

## Determination the effects of Laser radiation on adhesion and biofilm formation of some gram-negative bacteria in Al-Diwaniyah and Babylon slaughterhouse.

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**Submitted:** June 8, 2025

**Revised:** August 8, 2025

**Accepted:** August 9, 2025

**Abstract** This study was conducted to understand the effect of laser radiation on *E. coli* and *Klebsiella pneumoniae* bacteria found on the walls, floors, knives, and equipment of slaughterhouses. The current study involved collecting 160 clinical samples from slaughterhouses in Al-Diwaniyah and Babylon Governorates, Iraq. To ascertain how laser treatment affects bacterial adherence and biofilm formation, *K. pneumoniae* and *E. Coli*. The outcomes for *E. Coli* and *k. pneumoniae* showed that laser irradiation affected the production of biofilms and bacterial adhesion, especially when the total number of hits rose. The impact was greatest at 10 hits with 300Jw energy. Overall, the findings demonstrated that lasers had reduced the bacterial biofilm and adhesion formation.

**Keywords:** Laser, adhesion, biofilm, slaughterhouse

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**Introduction** Animals are murdered in slaughterhouses to satisfy the need for meat for consumption by humans. Animal byproducts make up the majority of wastes. Slaughterhouse waste contributes to the pollution as the population grows. Many operations, including keeping the animal, killing it, cleaning the corpse, dressing it, and getting rid of and cleaning the stomach, internal organs, unsuitable for eating meat and fats, waste products, includes blood, etc., result in a huge volume of solid and liquid waste. Based on the amount of waste they generate, slaughterhouses and packinghouses (slaughtering and meat handling) can be categorized into two classes (1). Types of Slaughterhouses Simple abattoir: Only a tiny amount of the animals' wastes is dealt with at this facility after they are killed (1) and Complex slaughterhouse kills animals and thoroughly processes their waste. Rendering, managing paunch and internal organs, processing blood, and processing hide and hair are all common procedures. Abattoir waste management is a significant issue on a global scale, and legislation pertaining to its treatment is currently patchy. The dangers to human and animal health from infections and environmental deterioration, including the potential for surface and groundwater pollution, are increased when abattoir waste is disposed of directly. Like other wastes like

plastic, aquatic vegetation, and municipal trash, it also clogs the drainage system. (2).

Any animal that produces meat must be killed by removing its meat from two contaminating surfaces: the hide and the digestive system. It is unavoidable that part of the meat will become contaminated with microorganisms, some of which might cause food-borne diseases in people, regardless of how thoroughly this process is carried out. Therefore, minimizing bacterial contamination of the carcasses and eliminating any existing contamination are the slaughter industry's main objectives for food safety (3). Meat, an important component in daily meals, has a number of health benefits (4).

Because of their high protein, water, fat, mineral, and Due to their vitamin content, all varieties of meat are classified as decomposable foods. This rendered it susceptible to internal enzymatic activity, increasing its vulnerability to oxidation and bacterial invasion (5). Meat serves as an ideal substrate for bacterial growth and reproduction due to its requisite growing factors: neutral pH, elevated moisture content, and mineral constituents. Bacteria R.K. may infect meat and meat products during slaughtering and processing, as well as from blades, air, personnel, carts, containers, and equipment in general. Pathogenic and spoilage microorganisms are the two principal categories of bacteria. Although no health hazards are identified

with decaying bacteria, their existence may lead to a deterioration in food quality and freshness (8). The two predominant foodborne pathogenic bacteria responsible for bacterial illnesses in humans are *E.coli* and *Klebsiella spp.* (9). Red meat contains *Klebsiella* and *E. Coli*. Meat must be handled, stored, and consumed with prudence because it spoils easily and can get infected with dangerous bacteria and toxins produced by bacteria that humans may contract by eating it under certain conditions (7). A laser is a machine that uses optical amplifiers that rely on stimulated release of electromagnetic energy to produce light. Originally, the term "laser" meant light amplification by stimulating the release of light(9).

The applications like lithography, because the coherent light emitted by a laser's beam sets it apart from other types of light. It is also utilized in lidar, laser pointers, and free-space optical communications because it enables a laser beam to remain narrow over long distances (collimation). Additionally, lasers may produce light with a very narrow frequency spectrum due to their great temporal coherence. Ultrashort bursts of broad-spectrum light, which can last as few as one attosecond, can also be produced using this temporal coherence (11). Fiber-optic and free-space communication through optical fibers, optical disc drives, laser printers, barcode scanners, manufacturing of semiconductors (photolithography and etching), laser treatment, skin therapy, cutting and welding materials, target marking tools for the military and legal system, speed and range determination, and entertainment laser lighting displays are just a few of the many uses for lasers (12,13).

A gain medium, an energizing mechanism, and an optical feedback mechanism make up a laser. Anthony E. Siegman. (1986) A material known as the gain medium is defined by its capacity to intensify light via stimulated emission. When a specific wavelength of radiation passes through the gain medium, it is amplified, which raises the power. The optical frequency that corresponds to the tip of the gain-frequency curve is amplified as a result of feedback facilitating stimulated emission. A coherent beam will arise as a result of one frequency eventually dominating all others as stimulated emission rises (14).

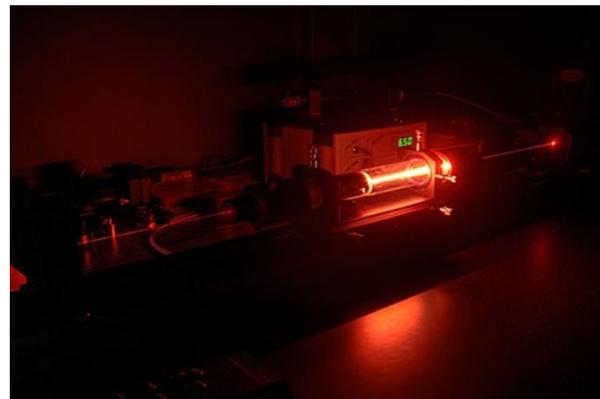


Figure (1): Typical laser components

*Escherichia coli* or *E.Coli* On MacConkey medium, *E. Coli* is pink, rod-shaped, and gram-negative. *E. Coli* is a member of the Enterobacteriaceae family. The Enterobacteriaceae family includes *E. Coli*. A Gram-negative rod known as *Bacteria coli* commune is frequently found in the flora of healthy people's digestive tracts, according to research done in 1885 by German physician Theodor Escherich. Later, these rods were dubbed *Escherichia coli* in his memory. A few decades later, in 1935, they were first identified as pathogens after being connected to a severe episode of gastroenteritis known as "cholera infantum," which primarily affected infants (15). Numerous studies have been conducted on *E.Coli*, describing it from a range of perspectives, including molecular, genetic, antibiotic resistance, and serological analysis. In his honor, the scientific name *Escherichia coli* was later applied to these rods. A few decades later, in 1935, they were first identified as pathogens after being connected to a severe gastroenteritis outbreak known as "cholera infantum," which primarily afflicted newborns (15). As a result, it is today regarded as one of the best-characterized bacteria. Many elements of this bacteria remain unknown, despite the fact that it continues to cause devastating illnesses such as meningitis, pneumonia, diarrhea, wound infections, septic shock, and urinary tract infections in both healthy and immunodeficient people (16).

*The Klebsiella pneumoniae* It was discovered in 1834 by the German microbiologist Edwin Klebs, who also gave it its present name. Except for *Klebsiella mobilis*, Fried Landers was the first to discover *Bacillus klebsii*, commonly known as the Fried Landers bacteria. Some strains produce a bactericidal chemical known as klebsin, and it is non-sporulating (18). The entire cell

surface of gram-negative bacteria is covered in a polysaccharide shell. Because of their relatively thick shell, the colonies seem mucoid and shimmering when seen on agar plates. The enteric and extraintestinal pathotypes of *E. Coli* isolates have similar virulence factors and tactics (19). In addition, the Biofilm In their natural environments, bacteria commonly grow and self-organize into multicellular structures called biofilms (44). Biofilms form when bacteria attach onto a solid surface and divide while embedding themselves in a matrix of extracellular polymeric substances (EPS) (45).

Numerous every day, medical, and industrial environments include biofilms, which can contaminate tools and endanger human life. Bacteria may exist in a static 'biofilm' form, where cells exchange resources and offer protection from adverse conditions, or in a movable, 'planktonic' state, where they spread out freely in pursuit of nutrients (26). The way that each species reacts to specific environmental conditions determines the elements that lead bacteria to change from a moving form to a biofilm lifestyle. These elements include the generation of certain chemical signals, the availability of nutrients, and cellular traits including microbial concentration, growth rate, and diffusing behavior. (27).

Moreover, Adhesion the first stage of colonizing and biofilm formation is known as microbial adhesion, and it is defined by the accumulation of bacteria and extracellular materials on a solid basis. Biofilms have the potential to endanger both industrial processes and human health by causing infections connected to medical implants[28], pathogen-host cell interaction [29], [30], periodontitis or dental caries [31], [32], food contamination from processing equipment [33], [34], increased metal corrosion [35], marine biofilms on ship hulls [36], and more. On the other hand, microbial adhesion can be advantageous, such including the degradation of ecologically toxic compounds in sand [37], [38], bioreactors for wastewater treatment [39], or off-gas treatments [13], as well as the agricultural applications of root nodules as microbes in the rhizosphere [40].

#### **Materials and Methods**

##### **Ethical approval**

The project was approved ( 226 in 15/1/2025 ) by the Committee for Research Ethics at the College of Veterinary Medicine, University of Al-Qadisiyah, Iraq.

##### **Techniques:**

A sample is taken from the slaughterhouse and cultured in a culture medium and LB broth. Then, it is incubated at 37°C for 16 to 18 hours. After colonies appear, they are cultured in sub-plates, after which biochemical tests are performed.

#### **Biochemical tests for identification of bacterial isolates**

##### **Oxidase test**

The test had been conducted by spreading a colony of the organism on a filter paper, followed by the addition of drops of the oxidase reagent. A color change from colorless to purple had indicated a positive oxidase test within 10 seconds.

##### **MacConkey agar test**

MacConkey agar is a kind of selective medium that is used for the purpose of isolating gram-negative enteric bacteria and differentiating between strains that are capable of lactose fermenting and non-lactose fermenting strains. The bacterial culture was started by streaking it onto MacConkey agar plates and then incubating it at 37°C for 24 hours to promote growth (46).

##### **Chromogenic Test :**

Use to detect the *E.coli* and *K.pneumoniae*, Brilliance ESBL agar (Oxoid; Thermo Fisher Scientific, UK) is a selective and differential chromogenic agar for the detection of ESBL-producing organisms. Brilliance ESBL agar contains two chromogens that are targeted by specific enzymes: glucuronidase and  $\beta$ -galactosidase. The presence of glucuronidase and  $\beta$ -galactosidase enzymes in *E. coli* results in a blue colony; however,  $\beta$ galactosidase negative *E. coli* will produce a pink colony. *K. pneumoniae* produces  $\beta$ -galactosidase, resulting in green colonies. Using the microtiter plate method for biofilm assay:

The assay was performed for 20 isolates based to a method performed by Piechota et al., 2018 with little modification. freshly pure colonies from each isolate was put on Luria Bertani broth supplemented with dextrose 0.5% and incubated at 37°C for 24 hours without shaking. After the incubation procedure an inoculum of bacterial cell suspension (by dilution with sterile Luria Bertani broth) was ready to be matched with 0.5 McFarland standard that equivalent to 108CFU/ml. Then 200  $\mu$ l of the suspension of each isolate was passed on into wells of 96-well flat-bottom polystyrene plate and incubated without shaking at 37°C for 24 hours, after the second incubation, the excessive medium was eliminated and washed 2 times with 200  $\mu$ l of sterile PBS (pH 7.4) to eliminate

non-attached bacterial cells, the following step was fixation of biofilm by heat, the plate was retained in oven at 60°C for one hour, then stained with 200 µl of crystal violet 1% for 5 minutes. after this, the plate was washed with PBS and air-dried for one hour. Colorant was solved in 96% ethanol and absorption was measured by microplate reader at 490 nm (BioTeck, USA), each test was conducted in duplicate to calculate the average results. Sterile LB broth was used as a negative control. The following formula was used to determine the rate of biofilm formation:

The formula is  $BR = AT - ANC$ , where BR is the biofilm result, AT is the tested strain's absorbance as measured at OD490 nm, and AC is the OD490 nm of the negative control wells that contained only plain broth. A biofilm formation absorbance value of  $\geq 0.12$  was deemed positive, as were weak biofilm producers at  $< 0.2$ , moderate producers at 0.2-0.4, and strong producers at  $> 0.4$  (41).

### Results and discussion

The current study included the collection of 160 clinical specimens in Al-Diwaniyah and Babil Slaughterhouses during the period from October 2024 to April 2025, *E. Coli* and *K. pneumoniae* bacteria were determined as having the highest ratio in the aerobic microbial culture. Cleanliness, residential location of Slaughterhouses, and the use of certain chemical disinfectant are the primary causes of the variation in isolation rates. *K. pneumoniae* may have spread as a result of the isolation time (42). The unique morphological traits of the *K. pneumoniae* and *E. coli* isolates allowed for their identification. The strain of *E. coli* produced pink colonies when grown on MacConkey agar. Furthermore, characteristics of the bacteria, such as the structure and content of the cell wall, have a significant impact on the disinfection efficacy. For instance, compared to Gram-positive bacteria, which had thicker peptidoglycan layers, Gram-negative bacteria had extremely thin peptidoglycan cell walls. Gram-positive bacteria have so demonstrated greater resistance to physical and chemical stresses (43).

#### Laser effect on *E. coli* biofilm:

After completing the collection of samples from the slaughterhouses of Diwaniyah and Babylon and isolating and identifying some Gramnegative bacteria, it appeared that the highest percentage were *Escherichia coli* and *Klebsiella pneumoniae*. The procedure of forming biofilms for both *Escherichia*

*coli* and *Klebsiella pneumoniae* was completed to prepare them for treatment with laser rays. Under a known number of hits, known energy, and fixed frequency, the isolates were divided into three groups based on laser energy, number of hit, and frequency. Then were treated with laser. After treatment, a large difference in the results was found, as the effect of laser rays on the biofilms of *E. coli* and *Klebsiella pneumoniae*. (In the same way as before in the case of adhesion).

The obtained results showed that the bacterial biofilm formation was significantly  $P < 0.05$  affected by laser treatment, this effect was increased by the energy and the number of hits, the highest effect was at 10 hits in 300jw energy (0.03650), while the minimum effect was at 1 hits in 100 jw energy (0.0005000) with Mean (0.01134) and Standard Deviation (0.009029) and Standard Error (0.002071) As shown in Table(1,2) Table(1) OD<sub>490</sub> values of cultured *Escherichia coli*

<i>Escherichia coli</i>			
N0	Sample ID	Biofilm Before LASER	Biofilm After LASER
1	E2	0.026	0.0365
2	E3	0.077	0.0285
3	E15	0.024	0.0185
4	E12	0.0505	0.017
5	E1	0.024	0.015
6	E4	0.0165	0.014
7	E9	0.0185	0.0125
8	E13	0.0225	0.012
9	E10	0.0435	0.0095
10	E17	0.031	0.0085
11	E18	0.044	0.0085
12	E11	0.023	0.006
13	E16	0.0165	0.006
14	E19	0.0355	0.006
15	E6	0.0145	0.0055
16	E14	0.0365	0.0045
17	E8	0.0095	0.0035
18	E7	0.007	0.003
19	E20	0.0475	0.0005
20	E5	0.1105	-0.001

Table (2) Descriptive analysis of *E.coli* Biofilm:

	<i>Escherichia coli</i>	
	Biofilm Before LASER	Biofilm After LASER
Minimum	0.007000	0.0005000
25% Percentile	0.01650	0.005500
Median	0.02400	0.008500
75% Percentile	0.04350	0.01500
Maximum	0.07700	0.03650
Mean	0.02987	0.01134
Standard Deviation	0.01710	0.009029
Standard Error	0.003924	0.002071
Lower 95% CI	0.02162	0.006990
Upper 95% CI	0.03811	0.01569

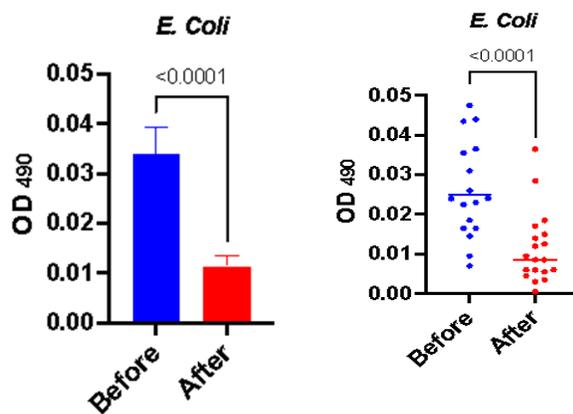


Figure (2) Statistics using GRAPH PAD PRISM 10 software:

**Laser effect on k. pneumoniae biofilm :**

The obtained results showed that the bacterial biofilm formation was significantly  $P < 0.05$  affected by laser treatment, this effect was increased by increase the energy and the number of hits, the highest effect was at 10 hits in 300jw energy( 0.05350), while the minimum effect was at 1 hits in 100 jw energy (0.003500) with Mean (0.01471) and Standard Deviation(0.01161) and Standard Error(0.002817) As shown in Table(3,4).

**Table(3) OD<sub>490</sub> values of cultured *Klebsiella pneumoniae***

	<i>Klebsiella pneumoniae</i>	
Sample ID	Biofilm Before LASER	Biofilm After LASER
K1	0.0095	0.007
K2	0.0445	0.0165
K3	0.0745	0.01
K4	-0.003	0.006
K5	0.0195	0.01
K6	0.016	0.009
K7	0.0075	-0.0005
K8	0.0255	0.016
K9	0.0505	0.0125
K10	0.0295	0.021
K11	0.0325	0.0175
K12	0.1025	0.0535
K13	0.086	0.026
K14	0.018	0.0085
K15	0.0155	0.005
K16	0.0445	0.0085
K17	0.005	0.0035
K18	0.027	0.009
K19	0.073	0.0165
K20	0.049	-0.001

**Table(4) Descriptive analysis:**

	<i>Klebsiella pneumoniae</i>	
	Biofilm Before LASER	Biofilm After LASER
Minimum	0.005000	0.003500
25% Percentile	0.01700	0.008500
Median	0.02950	0.01000
75% Percentile	0.06175	0.01700
Maximum	0.1025	0.05350
Mean	0.03962	0.01471
Standard Deviation	0.02876	0.01161
Standard Error	0.006975	0.002817

Lower 95% CI	0.02483	0.008734
Upper 95% CI	0.05440	0.02068

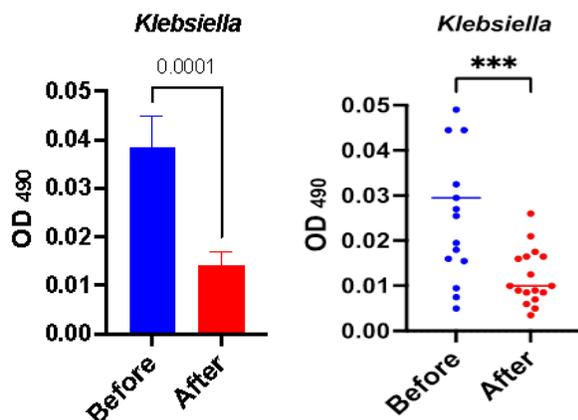


Figure (3) Statistics using GRAPH PAD PRISM 10 software :

**Laser effect on E. coli adhesion:**

The present study, showed that the bacterial adhesion was significant statically  $P < 0.05$  affected by Laser treatment, this effect was increased by increase the energy and the number of hit, the highest effect was at 10 hits in 300jw energy as Strong adhesion (+++), while the minimum effect was at 1 hit in 100 jw energy (+or 0) shown Table(5,6,7,8).

Table(5) Adhesion of *E. coli* before the exposure to the laser:

Isolate	Dilution (CFU/ml)					
	1.5 X 10 <sup>8</sup>	7.5 X 10 <sup>7</sup>	3.7 X 10 <sup>6</sup>	1.8 X 10 <sup>6</sup>	9 X 10 <sup>6</sup>	4 X 10 <sup>6</sup>
E01	+++	+++	+++	++	+	0
E02	+++	+++	+++	++	+	0
E03	+++	+++	+++	++	+	0
E04	+++	+++	+++	+++	++	+
E05	+++	+++	+++	++	+	0
E06	+++	++	+	0	0	0
E07	+++	+++	+++	+++	++	+
E08	+++	+++	+++	++	+	

E09	+++	+++	++	+	0	0
E10	+++	+++	++	+	0	0

Table (6) Adhesion of *E. coli* after the exposure to the laser

Isolate	Dilution (CFU/ml)					
	1.5 X 10 <sup>8</sup>	7.5 X 10 <sup>7</sup>	3.7 X 10 <sup>6</sup>	1.8 X 10 <sup>6</sup>	9 X 10 <sup>6</sup>	4 X 10 <sup>6</sup>
E01	+++	++	+	0	0	0
E02	+++	++	+	0	0	0
E03	+++	+++	++	+	0	0
E04	+++	++	+	0	0	0
E05	+++	++	+	0	0	0
E06	+++	+++	++	+	0	0
E07	+++	++	+	0	0	0
E08	+++	+++	++	+	0	0
E09	+++	++	+	0	0	0
E10	+++	++	+	0	0	0

Table(7) Interpretation of the results

+++	Strong adhesion
++	Moderate adhesion
+	Weak adhesion
0	No adhesion

Table (8) *E. coli* (Mean ±SE)

Type of treatment	Adhesion score
Before exposure	2.90±0.06a
Laser 100 J.W energy 1 bits	1.35±0.13b
Laser 200 J.W energy 5 bits	0.350±0.13c
Laser 300 J.W energy 10 bits	0.05±0.05d
LSD(P<0.05)	0.28

**Laser effect on k. pneumoniae adhesion:**

The present study, showed that the bacterial adhesion was significant statically  $P < 0.05$  affected by Laser treatment, this effect was increased by increase

the energy and the number of hits, the highest effect was at 10 hits in 300jw energy as Strong adhesion (+++), while the minimum effect was at 1 hit in 100 jw energy (+or 0) shown Table (9,10,11).

**Table (9) Adhesion of *Klebsiella pneumonia* before the exposure to the laser**

Isolate	Dilution (CFU/ml)							
	1.5 X 10 <sup>8</sup>	7.5 X 10 <sup>7</sup>	3.7 X 10 <sup>6</sup>	1.8 X 10 <sup>6</sup>	9 X 10 <sup>6</sup>	4 X 10 <sup>6</sup>	2 X 10 <sup>6</sup>	1 X 10 <sup>6</sup>
K01	++ +	++ +	++ +	++ +	++ +	++ +	++ +	0
K02	++ +	++ +	++ +	++ +	++ +	++ +	++ +	+
K03	++ +	++ +	++ +	++ +	++ +	++ +	++ +	+
K04	++ +	++ +	++ +	++ +	++ +	++ +	++ +	0
K05	++ +	++ +	++ +	++ +	++ +	++ +	++ +	+
K06	++ +	++ +	++ +	++ +	++ +	++ +	++ +	++
K07	++ +	++ +	++ +	++ +	++ +	++ +	++ +	++
K08	++ +	++ +	++ +	++ +	++ +	++ +	++ +	++
K09	++ +	++ +	++ +	++ +	++ +	++ +	++ +	++
K10	++ +	++ +	++ +	++ +	++ +	++ +	++ +	+

**Table(10) Adhesion of *Klebsiella pneumonia* after the exposure to the laser**

Isolate	Dilution (CFU/ml)							
	1.5 X 10 <sup>8</sup>	7.5 X 10 <sup>7</sup>	3.7 X 10 <sup>6</sup>	1.8 X 10 <sup>6</sup>	9 X 10 <sup>6</sup>	4 X 10 <sup>6</sup>	2 X 10 <sup>6</sup>	1 X 10 <sup>6</sup>
K01	++ +	++ +	++ +	++ +	++	+	0	0
K02	++ +	++ +	++ +	++ +	++	+	0	0
K03	++ +	++ +	++ +	++ +	++	+	0	0
K04	++ +	++ +	++ +	++ +	++	+	0	0

K05	++ +	++ +	++ +	++ +	++ +	++ +	0	0
K06	++ +	++ +	++ +	++ +	++ +	++ +	+	0
K07	++ +	++ +	++ +	++ +	++ +	++ +	+	0
K08	++ +	++ +	++ +	++ +	++ +	++ +	+	0
K09	++ +	++ +	++ +	++ +	++ +	++ +	+	0
K10	++ +	++ +	++ +	++ +	++ +	++ +	+	0

**Table (11) *Klebsiella* (Mean ±SE)**

Type of treatment	Adhesion score
Before exposure	3±0a
Laser 100 J.W energy 1 bits	1.30±0.14b
Laser 200 J.W energy 5 bits	0.400±0.11c
Laser 300 J.W energy 10 bits	0±0d
LSD(P<0.05)	0.26

**Conclusions**

The results showed that the highest percentage of bacteria isolates was *E. coli*, followed by *K. pneumoniae*. And The Laser rays effect of the adhesion of *E. coli* and *Klebsiella* bacteria, and its effect increased with energy and number of hits. The Laser rays effect of on biofilm formation of *E. coli* and *K. pneumoniae* increases with energy and number of hits.

**Acknowledgment:**

I would like to extend my special thanks and appreciation to the Head and faculty members of the Department of Public Health, College of Veterinary Medicine, Al-Qadisiyah University, for the facilities they provided me to conduct the research.

**Conflict of interest**

No conflict of interest is found for the present study.

**Funding source**

This research had no specific fund; however, it was self-funded by the authors

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