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Influence of Cold Atmospheric Plasma on *Acinetobacter baumannii*

Reyam Radi Atta ***Halah Al Haideri*** ***Hamid H Murbat***

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Abstract:

A. baumannii is an aerobic gram negative coccobacilli, it is considered multidrug resistance pathogen (MDR) and causes several infections that are difficult to treat. This study is aims to employ physical methods in sterilization and inactivation of *A. baumannii*, as an alternative way to reduce the using of drugs and antibiotics.

Cold Atmospheric Plasma was generated by one electrode at 20KV, 4 power supply and distance between electrode and sample was fixed on 1mm. *A. baumannii* (ATCC 19704 and HHR1) were exposed to Dielectric Barrier Discharge type of Cold Atmospheric Plasma (DBD-CAP) for several periods of time (15, 30, 45, and 60 sec.) . After sterilization test, several methods were done to analyze the effect of DBD-CAP on bacterial morphology, proteins and DNA. Change in morphology was assessed by cover slid method. Damaged DNA was investigated by PCR technique, and DNA sequencing. The impact of DBD-CAP on the entity of proteins was detected by SDS-PAGE. The observed inactivation of bacterial colony on agar plates has been quantified by measuring the inactivation diameter.

The important conclusion that HHR1 more resistance to DBD-CAP than ATCC 17904 because it is more virulence than standard strain; thus, the growth of both strains is largely affected by plasma and this influence is increased by increasing the time of exposure, also the plasma affects the DNA especially on standard strain as it is explained in sequencing result, so it causes more deletion in DNA sequence. In addition, plasma also has been showed to damage proteins and morphology thus, the bacterial cells transform from cocco-bacillus to bacillus.

Key words: *Acinetobacter baumannii ATCC17901, Cold Atmospheric Plasma, Dielectric barrier discharge, HHR1*

Introduction:

Acinetobacter baumannii is an aerobic, non-fermented Gram-negative coccobacilli, it is grown well on solid media with smooth grayish white colonies. It is identified as one of the main multidrug resistance (MDR) organisms, and it has been considered as a very important pathogen that causes several hospital and community infections, such as ventilator and community acquired pneumonia, meningitis, wound and trauma infections, and septicemia (1, 2). It is ubiquitous in nature and commonly associated with aquatic environments, such as water surface, and soil (3). It is found on the skin and it can be isolated in large numbers from the secretions of respiratory and oropharynx of infected person (4). Plasma in physics is the fourth state of matter as ionized gas, is the most matter of universe. It is composed of positive and negative charged ions, electrons and neutral compounds (atoms, molecules), in additional it is composed of radicals and UV-radiation (5). Plasma is divided into two types: thermal and cold atmospheric plasma (CAP). Thermal plasma contains heavy particles and electrons at the same temperature, and the other type called Cold Atmospheric Plasma (CAP) has heavy particles at lower temperature than electrons, less than 40˚C, always be at room temperature. Nowadays, there is an rise attention and work out on cold plasma processes, thus there are numerous means to manufacture the CAP like Dielectric Barrier Discharge (DBD), plasma needle, and plasma pencil, Atmospheric Pressure Plasma Jet (APPJ) (6). Moreover, Cold Atmospheric Plasma has been employed in several sides like, polymerization (7), sterilization/inactivation of microorganisms, this is more essential part since it is participate in keeping life for human beings. It has been depended on either physical or chemical process that destroys or reduces microorganisms, or

[1, 2] Department of Biology, College of Science for Women, University of Baghdad, Baghdad, Iraq.
[3] Department of Physics, College of Science for Women, University of Baghdad, Baghdad, Iraq.
*Corresponding author: reyam.radi@yahoo.com

both (8,9) and CAP has been proposed as a therapeutic method to treat cancer (10). In this study the dielectric barrier discharge (DBD) was used, it is well known as one of the plasma sources capable of working with diverse gasses at high pressures (higher than atmospheric pressure) (11). This study aims to employ Cold Atmospheric Plasma (CAP) in treatment of *A. baumannii* infections to reduce the using of drugs and antibiotics. In addition, the study focuses on the effect of CAP on *OmpA* gene of *A. baumannii*.

Materials and Methods:

Bacterial Sample Preparation

Two types of *A. baumannii* were used; standard strain *A. baumannii* ATCC17904 was purchased from (ATCC, USA), and *A. baumannii* HHR1(Accession number: MH6B5112.1) which was previously isolated from patient attending in Al-Kindi Teaching Hospital. The patient was suffering from urinary tract infection (UTI) and identified by Vitek and 16S rRNA (Accession number: MH6B5112.1). Both types of *A. baumannii* were routinely cultured on MacConkey agar and nutrient agar, and then incubated at 37˚C for 24 hrs. For liquid cultures, the strains were inoculated in Mueller Hinton (MH) broth and incubated aerobically at 37˚C for 24 hrs with gentle agitation at 180 rpm .The growth cultures of *A. baumannii* was monitored and adjusted till OD_{600} 0.5-0.6. A 100 µl of each culture was spread off on nutrient agar plate, and the plates were left to dry before being incubated at 37˚C for 24 hrs.

DBD Treatment

Plasma System

The DBD system used in this study has been designed and made locally by Dr. Murbat H H/ College of Science for Women/University of BaghdadDr. Hamid H. Murbat (University of Baghdad, College of Science for Women, Department of Physics) Fig.1. It is composed of one electrode with 50 mm in diameter and made of copper rod surrounding by Teflon for sequestered. Quartz sheet with one millimeter thickness was used as a dielectric substantial between two electrodes. The electrode was linked to high voltage convertor that its output voltages can vary between (1-20 KV). The applied voltage was fixed at (20 KV) in our experiment. Samples were placed on the stage and the distance between the electrode and samples was kept as 1mm. Finally, the plasma region diameter is 2.2 cm.

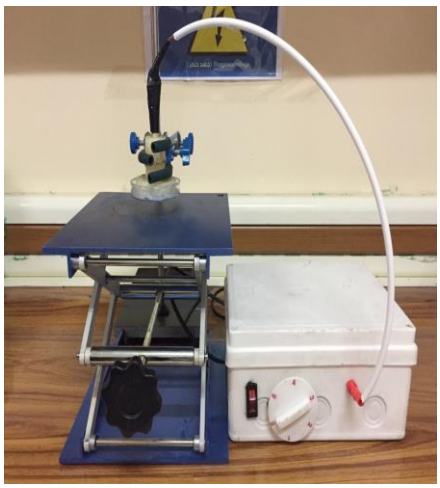


Figure 1. Locally design DBD plasma generator used in this study.

Sterilization tests were employed for both strains, by adding 100µl of overnight incubation suspension and poured on agar surface and left to dry at room temperature for 30 min. After that, sterilization is done by exposing bacteria to DBD-CAP for 15, 30, 45, and 60 sec. The experiment was conducted in open air under atmospheric pressure and at 14˚C, and one sample was left without exposure to plasma as a control. After treatment was completed, the plates were incubated at 37˚C for 24 hrs and the inactivation diameter was measured (12, 13).

Outer Membrane Extraction

The OMP fraction of *A. baumannii* cultures were prepared following a procedure described previously by Jyothisri, *et al*., (14) with some modifications**.** Each of overnight liquid culture was treated with DBD-CAP of both *A. baumannii* strains, ATCC 17904 and HHR1, and control was pelleted by centrifugation at 4000 rpm for 10 min. The pellet was resuspended in 10 mM of Tris-HCl pH 8, and then re-centrifuged as above. After final wash step, the pellet was sonicated for 15 sec pulses x6 at frequency amplitude of 16 microns using a soniprep 150 ultrasonic disintegrator (SANYO) and pelleted by centrifugation at 10.000 rpm for 10 min. To separate the outer membrane from the whole cells, the supernatant was discarded and the pellet was resuspended in Triton X-100 with gentle mixing for 30 min at 4˚C. The pellets were obtained by centrifugation at 4000 rpm for10 min and the outer membrane fraction was stored at -20˚C until use.

Periplasmic Fraction by Osmotic Shock Method

Both strains of *A. baumannii,* treated and untreated with DBD-CAP, were aerobically grown in 100 ml MH broth at 37˚C for 24 hrs with gentle shaking. The cells were harvested by centrifugation

at 8000 xg for 10 min at 4˚C and resuspended in 10 ml STE buffer (20 % [w/v] sucrose, 30 mM Tris-HCl pH 8, and 1mM EDTA) and then incubated with gentle shaking for 30 min at room temperature. The cells were pelleted by centrifugation (10,000 xg, 10 min at 25˚C), resuspended in 5 ml ice-cold 10 mM Tris-HCl pH 8.0, followed by incubation with gentle shaking (20 rpm, 2 hr, 4˚C). Finally, the periplasm fraction was obtained by centrifugation (15,000 xg, 25 min, and 4˚C) and then either stored at -20˚C, or mixed with 1X SDS loading dye and electrophoresed on 12 % SDS-PAGE. The preparation of periplasm is performed by isolation procedure for *Pseudomonas aeruginosa* (15).

Preparation of Cytoplasmic Fraction

Overnight cultures of *A. baumannii* strains (treated and untreated with DBD-CAP) were harvested by centrifugation (4000 xg, 10 min), and decant the supernatant. The pellet is resuspended in 2ml Tris-HcL pH 8 and mixed gently. The solution is sonicated four times, 15 sec. for each one. Finally, centrifugation for 10-15 min was done, and the supernatant was either used or stored at -20˚C(16).

Protein Manipulation

Determination of Protein Concentration

The concentration of soluble proteins, cytoplasm and periplasm proteins, was determined by using Bio-Rad Protein Assay (17). Protein samples were diluted in 800 µl of 4x Bio-Rad reagent and 200 µl dH_2O to reach the final concentration of 1x. Then, the solution was transferred to 1 ml volume cuvette, and 1-20 µl protein solution was added. Thereafter, the mixture was measured at OD_{595} nm using spectrophotometer (Optima, Japan). The protein concentration (mg ml^{-1}) was calculated using the formula: Protein concentration (mg ml^{-2}) = (OD_{595} x 15)/volume of protein (µl).

On the other hand, the concentration of insoluble protein (whole cells and membrane proteins) was determined using Lowry assay (18). Protein standard and samples were diluted in several solutions with ddH2O. Solution A (2% [w/v] sodium carbonate and 0.1N sodium hydroxide) was mixed with solution B (4 % [w/v] cupric sulphate pentahydrate and 1% potassium sodium tartraate) to give solution C. Solution C was added to each tube with protein standard and samples, and incubated at room temperature for 10 min. Folin reagent (solution D) was added to the tubes to final concentration 1X and incubated at RT for 30 min. The absorbance was measured at 600 nm in comparison to blank that does not contain protein samples.

SDS-PAGE (One Dimensional SDS-polyacrylamide Gel Electrophoresis)

SDS-polyacrylamide electrophoresis was done using Bio-Rad system (19). 12 % resolving gel and 4 % stacking gel were prepared from the same component while resolving and stacking gel with Tris-HCl pH 8.8 and Tris-HCl pH 6.8 respectively. Protein samples were prepared by mixing with 4x SDS loading dye and boiled at 95˚C for 5 min. After gel setting, the comb was removed from stacking gel, then gel was located in a gel tank with 1x SDS running buffer (25 mM Tris, 250 mM Glycine, and 0.1 % [w/v] SDS). The samples were then loaded into the gel with PageRuler™ Plus Unstained Rec. Protein Ladder (Promega). The gel was electrophoresed at a constant 100 v until the dye reached to the bottom or as desired.

Gel was stained with Coomassie blue (50 % [v/v] methanol, 10 % [v/v] glacial acetic acid, 0.1 % [w/v] Coomassie brilliant blue R (Sigma-Aldrich) and destained in (50 % [v/v] methanol, 10 % [v/v] glacial acetic acid) twice or until the proteins became visible.

Morphology Assay

The cell morphology of *A. baumannii* strains (ATCC 17904 & HHR1) and control were determined under light microscope after staining with gram stain (20).

Genomic DNA Extraction and Polymerase Chain Reaction

Plasma-treated culture for both strains (standard and HHR1) was centrifuged at 2,500 xg for 15min to obtain the pellet. Genomic DNA was then extracted from the pellet by Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA).The *OmpA* gene of *A. baumannii* for both strains was amplified by PCR using the *OmpA* primers (Forward primer 5′-ATGAAATTGAGTCGTATTGC-3′, and Reverse primer 5′-TTATTGAGCTGCTGCAG-3′).The concentration of each primer was adjusted to 10 µM using dH_2O. All reactions were performed with GoTaq Green Master mix (Promega). 50µl PCR reaction was used that contained 25µl Mastermix, 5µl of each primer, 2.5µl of genomic DNA as a template, and sterilized water to make final volume up to 50µl. Amplification program was conducted as follows: initial denaturation step at 95˚C for 1min, followed by 25 cycles at 95˚C 30sec for denaturation, annealing at 56˚C 30sec, extension at 72˚C 30sec, final extension at 72˚C for 1min, and holding at 4˚C. The PCR product was examined on agarose gel to confirm that there is a specific product with the desired size. The PCR product was sequenced using automated DNA sequencer

(AB13730XL) (Macrogen Company in Korea). The results were analyzed by BLAST website on NCBI (21).

Statistical Analysis

Statistical analyses were performed using GraphPad Prism v.7. In addition, quantitative assay was done by using linear regression and statistical significance was defined as P≤0.05.

Results and Discussion:

Effect of Cold Plasma on Bacterial Growth

Cultures of *A. baumannii* strains were exposed to DBD-CAP at fixed discharge voltage 20 kv, 4 power supply and fixed distant 1mm. Sterilization test for both strains (Standard and HHR1) showed the inactivation diameter is increased by increasing the exposure time Fig. 2. In *A. baumannii* ATCC17904, the inactivation gap was increased from 2.5 cm to 4 cm after 60 sec, while the HHR1isolated was less inhibited after 60 sec exposure time (Table 1).

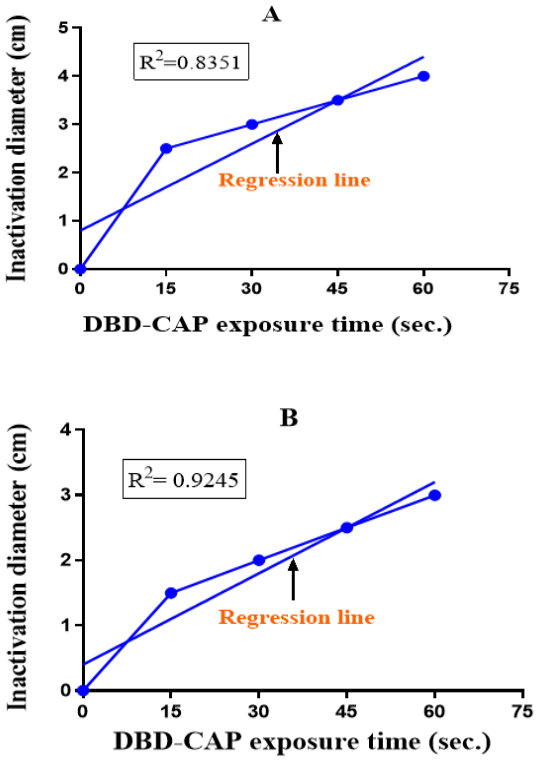


Figure 2. Plasma exposure timeline for *A. baumannii* (A) ATCC 17904, (B) HHR1. *A. baumannii* cultures were exposed to DBD-CAP at different time intervals. The regression line was plotted in according to the exposure time.

Table 1: Inactivation diameters of *A. baumannii* ATCC 17904 and HHR1 exposure to DBD-CAP.

DBD-CAP exposure time (sec.)	Inactivation diameter of ATCC 17904 (cm)	Inactivation diameter of HHR1 (cm)
0	0	0
15	2.5	1.5
30	3	2
45	3.5	2.5
60	4	3
P value	0.0300 *	0.0090 *
	*(P<0.05)	

These results proved that sterilization by DBD-CAP is efficient, it can be notified very well (22). The neutral and charge particles of plasma cause damage to cell, air plasma for example is a perfect source of reactive oxygen-based and nitrogen-based species (ROS and RNS) such as O, O_2, O_3, OH, NO, NO2, etc. and by chemical interaction with macromolecules such as membrane lipids, proteins and nucleic acids. These species cause rupture to cell membrane, followed by bacterial death (23, 24). Furthermore, Fig. 3 clearly shows that the bacteria were dead after exposure to DBD-CAP 20 kv for 60 sec. Thus, because of the charged particles (ions & electrons) of plasma play an important role in rending the outer membrane of bacterial cells when they accumulate on the cell surface and forming of electrostatic tensions which lead to cause the disturbance of the cell membrane. Moreover, plasma may catalyze the oxidation and peroxidation processes within the cell and in the external environment which would lead to inactivation of bacterial metabolism (24, 25).

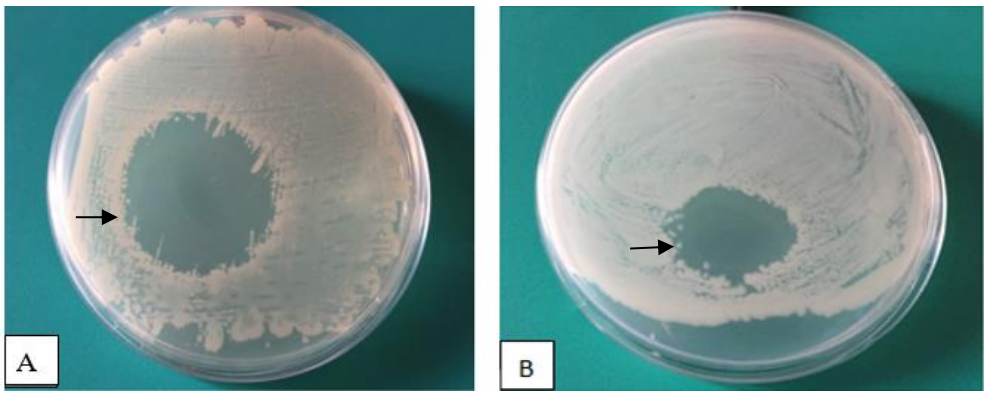


Figure 3. Effect of cold plasma on *A. baumannii* strains. Nutrient agar plates inoculated with 100 µl of *A. baumannii* strains and exposed to DBD-CAP for 60 sec. (A) ATCC 17904 (B) HHR1. The inhibited region is referred by black arrows.

Effect of Plasma on General Morphology

Light microscope images of standard strain treated by DBD-CAP and untreated cells stained with Gram stain are shown in Fig. 4. Smooth coccobacilus bacterial cells were observed in samples that untreated with plasma, following cells that exposed to plasma, as it seems below that the morphology of cells undergo some conformational changes after plasma treatment, and plasma may be deleterious to the cell wall structure.

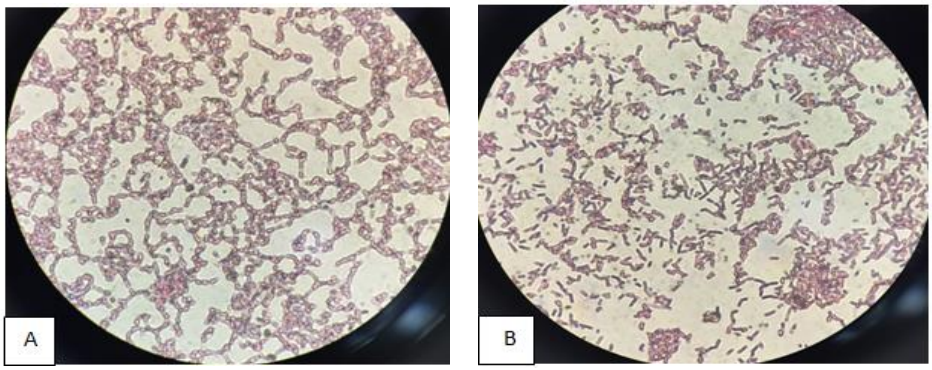


Figure 4. *A. baumannii* ATCC 17904 treated by sublethal dose of DBD-CAP under Light microscope with 40X magnification (A) before treatment (B) after treatment. In all cases, bacteria were stained with gram stain.

In addition, DBD-CAP has a potential effect on HHR1 morphology Fig. 5, there are obvious changes in the morphology after DBD-CAP treatment. The LM images for both strains with sub lethal dose demonstrated that the cells elongated with no clear appearance of cell lysis. It was reported that cell lysis may not be the essential mechanism for bacterial inactivation, or it may be performed at lethal dose of plasma (26, 27), and also plasma did not affect the acceptance of cells to the gram stain.

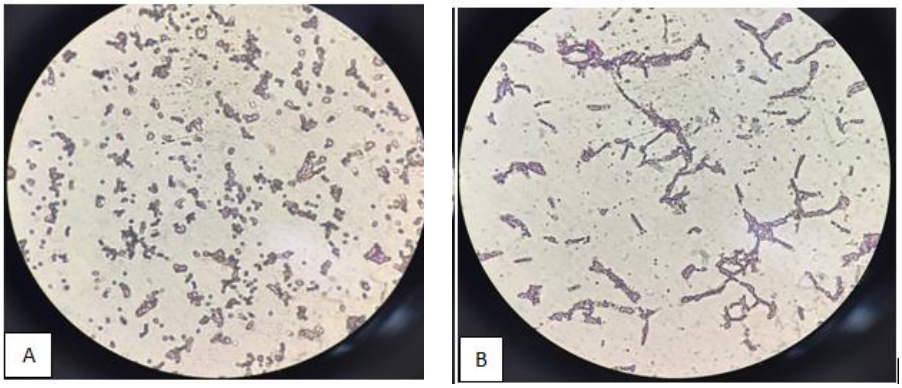


Figure 5. *A. baumannii* HHR1 treated by sublethal dose of DBD-CAP under Light microscope with 40X magnification (A) before treatment (B) after treatment. In both cases, bacteria were stained with Gram stain.

Effect of Plasma on DNA

The DNA was extracted from both treated and untreated cells of strains, then *OmpA* gene was amplified by PCR as described previously. The PCR products of DBD-CAP treated and untreated strains were electrophoresed on agarose gel electrophoresis Fig. 6A, thus the PCR product for all samples appeared as a clear band with corresponding size of 1071 bp, Fig. 6 B.

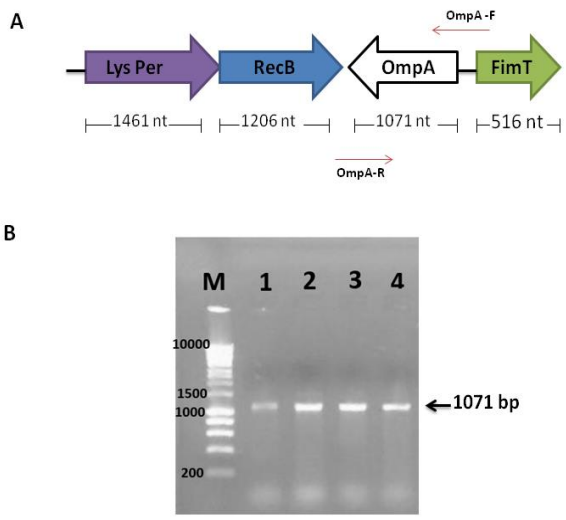
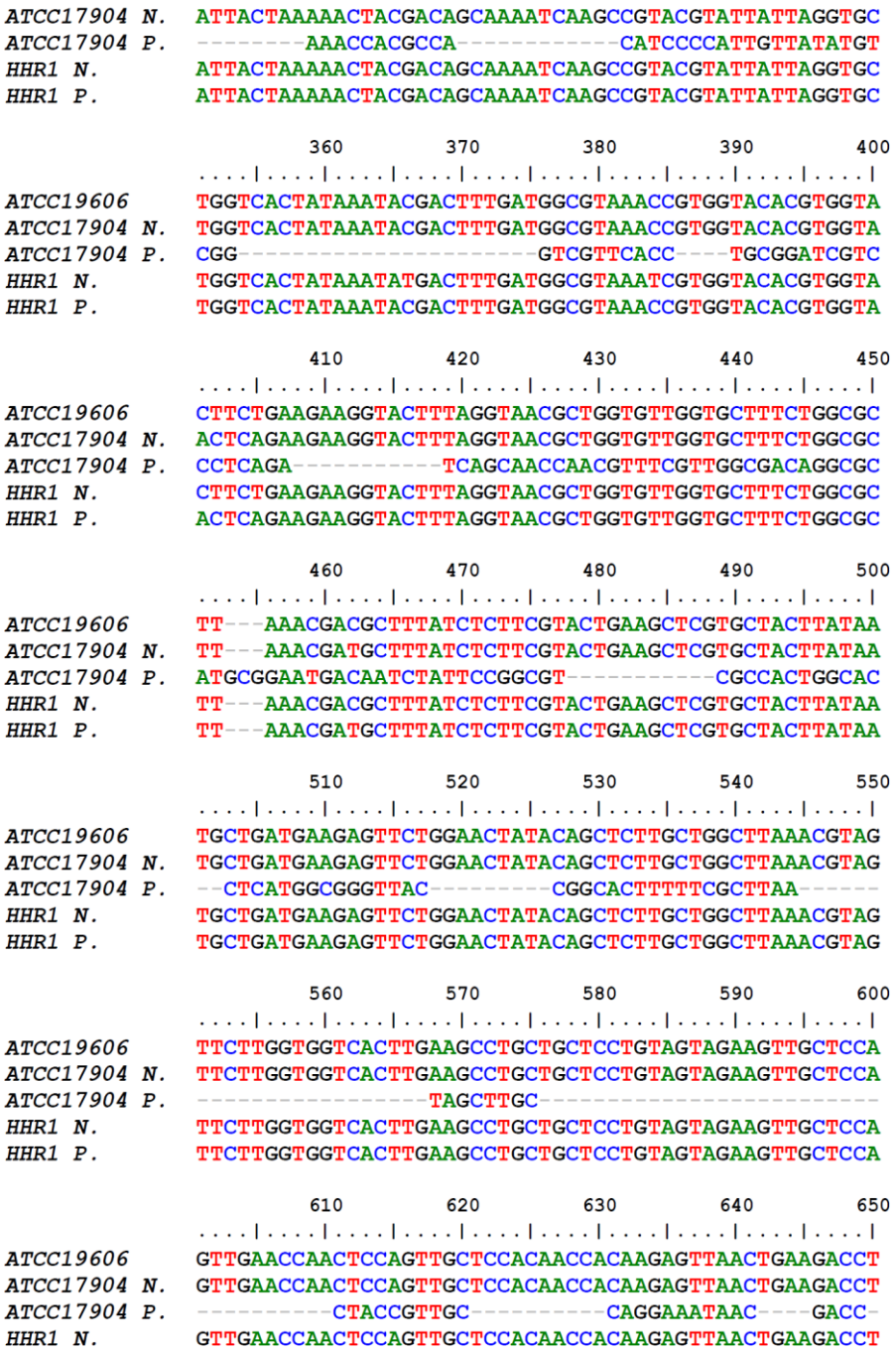


Figure 6. Detection of *OmpA* gene in *A. baumannii*. (A) *OmpA* gene organization map. The location of *OmpA* was determined and specific primers were designed. The gene of interest was PCR amplified using *OmpA*-F / *OmpA*-R primer pairs. (B) The PCR product was loaded on 1% agarose gel. The products size was 1071 bp (black arrow). lane M: HypperLadder™ I Molecular Marker (Biolone), lane 2: amplified *OmpA* gene in ATCC 17904 normal, lane 3: ATCC 17904 treated with plasma, lane 4: amplified *OmpA* gene in HHR1 isolate normal, lane 5: HHR1 treated with plasma.

Furthermore, sequencing of *OmpA* gene was performed to investigate the genetic variation of *A. baumannii* ATCC 17904 and HHR1 resulted from exposure to DBD-CAP treatment in comparison with the control. In Fig. 7, the sequence alignment of *OmpA* gene of both strains with the relative strain of *A. baumannii* ATCC 19606 (accession number: AY485227.1) shows a relative variation in DNA sequences among untreated strain *A. baumannii* ATCC 1790, treated strain *A. baumannii* HHR1 DBD-CAP and untreated strain *A. baumannii* HHR1. Whereas treated *strain A. baumannii* ATCC 17904 with DBD-CAP exhibited a huge variations with all aligned sequences. The *A. baumannii* ATCC 17904 strain displayed more sequence variation than *A. baumannii* HHR1 isolate. The sequence implies more deletion in DNA sequence in comparison with control and *A. baumannii* HHR1 isolate. The result suggests that the DNA damage is dependent on the type of bacteria (28). In addition, several studies reported that one of most inactivation mechanism of plasma is the UV radiation, which caused damage to DNA by inducing the formation of thymine dimmers (29). Moreover, the reactive species generated by plasma, hydrogen peroxide, single oxygen and ozone have strong oxidative effect that cause damage to DNA, as well as peroxynitrite that formed due to the reaction of nitric oxide and superoxide (21,30).


ATCC17904 N. ATTACTAAAAACTACGACAGCAAAATCAAGCCGTACGTATTATTAGGTGC
ATCC17904 P. --------AAACCACGCCA----------CATCCCCATTGTTATATGT
HHR1 N. ATTACTAAAAACTACGACAGCAAAATCAAGCCGTACGTATTATTAGGTGC
HHR1 P. ATTACTAAAAACTACGACAGCAAAATCAAGCCGTACGTATTATTAGGTGC
360 370 380 390 400
ATCC19606 TGGTCACTATAAATACGACTTTGATGGCGTAAACCGTGGTACACGTGGTA
ATCC17904 N. TGGTCACTATAAATACGACTTTGATGGCGTAAACCGTGGTACACGTGGTA
ATCC17904 P. CGG----------GTCGTTCACC----TGCGGATCGTC
HHR1 N. TGGTCACTATAAATATGACTTTGATGGCGTAAATCGTGGTACACGTGGTA
HHR1 P. TGGTCACTATAAATACGACTTTGATGGCGTAAACCGTGGTACACGTGGTA
410 420 430 440 450
ATCC19606 CTTCTGAAGAAGGTACTTTAGGTAACGCTGGTGTTGGTGCTTTCTGGCGC
ATCC17904 N. ACTCAGAAGAAGGTACTTTAGGTAACGCTGGTGTTGGTGCTTTCTGGCGC
ATCC17904 P. CCTCAGA----------TCAGCAACCAACGTTTCGTTGGCGACAGGCGC
HHR1 N. CTTCTGAAGAAGGTACTTTAGGTAACGCTGGTGTTGGTGCTTTCTGGCGC
HHR1 P. ACTCAGAAGAAGGTACTTTAGGTAACGCTGGTGTTGGTGCTTTCTGGCGC
460 470 480 490 500
ATCC19606 TT---AAACGACGCTTTATCTCTTCGTACTGAAGCTCGTGCTACTTATAA
ATCC17904 N. TT---AAACGATGCTTTATCTCTTCGTACTGAAGCTCGTGCTACTTATAA
ATCC17904 P. ATGCGGAATGACAATCTATTCCGGCGT----------CGCCACTGGCAC
HHR1 N. TT---AAACGACGCTTTATCTCTTCGTACTGAAGCTCGTGCTACTTATAA
HHR1 P. TT---AAACGATGCTTTATCTCTTCGTACTGAAGCTCGTGCTACTTATAA
510 520 530 540 550
ATCC19606 TGCTGATGAAGAGTTCTGGAACTATACAGCTCTTGCTGGCTTAAACGTAG
ATCC17904 N. TGCTGATGAAGAGTTCTGGAACTATACAGCTCTTGCTGGCTTAAACGTAG
ATCC17904 P. --CTCATGGCGGGTTAC----------CGGCACTTTTTCGCTTAA------
HHR1 N. TGCTGATGAAGAGTTCTGGAACTATACAGCTCTTGCTGGCTTAAACGTAG
HHR1 P. TGCTGATGAAGAGTTCTGGAACTATACAGCTCTTGCTGGCTTAAACGTAG
560 570 580 590 600
ATCC19606 TTCTTGGTGGTCACTTGAAGCCTGCTGCTCCTGTAGTAGAAGTTGCTCCA
ATCC17904 N. TTCTTGGTGGTCACTTGAAGCCTGCTGCTCCTGTAGTAGAAGTTGCTCCA
ATCC17904 P. ----------TAGCTTGC----------
HHR1 N. TTCTTGGTGGTCACTTGAAGCCTGCTGCTCCTGTAGTAGAAGTTGCTCCA
HHR1 P. TTCTTGGTGGTCACTTGAAGCCTGCTGCTCCTGTAGTAGAAGTTGCTCCA
610 620 630 640 650
ATCC19606 GTTGAACCAACTCCAGTTGCTCCACAACCACAAGAGTTAACTGAAGACCT
ATCC17904 N. GTTGAACCAACTCCAGTTGCTCCACAACCACAAGAGTTAACTGAAGACCT
ATCC17904 P. ----------CTACCGTTGC----------CAGGAAATAAC----GACC-
HHR1 N. GTTGAACCAACTCCAGTTGCTCCACAACCACAAGAGTTAACTGAAGACCT

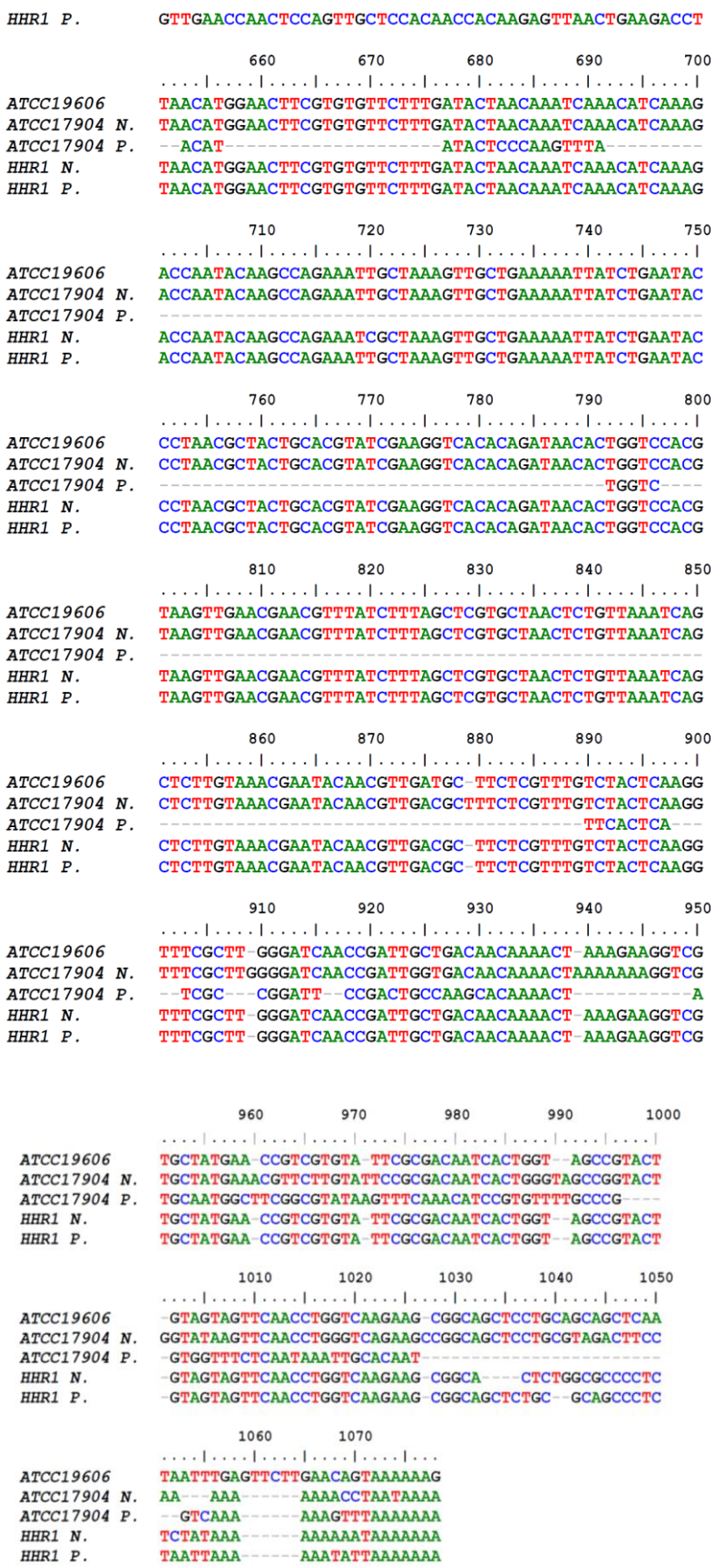


Figure 7. Sequence alignment of *OmpA* gene for *A. baumannii* strains ATCC 17904 strain and *A. baumannii* HHR1 isolates. Both strain and isolate were exposed to DBD-CAP and their sequenced were aligned with the corresponding strain ATCC 19606 (Sequence ID: AY485227.1) from NCBI.
N; is referred to normal (control or untreated), P; represents the plasma treatment. The sequence alignment was done by MEGA program, and the graphic view was displayed by Bioedit service.

Does Cold Plasma Disrupt the Bacterial Plasma Membrane and Cell Wall?

To unequivocally determine the ability of cold plasma to damage the cell wall and penetrate to the cytoplasm, *A. baumannii* DBD-CAP treated and untreated cells were fractionated into cytoplasm (Cyt), periplasm (PM), and outer membrane (OM), and all fractions were mixed with 1X SDS loading dye and resolved on 12 % SDS-PAGE before being visualized by UV. Figure 7 showed that the DBD-CAP is fairly damage the cell wall and penetrate to the cytoplasm and target the DNA, where *A. baumannii* ATCC 17904 is more sensitive to DBD-CAP than *A. baumannii* HHR1. As shown in Fig. 8 A, there is no change in OM, PM and cytoplasm of standard strain. This perhaps proves that cold plasma affects proteins only at lethal dose instead of sub-lethal does. Fig. 8B did not show any damage in the cytoplasm or in somehow PM and OM, however, PM is slightly smear and unclear in comparison to the control, and thus might due to the technical trouble shoot in SDS-PAGE and for CAP in particular. The effect of cold plasma on the cell wall integrity was stated in previous study which reported that a partial or complete destruction of plasma membrane architecture occurred when *E.coli* cells are treated with cold plasma, and triggers all the intracellular material out of the cells and ultimately death (31).

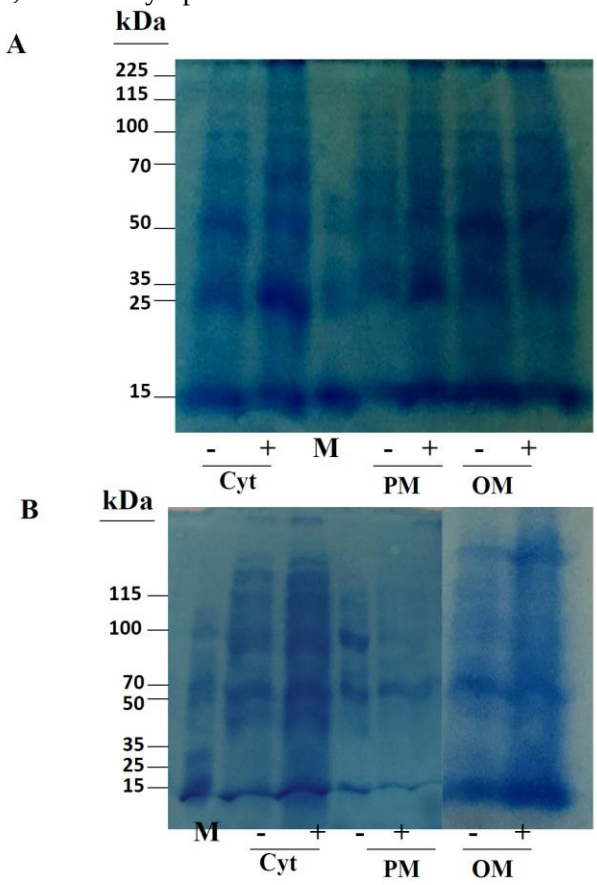


Figure 8. One dimensional SDS-PAGE of *A. baumannii* fractions. Overnight cultures of *A. baumannii* were fractionated into periplasm by osmotic shock procedure, outer membrane by Triton X-100 and cytoplasm. All fractions for both treated and untreated cells were mixed with 1X SDS loading dye and subjected to 12 % SDS-PAGE. A: Coomassie blue staining of *A. baumannii* ATCC 17904 fractions; B; SDS-PAGE for *A. baumannii* HHR1isolate. In all cases: Lane M: is the PageRuler™ Plus Unstained Rec. Protein Ladder (Promega). The sign – refers to the untreated cells with cold plasma; the sign + is represents the cells treated with CAP; Cyt: is the cytoplasm, PM is periplasm and OM is the outer membrane. ~35µg of proteins were loaded into each well.

Conclusion:

The growth of both strains (ATCC 17904 and HHR1) of *A. baumannii* is affected by exposure to DBD-CAP for several time intervals and the inactivation diameter is increased via increasing the time of exposure. Moreover, *A. baumannii* HHR1 isolate was more resistant to the effect of DBD-CAP than *A. baumannii* ATCC 17904; the inactivation diameter for the later was greater than that for the *A. baumannii* HHR1.

Conflicts of Interest: None.

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دراسة تأثير البلازما الباردة على بكتريا الراكدة البومانية

ريام راضي عطا **هاله الحيدري** **حامد مربط**

[2،1] قسم علوم الحياة، كلية العلوم للبنات، جامعة بغداد، بغداد، العراق.
[3] قسم الفيزياء، كلية العلوم للبنات، جامعة بغداد، بغداد، العراق.

الخلاصة:

تهدف هذه الرسالة الى توظيف طرائق فيزيائية في معالجة الراكدة البومانية وذلك لغرض التقليل من استخدام الادوية والعقاقير. تضمنت التجربة تعريض سلالتين من الراكدة البومانية (السلالة العالمية والسلالة المحلية) الى البلازما الباردة بقدرة 20 كيلو فولت وعلى الاوقات التالية (15 و30 و45 و60) ثانية، ثم بعد ذلك اجريت العديد من الاختبارات لغرض دراسة تأثير البلازما على البروتين والحامض الرايبوزي منقوص الاوكسجين وايضا على الشكل الخارجي، ان تأثير البلازما على نمو البكتريا تم حسابه عن طريق قياس قطر التثبيط. أثبتت النتائج ان قطر التثبيط يزداد بزيادة زمن التعرض الى البلازما ايضا اثبتت ان السلالة المحلية هي اكثر مقاومة الى البلازما من السلالة العالمية.

الكلمات المفتاحية: الراكدة البومانية السلالة العالمية، البلازماالباردة، حاجز ثنائي القطب، السلالة المحلية .