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Clinical, Radiographic and Microbiological Evaluation of Antimicrobial Photodynamic Therapy, LASER Versus Chlorhexidine, for Root Canal Disinfection in Primary Molars

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ABSTRACT

Background: Primary teeth preserve arch length and guide permanent teeth, making pulpectomy a key treatment to maintain space and function. It also enhances esthetics, mastication, speech, and overall health while preventing psychological effects of tooth loss.

Aim: To evaluate the antimicrobial efficacy of Photodynamic Therapy (PDT) and 980 nm Diode Laser versus 2% Chlorhexidine (CHX) irrigation for root canal disinfection in infected primary molars against *Enterococcus faecalis*.

Materials and methods: The study included 30 Egyptian children (4-6 years) at the Pedodontics Department, Faculty of Dental Medicine for Girls, Al-Azhar University. Patients were divided into three equal groups (10 in each group): Group A (2% chlorhexidine), Group B (diode laser 980 nm), and Group C (photodynamic therapy with 650 nm laser and methylene blue). Parental consent was obtained before participation.

Results: Intergroup comparison showed no significant difference before treatment ($P = 0.967$) but a significant difference after treatment ($P < 0.005$), with PDT having the lowest bacterial count, followed by the diode laser, while chlorhexidine had the highest. Intragroup comparison ($n = 10$) showed a significant bacterial reduction ($P < 0.05$) in all groups: chlorhexidine (1160.0 ± 811.3), diode laser (1080.0 ± 614.2), and PDT (1100.0 ± 611.0).

Conclusion: PDT, diode laser (980 nm), and 2% CHX effectively reduce bacteria in primary tooth root canals, with PDT showing the highest efficacy. The diode laser offers a viable alternative to CHX, and combining these methods may improve treatment outcomes.

Keywords: *Chlorhexidine; Photodynamic Therapy; Antimicrobial Photodynamic Therapy.*

1. INTRODUCTION

Primary teeth maintain the arch length and form by maintaining proper guidance for permanent teeth. Pulpectomy is one of the treatment options available to preserve the tooth and thus helps in maintaining normal space maintainers. Other objectives of preserving primary teeth are to enhance esthetics and mastication, aid in speech, and prevent the psychological effects associated with tooth loss and preserve general health of the individual [1].

Despite the progress made in preventive dentistry, dental caries and its sequelae are among the foremost problems affecting the standard of life in children [2].

The American Academy of Pediatric Dentistry indicated pulpectomy in a primary tooth with irreversible pulpitis within which the radicular pulp exhibits the clinical signs of pulp necrosis or shows evidence of chronic inflammation [3].

Lasers have been regarded as a novel technique for the purposes of clinical use in the dental field. Recently, numerous studies have been conducted on the potential applications of laser therapy in endodontics. Moreover, due to their ablation, penetrability, and disinfection capabilities, lasers have performed well with respect to endodontic treatments, including root canal treatment, vital pulp therapy (pulp capping and pulpotomy), dentinal hypersensitivity treatment, and management of dental pain related to pulp and periradicular disease [4].

Chemo-mechanical instrumentation is the basic technique in eradication of bacteria biofilm, necrotic, and vital tissue. But it is not sufficient for complete removal of microorganisms in the infected root canals, especially at apical third of the root canal [5].

The endodontic disinfection solutions cannot reach and effectively eliminate the pathogenic microorganisms that have penetrated the root dentine [6].

During chemo-mechanical preparation, irrigation solutions act as disinfectant, lubricant and cleaning agents that assist in neutralizing microorganisms, their byproducts tissue remnant and elimination which have been produced by dentin cutting instruments [7].

Irrigation with conventional syringe has been reported to be inefficient in cleaning the more apical portions of the root canal system [8].

The aim of the study was to evaluate the antimicrobial efficacy of Photodynamic Therapy (PDT) and 980 nm Diode Laser versus 2% Chlorhexidine (CHX) irrigation for root canal disinfection in infected primary molars against *Enterococcus faecalis*.

2. MATERIAL AND METHODS

The study was conducted at the outpatient clinic of the Pedodontics and Oral Dental Health Department, Faculty of Dental Medicine for Girls, Al-Azhar University. A total of 30 Egyptian children (both sexes) aged 4-6 years participated in the study. Verbal and written consent was obtained from the parents before the study commenced.

The 30 patients were equally divided into three groups based on the disinfectant system used: Group A (control) received 2% chlorhexidine irrigation, Group B was treated with a diode laser at a 980 nm wavelength, and Group C underwent photodynamic therapy using a 650 nm wavelength laser with methylene blue as a photosensitizing agent.

Randomization: Participants were randomly assigned to one of three groups using a computer-generated system (Block Stratified Randomization System Version 6).

Sample size estimation and statistical power [9]: The sample size was determined based on a statistical formula, considering the standard deviation estimated from a previous study, the level of significance, and the effect size between the samples. Accordingly, 10 patients were selected per group, with a total sample of 30 patients.

Inclusion Criteria [10]: Children aged 4-6 years with no systemic diseases were included in the study. Eligibility criteria required no recent antibiotic use (last 2 weeks), antimicrobial mouth rinse (last 12 hours), or topical fluoride treatment (last 4 weeks). Primary molars had to be indicated for pulpectomy with sufficient tooth structure for stainless steel crown restoration. Radiographic criteria included the absence of root or bone resorption, a healthy periodontium, and no periapical infection.

Exclusion Criteria [10]: Medically compromised patients were excluded from the study. Additional exclusion criteria included evidence of root resorption, interradicular bone loss, radiographic signs of calcific globules in the pulp chamber, and caries penetrating the floor of the pulp chamber.

Materials: Various materials were used in the study, including irrigants like 2% Chlorhexidine (Egypt) and cavity preparation instruments such as sterile absorbent paper points (Korea), burs, and files (China, Japan, USA). Restorative materials included Metapex (Korea), glass ionomer (Australia), and stainless-steel crowns (3M, USA). Media included Selective Enterococcus agar (USA), while devices used were the TriAuto Mini Morita Endomotor (Japan), diode laser (China), and an incubator (Germany). Other products included methylene blue, Petri dishes, and Bio 5 ml tubes (USA).

Pulpectomy Procedure and Disinfection Protocol

A preoperative periapical radiograph confirmed the diagnosis before pulpectomy. Local anesthesia was administered, and isolation was done using a rubber dam. Caries was removed, and access cavity preparation was performed with a sterile round bur, followed by coronal pulp removal [11]. The working length was determined 1 mm short of the anatomic apex using

a digital radiograph, and rotary NiTi files (size 30) were used with an endomotor at 300 RPM [12,13]. Root canal disinfection followed the assigned protocol [14]. Group A (Control Group) used 20 mL of 2% chlorhexidine irrigant [15]. Group B (Laser Group) used a 980 nm diode laser (1.5W). Group C (Photodynamic Therapy Group) received 0.5 mL methylene blue followed by a 650 nm diode laser (1.5W) applied in a circular motion for two cycles of 15 seconds each. Microbiological samples (S1) were collected before and (S2) after disinfection using sterile paper points [16]. For patient protection [1112], all involved wore protective eyeglasses, and laser equipment was covered with disposable plastic sleeves and disinfected between sessions using UNISEPTA PLUS®. Samples were transported aseptically to the microbiology lab, cultured on Selective Enterococcus Agar, incubated at 37°C for 24 hours, and analyzed for colony-forming units per mL (CFU/mL).

Restoration Procedures and Clinical Evaluation: Following disinfection and microbiological sampling, a thin mix of Metapex was inserted into each root canal [15]**.** The pulp chamber was sealed with a layer of glass ionomer cement, followed by resin-modified glass ionomer as the final restoration. Each tooth was then restored with a stainless-steel crown [12]**.** The treatment prognosis was evaluated clinically and radiographically after three months [17]**.** Clinical success was defined by the absence of persistent pain, fistula, intraoral swelling, or mobility, while radiographic success was determined by the absence of internal resorption, furcal, or periapical radiolucencies. Data were recorded as good prognosis = 0 and Poor prognosis = 1 for both clinical and radiographic assessments.

Ethical consideration

Ethical approval was obtained from the Research Ethics Committee of the Faculty of Dental Medicine for Girls, Al-Azhar University (P-PE-22-01), ensuring compliance with ethical standards. Participants were recruited through medical record screening, and eligible candidates were informed about the study. A detailed written informed consent, approved by the ethics committee, was obtained from parents or legal guardians, outlining the study's objectives, procedures, potential risks, benefits, and the voluntary nature of participation.

Statistical Analysis

Collected data was reviewed, coded, and analyzed using SPSS software (version 16, SPSS Inc., Chicago, IL, USA) for Microsoft Windows (142). Descriptive statistics included mean and median for central tendency and standard deviation for data dispersion. Analytic statistics involved different tests for comparing groups: the paired t-test for differences between two dependent parametric means, the Wilcoxon test for two dependent non-parametric means, the ANOVA test for more than two independent parametric means, and the Kruskal-Wallis test for more than two independent non-parametric means. Statistical significance was set at $p < 0.05$, with results presented in tables and figures.

3. RESULTS

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

Intergroup comparison between treatments have shown no statistically significant difference before treatment ($P = 0.967$). Intergroup comparison between treatments has shown statistically significant difference after treatment ($P < 0.005$). Photodynamic therapy showed the least bacterial count followed by diode laser while chlorhexidine, had the highest bacterial count after treatment (Table 1).

Table (1): Log [(10)] of CFU of bacterial count (Mean ± SDs) between all groups before and after treatment

	Chx Group	Laser Group	PDT Group	Significance test
Before irrigation				
Mean± SD	1160.0± 811.3	1080.0± 614.2	1100.0± 611.0	P value= 0.967
Median	900.00	950.0	775.00	
After irrigation				
Mean± SD	135.0± 70.9	80.0± 42.1	30.0± 34.9	P value= 0.002*
Median	150.0	100.0	25.0	
Percent reduction				

Mean± SD	80.8± 16.59	91.1±6.1	95.9±5.4	P value= 0.010*
Median	89.68	92.2	97.5	

*significant difference (p value<0.05).

Intragroup comparison within each group regarding bacteria colony forming units (CFU/ml) before and after irrigation:

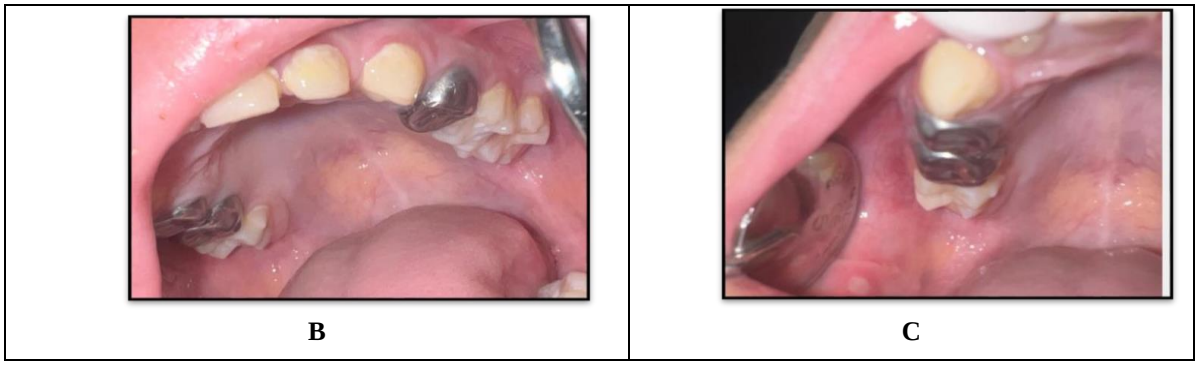
Intragroup comparison within chlorhexidine, diode laser and PDT with equally distributed sample size, that is, (n = 10) have shown statistically significant reduction of bacterial count (P <0.05). Chlorhexidine decreased bacterial count with (P <0.05) with mean difference of Log $^{(10)}$ of CFU (1160.0± 811.3). Diode laser decreased bacterial count with (P <0.05) with mean difference of Log $^{(10)}$ of CFU (1080.0± 614.2). PDT decreased bacterial count (P <0.05) with mean difference of Log $^{(10)}$ of CFU (1100.0± 611.0) (Table 2).

Table (2): Log $^{(10)}$ of CFU of bacterial count (Mean ± SDs) within each group before and after irrigation:

	Before Irrigation	After Irrigation	Significance Test
Chx Group:			
Mean± SD	1160.0± 811.3	135.0± 70.9	P value= 0.002*
Median	900.00	150.0	
Laser Group:			
Mean± SD	1080.0± 614.2	80.0± 42.1	P value= 0.001*
Median	950.0	100.0	
PDT Group:			
Mean± SD	1100.0± 611.0	30.0± 34.9	P value= 0.005*
Median	775.00	25.0	

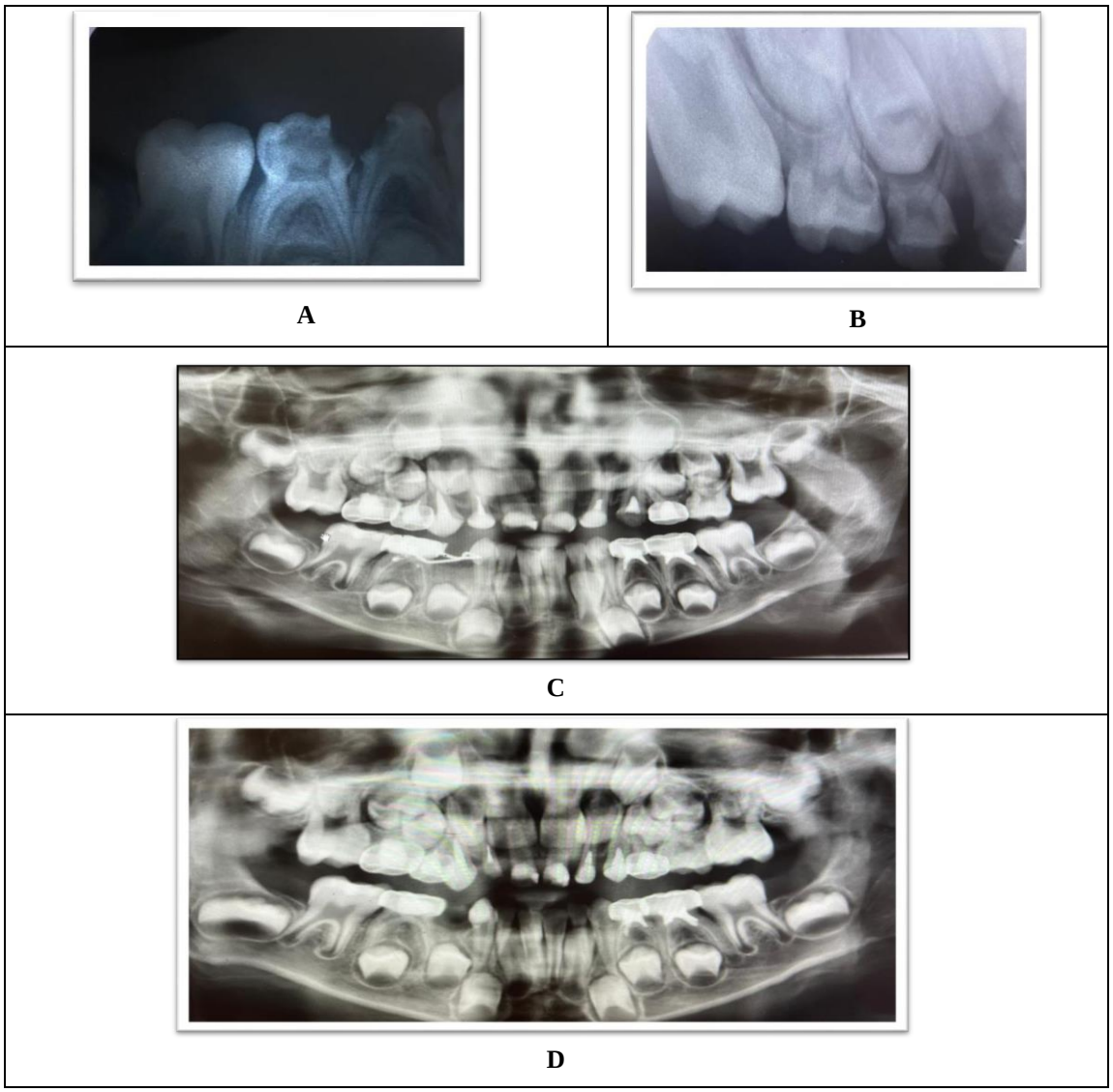
*significant difference (p value<0.05).

A



B C

Figure (1): (A): Decayed teeth in the upper arch before treatment (B, C): Primary molars after pulpectomy treatment using diode laser.



A B C D

Figure (2): (A, B): X-ray before pulpectomy treatment (C): Panoramic view after full mouth treatment using diode laser (D): Panoramic view after 3 months follows up using diode laser.

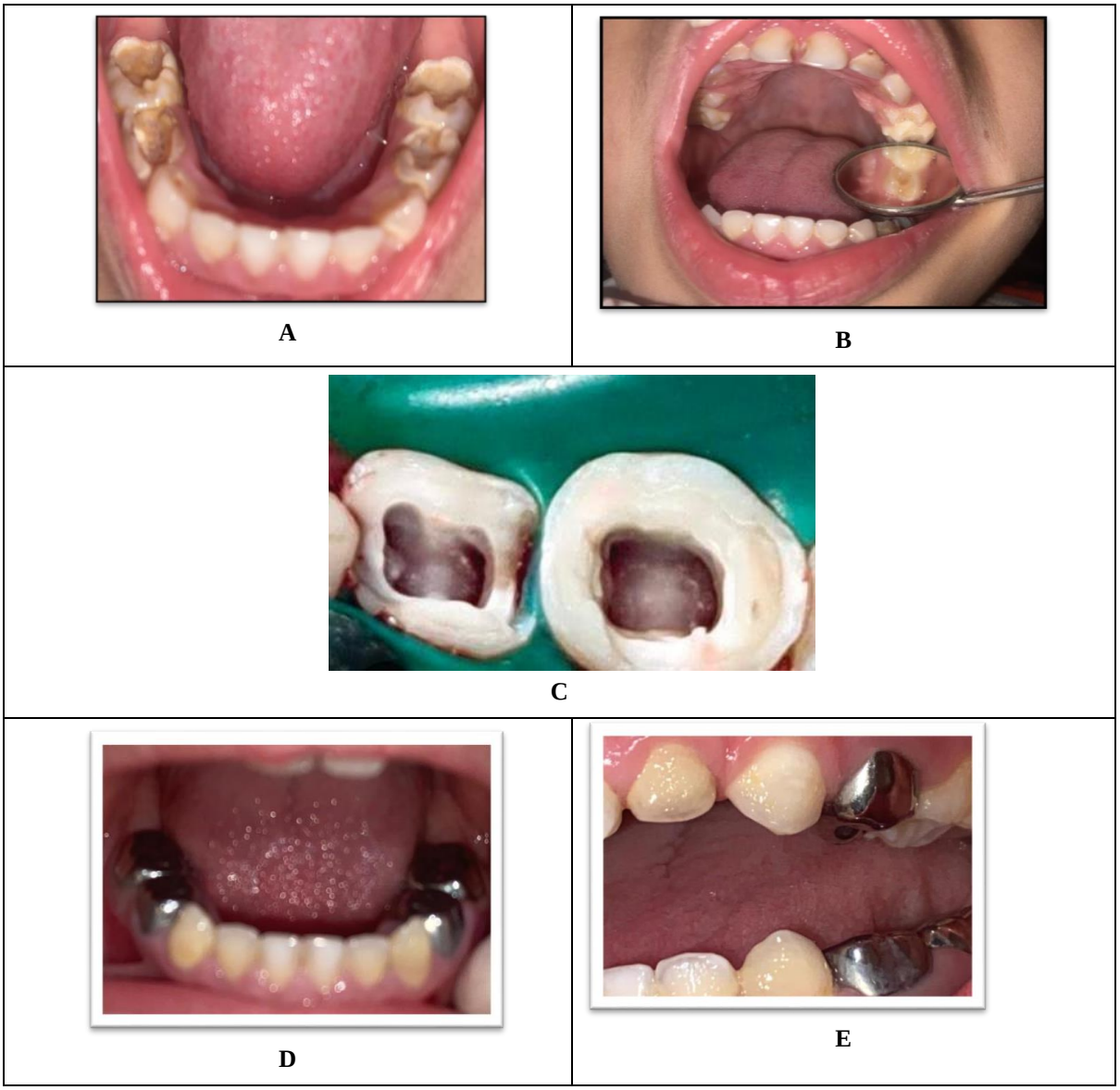
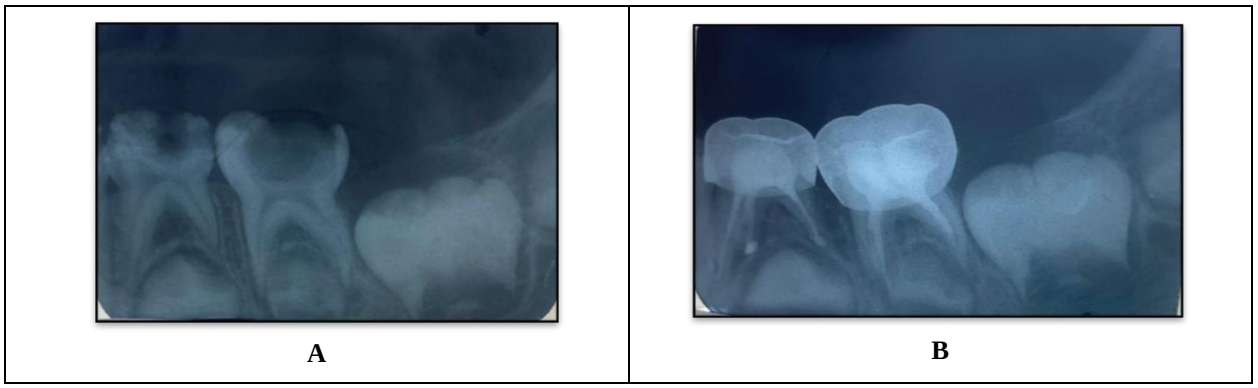


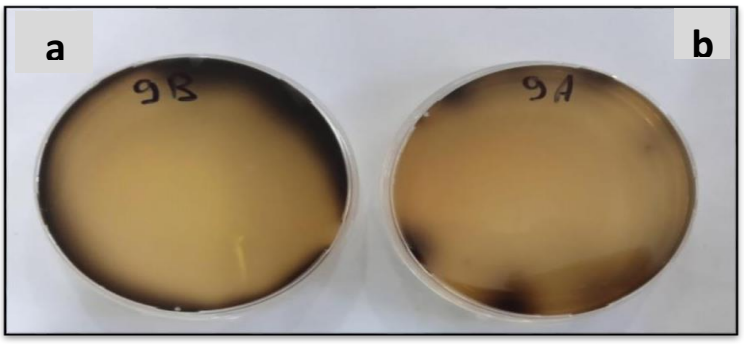
Figure (3): (A, B): Showing decayed teeth in both arches (C): Access cavity in primary molars (D, E): A photograph after treatment using Photodynamic therapy.



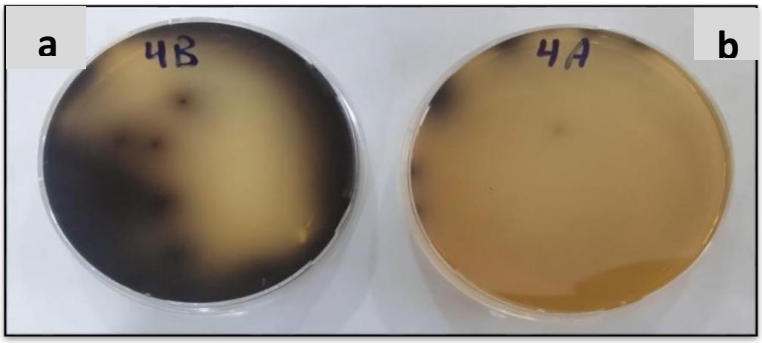
C

D

Figure (4): (A, B): Showing radiographic x-ray before and after treatment using PDT. (C): Panoramic view after full mouth treatment using PDT. (D): Panoramic view after 3 months follows up using PDT.



A



B

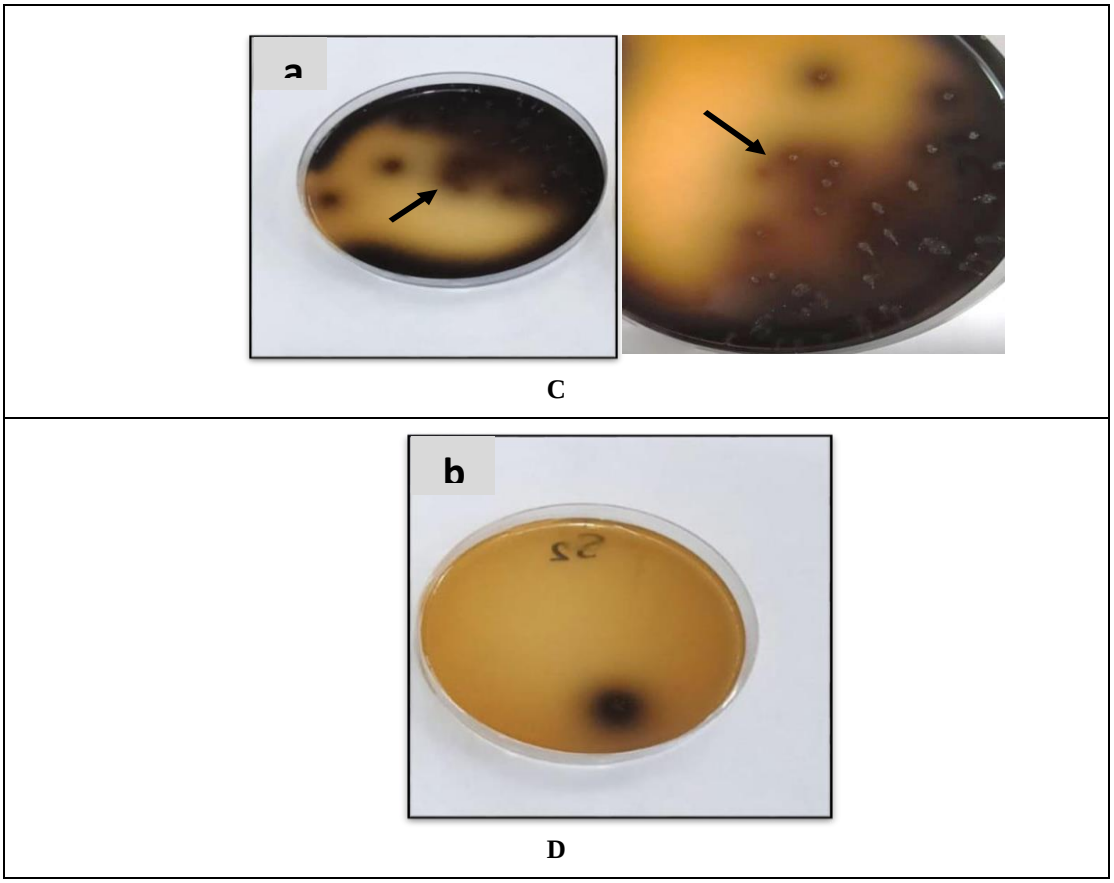


Figure (5): (A): Bacterial colonies of *Enterococous Feacalis*. (a): Before chlorohxidine irrigation (b): After chlorohxidine irrigation. (B): Bacterial colonies of *Enterococcus Faecalis*. (a)Before diode laser. (b): After diode laser. (C): Bacterial colonies of *Enterococcus faecalis* on plates. (a): Before photodynamic therapy. (b): After photodynamic therapy.

4. DISSCSSION

In recent years, there has been a notable shift in the approach to root canal treatment for primary teeth. This change is largely due to the understanding that mechanical instrumentation alone may not effectively eliminate bacteria from the root canal system. Traditional mechanical methods, such as hand instrumentation, have been shown to leave residual bacteria in the complex root canal systems of primary teeth. The intricate anatomy of these teeth, including multiple canals and variations in curvature, makes it challenging to achieve complete disinfection through mechanical means alone [18].

Regarding subject selection, patients were selected with an age range of 4 to 6 irrespective of their sex because they are at a high risk for dental caries, which can lead to pulpitis or necrosis. This age group often experiences early childhood caries, making pulpectomy a necessary intervention to treat severely decayed teeth and preserve them until they naturally exfoliate [19].

The first approach used in the current study was chlorhexidine as the control group because it is the gold standard irrigant solution and has a long- standing reputation as a reliable disinfectant due to its higher antibacterial capacity study as it has broad spectrum antimicrobial activity, substantively, low toxicity and water solubility have increased the interest in its use in endodontic and safe for the successor in primary teeth in accordance with **Alvarado Rodríguez PY et al.,2022** [20] and **Mohammadi Z et al**..2021[21]

In the present study 2% concentration of CHX was used in accordance to Briseño- Marroquín B et al., 2022(162) study that found that the inhibition zone of *E. faecalis* diameter increased with increasing concentration to 2%.

The second approach used in the current study was diode laser 980 nm wavelengths as a modern laser technology provides paradigm shift in endodontic therapy as it has great benefit of reaching areas that are not accessible to conventional rinsing solutions, **Saydjari Y. et al.,2017**[22] investigated the antibacterial effect of a diode laser in deep root canal dentin. The study showed a mean bacterial reduction of 74% was achieved even with a 500-µm thick slice. This study in accordance with the current study indicated that the diode laser radiation reduced the number of bacteria in deep layers of infected root canal wall

dentin.

In this study diode laser irradiation was performed with an output of 1.5 Watt for 15 s followed a 10 s interval as the thermal effect is the most important point to be considered in laser applications. A temperature rise to a critical level could have deleterious effects on the tissues surrounding the tooth. The temperature increases by approximately 10 °C and treatment duration of 1 min can cause irreversible injury to periodontal tissues. **Shehab NF et al., 2020** [16] demonstrated that diode laser irradiation for 5 s, with 10 s of resting time, should be considered to avoid a temperature rise to an undesired level.

The third approach was photodynamic therapy as a disinfectant procedure as it has emerged a promising alternative to traditional disinfectants like chlorhexidine and laser treatments in the context of pulpectomy in pediatric dentistry. PDT utilizes a photosensitizing agent that, when activated by a specific wavelength of light (low level laser therapy 650nm), produces reactive oxygen species (ROS) that effectively kill bacteria. This mechanism not only targets the bacteria directly but also minimizes the risk of developing antibiotic resistance, a significant concern with conventional antimicrobial agents like chlorhexidine [23].

In the current study methylene blue was chosen in PDT as a disinfectant during pulpectomy procedures due to its effective properties as a photosensitizer that, when exposed to light, generates reactive oxygen species, particularly singlet oxygen. This process is crucial for targeting and eliminating bacteria within the root canal system during pulpectomy, enhancing the disinfection process [24].

The results in this study showed that the intergroup comparison of bacterial colony-forming units (CFU/ml) before and after treatment provide valuable insights into the effectiveness of various therapeutic modalities in reducing bacterial load. Prior to treatment, the absence of statistically significant differences ($P = 0.967$) indicates that the initial bacterial counts were comparable across all treatment groups. This similarity in baseline levels is crucial for validating the results post-treatment, suggesting that any observed differences can be attributed directly to the efficacy of the treatments administered.

After treatment, the analysis revealed a statistically significant difference ($P < 0.005$) among the groups, highlighting the effectiveness of the different therapeutic approaches. Notably, photodynamic therapy (PDT) demonstrated the most significant reduction in bacterial counts, consistent with recent literature that underscores its potential as a powerful antimicrobial treatment. PDT utilizes light- activated photosensitizers to generate reactive oxygen species, which can effectively destroy bacterial cells while minimizing damage to surrounding tissues **Matsumoto et al., 2024** [25] This mechanism not only facilitates the targeted elimination of pathogens but also reduces the likelihood of resistance development, a critical concern in antimicrobial therapy.

5. CONCLUSION

The findings of this study suggest that photodynamic therapy (PDT) using methylene blue, diode laser (980 nm), and 2% chlorhexidine (CHX) are all effective strategies for bacterial reduction in root canal disinfection of primary teeth. Notably, PDT demonstrated superior efficacy compared to the other methods. Additionally, the application of a diode laser presents a viable alternative to CHX irrigation. Integrating or combining these disinfection techniques may further enhance the overall effectiveness of root canal treatments in clinical settings, allowing for tailored approaches based on specific patient needs.

6. RECOMMENDATIONS

Photodynamic therapy and diode laser can be recommended as effective methods for root canal disinfection in primary teeth. However, further studies are needed to evaluate their effectiveness against other microorganisms and to confirm their biological safety in pediatric applications. Additionally, future research should explore the potential benefits of integrating Low-Level Laser Therapy (LLLT) with other supportive or preventive interventions to enhance overall treatment outcomes.

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