



**UNIVERSIDADE
FERNANDO
PESSOA**

COMPARATIVE EFFICACY OF DIODE LASERS AND SODIUM HYPOCHLORITE AGAINST ENTEROCOCCUS FAECALIS DISINFECTION: INTEGRATIVE REVIEW

[Eficácia comparativa de lasers de díodo e hipocloreto de sódio contra a desinfeção por enterococcus faecalis: revisão integrativa]

Dissertação de Mestrado

[Mestrado Integrado em Medicina Dentária]

Alexandre Cerutti

Orientadora:

Mestre Isabel Alexandra da Silva Martins

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ACKNOWLEDGEMENTS

Em primeiro lugar, gostaria de agradecer a Portugal e aos seus habitantes por me terem acolhido durante estes 6 belos anos. Foi aqui que me formei para seguir a profissão que sempre me apaixonou, mas foi também aqui que descobri a minha vida de jovem adulto num país muito hospitaleiro. Guardarei sempre um carinho especial e apenas boas memórias deste país e desta cidade.

De seguida, quero expressar a minha gratidão à Universidade Fernando Pessoa, bem como a todos os professores e demais colaboradores da faculdade, por me terem transmitido o conhecimento e por me terem formado como médico dentista. Gostaria de agradecer em especial à minha orientadora deste trabalho, Isabel Alexandra da Silva Martins, que soube sempre encontrar tempo para responder às minhas questões ao longo deste percurso, e que, além disso, foi sempre pedagógica durante os ensinamentos clínicos.

Je tiens tout particulièrement à remercier mes parents pour l'amour et le dévouement qu'ils nous témoignent chaque jour, à mes deux sœurs, mon frère et moi. Merci de nous accompagner sur nos chemins de vie, aussi différents soient-ils, qu'ils soient sportifs ou professionnels, et de nous aider à poursuivre nos objectifs et à réaliser nos rêves.

Merci également au reste de ma famille pour leur présence, leurs visites, et leur soutien sans faille tout au long de cette aventure.

Merci aussi à mes amis, les FDG, d'être venus à de multiples reprises durant mon cursus. Tous ces séjours resteront gravés comme des souvenirs inoubliables.

Merci à ma copine qui, durant plus de cinq ans, malgré la distance, a su me témoigner tout son amour.

Pour finir, merci à mes colocataires : Alix, Héloïse, Enzo et les Victors, avec qui j'ai, sans nul doute, vécu mes meilleurs moments au Portugal.

ABSTRACT

Failures in endodontic treatment are frequently attributed to persistent microbial infections within the complex anatomy of the root canal system, making effective disinfection essential for successful clinical outcomes. This integrative review aims to study the antibacterial efficacy of a 5.25% sodium hypochlorite solution, with diode laser treatment in continuous mode or their combination (hypochlorite solution/diode laser) in eliminating *Enterococcus faecalis* from infected root canals. A targeted literature search using PubMed and ScienceDirect identified five relevant in vitro and ex vivo studies that met defined inclusion criteria. The available evidence shows that sodium hypochlorite irrigation alone consistently achieves high levels of bacterial reduction, often outperforming diode laser treatment when the LASER is used alone. Diode laser disinfection demonstrated variable effectiveness, depending on parameters such as wavelength, power, and exposure time. Although two studies have evaluated the combined use of sodium hypochlorite and a diode laser, neither demonstrated a significantly superior antibacterial effect compared to the use of sodium hypochlorite alone. These findings support the potential role of diode laser technology as a complementary adjunct to conventional chemical irrigation. They also underscore the need for further well-controlled studies to evaluate the possible benefits of combining both methods and to refine clinical disinfection protocols in endodontics.

Keywords: “Enterococcus faecalis”; “diode laser”; “sodium hypochlorite”; “root canal disinfection”; “antimicrobial efficacy”.

RESUMO

Falhas no tratamento endodôntico são frequentemente atribuídas a infecções microbianas persistentes na complexa anatomia do sistema de canais radiculares, tornando a desinfecção eficaz essencial para resultados clínicos bem-sucedidos. Esta revisão integrativa tem como objetivo estudar a eficácia antibacteriana de uma solução de hipoclorito de sódio a 5,25%, com tratamento a laser de dióxido em modo contínuo ou sua combinação (solução de hipoclorito/laser de dióxido) na eliminação de *Enterococcus faecalis* de canais radiculares infetados. Uma busca bibliográfica direcionada utilizando *PubMed* e *ScienceDirect* identificou cinco estudos relevantes *in vitro* e *ex vivo* que atenderam aos critérios de inclusão definidos. As evidências disponíveis mostram que a irrigação com hipoclorito de sódio isoladamente atinge consistentemente altos níveis de redução bacteriana, frequentemente superando o tratamento com laser de dióxido quando o LASER é usado isoladamente. A desinfecção com laser de dióxido demonstrou eficácia variável, dependendo de parâmetros como comprimento de onda, potência e tempo de exposição. Embora dois estudos tenham avaliado o uso combinado de hipoclorito de sódio e laser de dióxido, nenhum deles demonstrou efeito antibacteriano significativamente superior ao uso de hipoclorito de sódio isoladamente. Esses achados corroboram o potencial papel da tecnologia do laser de dióxido como adjuvante complementar à irrigação química convencional. Eles também ressaltam a necessidade de mais estudos bem controlados para avaliar os possíveis benefícios da combinação de ambos os métodos e refinar os protocolos de desinfecção clínica em endodontia.

Palavras-chave: “*Enterococcus faecalis*”; “laser de dióxido”; “hipoclorito de sódio”; “desinfecção do canal radicular”; “eficácia antimicrobiana”.

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LIST OF ABBREVIATIONS, ACRONYMS, AND SYMBOLS

°C	Degree Celsius
ATCC	American Type Culture Collection
CFU	Colony-Forming Units
CFU/mL	Colony-Forming Units per Milliliter
DNA	Deoxyribonucleic Acid
<i>E. faecalis</i>	<i>Enterococcus faecalis</i>
h	Hour
Hz	Hertz
L	Length
LASER	Light Amplification by Stimulated Emission of Radiation
mL	Milliliters
mm	Millimeters
ms	Millisecond
n	Number
NaOCl	Sodium Hypochlorite
nm	Nanometers
PICO	Population, Intervention, Comparison, Outcome
PRISMA Analyses	Preferred Reporting Items for Systematic Reviews and Meta-
W	Watt

1. INTRODUCTION

Teeth are complex biological structures resulting from the interaction between highly mineralized tissues and vital soft components. Among them, dental enamel, which originates from epithelial cells during development, is the hardest tissue in the human body due to its high degree of mineralization and unique hierarchical structure that give it exceptional mechanical strength (Habelitz et al., 2019). Just beneath the enamel and dentin layers lies the dental pulp, a soft connective tissue well protected by these mineralized barriers. These hard tissues form a tight and rigid shield against physical and microbial aggression, while pulp cells are capable of detecting external stimuli and initiating defense responses (Goldberg et al., 2008). This intricate structure not only allows for sensory and protective functions but also makes teeth true living organs, capable of detecting, reacting, and healing. However, this complexity also creates ecological niches within the dentinal tubules, where bacteria may remain sheltered. Experimental evidence has shown that viable bacteria can persist deep within dentinal tubules even after root canal disinfection procedures (Parmar et al., 2011).

Dental caries, through their insidious and progressive nature, create an entry point for a wide range of oral microorganisms. These bacteria initiate the demineralization of enamel and dentin, gradually advancing toward the pulp chamber once the protective barriers are breached (Nair, 2004). Upon microbial infiltration into the pulp chamber, an innate immune response is rapidly initiated, involving odontoblasts, dendritic cells, macrophages, and lymphocytes. These cells recognize microbial components through pattern recognition receptors, leading to the secretion of pro-inflammatory cytokines such as TNF- α and IL-17, which orchestrate leukocyte recruitment and tissue defense (Kritikou et al., 2021). As the inflammatory response intensifies, oxidative stress and extracellular matrix degradation contribute to tissue damage. If the microbial insult persists and exceeds the pulp's defense capacity, the inflammation becomes irreversible, compromising pulp vitality and initiating necrosis. At this stage, clinical intervention through endodontic therapy becomes essential to remove necrotic tissue and prevent periapical complications (Hahn & Liewehr, 2007). Furthermore, the anatomical complexity of the root canal system complicates complete bacterial removal, reinforcing the need for both mechanical and chemical disinfection strategies.

In the context of root canal infections, *Enterococcus faecalis* is recognized as one of the most persistent and problematic microorganisms involved in endodontic failure. It is frequently isolated from previously treated teeth that exhibit persistent or secondary infection, and its resistance mechanisms make it particularly difficult to eliminate (Ashofteh et al., 2014). One of its main survival strategies is its ability to form structured biofilms, which are dense bacterial communities embedded in an extracellular matrix composed of polysaccharides, proteins, and DNA. This matrix provides a high level of protection against antimicrobial agents and mechanical cleaning methods (Castelo-Baz et al., 2012).

At the same time, the mechanical instrumentation of the root canal generates a smear layer, which is a compact film made up of organic and inorganic debris that covers the dentinal walls. This layer not only traps bacteria inside the dentinal tubules but also limits the penetration and action of disinfecting solutions (Hegde et al., 2015). The combination of biofilms and the smear layer creates a complex barrier that significantly reduces the effectiveness of conventional disinfection methods. As a result, additional strategies are required to achieve better cleaning and long-term success in endodontic treatment.

While devitalization is essential to stop the progression of infection, mechanical instrumentation alone has clear limitations. Even with modern rotary systems and advanced techniques, the complex anatomy of the root canal system, including lateral canals, curvatures and isthmuses, makes it difficult to access all contaminated areas. Experimental studies have shown that bacteria can survive in untouched regions of the canal and deep within dentinal tubules despite thorough instrumentation, which underlines the limits of mechanical cleaning (Parmar et al., 2011). In addition, the formation of a smear layer during canal preparation creates a physical barrier that can prevent disinfectants from reaching deeper structures (Hegde et al., 2015). As a result, residual infection may persist, increasing the risk of reinfection. To address these challenges, chemical irrigants and complementary techniques have been developed to enhance disinfection and improve treatment outcomes.

This complex context forms the basis for the main objective of this study. This study aims to enhance root canal disinfection in anatomically challenging areas where conventional methods are insufficient. Sodium hypochlorite (NaOCl), especially at a concentration of 5.25%, is well known for its strong antimicrobial effects and tissue-dissolving properties, and is widely regarded as the gold standard for chemical root canal irrigation. In contrast,

the effectiveness of diode lasers has shown more variable results, depending on parameters such as wavelength, power output, and exposure time (Sohrabi et al., 2016). Some studies, including Castelo-Baz et al. (2012) and Cretella et al. (2017), have explored the possibility of combining both techniques, suggesting potential benefits. Each method operates through different mechanisms and presents specific advantages and limitations. Sodium hypochlorite has been widely used in endodontics for several decades due to its strong antimicrobial properties and its ability to dissolve organic tissue (Zehnder, 2006). Its mechanism of action involves oxidative damage to bacterial proteins and cell membranes, which leads to bacterial death and disruption of biofilm structure (Siqueira et al., 2000). In addition to its antimicrobial effect, NaOCl can dissolve necrotic tissue and facilitate the removal of debris from the canal system (Zehnder, 2006). However, its use is not without risks. The solution is highly cytotoxic to periapical tissues if extruded beyond the apex, and it shows limited penetration into the dentinal tubules, potentially allowing residual microorganisms to persist (Sohrabi et al., 2016).

Sodium hypochlorite (NaOCl) is widely used in endodontics for its ability to dissolve organic tissues and eliminate microbial biofilms. Its clinical effectiveness, however, depends heavily on the balance between concentration, temperature, and exposure time. These factors influence the depth of penetration into dentinal tubules and the overall antimicrobial activity. Studies have shown that increasing any of these parameters enhances the diffusion and efficacy of NaOCl, although the gains diminish beyond certain thresholds (Zou et al., 2010). While a 5.25% solution is traditionally preferred for its strong antibacterial effect, lower concentrations such as 2% can achieve similar outcomes when heated to 60°C, with the added benefit of reduced cytotoxicity to periapical tissues (Liu et al., 2023). Longer exposure times further improve disinfection, even without activation, as shown in comparisons between short and extended irrigation protocols (Zou et al., 2010; Liu et al., 2023). Nevertheless, the chemical aggressiveness of NaOCl must be managed carefully. Higher concentrations and longer exposures can compromise dentin structure by degrading its collagen matrix and reducing mechanical strength (Wang et al., 2017). Altogether, these findings highlight the importance of standardizing not only NaOCl concentration but also its method of application, in order to optimize antimicrobial efficacy while preserving tissue integrity.

The use of diode lasers in endodontics has emerged as a complementary technique to overcome some of the limitations associated with sodium hypochlorite (Sohrabi et al., 2016).

Diode lasers emit light at specific wavelengths that generate heat when absorbed by tissue. This localized thermal effect not only destroys microorganisms and disrupts biofilm structure (Sohrabi et al., 2016), but also enhances energy diffusion into dentinal tubules, areas often inaccessible to conventional irrigants. To prevent excessive heat and potential damage to surrounding tissues, these effects must be carefully managed by adjusting parameters such as wavelength, power, exposure time, emission mode, and application technique (Sohrabi et al., 2016). The effectiveness of laser disinfection thus depends on a complex interplay of these same parameters, which collectively determine how energy is delivered and how deeply it penetrates the dentinal walls. Thamer & Alkurtas (2022) showed that an 810 nm diode laser achieved significantly greater bacterial reduction than a 980 nm laser (70.8% vs 29.1%) under identical power settings, despite the latter's theoretically higher absorption in water. This unexpected outcome may be explained by factors such as light scattering in dentin or differences in experimental protocols. Similarly, Kıvanç et al. (2017) observed no significant differences in CFU reduction between 1.2 W, 2 W, and 3 W settings, although higher power levels generated more heat, highlighting the need for thermal control. In our review, Hegde et al. (2015) used a 908 nm laser in continuous mode with spiral motion and reported complete disinfection, in contrast to Cretella et al. (2017) and Sohrabi et al. (2016), who used similar power lasers but achieved less favorable outcomes. These inconsistencies may be attributed to variations in fiber positioning and movement, factors that are seldom standardized. A fiber placed closer to the apex and moved in a helical manner may enhance surface contact and energy distribution (Kıvanç et al., 2017). Additionally, the emission mode itself plays a critical role: while continuous mode delivers more energy in a shorter time, it also increases the risk of overheating, whereas pulsed mode reduces heat accumulation and may allow deeper penetration within safe limits. Altogether, these findings emphasize that laser efficacy is influenced not only by the device's technical specifications, but more importantly by how it is applied within the root canal system. A detailed understanding and reporting of these variables are essential for explaining the performance variations observed across studies and for optimizing clinical outcomes.

Despite these limitations, diode lasers remain a promising tool to enhance root canal disinfection when used alongside chemical irrigants (Ashofteh et al., 2014; Cretella et al., 2017; Mehrvarzfar et al., 2011)

The combination of sodium hypochlorite and diode laser has been proposed as a synergistic strategy that combines the chemical action of NaOCl with the physical biofilm-disrupting effects of laser irradiation. The rationale behind this technique is to first use NaOCl to dissolve organic material, reduce bacterial load, and begin the removal of the smear layer. This is followed by diode laser application, which allows deeper penetration into dentinal tubules and further disruption of biofilms that may have survived irrigation (Castelo-Baz et al., 2012; Ashofteh et al., 2014; Cretella et al., 2017). This protocol addresses the limitations of each method when used alone and may result in a more thorough microbial elimination and improved canal conditions for final obturation. Several experimental studies have reported significant bacterial reduction when using this combined technique, suggesting that it may enhance clinical outcomes in endodontic treatment (Ashofteh et al., 2014; Cretella et al., 2017; Mehrvarzfar et al., 2011; Castelo-Baz et al., 2012).

Beyond the diversity of available techniques, questions remain about how certain disinfection methods might complement each other under controlled in vitro conditions. Although these experimental settings do not fully reflect clinical complexity, they provide valuable insights into the potential interactions between chemical and physical approaches. This review does not seek to offer definitive conclusions, but rather to shed light on current in vitro findings, underline existing uncertainties, and explore whether a synergistic use of these methods could offer meaningful advantages in the context of endodontic disinfection.

Comparative efficacy of diode lasers and sodium hypochlorite against enterococcus faecalis disinfection:
integrative review

2. MATERIALS AND METHODS

2.1. Sources of Research

A bibliographic search was conducted in two electronic databases: PubMed and ScienceDirect. The search aimed to identify studies investigating the antibacterial effects of sodium hypochlorite and diode laser treatment in the disinfection of root canals infected with *Enterococcus faecalis*. The same set of keywords was used in both databases: *Enterococcus faecalis*, sodium hypochlorite, diode laser, root canal, disinfection, and biofilm.

Although the core search terms remained identical, the structure of the queries was slightly adapted to match the technical requirements of each platform. For instance, PubMed requires field-specific tags such as "Title/Abstract," while ScienceDirect does not support them in the same way. These adjustments ensured optimal retrieval of relevant studies without altering the conceptual basis of the search.

2.1.1. PubMed

```
((("Enterococcus faecalis"[Title/Abstract] OR "E. faecalis"[Title/Abstract]) AND ("sodium hypochlorite"[Title/Abstract] OR "NaOCl"[Title/Abstract]) AND ("diode laser"[Title/Abstract] OR "laser diode"[Title/Abstract]) AND ("root canal"[Title/Abstract]) AND ("disinfection"[Title/Abstract] OR "biofilm"[Title/Abstract]))
```

2.1.2. ScienceDirect

```
((("Enterococcus faecalis" OR "E. faecalis") AND ("sodium hypochlorite" OR "NaOCl") AND ("diode laser" OR "laser diode") AND "root canal" AND ("disinfection" OR "biofilm"))
```

This search strategy was used to retrieve relevant studies assessing the bactericidal effects of 5.25% sodium hypochlorite and diode lasers operating in continuous mode at wavelengths between 830 and 980 nm. These parameters correspond to those found in the final selection of included studies.

2.2. Study Selection Criteria

The selection process involved an initial screening of titles and abstracts, followed by a full-text review of potentially relevant articles. Studies were included if they met all of the following criteria:

2.2.1. Inclusion criteria

- In vitro or ex vivo studies conducted on extracted human adult teeth with a single root canal.
- Evaluation of the antibacterial efficacy of diode laser treatment, used alone or in combination with 5.25% sodium hypochlorite, with the laser operating in continuous mode within a wavelength range of 830 to 980 nm.
- Quantitative reporting of outcomes, such as bacterial count reduction (e.g., CFU or percentage reduction).
- Full-text articles published in English, using standardized disinfection protocols.

2.2.2. Exclusion criteria

- Studies performed on animal models, primary teeth, multi-rooted teeth, or dental implants.
- Clinical in vivo studies.
- Studies not specifically targeting *Enterococcus faecalis*, or lacking measurable disinfection-related outcomes.
- Articles with incomplete methodologies or lacking standardization.

2.3. PICO Question

- **P (Population):** Extracted human adult teeth with a single root canal infected by *Enterococcus faecalis*.
- **I (Intervention):** Disinfection using a diode laser (wavelength between 940 and 980 nm), either alone or in combination with 5.25% sodium hypochlorite.

- **C (Comparison):** Disinfection using 5.25% sodium hypochlorite alone.
- **O (Outcome):** Reduction of bacterial load (measured by colony-forming unit [CFU] counts or percentage reduction).

2.3.1. Combined PICO Question

In extracted human adult teeth with a single root canal infected by *Enterococcus faecalis* (P), how does disinfection using a diode laser alone or in combination with 5.25% sodium hypochlorite (I) compare to 5.25% sodium hypochlorite alone (C) in terms of bacterial load reduction (O)?

2.4. Data Extraction and Analysis

Data were extracted from the selected studies regarding:

2.4.1. Study Characteristics

Design (in vitro or ex vivo), sample size, tooth type, and canal configuration.

2.4.2. Intervention Details

Laser parameters (e.g., wavelength, power, pulse duration, total exposure time) and details of NaOCl usage (concentration of 5.25% and volume).

2.4.3. Outcome Measures

CFU counts, percentage reduction in bacterial load, and any reported measures of disinfectant penetration into dentinal tubules.

2.4.4. Key Findings

Main results and conclusions regarding the efficacy of the disinfection methods.

The extracted data were tabulated for comparative analysis, and a narrative synthesis was performed to highlight trends, similarities, and differences among the studies.

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3. RESULTS

3.1. Study Selection

A total of 40 articles were identified from PubMed and 155 from ScienceDirect, yielding 195 articles overall. After removing 12 duplicates, 183 unique articles remained.

The titles of these 183 articles were screened, leading to the exclusion of 139 that did not meet the inclusion criteria (e.g., clinical in vivo studies, studies on primary or multi-rooted teeth, or studies lacking quantifiable outcomes). This resulted in 44 articles retained for abstract review.

After careful evaluation of the abstracts, 9 studies were considered potentially relevant. A full-text analysis of these 9 articles was then conducted, and 5 articles met all inclusion criteria and were included in the final synthesis.

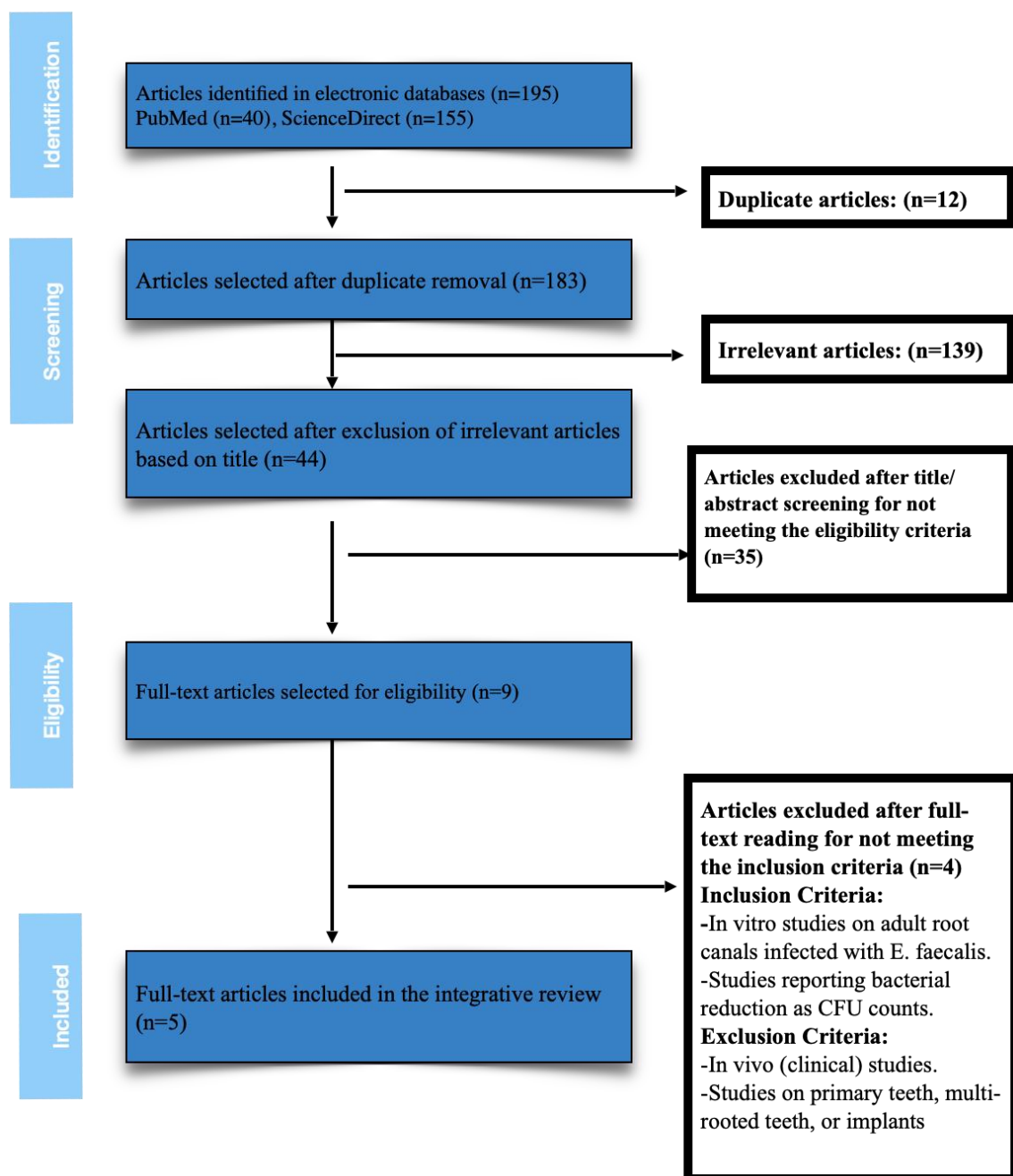
A PRISMA flow diagram was created to illustrate the study selection process.

The studies selected and considered in this review are as follows:

Castelo-Baz et al. (2012); Ashofteh et al. (2014); Hegde et al. (2015); Sohrabi et al. (2016); Cretella et al. (2017)

Figure 1

Graphical representation of the PRISMA diagram



3.2. Characteristics of the Selected Studies

All five studies included in this review are in vitro investigations conducted on extracted human permanent teeth with single root canals. Despite variations in methodology, they share a common goal: evaluating the antibacterial efficacy of diode laser irradiation and 5.25% sodium hypochlorite (NaOCl), either alone or in combination, in eliminating

mature biofilms of *Enterococcus faecalis* from infected root canals.

3.2.1. Sample Origin and Type

The selected teeth were permanent single-rooted teeth, mainly incisors and premolars, free of caries, restorations, fractures, or calcifications. Ashofteh et al. (2014), Hegde et al. (2015) and Sohrabi et al. (2016) used mandibular premolars or anterior teeth. Castelo-Baz et al. (2012) and Cretella et al. (2017) selected maxillary central and lateral incisors. In all studies, the crowns were removed to standardize working lengths, typically between 12 and 16 mm, and root canals were instrumented using rotary systems. Sterilization of external surfaces was achieved via autoclaving in all cases. Hegde et al. (2015) additionally used gamma irradiation to ensure sterility.

3.2.2. Sterilization and Biofilm Formation

After preparation, the canals were inoculated with a standardized suspension of *E. faecalis* (approximately 1.5×10^8 CFU/mL, adjusted to a 0.5 McFarland standard). The teeth were incubated at 37 °C for periods ranging from 14 to 21 days to allow biofilm maturation. Hegde et al. (2015) extended the incubation period to 21 days, while the other studies used an average of two weeks. Bacterial quantification post-treatment was generally based on CFU counts, as in the studies by Castelo-Baz et al. (2012), Ashofteh et al. (2014), Hegde et al. (2015) and Sohrabi et al. (2016). Cretella et al. (2017) used a semi-quantitative scoring system based on culture density after 24 hours.

3.2.3. Disinfection Protocols

Each study compared at least two disinfection methods: diode laser treatment and 5.25% NaOCl irrigation, either used alone or in combination. Castelo-Baz et al. (2012) used a 940 nm diode laser with specific pulse settings (0.05 ms pulse duration, 0.2 ms interval) delivered in four 15-second cycles for a total of one minute. The study also tested sequential use of NaOCl followed by laser irradiation. Sohrabi et al. (2016) employed a 980 nm diode laser at an output of 2.5 W in continuous mode and directly compared its efficacy with NaOCl irrigation alone. Hegde et al. (2015) used a 908 nm diode laser for intracanal irradiation and reported complete disinfection (0 CFU) when the laser was

applied as a stand-alone treatment. In that same study, NaOCl was applied via the EndoVac irrigation system, which uses apical negative pressure. This may limit direct comparison with other studies using standard syringe irrigation. Ashofteh et al. (2014) used an 830 nm diode laser in continuous mode and evaluated three approaches: laser alone, NaOCl alone, and their combination. Cretella et al. (2017) tested a 980 nm diode laser with NaOCl, examining both individual and combined applications. In all protocols, the laser fiber was inserted approximately 1 mm short of the working length and moved in a controlled oscillatory or spiral motion to ensure uniform exposure of the canal walls. The volume of NaOCl used varied across studies, typically between 1 and 5 mL. The irrigant was not always activated during application.

3.2.4. Outcome Measures and Data Extraction

The primary outcome in all studies was the reduction in bacterial load, usually measured through colony-forming unit (CFU) counts per milliliter. Ashofteh et al. (2014), Castelo-Baz et al. (2012) and Hegde et al. (2015) measured CFU reduction immediately after treatment. Castelo-Baz et al. (2012) and Sohrabi et al. (2016) also included delayed evaluations at 24 hours and 7 days to assess bacterial regrowth. Cretella et al. (2017) used a semi-quantitative scoring method based on bacterial density observed 24 hours after treatment. Only data concerning diode laser treatment and 5.25% NaOCl whether used alone or in combination were extracted for this review to ensure consistency and comparability among the included studies.

3.2.5. Experimental Consistency and Observed Differences

Although the overall design and objectives of the five studies are consistent, there are minor differences in laser parameters (wavelength, power settings, pulse duration, and exposure cycles) as well as in the NaOCl delivery protocols, including the volume used, application time, and irrigation method. Hegde et al. (2015) demonstrated that the 908 nm diode laser achieved complete bacterial elimination when used as a stand-alone method. However, their protocol for NaOCl involved EndoVac negative-pressure delivery, which may account for the lower disinfection performance compared to other studies using conventional irrigation. Castelo-Baz et al. (2012) showed that the sequential use of 5.25% NaOCl followed by a 940 nm diode laser led to greater bacterial reduction

than either method alone. Sohrabi et al. (2016) found that NaOCl irrigation alone was more effective than the 980 nm diode laser used on its own. Ashofteh et al. (2014) also compared three groups: NaOCl alone, diode laser alone, and the combination. Their results showed that the combined approach led to a greater bacterial reduction than the laser alone, but not significantly more than NaOCl. Cretella et al. (2017) used a semi-quantitative scoring system and observed better disinfection when both methods were combined, though the difference was not dramatic compared to the single use of NaOCl.

3.2.6. Additional Comparative Findings

Three of the five studies (Ashofteh et al. 2014; Hegde et al. 2015; Sohrabi et al. 2016) specifically compared the effects of diode laser versus NaOCl used individually. Two studies (Castelo-Baz et al. 2012 and Cretella et al. 2017) included a group where both treatments were combined sequentially. Although the idea of a synergistic effect is supported by some data, the overall evidence remains limited. The fact that only two studies explicitly evaluated the combined use of NaOCl and diode laser restricts the ability to draw strong conclusions. Therefore, any potential advantage of integrating both approaches should be interpreted with caution and validated by further research.

3.3. Summary

The five studies demonstrate a high degree of methodological consistency in terms of sterilization procedures, biofilm formation, and the structure of disinfection protocols. This uniformity provides a solid basis for comparison. Despite differences in laser wavelength, tooth count, output, and irrigation techniques, the results converge on several key observations. Hegde et al. (2015) reported complete disinfection using a 908 nm diode laser. However, the NaOCl used in their study was delivered via the EndoVac system, which may explain the lower efficacy observed. Castelo-Baz et al. (2012), Ashofteh et al. (2014) and Sohrabi et al. (2016) all found that NaOCl alone was generally more effective than diode laser alone. Cretella et al. (2017), along with Castelo-Baz et al. (2012), suggested that the combined approach may enhance disinfection, although the evidence remains limited. Overall, NaOCl appears to be the most consistently effective single agent, while diode laser treatment shows promise as a complementary technique.

Table 1*Characteristics of the Studies Selected for Analysis*

	Study Type	Sample Size & Tooth Type	Incubation Duration (Biofilm)	Laser Parameters (Wavelength, Power, Mode, Time)	NaOCl Protocol	Outcome Measures
Hegde et al., 2015	In vitro	18 single-rooted mandibular premolars	Biofilm matured over ~21 days	980 nm diode laser; continuous	5.25% NaOCl with a standard irrigation protocol	CFU reduction; % bacterial removal
Sohrabi et al., 2016	In vitro	18 single-rooted mandibular premolars (experimental groups, n=8)	2 weeks incubation	980 nm diode laser; continuous; parameters provided as per study	5.25% NaOCl used for irrigation	CFU counts; statistical analysis (t-test)
Ashofteh et al., 2014	In vitro	135 extracted single-rooted human teeth	4 weeks contamination	830 nm diode laser, 1.5 W, pulsed mode, 20 Hz, 50 ms pulse duration, 4 cycles of 15 sec each	5.25% NaOCl,	CFU counts from root canal samples and dentinal shavings (CFU/mg)
Castelo-Baz et al., 2012	In vitro	68 single-rooted maxillary central incisors	2 weeks (mature biofilm)	940 nm diode laser; continuous; parameters not fully detailed	5% NaOCl (considered comparable to 5.25%)	CFU counts at 24h and 7 days
Cretella et al., 2017	Ex vivo	120 extracted human single-rooted teeth (infected with <i>E. faecalis</i>)	21 days contamination with <i>E. faecalis</i> ATCC 29212	Diode laser 810 nm, 2.5 W, 3 cycles of 30 sec, intracanal irradiation with optical fibers (200 µm and 300 µm)	5.25% NaOCl	CFU counts classified on a 3-point scale (2.5×10^4, $2.5 - 7 \times 10^4$, $> 7 \times 10^4$ CFU/mL)

Table 2*Results of the Studies Selected for Analysis*

Reference (Author, Year)	Comparison Focus	NaOCl Alone Outcome (CFU Reduction)	Diode Laser Alone Outcome (CFU Reduction)	NaOCl + Diode Laser Outcome (CFU Reduction)	Conclusion
Hegde et al., 2015	Evaluated disinfection in mature biofilms over an extended period (~21 days).	Endovac: 10 ³ CFU/mL (~50% disinfection) Stropko: 10 ⁵ CFU/mL (~20% disinfection)	0 CFU/mL (100% disinfection)	Not evaluated.	Laser alone achieved complete disinfection, significantly outperforming NaOCl alone delivered via Endovac or Stropko (50% and 20%, respectively).
Sohrabi et al., 2016	Direct numerical comparison of disinfection outcomes.	14.5 ± 3.66 CFU/mL; 99.87% bacterial reduction	405.37 ± 395.08 CFU/mL; 96.56% bacterial reduction	Not evaluated.	5.25% NaOCl is significantly more effective than the 980-nm diode laser (P=0.027).
Ashofteh et al. 2014	Antibacterial effect of 5.25% NaOCl and 830 nm diode laser on <i>E. faecalis</i> -contaminated root canals and dentin.	99.97% reduction in canal samples.	97.56% reduction in canal samples.	Not evaluated.	NaOCl achieved the highest bacterial reduction. Diode laser was effective but less so than NaOCl.

Reference (Author, Year)	Comparison Focus	NaOCl Alone Outcome (CFU Reduction)	Diode Laser Alone Outcome (CFU Reduction)	NaOCl + Diode Laser Outcome (CFU Reduction)	Conclusion
Castelo-Baz et al., 2012	Evaluated NaOCl, diode laser, and their combination in mature biofilms at different time points.	<p>Group B (NaOCl Alone):</p> <ul style="list-style-type: none"> • After 24 h: 1 out of 20 teeth (5%) showed bacterial growth (264×10^7 CFU) • At 7 days: 20% of samples infected (range: $120-232 \times 10^7$ CFU) 	<p>Group A (Laser Alone):</p> <ul style="list-style-type: none"> • After 24 h: 30% of teeth infected (range: $11-167 \times 10^7$ CFU) • At 7 days: 70% of samples infected (range: $12-230 \times 10^7$ CFU) 	<p>Group C (Combined):</p> <ul style="list-style-type: none"> • After 24 h: No bacterial growth observed (0% infection) • At 7 days: Only 10% of samples infected (values: 120 and 136×10^7 CFU) 	Combined treatment led to complete disinfection at 24 h and very low infection rates at 7 days, demonstrating synergy. Laser alone left high residual infection, while NaOCl alone was effective but less complete.
Cretella et al., 2017	Compared the antibacterial effects of 5.25% NaOCl, diode laser alone, and their combination in <i>E. faecalis</i> -infected canals.	No bacterial growth detected (score 0, $<2.5 \times 10^4$ CFU/mL)	Moderate bacterial load remaining (score 1, $2.5-7 \times 10^4$ CFU/mL)	No bacterial growth detected (score 0, $<2.5 \times 10^4$ CFU/mL)	NaOCl alone and NaOCl + laser both achieved total decontamination. Laser alone did not significantly reduce bacterial load compared to control.

3.4. Results of the Selected Studies

Five in vitro studies were included in this review to assess the antibacterial efficacy of 5.25% sodium hypochlorite (NaOCl), diode laser irradiation, and their combination in disinfecting root canals infected with *Enterococcus faecalis*. While methodologies varied across studies, all focused on quantitative measurement of bacterial reduction through CFU analysis and aimed to determine whether the diode laser could serve as a reliable alternative or adjunct to chemical irrigation.

3.4.1. Sohrabi et al. (2016)

Sohrabi et al. (2016) evaluated 18 mandibular premolars after 14 days of contamination. Two groups were tested: 5.25% NaOCl irrigation vs. 980 nm diode laser (2.5 W, continuous mode).

- **NaOCl group:** Mean CFU = 14.5 ± 3.66 → 99.87 % reduction
- **Laser group:** Mean CFU = 405.37 ± 395.08 → 96.56 % reduction
- **Statistical significance:** The NaOCl group performed significantly better than the laser group ($p = 0.027$)

Conclusion: While both techniques significantly reduced bacterial load, NaOCl was more reliable under the tested conditions.

3.4.2. Castelo-Baz et al. (2012)

Castelo-Baz et al. (2012) used 68 maxillary central incisors and evaluated three groups: laser alone (940 nm), NaOCl alone (5.25 %), and a combination of NaOCl followed by laser.

At 24 hours:

- **Laser alone:** 30 % of samples positive (11×10^7 to 167×10^7 CFU/mL)
- **NaOCl alone:** 5 % positive, with one sample at 264×10^7 CFU/mL
- **Combination:** 0 % bacterial growth – complete disinfection

At 7 days:

- **Laser alone:** 70 % reinfection (12×10^7 to 230×10^7 CFU/mL)

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- **NaOCl alone:** 20 % reinfection (120×10^7 to 232×10^7 CFU/mL)
- **Combination:** 10 % reinfection (120×10^7 and 136×10^7 CFU/mL)

Statistical analysis:

- Combination vs. laser: significant at both 24 h ($p = 0.002$) and 7 d ($p = 0.000$)
- Combination vs. NaOCl: not significant at 24 h ($p = 1$), but significant at 7 d ($p = 0.04$)

Conclusion: The combination therapy showed the most durable effect over time.

3.4.3. Ashofteh et al. (2014)

Ashofteh et al. (2014) investigated 135 extracted teeth contaminated with *Enterococcus faecalis* for 28 days. The tested groups relevant to this review were: NaOCl 5.25 %, diode laser 830 nm (continuous mode), and their combination.

- **NaOCl alone:** 99.97 ± 0.14 % CFU reduction
- **Laser alone:** 97.56 ± 6.36 % CFU reduction
- **NaOCl + laser:** 99.99 ± 0.03 % CFU reduction

Statistical analysis: Significant differences between all groups ($p < 0.05$)

Conclusion: The combination enhanced bacterial elimination more effectively than either method alone.

3.4.4. Cretella et al. (2017)

Cretella et al. (2017) analyzed 128 extracted human anterior maxillary teeth with single canals. Among them, 120 were inoculated with *Enterococcus faecalis* for 21 days. The remaining 8 non-inoculated teeth were used as negative controls.

Bacterial growth was evaluated after treatment using a semi-quantitative 3-level scale based on CFU/mL:

- **Score 0:** $< 2.5 \times 10^4$ CFU/mL (complete disinfection)
- **Score 1:** $2.5 \times 10^4 - 7 \times 10^4$ CFU/mL (partial disinfection)
- **Score 2:** $> 7 \times 10^4$ CFU/mL (no disinfection)

Group 1 - Positive control (saline only)

→ All 24 samples remained at score 2, indicating no disinfection.

Group 2 - Sodium hypochlorite (5.25 %) alone

→ All 24 samples achieved score 0, indicating complete disinfection.

Group 3 - Diode laser alone (810 nm, 2.5 W)

→ All 24 samples scored 1, showing partial bacterial reduction but no complete disinfection.

Group 4 - NaOCl (5.25 %) activated by diode laser (810 nm)

→ All 24 samples also achieved score 0, indicating complete disinfection.

Conclusion: Laser alone was ineffective. The combination was equivalent to NaOCl alone, without added benefit.

3.4.5. Hegde et al. (2015)

Hegde et al. (2015) used 18 single-rooted mandibular premolars inoculated with *Enterococcus faecalis* and incubated for 21 days. A 908 nm diode laser in continuous mode was used, with the fiber inserted 1 mm short of the apex and moved in a spiral motion.

- **Laser alone:** All samples demonstrated complete disinfection (0 CFU/mL)
- **NaOCl alone (EndoVac):** Median CFU reduction of approximately 50 %
- **Stropko system (NaOCl + positive pressure):** Median CFU reduction of approximately 20 %

Conclusion: The diode laser, under these specific parameters, achieved total bacterial eradication. However, the use of activated irrigation systems with NaOCl limits comparability with studies using standard syringe irrigation.

3.5. Synthesis of Findings

Although the five studies do not all reach the same conclusions, they show a general consistency that allows for a common direction to emerge. Taken together, they offer a useful foundation for further analysis of the effectiveness and complementarity of the three disinfection methods explored.

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4. DISCUSSION

This review draws on *in vitro* and *ex vivo* studies, which provide a controlled environment for examining how disinfection protocols interact with the root canal system. By analyzing the results across these studies, it becomes possible to identify recurring patterns, formulate hypotheses about the reliability and performance of the different strategies, and begin to understand the factors that may explain their varying levels of effectiveness.

Across all five studies, NaOCl at a concentration of 5.25% demonstrated consistently strong antibacterial performance. Sohrabi et al., (2016) reported a 99.87% reduction in CFU, while Castelo-Baz et al. (2012) observed minimal bacterial regrowth in only 5% of samples 24 hours post-treatment. Ashofteh et al. (2014) similarly reported a 99.97% reduction. These results reaffirm NaOCl's long-standing status as the gold standard irrigant in endodontics, primarily due to its ability to dissolve necrotic tissue and disrupt bacterial membranes. However, its efficacy is limited by the presence of the smear layer, which acts as a mechanical barrier that prevents penetration into the dentinal tubules and deeper bacterial biofilms.

It is also worth noting that the method of NaOCl application varied across studies. Hegde et al. (2015) used the EndoVac and Stropko irrigation systems, which rely on negative and positive pressure, whereas the other studies applied NaOCl manually using syringes. Such variations can significantly influence irrigant dynamics, apical penetration, and interaction with residual bacteria, further complicating cross-study comparisons.

The smear layer, created during instrumentation, consists of both organic and inorganic debris. Its presence is not only a physical obstacle but also a potential reservoir for bacterial entrapment. Effective smear layer removal is essential to improve the diffusion of irrigants and the overall success of disinfection. Studies like Castelo-Baz et al. (2012) highlight that adjunctive methods such as diode laser irradiation or activation systems can aid in modifying or removing this layer, making the canal system more accessible to chemical agents like NaOCl.

The use of diode lasers as a stand-alone treatment revealed heterogeneous results, with variable outcomes among the studies. Ashofteh et al. (2014) observed a 97.56% CFU reduction, which, while impressive, was lower than NaOCl alone or in combination.

Cretella et al. (2017) and Sohrabi et al. (2016) reported significantly higher residual bacterial loads, indicating that diode laser alone is not reliably sufficient for complete disinfection. In contrast, Hegde et al. (2015) reported total bacterial elimination (0 CFU/mL) with diode laser alone. However, this finding is inconsistent with the broader literature. Importantly, Hegde's study did not compare the laser to standard NaOCl irrigation but rather to alternative systems like EndoVac and Stropko. These systems utilize negative pressure or controlled delivery, which may alter irrigant behavior and reduce its impact. This divergence, combined with the absence of a direct NaOCl group and the study's unusually high laser efficacy, suggests that these results should be interpreted with caution.

In addition to methodological design, discrepancies in laser performance across studies can be linked to differences in technical parameters. The five studies used wavelengths ranging from 810 nm (Cretella et al., 2017) to 980 nm (Sohrabi et al., 2016), with variation in output power, emission mode (continuous or pulsed), and exposure time. The way the laser fiber was positioned and moved inside the canal whether in spiral motion, up-and-down, or stationary — was also inconsistently described. Hegde et al. (2015), for instance, applied the diode laser in spiral motion close to the apex, which could explain the superior results obtained.

Moreover, variations in sample type and incubation duration also impact results. While Hegde et al. (2015) and Cretella et al. (2017) used 21-day contamination models to simulate mature biofilms, Castelo-Baz et al. (2012) and Sohrabi et al., (2016) incubated for only 14 days. Ashofteh et al. (2014) extended incubation to 28 days, likely increasing bacterial resistance. Although all five studies were conducted exclusively on single-rooted teeth, the tooth types used differed: anterior maxillary incisors in some studies, mandibular premolars in others. These anatomical variations influence canal size, curvature, and dentin thickness, which may affect both laser energy penetration and irrigant efficacy.

Beyond these morphological considerations, another important but often overlooked factor is the number of teeth used in each study. For instance, Hegde et al. (2015) included only 18 samples, whereas Ashofteh et al. (2014) worked with 135. Such a large gap in sample size inevitably affects the statistical reliability of the findings. Small sample groups, like in Hegde et al. (2015), are more susceptible to extreme or isolated results. The complete absence of bacteria (0 CFU/mL) reported in that study might reflect a

random outcome rather than a consistent effect. On the other hand, larger sample sizes, like those in Ashofteh et al. (2014) or Cretella et al. (2017) with 120 teeth, provide more stable averages but might also dilute subtle differences that could exist between groups. Interestingly, Cretella et al. (2017) did not observe any added benefit from the laser treatment, despite using a large cohort. This suggests that other factors, such as rigid protocols or less sensitive evaluation methods, might have influenced the results more than the sample size itself.

Combining NaOCl with diode laser treatment has emerged as a promising strategy, aiming to compensate for the limitations of each individual approach. NaOCl rapidly reduces bacterial load and disrupts the smear layer, potentially allowing the laser to penetrate deeper and thermally inactivate residual microorganisms. This sequential synergy was observed in Castelo-Baz et al. (2012) and Ashofteh et al. (2014), with the latter reporting a 99.99 % bacterial reduction and the lowest CFU levels in dentinal tubules. However, not all findings support a synergistic effect. Cretella et al. (2017) observed no additional benefit from combining NaOCl and diode laser compared to NaOCl alone. The disparity in outcomes may be attributed to differences in study design, sample preparation, or evaluation techniques, highlighting the need for further high-quality research to confirm any additive or synergistic effects.

Another crucial factor is the maturity and structure of the biofilm. Mature biofilms exhibit increased resistance due to their dense extracellular matrix, which acts as a protective barrier against antimicrobial agents. While NaOCl is effective against planktonic bacteria, its limited penetration into biofilm structures can reduce its efficacy. Diode lasers, through thermal action, may help overcome this limitation by enhancing disinfection within dentinal tubules. Ashofteh et al. (2014) addressed this by measuring CFU not only from the canal lumen but also from dentin shavings, thus providing a more detailed picture of residual contamination.

While in vitro insights are valuable, their translation to clinical settings requires further contextual evaluation. To assess the true clinical relevance of current findings, it would be important to investigate their outcomes in in vivo conditions. While in vitro studies consistently demonstrate the potential of sodium hypochlorite and diode lasers in reducing bacterial load, these results may not fully translate to clinical scenarios, where factors such as canal anatomy, tissue remnants, and operator variability can influence outcomes. Practical aspects like heat generation and precise application technique also

play a role that laboratory settings may not capture adequately. Therefore, further in vivo research would be valuable to validate the effectiveness and safety of these disinfection methods under real clinical conditions.

Finally, while diode lasers show potential in enhancing disinfection, their safe implementation depends on precise parameter control. Improper use can lead to undesirable thermal damage to dentinal structures and surrounding tissues. The integration of diode lasers in daily clinical practice also presents practical challenges. Operator training, familiarity with laser physics, and cost of equipment are all barriers to widespread adoption. A lack of standardized protocols may also prevent consistent outcomes in non-specialized environments. Future developments in laser technology may enable better control of output settings and integration with irrigation protocols, thereby making this combined approach more practical and predictable in clinical endodontics.

While no single protocol guarantees success, acknowledging the limits of each and striving for better standardization may prove more valuable than the search for a universal solution.

5. CONCLUSION

This integrative review confirms that 5.25% sodium hypochlorite remains the most consistently effective method for disinfecting root canals infected with *Enterococcus faecalis*. Its strong antimicrobial properties and ability to dissolve organic tissue continue to make it the gold standard in endodontic irrigation.

In this study, we compared it to diode laser therapy, which appeared less predictable and often less effective as a stand-alone method. Even in combination, laser and NaOCl did not consistently outperform sodium hypochlorite used alone. This suggests that, despite their potential, these newer approaches have yet to demonstrate clear superiority.

What emerged clearly, however, is that both methods are influenced by multiple parameters such as concentration, exposure time, temperature or power settings, and application techniques. These variables significantly impact performance and may explain the inconsistencies seen across studies. Rather than seeking a single universal solution, it may be more relevant to adapt disinfection strategies to the specific anatomy of the tooth and the clinical context of each case. Future studies exploring how to personalize protocols according to tooth morphology or patient factors could help define when one technique might be preferable to another.

Moreover, both sodium hypochlorite and diode laser therapy carry inherent risks. While NaOCl is known for its cytotoxic potential when extruded beyond the apex, laser misuse can lead to unwanted thermal damage. Combining the two methods might not only enhance disinfection but also offer a way to limit the drawbacks of each by allowing the use of lower concentrations or shorter exposure times, thereby improving overall safety without sacrificing efficacy.

Finally, these technologies demand new skills from practitioners, and their integration into daily practice will require both technical training and stronger clinical evidence. Future *in vivo* research assessing long-term success rates, patient outcomes and real-world applicability will be essential to determine how these tools can best complement established disinfection protocols.

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