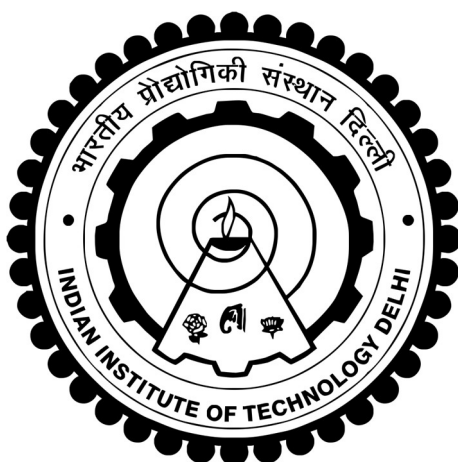


**ANTIMICROBIAL EFFICACY OF COLD ATMOSPHERIC  
PRESSURE PLASMA JET AGAINST CLINICAL BACTERIAL  
ISOLATES**

**SARTHAK DAS**



**SCHOOL OF INTERDISCIPLINARY RESEARCH  
INDIAN INSTITUTE OF TECHNOLOGY DELHI**

**FEBRUARY 2025**

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*by*

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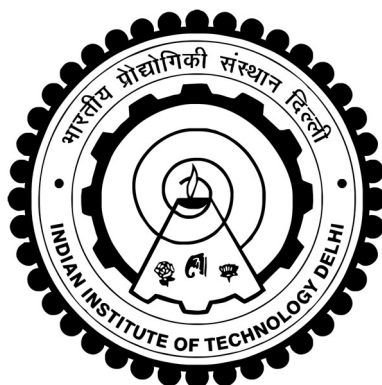
**School of Interdisciplinary Research**

*Submitted*

*in fulfilment of the requirements of the degree of*

**DOCTOR OF PHILOSOPHY**

*to the*



**INDIAN INSTITUTE OF TECHNOLOGY DELHI**

**FEBRUARY 2025**

*Dedicated to*

*Budha Bapa*

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# *Undertaking*

---

I hereby declare that the work presented here in the thesis has been carried out by me towards the partial fulfilment of the requirement for the award of the degree of **Doctor of Philosophy** at the Indian Institute of Technology Delhi. The content of this report, in full or in parts, have not been submitted to any other institute or university for the award of any degree.

*Sarthak Das*

Sarthak Das

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## *Certificate*

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This is to certify that the thesis entitled, “**Antimicrobial efficacy of cold atmospheric pressure plasma jet against clinical bacterial isolates**”, submitted by **Mr. Sarthak Das**, to the Indian Institute of Technology Delhi for the award of the degree of **Doctor of Philosophy**, is a bonafide record of the research work done by him under our supervision.

In our opinion, the thesis has reached the standard of fulfilling the requirement of all the regulations regarding the degree. The results contained in this thesis have not been submitted, in part or in full, to any other university or institute to award any degree or diploma.

*Satyananda Kar*

**Prof. Satyananda Kar**  
**Department of Energy Science**  
**and Engineering**  
**IIT Delhi, New Delhi, India**



**Prof. Sarita Mohapatra**  
**Department of Microbiology**  
**AIIMS New Delhi,**  
**New Delhi, India**



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**Sarthak Das**

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## *Abstract*

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The alarming rise of antimicrobial resistance (AMR) necessitates the development of novel antimicrobial strategies. In this aspect, cold atmospheric pressure plasma jet (CAPJ) has sparked research interest for its novel antimicrobial properties and has found its applicability in sterilization, disinfection, and decontamination. The CAPJ discharges are composed of reactive species such as reactive oxygen and nitrogen species (RONS), electrons, ions, and excited atoms and molecules, which incorporate an antimicrobial nature. Although CAPJ has proven highly efficient in bacterial load reduction, certain unresolved problems must be addressed systematically to establish CAPJ as a prominent antimicrobial technique. Firstly, CAPJ's efficacy against clinically isolated multidrug-resistant (MDR) bacteria has not been adequately studied. Secondly, to achieve optimal productivity, it is imperative to systematically characterize the developed CAPJ device based on operational parameters concerning antimicrobial activity against MDR isolates. Thirdly, it is important to assess the time-dependent bacterial inactivation process that CAPJ reactive species follow. Fourthly, there has been limited studies on CAPJ's efficacy in eliminating bacterial load from inanimate surfaces, along with any alteration in surface property. Lastly, there is a dearth of research comparing the antimicrobial activity of various CAPJ activated liquids (CAPJALs) and comprehending the specific parameters required for enhanced efficacy. The goals of the current study were to address the aforementioned problems.

In the thesis, CAPJ powered by a low-frequency AC source was designed and developed for antimicrobial applications. A thorough investigation of the CAPJ's operational parameters (intrinsic and extrinsic) and their influence on discharge characteristics, reactive species composition, and antimicrobial activity was carried out. Various reactive species such as excited atoms (Ar I, He I, O I, etc.), ions ( $\text{Ar}^+$ ,  $\text{N}_2^+$ ,  $\text{N}^+$ , etc.), radical RONS ( $\text{OH}\cdot$ ), and non-radical RONS ( $\text{OH}^+$ ,  $\text{NO}^+$ ,  $\text{N}_2\text{O}_3^-$ ,  $\text{NO}_3^-$ , etc.) were observed to vary in the discharge depending on the intrinsic settings (voltage, frequency, gas flow rate, and operating gas). This variance in reactive species in relation to intrinsic settings was associated with varying bacterial inactivation area. Extrinsic parameters such as exposure distance, exposure time, bacterial concentration, and type of target bacteria also influenced the antimicrobial activity of CAPJ.

The study also examined the induced probable bacterial inactivation path by Ar CAPJ. A greater than 6  $\log_{10}$  reduction of *E. coli* and *S. aureus* within 60 and 120 s CAPJ exposure was noticed, respectively, along with low *D*- values. The correlation between the inactivation curve

and the time-dependent damage to bacterial cells revealed varying inactivation processes among isolates. The alteration of Fourier transform infrared spectra (FTIR) and Raman microspectra signals of post-CAPJ exposed bacteria demonstrated the degree of destruction at the molecular level, such as lipid peroxidation, protein oxidation, bond breakages, etc. Further, the transmission electron microscopy (TEM) images of exposed bacteria indicated incurred damages on cell morphology by CAPJ reactive species.

A greater than  $5 \log_{10}$  reduction of *E. coli* and  $\sim 3.4 - 4.6 \log_{10}$  reduction of *S. aureus* along with *D*- value in the range 27 – 63 s was observed over the material test surfaces on Ar CAPJ exposure. In addition, the study examined the effect of repeated CAPJ exposure on surface property by replicating hospital surface decontamination. A non-linear variation in the surface properties, such as wettability, roughness, and elemental composition was noticed. Specifically, the CAPJ reactive species would play a significant role in bacterial load reduction and any surface property alteration occurring.

The Ar CAPJ was used to activate liquids such as deionized water (DI-W), drinking water (DW), tap water (TW), and normal saline (NS), and its antimicrobial property was evaluated against *E. coli* and *S. aureus*. The computed *D*- value followed the trend – DI-W  $\approx$  NS > DW > TW. An optimal setting for liquid activation by CAPJ and CAPJAL – bacterial interaction time was noticed to achieve higher bactericidal efficacy. Further, the pace at which the physicochemical parameters (electrical conductivity (EC), total dissolved solids (TDS), pH, and reactive species concentration ( $\text{H}_2\text{O}_2$ ,  $\text{NO}_3^-$ , and  $\text{NO}_2^-$ )) changed within the liquid differed. The interaction of CAPJ reactive species with the liquid would lead to the generation of liquid phase reactive species ( $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{H}^+$ ,  $\text{H}_2\text{O}_2$ , etc.), contributing to physicochemical properties variation and incorporation of antimicrobial nature.

An in-depth grasp of controlling CAPJ parameters involving antimicrobial activity and the emergence of unique pathways for bacterial inactivation could be advanced by this research. The study on CAPJ's efficiency in decontaminating inanimate surfaces and CAPJALs insights CAPJ to be used as a point-of-care antimicrobial technique. The findings of this thesis would be a great asset to multidisciplinary researchers.

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# सार

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रोगाणुरोधी प्रतिरोध (AMR) की चिंताजनक वृद्धि के कारण नवीन रोगाणुरोधी रणनीतियों के विकास की आवश्यकता है। इस पहलू में, ठंडे वायुमंडलीय दबाव प्लाज्मा जेट (CAPJ) ने अपने उपन्यास रोगाणुरोधी गुणों के लिए अनुसंधान रुचि जगाई है और जीवाणु-नाशन, कीटाणुशोधन और शुद्धीकरण में इसकी प्रयोज्यता पाई है। CAPJ डिस्चार्ज प्रतिक्रियाशील प्रजातियों जैसे प्रतिक्रियाशील ऑक्सीजन और नाइट्रोजन प्रजातियों (RONS), इलेक्ट्रॉनों, आयनों और उत्तेजित परमाणुओं और अणुओं से बने होते हैं, जिनमें रोगाणुरोधी प्रकृति शामिल होती है। हालाँकि CAPJ बैक्टीरिया के भार को कम करने में अत्यधिक कुशल साबित हुआ है, CAPJ को एक प्रमुख रोगाणुरोधी प्रविधि के रूप में स्थापित करने के लिए कुछ अनसुलझे समस्याओं को व्यवस्थित रूप से संबोधित किया जाना चाहिए। सबसे पहले, चिकित्सकीय रूप से पृथक मल्टीड्रग-प्रतिरोधी (MDR) बैक्टीरिया के खिलाफ CAPJ की प्रभावकारिता का पर्याप्त अध्ययन नहीं किया गया है। दूसरे, इष्टतम उत्पादकता प्राप्त करने के लिए, MDR आइसोलेट्स के खिलाफ रोगाणुरोधी गतिविधि से संबंधित परिचालन मापदंडों के आधार पर विकसित CAPJ डिवाइस को व्यवस्थित रूप से चिह्नित करना अनिवार्य है। तीसरा, समय-निर्भर जीवाणु निष्क्रियता प्रक्रिया का आकलन करना महत्वपूर्ण है जिसका CAPJ प्रतिक्रियाशील प्रजातियाँ पालन करती हैं। चौथा, सतह की संपत्ति में किसी भी बदलाव के साथ-साथ निर्जीव सतहों से बैक्टीरिया भार को खत्म करने में CAPJ की प्रभावकारिता पर सीमित अध्ययन हुए हैं। अंत में, विभिन्न CAPJ सक्रिय तरल पदार्थों (CAPJAL) की रोगाणुरोधी गतिविधि की तुलना करने और बढ़ी हुई प्रभावकारिता के लिए आवश्यक विशिष्ट मापदंडों को समझने वाले शोध की कमी है। वर्तमान अध्ययन का लक्ष्य उपरोक्त समस्याओं का समाधान करना था।

थीसिस में, कम आवृत्ति वाले एसी स्रोत द्वारा संचालित CAPJ को रोगाणुरोधी अनुप्रयोगों के लिए डिजाइन और विकसित किया गया था। CAPJ के परिचालन मापदंडों (आंतरिक और बाहरी) और डिस्चार्ज विशेषताओं, प्रतिक्रियाशील प्रजातियों की संरचना और रोगाणुरोधी गतिविधि पर उनके प्रभाव की गहन जांच की गई। विभिन्न प्रतिक्रियाशील प्रजातियाँ जैसे उत्तेजित परमाणु (Ar I, He I, O I, आदि), आयन (Ar<sup>+</sup>, N<sub>2</sub><sup>+</sup>, N<sup>+</sup>, आदि), रेडिकल RONS (OH•), और नॉनरेडिकल RONS (OH<sup>+</sup>, NO<sup>+</sup>, N<sub>2</sub>O<sub>3</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>, आदि) को आंतरिक सेटिंग्स (वोल्टेज, आवृत्ति, गैस प्रवाह दर और परिचालन गैस) के आधार पर डिस्चार्ज में भिन्न देखा गया। आंतरिक सेटिंग्स के संबंध में प्रतिक्रियाशील प्रजातियों में यह भिन्नता विभिन्न जीवाणु निष्क्रियता क्षेत्रों से जुड़ी थी। बाहरी पैरामीटर जैसे एक्सपोजर दूरी, एक्सपोजर समय, बैक्टीरिया एकाग्रता और लक्ष्य बैक्टीरिया के प्रकार ने भी CAPJ की रोगाणुरोधी गतिविधि को प्रभावित किया।

अध्ययन में Ar CAPJ द्वारा प्रेरित संभावित जीवाणु निष्क्रियता पथ की भी जांच की गई। क्रमशः 60 और 120 सेकेंड CAPJ एक्सपोजर के भीतर *E. coli* और *S. aureus* में  $6 \log_{10}$  से अधिक की कमी देखी गई, साथ ही कम *D-value* भी। निष्क्रियता वक्र और जीवाणु कोशिकाओं को समय-निर्भर क्षति के बीच सहसंबंध से आइसोलेट्स के बीच अलग-अलग निष्क्रियता प्रक्रियाओं का पता चला। पोस्ट- CAPJ एक्सपोजर बैक्टीरिया के फूरियर-ट्रंसफॉर्म इंफ्रारेड स्पेक्ट्रा (FTIR) और रमन माइक्रो-स्पेक्ट्रा संकेतों के परिवर्तन ने आणविक स्तर पर विनाश की परिमाण का प्रदर्शन किया, जैसे कि लिपिड पेरोक्सीडेशन, प्रोटीन ऑक्सीकरण, बंधन टूटना, आदि। इसके अलावा, संचरण उजागर बैक्टीरिया की इलेक्ट्रॉन माइक्रोस्कोपी (TEM) छवियां CAPJ प्रतिक्रियाशील प्रजातियों द्वारा कोशिका आकृति विज्ञान पर हुए नुकसान का संकेत देती हैं।

Ar CAPJ एक्सपोजर पर सामग्री परीक्षण सतहों पर 27 - 63 s की सीमा में *D-value* के साथ-साथ *E. coli* की  $5 \log_{10}$  से अधिक कमी और *S. aureus* की  $\sim 3.4 - 4.6 \log_{10}$  कमी देखी गई। इसके अलावा, अध्ययन ने अस्पताल की सतह के परिशोधन की नकल करके सतह की संपत्ति पर बार-बार CAPJ एक्सपोजर के प्रभाव की जांच की। सतह के गुणों जैसे गीलापन, खुरदरापन और मौलिक संरचना में एक गैर-रैखिक भिन्नता देखी गई। विशेष रूप से, CAPJ प्रतिक्रियाशील प्रजातियां जीवाणु भार में कमी और किसी भी सतह संपत्ति परिवर्तन में महत्वपूर्ण भूमिका निभाएंगी।

Ar CAPJ का उपयोग विआयनीकृत पानी (DI-W), पीने के पानी (DW), नल का पानी (TW), और सामान्य खारा (NS) जैसे तरल पदार्थों को सक्रिय करने के लिए किया गया था, और इसकी रोगाणुरोधी संपत्ति का मूल्यांकन *E. coli* और *S. aureus* के खिलाफ किया गया था। परिकल्पित *D-value* प्रवृत्ति का अनुसरण करता है -  $DI-W \approx NS > DW > TW$ । CAPJ और CAPJAL द्वारा तरल सक्रियण के लिए एक इष्टतम सेटिंग - उच्च जीवाणुनाशक प्रभावकारिता प्राप्त करने के लिए जीवाणु संपर्क समय देखा गया। इसके अलावा, जिस गति से तरल के भीतर भौतिक रसायन पैरामीटर (विद्युत चालकता (EC), कुल घुलनशील ठोस (TDS), pH, और प्रतिक्रियाशील प्रजातियों की एकाग्रता ( $H_2O_2$ ,  $NO_3^-$ , और  $NO_2^-$ ) में बदलाव आया, वह अलग-अलग था। तरल के साथ CAPJ प्रतिक्रियाशील प्रजातियों की परस्पर क्रिया से तरल चरण प्रतिक्रियाशील प्रजातियों ( $NO_3^-$ ,  $NO_2^-$ ,  $H^+$ ,  $H_2O_2$ , आदि) की उत्पत्ति होगी, जो भौतिक रासायनिक गुणों में भिन्नता और रोगाणुरोधी प्रकृति के समावेश में योगदान करेगी।

इस शोध से रोगाणुरोधी गतिविधि से जुड़े CAPJ मापदंडों को नियंत्रित करने और जीवाणु निष्क्रियता के लिए अद्वितीय मार्गों के उद्भव की गहराई से समझ विकसित की जा सकती है। निर्जीव सतहों और CAPJALs को कीटाणुरहित करने में CAPJ की दक्षता पर अध्ययन से पता चलता है कि CAPJ को एक

बिंदु-देखभाल रोगाणुरोधी तकनीक के रूप में उपयोग किया जा सकता है। इस थीसिस के निष्कर्ष बहु-विषयक शोधकर्ताओं के लिए एक बड़ी संपत्ति होंगे।



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## *List of abbreviations*

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- AMR:** Antimicrobial resistance
- WHO:** World Health Organization
- MDR:** Multidrug-resistant
- CAP:** Cold atmospheric pressure plasma
- RONS:** Reactive oxygen and nitrogen species
- DBD:** Dielectric barrier discharge
- CAPJ:** Cold atmospheric pressure plasma jet
- SMD:** Surface microdischarge
- RF:** Radiofrequency
- MW:** Microwave
- DC:** Direct current
- AC:** Alternating current
- CFU:** Colony forming units
- lpm:** liters per minute
- D- value:** Decimal reduction time
- DNA:** Deoxyribonucleic acid
- CAPAL:** Cold atmospheric pressure plasma activated liquid
- DI-W:** De-ionized water
- OES:** Optical emission spectroscopy
- FTIR:** Fourier transform infrared spectroscopy
- TEM:** Transmission electron microscopy
- CAPJAL:** Cold atmospheric pressure plasma jet activated liquid
- AST:** Antimicrobial susceptibility testing
- CLSI:** Clinical and Laboratory Standards Institute
- MF:** McFarland standards
- MHA:** Mueller-Hinton agar
- IR:** Infrared
- GNB:** Gram-negative bacillus
- GPC:** Gram-positive cocci
- rpm:** revolutions per minute
- ANOVA:** Analysis of variance

**AFM:** Atomic force microscopy  
**RMS:** Root mean square  
**SEM:** Scanning electron microscopy  
**EDX:** Energy dispersive X-ray  
**MBMS:** Molecular Beam Mass Spectrometer  
**PG:** Peptidoglycan  
**SS:** Stainless steel  
**Al:** Aluminium  
**TW:** Tap water  
**NS:** Normal saline  
**TDS:** Total dissolved solids  
**ISA:** Ionic strength adjuster

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## *List of Notations*

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**T<sub>e</sub>**: Electron temperature

**T<sub>i</sub>**: Ion temperature

**T<sub>g</sub>**: Gas temperature

**P<sub>a</sub>**: Average plasma discharge power

**V**: Voltage

**Q**: Charge

**Y<sub>i</sub>**: Count intensity of specific ion

**f**: Frequency

**t**: CAPJ exposure time

**d**: CAPJ exposure distance

**C**: Bacterial concentration

**T<sub>t</sub>**: Target temperature

**T<sub>p</sub>**: Plume temperature

**at. %**: Atomic percentage

**P<sub>app</sub>**: Average applied power



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## *List of bacteria*

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- E. faecium: Enterococcus faecium*  
*S. aureus: Staphylococcus aureus*  
*K. pneumoniae: Klebsiella pneumoniae*  
*A. baumannii: Acinetobacter baumannii*  
*P. aeruginosa: Pseudomonas aeruginosa*  
*E. coli: Escherichia coli*  
*B. stearothermophilus: Bacillus stearothermophilus*  
*B. subtilis: Bacillus subtilis*  
*M. luteus: Micrococcus luteus*  
*S. typhimurium: Salmonella typhimurium*  
*L. monocytogenes: Listeria monocytogenes*  
*E. cloacae: Enterobacter cloacae*  
*E. faecalis: Enterococcus faecalis*  
*S. epidermidis: Staphylococcus epidermidis*  
*B. atrophaeus: Bacillus atrophaeus*  
*B. pumilus: Bacillus pumilus*  
*S. heidelberg: Salmonella heidelberg*  
*B. cereus: Bacillus cereus*  
*L. innocua: Listeria innocua*  
*A. hydrophila: Aeromonas hydrophila*  
*S. putrefaciens: Shewanella putrefaciens*  
*P. fluorescens: Pseudomonas fluorescens*  
*V. cholerae: Vibrio cholerae*