

A PILOT STUDY FOR THE TREATMENT OF MICROBIAL SKIN INFECTIONS CAUSED BY BACTERIA *PSEUDOMONAS AERUGINOSA* BACTERIA THAT RESISTANCE TO ANTIBIOTIC IN HUMAN AND ANIMAL BY (ND: YAG LASER) TECHNOLOGY

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ABSTRACT

Pseudomonas Aeruginosa is the second most important of the Gram-negative pathogenic bacteria for humans and animals. They cause high mortality rates in hospitals because of their resistance to detergents and a large number of chemicals and antibiotics. This study was conducted to demonstrate the effectiveness and impact of the laser (ND: YAG laser) technology to kill the bacteria *Pseudomonas Aeruginosa*. Use most common laser And yak (ND: YAG laser) with a wavelength of 532 nm and strongly 200 megawatts, we shed light of laser on the dishes of bacterial implant of bacteria *Pseudomonas Aeruginosa* free of antibiotics or containing bacterial colonies only for periods of time (1 to 6) minutes and then shed light of laser on the dishes implant bacterial used to examine resistant bacteria to antibiotics and measure the diameter of the constituent circle around each antibiotic and then measure the diameter Circle Counting shed laser on these dishes and the presence of antibiotics together also for periods of time (1 to 6) minutes, the result show that not killed *Pseudomonas Aeruginosa* in the case of the use of laser alone wavelength and strength of the above for the time periods mentioned above, and in the case of the use of antibiotics alone. While when the combination of antibiotic treatment and use of laser was the best result as bacteria began to weaken and increased sensitivity to antibiotics when using laser light for one minute and the presence of antibiotic and weakened bacteria more and killed about half the number of colonies when the light of laser shed for (3 minutes) and the use of antibiotic also the sensitivity of bacteria to antibiotics increased to (100%) where bacteria killed and the elimination of all colonies when using antibiotics and laser irradiation for (6 minutes) in one. We use an antibiotic (Penicillin, terramycine, ciprofloxacin, azactam, maxipime, cephalixin, amikacin, gentamicin).

The result was better when using gentamicin antibiotic than the rest of antibiotics and amikacin.

Keywords: Skin infections, *Pseudomonas Aeruginosa*, Resistance, Antibiotics, Laser, Animals.

INTRODUCTION

The external surface of the natural skin provides an environment conducive to the growth of bacteria through sweating; moisturizing the skin, the presence of dead tissue and the skin is a mechanical barrier penetrates the skin due to the occurrence of normal wounds, surgical wounds, accidents or burns. Skin, or external environment, wound infections, especially after surgery and various accidents, are a major problem for many people and animals are often exposed to a bacterial infection during a visit to the hospital to make a change the surgical spare or stay in hospital after surgery for humans and contaminants of the surrounding environment for animals. Therefore, the bacteria that cause inflammation of the wounds, especially *Pseudomonas Aeruginosa*, have been identified as the most common cause of this condition. The infection becomes more acute by increasing the number of germs present in the outer and gaseous area of the skin and beyond immunological defenses (Kobayashi, Itoh *et al.* 2002).

The process of wound injuries goes through several stages (vasodilation, interleukocytosis, pus formation and then healing). Burns are usually antiseptic after burning directly but are quickly contaminated with microorganisms. This is due to the breakdown of the mechanical barrier of the body also due to a change in the function of neutral

white blood cells(neutrophils) and the effect of the immune response(Simpson, Ramphal *et al.* 1995). The *P.aeruginosa* germinates in severe burns after entry the patient to the hospital immediately intervention due to weak resistance due to damaged tissues with a large area. *Pseudomonas Aeruginosa* was first named by the world Schroeter in 1882 when it was isolated for the first time in a pure farm by Gessard in 1882 of skin lesions that had a blueish green color. Gessard has conducted several studies since (1892,1891,1890) show the bacteria has two pigments, one of which is Polyamine, derived from Pyocyanus, which means blue pus that characterizes *P. aeruginosa*, a non-fluorescent blue dye that is dissolved in chloroform, water, and other fluorescent green fluorescents (Simpson, Ramphal *et al.* 1995).

Pseudomonas Aeruginosa, *P. orizihabitans*, and *P. plecoglossicida* are abundant in hospital environments and the environment adjacent to the animal's living place. These pathogens may be the result of proteins separated by *p aeruginosa* releasing devices and produce many extracts that are important in the pathogenesis mechanism(Samad, Ahmed *et al.* 2017). *Pseudomonas Aeruginosa* is classified as one of the opportunistic infections associated with the medical field. Several epidemiological studies suggest that resistance to antibiotics is an increase in medical isolates(Eldere 2003).

Bacteria have several mechanisms for adhesion, growth and colonization of host tissues based on virulence, family health status, and the ability of bacteria to enter (Standards 2000). Bacteria *P. aeruginosa* have many factors of virulence to colonize host cells including: papillae, secrete polysugared sugars and extracellular factors such as exotoxine A, the most toxic protein produced by the microbial which inhibits the process of protein synthesis in host and toxin cells Intestinal, which works on the intestinal tract leads to the loss of electrolyte, causing diarrhea and vomiting. Multi-surface polysaccharides (alginate) also play different roles in the life of a microbial, where they act as a barrier between the cellular wall and the external environment (Koopmann-Holm and David Matsumoto 2011; Abbas, Al-Yasseen *et al.* 2017; Ermawati and Wibisono 2017; Preeti and Sharma 2017).

The aim of the study is to eliminate the wound infections and skin burns caused by antibiotic-resistant bacteria *P. aeruginosa* using laser technology (ND: YAG laser) and to note which treatment is best for antibiotics or laser technology.

The method of work

Collection of samples: Twenty-five swabs were collected from patients suffering from wound inflammation, ulcers and skin burns who were visiting Yarmouk and Kadhimiya hospital in Baghdad and Al Hussein Medical hospital in the holy governorate of Karbala, who were treated with different types of antibiotics and were not cured. Also seventy five swabs were collected from inflamed wounds of cattle, goats, dogs and cats were brought to the veterinary clinic in the yard of Aden in Baghdad and from two deers in the zoo in Baghdad, one was carried out a caesarean section and the other conducted a cleanup of the stomach to swallow Nylon bags thrown by visitors the zoo .

A - Bacterial diagnosis and bacterial transplant:

The bacterial infection was diagnosed by direct examination of the swab by placing it on the glass slide (slide) to making smear then stained by gram stain to observing the red cylindrical shape bacteria as in Figure 1 and then planting them on the basic plant media (nutrient, MacConkey and Blood agar) to observation of the growth of the bluish-green bacterial colonies as shown in Fig. 2 and the observation of blood decomposition on blood agar as shown in Figure 3.

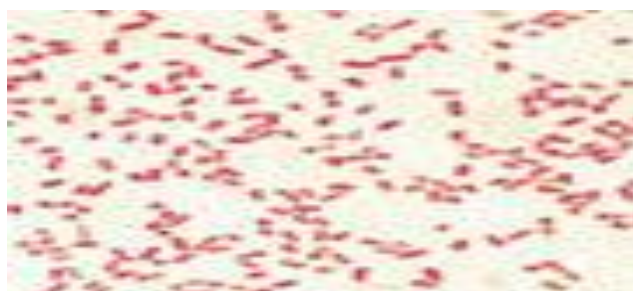


Figure 1: The red cylindrical bacteria that negative to gram stain



Figure 2: The bluish green color of the *Pseudomonas Aeruginosa* colonie



Figure 3: colonies of the *Pseudomonas Aeruginosa* on blood agar

B- Serological examination: The serological tests of bacterial growth were carried out to ensure the isolation of the *Pseudomonas Aeruginosa* bacteria that caused dermatitis (inflammation of skin) as shown in Table 1.

Table 1: the types of serological tests and their results for all samples to confirm the type of bacteria *Pseudomonas Aeruginosa*

Biochemical test	Result
Oxidase	+
Lactose fermentation	-
Citrate utilization	+
Catalase	+
H ₂ S formation	-
Methyl red	+

Indole -	-
Growth at 42 °C	+
Voges-proskaure -	-
Kligler test	A/ K/ -/-

Key (+): positive reaction, (-) negative reaction.

2 - Test the sensitivity of bacteria to antibiotics:

After the bacterial transplantation and the confirmation of the cause of the psoriasis and the identification of the types of antibiotics used in the treatment of patients with inflamed wounds and owners of animals taken from bacterial swabs did not match the wound to heal we examined the sensitivity of these antibiotics and the extent of resistance to them. Antibiotics used were Penicillin, terramycin, ciprofloxacin, azactam, maxipime, cephalixin, amikacin, and gentamicin as shown in Figs. 4, 5.

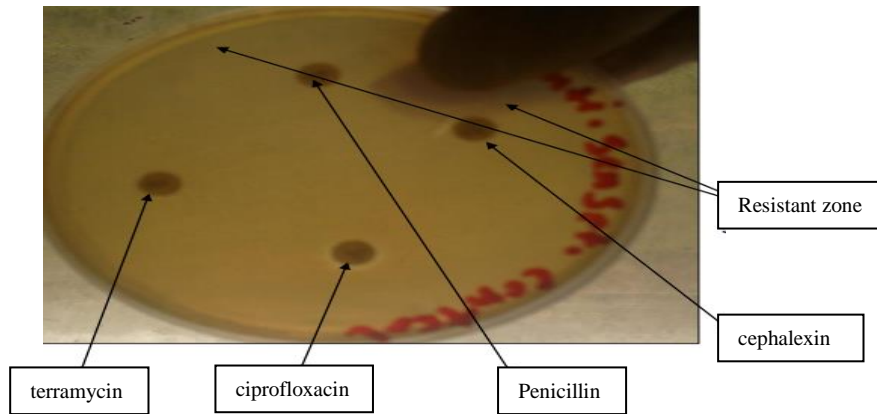


Figure 4: The sensitivity of bacteria to antibiotics

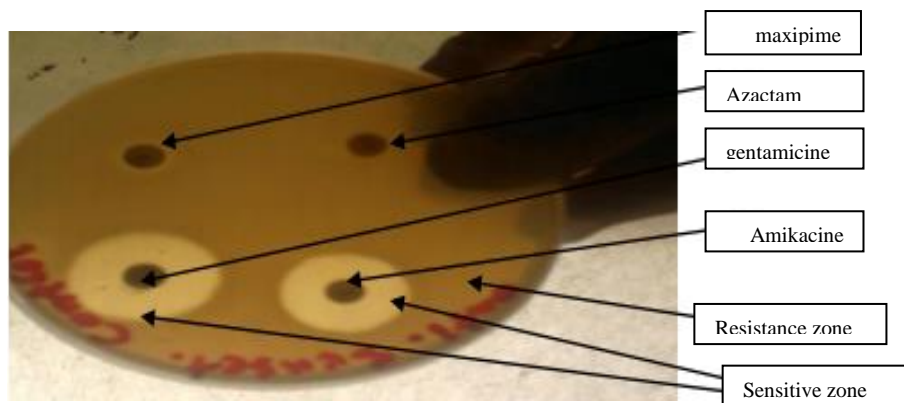


Figure 5: The sensitivity of bacteria to antibiotics

3 - Method of the use of laser technology (ND-YAG laser): After planting the bacterial culture dishes were incubated for 24 hours at a temperature of (37°C), the irradiation circles are subjected to laser diode (532 nm) and strongly (200) megawatts, the most common laser use (Pirt 1983). The laser diode is applied to these bacterial culture dishes that free of antibiotic then applied to the dishes on

the bacterial colonies only. We applied the light of laser at intervals of time (1 to 6) minutes, and then applied the light of laser on the microbial dishes used to test the resistance Bacterium antibiotic The diameter of the constituent circuit is measured around antibiotic then measure the diameter of circle after shedding a laser on these dishes and the

presence of antibiotics with laser together for periods of time (1 to 6) minutes.

RESULTS AND DISCUSSION

One of the worrisome characteristics of *Pseudomonas aeruginosa* it is resistance to the susceptibility to antibiotics. This property is due to the pumps located at the level of the cell membrane, which pump several drugs, including antibiotics, to the outside of the cell. Besides that easily develops acquired resistance, through genetic mutations of chromosome genes, or by converting antibiotic resistance genes horizontally from cell to cell (Standards 2000).

The result of current study was found that the most effective antibiotics were gentamycin and amikacin then (azactam, spirofloxacin, maxipim, cephalaxin, teramycin, penicillin) respectively, this is consistent with (Pirt 1983) as shown above in Figures (4, 5). The laser was then irradiated with a wavelength of (532 nm) with a force of (200 maw) on the microbial cultures of the bacterial colonies. The results were as follows: When the laser was applied for a minute, the bacterial sensitivity to the laser was very low and the resistance was high as shown in Figure 6.

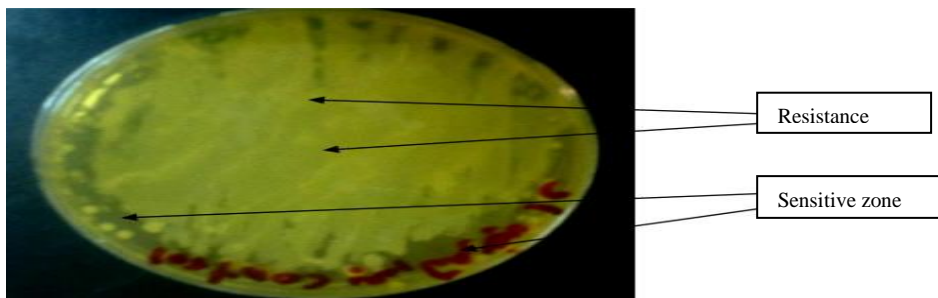


Figure 6: the extent of the resistance of the pseudo-violet bacteria to the laser When exposed for 1 minute

When the time was doubled to (3) minutes, the sensitivity was more and the resistance was less, as

shown in Figure 7. This is consistent with (Anwer and Husien 2007).

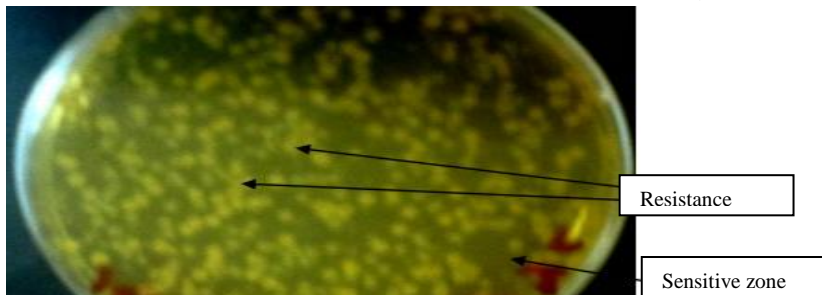


Figure 7: the sensitivity of pseudomonas aeruginosa to exposure to the laser for 3 minutes

When the time was doubled to 6 minutes the sensitivity was more and very strong while the resistance is very weak so all colonies of bacteria are

died as shown in Figure 8. This is consistent with (Anwer and Husien 2007)

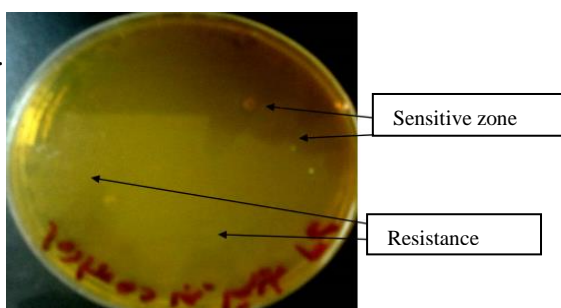


Figure 8: the weakness of the resistance of pseudomonas aeruginosa to exposure to the laser for 6 minutes

We found that the ability to kill bacteria increases when using laser with an antibiotic tablet,

the combination of antibiotic treatment and laser therapy together more strong than the use of antibiotic alone or laser radiation alone we also observed the relationship between time and the area of sensitivity increases with time increase so we saw at the sixth minute of applied to the light of the laser there was no resistance zone and all bacterial colonies were eliminated as shown in Figures 9-11.

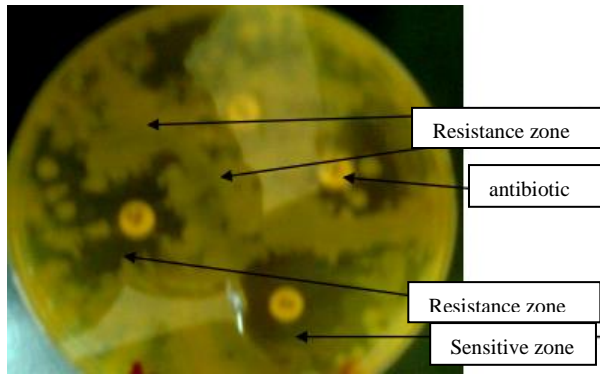


Figure 9: The response of weak *Pseudomonas Aeruginosa* to the laser with presence of antibiotic when exposed to laser radiation for 1 minute

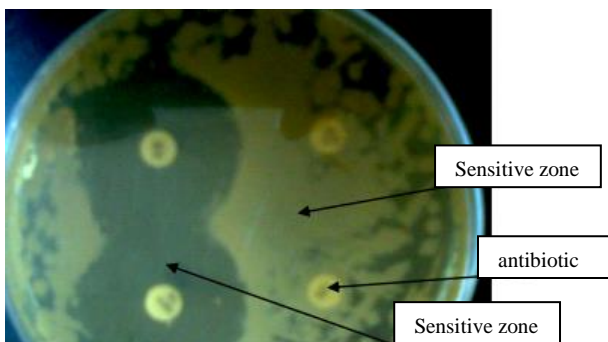


Figure 10: The increased response of the antibiotic when exposed to laser radiation and doubling the exposure time to 6 minutes

Pseudomonas Aeruginosa to the laser with presence of antibiotic at exposure to the laser and the doubling of the exposure time of the radiation for 3 minutes.

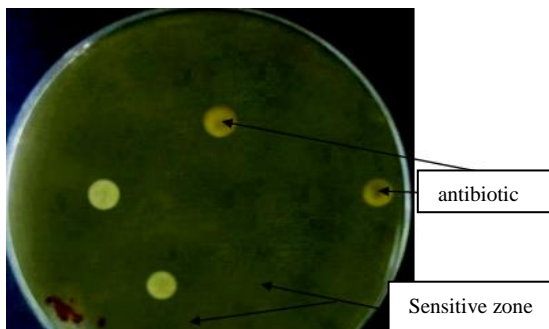


Figure 11: An increase in the response of 100%

Pseudomonas Aeruginosa with the presence of

We also noticed that the best antibiotic used for treatment with laser radiation is gentamicin than amyacycin and other antibiotics we used this is consistent with(Forestier, Guelon *et al.* 2008). The most important factors affecting cell growth is a temperature, the optimum degree of growth of human parasitic bacteria is (37 ° C). Above the maximum temperature (upper) and less than (37 °C) affect the bacterial growth. Heat also kills microorganisms because they change the nature of

proteins and involve changes in the chemical or physical properties of the protein. The mutant includes structural alterations because of the destruction of the chemical bonds of the amino acids of the proteins. In the three dimensional form, proteins return to a two-dimensional structure, Its function changed(Anwer and Husien 2007).

These results suggest that, in fact, there is a genetic basis behind the resistance to bacterial antibiotics, rather than the simple biomarker acting as a barrier to the spread of antibiotics.

Some recent studies have shown that the typical resistance to biofilm formation or the emergence of small and varying colonies may be necessary to respond to the extent and impact of pseudoephenia with antibiotic therapy. Some recent studies have shown observational phenomena associated with the formation of the biomembrane or the emergence of small colony species that may be important in the pseudomonas aeruginosa response to antibiotic treatment(Standards 2000).

Conclusion:

The result show that not killed *Pseudomonas Aeruginosa* in the case of the use of laser alone wavelength and strength of the above for the time periods mentioned above, and in the case of the use of antibiotics alone. While when the combination of antibiotic treatment and use of laser was the best result as bacteria began to weaken and increased sensitivity to antibiotics when using laser light for one minute and the presence of antibiotic and weakened bacteria more and killed about half the number of colonies when the light of laser shed for (3 minutes) and the use of antibiotic also the sensitivity of bacteria to antibiotics increased to (100%) where bacteria killed and the elimination of all colonies when using antibiotics and laser irradiation for (6 minutes) in one. Furthermore using an antibiotic (Penicillin, terramycin, ciprofloxacin, azactam, maxipime, cephalixin, amikacin,gentamicin).

The result was better when using gentamicin antibiotic than the rest of antibiotics and amyacsin.

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