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# Study the antibacterial Effect of $CO_2$ Laser againstMultidrug Resistant Biofilm formation*Acinetobacterbaumannii*Isolated from Different Samples

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**Abstract**

Acinetobacterbaumanniihas become known as an imperative healthcare associated and multidrug-resistant microorganismwarrants the training of novel methodologies for prevention and treatment. This report aimed to estimate the antimicrobial properties of$CO_2$laser at 10600 nm on *A.baumannii* isolates. Two hundred specimens were collected from patients suffering from wound infections, urinary tract infections, respiratory infections and medical equipment samples. 50 samples for each. These samples were gathered from diverse hospitals in Baghdad\Iraq. The collected specimens were streaked directly on CHROM agar Acinetobacter. The positive culture results were diagnosed genotypicaly, *recA* gene (a house keeping gene) was used for this purpose. All isolates were tested for antibiotic sensitivity testing and 13 divers of antibiotics were used. Also, the ability of biofilm formation was detected. $CO_2$laser 10600 nm at power densities (1000,1500,2000 and 2500) W/cm2 with exposure time (15,30, 45 and 60) second was used for the irradiation experiment.17 isolates were positive to *A. baumannii* which were distributed as follows 4 ,6, 3 and 4 isolates from wounds,urine , sputum and medical equipments samples respectively. Antibiotic sensitivitytest results considered ten of *A.baumannii*as MDR isolates because of its capability to resist ten antibiotics belong to cephalosporins, carbapenems,ampicillin-sulbactam, fluoroquinolones, and aminoglycosides groups. In additions 14(82.35%) out of the 17 isolates were biofilm producer ranged from weak, moderate to strong biofilm produer. Four isolates, one from four different isolation sources were chosen for $CO_2$ laser irradiation each isolate resisted to all antibiotics used in this study and strong biofilm producer. In general, in this study the results showed that when using power densities (2000 and 2500 $W/cm^2$ at exposure time 30s and 45s) of $CO_2$ laser irradiations the maximum rate of killing percentage was ranged from 97% to 100% for *A.baumannii* isolated from clinical samples while more exposure time were needed (45s and 60s) to get the same killing percentage for *A.baumannii* isolated from medical equipments. Consequently, the use of $CO_2$ laser 10600 nm which is independent towards the antibacterial agents resistance pattern of MDR *A.baumannii* could verify advantageous in treatment of various types of infections caused by these bacteria in addition it may be used in sterilization of some medical equipment surfaces.

***Keywords:****A.baumannii, antibacterial effects, biofilm formation, $CO_2$ Laser 10600nm*

**الخلاصة:**

لقد اصبحت بكتريا *Acinetobacterbaumannii*تعرف بمقاومتها المتعدده للمضادات الحيويه مما دعى الى تجربه طرق جديده لعلاجها وتجنب الاصابه بها .كان الهدف من هذا البحث هو الكشف عن مدى تأثير ليزر ثاني اوكسيد الكربون 10600 نانوميتر على هذا النوع من البكتريا .تم جمع 200 نموذج من المرضى المصابين بالتهابات الجروح والمجاري البوليه والتهابات الجهاز التنفسي اظافة الى نماذج من العدد الطبيه المختلفه .50 نموذج لكل نوع.جمعت هذه النماذج من مستشفيات مختلفه في مدينة بغداد/العراق.زرعت النماذج التي تم جمعها مباشرة على وسط استنوبكتر كروم اكار .شخصت النماذج الزرع الموجبه جينيا واستخدم لهذا الغرض *recA* جين احد انواع الجينات المصنفه للنوع في هذه البكتريا .اختبرت حساسيه كافة العزلات الى 13 نوع مختلف من المضادات الحيويه كذلك تم الكشف عن قابلية هذه العزلات على تكوين الغشاء الحيوي.اجريت تجربة التشعيع باليزر نوع ثاني اوكسيد الكربون 10600 نانوميتر وعند قوة

1000,1500,2000و2500 واط/سم2 وباوقات زمنيه 15,30و45 ثانيه. تم عزل وتشخيص 17 عزله من بكتريا ووزعت على التوالي الى 4,6,3و4 عزلات تم عزلها من نماذج الجروح،البول،القشع والعدد الطبيه. اظهرت نتائج فحص الحساسيه الى ان 10 عزلات من اصل 17 عزله كان لها القدره على مقاومة 10 مضادات حيويه تنتمي الى مجاميع مختلفه.اضافة الى ذلك كانت 14 (82.35%) من اصل 17 عزله منتجه للغشاء الحيوي بدرجة ضعيف-متوسط الى قوي. . تم اختيار عزله واحده لكل مجموعه من العينات تميزت بتعدد مقاومتها للمضادات الحيويه وقدرتها العاليه على انتاج الغشاء الحيوي.
اضهرت نتائج هذه الدراسه الحصول على اعلى نسبة قتل لبكتريا *A.baumannii* المعزوله من النماذج المرضيه تراوحت من 97%الى 100% عند 200 الى 2500 واط/سم2 وزمن تشعيع 30-45 ثانيه بينما احتاجت عزلات *Abaumannii* المعزوله من العدد الطبيه الى وقت اطول تراوح من 45-60 ثانيه .استنادا الى هذه النتيجه ،يمكن ان نعتمد استخدام التشعيع بالليزر نوع ثاني اوكسيد الكربون 10600 نانوميتر كعلاج من الامراض المختلفه او لتعقيم بعض انواع العدد والمستلزمات الطبيه ضد هذا النوع من البكتريا المتعدد المقاومه للمضادات الحيويه والنتج للغشاء الحيوي .

## Introduction:

Acinetobacter baumannii is a Gram-negative, non-motile, obligate aerobic coccobacilli that is omnipresent in nature and have been found in soil, water,sewage, animals and humans and in healthcare settings,this bacteria non-fastidious and be able to grow in ordinary laboratory culture media.[1], in the earlier, it is decades, has evolved from an microrganism of problematic pathogenicity to one of the most essential pathogens causing hospital-acquired infections, mainly in intensive care units (ICUs) [2].Several strains of Acinetobacter can stay alive for 30-45 days in the atmosphere, promoting spread within the hospital settings [3]. *A. baumannii* was isolated from the skin, throat, rectum,urethra and respiratory tract of humans. *A. baumannii* financial records for almost 80% of reported Acinetobacter infections [4]. Thecontagionwith *A. baumannii* in healthcare settings are associated with the employ of invasive actions (automatic ventilation, vascular catheters) and patient's fundamental conditions [5]. This characteristic in company with antibiotic resistance, biofilm formation, multiple virulence factors and directextend are the main challenges for deterrence and manage activities [6].

The majorityurgent risk factors for *obtainingA. baumannii* infections are: previous antibiotic use (third-generation cephalosporins, fluoroquinolones or carbapenems), *A.baumannii* has obtainresistance to lately urbanized antimicrobial agents; these strains are identified as multidrug resistant MDR*A.* baumannii. It became common in numerous hospitals everywhere all through the worldand has been lately documented as a leading nosocomial pathogen [7].MDR Acinetobacter spp. can allude to being resistant to a minimum of three classes of antimicrobial drugs e.g. all penicillins and cephalosporins fluoroquinolones, and aminoglycosides. The management of these infections has become progressively morecomplicated due to the surfacing of extremely resistant strains [8]. In addition, the ability of *A.baumannii* to contribute in biofilm formation promotes stability in surfaces and could be a factor to continuation of environmental incidence[5]. It is correctly noticeable that the biofilm-forming capability can be considered a standout amongst the most vital virulence factors wide spreadin a great number of *A. baumannii* medical isolates[9,10].

Present researches have started to embrace the expansion of alternative therapies instead of antibacterial drug to help patients in convalescing from their infections because the MDR bacterial infections are progressively widespread and complicated to treat. Potential of old-antibiotics types to address the current requirement for new antibiotics. One of these methods, the employ of laser light in the management of infectious disease and diverse medical field in. $CO_2$ laser has been extensively used in the next two decades as an incisive tool in increasingly wide areas, such as neurosurgery, dermatology and plastic surgery, dental treatments, general surgery and for schemingbacterial infections[11,12,13].The bactericidal effect of 10,600 nm CO2 (carbon-dioxide) laser, on gram positive andgram-negative bacteria

was recognized by different authors' worldwide [12,13,14,15],they werereported that $CO_2$ laser has bactericidal effect on different pathogenic bacteria isolated from divers clinical samples. According to our knowledge,no .Previous data are reported about the impact of laser on *A. baumannii* , therefore this study designed to prevalence the effect of $CO_2$ laser (10600nm) on this bacteria isolates from different samples at different power densities and different exposure times.

## Material and Methods:

**Collection and diagnosis of bacterial isolates:**

Two hundredspecimens were collected from patients suffering from wound infections, urinary tract infections, sputum and medical equipments samples. 50 samples for each.These samples were collected from different hospitals in Baghdad \Iraq.Samples were collected from the same hospitals in the same period.The collected specimens were streaked directly on CHROM agar Acinetobacter (this media was prepared according to the manufacturer's instructions. Chromagar/Paris-France) then incubated for 18-24.Hours at 37ºC. The positive culture results were examined for presumptive colonies in agreement with the manufacturer's recommendations. Further identification tests included the morphological characteristics and biochemical tests were carried out depending on[16].

**Genotyping detection for bacterial isolates:**

*recA*gene(a house keeping gene ) was used for genotypic diagnosis. Specific primers listed in table(1) wereemployed and the amplified size was 425bp.Template DNA was prepared by boiling method by [17].Concisely, few isolated colonies of overnight growth bacteria were suspended thoroughly in 1 mL distilledwater and boiled in a water bath for 10 min. After centrifugation, the supernatant was used as template DNA.PCRmixture of composed of 12.5 of GoTaq®Green Master Mix (2x), 5 µl template DNA, 1.5 µl primers (foreach) final concentration (0.6pmol/µl),and nuclease free water up to 25 µl (4.5 µl).

**Table-1: The oligonucleotide PCR primers used in this study**.

| Primer | Sequences (5'_3') | Annealing temp.°Ctime sec | Product size bp | Reference |
|---|---|---|---|---|
| *RecA* | F- CCTGAATCTTCYGGTAAAAC<br>R- GTTTCTGGGCTGCCAAACATTAC | 54°Cfor 30 sec. | 425 | [17] |

Resultswere interpreted based on the instruction provided by Clinical Laboratory and Standard Institute (CLSI 2014) Guidelines, and use *Pseudomonas aeruginosa* ATCC® 27853 and *Escherichia coli* ATCC® 25922 as a quality controlfor tetracyclines and trimethoprim-sulfamethoxazole **[17].**

**Biofilm detection assay:**

This quantitative test described by [18, 19] considered the standard method for biofilm detection all isolated from fresh agar plates were inoculated in 10 ml of trypticase soy broth with 1% glucose w/v. Bacterial broths were incubated at 37Cº for 24 hours. The culture was then diluted 1:100 with

**Antibiotic sensitivity testing:**

All isolates were tested for antibiotic sensitivity with (AK) Amikacin (30 µg), , (CAZ) Ceftazidime (30µg) , (A/S) Ampicillin-sulbactam (10/10µg), (IPM) Imipenem (10µg) , (MEM) Meropenem (10µg) , (PI) Piperacillin (100µg) , (TI) Ticarcillin (75µg ) , (TE) Tetracycline (30µg) , (CPM) Cefepime (30µg) , (CRO) Ceftriaxone (30µg) , (CTX) Cefotaxime (30µg) , (LEV) Levofloxacin (5µg), (SXT) Trimethoprim-sulfamethoxazole (1.25/23.75µg) antibiotic agents (Bioanalys ,Turkey ) All of the inoculated plates were aerobically incubated at 37°C for 18-24 h in an aerobic atmosphere.

containing 9 ml of normal saline, then mixed by vortex to get homogenous suspension compared with the McFarland solution ($1.5\times10^8$ CFU/ml). Standard suspension ofbacterial growth with dilution of ($10^{-6}$ viable cell/ml) is chosen from the other serial dilutions for *P. aeruginosa* irradiation, 400 µl ofthissuspension was placed in a sterile Eppndroff tube. The irradiation Experimentswere done in sterilized hood and the hand piece of $CO_2$ laser was fixed. perpendicular on the opening of the Eppndroff tube. Thebacterial suspension was subjected to laser irradiation experiment at different power densities (1000, 1500,2000 and 2500 W/cm$^2$) and exposure time (15,30,45 and 60) second. After irradiation, 100 µl of the irradiated suspension was spread on the surface of brain, heart infusion agar plates for each isolate and incubated aerobically at 37 ºC for (18-24) hrs, 3 replicates were used for each bacterial isolate. After incubation, the viable cells count CFU/ml was determined by using of digital colony counter [20].

**Statistical analysis**

Differences between groups were calculated by one-way analysis of variance where appropriate using (Minitab VERSION 11) Values are expressed as mean ±SD. LSD (Least Significant Difference) a,b,c,d for rows ,similar letters mean the absence of significant differences and different letters mean the presence of significant differences .A p value of less than 0.05 was considered statistically significant.

## Results and Discussion:

In the up to datestudy,17 isolates of *Acinetobacter baumannii* out of 65 gram negative and positive bacterial fresh medium and inoculated individual wells of sterile 96 well- flat bottom polystyrene tissue culture plate. Negative control wells contained inoculated sterile broth. The plates were incubated at 37Cº for 24hrs. After the incubation content of each well was removed by gentle tapping. The wells were washed with Deionized water once. This removed free floating bacteria. Biofilm formed by bacteria adhere to the wells were stained by (0.1%) w/v crystal violet for 15 min. Excess stain was removed by using Deionized water and plates were kept for drying. Optical density (OD) of stained adherent biofilm was obtained by using a micro ELISA auto reader (Human reader ELISA HS) at wavelength 630 nm, and the interpretation of the results was calculated according to [18] The experiment was performed in triplicate and repeated three times.

**$CO_2$ Laser System**

The CW $CO_2$ laser system (DS-40 U, Daeshin Enterprise co., Ltd., Korea) emitting laser light at 10600 nm, IR light. The power densities are (1000, 1500,2000 and 2500) W/cm² and the exposure time is (15,30 and 45) second.

**Selection of Bacterial Isolates for $CO_2$ Laser Irradiation**

Fourisolates of ***Acinetobacter baumannii*** were chosen for$CO_2$ laser irradiation according to the results of antibiotic susceptibility test and biofilm formation, one isolate from each different clinical sourcesand one isolatefrom the medical equipments samples,all selected isolates are (MDR) and biofilm producerin order to contrast the effect of $CO_2$ laser 10600 nm irradiation on the same bacterial species but isolated from different sources.

**Irradiation Procedure**

Bacterial colonies are selected from the brain heart infusion agar to a test tube

According to table 1, out of 200 specimens of wound infections, urinary tract infections, respiratory infections and medical equipments samples,17 isolates were positive to *A. baumannii* which were distributed as follows4 ,6 and 3 isolated from wounds,urine and sputum respectively.Then again4 isolates of these bacteria were isolated from 50 medical equipments samples.

speciesisolated from all positive culture samples.The identification of this bacteria was confirmed by phenotypic and genotypic methods.On CHROMagarAcinetobacter, Acinetobacter species seem as bright salmon-red colonies after 24 hours of growth. The molecular detection shows a positive result of the recA gene presence for all suspected *A. baumannii*isolated in our study as shown in figure 1.

**Table-2: Number and percentage distribution of *Acinetobacter baumannii* isolated from different sources**

| Type of specimens | Total number of specimens Total no.(200) | Symbol | No(%) of positive *A. baumannii* isolates |
|---|---|---|---|
| Wound infections (wound swap) | 50 | Abw | 4(8) |
| Urinary tract infections(urine) | 50 | Abu | 6(12) |
| Respiratory infections(sputum) | 50 | Abs | 3(6) |
| Medical equipments | 50 | Abme | 4(8) |

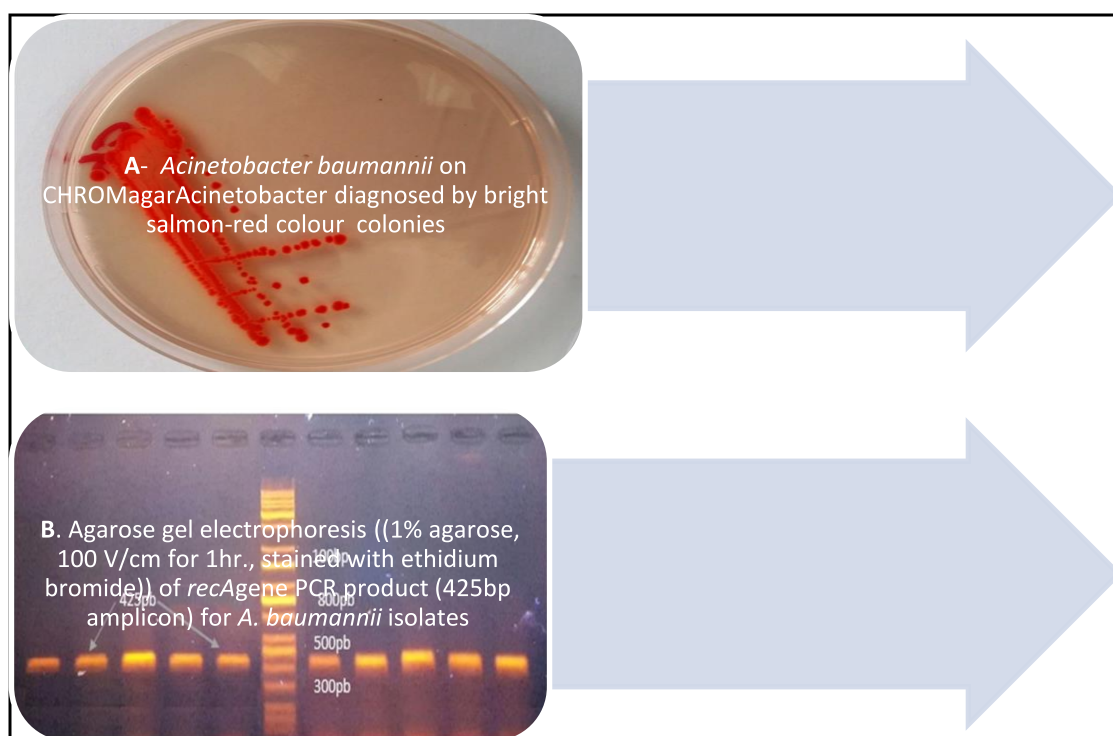


**Figure-1:Phenotypic and genotypic detection of *Acinetobacter baumannii***

*A. baumannii* it's a goodreasonfor infections in both healing center settings and in population. They are the second most frequently isolated non-fermenters in human samples, after *P. aeruginosa*. About 1-3% of health care-associated infections are caused by Acientobacter spp.One important feature of *A. baumannii* is its capability to createoutbreaks, which is in correlated to antimicrobial resistance and resistance to dehydration [22].Extensive use of antimicrobial agents has contributed to appearance and dissemination of nosocomial *A. baumannii* infections. These infections are tricky to treat owing to the microrganism's multidrug-resistant (MDR) phenotype [23], and increase in the number of *A. baumannii* strains resistant to a extensivevariety of antibiotics, include aminoglycosides, fluoroquinolones [24], tetracyclines, broad-spectrum β-lactam antibiotics [25], aminopenicillins, ureidopencillins, chloramphenicol and more recently carbapenemes [26].In the current study the *A. baumannii* Iraqi isolates 2Abw,5Abu,7Abu,10Abu, 11Abs,12Abs,16Abme and 17Abme considered as MDR isolates depend on the **[27].**

Data accessible in table 3 shows the resistance number and percentage of *A. baumannii*isolates to the antibiotics used in update study, we discovered that all *A. baumannii* clinical and medical equipmentsisolates had 17(100%) resistance to Ceftriaxone, Piperacillin and gentamicin. This study also showed a high resistance 16(94%) to Cefepime and Ceftazidime followed12(71%) to Ticarcillin and Levofloxacin, while 11(64.7%) showed moderate resistance to Ampicillin-sulbactam combination followed by Meropenem, Imipenem 10(59%) and Amikacin 8(47%).4(23.5%) isolates were resistedto Trimethoprim-sulfamethoxazole and Tetracycline.The current study found that there were some differences in the number of antibiotics that each isolate of*A. baumannii*can resist as clear in table 3, figure 2,some of them like no 2Abw,5Abu,7Abu,10Abu,11Abs,12Abs,16 Abme and 17Abme were able to resist from 11 to 13 antibiotics belong to diverse groups. While *A. baumannii*isolates number 3W,4W,6Abu,8Abu,9Abu,13Abs,14Abme and 15Abme wereable to resist from 7 to 10 antibiotics.

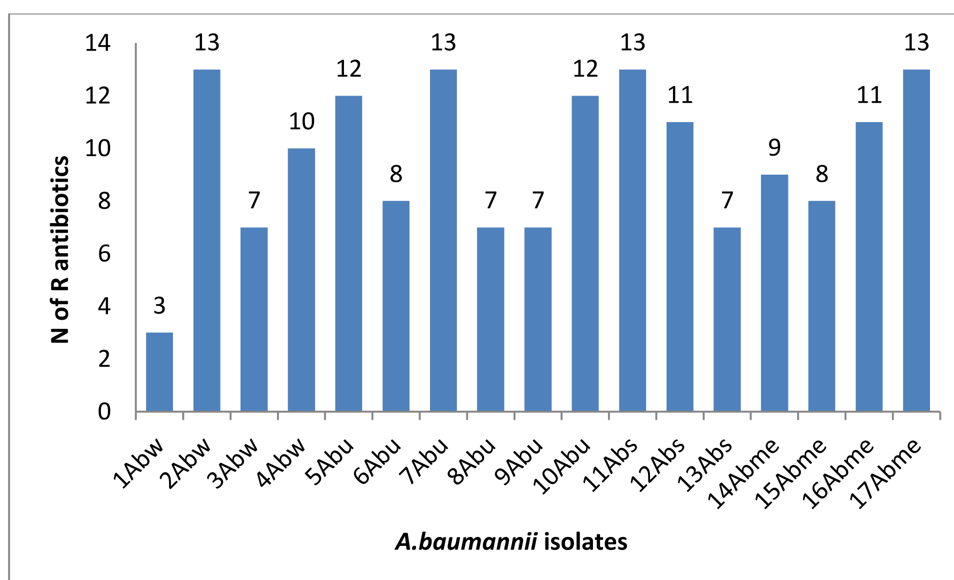


**Figure-2:Number of resisting antibiotics by*A.baumannii* isolates**

**Table-3: Antibiotic Susceptibility of 17 Acinetobacter baumannii isolates.**

| ^Isolate no (Source) | A/S | CRO | TS | CPM | TZ | AK | TI | TE | PI | MEM | LEV | IP | GN | N of R antibiotics |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1Abw** | S | R | S | S | S | S | S | S | R | S | S | S | R | 3 |
| **** Abw** | R | R | R | R | R | R | R | R | R | R | R | R | R | 13 |
| **3 Abw** | S | R | S | R | R | S | R | S | R | S | R | S | R | 7 |
| **4 Abw*** | R | R | S | R | R | S | R | S | R | R | R | R | R | 10 |
| **5 Abu **** | R | R | S | R | R | R | R | S | R | R | R | R | R | 12 |
| **6 Abu** | R | R | S | R | R | S | R | S | R | S | R | S | R | 8 |
| **7 Abu **** | R | R | R | R | R | R | R | R | R | R | R | R | R | 13 |
| **8 Abu*** | S | R | S | R | R | S | R | S | R | S | R | S | R | 7 |
| ***9 Abu** | S | R | S | R | R | S | R | S | R | S | R | S | R | 7 |
| **10 Abu **** | R | R | S | R | R | R | R | S | R | R | R | R | R | 12 |
| **11 Abs**** | R | R | R | R | R | R | R | R | R | R | R | R | R | 13 |
| ****12 Abs** | R | R | S | R | R | R | R | S | R | R | R | R | R | 11 |
| **13 Abs **** | S | R | S | R | R | S | R | S | R | S | R | S | R | 7 |
| **14Abme*** | S | R | S | R | R | S | R | S | R | R | R | R | R | 9 |
| ***15 Abme** | R | R | S | R | R | S | R | S | R | S | R | S | R | 8 |
| **16 Abme **** | R | R | S | R | R | R | R | S | R | R | R | R | R | 11 |
| **17 Abme **** | R | R | R | R | R | R | R | R | R | R | R | R | R | 13 |
| **RN(%)** | 11 **(64.7)** | 17(**100**) | 4(**23.5**) | 16(**94.**) | 16(**94**) | 8(**47**) | 12(**71**) | 4(**23.5**) | 17(**100**) | 10(**59**) | 12(**71**) | 10(**59**) | 17(**100**) | ------ |
| **SN(%)** | 6(**35.3**) | 0(**0**) | 13(**76.5**) | 1(**6**) | 1(**6**) | 9(**53**) | 1(**29**) | 13(**76.5**) | 0(**0**) | 7(**41**) | 1(**29**) | 7(**41**) | 0(**0**) | ------- |

^W:Wound, U:Urin, S:Sputum, me :medical equibments , RS:total of resisted isolates, SN: total of sensitive isolates. (A/S) Ampicillin-sulbactam (10/10μg), (CRO) Ceftriaxone (30μg) , (TS) Trimethoprim-sulfamethoxazole (1.25/23.75μg), (CPM) Cefepime (30μg) , (TZ) Ceftazidime (30μg) , (AK) Amikacin (30 μg), (TI) Ticarcillin (75μg ) , (TE) Tetracycline (30μg) ,( PI) Piperacillin (100μg) , (MEM) Meropenem (10μg) , (LEV) Levofloxacin (5μg), (IP) Imipenem (10μg) , GN:gentamicin (30 μg) .,(*) Weak to Moderate -biofilm producer ,(**)Strong- biofilm producer.

management of these infections has become a public health challenge in many countries. Nowadays, the most serious problem in the treatment of *A.bumannii* infection is acquired multidrug-resistance and biofilom formation ability and other important virulence factors, leaving only few antimicrobial agents as treatment options[29]

Four isolates from four different isolation sources were chosen for $CO_2$ laser irradiation according to the results of antibiotic sensitivity and biofilm formation results. The selected *A. baumannii* isolates were (2Abw,7Abu,11Abs,17Abme) each isolate resisted to all 13 examined antibiotics and strong biofilm producer as mentioned above in table 3.

exposure times 15-45s (15 sec. increment) corresponding to power densities 1000,1500,2000 and 2500 W/$cm^2$ the results have shown a reduction in CFU/ml of *A. baumannii*clinical isolates[ 2Abw]isolated from wound infectionascompared with control group(87CFU/mL) as exposed in table (4) and figure (3).In the curent study, the $CO_2$ laser killed 99.4% and 100% of this bacteria at 2000 W/$cm^2$ and 2500W/$cm^2$ respectivly at exposure times 45s while it killed 97.7 % and 96.6% of bacteria at 2500 and 1500 W/$cm^2$ at exposure time 30s and 45s, whereas the lowest percentage reached 94.3% at exposure time 45s when using power density 1000 W/$cm^2$ as revealed in table (4).

It is worthy to notice that these resisted isolates not easy to treat, especially if they can produce bifilm as clearly noticed in the table 3 that 14(82.35%) out of the 17 isolates were biofilm producer ranged from weak,moderate to strong biofilm produer isolates while only 3(27.65%) were provide negative outcome in the quantitative analyze for biofilm formation.

Acinetobacter can pollute many surfaces and medical apparatus, such are: suctioning equipment, washbasins, bedrails, bedside tables, ventilators, cushions, sleeping pads, hygroscopic swathes and revival gear [28]. The capability of this bacteria to contribute in biofilm formation promotes durability in surfaces and may contribute to the continuation of an environmental presence during outbreaks [5]. As a result, the clinical

**$CO_2$ Laser irradiation results**

**1-The effect of $CO_2$ laser 10600 nm irradiation on the (CFU/ml)of *A. baumannii* isolated from clinical sources.**

According to the results of statistical analysis by using analysis of variance and Least Significant difference-LSD test,it was found that there were statistical significant differences in the viable bacterial number (CFU/ml) amongdiverse power densities and different exposure times for all MDR biofilm producer *A.baumannii* isolates.The highest statistically important differences ($p< 0.001$) were detected between different exposure times compared with each other and with control when the power densities were considered a constant.

After $CO_2$ laser irradiation by using different power densities and different

**Table -4: Antibacterial effect of $CO_2$ laser 10600 nm against *A. baumannii* 2Abw at different exposure time and power densities**

| Power densities W/cm² | Control Mean±SD * CFU/ml | Exposure time/second CFU/ml (killing percentage) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 15 sec | | 30 sec | | 45 sec | |
| | | Mean±SD | ( %) | Mean±SD | ( %) | Mean±SD | ( %) |
| 1000 | 87 | 55±3 a | 36.8 | 29±6 b | 66.7 | 5±4 c | 94.3 |
| 1500 | 87 | 48±5 a | 44.8 | 13±4 b | 85.1 | 3±7 c | 96.6 |
| 2000 | 87 | 41±3 a | 52.8 | 6±2 b | 93.1 | 0.5±5 c | 99.4 |
| 2500 | 87 | 36±4 a | 86.6 | 2±5 b | 97.7 | 0 c | 100 |

* CFU/ml=mean×10 [7] // a < 0.05 , b <0.01 , c< 0.001.

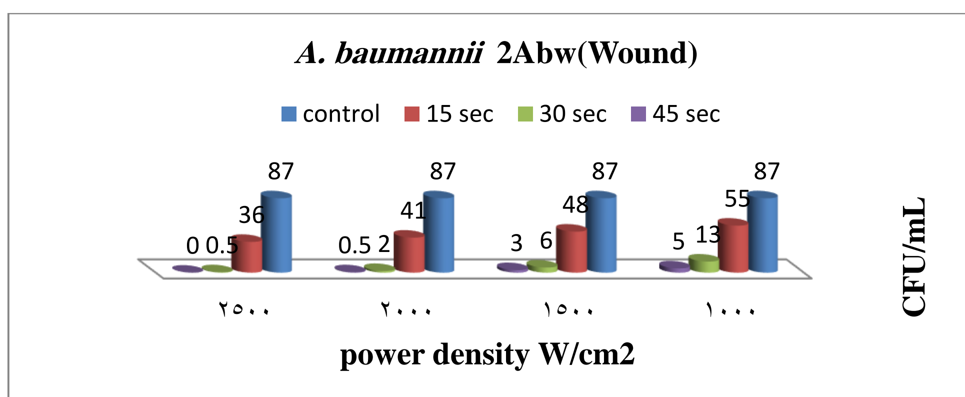


**Figure-3:The reduction in CFU/mL of *A. baumannii* 2Abw(Wound)after $CO_2$ laser irradiation corresponding to power densities 1000,1500,2000 and 2500 w/cm$^2$ with exposure times 15-45sec (15 sec. Increment)**

power densities as illustrated in table 5 and figure 4.The present study recorded high percentage of killing 100% at exposure time 45s at 2000 and 2500 W/cm$^2$ while 98.9% at 2500 W/cm$^2$ at 30s and 1500 W/cm$^2$ at 45 s, also 96.8% at 30s at 2000 W/cm$^2$ while 92.4% at 1500 W/ cm$^2$ for 45s.

The reduction in the mean value of MDR strong biofilm producing*A. baumannii* 7Abu (urine) isolated from UTIs infection after irradiation with $CO_2$ laser was observed when compared with the mean value of bacteria before laser irradiation. A reduction in the CFU/mL was observed with the increase of the exposure times and

**Table (5): Antibacterial effect of CO2 laser 10600 nm against *A.* baumannii7Abu(urine)in different exposure time and power densities**

| Power densities W/cm² | Control Mean±SD * CFU/ml | Exposure time/second CFU/ml(killing percentage) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 15 sec | | 30 sec | | 45 sec | |
| | | Mean±SD | ( %) | Mean±SD | ( %) | Mean±SD | ( %) |
| 1000 | 93 | 53±8 a | 43 | 18±4 b | 80.6 | 7±4 c | 92.4 |
| 1500 | 93 | 43±6 a | 53.7 | 13±7 b | 86.2 | 1±2 c | 98.9 |
| 2000 | 93 | 25±9 a | 73.1 | 3±8 b | 96.8 | 0 c | 100 |
| 2500 | 93 | 9±7 a | 90.3 | 1±2 b | 98.9 | 0 c | 100 |

* CFU/ml=mean×10 $^{7}$ // a < 0.05 , b <0.01 , c< 0.001.

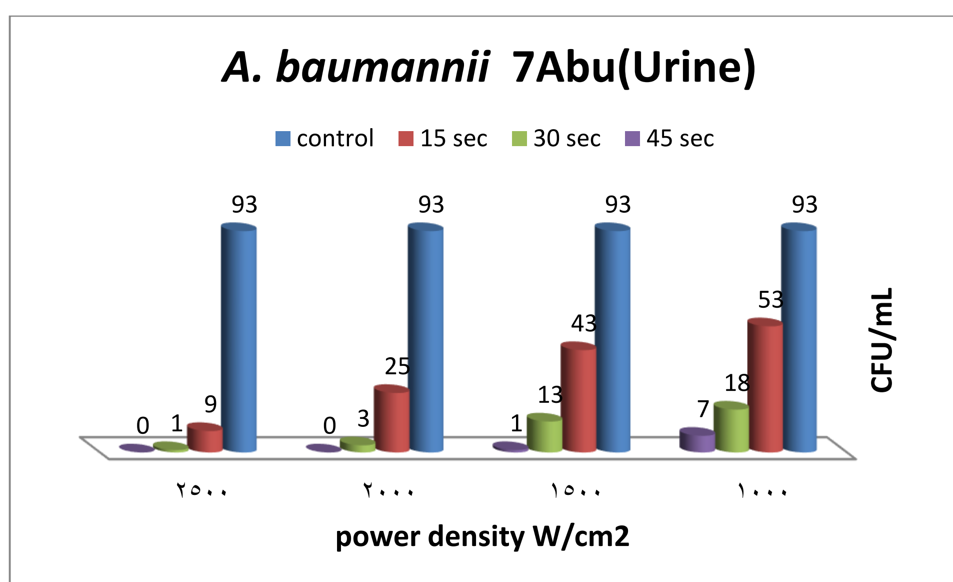


**Figure-4: The reduction in CFU/mL of *A. baumannii* 7Abu (urine)after $CO_2$ laser irradiation corresponding to power densities 1000,1500,2000 and 2500 w/cm² with exposure times 15-45sec (15 sec. Increment)**

number decrease was achieved with 500 W/cm² $CO_2$ laser irradiation at 45s whereas the complete killing 100% observed with 2500 W/cm²at exposure time45s, the reduction of mean value had decreased to 97.5 CFU/ml at 1500,2000,2500 W/cm² at exposure times30s and 45s as publicized in table(6).

There were the maximum reductions in number of mean values of viability of ***A. baumannii***11Abs(Sputum) isolated from respritory tract infections after $CO_2$ laser irradiation when compared with control group 79 CFU/ml by using diverse power densities and diverse exposure times as shown in table (6) and figure (5). In the present work 96.2% of the bacterial

**Table (6): Antibacterial effect of CO2 laser 10600 nm against *A. baumannii* 11Abs(Sputum) in different exposure time and power densities**

| Power densities W/cm² | Control Mean±SD * CFU/ml | Exposure time/second CFU/ml (killing percentage) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 15sec | | 30 sec | | 45 sec | |
| | | Mean±SD | ( %) | Mean±SD | ( %) | Mean±SD | ( %) |
| 1000 | 79 | 39±5 a | 50.6 | 21±8 b | 73.4 | 3±9 c | 96.2 |
| 1500 | 79 | 33±7 a | 58.2 | 16±2 b | 79.7 | 2±5 c | 97.5 |
| 2000 | 79 | 24±2 a | 69.6 | 2±7 b | 97.5 | 1± 0 c | 98.7 |
| 2500 | 79 | 11±5 a | 86.1 | 2±5 b | 97.5 | 0 c | 100 |

* CFU/ml=mean×10 $^{7}$ // a < 0.05, b <0.01, c< 0.001.

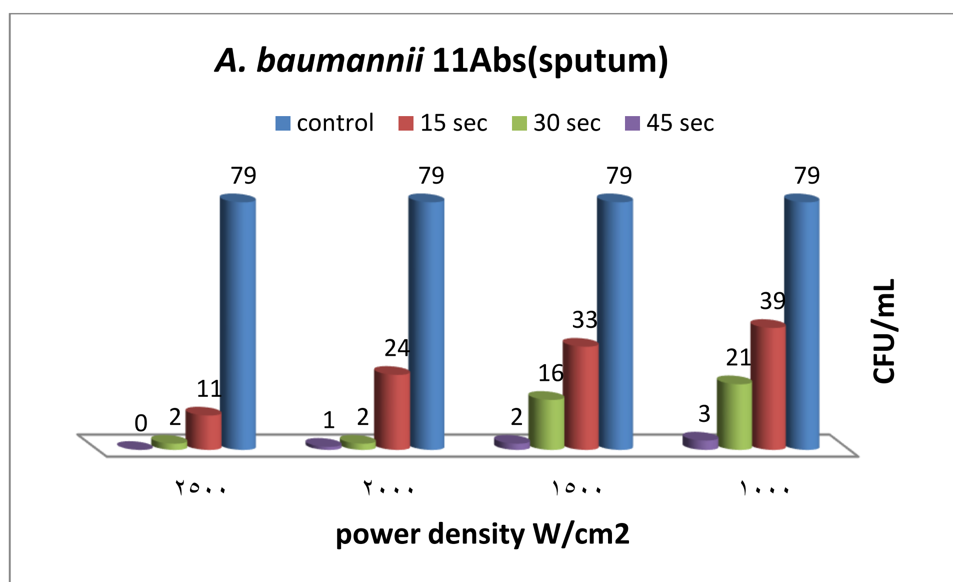


**Figure 5 : The reduction in CFU/mL of *A. baumannii* 11Abs(Sputum) after $CO_2$ laser irradiation corresponding to power densities 1000,1500,2000 and 2500 w/cm$^2$ with exposure times 15-45sec (15 sec. Increment)**

**2-The effect of $CO_2$ laser 10600 nm irradiation on the CFU/mLof *A. baumannii*isolatedfrom medical equipment**

The outcome of the current work demonstrated that there was a statistical significant difference (p<0.001) among different power densities and exposure times.The mean value of *A. baumannii* 17Abme isolatedfrommedical equipment**.**After laser irradiation had been reduced when compared with control group(85CFU/Ml) (without laser irradiation) as listed in table (6) and figure (5). On the other hand, the highest percentage killing of $CO_2$ laser on *A. baumannii* 17Abme was observed 97.6%, 99.4%and 100% only by using1500,2000 and 2500 W/cm$^2$respectivlyat exposure time 60s,this isolate needs more exposure time inorder to reach to full killed, when the exposure time 45s the percentage was

94.1% and 97.6 at 2000 ,2500 W/$cm^2$ and 85.8% at 1500 W/$cm^2$at the same exposure time 45s table (7) figure 6.

**Table (6): Antibacterial effect of CO2 laser 10600 nm against *A. baumannii* 17Abme (medical equipment)in different exposure time and power densities**

| Power densities W/cm² | Control Mean±SD * CFU/ml | Exposure time/second CFU/ml (killing percentage) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 15 sec | | 30 sec | | 45 sec | | 60 sec | |
| | | Mean±SD | ( %) | Mean±SD | ( %) | Mean±SD | ( %) | Mean±SD | ( %) |
| 1000 | 85 | 76±9 a | 10.5 | 69±3 a | 18.8 | 33±5 c | 61.2 | 19±5 c | 77.6 |
| 1500 | 85 | 73±5 a | 14.1 | 57±5 b | 32.9 | 12±9 c | 85.8 | 2±4 c | 97.6 |
| 2000 | 85 | 66±9 a | 22.3 | 43±2 b | 49.4 | 5±2 c | 94.1 | 0.5±5 c | 99.4 |
| 2500 | 85 | 58±7 a | 31.7 | 29±4 b | 65.9 | 2± 6 c | 97.6 | 0 c | 100 |

* CFU/ml=mean×$10^7$ // a < 0.05 , b <0.01 , c< 0.001.

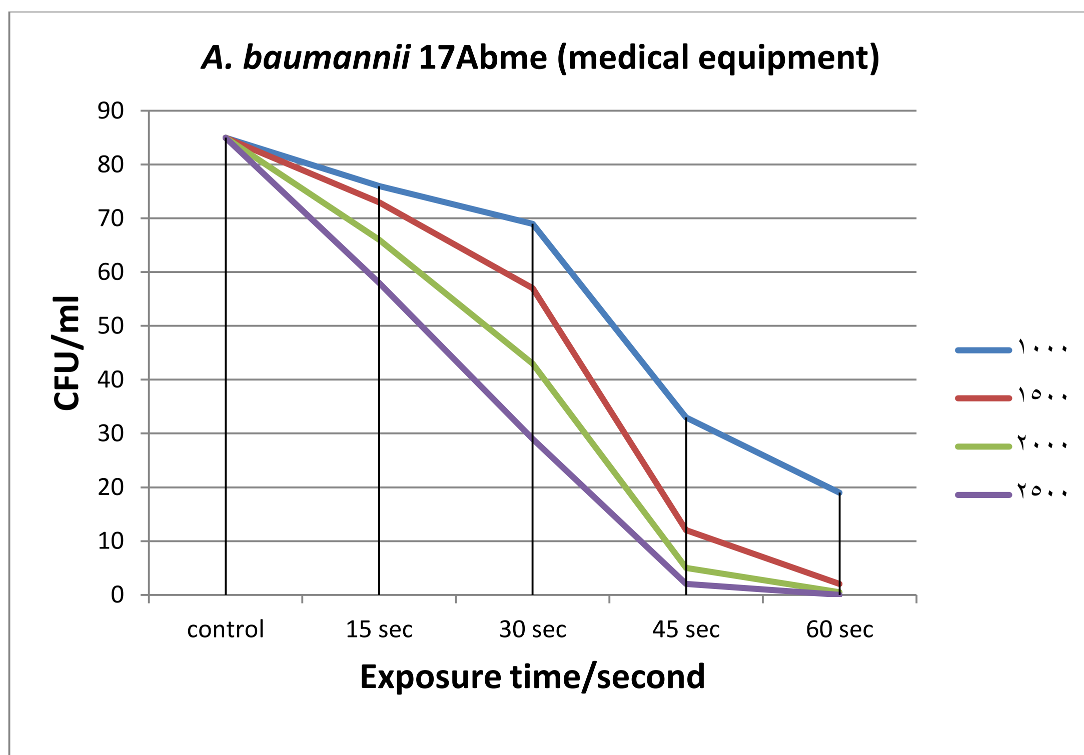


**Figure-6: The reduction in CFU/mL of *A. baumannii* 17Abme (medical equipment) after $CO_2$ laser irradiation corresponding to power densities 1000,1500,2000 and 2500 w/$cm^2$ with exposure times 15-60sec (15 sec. increment)**

Generally, in this search the results showed that when using power densities (2000 and 2500 W/$cm^2$ at exposure time 30s and 45s) of $CO_2$ laser irradations the maximum rate killing percentage was reached 97% to 100% for *A.baumannii* isolated from clinical samples as revealed in tables (4,5 and 6) and while more exposure time were needs( 45s and 60s) to get the same killing percentage for

*A.baumannii* isolated from medical equipment, the temperature ranging from 45 -75℃ recorded by thermocouple device, this may be explained by the photo thermal relations mechanism of $CO_2$ laser. When the laser light is wrapped up by bacterial cell wall, the photon energy of laser light is absorbed by bacterial cell structure (in which the water is most important element affected) it will be transformed into heat energy and the second will lead to changes in the permeability of the cell wall or may change enzymes activity, follow-on in decrease of energy transport within the bacterial cell and lead to cell immobility or may lead to denaturation of protein as a result the bacteria is killed. [30] Non spore forming *A.baumannii* possibly killed at 72℃ at high power densities with long exposure times were needed, especially for an MDR strong biofilm producer bacterial isolates of *A.baumannii*. Hamzah *et al* [12] mentioned in their reports that sensitive bacteria to antibiotics need less power density and exposure time than the resistance isolates belonging to the same species,this study conducted on *S. oralis, S. aureus* and *S. epidermis* isolated from peri-implant infections. Naji *et al* [13] reported that there were bactericidal effects on *P. aeruginosa* isolated from wounds and burned infections. Watson *et al* [15] revealed that Laser scanning systems improved methods of bacterial inactivation and Sterilization on different surfaces. There are many studies proved that $CO_2$ laser had a bactericidal effects on bacteria isolated from different sources but non of these studies noted to its effects on *A.baumannii*. The results of this study proved that irradiation by CO2 laser 10600 nm had a highest killing effect 100% of the all *A.baumannii* MDR biofilm producer isolates. The maximum effect of CO2 laser on this bacteria which isolated from clinical and medical equipments sample was reached when the duration of the exposure time was increased during irradiation at 2000 and 2500 W/cm$^2$. consequently, the use of CO2 laser 10600 nm which is independent towards the antibacterial agents resistance pattern of MDR *A.baumannii* could verify advantageous in treatment of different type of infections caused by this bacteria in addition it may used in sterilization of some medical equipment surfaces.

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