Journal of
Medical Microbiology & Diagnosis

Azim, et al., J Med Microb Diagn 2016, 5:4
DOI: 10.4172/2161-0703.1000245

Research Article | Open Access

Assessment of Microbicidal Activity of Atmospheric Pressure Non-Thermal Plasma Against Planktonic and Biofilm Forms

Shymaa Abdel Azim[1*], Refat Sadeq[2], Mohamed El Shaer[3] and Heba Mohamed[1]

[1]*Department of Medical Microbiology and Immunology, Faculty of Medicine, University of Zagazig, Zagazig, Egypt*

[2]*Department of Medical Microbiology and Immunology, Port Said University, Egypt*

[3]*Department of Plasma Physics, Faculty of Engineering, Zagazig University, Egypt*

***Corresponding author:** Shymaa Abdel Azim, Department of Medical Microbiology and Immunology, Faculty of Medicine, University of Zagazig, Zagazig, Egypt, Tel: 201008118736; E-mail: shymaa_abdelazim@yahoo.com

Rec Date: Jul 09, 2016; **Acc Date:** Nov 22, 2016; **Pub Date:** Nov 29, 2016

Copyright: © 2016 Azim SA, et al. This is an open-access article distributed under the terms of the creative commons attribution license, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract

Background: Atmospheric pressure non-thermal plasma (APNTP) is a promising, relatively novel method for destroying microorganisms either in planktonic or biofilm form, alternative to “conventional” methods which have numerous drawbacks.

Aim of the work: To assess the microbicidal activity of atmospheric pressure non-thermal plasma (APNTP) on planktonic and biofilm forms.

Subjects and methods: This study was performed on *Staphylococcus aureus* (*S. aureus*), coagulase negative *staphylococci* (*CoNS*), *P*seudomonas (P.) *aeruginosa* and *Escherichia* (E.) *coli* isolates from patients with indwelling medical devices associated infections in different intensive care units (ICUs), Zagazig University Hospitals. Detection of biofilm forming ability of these isolates was done by tube method (TM). Planktonic and biofilm counterpart of selected biofilm forming isolates were exposed to APNTP for different durations to assess the biocidal efficacy of plasma on both microbial forms by colony forming unit (CFU) count and/or 2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide (XTT), assay. APNTP morphological changes in *E. coli* and *S. aureus* were assessed by transmission electron microscopic (TEM) imaging.

Results: APNTP treatment of *S. aureus*, *E. coli* suspensions caused progressive reduction in surviving bacterial count and metabolic activity with increasing treatment duration and at 180 seconds of exposure complete sterilization achieved. Similar but more prolonged effect was detected on CoNS and *P. aeruginosa* suspensions. Its exposure for 240 seconds was needed for their complete sterilization. There was no difference between bacterial percentage reduction calculated by CFU count and XTT assay except in *P. aeruginosa* suspension for 60 seconds. No observed difference between APNTP effect on planktonic gram positive (GP) and gram negative (GN) bacteria. On the other hand, GN bacterial biofilm was more resistant to APNTP than GP bacterial biofilm. TEM showed that in both *S. aureus* and *E. coli* there were significant morphological changes after exposure to plasma.

Conclusion: The efficacy of APNTP was proved for *in vitro* decontamination of planktonic and biofilm forms of S. aureus, CoNS, *P. aeruginosa* and *E. coli* that are responsible for many healthcare-acquired infections (HCAIs).

Keywords: Non-thermal plasma; Planktonic; Biofilm

Introduction

Biofilm can be characterized as a surface-attached group of microbes that become inserted in a self-synthesized network of paste like substance called extracellular polymeric substances (EPS) [1]. Around 99% of the world's populace of micro-organisms is found as a biofilm at different phases of development [2].

The resistance of microorganisms to disinfection is frequently associated with the presence of biofilms on surfaces [3]. The mechanisms of biofilm insusceptibility to disinfectants include: diffusion limitation of disinfectants in biofilms, gene transfers and mutations and pathogen protection in multispecies biofilms [4].

Plasma “ionized gas” is the fourth state of matter which is much more ubiquitous than the other states [5]. There are two types of plasma; thermal (hot) and non-thermal (cold) plasma. Atmospheric pressure non-thermal plasma (APNTP) is a promising, relatively novel method for destroying microorganisms either in planktonic or biofilm forms [6].

There are many challenges in healthcare environment which consists of very vulnerable patients, inaccessible or challenging items of equipment to decontaminate and microbes that have adapted to be able to replicate and spread in this hospitable niche [7].

Atmospheric pressure non-thermal plasma (APNTP) application is able to fulfil these requirements as it is portable, easy to operate and suitable for treatment of heat-sensitive items. This allows large scale application in health care sittings as an alternative to “conventional” methods which have numerous drawbacks. Moreover, due to other

Citation: Azim SA, Sadeq R, El Shaer M, Mohamed H (2016) Assessment of Microbicidal Activity of Atmospheric Pressure Non-Thermal Plasma Against Planktonic and Biofilm Forms. J Med Microb Diagn 5: 245. doi:10.4172/2161-0703.1000245

advantages of APNTP, it is non-allergic or toxic, painless and non-invasive technique [8].

There are ongoing researches to evaluate APNTP efficacy in a routine and busy clinical environment, in terms of reduced surface bacterial counts and reduced numbers of hospital acquired infections [9].

Materials and Methods

This study included 175 samples from patients with indwelling medical devices associated infection in different ICUs in Zagazig University Hospitals. Seventy-five urine samples from catheterized patient, 50 endotracheal aspirate samples and 50 intravenous cannulas.

All samples were subjected to the following: microscopic examination of direct smears, cultivation on the suitable culture media: Nutrient agar (Oxoid, UK), 5% blood agar, MacConkey's agar (Oxoid, UK).

Identification of isolates by the following methods

Colonial morphology, microscopic examination of gram-stained films, conventional biochemical reactions: for identification of gram-positive cocci: catalase test and tube coagulase test were done. For gram-negative bacilli: oxidase test, (Oxoid, UK), triple sugar iron (Oxoid, UK), indole test (Oxoid, UK), citrate utilization test and demonstration of the motility using semi-solid agar.

Assessment of biofilm formation among the clinical isolates by tube method (TM)

For *Staphylococcus aureus* (*S. aureus*), *Coagulase negative staphylococci* (*CoNS*), *Escherichia coli* (*E. coli*) and *Pseudomonas* (*P.*) *aeruginosa* [10,11].

Antimicrobial Susceptibility Testing (AST)

All isolates were subjected to disc diffusion method for comparing the susceptibility pattern to antibiotics between biofilm forming and biofilm non-forming microbes [12].

Atmospheric pressure non-thermal plasma (APNTP) generation by plasma jet device

This device was designed and constructed by staff members of physical and mathematical engineering department, Faculty of Engineering, Zagazig University (Figure 1). It consists of three major components called high power voltage supply, electrodes and dielectric tube. Plasma jet was generated using 99.5% helium and 0.5% oxygen. It was indirect plasma as regarding its mode of generation and operated at 9 kV, 16 kHz. Also, it was no-thermal which was proved by the infrared thermometer where plasma temperature was 21.5°C.

Atmospheric pressure non-thermal plasma (APNTP) treatment of planktonic and biofilm counterpart of the same isolate

The microtitre plate wells containing the planktonic and biofilm forming isolates and quartz tube outlet was put at a distance of 20 mm. Different wells were exposed to the gas discharge plasmas for various exposure times. Negative control without plasma treatment i.e. 0-second exposure time was included. A helium control was included on testing biofilms forming isolates in XTT assay to avoid the cidal effect of gas flow without plasma ignition and it was showed minimal affection by gas flow treatment without plasma ignition for 240 seconds (Figure 2a).

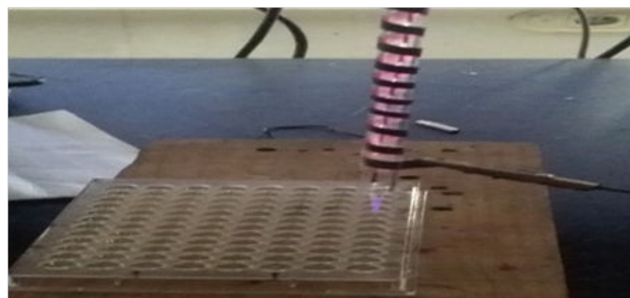

Figure 1: Plasma jet device.

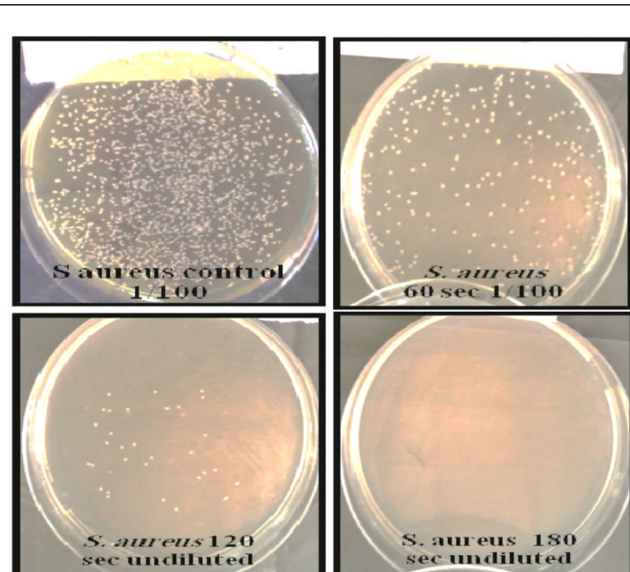

Figure 2a: Plates of CFU count of planktonic *S. aureus*.

Quantification of viable cells

By colony forming unit (CFU) count for planktonic form [13] and XTT assay for both planktonic [13] and biofilm forms [14] of the same isolate.

Colony forming unit (CFU) count

- CFU $\log_{10}$ reduction was calculated as follows:

  CFU $\log_{10}$ reduction= $\log N_0 - \log N_s$

- CFU Percentage reduction was calculated as follows:

  CFU percentage reduction= $[1- (N_S/N_0)]$ X100%

  N_0: the initial population count (count of untreated suspension).

  N_s: the surviving population count.

XTT assay

Using *in vitro* Toxicology Assay Kit, XTT based (Sigma-Aldrich Co., Ireland).

- Normalized absorbance values were calculated by the following equation:

Normalized absorbance values (A_N)= A_{APNTP}-A_C

A_{APNTP}: the absorbance of APNTP treated sample

A_C: the absorbance of negative control

- XTT percentage reduction was calculated by the following equation:

XTT percentage reduction= $[1- (A_N / A_0)] \times 100\%$

A_0: the absorbance of untreated control sample

A_N: normalized absorbance value of treated sample

Transmission electron microscopic (TEM) imaging

It was done at the regional center for mycology and biotechnology (RCMB), Al-Azhar University. It was performed for detection of bacterial cell integrity and morphological structure changes (Figure 2b).

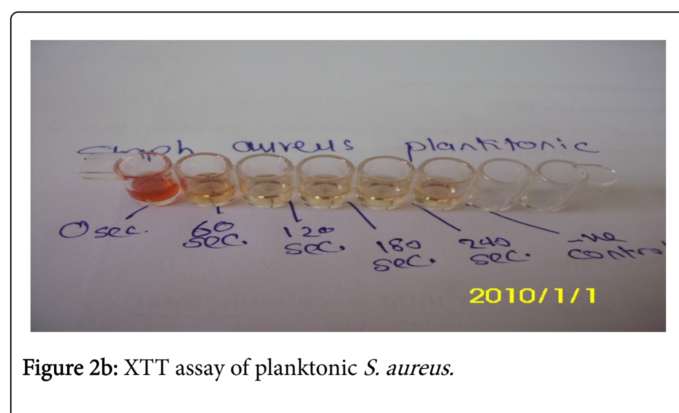

Figure 2b: XTT assay of planktonic *S. aureus.*

Statistical Analysis

All patients' data were tabulated, and then processed using statistical package for sciences and society (SPSS 19.0) (SPSS Inc., Chicago, IL, USA). Data were expressed as number and percentage for qualitative variables and mean ± standard deviation (SD) for quantitative one. Chi-square test (X^2) is the test of significance in qualitative variables and comparisons with $P<0.05$ were considered significantly different. Z test of proportion was used for comparing two percentages. It is used for comparing percentage reduction of planktonic and biofilm forms after plasma treatment for different durations using CFU count method and XTT assay and XTT assay alone; respectively. Z test is significant at 0.05 level if the result >2.

Results

Out of 175 samples, 162 (92.6%) are culture positive. There was a statistically significant difference regarding mixed infection of different samples ($p<0.001$); urine samples having the highest percentage of mixed infection (54.6%). *CoNS* was statistically significant more frequently isolated from intravenous cannulas ($p=0.003$) as well as *E. coli* more frequently isolated from urine samples ($p<0.001$).

The result of isolates screening for biofilm formation was that 72 out of 128 (56.2%) identified isolates are biofilm former. The biofilm formers in CAUTI (45.8%) were less than that reported in other samples (69.4% and 60.6% for endotracheal aspirate and intravascular cannula respectively) but without statistical significance ($p=0.07$).

There was a higher antibiotic resistance to all tested antibiotics among biofilm formers than non-formers except to vancomycin and linezolid in *S. aureus* and *CoNS* to which none of the isolates are resistant. On the other hand, there was a higher antibiotic resistance to all tested antibiotics among biofilm formers than non-formers in *P. aeruginosa* and except to imipenem in *E.coli* to which none of the isolates are resistant.

Table 1, By Z test, no significant difference and no p-value between bacterial percentage reduction calculated by CFU count and XTT assay except in *P. aeruginosa* suspension APNTP treatment for 60 seconds; by Z test there is significant difference but there is no p-value.

	CoNs						*S. aureus*				
	CFU			XTT			CFU			XTT	
Treatment time	Mean ± SD	Log reduction	Percentage reduction	Normalized reading Mean ± SD	Percentage reduction	Treatment time	Mean ± SD	Log reduction	Percentage reduction	Normalized reading Mean ± SD	Percentage reduction
0 Sec	2.75*106 ± 0.37*106	0	0	1.673 ± 0.41	0%	0 Sec	3.3*106 ± 0.39*106	0	0	1.443 ± 0.36	0%
60 Sec	2.6* 105 ± 0.54*305	1.02	90.5	0.267 ± 0.05	88%	60 Sec	2.85*105 ± 0.24*105	1.06	91.4%	0.173 ± 0.03	88%
120 Sec	3.9*103 ± 0.46*103	2.8	99.85	0.016 ± 0.002	99%	120 Sec	2.2*102 ± 0.3*102	4.18	99.99%	0	100%
180 Sec	3.3*102 ± 0.85*102	3.92	99.98	0	100%	180 Sec	0	6.52	100%	0	100%
240 Sec	0	6.44	100	0	100%						
	E. coli						*P. aeruginosa*				

	CFU			XTT			CFU			XTT	
Treatment time	**Mean ± SD**	**Log reduction**	**Percentage reduction**	**Normalized reading Mean ± SD**	**Percentage reduction**	**Treatment time**	**Mean ± SD**	**Log reduction**	**Percentage reduction**	**Normalized reading**	**Percentage reduction**
0 Sec	2.95*106 ± 0.45*106	0	0	1.840 ±0. 32	0%	0 Sec	2.9*106 ± 0.26*106	0	0	1.870 ± 0.26	0%
60 Sec	8*104 ± 1.64*104	1.6	97.2	0.09 ± 0.002	95.1%	60 Sec	2.9*105 ± 0.34*105	1	90	0.187 ± 0.01	77.4%
120 Sec	4.2*102 ± 0.66*102	3.84	99.98	0	100%	120 Sec	4.9*103 ± 0.43*103	2.77	99.83	0.004	99.78%
180 Sec	0	6.47	100	0	100%	180 Sec	1.7*102 ± 0.34*102	4.23	99.99	0	100%
						240 Sec	0	6.46	100	0	100%

Table 1: Comparison between percentage reduction of planktonic *S. aureus*, CoNS, *E. coli* and *P. aeruginosa* after plasma treatment for different durations using CFU count method and XTT assay.

Figure 1, Detection of effect of plasma treatment on planktonic *S. aureus* by a: CFU count and b: XTT assay. Figure 3, By Z test no significant difference and no p-value between APNTP effect on planktonic Gram positive and Gram negative bacteria.

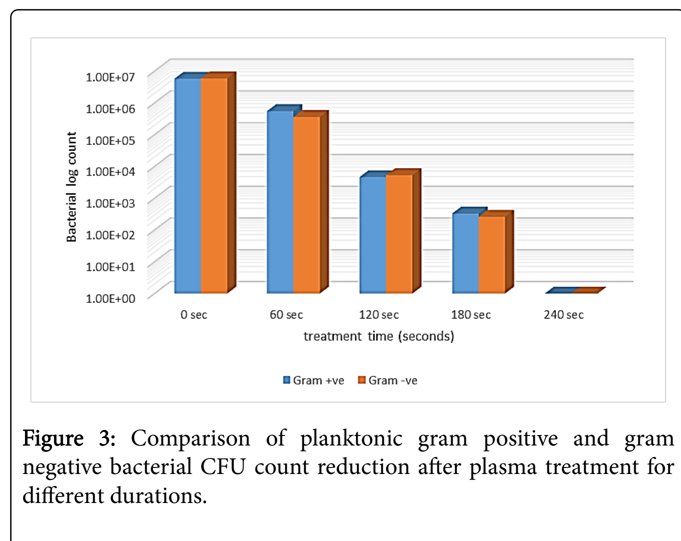

Figure 3: Comparison of planktonic gram positive and gram negative bacterial CFU count reduction after plasma treatment for different durations.

Figure 4, XTT assay of different bacterial biofilm formers.

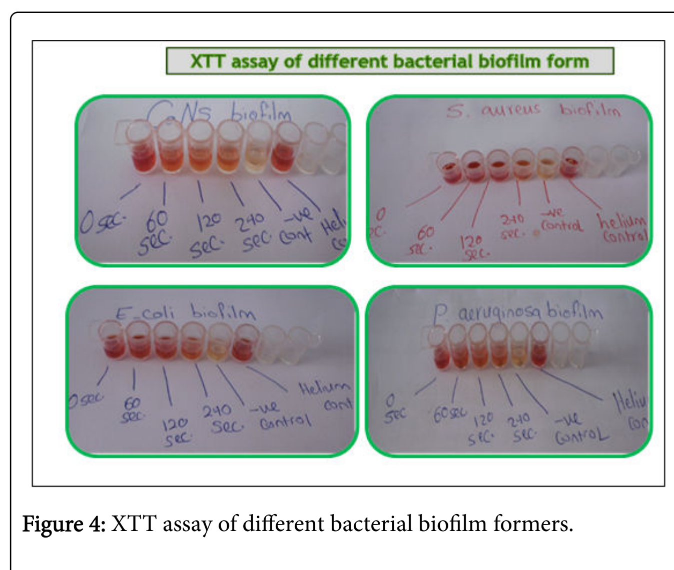

Figure 4: XTT assay of different bacterial biofilm formers.

Table 2 and Figure 5: *P. aeruginosa* biofilm has the least percentage reduction in all APNTP treatment. Gram negative bacterial biofilm was more resistant to APNTP than Gram positive bacterial biofilm.

		S. aureus	**CoNS**	*P. aeruginosa*	*E. coli*
0 second	XTT	1.775 ± 0.33	1.755 ± 0.36	1.821 ± 0.40	1.729 ± 0.33
	% reduction	0	0	0	0
60 second	XTT	0.625 ± 0.05	0.756 ± 0.038	1.331 ± 0.041	1.259 ± 0.052
	% reduction	64.80%	56.90%	26.90%	36.10%
120 second	XTT	0.444 ± 0.006	0.611 ± 0.009	1.056 ± 0.01	0.868 ± 0.009
	% reduction	75%	65.20%	42.10%	49.80%
240 second	XTT	0.351 ± 0.001	0.433 ± 0.002	0.758 ± 0.005	0.689 ± 0.004

	% reduction	80.20%	75.30%	58.40%	60.10%
Gas control 240 sec	XTT	1.713	1.667	1.748	1.677
	% reduction	3.50%	5%	4.10%	3.10%

Table 2: Comparison between biofilm cell percentage reductions of different bacteria using XTT assay.

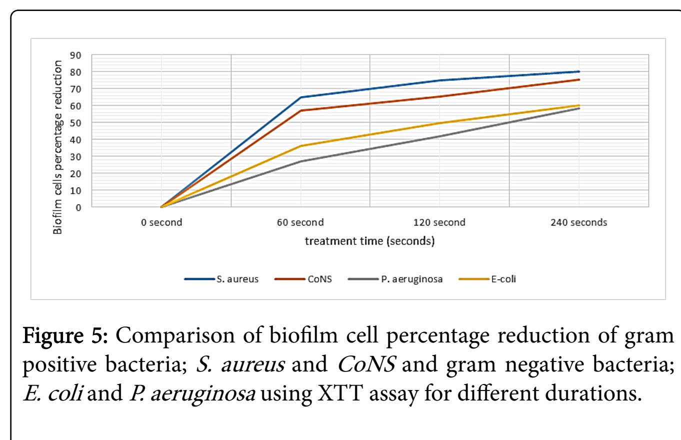

Figure 5: Comparison of biofilm cell percentage reduction of gram positive bacteria; *S. aureus* and *CoNS* and gram negative bacteria; *E. coli* and *P. aeruginosa* using XTT assay for different durations.

Figure 6, transmission electron microscopic (TEM) shows the morphological changes of *S. aureus* after plasma treatment. (A) The control untreated cells have spherical or elliptical outline, with intact cell envelope and smooth surface (black arrow). (B) After plasma treatment the cell envelope is disrupted (green arrow) with release of the cytoplasm contents (blue arrow).

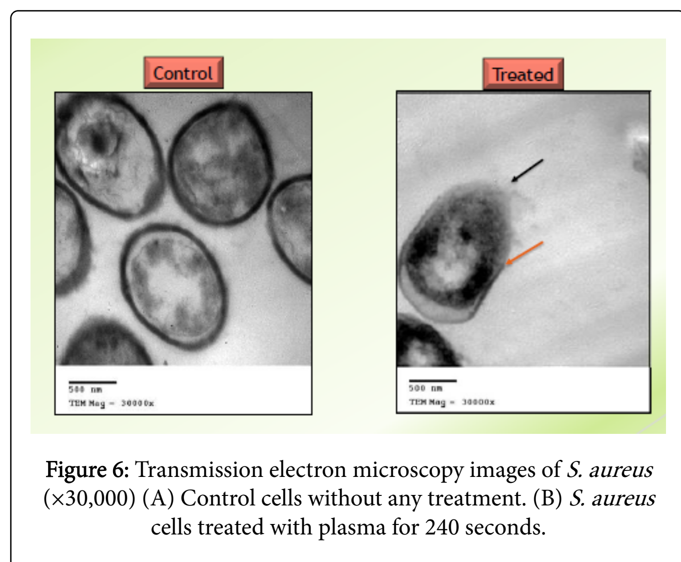

Figure 6: Transmission electron microscopy images of *S. aureus* (×30,000) (A) Control cells without any treatment. (B) *S. aureus* cells treated with plasma for 240 seconds.

Figure 7, shows the morphological changes of *E. coli* after plasma treatment. (A) The control untreated cells of *E. coli* have a normal cell shape with an undamaged structure of the inner membrane (red arrow) and an intact, slightly waved outer membrane (black arrow). The periplasmic space was thin and had a uniform appearance (green arrow). (B) After plasma treatment, the cell envelope is partially disrupted with release of the cytoplasm contents with loss of well demarked cell outlines found in control cells (green arrow). Lysis of the treated cells (black arrow) and fragmentation (red arrows) is also detected.

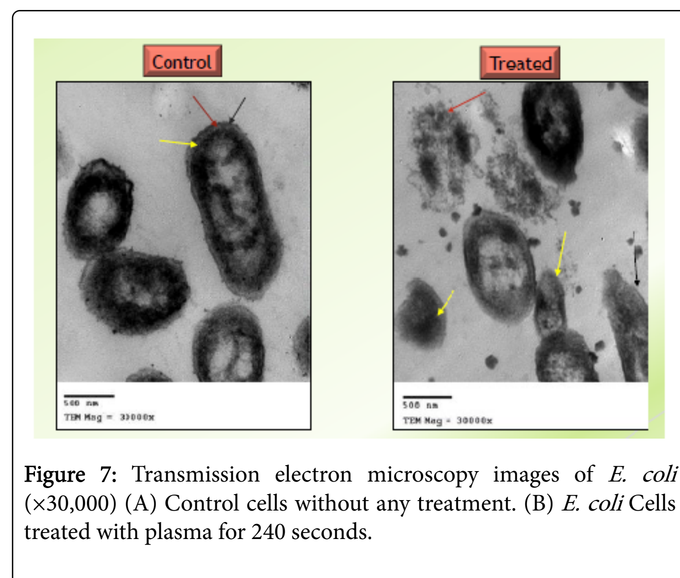

Figure 7: Transmission electron microscopy images of *E. coli* (×30,000) (A) Control cells without any treatment. (B) *E. coli* Cells treated with plasma for 240 seconds.

Discussion

In most health care facilities, especially ICUs, device related infection (DRI) is responsible for increasing morbidity and failure of therapy irrespective of the severity of underlying illness. This may be partially due to resistant strains in the hospital environment.

In this study, the biofilm formers in CAUTI were less than that reported in other samples that were concomitant with higher prevalence of mixed infection in the CAUTI isolates. This may indicate that biofilms on urinary catheters might initially be composed of a single species, but longer use duration inevitably lead to multispecies biofilms.

Concerning comparison between percentage of antibiotic resistance in biofilm former and non-former of *E. coli*, gentamicin resistance among biofilm formers was significantly higher than non-formers. Higher antibiotic resistance to all tested antibiotics except imipenem among biofilm producers than non-producers was also detected. This is consistent with Sharma et al. [15].

Using APNTP for inactivation of bacteria in planktonic and biofilm forms have drawn increasing attention in the study of biological decontamination.

Ehlbeck et al. mentioned that the comparison of microbiocidal results for different experiments is very difficult. This is probably due to different factors affecting interaction between microbes and plasma discharge including microbial, plasma generation conditions and experimental factors [16].

As regards microbial factors affecting microbicidal results of plasma, they are different bacterial strains [17], bacterial suspension concentration [18] and the formulations of nutrient macromolecules of fluid in which the bacterial cells were suspended [19].

On the other hand, microbicidal results of plasma are affected by plasma generation condition. For example, difference in frequency and voltage of the applied electric current, construction of the cathode and anode configuration, and sustainment of the electric field leads to difference in physical parameters and composition of any generated plasma discharge [20,21].

Other factors that determine the efficacy of plasma treatment are difference in working gas and subsequent creation of downstream molecules and free radicals. Different gases (most commonly argon, helium, nitrogen, air or oxygen) or gas mixtures may be used for plasma generation [22].

Moreover, the active species generated by the discharge are also strongly affected by the surrounding conditions e.g. humidity, gas temperature and pressure. Exposure of the bacterial samples to the plasma glow is always more effective than exposure to plasma afterglow as in the plasma glow the short-living species as well as long-living species have a high probability to reach the treated sample [23].

In this study concerning cell percentage reduction values of *S. aureus*, *CoNS*, *P. aeruginosa* and *E. coli* calculated based on XTT assay in comparison with CFU count method, there was no marked difference in different treatment durations except at the 60-seconds exposure point in *P. aeruginosa*. This finding may indicate that plasma exposure for this period of time provides a sub-lethal dose which may render some of the bacterial cells non-culturable but still viable which could be detected by XXT assay which depends on assaying the metabolic activity and the viability status of the bacterial population and clinically, this may have catastrophic consequences if microorganisms that are assumed dead, are pathogenic ones who may retain virulence even when they are non-culturable [24].

In different studies, when gram-positive and gram-negative bacteria were compared, the results were controversial. In some cases, no differences were detected [17,19] which suggested that there is no selectivity in the action of APNTP generated species based on the bacterial cell wall structure. In this study, no observed difference between APNTP effect on planktonic gram positive and gram negative bacteria.

Whilst other researchers found that gram-positive bacteria were more resistant than gram-negative bacteria, due to their thicker peptidoglycan layer in cell wall [13,19]. On the other hand, Lazovic et al. found that gram-negative bacteria are more resistant than gram-positive, due to their extra proteins and lipopolysaccharide in their additional outer membrane which acts as a barrier [25].

Compared with isolated bacterial cells, biofilms represent a major form of bacterial persistence on the surface of both medical equipment and chronic wounds to antimicrobial treatments [26].

Gilmore, studied helium/oxygen plasma jet and recorded considerable resistance of mucoid strains biofilm (characterized by producing extensive extracellular matrix) to plasma inactivation in comparison with non-mucoid strain biofilm, indicating that the biofilm matrix is directly influencing plasma species *in vitro*, attenuating the bactericidal activity of APNTP [27].

On comparison between different bacterial biofilm susceptibility to non-thermal plasma, Gram negative bacterial biofilms were more resistant than Gram positive bacteria. This may be due to the variation in thickness, composition and quantity of the EPS depending on the type of microorganisms, which could consequently impact ACP inactivation efficacy [6].

This is consistent with study by Alkawareek et al. who found that *E. coli* and *P. aeruginosa* biofilm more resistant than *S. aureus* and *B. cereus* biofilm. The decreased *P. aeruginosa* susceptibility might be explained by the presence of certain matrix constituents that may have an ability to neutralize the reactive species responsible for bacterial killing. In this regard, the exopolysaccharide alginate produced by *P. aeruginosa* was found to be able to scavenge the oxidant free radicals known to be released by human leucocytes, which are basically similar to those produced in plasma discharge [28].

Regarding the effect of APNTP on bacterial planktonic and biofilm forms, the current study demonstrated that biofilm form is more resistant to plasma treatment than planktonic form.

This is consistent with results obtained by Gilmore, who studied helium/oxygen plasma jet (6 kV, 20 kHz) effect on *P. aeruginosa* and found that all biofilms exhibited increased resistance to plasma exposure when compared to their planktonic counterparts [27].

This was explained by biofilm cells organization into overlapping layers make its plasma induced inactivation mechanism more complicated than planktonic counterpart, as plasma reactive species have to penetrate biofilm structure layer by layer, meaning that destruction of the upper layers of bacterial biofilm cells that are exposed to plasma, then plasma had to etch and penetrate layers of cell debris and dead cells before reaching the inner layers of the biofilm [29].

As regards using transmission electron microscope (TEM) imaging for observation of the effects of the plasma on bacterial cell morphology, TEM showed that in both *S. aureus* and *E. coli* there had significant morphological changes after exposure to plasma. After plasma treatment, there was disruption and loss of bacterial cell envelop and leakage of the bacterial cytosol into surrounding medium.

This is consistent with Park et al. who studied effect of non-thermal plasma on *S. aureus* and observed cell surface damage by scanning electron microscope [30].

Lysis and fragmentation of *E. coli* cells after treatment was also detected. Ulbin-Figlewicz et al. showed that helium plasma caused *E. coli* cells disruption and lysis when visualized by scanning electron microscope [31]. These morphological changes and erosion of bacterial cells envelope can be attributed to the etching activity of plasma generated radicals and charged particles [32].

Conclusion

The efficacy of APNTP was proved for *in vitro* decontamination of planktonic and biofilm

forms of *S. aureus*, *CoNS*, *P. aeruginosa* and *E. coli* that are responsible for many HCAIs. This finding is promising and raising the possibility of its use for sterilization *in vitro* on large scales.

Recommendations

Performing intensified studies on one species of bacteria to test if bacterial strain in conjunction with its biofilm matrix composition have an effect on APNTP efficacy. Further studies to improve efficiency of plasma jet used in this study (by decreasing the exposure time as well as the cost) by variation in applied electric current frequency and voltage as well as use of gas mixtures.

References

1. Shirtliff ME, Mader JT, Camper AK (2002) Molecular interactions in biofilms. Chem Biol 9: 859-871.
2. Gautam CK, Srivastav AK, Bind S, Madhav MU, Shanthi V (2013) An insight into biofilm ecology and its applied aspects. Int J Pharm Pharm Sci 5: 597-620.
3. Bressler D, Balzer M, Dannehl A, Flemming HC, Wingender J (2009) Persistence of Pseudomonas aeruginosa in drinking-water biofilms on elastomeric material.Water Sci Technol 9: 81-87.
4. Bridier A, Briandet R, Thomas V, Dubois-Brissonnet F (2011) Resistance of bacterial biofilms to disinfectants, A review. Biofouling 27: 1017-1032.
5. Friedman A, Chirokov A, Gutsol A (2005) Non-thermal atmospheric pressure discharges. J Phys D Appl Phys 38: 1-24.
6. Ziuzina D, Boehm D, Patil S, Cullen PJ, Bourke P (2015) Cold plasma inactivation of bacterial biofilms and reduction of quorum sensing regulated virulence factors. PLoS One 10: e 0138209.
7. Liang Y, Wu Y, Sun K, Chen Q, Shen F, et al. (2012) Rapid inactivation of biological species in the air using atmospheric pressure non-thermal plasma. Environ Sci Technol 46: 3360-3366.
8. Heinlin J, Isbary G, Stolz W, Zeman F, Landthaler M, et al. (2013) A randomized two-sided placebo-controlled study on the efficacy and safety of atmospheric non-thermal argon plasma for pruritus. J Eur Acad Dermatol Venereol 27: 324-331.
9. Napp J, Daeschlein G, Napp M, Von Podewils S, Gümbel D, et al. (2015) On the history of plasma treatment and comparison of microbiostatic efficacy of a historical high-frequency plasma device with two modern devices. GMS Hyg Infect Control 10: 1-7.
10. SarojGolia D, Hittinahalli V, Karjigi SK, Reddy KM (2012) Correlation between biofilm formation of uropathogenic Escherichia Coli and Its antibiotic resistance pattern. J Med Dent Sci 1: 166-176.
11. Hassan A, Usman J, Kaleem F, Omair M, Khalid A, et al. (2011) Evaluation of different detection methods of biofilm formation in the clinical isolates. Braz. j. infect. dis 15: 305-311.
12. Bauer AW, Kirby WM, Sherris JC, Turck M (1966) Antibiotic susceptibility testing by the standard single disc method. Am J Clin Pathol 45: 493-496.
13. Joshi SG, Paff M, Friedman G, Fridman G, Fridman A, et al. (2010) Control of methicillin-resistant Staphylococcus aureus in planktonic form and biofilms, Abiocidal efficacy study of nonthermal dielectric-barrier discharge plasma. Am J Infect Control 38: 293-301.
14. Peeters E, Nelis HJ, Coenye T (2008) Comparison of multiple methods for quantification of microbial biofilms grown in microtiter plates. J Microbiol Methods 72: 157-165.
15. Sharma S, Mahajan B, Patidar RK, Sahare KN, Khare M, et al. (2013) Molecular detection of antimicrobial resistance genes in biofilm forming Escherichia Coli. AJPHR 6: 2-12.
16. Ehlbeck J, Schnabel U, Polak M, Winter J, Von Woedtke T, et al. (2010) Low temperature atmospheric pressure plasma sources for microbial decontamination. J Phys Appl Phys 44: e 013002.
17. Venezia RA, Orrico M, Houston E, Yin SM, Naumova YY (2008) Lethal activity of nonthermal plasma sterilization against microorganisms. Infect Control Hosp Epidemiol 29: 430-436.
18. Lazovic S, Puač N, Miletić M, Pavlica D, Jovanović M, et al. (2010) The effect of a plasma needle on bacteria in planktonic samples and on peripheral blood mesenchymal stem cells. New J Phys 12: 083037.
19. Ferrell JR (2013) Effects of non-thermal plasma on prokaryotic and eukaryotic cells (Doctoral thesis, Kent State University,Ohio, USA).
20. Conrads H, Schmidt M (2000) Plasma generation and plasma sources. Plasma Sources Sci Technol 9: 441- 450.
21. Friedman G, Friedman G, Gutsol A, Shekhter AB, Vasilets VN, et al. (2008) Applied plasma medicine. Plasma Process Polym 5: 503-533.
22. Mai-Prochnow A, Bradbury M, Ostrikov K, Murphy AB (2015) Pseudomonas aeruginosa biofilm response and resistance to cold atmospheric pressure plasma is linked to the redox-active molecule Phenazine. PLoS One 10: e0130373.
23. Hoffmann C, Berganza C, Zhang J (2013) Cold atmospheric plasma, methods of production and application in dentistry and oncology. Med Gas Res 13: 3-21.
24. Alkawareek MY, Gorman SP, Graham WG, Gilmore BF (2014) Potential cellular targets and antibacterial efficacy of atmospheric pressure non-thermal plasma. Int J Antimicrob Agents 43: 154-160.
25. Lazovic S, Puač N, Miletić M, Pavlica D, Jovanović M, et al. (2010) The effect of a plasma needle on bacteria in planktonic samples and on peripheral blood mesenchymal stem cells. New J Phys 12: 083037.
26. Hall-Stoodley L, Costerton JW, Stoodley P (2004) Bacterial biofilms, from the natural environment to infectious diseases. Nat Rev Microbiol 2: 95-108.
27. Gilmore BF (2013) Non thermal plasmas-new frontiers in control of biofilm infections. Paper presented at the 32nd international conference on phenomena in ionized gases, Iasi, Romania.
28. Alkawareek MY, Algwari QT, Gorman SP, Graham WG, O'Connell D, et al. (2012) Application of atmospheric pressure nonthermal plasma for the in vitro eradication of bacterial biofilms. FEMS Immunol Med Microbiol 65: 381-384.
29. Abramzon N, Joaquin JC, Bray J, Brelles-Marino G (2006) Biofilm destruction by RF high-pressure cold plasma jet. IEEE Trans Plasma Sci 34: 1304-1309.
30. Park JH, Kumar N, Park DH, Yusupov M, Neyts EC, et al. (2015) A comparative study for the inactivation of multidrug resistance bacteria using dielectric barrier discharge and nano-second pulsed plasma. Sci Rep 5: 13849.
31. Ulbin-Figlewicz N, Jarmoluk A, Marycz K (2015) Antimicrobial activity of low-pressure plasma treatment against selected foodborne bacteria and meat microbiota. Ann Microbiol 65: 1537-1546.
32. Laroussi M, Mendis DA, Rosenberg M (2003) Plasma interactions with microbes. New J Phys 5: 41- 46.