RESEARCH ARTICLE

Study the Effect of Laser Irradiation on Bacterial Susceptibility of *Pseudomonas euroginosa* Isolated from Different Source Infections

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Received: 15th February, 2022; Revised: 18th April, 2022; Accepted: 12th May, 2022; Available Online: 25th June, 2022

ABSTRACT

Pseudomonas aeruginosa is an opportunistic microorganism that does not infect healthy individuals but does cause a broad variety of severe infections in immunocompromised patients. Samples were collected from 345 patients who suffered from diabetic foot infection (115), wounds and burns infection (115), and abortion cases (115) by use of the swabbing method; the diagnosis of samples depending on the morphological examinations, biochemical tests and culturing on selective or suitable media, to confirm the use of the API system was done. These samples include 52 isolates of *P. aeruginosa*, which consider the aims of this study at a rate of (15.07%) from the total number of samples (345).

The current study demonstrates that the age group (31–40) years had a higher rate of isolation 52.9% while a reduced number of isolation occurred in the age group (61–70) year at rate of 5.88%. The sensitivity of *P. aeruginosa* before irradiation to a number of antibiotics gives high resistance to the antibiotics used in this study. There is a significant decrease in the viability of the bacterial cell where the Laser effect on the bacterial isolates by increasing the dose, while the killing of bacteria occurs after 30 minutes and more. We conclude that bacterial isolates of *P. aeruginosa* were affected with exposure to irradiation of laser with 820 and 915 nm at 50–250 MW using different times.

Keywords: Inhibition zone, Killing bacteria, Laser, Pseudomonas aeruginosa, Wavelength.

International Journal of Drug Delivery Technology (2022); DOI: 10.25258/ijddt.12.2.28

How to cite this article: Nahab HM. Study the Effect of Laser Irradiation on Bacterial Susceptibility of *Pseudomonas euroginosa* Isolated from Different Source Infections. International Journal of Drug Delivery Technology. 2022;12(2):629-633.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Pseudomonas aeruginosa is a gram-negative bacterium, non-spore-forming bacillus about 3×0.5 µm, belonging to the γ-Proteobacteria group. Like most pseudomonas, is motile by polar flagella, the colonies of *P. aeruginosa* were smooth with flat edge giving grape like odor. Oxidase test is positive, catalase-positive, mesophilic and has great nutrient diversity, Do not ferment carbohydrate (glucose, sucrose, lactose), on the blood agar, the isolates colonies were dark in color with producing transparent zones around the colonies show the beta hemolysis, many strain can also produce water-soluble pigments include pyocyanin, pyoverdin (Fluorescent), pyorubin and pyomelanin, that diffuse into the medium and change its color.[1]

P. aeruginosa grows in moist environments within a wide range of temperatures, the optimum temperature for its growth is 37°C, but it is capable of growing at high temperatures as 42°C. It plays an important role as an etiological agent of severe infections in patients with wounds.[2] And has been estimated that at least 50% of all cases of deaths caused by burns, Diabetic foot ulcers (DFUs) are a serious of medical disease. The lifetime risk of developing a foot ulcer in diabetic patients may be as high in recent studies as 25%.[3] The most specific major populations of bacteria evident in the DFUs include *Staphylococcus, Pseudomonas*, *Enterobacter*, *Klebsiella*, *Proteus*, *Citrobacter*, *Escherichia coli* and others.[4]

There are a lot of species of the genus Pseudomonas, is play a great role as human opportunist bacteria in the infection of diabetic foot. It may be responsible for a broad spectrum of presentations from superficial colonization of ulcers to extensive tissue damage, including bacteremia, osteomyelitis, and septic arthritis.[2] *P. aeruginosa* is an opportunistic microorganism that does not infect healthy individuals but does cause a wide variety of severe infections in immunocompromised patients. For that reason, *P.aeruginosa* is categorized as an opportunistic pathogen. Nosocomial pneumonia with it is now classified as second after *S. aureus*.[5] In medicine and veterinary, the abortion word refers to any process that leads to a pregnancy ends with the removal or expulsion of the fetus and death.[6]

The laser may be defined as an acronym for light amplification by stimulated emission of radiation; common usage recent is to use the word as a noun (laser) rather than

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as an acronym.[7] It is a type of electromagnetic radiation that includes infrared radiation, radio waves, microwaves, visible-ray, UV–rays, x–rays, and gamma rays, which has become an important tool in various fields, starting with medicine until communications. It is considered a light source but it is very high difference from many traditional light sources.[8] Microorganism considered as susceptible to the antibiotics were inhibited from growing in a clear zone around the antibiotic disk. The inhibition zone diameter produced by a control strain was used to compare interpreting the antimicrobial sensitivity.[9]

MATERIALS AND METHODS

Patient and Sampling

Three hundred and 45 specimens were collected from patients admitted in Al- Hussain Teaching Hospital, Al–Muthanna Province, complaining from diabetic foot infections (115), wounds and burns infection (115), and abortion cases where the last Samples collected from 115 women lying in the Obstetric and Pediatric Hospital in Samawa city, suffering from abortion. The collected samples were transferred to the laboratory under cool conditions to isolate the bacteria Using sterilized cotton swabs and vaginal swab.

Identification of Isolates

After collection of specimens from different source infections, the identification of samples was done according to Berge's manual using different properties of morphology, and hemolysis Detection, which means the appearance of clear zones around the colonies duo to RBCs lyses considered as a positive result, in addition to biochemical tests and analytical profile index (API) system.[10] API system is an identification test for bacteria; it contains standardized and miniaturized biochemical test and database. The reaction was read according to the identification and reading table obtained by referring to the API.

Antimicrobial Sensitivity Tests

Antimicrobial sensitivity tests were done by (Kirby - Bauer) technique.[11] The inhibition zone diameter produced by a control strain was used to compare and interpret the antimicrobial sensitivity. Levels of sensitivity recognized are sensitive (S), intermediate (I), and resistant (R) by comparison of the bacterial inhibition zone.

Bacterial Count

The method of plate count is done by dilution method of the original sample in serial dilution tubes. The colony-forming units (CFUs) is counted and assumed that every colony in culture media is separate and founded by a single viable microbial cell, so the total counts of colony occurred in CFU from the incubated agar petridis and the respective dilution factor used can then be combined in order to calculate the microorganism's original number in the sample.[12]

Preparation of Bacterial Samples

A loop full from culture was transferred from the Trypanosoma agar slants (Bacterial Preservation) to a test tube containing brain–heart infusion broth and incubated at 37°C for 24 hours then The suspension was centrifuged (cold centrifuge) at (6000 rpm) for 10 minutes, the supernatant was removed, and the bacterial pellet was re suspended using normal physiological saline, a serial of dilutions made until the solution has turbidity. The bacterial broth was compared with McFarland tubes to determine the number of bacteria equal to 10^8 CFU/mL.

Laser Diode

The experimental laser in this study was the (Omega) Diode laser. It had wavelengths lies between (600–1000 nm), with a series of probes, and maybe defined as CW laser and had the properties as: - (820 and 915) nm in wavelength, Frequencies (1, 5, and 10) kHz, time of exposure which is different (10, 15, 20, 30, 40 and 50) minutes and power density (PD): 240, 480 and 960 J/cm^2.min. Then take 1-mL of the diluted bacterial suspension of *P. aeruginosa* and place in a sterile Eppendorf tube later the specimen was exposed to the diode laser with an exposure time 10, 20, 30, 40, and 50 minutes, and different frequencies (5 and 10) KHz. After irradiation, samples were immediately re-cultured on some media such as Muller Hinton agar and Blood agar media, incubation at 37ºC for 24 hours. To determine the effect of laser on the bacteria, observing that by antibiotic sensitivity, bacterial count and Hemolysis production. Non-irradiated samples used as control. This method is represented by live attenuated and killed bacteria.

RESULTS AND DISCUSSION

Collections of Samples

In this study, 345 samples were collected from different sources as 115 patients complained of diabetic foot infections, 115 burns and wound infections, and 115 abortion case in pregnant women. These samples include 52 isolates of *P. aeruginosa* at a rate of 15.07% of the total number of samples (345), (Table 1).

P. aeruginosa is considered as an opportunistic pathogen and is broadly known to cause chronic biofilm infections. They had an expression as the number of virulence factors and proteins with the surface of a bacterial cell which promotes its adherence with damaged tissue and neutrophils was decreased functions and the host immune system and tolerance to antimicrobial treatments. In this study, in diabetic foot disease, the occurrence of *P.aeruginosa* was 14.78%; these results were incompatible with those obtained by,[13] whose results were

Table 1: Numbers and percentages of *P. aeruginosa* isolated from different samples.

No.	*Type of specimens*	*Total No. of specimens*	*No. of P. aeruginosa*	*Per %*
1	diabetic foot infections	115	17	14.78
2	burns and wound infections	115	31	26.95
3	Abortion infection	115	4	3.47
	Total	345	52	15.07

52.2%. The samples of diabetic foot including 66 male at a rate of 57.5%, and 49 female with percentage 42.6%, while in the infection of burns and wound 45 male with 39.13% and 70 female at a rate of 60.8%. In abortion cases of pregnant women, *Pseudomonas aeruginosa* was isolated in 4 aborted women at a rate of 6.08% as shown in Table 2.

The percentages of female samples were higher than the males, where those ratios 32.17% male, 67.8% female, and these results were to approach the results which obtained by Chaya.[14] In the case of diabetic foot; the age group 31 to 40 years has a higher rate of isolation 52.9%, while the reduced number of isolation occurs in age group 61 to 70 years at rate of 5.88%, (Table 3) which also explain the higher rate of isolation in Burns and wound infection happened in the age group 31 to 40 year at a rate of 45.1% but the lowest number occurred in patient which age of them lies between 61 to 70 year. Abortion case registered a small number of isolation as Table 3.

Identification of Bacteria

P. aeruginosa has a feature as an obligate aerobic microorganism that grows on different types of culture media, as morphological properties; it has a sweet or odor like - grape or corn taco, and hemolysis of blood. *P euroginosa* characterized by smooth round colonies with a fluorescent greenish color. It produces (non-fluorescent) bluish pigment (pyocyanin), pyorubin, and pyomelanin, which diffuse into the culture media. Microscopic Examination of *P. aeruginosa* showed as motile, rod shape and measuring about (0.6 x 2) m. It is often occurs in single bacteria or in pairs, and occasionally in short chains. Figure 1.

Biochemical tests are often required to identify the pathogenic bacteria, including using substrates and sugars and enzymatic and fermentation reactions, as shown in Table 4. The results of API 20 NE system came to ensure the biochemical identification of *P. aeruginosa*.

Antibacterial Susceptibility Testing

The antimicrobial agent activities against the pathogenic isolates of bacteria were variable, depending on the species of bacteria and the action mode of each agent. The results showed that the isolates of *Pseudomonas aeruginosa* were resistant to list of the antibiotics used in this study before irradiation, as shown in Table 5.

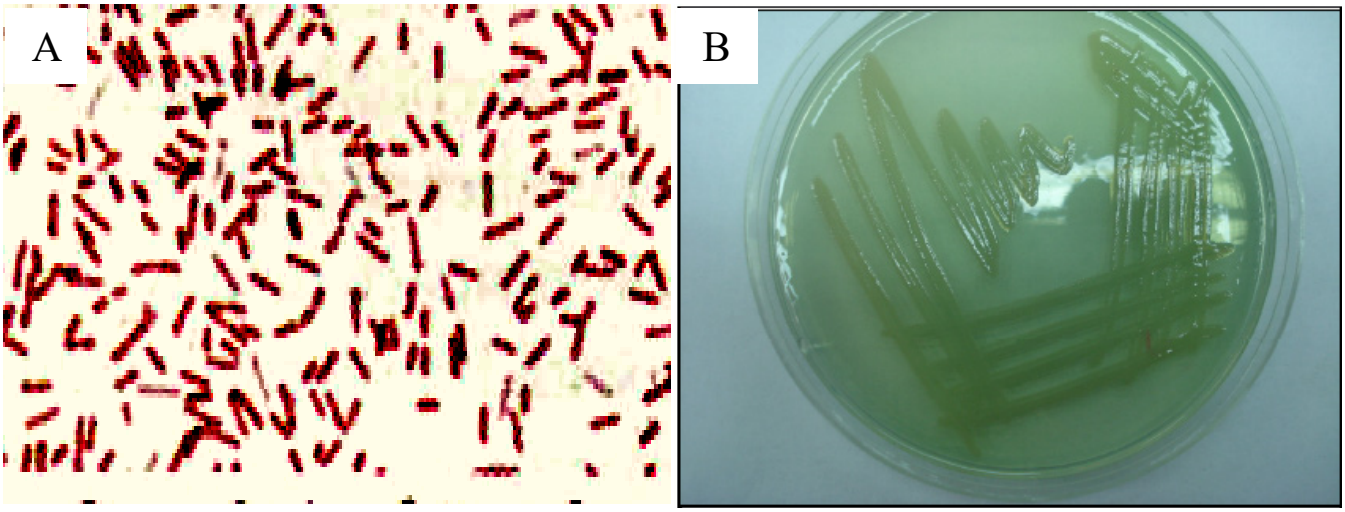

Figure 1: A- *Pseudomonas aeruginosa* on nutrient agar with greenish- blue pigment. B- *Pseudomonas aeruginosa* microscopically stained with gram stain.

Table 2: Numbers and percentages of patient sex isolated from different samples.

No.	*Type of infection*	*Male*	*Per%*	*Female*	*Per%*	*Total No.& %*
1	Diabetic foot	66	57.5	49	42.6	115 (100%)
2	Burns and wound	45	39.13	70	60.8	115 (100%)
3	Abortion	-	-	115	100	115 (100%)
Total		111	-	234		345 (100%)

Table 3: Distribution of *P. aeruginosa* in different samples according to age group

		Age group					
No.	*Type of infection*	*20–30*	*31–40*	*41–50*	*51–60*	*61–70*	*Total*
1	Diabetic foot	3	9	2	2	1	17
2	Burns and wound	5	14	6	4	2	31
3	Abortion	1	2	1	0	0	4
Total		9	25	9	6	3	52

Table 4: Biochemical tests of *P. aeruginosa* isolates.

Biochemical test	*Result*	*Biochemical test*	*Result*
hemolysin production	+	Oxidase	+
Mannitol fermentation	/	Catalase	+
Gelatin	+	Coagulase	/
Pyoverdin produced	+	Urease	-
Growth at 42c˚	+	Motility	+
Oxidation of lactose	+	Kligler test or (TSI)	AK/NC ($-H_2S$), (-gas)
Oxidation of glucose	+	Lactose fermentation	+
Oxidation of manitole	-	Indol	-
Oxidation of maltose	+	Methyl red	+
Arginine dihydrolase	-	Voges Proskauer	-
Oxidation of manitole	+	Citrate utilization	+
H2s	-	Nitrate reduction	+
Indol	-	Lysine decarboxylase	-
Oxidation of manitole	+		

Key: (+); positive test , (-); negative test , (A/A) ;Acidic/ Acidic , (AK/A) ;Alkaline /Acidic , (AK/NC); Alkaline / No Change, (+S) ; swarming motility , (/); No test .

Table 5: The results of the antibacterial susceptibility test of *P. aeruginosa* before irradiation.

Antibiotics	*Symbol*	*Conc. µg/ disc*	*Result*
Amikacin	(AK)	30	S
Chloramphenicol	(C)	30	R
Rifampin	(RA.)	5	R
Gentamicin	(GN.)	10	I
Ciprofloxacin	(CIP)	5	S
Vancomycin	(VA)	30	R
Oxacillin	(OX.)	1	R
Erythromycin	(E)	15	R
Cefoxitin	(FOX)	30	R
Nalidixic acid	(NA)	30	s
Ceftazidime	(CAZ)	30	R
Lincomycin	(L)	2	R
Cephalothin	(F)	30	**S**
Azithromycin	(AZM)	10	**S**

Key : (R): resistance, (S): sensitive, (I): Intermediate.

The bacterial resistance of *Pseudomonas aeruginosa* is represented by different mechanisms of action: cell wall synthesis inhibitors (Vancomycin, Cefoxitin, and Oxacillin), protein synthesis inhibitors which include (Erythromycin), and RNA synthesis inhibitors such as (Rifampicin).[15]

After irradiation by omega diode laser for different frequencies and exposure times, using the (820 and 915) nm in wavelength, there are significant changes in pathogenic isolates' sensitivity of *P. aeruginosa* antibiotics (Table 6).

Table 6: The results of the antibacterial susceptibility test of *P. aeruginosa* after irradiation.

Antibiotics	*Symbol*	*Conc. µg/ disc*	*Result*
Amikacin	(AK)	30	S
Chloramphenicol	(C)	30	S
Rifampin	(RA.)	5	I
Gentamicin	(GN.)	10	S
Ciprofloxacin	(CIP)	5	S
Vancomycin	(VA)	30	R
Oxacillin	(OX.)	1	S
Erythromycin	(E)	15	I
Cefoxitin	(FOX)	30	R
Nalidixic acid	(NA)	30	S
Ceftazidime	(CAZ)	30	S
Lincomycin	(L)	2	S
Cephalothin	(F)	30	S
Azithromycin	(AZM)	10	S

The Irradiation of *P. aeruginosa* in different (time and frequencies) showed a notable decrease in the bacterial viability with increasing the dose. *Pseudomonas aeruginosa* remained to survive until 27 minutes. for all Wavelengths, while killing bacteria occurred at exposure time 30-40 minutes by using 10 kHz. The bacterial isolates of *Pseudomonas aeruginosa* after irradiation showed that hemolysis production was lost or decreased at wavelength 915 nm with 20 minutes (lost), 10 kHz frequency, and power 250, While in the 820 nm the results of absence of hemolysis occurred in 30 minutes. The result of pyocyanin pigment loss is also affected by laser irradiation, as shown in Table 7.

When using 820 nm, (5 and 10 kHz), for 30 minutes, the bacteria just attenuated. But there are the different thing at the wavelength (915) nm, (10) kHz, and 20 minutes, where the bacteria was killed; in the process killing bacteria, the bacterial counting lost while a decrease in the case of preparation of attenuated bacteria, and this result not agreed with.[16] Who revealed that *P. aeruginosa* was eradicated after irradiation with diode laser 14.14×10-3 J/cm2 power density at 5,10 and 15 minutes exposure time. While[17] found that *P. aeruginosa* absorbed light at visible range where the maximum was 537 nm for prodigiosin and (690) nm for pyocyanin, and maybe act as photosensitizers, causing a reduction 62%, of *P. aeruginosa*.

Many factors such as the capacity of light absorption, the wavelength of the laser, bacterial physiology, and emission from the laser, in addition to exposure time, pH medium, water, thermal conductivity, and the organic matrix play important roles with the effectiveness of laser, (Hayek *et al*., 2005).[18]

To confirm, the irradiated bacterial suspension re cultured, the viable bacterial counting decreased and lost by using this wavelength in this study at the preparation of attenuated and killed bacteria, respectively.

Table 7: The results of irradiation laser on the bacterial growth.

Wavelengths (nm)	*Frequency (kHz)*	*Time(min.)*	*Power mW*	*Result of growth*	*Result of hemolysis*	*Result of pigment production*
820 nm	5 kHz	10	50	+++	+++	++
		20	50	++	+	+
		30	50	+	-	-
		40	50	-	-	-
	10 kHz	10	250	++	++	+
		20	250	+	+	+
		30	250	-	-	-
		40	250	-	-	-
915 nm	5 kHz	10	50	++	++	+
		20	50	+	+	-
		30	50	+	-	-
		40	50	-	-	-
	10 kHz	10	250	+	+	+
		20	250	+	-	-
		30	250	-	-	-
		40	250	-	-	-

Key : (+++) growth , (++) weak , (+) very weak, (-) no result.

REFERENCES

1. Quinn PJ, Carter ME, Markey B, Carter GR. Clinical Veterinary Microbiology. Mosby. 2004;237-242.
2. Catherine SM, Marisela V, Fralick JA. Phage Therapy of Pseudomonas aeruginosa Infection in a Mouse Burn Wound Model. Antimicrobial agents and chemotherapy. 2007;51(6): 1934–1938.
3. Kaya A, Aydin F, Taskin A, Hasan Q. and Karakuzu, G. Can major amputation rates be decreased in diabetic foot ulcers with hyperbaric oxygen therapy? International Orthopaedics. 2009; 33(7):441–446.
4. Dowd SE, Sun Y, Secor R, Rhoads D, Wolcott M, James A, Wolcott RD. Survey of bacterial diversity in chronic wounds using pyrosequencing, DGGE, and full ribosome shotgun sequencing. BMC Microbiology, 2008;8(43).
5. Sadikot RT, Blackwell TS, Christman JW, Prince AS. Pathogen-host interactions in Pseudomonas aeruginosa pneumonia. Am. J. Respir. Crit. Care Med. 2005;171: 1209-1223.
6. Grimes DA, Benson J, Singh S *et al*. "Unsafe abortion: the preventable pandemic" .Lancet (2006;368 (9550): 1908–19.
7. Hedaa M, Nahaab, Ihsan F. Rostum. February. Preparation of Vaccine for pathogenic bacteria causing abortion using low level diode laser: AL- Muthanna Journal of Pure Sciences, 2013;Number (2;Volume (1).
8. Avadhanulu MN. An Introduction to lasers Theory and Applications. Published by S.Chand &Company Ltd, .7361, Ramnagar, New Delhi-110055.ISBN:81-219-2071-X, An ISO 9001:2000Company). 2009;P. 166-174.
9. Angus, J. R. and Olila, D. Drug sensitivity patterns of bacterial isolates from septic post-operative wounds in a regional referral hospital in Uganda. African Health Sciences, 2007;7(3): 148 – 154.
10. Hopkins T. Guide to LAB notes and Diagnostic tests. 1st ed. F. A. Davis, 2005;Pp: 103 – 105.
11. NCCLS (National Committee for Clinical Laboratory Standards). Approved Standard M100-S12. Wayne. PA, NCCLS. Methods for dilution antimicrobial susceptibility tests of bacteria that grow aerobically, 2002.
12. Goldman E, Green LH. Quantitation of Microorganisms: Practical Handbook of Microbiology. 2nd ed. Chap. (2). Taylor & Francis Group, 2009;LLC. Pp: 18 – 31.
13. Fazli, M. ; Bjarnsholt, T, Klaus, K.M. ; Jorgensen, B. ; Andersen, A. S, Krogfelt, K.A, Givskov,A. and Nielsen, T. T. Nonrandom Distribution of Pseudomonas aeruginosa and Staphylococcus aureus in Chronic Wounds. Journal of Clinical Microbiology, (2009;47 (12): 4084–4089.
14. Chaya, P. ; Mabula, J. ;Dass, M. ;Kabangila, R. ;Jaka, H. ; Mchembe, H. ; Kataraihya, J. ; Mbelenge, N. and Gilyoma, M. Surgical management of Diabetic foot ulcers: Tanzanian university teaching hospital experience. BMC Research Notes. 2011;4(365).
15. Cerca, N, Martins, S, Cerca, F, Jefferson, K, Gerald, B, Oliveira, R. and Azeredo, J. Comparative assessment of antibiotic susceptibility of coagulase-negative staphylococci in biofilm versus planktonic culture as assessed by bacterial enumeration or rapid TXT colorimetry. J. Antimicrob Chemother, 2005;56(2): 331–336.
16. Kadim, N. A. Effect of 337.1 nm and 805 nm lasers on the biochemical characteristics of Pseudomonas aeruginosa. M.Sc. Thesis, Institute of laser for post graduate studies. University of Baghdad, 2007.
17. Lipovsky, A, Nitzan,Y. and Lubart, R. A Possible Mechanism for Visible Light-Induced Wound Healing. Lasers in Surgery and Medicine, 2008;40:509–514.
18. Hayek, R, Araujo, N, Gioso, M, Ferreira, J, Yamada, A. and Ribeiro, M. Comparative Study between the Effects of Photodynamic Therapy and Conventional Therapy on Microbial Reduction in Ligature-Induced Peri-Implantitis inDogs. Photodynamic Therapy Compared to Conventional Therapy, 2005;76 (8):1275-1281.