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Experimental studies on cold atmospheric pressure argon plasma jet as a tool for antibacterial activity

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In recent days, cold atmospheric pressure plasmas are gaining attention due to their chemical features suitable for various biomedical applications^[1]. The present study aimed to evaluate the use of cold plasma as an effective disinfectant against Multidrug resistant (MDR) organisms without altering the experimental input parameters. For this, a portable cold atmospheric pressure argon plasma jet source is developed. With an application of a sinusoidal high voltage of 6 kV_{p-p}, 25 kHz frequency, and 3 lpm Argon feed gas produces a plasma jet of length ~ 2.5 cm in the ambient air. The developed plasma jet is characterized thoroughly using voltage and current probes for electrical discharge parameters and utilizing optical emission spectra for the estimation of plasma parameters (electron excitation temperature (T_{exc}) , electron density (n_e) , and for identification of reactive species present in the plasma (OH, N2⁺, O, etc). The obtained plasma parameters T_{exc} and n_e are in the order of 0.3 eV and 2 x 10¹¹ cm⁻³ respectively. Further, the gas temperature of the generated plasma jet is estimated using an insulated thermocouple and found to be around 40°C.

By using the developed plasma source, the inactivation of multidrug resistant bacteria experiments are performed on different MDR bacteria isolates. These MDR organisms such as Escherichia coli, Klebsiella pneumoniae. Pseudomonas spp., Acinetobacter baumannii, Methicillin resistant Staphylococcus aureus, and Vancomycin resistant enterococci are the most prioritized bacteria by WHO causing significant morbidity and mortality in healthcare settings. In the present experiments, 8 MDR isolates (viz., E. coli, K. pneumoniae, P. aureginosa, A. baumannii, S. aureus, and E. faecium) which are identified and selected from clinical samples i.e., sputum, blood, and urine specimen. The details of bacteria were identified by MALD-TOF and antibiotic susceptibility testing by VITEK 2 system. The samples of concentrations from 10⁵ to 10¹ CFU/ml are tested for plasma exposure for 5 min covering an area of about 12 cm² using the solid agar plates. The experiments are also carried out in the liquid media with plasma exposure for 1 minute and the viable growth was detected using ATP Luminometer and subculture on solid media. The experiments are repeated 3 times to ensure the reproducibility of the outcomes and the inactivation efficiency of plasma treatment is calculated against various isolates at different concentrations.

A significant decrease in the growth after plasma treatment is observed for all the concentrations in both solid and liquid media. Using solid media, the mean percentage inhibition at the highest dilutions for gram-positive and gram-negative isolates was 55.8% and 79.4%, respectively whereas for lower dilutions either negligible growth or zero growth is observed. Moreover, no growth found from the subculture of plasma exposed liquid media determines its higher antibacterial efficacy in liquid media than solid media. Thus these preliminary experimental outcomes support the concept of using plasma as an alternate tool for decontamination of medical devices and inanimate surfaces in hospital settings.

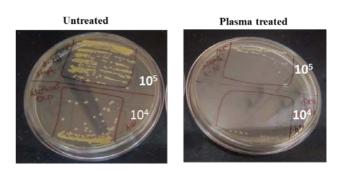


Figure:1 Representative images of Kleb. pneumoniae with concentration of 10^5 (top) and 10^4 (bottom) CFU/ml untreated (left) and treated (right) for 5 mins covering an area of around 12 cm²

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References

[1] Laroussi, M. Plasma Medicine: A Brief Introduction. Plasma 1, 47-60, 2018,