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Review Article

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Disinfectant effectiveness of chlorhexidine gel compared to sodium hypochlorite: a systematic review with meta-analysis

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Conflict of Interest
No potential conflict of interest relevant to this article was reported.

Author Contributions
Conceptualization: Weissheimer T, Só MVR; Data curation: Weissheimer T, Pinto KP, da Silva EJNL, Só MVR; Formal analysis: Weissheimer T, Pinto KP; Investigation: Weissheimer T, Pinto KP; Methodology:

ABSTRACT

This study aimed to compare the disinfectant ability of chlorhexidine (CHX) gel and sodium hypochlorite (NaOCl). Systematic searches were conducted from inception until December 8th, 2022 (MEDLINE/PubMed, Cochrane Library, Web of Science, Scopus, Embase, and Grey Literature databases). Only randomized clinical trials were included. The revised Cochrane risk of bias tools for randomized trials were used to assess the quality of studies. Meta-analyses were performed. The overall quality of evidence was assessed through the Grading of Recommendations Assessment, Development, and Evaluation tool. Six studies were included. Five had a low risk of bias and 1 had some concerns. Three studies assessed bacterial reduction. Two were included in the meta-analysis for bacterial reduction (mean difference, 75.03 [confidence interval, CI, −271.15, 421.22], $p = 0.67$; $I^2 = 74\%$); and 3 in the meta-analysis for cultivable bacteria after chemomechanical preparation (odds ratio, 1.03 [CI, 0.20, 5.31], $P = 0.98$; $I^2 = 49\%$). Five studies assessed endotoxin reduction. Three were included in a meta-analysis (mean difference, 20.59 [CI, −36.41, 77.59], $p = 0.48$; $I^2 = 74\%$). There seems to be no difference in the disinfectant ability of CHX gel and NaOCl, but further research is necessary.

Keywords: Bacteria; Chlorhexidine; Endotoxin; Sodium hypochlorite; Systematic review

INTRODUCTION

The main purpose of root canal treatment in necrotic cases is to reduce the infectious content load to subcritical levels compatible with the healing process [1,2]. The greatest challenge in achieving this purpose is the association of the complexity of the root canal morphology with the limited range of action of the instruments during root canal preparation [3-5]. These

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limitations can imply bacterial maintenance and its sub-products within the root canal, which can lead to treatment failure [5,6].

In order to ensure maximum reduction of the infectious content load, several substances have been tested as irrigants to be used during chemomechanical preparation [7-9]. Among these, the most used are sodium hypochlorite (NaOCl) and chlorhexidine (CHX) digluconate. NaOCl is a time/volume/concentration dependent substance that possesses antibacterial properties, fat and organic tissue dissolution capabilities [10-14]. Regarding NaOCl antibacterial mechanism of action, its effectiveness is based on its high pH, influenced by the release of hydroxyl ions, interfering in the cytoplasmic membrane integrity, causing an irreversible enzymatic inhibition, biosynthetic alteration in cellular metabolism and phospholipid degradation which can be observed in lipidic peroxidation [10]. An amino acid chlorination reaction occurs, forming chloramines, that interfere with cellular metabolism. In addition, an oxidation promotes an irreversible bacterial enzymatic inhibition replacing hydrogen with chlorine, causing this inactivation, which can be observed in the reaction of chlorine with amino groups and an irreversible oxidation of sulfhydryl groups of bacterial enzymes, like cysteine [10].

CHX, usually used in a liquid formulation, is a cationic bisbiguanide that presents an antifungal effect, and is a potent bacteriostatic in low concentrations (0.2%) and bactericidal in higher concentrations (2%) [15]. The primary characteristic of this substance is its capacity to adhere to dentin and deliver a sustained antimicrobial effect (substantivity) [15,16]. Nevertheless, the substantivity of CHX has yet to be proven in clinical conditions [17]. Its antibacterial activity occurs when its cationic molecule binds to the extracellular complexes and negatively charged bacterial cell walls, altering the osmotic equilibrium of the bacterial cells [16]. This reaction increases the permeability of the cell wall, allowing the CHX molecule to penetrate into the bacteria [15]. The use of this substance during chemomechanical preparation is based on the concept that CHX may be less toxic to the periapical tissues when compared to NaOCl [18]. However, this premise presents divergent results in the literature [19,20]. On the other hand, CHX does not present the ability to dissolve necrotic tissue remnants or to disrupt biofilm structures [14,21]. Nevertheless, regarding infectious content reduction, it demonstrates satisfactory results [22,23].

Some authors proposed CHX on its gel formulation to be used as an intracanal dressing alone or combined with calcium hydroxide [24-26]. Additionally, the use of CHX on its gel formulation during chemomechanical preparation has been proposed. In these situations, its usage presupposes that, despite presenting the same properties as the liquid formulation, the gel formulation would promote a reduction of the smear layer formation due to its rheological action, presenting a greater lubricant activity, reducing the friction between the instrument and the dentin surface, facilitating the removal of organic tissues and reducing the incidence of instrument fracture [16,27].

Previous *in vitro* studies investigating the disinfection capacity of CHX gel compared to liquid CHX and NaOCl have shown that CHX gel takes a longer time to eliminate bacteria [28,29]. However, these results are based on methodologies that directly immersed single-species biofilm membranes on tubes containing the investigated solutions, which does not necessarily reflect the conditions of clinical practices [28,29]. Additionally, previous systematic reviews evaluating the capacity of NaOCl and CHX on the reduction of the infectious content already exist, none of these studies specifically evaluate CHX in its gel form, which could potentially overestimate the disinfectant effectiveness of this formulation, when compared to the liquid form [30-33]. When liquid CHX is used during chemomechanical preparation, the root canal is constantly flooded by the solution, exerting its effects constantly during treatment. However, when using CHX gel, the working method involves the use of a small amount of CHX gel during instrumentation, followed by irrigation with an inert solution. Based on this, it is possible to hypothesize that the short time of action exerted by CHX gel would not generate the desired decontamination effects. Additionally, it is important to emphasize that CHX in its gel form is viscous, therefore, it may not be appropriately delivered through the entire root canal, which could also hamper its disinfection capability.

Since the reduction of the infectious content (bacterial and endotoxins) is mandatory to achieve success during endodontic therapies, it is necessary to evaluate, prior to clinical application, the available evidence regarding the performance of CHX gel on such aspects. Hence, a systematic review that only evaluates the capacity of CHX gel, when compared to NaOCl, on bacterial and endotoxin reduction is necessary. For this reason, the current systematic review aimed to answer the following question: "Is CHX gel as effective as NaOCl in disinfecting the root canal system?"

MATERIALS AND METHODS

Protocol and registration

This systematic review followed the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA)recommendation (http://wwwprisma-statement.org) and was registered on the PROSPERO database under the number CRD42020211315 [34].

Search strategy

The search was performed independently by 2 examiners (T.W. and M.V.R.S.) in the following electronic databases: MEDLINE/PubMed, Cochrane Library, Web of Science, Scopus, Embase, and Grey Literature Reports. The search was conducted from inception up to December 8th, 2022, without language and year restriction. The electronic search strategy was developed using the most cited descriptors in previous publications on this theme combining Medical Subject Heading terms and text words (tw.). For each database, the following terms were combined: "Root canal", "Root canal therapy", "Root canal treatment", "Endodont*", "Chlorhexidine", "CHX", "Chlorhexidine gel", "CHX gel", "Irrigant", "Root canal irrigant", "Sodium hypochlorite", "NaOCl", "Microb*", "Microbial consortia", "Disinfection", "Bacteria" "Bacterial reduction", "Microorganism", "Endotoxin", "Lipopolysaccharide", "LPS", "Lipoteichoic acid", "LTA". The Boolean operators 'AND' and 'OR' were applied to combine the terms and create a search strategy.

The search strategies for each database and the following findings are summarized in **Supplementary Table 1**. An additional screening of the references of the selected studies was performed, and the related articles were searched in the PubMed database. All articles selected were imported into the Mendeley© (Mendeley Ltd., London, United Kingdom) reference manager to catalogue the references and facilitate the exclusion of duplicates.

Eligibility criteria

The eligibility criteria were based on the PICOS strategy (PRISMA-P 2015), as follows [34-36]:

- Population (P): Adult patients subjected to root canal treatment or retreatment presenting symptomatic or asymptomatic apical periodontitis;
- Intervention (I): Root canal treatment using CHX gel as an adjunct substance during root canal preparation;
- Comparison (C): Root canal preparation using liquid NaOCl;
- Outcome (O): Primary: bacterial and/or endotoxin reduction;
  Secondary: periapical healing.
- Study design (S): Only randomized clinical trials (RCTs).

Study selection

The first stage consisted of excluding the duplicated studies, considering only once, and examining the retrieved titles and abstracts of the selected studies by 2 independent authors (T.W. and M.V.R.S.). When it was not possible to judge the studies by title and abstract, the full text was accessed and read for the final decision. The second stage consisted of reading the potentially eligible studies' full texts based on the eligibility criteria through the PICOS strategy. Disagreements on study inclusion were solved by a consensus with a third author (E.J.N.L.S.)

Data extraction

Two authors (T.W. and M.V.R.S.) independently collected the data from the included studies. Disagreements were solved by a third author (E.J.N.L.S.). The following data were extracted from the included studies: author(s), year of publication, assessed outcome, sample size (per group), teeth evaluated, intervention, irrigants, concentration, volume, method of endotoxin collection, method of endotoxin measurement, method of bacterial collection, method of bacterial measurement, moments of evaluation, outcomes, main findings. In cases of missing data, the authors were contacted 3 times by e-mail.

Quality assessment

The methodological risk assessment of bias for each study was performed by 2 independent authors (T.W. and M.V.R.S.), and, in case of disagreement, it was resolved by a third author (E.J.N.L.S.).

The qualitative analysis of the randomized studies was performed using the Cochrane risk of bias tool for RCTs (Cochrane Risk of Bias 2 - RoB2): 'Bias Risk Assessment of Randomized Controlled Studies' – Cochrane Handbook 6.0 [37]. The following domains were considered: randomization process; deviations from intended interventions; missing outcome data; measurement of the outcome; selection of the reported results.

Each included study was judged as 'high' risk of bias for negative domain response (red), 'low' risk of bias for positive domain response (green), and risk of 'some concerns' bias (yellow) when the response was not clear. When the study was judged as 'some concerns', the authors were contacted by email at least 3 times for more information and allowed to be classified as 'low' (green) or 'high' (red) risk of bias. Once this information was not possible to be acquired, the articles remained with some 'some concerns' bias risks. Overall quality was based on the scores in individual domains. When it was verified a low risk of bias for all domains, the overall quality was of low risk of bias. When at least 1 domain was of some concerns risk, the overall quality was some concerns risk of bias. Also, the assessment of at least 1 domain as high risk or 3 or more domains as some concerns risk resulted in an overall quality of high risk of bias.

Quantitative analysis

Meta-analysis was performed on the included studies that provided data regarding bacterial reduction, samples presenting cultivable bacteria after chemomechanical preparation, and endotoxin reduction, using RevMan software (version 5.3, The Cochrane Collaboration). The mean difference was used to assess bacterial reduction and endotoxin reduction (by entering means and standard deviations for continuous data), and odds ratio was used to assess the presence of cultivable bacteria after chemomechanical preparation (by entering the number of positive samples in each of the intervention groups for dichotomous data) [38]. In case of missing data, the authors were contacted to provide additional information. The statistical heterogeneity was assessed using the T^2, Cochran Q test and I^2 statistics. An I^2 statistic below 30% was considered as not important, between 30% and 60% was considered as moderate heterogeneity, between 50% and 90% as substantial heterogeneity, and over 75% was considered as considerable heterogeneity [38,39]. If possible, sensitivity analysis was performed excluding studies presenting 'some concerns' or 'high' risk of bias and studies that performed root canal treatments or retreatments. Publication bias will be visually assessed by the generation of funnel plots only when 10 or more studies are included in a meta-analysis [40].

Strength of evidence

The strength of the evidence of the included studies was assessed using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) tool (GRADEpro GDT: GRADEpro Guideline Development Tool [Software]). McMaster University, 2015 (developed by Evidence Prime, Inc.), available from gradepro.org: https://gdt.gradepro.org/app/handbook/handbook.html#h.rkkjpmwb6m6z [41]. The GRADE tool has 5 domains that can be downgraded and reduce the quality of the evidence. The following domains were considered to assess the strength of the evidence: risk of bias; inconsistency; indirectness; imprecision; other considerations.

RESULTS

Study selection

Initial screening of databases resulted in 2.765 studies identified, where 1.305 were excluded as they were duplicates, as presented in **Figure 1**. After title and abstract reading, 1.451 records were excluded and 9 studies matched the inclusion criteria [23,42-49].

After full-text reading, 3 studies were excluded [23,42,43]. One study for not presenting the data after chemomechanical preparation; 1 study for using liquid CHX instead of CHX gel; and 1 study for not comparing the results with a NaOCl group [23,42,43]. Therefore, 6 studies were included for analysis in the present systematic review [44-49].

Data extraction

Table 1 presents the characteristics and main findings of the included studies.

Regarding the assessed outcomes, 1 study assessed bacterial reduction only; 3 studies assessed endotoxin reduction only; and 2 studies assessed bacterial and endotoxin reduction [44-49]. Of the studies that evaluated endotoxin reduction, only 1 study assessed lipoteichoic

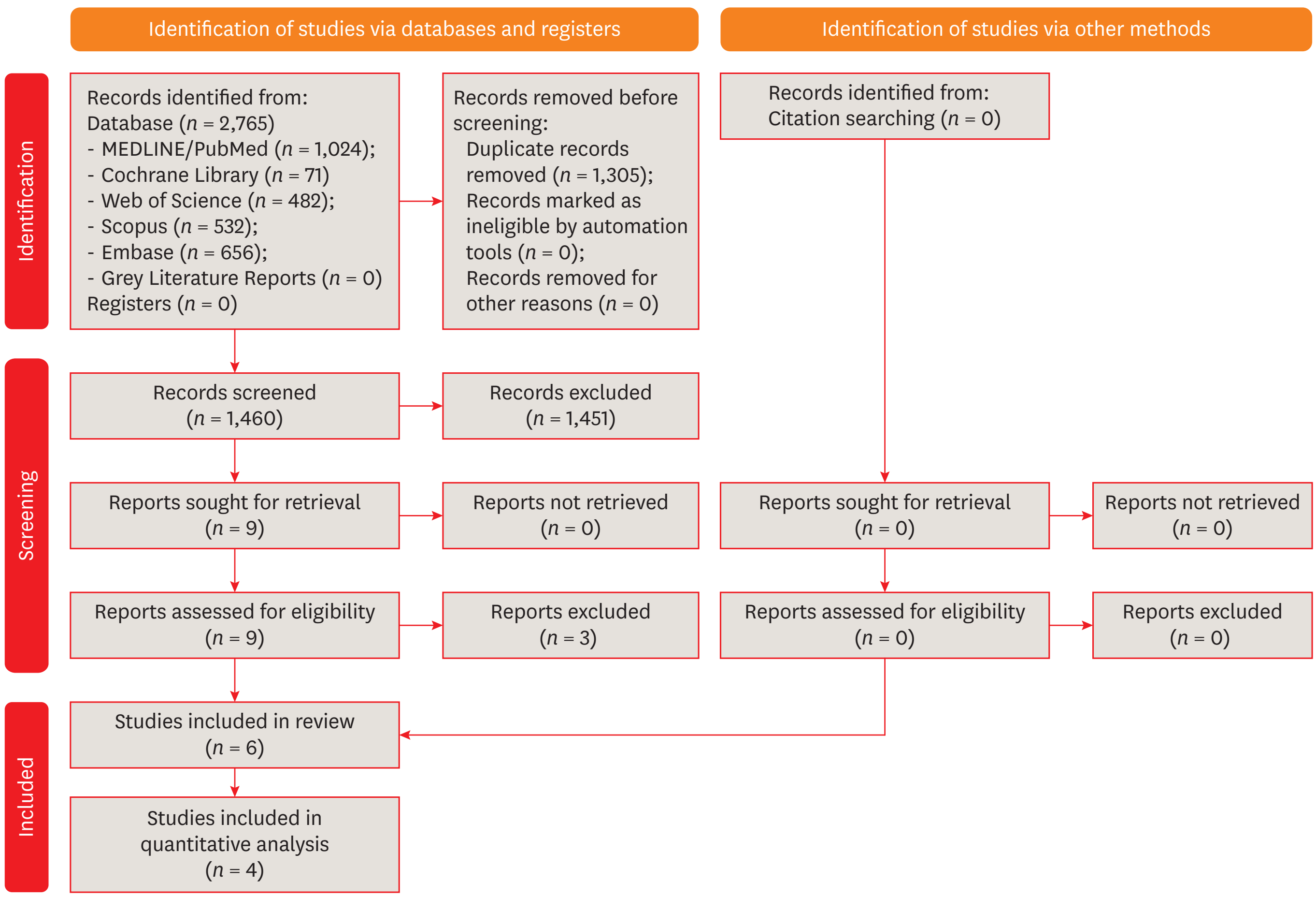

Figure 1. Flow diagram of the systematic search according to Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines.

acid (LTA) reduction; and 4 studies assessed lipopolysaccharide (LPS) reduction [45-49]. None of the included studies evaluated periapical healing.

In relation to the group of teeth evaluated, all studies included only uniradicular teeth with a single root canal [44-49]. Most of the studies included only patients with a diagnosis of asymptomatic apical periodontitis; and only 1 study included patients presenting a diagnosis of symptomatic and asymptomatic apical periodontitis [44-49]. As for the interventions, 1 study performed non-surgical root canal retreatment; and 5 studies [44-49] performed non-surgical root canal treatments.

Regarding bacterial assessment, 2 studies reported using sterile paper points for bacterial collection; and 1 study reported using sterile/apyrogenic paper points [44,45,49]. Two studies reported using the colony forming unit (CFU) technique for bacterial measurement; and 1 study CFU technique and real-time quantitative-polymerase chain reaction using 2 different assay chemistries (SYBR Green PCR Master Mix, and TaqMan PCR Master Mix; Applied Biosystems, Waltham, MA, USA) [44,46,49].

As for endotoxin assessment, 1 study reported using sterile paper points for endotoxin collection; and 4 studies reported using sterile/apyrogenic paper points [44-49]. One study reported using the enzyme-linked immunosorbent assay (ELISA) for endotoxin

Table 1. Characteristics and main findings of the included studies

Author(s) (year of publication)	Assessed outcome	Sample size (per group)	Teeth evaluated/ diagnosis	Intervention	Irrigants/ concentration/ protocol	Method of endotoxin collection	Method of endotoxin measurement	Method of bacterial collection	Method of bacterial measurement	Moments of evaluation	Outcomes	Main findings
Barbosa-Ribeiro *et al.* (2016) [49]	Bacterial and endotoxin (LTA) reduction	20 (CHX gel – *n* = 10; NaOCl – *n* = 10)	Uniradicular teeth with a single root canal/ symptomatic or asymptomatic apical periodontitis	Non-surgical retreatment	CHX gel: 2%/1 mL before each instrument and 5 mL saline solution rinse afterward with EndoVac System; NaOCl: 6%/1 mL before the use of each instrument and 5 mL rinse afterward with EndoVac System	Sterile paper point	Enzyme-linked immunosorbent assay	Sterile paper point	CFU technique	S1: Before; S2: and after chemomechanical preparation; S3: After 30 days of intracanal medication ($Ca(OH)_2$ + 2% CHX gel)	S1: All samples presented cultivable bacteria and LTA; S2: 2% CHX gel reduced significantly more bacteria (99.3%–92.1%) and LTA (26.9%–22.6%) than 6% NaOCl; S3: Intracanal medication significantly decreased cultivable bacteria (99.5%) and LTA (38.6%), without differences between groups	Reduction of cultivable bacteria was greater than the reduction of LTA in all phases of endodontic retreatment; intracanal medication favored a greater bacterial and LTA removal
Gomes *et al.* (2009) [45]	Endotoxin (LPS) reduction	54 (CHX gel – *n* = 27; NaOCl – *n* = 27)	Uniradicular teeth with a single root canal/ asymptomatic apical periodontitis	Non-surgical root canal treatment	CHX gel: 2% /1 mL CHX gel + 4 mL of sterile saline solution before each instrument; NaOCl: 2.5%/5 mL following each instrument used	Sterile/ apyrogenic paper point	LAL method, a modified LAL and a synthetic color-producing substrate to detect endotoxin chromogenically	-	-	S1: Before; S2: and after chemomechanical preparation	S1: Endotoxin was present in all samples; S2: Endotoxin reduction per group: 2,5% NaOCl – 57.98%; 2% CHX gel – 47.126%	Both substances were not effective in completely removing endotoxins from primarily infected root canals; NaOCl presented a significantly higher reduction when compared to CHX gel
Marinho *et al.* (2014) [47]	Endotoxin (LPS) reduction	30 (CHX gel – n: 10; NaOCl – n: 10; Saline solution – n: 10)	Uniradicular teeth with a single root canal/ asymptomatic apical periodontitis	Non-surgical root canal treatment	CHX gel: 2%/1 mL CHX gel + 5 mL of sterile saline solution following each instrument used; NaOCl: 2.5%/5 mL following each instrument used; Saline Solution (SS): 5 mL following each instrument used	Sterile/ apyrogenic paper point	Turbidimetric kinetic limulus amoebocyte lysate (LAL) assay	-	-	S1: Before; S2: and after chemo-mechanical preparation; S3: After 3 minutes of 17% EDTA; S4: After 30 days of intracanal medication ($Ca(OH)_2$ + saline solution)	S1: Endotoxins were present in all samples; S2: Reduction occurred in all groups without differences: 2,5% NaOCl – 99.65%; 2% CHX gel – 94.27%; SS – 96.79%; S3: 17% EDTA did not significantly decrease endotoxins; S4: Intracanal medication significantly reduced residual endotoxins compared to S3 (2,5% NaOCl – 90%; 2% CHX gel – 88.8%; SS: 85.7%), but without differences compared to S2	No differences were found among substances used during root canal preparation; the use of intracanal medication for 30 days contributed for improvement of the endotoxin reduction

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Table 1. (Continued) Characteristics and main findings of the included studies

Author(s) (year of publication)	Assessed outcome	Sample size (per group)	Teeth evaluated/ diagnosis	Intervention	Irrigants/ concentration/ protocol	Method of endotoxin collection	Method of endotoxin measurement	Method of bacterial collection	Method of bacterial measurement	Moments of evaluation	Outcomes	Main findings
Marinho *et al.* (2015) [48]	Endotoxin (LPS) reduction	30 (CHX gel – n: 10; NaOCl – n: 10; Saline solution – n: 10)	Uniradicular teeth with a single root canal/ asymptomatic apical periodontitis	Non-surgical root canal treatment	CHX gel: 2% / 1 mL CHX gel + 5 mL of sterile saline solution following each instrument used; NaOCl: 2.5%/5 mL following each instrument used; Saline Solution (SS): 5 mL following each instrument used	Sterile/ apyrogenic paper point	Turbidimetric kinetic LAL assay	-	-	S1: Before; S2: and after chemo-mechanical preparation; S3: After 3 minutes of 17% EDTA; S4: After 30 days of intracanal medication ($Ca(OH)_2$ + saline solution); S5: Before root canal obturation	S1: Endotoxin was present in all samples; S2: Significant higher reduction was achieved with 2,5% NaOCl when compared to 2% CHX gel and SS; S3: 17% EDTA significantly improved endotoxin reduction in 2% CHX gel and SS groups, without differences between them; S4: Intracanal medication for 30 days significantly reduced endotoxins; S5: No differences were found between 2,5% NaOCl and 2% CHX gel groups, only when compared to SS group	The greatest endotoxin reduction occurred after chemomechanical preparation; intracanal medication enhanced endotoxin reduction
Vianna *et al.* (2006) [44]	Bacterial reduction	32 (CHX gel – *n* = 16; NaOCl – *n* = 16)	Uniradicular teeth with a single root canal/ asymptomatic apical periodontitis	Non-surgical root canal treatment	CHX gel: 2%/1 mL CHX gel + 4 mL of sterile saline solution after each instrument; NaOCl: 2.5%/5 mL following each instrument used	-	-	Sterile paper points		S1: Before; S2: and after chemomechanical preparation	<u>RTQ-PCR results:</u> S1: Bacterial loads of both groups were comparable; S2: Bacterial reduction was significantly different between groups, irrespective of the detection system (SYBR Green/TaqMan): NaOCl group -99.99%/99.63%; CHX gel group - 96.62%/96.60%; <u>CFU results:</u> S1: Microorganisms were detected in all samples; S2: Mean bacterial reduction was similar in both groups: NaOCl group -99.93%; CHX gel group – 99.69%	NaOCl has presented a higher capacity to kill microorganisms and to remove cells from the root canal

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Table 1. (Continued) Characteristics and main findings of the included studies

Author(s) (year of publication)	Assessed outcome	Sample size (per group)	Teeth evaluated/ diagnosis	Intervention	Irrigants/ concentration/ protocol	Method of endotoxin collection	Method of endotoxin measurement	Method of bacterial collection	Method of bacterial measurement	Moments of evaluation	Outcomes	Main findings
Xavier *et al.* (2013) [46]	Endotoxin (LPS) and bacterial reduction	48 (CHX gel – *n* = 12; CHX gel + calcium hydroxide dressing – *n* = 12; NaOCl – *n* = 12; NaOCl + calcium hydroxide dressing – *n* = 12)	Uniradicular teeth with a single root canal/ asymptomatic apical periodontitis	Non-surgical root canal treatment	CHX gel: 2%/1 mL CHX gel + 4 mL of sterile saline solution before each instrument; NaOCl: 1%/5 mL following each instrument used;	Sterile/ apyrogenic paper points	Kinetic chromogenic LAL assay	Sterile/ apyrogenic paper points	CFU technique	1-visit groups: S1: Before; S2: and after chemomechanical preparation 2-visit groups: S1: Before chemomechanical preparation; S2: after calcium hydroxide removal	Endotoxins: Detected in all samples; 1- and 2-visit treatment significantly reduced endotoxins: 1% NaOCl: 86.33%;/98.01%; 2% CHX gel: 84.77%/96.81%; 2-visit treatment reduced more endotoxins than 1-visit Bacteria: Detected in all samples; 1- and 2-visit treatment significantly reduced bacteria, without differences: 1% NaOCl: 99.97%/99.90%; 2% CHX gel: 99.75%/96.62%	1- and 2-visit root canal treatment protocols were effective in reducing bacteria and endotoxins, but did not completely eliminate them; 2-visit treatment was more effective in reducing endotoxins than 1-visit treatment.

LTA, lipoteichoic acid; CHX, chlorhexidine; NaOCl, sodium hypochlorite; LPS, lipopolysaccharide; CFU, colony forming unit; LAL, limulus amoebocyte lysate; EDTA, ethylenediaminetetraacetic acid; RTQ-PCR, real-time quantitative-polymerase chain reaction.

measurement; 1 study reported using a limulus amoebocyte lysate (LAL) method, a modified LAL and a synthetic color-producing substrate to detect endotoxin chromogenically; 2 studies reported a turbidimetric kinetic LAL assay; and 1 study a kinetic chromogenic LAL assay for endotoxin measurement [45-49].

After chemo-mechanical preparation, 1 study reported a significantly higher bacterial and endotoxin reduction for the CHX gel group when compared to the NaOCl group; 2 studies reported a significantly higher endotoxin reduction for the NaOCl group; 1 study reported a significantly higher bacterial reduction for the NaOCl group; 2 studies reported no statistical differences between substances for endotoxin reduction; and 1 study reported no statistical differences between substances for bacterial reduction [44-49].

Quality assessment

Risk of bias in the included studies is summarized in **Figure 2** [50]. From the 6 included studies, 5 studies were considered as presenting a low risk of bias, with all domains classified as low risk of bias [44,45,47-49]. Only 1 study was considered as presenting some concerns risk of bias, with 1 domain (randomization) classified as some concerns risk [46].

Quantitative analysis

Meta-analysis was performed on 2 studies for bacterial reduction (**Figure 3A**); 3 studies for the presence of cultivable bacteria after chemomechanical preparation (**Figure 3B**); and 3 studies for endotoxin reduction (**Figure 3D**) [44-46,48,49]. Additional data regarding the values of endotoxin reduction from 2 studies, and additional data regarding samples with cultivable bacteria after chemomechanical preparation from 1 study were obtained through the corresponding author of these studies [45,47,49]. Authors from another study were contacted for additional data regarding endotoxin reduction and although a response was obtained, no additional data was received and for this reason, the study was not included in the quantitative analysis of endotoxin reduction [46]. Sample sobreposition was observed in 2 studies and, for this reason, only the latter was included in the quantitative analysis [47,48]. Additionally, since only 6 studies were included in the meta-analyses, no funnel plot was generated to detect publication bias.

The authors used random-effects meta-analysis to produce the overall summaries due to the high heterogeneity. In relation to the bacterial reduction (**Figure 3A**), the forest plot indicates that the data are considerably heterogeneous ($I^2 = 74\%$) and that there were no statistically significant differences ($p = 0.67$; CI, 75.03 [−271.15, 421.22]) among the irrigants. For the presence of cultivable bacteria after chemomechanical preparation (**Figure 3B**), the forest plot indicates that the data presented moderate heterogeneity ($I^2 = 49\%$), and the overall

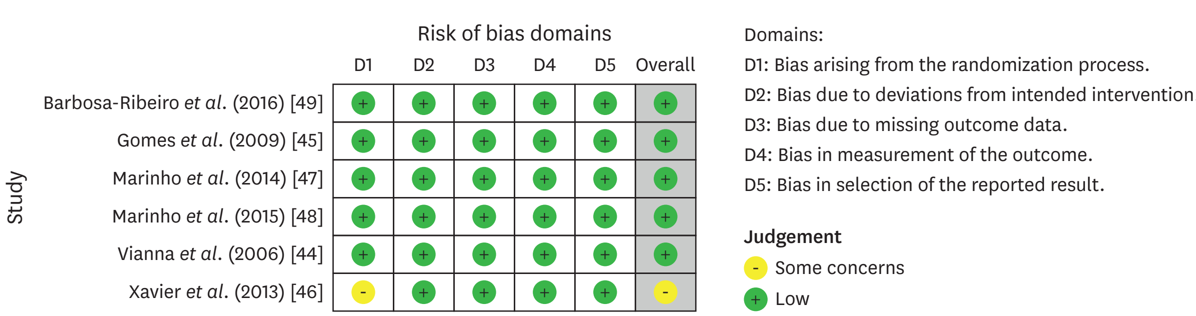

Figure 2. Quality assessment of the included studies according to the Cochrane Collaboration common scheme for bias and RoB 2 tool.

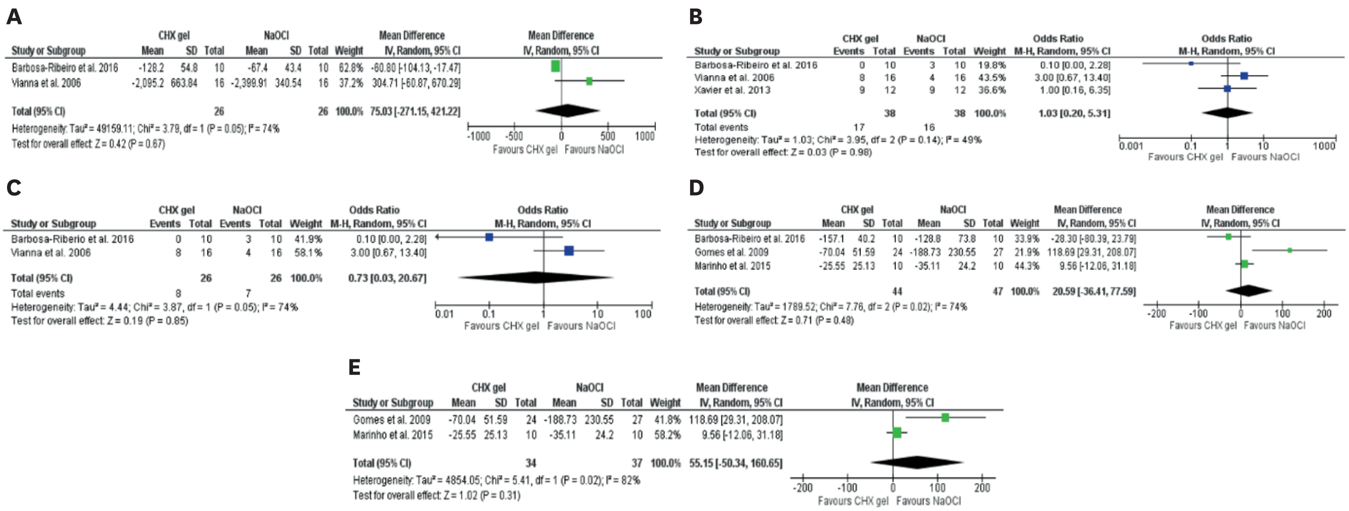

Figure 3. Meta-analysis results. (A) Forest plot of bacterial reduction after chemomechanical preparation with chlorhexidine gel and sodium hypochlorite; (B) Forest plot of cultivable bacteria after chemomechanical preparation with chlorhexidine gel and sodium hypochlorite; (C) Forest plot of cultivable bacteria after sensitivity analysis that excluded values from Xavier *et al.* [46]; (D) Forest plot of endotoxin reduction after chemomechanical preparation with chlorhexidine gel and sodium hypochlorite; (E) Forest plot of endotoxin reduction after sensitivity analysis that considered only studies that performed root canal treatments. CHX, chlorhexidine; NaOCl, sodium hypochlorite; SD, standard deviation; CI, confidence interval.

effect also demonstrated no statistically significant differences among irrigants ($p = 0.98$; CI, 1.03 [0.20, 5.31]). A sensitivity analysis was performed by removing the study of Xavier *et al.* [46]. Results (**Figure 3C**) indicate an overall high heterogeneity ($I^2 = 74\%$) and the overall effect did not demonstrate statistically significant differences ($p = 0.85$; CI, 0.73 [0.03, 20.67]). Regarding endotoxin reduction (**Figure 3D**), the forest plot indicates that data were considerably heterogeneous ($I^2 = 74\%$). Also, the overall effect demonstrated no statistically significant differences among irrigants ($p = 0.48$; CI, 20.59 [−36.41, 77.59). A sensitivity analysis was performed by considering only studies that performed root canal treatments (**Figure 3E**). Again, results indicate a high heterogeneity ($I^2 = 82\%$) and an overall effect that do not indicate statistically significant differences among irrigants ($p = 0.31$; CI, 55.15 [−50.34, 160.65]).

Strength of evidence

GRADE results are presented in **Table 2**.

The GRADE tool demonstrated a very low quality of evidence for the included studies [44-49]. The studies received the "not serious" classification for risk of bias and indirectness, "serious" classification for inconsistency, and "very serious" classification for imprecision. Other considerations domain did not influence the quality of evidence.

Table 2. Assessment of quality of evidence of the included studies

No. of studies (study design)	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Overall certainty of evidence
6 randomized trials	Not serious	Serious*	Not serious	Very serious†	None	⨁◯◯◯ VERY LOW

*Substantial heterogeneity was observed among studies; †Optimal information size was not met (pooled sample size of 300) and confidence intervals were under 0.75 or above 1.25.

DISCUSSION

This is the first systematic review that sought to encompass only studies that investigated the disinfectant effectiveness of CHX on its gel formulation compared to NaOCl. So far, previous systematic reviews evaluating the disinfecting effectiveness of NaOCl and CHX comprise both liquid and gel formulations in the same analyses, which prevents direct comparison of results [30-33]. Therefore, it is not possible to determine whether the CHX gel could exert the same disinfectant effectiveness compared to NaOCl.

In this systematic review, both bacterial reduction and cultivable bacteria after chemomechanical preparation were evaluated, and no statistical differences were observed in both methods ($p = 0.67$ and $p = 0.98$, respectively). Additionally, 1 sensitivity analysis was performed for cultivable bacteria, excluding the study by Xavier *et al.* [46], since this had a "some concerns" risk of bias. Again, sensitivity analysis also pointed to no statistical differences ($p = 0.85$). Although slight differences can be observed in the methodology adopted by the included studies, these results suggest that both substances can successfully reduce the bacterial content of the root canal during chemomechanical preparation [44,46,49].

As for the detoxification (endotoxins –LPS or LTA - removal) activity of both substances, previous studies have shown limited efficacy for both CHX and NaOCl [51-55]. Only 1 study demonstrated that NaOCl in higher concentrations (5.25%) was able to induce loss of detection of lipid A peaks and no detection of LPS bands in an *in vitro* model, suggesting a concentration-dependent detoxification activity [55]. However, based on the included studies, the available evidence suggests that the major content of endotoxin removal occurs through the mechanical debridement of the root canal, irrespective of the irrigant solution used ($p = 0.48$) [44-49].

Nevertheless, these findings present major limitations. A moderate to high heterogeneity was observed (37%–82%). This may be related to the small number of studies included in the meta-analyses or to clinical diversities that are not possible to be determined through the information presented by the studies [38]. Additionally, another important limitation is that none of the included studies had presented a sample size calculation [44-49]. Sample size calculation ensures that the sample size is adequate for the study, otherwise, it may be not possible to detect true differences in outcomes between investigated groups (type II error), or wrongly conclude that 1 intervention is more effective than the other when, in fact, it is not (type I error) [56]. Thus, increasing the heterogeneity shown by the presented forest plots.

All studies performed their evaluations only in uniradicular teeth with a single root canal [44-49]. It is well known that the anatomic complexity of the root canal system associated with the biofilms' organization and its defensive capacity against chemical solutions are important factors that can predict treatments failure [57,58]. Also, CHX does not present the capacity to dissolve necrotic tissue remnants or to disrupt biofilm structures [8,14,21,59]. Therefore, its disinfectant ability may be impaired in complex anatomies, such as isthmus, flattened root canals and apical ramifications. Although mechanical debridement can significantly reduce the infectious content from the root canal, the instruments action is limited, especially in complex anatomies, regardless of the instrument used [5,60]. Thus, the present findings may overestimate the true effectiveness of the solution, especially for having evaluated teeth with little anatomical complexities.

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Second, none of the included studies reported for retention time of irrigants inside the root canal during chemomechanical preparation. This fact, associated with the low volumes of irrigants used among the included studies, can be considered a limitation, since it is reported that a longer exposure time and a higher volume of NaOCl during chemomechanical procedures can promote a significantly greater bacterial elimination [11,44-49,61].

In addition, none of the studies evaluated the periapical healing of the tested treatment protocols. Periapical healing is directly related to the long-term success of root canal therapy and, thereby, can be considered as the true clinical outcome. Since, so far, no study has verified periapical healing when using CHX gel, it is not possible to determine whether the use of this substance can influence or not the long-term success of the therapy.

It is important to emphasize that 5 studies had a low risk of bias, and only 1 study had some concerns due to bias arising from the randomization process [44-49]. In this study, the authors stated that a randomization was performed, but did not describe the randomization and nor the allocation concealment method [46].

As for the overall quality of evidence, the classification provided by the GRADE tool was of very low quality. The domain 'risk of bias' received the not serious classification, because none of the included studies had a high risk of bias [62]. The domain 'inconsistency' received a serious classification, since a substantial heterogeneity was verified among the included studies, as presented in the meta-analysis results [63]. The domain 'indirectness' received the not serious classification, since no included studies performed indirect comparisons or presented indirect results [64]. The domain 'imprecision' received a very serious classification, because the optimal information size (pooled sample size of 300) was not met and confidence intervals were under 0.75 or above 1.25 [65]. Regarding the domain 'other considerations', none of the domains' criteria (publication bias, large effect, plausible confounding and dose-response gradient) were observed in the included studies [66].

This systematic review confirms the need for well-conducted research, with a great need for studies evaluating the disinfectant ability of CHX gel on teeth presenting anatomical complexities and long-term studies evaluating the periapical healing of teeth subjected to root canal treatment where CHX gel is used during chemomechanical preparation, prior to its application in clinical practice. So far, although the results of the present systematic review suggest no differences in the disinfectant ability of CHX gel and NaOCl, this suggestion is based on studies presenting important limitations and in a very low quality of evidence. Thus, the use of CHX gel during clinical practice should not be recommended in the absence of better-quality information.

CONCLUSION

This study aimed to verify the capacity of CHX gel compared to NaOCl on bacteria and endotoxins reduction and, based on the findings of this systematic review, there seem to be no differences in the disinfectant ability of both substances. However, these findings must be cautiously interpreted since are based on limited studies with very low certainty of evidence.

SUPPLEMENTARY MATERIAL

Supplementary Table 1

Search strategy in each database

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