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Antibacterial Efficacy of Diode laser 810nm on Enterococcus Faecalis on Primary Teeth, an invitro study

Zienab A. Ghonem

Mohamed H. Mustafa

Shaymaa A. El Shishiny

Antibacterial Efficacy of Diode Laser 810 nm on *Enterococcus faecalis* on Primary Teeth: An *In Vitro* Study

Zienab A. Ghonem*, Mohamed H. Mustafa, Shaymaa A. El Shishiny

Department of Pedodontics and Oral Health, Faculty of Dental Medicine for Girls, Al-Azhar University, Cairo, Egypt

Abstract

Objectives: This study was done to compare the efficacy of diode laser versus chlorhexidine (CHX) as root canal disinfectant in primary teeth. **Patients and methods:** A total of 30 extracted single rooted freshly extracted teeth divided into three groups as the following: group I: contain 10 teeth in which they were irrigated with saline solution. Group II: contain 10 teeth in which they were irrigated with CHX 2%. Group III: contain 10 teeth in which they were irradiated with diode laser (810 nm). The colony forming unit (CFU) was counted by multiplication of amount of colonies/plate. **Results:** After irrigation: there was a statistically a significant difference in mean % of reduction of CFU of *E. faecalis* in the three groups ($P < 0.001$*). CHX and Laser group showed higher % of reduction of CFU of *E. faecalis* than Saline group. **Conclusion:** Bacterial count decrease by both techniques showing no statistical differences against *Enterococcus faecalis* between 810 diode laser and 2% CHX.

Keywords: Diode laser, *Enterococcus faecalis*, Primary teeth

1. Introduction

Complete removal of microorganisms and remnants of necrotic pulp tissue from the root canal system is the major objective of endodontic therapy [1]. The most typical bacteria cultivated from original endodontically infected patients as well as necrotic root canals that receive retreatment is *Enterococcus faecalis*. It is a gram-positive, facultative anaerobic bacterium that develops through the creation of a biofilm, can withstand chemomechanical cleaning and root canal medicine, and can drive to the failure of root canal therapy following obturation [2].

Most bacterial species are removed after mechanical instrumentation, however specific bacteria may still be present and cause a root canal treatment to fail [3]. Additionally, bacteria in the root canal system cannot be entirely removed by biomechanical preparation; each method has certain limits. To ensure the effectiveness of endodontic therapy, root canal system cleaning is crucial [4].

To reach the effectiveness of endodontic therapy it's important to disinfect the root canal system. Sodium hypochlorite (NaOCl), chlorhexidine (CHX), hydrogen peroxide (H_2O_2), and normal saline solution (NSS or NaCl) are the most frequently utilized intracanal irrigation solutions [5]. CHX is available in concentration of 2% and 0.12% and is effective in reducing microbial flora [6]. Intense irrigation considerably reduces bacterial colonies in the root canal [7].

On the other hand, diode lasers provide a paradigm change in root canal disinfection. A diode laser (810 nm) has a bactericidal effect that bacteria cannot become resistant to laser irradiation based on its thermal characteristics. Bacteria in the intracanal space were decreased using a diode laser with a Picasso 810 nm wavelength (2 W power, every time for 5 s) [5]. To get rid of *E. faecalis*, a diode laser operating in continuous mode (CW) at 3 W with an 810 nm wavelength was utilized [5]. The purpose of the current study is to contrast the effectiveness of diode laser and CHX as root canal

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* Corresponding author at: Al-Azhar University, Faculty of Dental Medicine for Girls, Nasr city, Cairo, 11754, Egypt.
E-mail address: Zienab.ghonem@hotmail.com (Z.A. Ghonem).

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disinfectants in primary teeth against *E. faecalis* (*in vitro* study).

2. Patients and methods

The teeth utilized in this investigation were collected from pediatric patients (aged 2 to 6), totaling thirty freshly removed single-rooted teeth due to Trauma which results in avulsion, patients with systemic disease such as diabetes and papillon Lefever syndrome.

These teeth were gathered over a 6-month period from hospitals, and Faculty of dentistry University. In a previous study by Dai *et al.* in 2018 the response within the negative control was normally distributed with standard deviation 4.74. If the true difference in the experimental and control means is 6, we will need to study 10 specimens per group to be able to reject the null hypothesis that the population means of the experimental and control groups are equal with probability (power) 0.8. The Type I error probability associated with this test of this null hypothesis is 0.05. Sample size was calculated using PS (Power and sample, version 3.1.6 for windows) using independent *t*-test.

This study has been approved by the Research Ethics Committee (REC-PE-23-05).

All teeth samples were selected according to the following criteria:

2.1. Inclusion criteria

Deciduous anterior teeth, single-rooted, free of caries, complete root formation.

2.2. Exclusion criteria

Permanent, posterior, multirooted (mostly resorbed at the time of extraction), carious teeth. Physiologic or pathologic root resorption.

2.3. Bacterial reference strain

The regional center of mycology and biotechnology at Azhar University in Egypt generously provided the *E. faecalis* reference strain ATCC 19433 for use in this work. Its name is *E. faecalis*, and Streptococcus faecalis is a synonym for it. It was provided as an active culture on slope agar.

Bacterial suspension (1 ml) was injected inside each root canal by sterile plastic syringe under pressure to make sure that it reached to the full working length, and then these samples were placed individually inside Eppendorf tubes and submerged with 2 ml of brain heart infusion broth, closed, and inserted inside a rack, then incubated at 37°c for 24 h for allowing bacteria to multiply and proliferate.

2.4. Teeth preparation

Teeth surface preparation: The teeth were scaled from the outside to remove any debris from the soft or hard tissues, washed with water, disinfected with 1% NaOCl, and then kept in 0.1% thymol solution until usage.

2.5. Mechanical preparation of teeth

Gaining access in all teeth was provided by using round bur and tapered diamond bur till reaching the orifices of canals, all pulp tissue debris was removed with H-file, and the root canals of all teeth were prepared with Kidzo rotary files (manufactured in, Hong Kong; China) using crown down technique. To allow the bacteria to penetrate the dentinal tubules, 1 ml of 1% NaOCl and 17% EDTA were employed as irrigants to remove the organic debris of the pulp tissue and the inorganic smear layer of the root dentin, respectively. To eliminate any remaining irrigants, the canals were then cleansed with 0.9% saline.

2.6. Teeth decoronation

All teeth decoronated below the level of cementoenamel junction until having a standardized root length of 8 mm [8] of all teeth.

2.7. Teeth sterilization

After preparation of all teeth, they were packed in sterilization bags and sterilized in an autoclave (at 121 °C, for 30 min).

2.8. Grouping of the teeth samples

The samples were divided into three groups as the following: group I: include 10 teeth that were irrigated with saline, as a negative control [9]. Group II: include 10 teeth in which they were irrigated with the CHX 2%. Group III: includes 10 teeth that were irradiated with a diode laser (810 mm).

2.9. Estimation of bacterial counting

Three sterile absorbent paper points of sizes #30, #35, and #40 were inserted into each root canal to acquire the initial microbial sample (S1), and each paper point was allowed to get saturated with the bacterial solution for 1 min [9].

Using sterile tweezers, the paper point samples were taken out of the canal and put in a sterile falcon tube with 1 ml of saline. Prepare successive 10-fold dilutions of the bacterial suspension in sterile saline using a micropipette (1/10, 1/100, 1/1000, 1/10000, and 1/100000). A bacteriologic loop was used to pipette 0.1 ml of each dilution onto bile auscline agar, where the combination was then incubated for 24 h in an aerobic environment at 37 °C.

The colony forming unit (CFU) was calculated by dividing the number of colonies on a plate by the dilution and volume factor $(10 \times 10^4 \times 2) = 200{,}000$/organisms/ml.

2.10. Application of irrigant solution

2.10.1. Group I

After the incubation of 10 root canals with the bacterial suspension, they were irrigated with 1 ml of 0.9% sterile saline solution (the negative control) and left in the root canals for 5 min then insert three sterile paper points inside the root canals to take the second sample [9].

2.10.2. Group II

After the incubation of 10 root canals with the bacterial suspension, they were irrigated with 5 ml/2% CHX solution (the test material) and irrigated for 1 min then three sterile paper points were inserted by sterile tweezer inside the root canals to take the second sample.

2.10.3. Group III

After the incubation of 10 root canals with bacterial suspension, they were treated by diode laser where teeth were dried and irradiated by diode laser and output power of 2w. For 5 s and a wavelength of 810 nm in continuous mode. An optic fiber apparatus 200 μm in diameter was inserted into the canal remain at 1 ml short of working length irradiation repeated four times at 10 s intervals. Then the bacterial counting was performed as mentioned before (Estimation of bacterial counting).

3. Results

3.1. Statistical analysis

Intergroup comparison between continuous data was performed using one-way analysis of variance followed by tukey *post-hoc* test, while intragroup comparison was performed using paired *t*-test. A P value less than or equal to 0.05 will be considered statistically significant and all tests will be two-tailed.

3.2. Intergroup comparison regarding bacteria colony forming units (CFU/ml) before and after treatment

Intergroup comparison between CFU of bacterial counts of treatments has shown no statistically significant difference before treatment ($P = 0.156$). Intergroup comparisons between treatments have shown statistically significant differences after treatment ($P < 0.001$). Diode laser showed the least bacterial count followed by CHX, while saline had the highest bacterial count after treatment as presented in Table 1.

Intergroup comparison between treatments have shown no statistically significant difference before treatment ($P = 0.094$). Intergroup comparison between treatments have shown statistically significant differences after treatment ($P < 0.001$). Diode laser showed the least bacterial count followed by CHX, while saline had the highest bacterial count after treatment, as presented in Table 2.

3.3. Intragroup comparison within each group regarding bacteria colony forming units (CFU/ml) before and after treatment

Intragroup comparison within CHX and diode laser have shown a statistically significant reduction of bacterial count ($P < 0.0001$), while saline have shown no statistically significant reduction of bacterial count ($P = 0.1835$). CHX decreased bacterial count after treatment ($P < 0.0001$) with a mean difference of $Log_{(10)}$ of CFU (-0.96 ± 0.11). Diode laser decreased bacterial count after treatment

Table 1. Colony forming unit of bacterial count (Mean ± SDs) between all groups before and after treatment.

Treatment Time	Saline		Chlorohexidine		Diode laser		P value
	Mean	SD	Mean	SD	Mean	SD	
Before	61.3×10^6	2.08×10^6	66.6×10^6	12.9×10^6	54.93×10^6	19.8×10^6	$P = 0.156$
After	$60 \times 10^{6\ a}$	1.73×10^6	$7.27 \times 10^{6\ b}$	0.72×10^6	$5.96 \times 10^{6\ c}$	2.57×10^6	$P < 0.001$*

Means that do not share a letter are significantly different.
*Corresponds to a statistically significant difference.

Table 2. $Log_{(10)}$ of colony forming unit of bacterial count (Mean ± SDs) between all groups before and after treatment.

Treatment Time	Saline		Chlorohexidine		Diode laser		P value
	Mean	SD	Mean	SD	Mean	SD	
Before	7.78	0.015	7.82	0.082	7.71	0.16	$P = 0.094$
After	7.78^{a}	0.017	6.86^{b}	0.040	6.71^{c}	0.26	$P < 0.001$*

Means that do not share a letter are significantly different.
*Corresponds to a statistically significant difference.

($P < 0.0001$) with a mean difference of $Log_{(10)}$ of CFU (−0.99 ± 0.23). Saline did not decrease bacterial count after treatment ($P = 0.1835$) with mean difference of $Log_{(10)}$ of CFU (−0.006 ± 0.005), as presented in Table 3.

3.4. Comparison between groups regarding percentage of reduction of bacteria colony forming units after treatment

Intergroup comparison between treatments have shown statistically significant differences ($P < 0.001$). Pairwise comparisons between CHX and saline, and diode laser and saline have shown statistically significant differences regarding the percentage of reduction of bacteria colony forming units after treatment, while comparison between CHX and diode laser have shown no statistically significant difference. Diode laser showed the highest reduction in bacterial count (−88.76%) treatment as presented in Fig. 1a and b, followed by CHX (−88.36%) as presented in Fig. 2a and b, while saline showed the least reduction in bacterial count (−2.15%) treatment as presented in Fig. 3a and b and as presented in Table 4.

4. Discussion

Bacteria and bacterial byproducts are the major reason for initiation and propagation of dental Caries, therefore endodontic treatment is needed to eliminate all bacteria. Root canal success depends on mechanical preparation, irrigation, and microbial control of the root canal system. *E. faecalis* can survive as a single organism in a root canal without support of other bacteria [10], therefore *E. faecalis* was used in the current study as it is the most associated species in failed endodontic treatment [11].

A variety of irrigation solutions have been used in attempt to eliminate or reduce bacteria such as sodium hypochlorite, CHX, saline, and natural

Table 3. $Log_{(10)}$ of colony forming unit of bacterial count (Mean ± SDs) within each group before and after treatment.

Treatment Time	Saline		Chlorohexidine		Diode laser	
	Mean	SD	Mean	SD	Mean	SD
Before	7.78	0.015	7.82	0.082	7.71	0.16
After	7.78	0.017	6.86	0.040	6.71	0.26
Log reduction	−0.006	0.005	−0.96	0.11	−0.99	0.23
P value	$P = 0.1835$		$P < 0.0001$*		$P < 0.0001$*	

*Corresponds to a statistically significant difference.

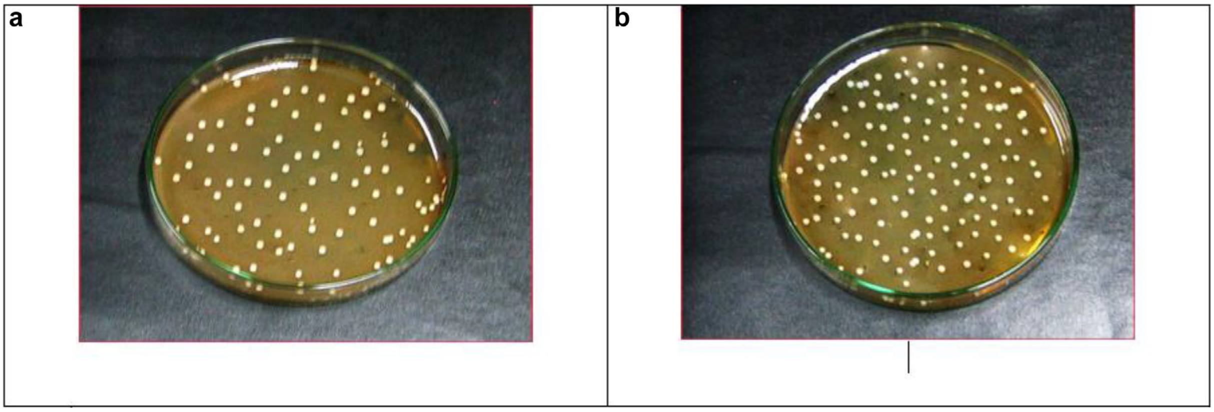

Fig. 1. (a): Bacterial colonies on brain heart infusion ager before irrigation. (b): bacterial reduction after saline irrigation (−2.15%).

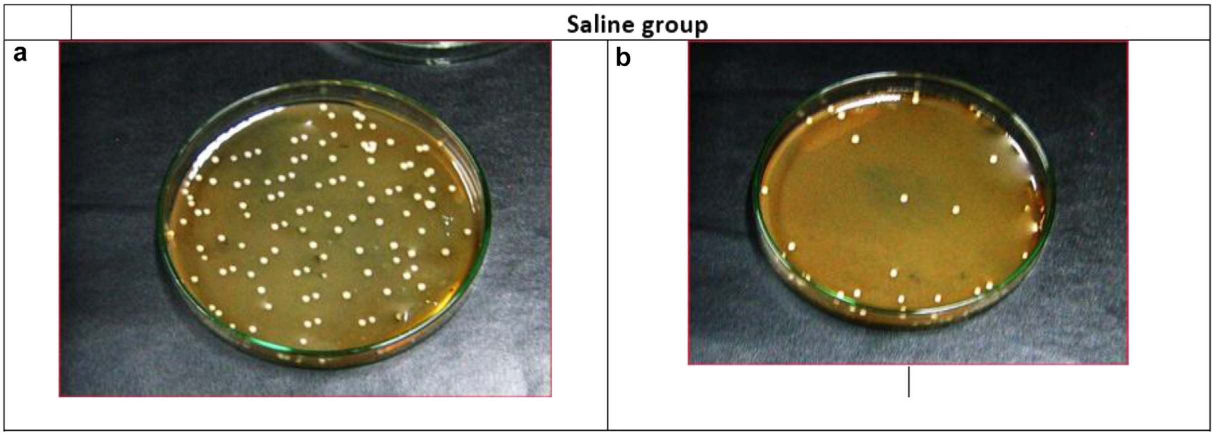

Fig. 2. (a): bacterial colonies on brain heart infusion ager before irrigation. (b): bacterial reduction after chlorhexidine irrigation (−88.36[b]).

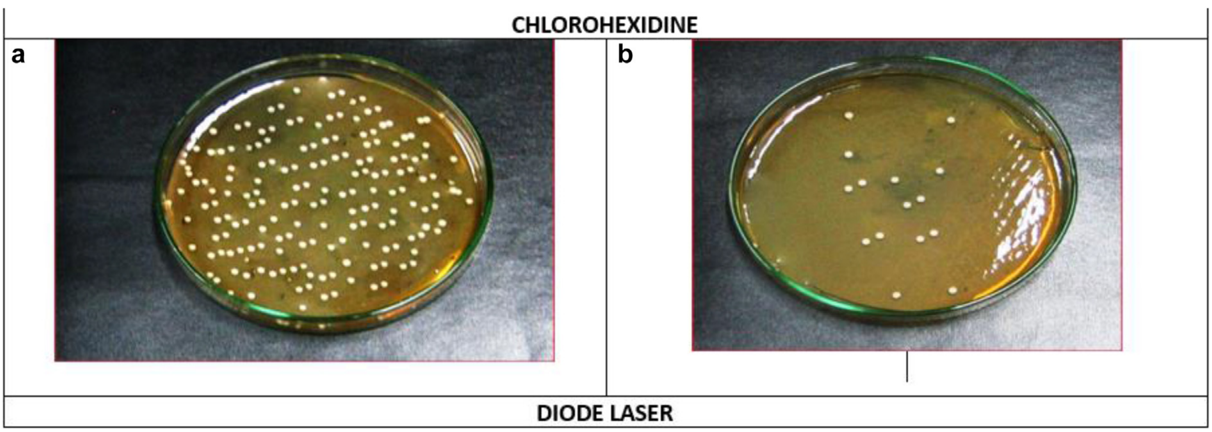

Fig. 3. (a): bacterial colonies on brain heart infusion ager before treatment. (b): bacterial reduction after diode laser treatment (−88.76%).

Table 4. Percentage of reduction of bacterial count (Mean±SDs) for all groups after treatment.

Treatment	Saline		Chlorohexidine		Diode laser		*P* value
	Mean	SD	Mean	SD	Mean	SD	
% of reduction	−2.15[a]	0.87	−88.36[b]	6.39	−88.76[b]	2.90	$P < 0.001$*

Means that do not share a letter are significantly different.
*Corresponds to a statistically significant difference.

irregants as chitosan, tea tree oil and miswak. The choice of irrigant in deciduous root canal treatment should be nonirritating to the periapical tissues [12,13].

In the present study we used CHX, it is used in endodontic procedures and is becoming more popular because of its characteristics, including broad-range antibacterial activity, substantivity, low toxicity, and water solubility [14].

As a result of the study's findings, CHX is used as an alternative irrigation agent in place of NaOCI as it has a unique property of substantivity; as the positive charges of the CHX molecule bind to the negative charges on dental surfaces resulting in prolonged adherence, which leads to long-lasting antimicrobial activity. The tested solutions demonstrated antibacterial activity. Due to its benign impact on the roughness and microhardness of root canal dentin [7], 2% CHX gluconate is an acceptable endodontic irrigating solution. Due to CHX's safe function as a root canal irrigant in primary teeth, it was chosen for the current investigation. The significant advantage of using contemporary laser technology in endodontic therapy is that it can access regions that traditional rinse solutions cannot. A new development is using a laser to disinfect the root canals. The increase of bactericidal impact of laser irradiation inside the dentinal tubules is caused by its deep light penetration. Deeper light penetration into the dentinal tubules is made possible by the inherent characteristics of laser irradiation, such as local intensity amplification, light scattering, and attenuation. In this work, the antibacterial effectiveness of 2% CHX and an 810 nm diode laser against *E. faecalis* attenuation is being assessed.

In this study, the effect of 2% CHX was compared with 810 nm diode laser on disinfection of the root canal and their effect on bacterial *E. faecalis*. Results of the present study showed that before irrigation: there was a statistically non-significant difference in mean CFU of *E. faecalis* in the three groups. After irrigation: there was a statistically significant difference in the mean CFU of *E. faecalis* in the three groups ($P < 0.001$*). CHX and Laser group showed higher % of reduction of CFU of *E. faecalis* than the Saline group. While comparison between CHX and diode laser has shown no statistically significant difference. Diode laser showed the highest reduction in bacterial count (−88.76%) followed by CHX (−88.36%), while saline showed the least reduction in bacterial count (−2.15%). These findings supported those made by other studies [15–17] who came to the same conclusion.

The results of the present study have shown that although a significant decrease in bacterial count was obtained with two groups, the laser has the highest bacterial reduction when compared with 2% CHX with no significant statistical difference between groups. The bactericidal effect of diode laser could be attributed to its greater depth of penetration (up to 1000 μm into dentinal tubules) when compared with the penetration power of chemical disinfectants, which is limited to 100 μm. Other study evaluated the horizontal depth of penetration of various irrigants into dentinal tubules using sodium hypochlorite, CHX and diode laser when used alone and in combination concluded that the horizontal depth of penetration was more when 2.5% NaOCl, 0.2% CHX gluconate in combination with 810 nm diode laser was used [18].

Additionally, there was an investigation on the antimicrobial effects of 2% cetrimide and 0.2% and 2% CHX in 2020 [16]. All have residual antimicrobial activity in root canals with *E. faecalis* infection, but final irrigation with 2% CHX showed more residual activity than 0.2% CHX and 2% cetrimide. Also supported the findings [19–23] of the existing study, which demonstrated that the diode laser had the capacity to eradicate *E. faecalis*. The increased bactericidal impact of laser irradiation inside the dentinal tubules is caused by this deeper light penetration.

The results of the present study showed that there is a significant decrease in bacterial count obtained by two groups, the laser has a comparable effect when compared with 2% CHX. In accordance with the study the results of another study showed that CHX solution (2%), CHX powder (0.2%) and diode laser has highest antibacterial effect from the control group and there is a difference between them but not significant [24] Other studies agreed with the present study in which they compared the effectiveness of laser sterilization against CHX for pulpectomy of primary teeth. The findings demonstrated that the bacterial count was reduced by both methods, but that the 980 nm diode laser performed better than the 2% CHX. However, the results of this study disagreed with other study showed that diode Laser and Brazilian Propolis are equally effective as CHX in cavity disinfection [25].

4.1. Conclusion

(a) Bacterial count decreases by both techniques showing no statistical differences against *E. faecalis* between the 810 nm diode laser and 2% CHX.
(b) The disinfection of saline solution is inferior to that of diode laser and CHX.

4.2. Recommendations

(a) Further studies are required to investigate the effectiveness of diode lasers against other micro-organisms.
(b) The biological safety of diode laser application requires more studies.

Ethics information

This study has been approved by the Research Ethics Committee the ethical code is (REC-PE-23-05).

Conflicts of interest

There are no conflicts of interest.

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Biographical information

The study was conducted in the outpatient clinics, Department of Endodontic Dentistry, Faculty of Dental Medicine for Girls Al-Azhar University, Cairo, Egypt.

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