juswono, D. J. D. H. Santjojo, R. A. Kasa, and M. Faisal, "Bacterial sterilization using non-thermal plasma method surface dielectric barrier discharge (SDBD): Effect of treatment duration on colony count, DNA, protease enzymes, and cell morphology," *Trends in Sciences*, vol. 20, no. 7, p. 6760, 2023. doi: 10.48048/tis.2023.6760.

[15] D. Battaggia, Y. Yao, M. N. Nierop Groot, T. Abee, and H. M. den Besten, "Impact of species and strain variability on non-thermal plasma decontamination efficacy," *Innovative Food Science & Emerging Technologies*, vol. 95, p. 103 674, Jul. 2024. doi: 10.1016/j.ifset.2024.103674.

[16] CLSI, "Performance standards for antimicrobial susceptibility testing," 2023, 33th edition. [Online]. Available: https://clsi.org/media/tc4b1paf/m10033_samplepages-1.pdf.

[17] N. Petrosillo, F. Taglietti, and G. Granata, "Treatment options for colistin-resistant *Klebsiella pneumoniae*: Present and future," *Journal of Clinical Medicine*, vol. 8, no. 7, p. 934, 2019. doi: 10.3390/jcm8070934.

[18] A. Lührmann, R. Matthes, and A. Kramer, "Impact of cold atmospheric pressure Argon plasma on antibiotic sensitivity of Methicillin-resistant *Staphylococcus aureus* strains *in vitro*," *GMS Hygiene and Infection Control*, vol. 11, Aug. 2016. doi: 10.3205/dgkh000277.

[19] L. Guo, R. Xu, Y. Zhao, D. Liu, Z. Liu, X. Wang, H. Chen, and M. G. Kong, "Gas plasma pre-treatment increases antibiotic sensitivity and persister eradication in methicillin-resistant *Staphylococcus aureus*," *Frontiers in Microbiology*, vol. 9, Mar. 2018. doi: 10.3389/fmicb.2018.00537.

[20] T. Gonçalves, A. T. Marques, V. Manageiro, L. Tanoeiro, J. S. Vital, A. Duarte, J. M. Vítor, M. Caniça, M. M. Gaspar, and F. F. Vale, "Antimicrobial activity of prophage endolysins against critical *Enterobacteriaceae* antibiotic-resistant bacteria," *International Journal of Pharmaceutics*, vol. 651, p. 123 758, Feb. 2024. doi: 10.1016/j.ijpharm.2023.123758.

[21] G. Guo, C. Guo, X. Qie, D. He, S. Meng, L. Su, S. Liang, S. Yin, G. Yu, Z. Zhang, X. Hua, and Y. Song, "Correlation analysis between Raman spectral signature and transcriptomic features of carbapenem-resistant *Klebsiella pneumoniae*," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 308, p. 123 699, Mar. 2024. doi: 10.1016/j.saa.2023.123699.

[22] J. Julák, E. Vaňková, M. Válková, P. Kašparová, J. Masák, and V. Scholtz, "Combination of non-thermal plasma and subsequent antibiotic treatment for biofilm re-development prevention," *Folia Microbiologica*, vol. 65, no. 5, pp. 863–869, 2020. doi: 10.1007/s12223-020-00796-3.

[23] Y. Li, L. Wei, J. Lin, Z. Xie, L. Lu, X. Pan, J. Xu, and R. Cai, "Nonthermal plasma air disinfection for the inactivation of airborne microorganisms in an experimental chamber and indoor air," *Journal of Applied Microbiology*, vol. 135, no. 4, lxae078, 2024. doi: 10.1093/jambio/lxae078.

[24] E. Vaňková, M. Válková, P. Kašparová, J. Masák, V. Scholtz, J. Khun, and J. Julák, "Prevention of biofilm re-development on Ti-6Al-4V alloy by cometary discharge with a metallic grid," *Contributions to Plasma Physics*, vol. 59, no. 2, pp. 166–172, 2018. doi: 10.1002/ctpp.201800044.

[25] K. Paul, P. Pandey, U. Rathore, and N. N. Gowda, "Applications of cold plasma technology in the food industry," in *Advanced Research Methods in Food Processing Technologies*, Apple Academic Press, 2024, pp. 23–37.

[26] J.-W. Lackmann and J. E. Bandow, "Inactivation of microbes and macromolecules by atmospheric-pressure plasma jets," *Applied Microbiology and Biotechnology*, vol. 98, no. 14, pp. 6205–6213, 2014. doi: 10.1007/s00253-014-5781-9.

[27] H. Lu, S. Patil, K. Keener, P. Cullen, and P. Bourke, "Bacterial inactivation by high-voltage atmospheric cold plasma: Influence of process parameters and effects on cell leakage and DNA," *Journal of Applied Microbiology*, vol. 116, no. 4, pp. 784–794, 2014. doi: 10.1111/jam.12426.

[28] V. Scholtz, E. Vaňková, P. Kašparová, R. Premanath, I. Karunasagar, and J. Julák, "Non-thermal plasma treatment of ESKAPE pathogens: A review," *Frontiers in Microbiology*, vol. 12, p. 737 635, Oct. 2021. doi: 10.3389/fmicb.2021.737635.

[29] A. Privat-Maldonado, D. O'Connell, E. Welch, R. Vann, and M. W. van der Woude, "Spatial dependence of DNA damage in bacteria due to low-temperature plasma application as assessed at the single cell level," *Scientific Reports*, vol. 6, no. 1, p. 35 646, 2016. doi: 10.1038/srep35646.

[30] J. A. Delben, C. E. Zago, N. Tyhovych, S. Duarte, and C. E. Vergani, "Effect of atmospheric-pressure cold plasma on pathogenic oral biofilms and *in vitro* reconstituted oral epithelium," *PLOS ONE*, vol. 11, no. 5, p. e0155427, 2016. doi: 10.1371/journal.pone.0155427.