The Cold Plasma: A Promising Tool to improve the Clinical Outcome of Iraqi Patients with Burn Infections

Geeran Al-Fahdawi Abdul-Rahman M.[1], Muhaidi M.J.[2]* and Hamad Mohammed. A.[2]
1. Department of Human Anatomy, College of Medicine, Anbar University, IRAQ
2. Department of Biotechnology, College of Applied Sciences, University of Falluja, IRAQ
*mjm20002014@uofallujah.edu.iq

Abstract

Forty (40) Iraqi patients with burns infections who were admitted to the burns center of Al-Ramadi Teaching Hospital from the period of January till April 2014 were included in the present study. Ethical permission to conduct this study as well as all information were taken from those patients and their relatives. Sterile cotton swabs were used to obtain the ideal clinical samples for cultivation on the media in the medical laboratories. After appropriate incubation conditions, the bacterial growth was identified by using API 20 E system.

Later on, all bacteria under study were exposed to the cold plasma by Dielectric Barrier Discharge (DBD) device with a certain voltage (25 kv) and a gap distance (2 mm) with different exposure times ranging from 30 - 150 seconds, the number of bacteria were counted after each DBD treatment. The results showed that all bacterial species under study undergo reduction in their numbers due to increase of the killing rate after DBD treatment; this is related to the direct effect of non-thermal plasma to the target bacteria due to generation of so many free radicals with bactericidal effect.

Keywords: Cold Plasma, Burns infections, DBD Treatment.

Introduction

Plasma is the ‘fourth state of the matter’ composed of electrons, positive and negative ions. Plasma is widely distributed in the world. It is attractive mode especially in medical applications such as management of burns infections; the most novel device to generate plasma is dielectric barrier discharge (DBD). Non-equilibrium, atmospheric pressure plasma treatment of living tissue is being used in a variety of processes collectively called plasma medicine[5]. Burn infections are associated with a high mortality rate. The common finding of the burn is the infection due to the ability of endogenous bacteria to colonize the site of the burns after 48 hrs because of the presence of necrotic tissue and protein rich exudates that act as a source of bacterial growth.

Many bacterial types cause these infections both gram positive and negative bacteria. The classification of burns depends upon tissue involved. The first and second degree burns with a partial thickness because only epidermis of the skin is involved. The third degree with full thickness involves both epidermis and dermis. The latter type is characterized by extensive loss of fluids and protein leading to the shock[11].

Material and Methods

Patients: Forty (40) Iraqi patients with burns infection who were admitted in the burns center of Al-Ramadi Teaching Hospital were included in this study from January to April 2014. They were 31 (77.5%) male and 9 (22.5%) female, their ages range from 16-37 years.

Samples collection: From those patients, sterile cotton swabs were used to obtain the clinical samples from the burn sites of the patient by the assistance of burn staff in the burns center. These samples were cultivated on MacConkey and blood agar plates at 37ºC for 24 hrs. After incubation, the bacteria were identified by means of API 20 E system. By using replica plating technique, five plates from each bacterial isolate were exposed to cold plasma (Dielectric Barrier Discharge DBD device) at different times 30, 60, 90, 120 and 150 seconds at applied voltage (25 kv) and a gap distance (2 mm). The percentages of bacterial killing were calculated for all bacteria under study. Regarding DBD device, the bacteria under study serve as a second active electrode of this DBD device (figure 1).

Results and Discussion

The results obtained from this study indicate the role of DBD treatment on medical applications. Figure 2 shows the distribution of burns infections according to the patient's gender. It is obvious that the males had more prevalent rate of burn infections as compared with females. They were 31(77.5%) males and just 9 (22.5%) females. Burn infections are the most common causes of increasing the mortality rate in the world. The results obtained from this study show the ratio of males to females (3:1). The males have a high percentage of burn infections compared with females. This is related to the occupational status of males make their jobs with direct contact with oil derivatives and electrical issues such as the workers of electrical generators making them under risk to get burns infections.

Figure 3 shows the types of bacteria that cause burn infections. It is clear that *E. coli* was the most common bacteria causing burn infections with a percentage 42.5% of the total isolates. It was followed by *Pseudomonas aeruginosa* and *Klebsiella pneumonia*e with percentages 32.5% and 25% respectively. *E. coli* is a member of the normal flora found with high numbers in the gastrointestinal tract GIT, so the probability of this bacteria to cause infections is high; this route is called endogenous route.

Another source of *E. coli* infection is hospital acquired infection[4]. *Pseudomonas aeruginosa* represents 32.5% of burns infections. It agrees with Al-Kabee 2013 who reported 40% of burns infections belonging to this bacteria[2]. This ratio is related to ability of this bacteria to resist, so many antimicrobial agents and disinfectants in the hospitals carry extrachromosomal elements of DNA (plasmids) that carry genes responsible for resistance of these agents and ability of this bacteria to vertical transmission to other bacteria that increase the probability of drug resistance[6,10]. It is the main cause for nosocomial infections[9].

In this study, *Klebsiella pneumoniae* represents 25% of the total isolates that causes burn infections, this finding agrees with Al-Begat 2007 who reported 23% of burn infections due to *Klebsiella pneumonia*[1]. Figure 4 shows the effect of cold plasma (CP) treatment on killing rate of *E. coli*. It is obvious that the treatment of such bacteria with DBD in different times increases the killing rate, the best exposure time is 150 second that kills 68% of these bacteria.

Figure 5 shows the effect of cold plasma treatment on killing rate of *Pseudomonas aeruginosa*. It is obvious that the treatment of such bacteria with DBD in different times increases the killing percentage; the best exposure time is 150 second that kills 66% of these bacteria.

Figure 6 shows the effect of cold plasma treatment on killing rate of *Klebsiella pneumoniae.* It is obvious that the treatment of such bacteria with DBD in different times increases the killing percentage; the best exposure time is 150 second that kills 54% of these bacteria. Regarding the effect of the cold plasma and its killing percentage on studied microorganisms, the electrode gap (the distance between the device and the sample under study) has a major influence on the properties of plasma operation; it is the most important factor in efficacy of plasma. It is easy to observe that through the plasma, brightness expands and spreads over the surface. At a distance of 2 mm, almost all of the plasma power is absorbed by the bacteria[7]. This is related to increase of the ionization rate in the plasma.

The mechanism of bacterial inactivation by the cold plasma can be identified due to many factors like heat, UV radiation and plasma chemistry. Regarding UV, it may be the most important factor in bacterial deactivation, because UV light is easily passed to the target (bacteria). UV in this range causes destruction of bacterial cells. The active radicals and anions like O, OH and HO_2 play the most important role in the destruction of micro-organisms in atmospheric pressure plasmas because of the ability of free radicals to destruct of cell membrane of bacteria causing them to die.

Another factor that contributes in bacterial killing is heat. The plasma temperature is completely based upon the gap distances between the device and the sample. Bacterial deactivation by heat can play a role only at plasma powers higher than 200mW where the temperature rises above 47°C. Well-localized voids with sharp edges indicate that the bactericidal agents have very short lifetimes in a liquid medium. These active species are fairly stable in the gas phase, because bacterial deactivation is observed even at quite long distances from the device (several millimeters).

Under these conditions, the glow, which corresponds to the active plasma zone, does not reach the sample. Bacteria are thus killed by species that are still present in the remote dark region. Once these particles cross the gas-liquid interface, they are immediately consumed in chemical reactions with the components of the solution. Their action in the liquid is thus strictly localized. These features suggest that plasma-produced species like radicals and (helium) meta-stables play an important role in bacterial deactivation. The mechanism of deactivation by meta-stables is not clear: they can either directly attack the bacterial membrane, or dissociate water to produce OH radicals, which further react with bacteria. Most probably there is no unique deactivation mechanism and different factors are important under different conditions[8].

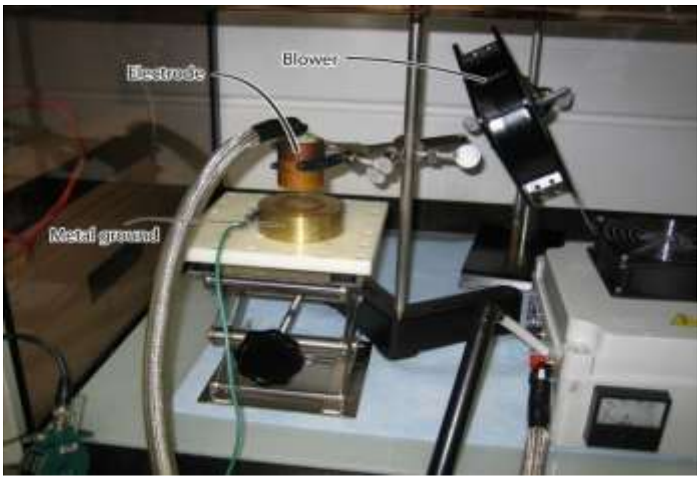

Figure 1: DBD system.

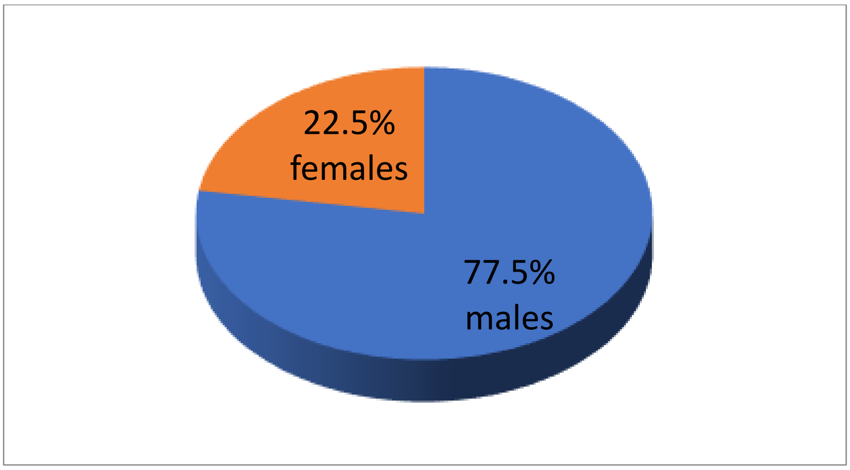

Figure 2: The distribution of burn infection according to patient's gender.

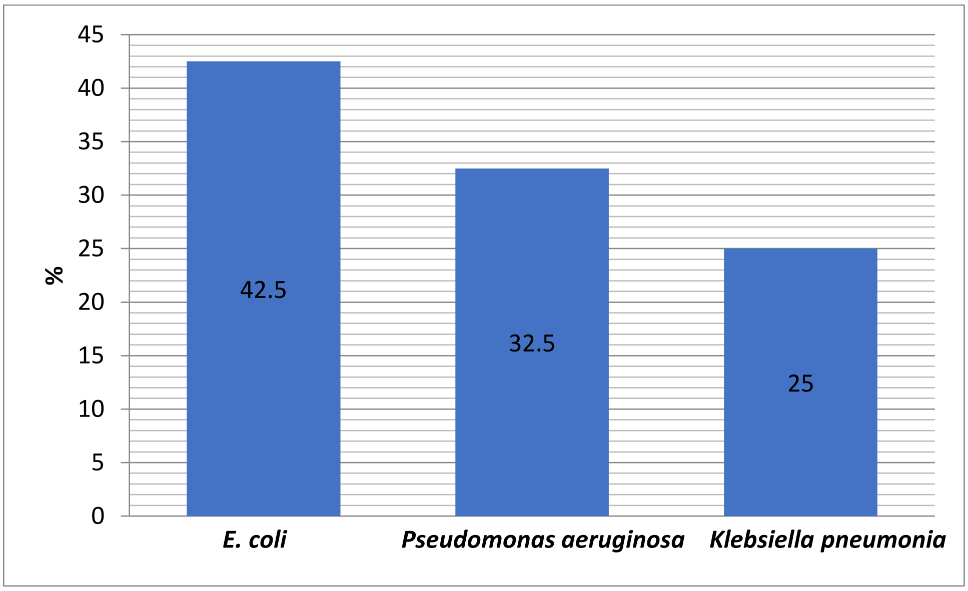

Figure 3: The types of bacteria causing burn infections and their percentages

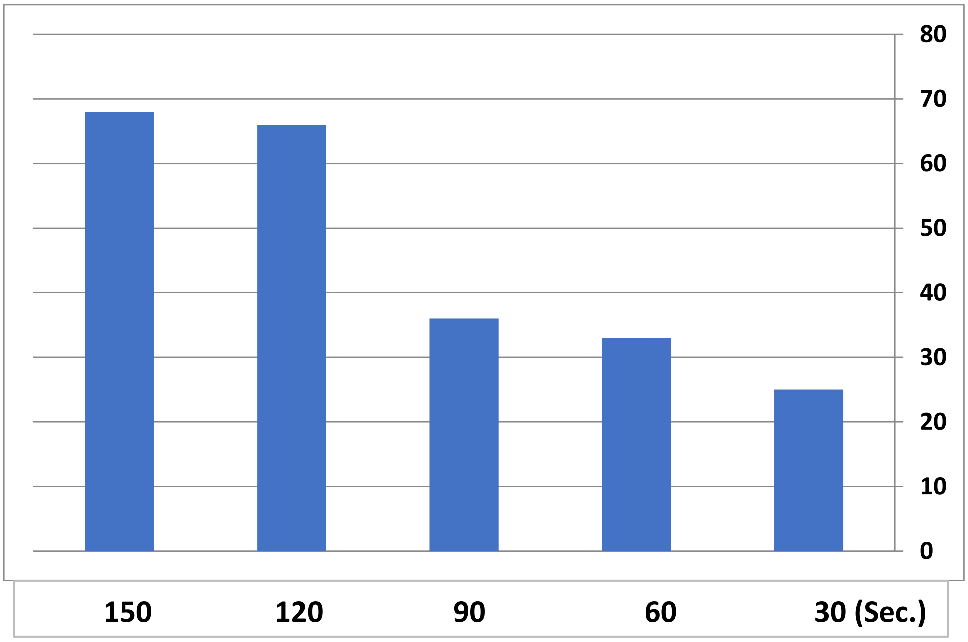

Figure 4: The effect of cold plasma treatment on killing rate of *E. coli*

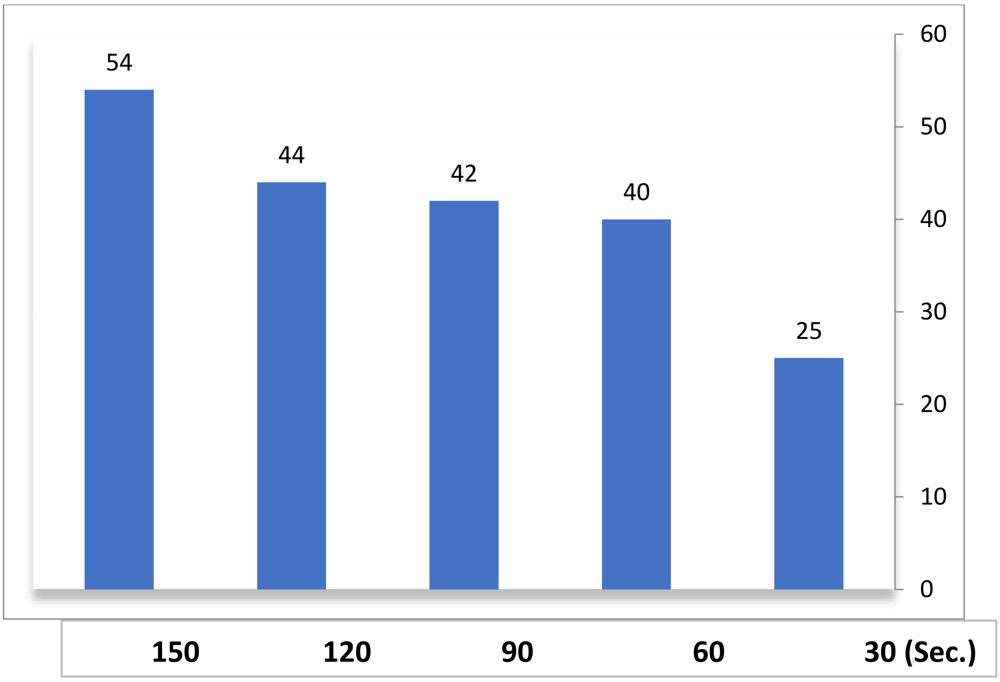

Figure 5: The effect of cold plasma treatment on killing rate of *Pseudomonas aeruginosa*

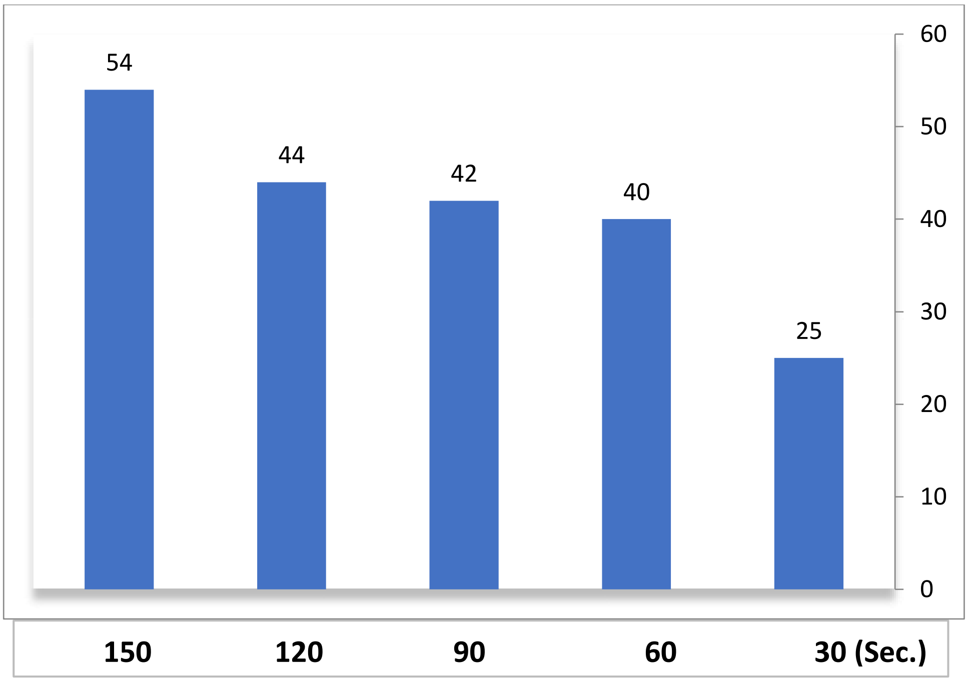

Figure 6: The effect of DBD treatment on killing rate of *Klebsiella pneumoniae*

Conclusion

The physical methods are very important in the treatment of infections caused by different types of organisms. Plasma technique is useful in the control of infections. This tool increases the quality of patient's life and increases the clinical outcome of those patients.

Acknowledgement

Our special thanks go to members of medical staff In Burns center at Al-Ramadi teaching hospital for their constant support and their cooperation to complete this work.

References

1. Al-Begat Saad Taha Mutlk Hmidon, Study of most commen aerobic bacteria causing lower urinary tract infection (UTI) in Ramadi general hospital, Thesis, College of Medicine-University of Al- Anbar **(2007)**

2. Al-kaabi Sadik Abdulridha Gatea, Bacterial Isolates and their Antibiogram of Burn Wound Infections in Burns Specialist Hospital in Baghdad, *J. Baghdad for Sci.*, **10(2)**, 331-340 **(2013)**

3. API 20 ETM identification system for Enterobacteriaceae and other non-fastidious gram-negative rod, USA **(2010)**

4. Church Deirdre, Sameer Elsayed, Owen Reid, Brent Winston and Robert Lindsay, Burn Wound Infections, Clinical Microbiology Reviews, *American Society for Microbiology*, **19(2)**, 403-434 **(2006)**

5. Graves D.B., Low temperature plasma biomedicine: a tutorial review, *Phys Plasmas*, **21**, e080901 **(2014)**

6. Koljali S., Naaber P. and Mikelsaar M., Antibiotic resistance as an indicator of bacterial chlorhexidine susceptibility, *J. Hosp. Infect*., **51**, 106-113 **(2002)**

7. Laroussi M., Tendero C., Lu X., Alla S. and Hynes W.L., Inactivation of Bacteria by the Plasma Pencil, *Plasma Processes and Polymers*, **3(6-7)**, 470-473 **(2006)**

8. Laroussi M., Mendis D.A. and Rosenberg M., Plasma interaction with microbes, *New Journal of Physics*, **5**, 41 **(2003)**

9. Muller G. and Kramer A., In vitro action of a combination of selected antimicrobial agents and chondroitin sulfate, C*hem. Bio. Interact. Journal*, **12(2)**, 77-85 **(2000)**

10. Strateva T. and Ouzounova-Raykova V., Markovab Problematic clinical isolates of *ps.aeruginosa* from the university hospitals in sofia, Bulgaria: current status antimicrobial resistance and prevailing resistance mechanisms, *J. Med. Microbiology*, **56**, 956-963 **(2007)**

11. Zedler S., Biol D., Bone R.C., Baue A.E., Donnersmarck G.H.V. and Faist E., T-cell reactivity and its predictive role in immunesuppression after burns, *Care Medicine*, **27**, 66-72 **(1999)**.