Iraqi Journal of Science, 2023, Vol. 64, No. 2, pp: 583-593
DOI: 10.24996/ijs.2023.64.2.8

ISSN: 0067-2904

The Effect of Diode Laser on Viability and Antibiotic Sensitivity of *Streptococcus mutans* Isolated From Dental Caries

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Received: 26/2/2022 Accepted: 12/7/2022 Published: 28/2/2023

Abstract

Streptococcus mutans is one of the major cariogenic microbial flora. In an attempt to determine the mutagenic effect of diode laser on the viability and antibiotic sensitivity of this bacteria; A total of 30 samples were collected from dental caries. The isolates were identified using ~~by~~ conventional identification methods and confirmed using VITEK2 system. Twenty-one isolates were recorded as *Streptococcus spp* and ten of them were identified as *Streptococcus mutans.* Antibiotic susceptibility profile for *Streptococcus mutans* isolates against ten antibiotics was tested. The results revealed that all the isolates were resistant to cefixime and cephalothin, nine of them were resistant to erythromycin, ampicillin, and nalidixic acid, seven isolates were resistant to tetracycline and bacitracin, whereas all isolates were sensitive to chloramphenicol, ciprofloxacin and gentamycin. The effect of the diode laser on the isolates (S5 and S10) was tested at different time intervals (1, 2, 5, and 10) min. The viability of *Streptococcus mutans* isolates was affected by the obvious change in the behaviour of the bacteria from resistance to sensitivity after Laser exposure

Keywords: *Streptococcus mutans*, antibiotic resistance, Laser technique, dental caries

تأثير ليزر الصمام الثنائي على حيوية وحساسية *المكورات العقدية* للمضادات الحيوية المعزولة من تسوس الأسنان

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الخلاصة

تعد المكورات العقدية من اهم مسببات تسوس الأسنان. في محاولة لتحديد تأثير الطفرات الجينية لليزر الثنائي على قابلية الحياة وحساسية هذه البكتيريا للمضادات الحيوية. تم جمع ما مجموعه 30 عينة من تسوس الأسنان. تم التعرف على العزلات باستخدام طرق تحديد تقليدية وتم تأكيدها باستخدام نظام VITEK2. تم تسجيل 21 عزلة على أنها Streptcoccus spp وتم تحديد عشر منها على أنها *Streptcoccus mutans*. تم اختبار ملف الحساسية للمضادات الحيوية لعزلات *Streptcoccus mutans* ضد عشرة مضادات حيوية. أظهرت النتائج أن جميع العزلات كانت مقاومة للسيفيكسيم والسيفالوثين ، تسعة منها كانت

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مقاومة للإريثرومايسين ، الأمبيسلين وحمض النالديكسيك ، سبع عزلات كانت مقاومة لمضادات التتراسيكلين والباسيتراسين ، بينما كانت جميع العزلات حساسة للكلورامفينيكول والسيبروفلوكساسين والجنتامايسين. تم اختبار تأثير ليزر الدايود على العزلات (S5 و S10) بتأثير ليزر الصمام الثنائي على العزلات (S5 و S10) على فترات زمنية مختلفة (1 ،2، 5 ، 10) دقيقة. تأثرت جدوى عزلات Streptcoccus mutans بالتغير الواضح في سلوك البكتيريا من المقاومة إلى الحساسية بعد التعرض بالليزر.

1. Introduction

The human mouth cavity is colonized by a highly diverse species of bacteria. Most of ~~the~~ them form complex accumulates termed as biofilms or dental plaques on the teeth surfaces [1]. *Streptococcus mutans* is colonized on the oral surfaces and constitute more than 75% of the cultivated bacteria that existed in the human dental plaque that leads to dental caries and may cause teeth loss in children and adults [2]. Antimicrobial activity of several compounds was tested against clinical isolates including *streptococcus mutans* collected from patients suffering from different dental diseases in Iraq [3]–[5]. Furthermore, mechanisms of actions of secondary metabolites are reviewed as the causative factors responsible for the antimicrobial activity of plants against *streptococcus mutans* clinical isolates [6], also *Leuconostoc mesenteroides* biofilm may be used as an antimicrobial agent [7]. Many factors accounted for the cariogenicity of *streptococcus mutans*such as short generation time, carbohydrates fermentation, and capability to resist lower pH. and their ability to production of extracellular glucan-homopolymers from sucrose, which have an important role in attachment, colonization and formation of biofilms on teeth surfaces [8]. Dental caries not only affects oral health, but may also correlate with other diseases such as diabetes, endocarditis, intravascular infections and bacteremia [9], therefore the treatment of dental caries by using laser technique is regarded as an important step to decrease this health risk [10].

Laser technique has been developed very rapidly and extended to use ~~it~~ in many applications starting from pure physics, biology, biotechnology, biochemistry and medicine [11]. Its characterized by its special properties that are not found in ~~the~~ normal light sources [12]. One of the characteristics of laser is conducted on its light waves ~~that~~ emits for ~~a~~ very long distances with ~~a~~ very low dispersion, these waves have a fixed phase that makes the bandwidth of laser light so narrow, highly powerful, and very easy to focus on a target object with high intensities [13]. Photodynamic inactivation of bacteria by low-power laser (He-Ne or diode laser) was used to kill the bacteria in the presence of suitable photosensitizer toluidine blue [14], [15].

2. Methods

2.1. Collection of samples

Thirty samples were collected by sterile swabs from a mouth cavity of individuals with dental caries in different dental clinics in Baghdad during period from November 2020 to February 2021.

2.2. Isolation and Identification of bacterial isolates

The swabs were taken from cases of dental caries, then streaked on Mitis Salivarius agar (MSA) plates and incubated overnight at 37˚C under anaerobic conditions in a candle jar. After incubation, the colony morphology was examined on selective media, and then the bacteria were stained by Gram stain.

2.3. VITEK2 system

The isolated bacteria were finally identified by using Vitek2 system kit

2.4. Antibiotic sensitivity test

The sensitivity of ten *Streptococcus mutans* isolates against 10 antibiotics was determined according to Kirby-Bauer method, in which a sterile cotton swab was immersed in the broth culture of *Streptococcus mutans* ~~at~~ compared with 0.5 McFarland's turbidity standard provided an optical density comparable to the density of bacterial suspension with a 1.5×10^8 colony forming unit (CFU/ ml). The swabs were streaked on the surface of Mueller-Hinton agar at different directions to ensure equal distribution, then disc for each antibiotic was fixed on the agar surface by a sterile forceps and the plates were incubated overnight at 37˚C. The diameter of the Inhibition zone was recorded, and the results were compared with national committee for clinical laboratory standard value (NCCLS 2020) with performance standard for antimicrobial susceptibility testing.

2.5. Irradiation of *Streptcoccus mutans* with diode laser

Continuous laser was used for irradiation of two *Streptococcus mutans* (S5and S10) isolates and it consisted of the following parameters: It propagates gamma light at wavelength 623nm, the spot size is approximately 2cm, it was fixed at 1000Hz [16]. Isolates of *Streptococcus mutans* were cultured in 5ml of brain heart broth and incubated at 37℃ for 24hrs, centrifuging at 6000 rpm for 5 min. The supernatant was removed and the cell deposit was diluted in a sterile PBS buffer PH7.2, then adjusted to a 0.5 McFarland's turbidity standard provided an optical density comparable to the density of bacterial suspension with a 1.5×10^8 colony forming unit (CFU/. Bacterial suspension in tubes was exposed to laser light at different times (1, 2, 5, and 10) min, the control tube contains bacterial suspension which was not exposed to laser light. Both irradiated suspension and control were grown on MSA overnight at 37˚C Figure 1.

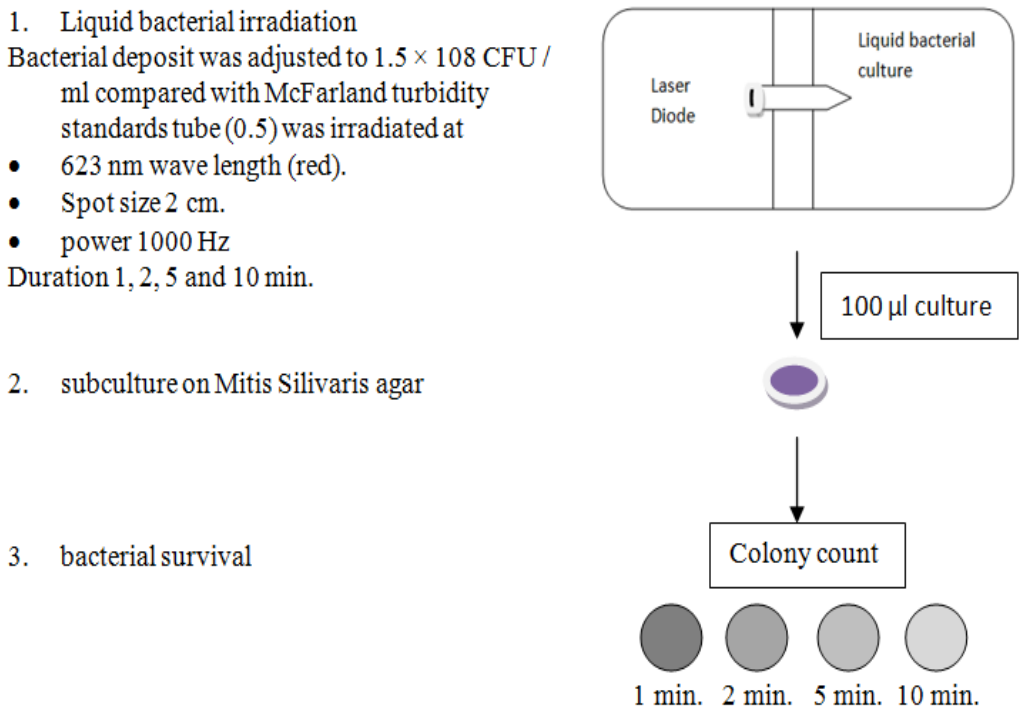

Figure 1: Diode laser irradiation experimental setup

3. Results and Discussion

3.1. Isolation and identification of *Streptococcus* species

The samples obtained from the mouth cavity of patient have ~~a~~ dental caries were streaked on mitis salivaris agar medium (MSA). This medium encourages the growth of *Streptococci* and inhibits the growth of other bacterial *spp*. because crystal violet and potassium tellurite act as an inhibitor for both gram-negative rods and other gram-positive bacteria except *Streptococci* [17]. Bacterial isolates were identified depending on their colony morphology on MSA medium that appeared as fine, smooth colonies. This result was agreed with Hata and Mayanagi [18], which noted that colonies of *Streptococcus spp.* appeared on MSA medium as small and adherent to the agar surface if it's transferred by loop, polysaccharide formation was observed as a drop glistening on the surface of the colony as shown in Figure 2.

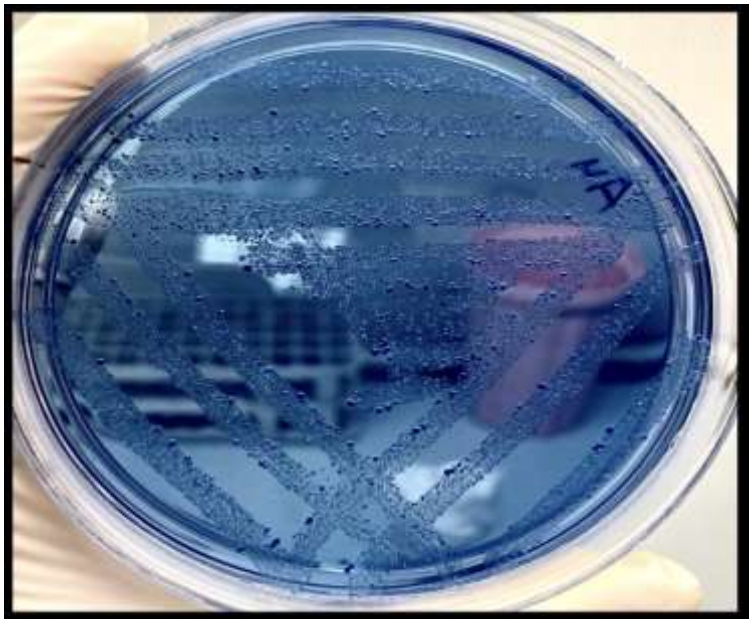

Figure 2: *Streptococcus* colonies on Mitis Salvaris agar medium

Streptococcus colonies have been recognized very easily after a longer incubation period in anaerobic conditions at 37˚C. This morphological characterization was agreed with that recorded by Murray and his colleagues [19]. In Gram stain, it appeared as Gram (+) cocci, spherical cells that are arranged in pairs or short chains, non-motile, non-spore forming, and it's compatible with that recorded by James and Natalie[20].

The results of the VITEK2 system revealed that 21 isolates were related to Streptococcus *spp*. and only 10 isolates were identified as *Streptococcus mutans*.

A study by Caprioglio and his colleagues in (2012) showed that *Streptococcus mutans* was the main species of Streptococci isolated from dental plaque which cause dental caries in infants and children [21]. Another study revealed that *Strept. mutans* was the first *bacterial species* that colonized among infants, shortly after their teeth erupt [22]. A study by Salman and Senthikumar, (2015) who collected samples from patients with dental caries and found that from a total of 50 samples, 20 were identified as *Streptcoccus mutans* while only 9 were recorded as *Streptococcus sobrinus* [23]. Haukioja and his colleagues in (2006) recorded that other pathogens like *Lactobacilli, Actinomyces spp*. and some other anaerobic bacteria also are considered as ~~an~~ important agents that may be involved in the development of dental caries [24].

3.2. Antibiotic susceptibility test for *Streptococcus mutans* isolates

Disc diffusion method was used to determine the sensitivity of *Streptococcus mutans* isolates against different antibiotics. The results revealed that all isolates were resistant for 2 antibiotics (cefixime and cephalothin), 90% of them were resistant to ampicillin, Naldixic acid and erythromycins, 70% of isolates were resistant to bacitracin and tetracycline, as shown in Table 1. While all isolates were sensitive to three antibiotics (gentamicin, ciprofloxacin and chloramphenicol) as shown in Figure 2.

A study by Chandrabhan and his colleagues reported that out of 120 samples collected from patients with dental plaque, 79 isolates that were characterized as *Streptococcus mutans* were examined for their sensitivity to penicillin, erythromycin, tetracycline and gentamycin. The results revealed that several isolates were resistant to penicillin and tetracycline [25]. Another study by Pasqnantonio and his colleagues recorded that out of 560 oral streptococci; 260 *Streptococcus mutans*, 120 *Streptococcus sanguis*, 100 *Streptococcus anginosus* and 80 *Streptococcus mitis* were isolated from patients with dental caries and tested for their sensitivity to 10 beta-lactam antibiotics and 7 non beta-lactam antibiotics, a reduced susceptibility to penicillin was recorded in 43.4% of cases [26]. Several *in vitro* studies revealed that the ability to transfer penicillin resistance could occur among related species.

Table 1: Antibiotics susceptibility of *Streptococcus mutans* isolated from dental caries

No. of Isolates	Antibiotics disc (µg/disc) / clear zone diameter in mm.									
	AM (10)	CFM (5)	KF (30)	CN (30)	B (10)	C (20)	TE (30)	CIP (5)	E (15)	NA (30)
S1	R	R	R	18mm	15mm	18mm	17mm	23mm	15mm	16mm
S2	R	R	R	15mm	R	14mm	15mm	24mm	R	R
S3	15mm	R	R	16mm	R	17mm	R	25mm	R	R
S4	R	R	R	16mm	15mm	18mm	R	27mm	R	R
S5	R	R	R	14mm	R	17mm	R	24mm	R	R
S6	R	R	R	15mm	14mm	16mm	R	26mm	R	R
S7	R	R	R	17mm	R	20mm	R	27mm	R	R
S8	R	R	R	15mm	R	19mm	R	25mm	R	R
S9	R	R	R	16mm	R	18mm	16mm	27mm	R	R
S10	R	R	R	17mm	R	21mm	R	26mm	R	R

R:resistance
KF: cephalothin, B: bacitracin, AM: ampicillin, CFM: cefixime, C: chloramphenicol,
E: erythromycin, TE: tetracycline, CN: gentamycin, CIP: ciprofloxacin, and NA: Nalidixic Acid

Figure 3: Antibiotic susceptibility test of *Streptococcus mutans* isolates.

Also, antibiotic pressure may lead to occurrence and transfer of penicillin resistance among oral Streptococci [27]. The results of the current study ~~were~~ agreed with a study reported by Jain & Pundir in India for evaluating the efficacy of fifteen antibiotics against three isolates of *Streptococcus mutans*. Gentamycin, followed by chloramphenicol was highly effective against these isolates. Many drugs such as tetracycline, cefixime, erythromycin, and methicillin have a moderate resistance [28].

A study recorded by Ten Cate and Zaura ~~in~~ (2012) revealed that *Streptococcus mutans* chromosomal DNA carries a heterologous gene responsible for resistance to erythromycin [29]. Brown and his colleagues noted that a high resistance rate to erythromycin was detected in *Streptococcus spp* that reach to 91% and may be associated with the occurrence of a new mechanism of erythromycin resistance-efflux in *Streptococcus spp*. The results also showed a high resistance ratio reach to (70%) against Bacitracin in *Strept. mutans* [9]. Hiromasa and his colleagues also found that *Streptococcus mutans* was resistant to Bacitracin at high ratio [30]. The isolation of *Streptococcus mutans* from human dental caries resistant to tetracyclines and macrolides may be a result of prolonged exposure to these antibiotics which are more commonly used [31]. The formation of microbial biofilms, such as dental plaque is one of the main reasons for the failure of antibiotics treatment and development of antibiotic resistance that mediated by biofilm which gives specific protection against oxidative stress, also the expression of biofilm-specific efflux that may pump antibiotics out [32]. The resistance may provide by matrix polysaccharides that can eliminate the diffusion of antibiotics and may play an important role in antibiotic resistance [33], [34].

3.3. Effects of diode laser on viability and antibiotic sensitivity of *Streptococcus mutans* isolates

The results showed that the effect of diode laser on the viability of *Strept*ococcus *mutans* and its ability to kill the bacteria was increased with increasing time of exposure to diode laser as shown in ~~the~~ Figure 4. Bargrizan and his colleagues reported that antibacterial photodynamic therapy (A-PDT) is able to kill oral bacteria in dental plaque [35]. A diode laser can diminish *Streptococcus mutans* in the carious dentin without elevating the temperature [36]. A study by Lee and his colleagues ~~in~~ (2006) determine the antimicrobial effect of diode laser on *Streptococcus mutans* through various thicknesses of human dentin

(400, 900, and 1800) µm. The results revealed that 7 W of laser power could kill 98% of bacteria through 400 µm dentin thickness, but the bactericidal efficiency was reduced when the thickness of dentin was increased [37].

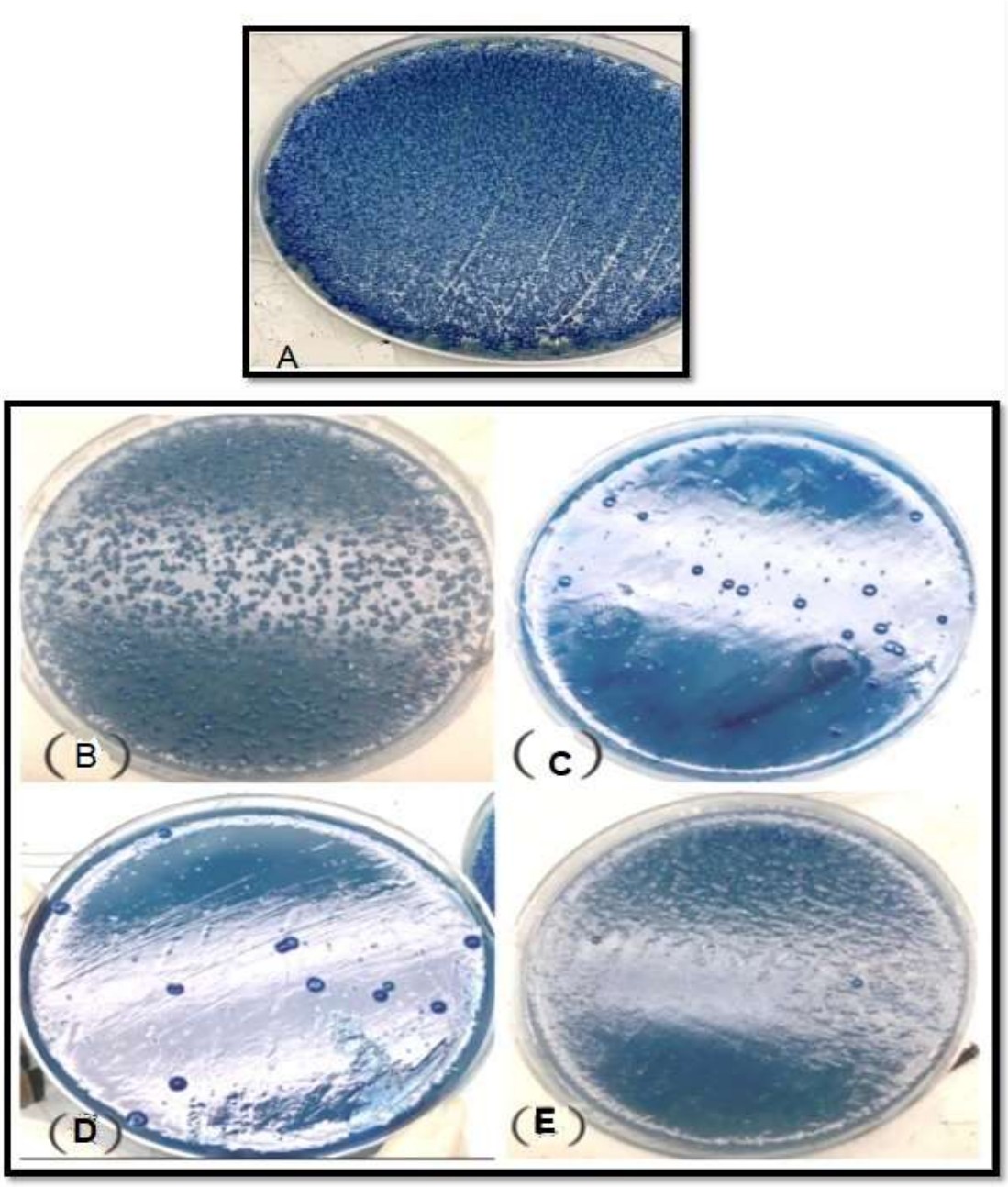

Figure 4: *Streptococcus mutans* S5isolates after exposure to diode laser for different times (A: Control – not exposed, B: 1 min, C: 2 min, D: 5 min, and E: 10 min)

Photodynamic therapy is used as a new method for decontamination of bacteria. *In vitro* study was done to evaluate the sensitivity of *Streptococcus mutans* to antibacterial photodynamic therapy by using 2 different photosensitizers and light sources. Suspensions of *Streptococcus mutans* were exposed to diode laser at 662 nm and Radachlorin or LED 630 nm synergistic with Toluidine blue (TBO). The results revealed that photodynamic therapy with (TBO) and Radachlorin lead to a high reduction in the viability of *Strept. mutans* [15]. A study by Ahmed and his colleagues on *Streptococcus mutans* isolated from patients with dental caries and exposed diode laser for different times (1, 3, 7, 10, and 12) min, then swabbed on the rabbit teeth. The bactericidal effect of the diode laser was detected after (7,10

and 12) min without any sign of curiosity found on the rabbit teeth [12].

When the microorganism is exposed to ~~a~~ stress like heat or stronger vibration on the cell walls, peptidoglycan layer has been solubilized rapidly by peptidoglycan hydrolases, leading to pores formation in the cell walls [38]. Laser light may also affect the normal cell division of *Streptococcus mutans* as it induce changes in mitotic activity in the irradiated monolayer cells which resulted in a decrease in the number of bacteria [35], [39], [40]. The bacteria exposed to laser have several changes in morphological features ranging from minor effects such as loss of their wall bands and presence of mini-cells to more severe damage such as disintegration and fusion of cells with pores on the cell wall [41].

Results also showed changes in sensitivity of *Streptococcus mutans* S5and S10isolates to many antibiotics after exposure to diode laser for different times (1,2,5, and 10) min. The sensitivity to gentamycin was slightly increased after (5 and 10) min of exposure to diode laser in both S5 and S10 isolates. The sensitivity of both isolates to chloramphenicol increased after 1 min and reach to higher sensitivity after 10 min of exposure to diode laser. Both isolates changes from resistance to sensitivity for the antibiotic tetracycline, nalidixic acid and cefixime after exposure to diode laser. The S10 isolate revealed sensitivity to tetracycline after 10 min of exposure to diode laser, while S5 isolate revealed sensitivity to tetracycline after 5 min of exposure to diode laser. Also, S10 isolates showed sensitivity to naldixic acid after exposure to diode laser for 5 min and increased sensitivity after 10 min, while S5 showed sensitivity to naldixic acid after 2 min and reach to higher sensitivity after 10 min of exposure to diode laser. Also, both isolates revealed sensitivity to cefixime after 10 min of exposure to diode laser as shown in Table 2.

Table 2: Antibiotic sensitivity of *Streptococcus mutans* isolates S5 and S10 after exposure to diode laser for different times

No. of Isolates	Time of exposure (min)	Antibiotics disc (µg/disc)									
		AM (10)	**CFM (5)**	**KF (30)**	**CN (30)**	**B (10)**	**C (20)**	**TE (30)**	**CIP (5)**	**E (15)**	**NA (30)**
S10	1	R	R	R	17 mm	R	23 mm	R	26 mm	R	R
S10	2	R	R	R	17 mm	R	25 mm	R	26 mm	R	R
S10	5	R	R	R	19 mm	R	28 mm	R	26 mm	R	15
S10	10	R	15 mm	R	20 mm	R	30 mm	16 mm	26 mm	R	18 mm
S5	1	R	R	R	14 mm	R	19 mm	R	24 mm	R	R
S5	2	R	R	R	14 mm	R	20 mm	R	24 mm	R	15 mm
S5	5	R	R	R	16 mm	R	22 mm	15 mm	24 mm	R	17 mm
S5	10	R	14 mm	R	17 mm	R	24 mm	15 mm	24 mm	R	20 mm

R: resistance. KF: cephalothin, B: bacitracin, AM: ampicillin, CFM: cefixime, C: chloramphenicol, E: erythromycin, TE: tetracycline, CN: gentamycin, CIP: ciprofloxacin, and NA: Nalidixic Acid

These result agreed with Rassam, who reported that exposure of bacteria to a diode laser reduce MIC values and increase the sensitivity of bacteria to antibiotics [42]. Lee and his

colleagues noted that the sensitivity of bacteria after exposure to diode laser was increased with increased the time and dose of diode laser [37]. The changes in sensitivity of bacteria to an antibiotic after exposure to diode laser are affected by ~~a~~ different laser aspects such as wavelength, the intensity of energy, time of exposure, and modality of propagation of laser irradiation [11], [21]. Also, exposure to diode laser may cause changes in bacterial efflux pump that accounted for bacterial resistance to antibiotics such as Beta-lactams and aminoglycoside [43]. In addition, it may cause bacterial failure in the production of target enzymes that chemically modified a special antibiotic leading to increased bacterial sensitivity to these antibiotics [13].

4. Conclusion

The results of this *in vitro* investigation have demonstrated the ability of diode laser as a technique to affect the viability of pathogenic *Streptococcus mutans* isolates an effect that can be detected through the change in the susceptibility profile of the isolates from sensitive to resistant.

5. Ethical Clearance

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

6. Conflict of Interest

The authors declare that they have no conflict of interest.

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