REVIEW ARTICLE

Antimicrobial Efficacy of Chlorhexidine and Sodium Hypochlorite in Root Canal Disinfection: A Systematic Review and Meta-analysis of Randomized Controlled Trials

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SIGNIFICANCE

Sodium hypochlorite and chlorhexidine are the most applied endodontic antimicrobial irrigants. However, it is unclear which of them is more effective. This meta-analysis conducted from randomized controlled clinical trials reveals that these irrigants have the same potent antimicrobial activity.

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ABSTRACT

Introduction: We aimed to compare the antimicrobial efficacy of chlorhexidine (CHX) and sodium hypochlorite (NaOCl), 2 irrigants routinely used in root canal therapy of permanent teeth. **Methods:** Electronic databases, including PubMed, EMBASE, Web of Science, and Cochrane Library, were searched for randomized controlled trials published until March 2020. The meta-analysis of relative risk (RR) and standardized mean difference (SMD) was performed using a random effects model with a 95% confidence interval (CI). Subgroup analysis was performed for culture and molecular methods of bacterial detection. **Results:** The literature search yielded 2110 records without duplicates. Eight studies were eligible for a systematic review. No significant differences in the incidence of samples with positive bacterial growth after irrigation (RR = 1.003; 95% CI, 0.729–1.380; $P = .987$) and mean bacterial number changes (SMD = 0.311; 95% CI, −0.368 to 0.991; $P = .369$) were observed between CHX and NaOCl in the culture and molecular subgroups. Heterogeneity in RR ($I^2 = 0.000\%$, $P = .673$) was low among studies, whereas considerable heterogeneity was observed in the analysis of SMD ($I^2 = 76.336\%$, $P = .005$). **Conclusions:** Our findings suggest that both CHX and NaOCl irrigation can reduce bacterial infections without any significant difference in antimicrobial efficacy between them, despite their difference in molecular mechanisms. Therefore, each can be used as the main antibacterial root canal irrigant. However, our results were limited by inconsistencies among retrieved articles and a lack of clinically relevant outcomes. Further well-designed clinical studies are warranted to supplement our results. *(J Endod 2020;46:1032–1041.)*

KEY WORDS

Chlorhexidine; endodontic treatment; meta-analysis; root canal irrigant; root canal disinfection; sodium hypochlorite

Bacteria and their by-products are the main etiologic factors for pulpal and periapical diseases[1–3]. The goals of endodontic treatment are to achieve complete disinfection and prevent reinfection in the root canal system and periapical tissues. Sterilization of root canals is limited by the presently available techniques, instruments, and irrigants[4]. Thus, the focus should be on reducing intracanal bacterial populations to levels that are compatible with periapical tissue healing[4]. Chemomechanical preparation, including both mechanical instrumentation and chemical irrigation, is crucial for decreasing bacterial population. Mechanical instrumentation alone is insufficient to yield effective disinfection[5] because the complexity of root canal anatomy[6,7] prevents the accessibility of instrumentation and provides a shelter for microorganisms[8]. The bacteria remaining in the root canal at the time of root filling cause persistent infection and treatment failure[9]. Therefore, to achieve adequate disinfection, mechanical instrumentation should be supplemented with chemical irrigation methods.

Sodium hypochlorite (NaOCl) is the most widely used irrigant during endodontic treatment[10] because of its effective antimicrobial[11,12] and tissue-dissolving properties[13]. Several concentrations of NaOCl ranging from 0.5%–5.25% were found in the endodontic literature, and the most widely used concentration is 2.5%[14]. Although higher concentrations of NaOCl may exert stronger antimicrobial activity[15] and tissue-dissolving properties[13], they can lead to increased cytotoxicity[16] and periapical tissue irritation[17].

Chlorhexidine (CHX) is an alternative irrigant to NaOCl because of its broad-spectrum antimicrobial activity[18,19] and considerably lower toxicity than NaOCl[20]. The most widely used concentration of CHX for root canal therapy is 2%. In contrast to NaOCl, high concentrations of CHX exert a bactericidal effect, whereas low concentrations provide only a bacteriostatic effect[21]. CHX can be used either as a gel or solution with the same effectiveness[15]. It exhibits the unique property of substantivity; the positive charges of the CHX molecule bind to the negative charges on dental surfaces resulting in prolonged adherence, which in turn leads to long-lasting antimicrobial activity[21,22]. However, as an endodontic irrigant, the lack of tissue-dissolving capacity of CHX is a considerable drawback[23].

Recent literature shows no agreement on the antimicrobial efficacy of CHX versus NaOCl because various studies presented contradictory results[24–26]. A previous systematic review on the comparison of antimicrobial efficacy of CHX and NaOCl concluded that the number of clinical studies was scarce and inconsistent[27] and proposed that additional well-designed randomized controlled trials (RCTs) should be conducted. A meta-analysis is required to provide robust evidence and improve clinical outcomes. Therefore, we performed a systematic review followed by a meta-analysis of available RCTs investigating the antimicrobial efficacy of CHX and NaOCl in root canal disinfection to improve the outcome of endodontic treatment.

MATERIALS AND METHODS

This study complies with the Preferred Reporting Items for Systematic Reviews and Meta-analysis Statement (PRISMA)[28]. PICOS is defined by the following characteristics: Population, participants with pulpal and/or periapical disease who received endodontic treatment in permanent teeth; Intervention, CHX irrigant; Comparison, NaOCl irrigant; Outcome, the primary outcome was reduction in the bacterial abundance and incidence of positive bacterial samples after irrigation, whereas the secondary outcome was an improvement of clinical symptoms, periapical tissue healing, and postoperative pain; and Study type, RCTs. The protocol was registered in PROSPERO a priori (registration number: CRD42019127651) (Supplemental Appendix S1 is available online at www.jendodon.com).

Search Strategy

A systematic search of electronic databases, including PubMed (via MEDLINE), EMBASE, Web of Knowledge, and Cochrane Library (CENTRAL), was conducted until March 2020. Reference lists from the identified records were also searched for all English-language articles. The following search queries in each database were based on PICO components combined with the Boolean operators and restricted to clinical trials for humans (Supplemental Appendix S2 is available online at www.jendodon.com).

1. Medical subject headings (MeSH) or entree terms for "Dental pulp cavity" or "Root canal therapy" and related terms
2. Use of chemical substances as endodontic irrigants
3. Irrigation or disinfection and synonyms
4. 1 AND 2 AND 3

Inclusion and Exclusion Criteria

The inclusion criteria were RCTs that used irrigants in root canal therapy of permanent teeth with pulpal and/or periapical disease. These studies compared the antimicrobial effects between CHX and NaOCl irrigants and reported the outcome as bacterial reduction using bacterial cultivation and/or molecular microbiological methods. Studies that did not compare the individual effects of NaOCl and CHX and those performed in primary teeth or open apex teeth were excluded from this meta-analysis.

Study Selection

All the records were imported to EndNote X8 (Clarivate Analytics, Philadelphia, PA). After the removal of duplicates, the titles and abstracts of the remaining records were screened independently for eligibility by 2 reviewers (K.R. and W.S.). In case of any disagreement, a third reviewer (Z.L.) was consulted to achieve a consensus.

Data Extraction

Two reviewers (K.R. and W.S.) independently extracted data from full-text studies that fulfilled the inclusion criteria by using a standardized data collection form. If multiple treatment groups were presented in a study, the data exclusively conforming with PICOS were collected. Moreover, 3 authors provided further information for a better risk of bias evaluation[25,26,29] and the mean with standard deviation values[26,29] through personal e-mail communication.

Quality Assessment

The quality of each RCT was assessed according to the Cochrane Risk of Bias Tool[30]. All 6 domains and the "other bias" domain, which was defined as a unit of randomization and irrigation protocol, were rated as "high," "unclear," and "low" risk of bias. To summarize the overall risk of bias, we considered only the following as key domains:

(1) randomization processes,
(2) allocation concealment,
(3) incomplete outcome data, and
(4) other bias.

The judgment was based on key domains and categorized as a "low" risk of bias when more than half of all the key domains were low. A study was categorized as a "high" risk of bias when there were at least 2 "high" key domains. Apart from these criteria, the overall result was considered "unclear."

Statistical Analysis

Relative risk (RR) was calculated for studies that reported the detection of samples showing positive and negative bacterial growth after irrigation. For studies reporting the number of bacteria before and after irrigation, the standardized mean difference (SMD) was calculated. The 95% confidence intervals (CIs) were calculated for RR and SMD to compare the antimicrobial efficacy of CHX and NaOCl. Subgroup analysis was conducted if sufficient data were obtained. The significance of any variation and degree of heterogeneity was determined by I^2 and chi-square statistics, respectively[31]. Pooled estimates were calculated with a random effects model using the DerSimonian-Laird method. Because of the low number of studies included, publication bias tests were not conducted. We attempted to perform trial sequential analysis to estimate the information size related to the imprecision of outcomes; however, it could not be performed because of low information size. Comprehensive Meta-Analysis Software Version 3 (Biostat, Englewood, NJ) was used to compute the RR and SMD.

Certainty of Evidence Assessment

The strength of evidence was evaluated according to the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) approach[32] using a summary of findings table constructed with GRADEpro Guideline Development Tool software (Evidence Prime,

Inc, Seattle, WA). Each GRADE criterion was assessed individually and then computed for the certainty of the evidence. To achieve transparency and implicity, the GRADE approach classifies the certainty of evidence into 1 of the following 4 grades: high, moderate, low, or very low.

RESULTS

Search and Selection of Studies

Figure 1 shows the flow diagram of the search and selection processes. The search strategy yielded 2763 records from the databases. One additional record[33] was identified in the reference list of a review article[34]. After removing duplicates, the remaining 2110 records were screened for titles and abstracts. A total of 2099 irrelevant records were found and removed. Finally, 11 articles were assessed for eligibility by full-text reviewing. At this stage, 3 articles were excluded because of the following reasons: 1 study reported only endotoxin level as an outcome[35], another study was a non-RCT[36], and the last one investigated only the effect of gaseous ozone in combination with NaOCl and CHX[37]. Finally, 8 studies were included for systematic review[24–26,29,33,38–40].

Although the 8 studies contained statistical data, 6 studies provided the number of samples with positive and negative bacterial growth[24–26,29,38,39] that could be included for RR analysis. One study reported only the number of visits that yielded negative culture[33], whereas another reported only secondary outcome parameters[40]; therefore, these 2 studies were not suitable for the quantitative analysis. Also, 1 of the 6 studies only reported selective bacterial strains[24], whereas another provided only the categorized data of bacteria[38]. Therefore, these 2 studies were not suitable for SMD analysis. Finally, 4 studies that revealed the number of bacteria before and after irrigation were eligible for SMD analysis[25,26,29,39].

Characteristics of the Studies Included

The characteristics of the RCTs are presented in Table 1. All the studies are single-center RCTs. They reported the concentrations and forms of irrigants. However, only 4 studies indicated the total amount of irrigants[26,29,38,40], whereas 3 studies reported the volume with instrumentation[24,25,39]. In addition, 1 study did not specify the volume of irrigants and vaguely mentioned "a copious amount of irrigant"[33].

All the studies used sterile paper points, either dry or soaked in transporting media, for sample collection. Only data taken from samples within the first visit were extracted. Of the 8 studies included in our meta-analysis, only 1 used both culture and molecular methods[25]. Three studies used molecular methods[26,29,40], whereas another 4 studies used culture methods[24,33,38,39]. However, none of the studies investigated fungi. Only 1 study assessed the clinical and radiographic outcomes after 1 and 4 years[40]; this was the follow-up of a previous study by Zandi et al[29].

Quality of Evidence

The risk of bias of the 8 RCTs is summarized in Figure 2. However, 1 pair of studies[29,40] was assessed as a single study because 1 of the studies was a continuation. Four studies were scored as an overall "low" risk of bias[25,26,29,40], although 2 of them[29,40] showed a "high" risk of bias of allocation concealment domain. Another 4 studies[24,33,38,39] were considered as an overall "unclear" risk of bias because none of them mentioned their randomization methods, allocation concealment, and irrigation in sufficient detail. In addition, 1 of them was assigned to "high" risk on the "other bias" domain[24]. Supplemental Appendix S3 (available online at www.jendodon.com) explains the risk of bias assessment for individual studies.

Meta-analysis

The forest plot shows the pooled RR of samples with positive bacterial growth after irrigation (Fig. 3). The results indicated no significant difference in the incidence of positive samples between CHX and NaOCl treatments (RR = 1.003; 95% CI, 0.729–1.380, $P = .987$; heterogeneity: $I^2 = 0.000\%$, $P = .673$). In addition, the results of the subgroup analysis, including culture (RR = 0.990; 95% CI, 0.649–1.509; $P = .962$; heterogeneity: $I^2 = 0.000\%$, $P = .408$) and molecular subgroups (RR = 1.020; 95% CI, 0.626–1.663; $P = .936$; heterogeneity: $I^2 = 0.000\%$, $P = .601$), showed no significant differences between CHX and NaOCl treatments. The heterogeneities of RR among studies were considered as low.

The forest plot comparing SMD is shown in Figure 4. The results indicated that the changes in the mean bacterial number were not significantly different between CHX and NaOCl treatments (SMD = 0.311; 95% CI, −0.368 to 0.991; $P = .369$). The data were considerably heterogeneous ($I^2 = 76.336\%$, $P = .005$). From this SMD forest plot, subgroup analysis for culture and molecular methods (Supplemental Figure S1 is available online at www.jendodon.com) was performed without calculating the overall result because the same patient population of 1 study was applied for both methods[25]. The forest plot revealed no significant differences among the 2 methods ($P = .880$), (culture method: SMD = 0.275; 95% CI, −0.765 to 1.315; $P = .605$; molecular method: SMD = 0.173; 95% CI, −0.636 to 0.982; $P = .675$). Substantial and considerable heterogeneities were observed in the culture ($I^2 = 69.449\%$, $P = .070$) and molecular ($I^2 = 81.463\%$, $P = .005$) subgroups, respectively.

Certainty of Evidence

The GRADE approach was used to rate the confidence of evidence obtained from our meta-analysis comparing the efficacy of the investigated irrigants on antibacterial parameters (Supplemental Table S1 is available online at www.jendodon.com). The total bacterial number reduction was graded as very low grade of evidence based on the serious inconsistency, indirectness, and imprecision domains. The incidence of samples with positive bacterial growth after irrigation was graded as low based on serious indirectness and imprecision domains.

DISCUSSION

In this study, we aimed to compare the antimicrobial efficacy of CHX and NaOCl irrigants in root canal therapy of permanent teeth. We found no significant differences in their antimicrobial efficacy.

Effective chemomechanical preparation using chemical substances can improve the clinical outcome and long-term success of endodontic treatment[41,42]. Our meta-analysis included only RCTs, which are considered as the high level in the hierarchy of evidence. Although numerous records were preliminarily retrieved from the 4 databases, only 8 RCTs remained eligible for a systematic review supplemented by the meta-analysis. However, inconsistent data (eg, infection types) were observed (Table 1) among the 8 RCTs. A single treated root canal with persistent infection can harbor a similar number of bacteria to that of untreated root canals with primary infection; however, the microbial diversity decreases after treatment in persistent infection[43]. Other factors such as tooth type, mechanical preparation, final canal enlargement and taper, irrigation protocols, and bacterial identification methods were also heterogeneous.

Various irrigation parameters of CHX and NaOCl were used in the included RCTs, especially concentrations and volume of irrigants. Their antimicrobial effect depends on the frequency[44] and contact time during irrigation[45]. Larger volume or longer contact time and frequent exchange of irrigants could

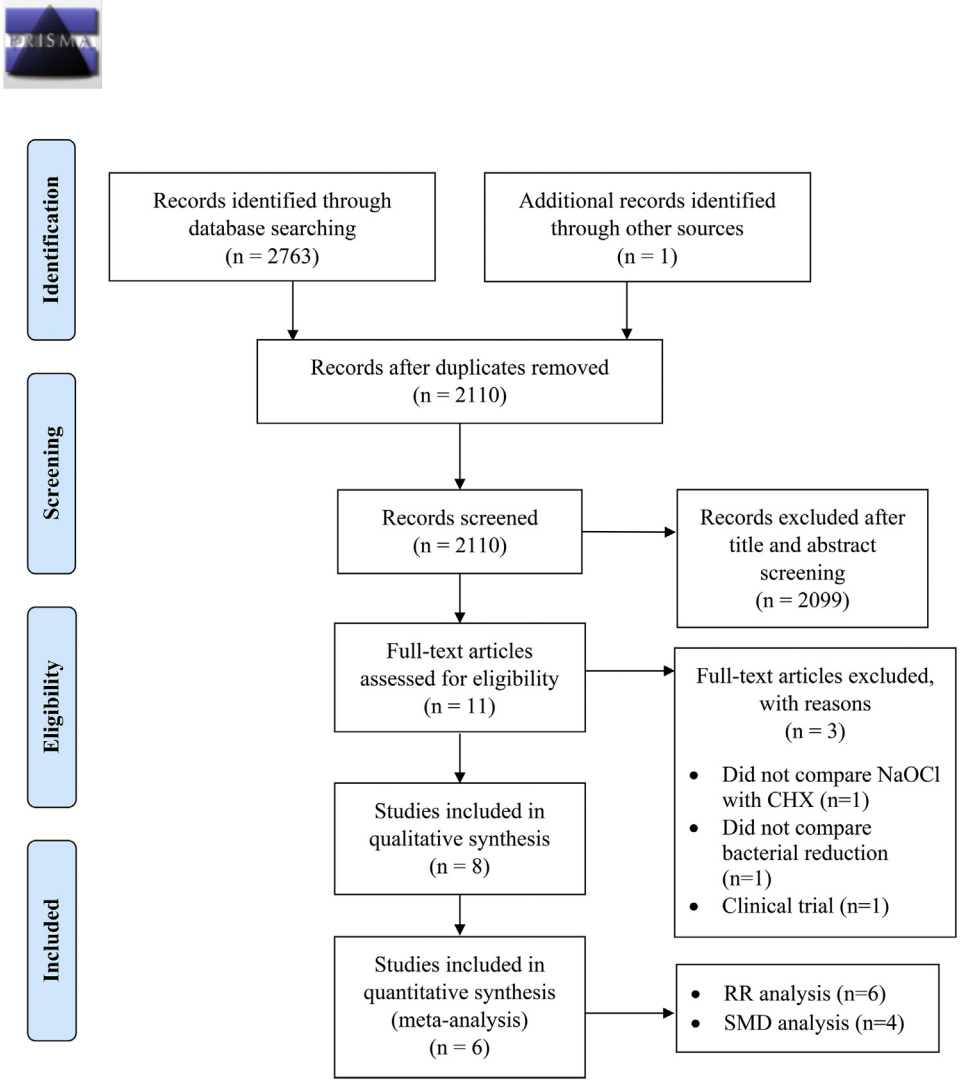

FIGURE 1 – A Preferred Reporting Items for Systematic Reviews and Meta-analysis Statement flowchart of the search and selection strategy.

compensate for the effects of smaller concentrations. However, these parameters were not precisely described in the trials. Furthermore, other factors, such as formulations[15], activation techniques and devices[46,47], and multivariable ratios of all parameters, are strongly related to the resulting antibacterial efficacy. Because of the limited data, we were not able to describe how these confounding factors affect our meta-analysis and perform further subgroup analysis. Because all these factors play a major role in the effectiveness of irrigants, future RCTs should ensure to publish all the details mentioned earlier.

Regarding sample collection, sterile paper points obtain bacteria only from the main root canal, and there is a lack of information on bacteria colonizing the hidden areas of the complex root canal[48,49]. Therefore, bacteria collected by this technique might not be perfect representatives of all bacterial populations in the entire root canal system. Nevertheless, at present, no better sampling method is available. Two bacterial detection methods after sample collection were described in the included studies. The first one is the culture method, which can estimate bacterial load and detect virulence factors or antibiotic susceptibility[50,51]. However, this method cannot characterize several microorganisms in parallel or identify uncultivable bacteria. The molecular method was introduced to overcome the limitations of semiqualitative culture techniques[43]. Studies in this meta-analysis used either the culture or molecular method, except for 1 study in which both the methods for bacterial quantification were applied[25]. In molecular methods, Taqman and SYBRGreen assays (Applied Biosystems, Foster City, CA) were also compared. However, the Taqman assay provides precise results when low target samples are used[25,52]. Consequently, we selected culture method results for RR analysis and molecular method results from the Taqman assay for SMD analysis. According to the varied sensitivity of culture and molecular methods, subgroup analysis for SMD was performed separately (Supplemental Figure S1 is available online at www.jendodon.com). The results indicated no significant difference in changes in the bacterial count after chemomechanical preparation between these 2 methods. This finding is in line with that of a recent *in vitro* study[53]. Thus, both methods are appropriate for endodontic bacterial detection.

The controversies among the results of the included RCTs might also be influenced by sample size because improper sample sizes will not give sufficient power to detect any

TABLE 1 - Characteristics of the Studies Included

Authors/publication year	Location and country of the study	Systemic antibiotic treatment	Tooth			Irrigation protocol			
			Number	Type	Infectious status	Type/concentration	Volume with instrumentation	Total volume	Exposure time
Ercan et al, 2004	NA	NA	30	Single-rooted teeth	Primary endodontic infection	2% CHX/5.25% NaOCl	2 mL CHX/2 mL NaOCl	NA	NA
Kuruvilla and Kamath, 1998	NA	Excluded	40	Single-rooted teeth	Primary endodontic infection	0.2% CHX/2.5% NaOCl		3 mL CHX/3 mL NaOCl	NA
Ringel et al, 1982	NA	NA	60	Single-rooted teeth	Primary endodontic infection	0.2% CHX/2.5% NaOCl	NA	Copious amount	Minimum 30 minutes
Rôças et al, 2016	Dental school, Brazil	Excluded	50	Single-rooted teeth	Primary endodontic infection	2% CHX/2.5% NaOCl	NA	15 mL CHX/15 mL NaOCl	NA
Vianna et al, 2006	Dental school, Brazil	Excluded	32	Single-rooted teeth	Primary endodontic infection	2% CHX gel/2.5% NaOCl	1 mL CHX/5 mL NaOCl	NA	NA
Xavier et al, 2013	Dental school, Brazil	Excluded	48	Single-rooted teeth	Primary endodontic infection	2% CHX gel/1% NaOCl	1 mL CHX/5 mL NaOCl	NA	NA
Zandi et al, 2016, 2019*	Dental school, Norway	Excluded	49	Single-rooted and multiple-rooted teeth	Persistent endodontic infection	2% CHX/1% NaOCl	NA	10 mL CHX/10 mL NaOCl	NA

Authors/year	Sampling technique	Primary outcome		Secondary outcome
		Culture method	Molecular method	
Ercan et al, 2004	Sterile paper point	Yes: *Enterococcus faecalis, Staphylococcus aureus, Streptococcus salivarius, Actinomyces israelii*	No	No
Kuruvilla and Kamath, 1998	Sterile paper point with distilled water	Yes: total bacteria	No	No
Ringel et al, 1982	Sterile paper point with reduced transport fluid	Yes: number of visits to yield negative culture	No	No
Rôças et al, 2016	Sterile paper point	No	Yes: total bacteria, *Streptococcus* species	No
Vianna et al, 2006	Sterile paper point	Yes: total bacteria	Yes: total bacteria	No
Xavier et al, 2013	Sterile paper point	Yes: total bacteria	No	No
Zandi et al, 2016, 2019*	Sterile paper point with sterile saline	No	Yes: total bacteria, *E. faecalis, Streptococcus* species	Yes: clinical and radiographic outcome (periapical status) after 1- and 4-year follow-ups

CHX, chlorhexidine; NA, not available; NaOCl, sodium hypochlorite.

*The study published in 2019 by Zandi et al. is the continuation of the study in 2016.

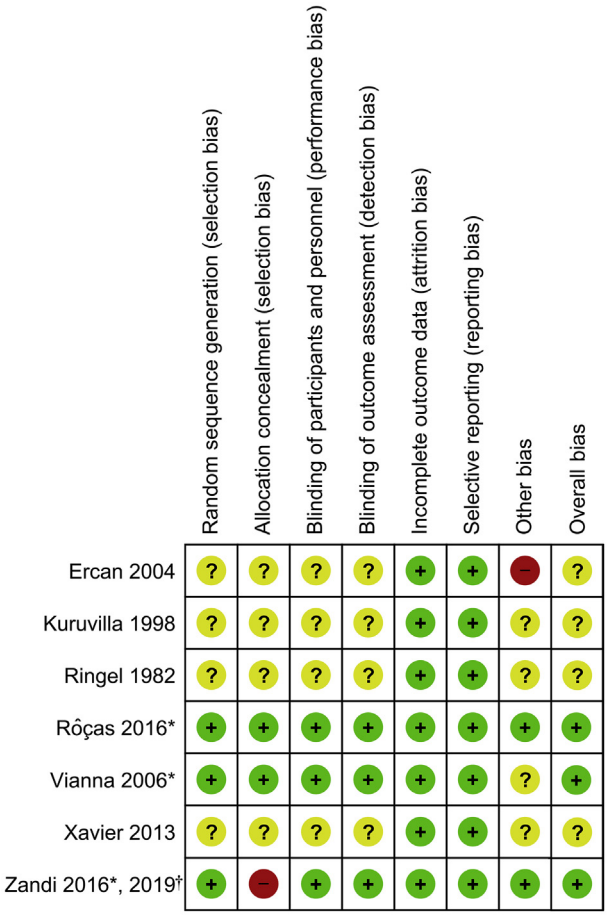

FIGURE 2 – A summary of the risk of bias of the included studies. *The authors provided further information requested by e-mail to evaluate the risk of bias. †The study published in 2019 by Zandi et al is the continuation of study from 2016.

differences between interventions[54]. Among the included RCTs, only 2 reported sample size calculation[26,29].

In the present meta-analysis using RR or SMD parameters, no significant difference was found in antibacterial efficacy between CHX and NaOCl treatments. Our analysis extended the basis of similar RR results in a previous meta-analysis published 8 years ago[34], which was based only on 2 articles[24,25]. Our findings of bacterial reduction also closely corresponded with those of another meta-analysis[55], which showed that intracanal endotoxin levels decreased compared with the initial levels after applying CHX and NaOCl. However, they found that NaOCl was more effective in the reduction of gram-negative bacterial endotoxin than CHX, but none of the gram-positive bacterial parameters were investigated.

Notably, in the included studies, more than half of the samples exhibited negative bacterial growth after irrigation with CHX or NaOCl, suggesting that neither of them could completely eliminate the bacterial population from the root canal. Although CHX and NaOCl showed similar antibacterial effectiveness, their molecular mechanisms of action were different. Clinicians should take other properties such as the necrotic pulp-dissolving capacity of NaOCl[23] or the substantivity of CHX[22] into consideration. Based on these properties, the combination of CHX and NaOCl may be recommended for endodontic irrigation. However, during the simultaneous application, their mixture can cause precipitate formation, which might occlude the dentinal tubules[56]. Thus, the consecutive application of NaOCl and CHX with intermediate flushes between each irrigant is needed.

The underlying discrepancy of the included studies might present certain limitations. Nonetheless, 4 studies retained an overall low risk of bias[25,26,29,40]. Although 2 of them did not perform the allocation

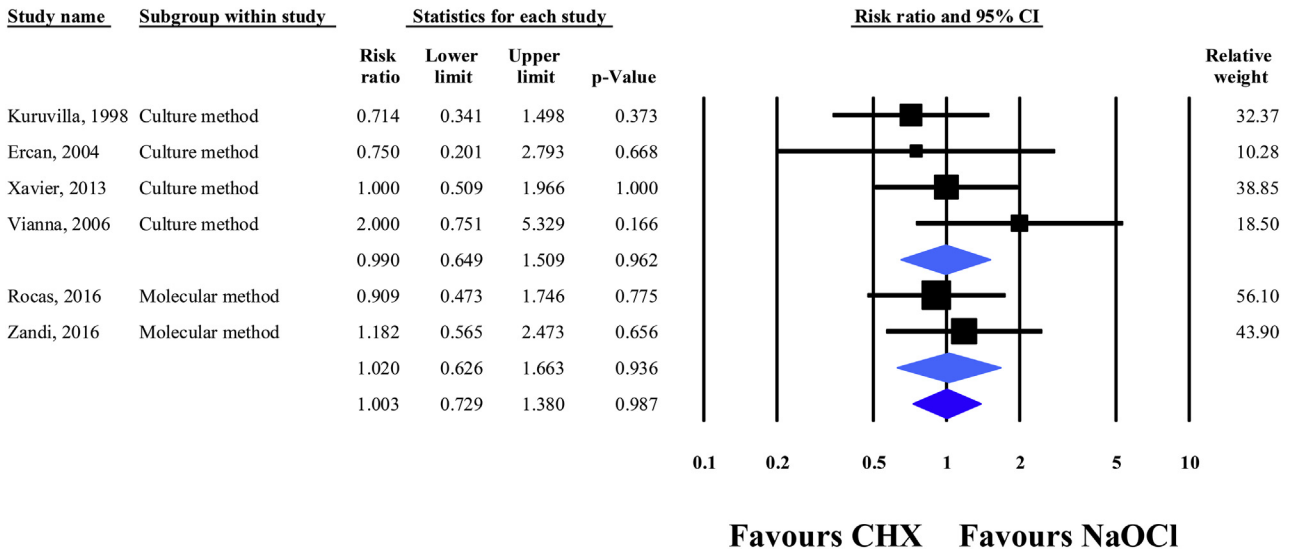

FIGURE 3 – The relative risk of samples with positive bacterial growth after irrigation with CHX versus NaOCl.

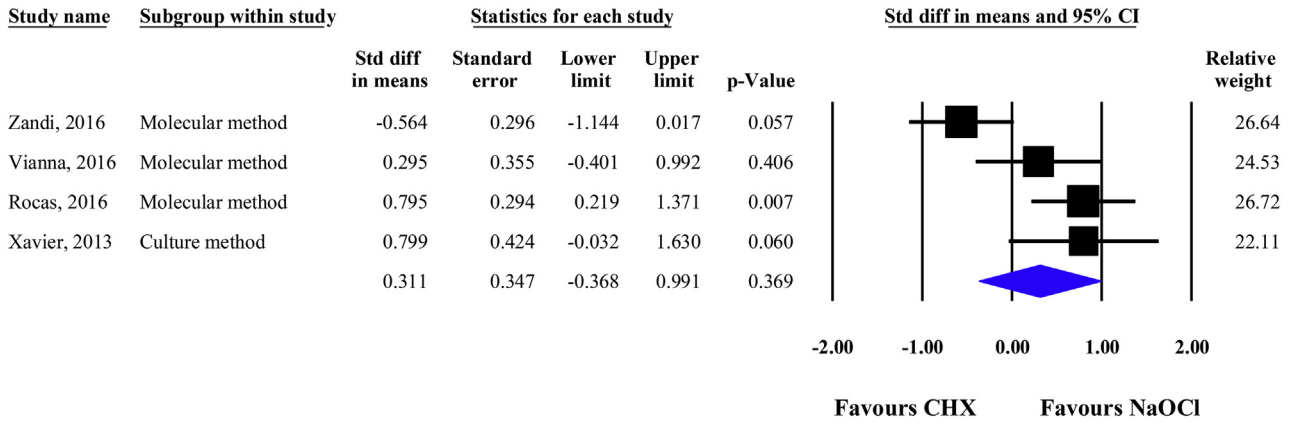

FIGURE 4 – The standardized mean difference of bacterial numbers after irrigation with CHX versus NaOCl.

concealment[29,40], the overall risk of bias resulted in low risk because of the prevalence of other low-risk key domains. The remaining 4 studies were considered unclear[24,33,38,39]. One of these studies showed a high risk of other bias[24] because it did not clearly describe the method used to randomize patients with multiple teeth, whether individual teeth or patients were the units of randomization. It is possible that these examinations are not independent, and the outcome may be subjected to a clustering effect. The investigated teeth might also be prone to cross contamination.

None of the included studies reported patient-relevant outcomes such as clinical symptoms and their disappearance, which are related to the effectiveness of the root canal irrigants, that would directly provide a recommendation to clinicians. Only 1 study showed the success rate based on radiographic outcome during endodontic retreatment[40]. Taking all of these reasons into account, it can be suggested that our meta-analysis is restricted by inconsistent and insufficient data from the included RCTs, resulting in the downgrading of certainty of our evidence. Therefore, further well-designed RCTs performed using different types of teeth and with proper sample size and all clinically relevant outcomes are required.

In conclusion, the obtained evidence suggested that both CHX and NaOCl significantly, but not completely, reduced endodontic infections during root canal therapy. They were found to be equally effective despite their different molecular mechanisms. Because the mixture of these 2 chemicals can cause precipitate formation, their consecutive application with intermediate flushes between each irrigant as well as the development of more potent antibacterial agents is proposed.

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The authors deny any conflicts of interest related to this study.

SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found in the online version at www.jendodon.com (https://doi.org/10.1016/j.joen.2020.05.002).

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SUPPLEMENTAL APPENDIX S1. PROSPERO AMENDMENT SUBMISSION ON 17/10/2019 (REGISTRATION NUMBER: CRD42019127651)

After we performed the final search and screening of the search results, we found 1 recent meta-analysis[1] that was associated with our study. This recent study reported endotoxin levels after irrigation with chlorhexidine and sodium hypochlorite, which is also 1 of our primary outcomes. While we were screening the search results against eligibility criteria, we found that the eligible articles related to the endotoxin level outcome were not different from the recent study. Therefore, we plan to exclude this outcome from our primary outcomes and do not investigate the endotoxin level. Although we excluded the endotoxin that was found in most gram-negative bacteria, we plan to investigate the total number of bacterial reduction, which covers all types of bacteria represented in the root canal system.

SUPPLEMENTAL APPENDIX S2. THE SEARCH STRATEGY IN EACH ELECTRONIC DATABASE

PubMed (MEDLINE)

The PubMed (MEDLINE) search query was as follows:

("Dental Pulp Cavity"[Mesh] OR "Root Canal Therapy"[Mesh] OR endodont* OR fill* OR tooth OR teeth) AND ("sodium hypochlorite" OR naocl OR chlorhexidine OR CHX OR edta OR mtad OR saline OR "etidronic acid" OR hebp OR "citric acid" OR ozon* OR "chlorine dioxide" OR ClO2) AND (irrigant* OR irrigation OR rinse OR disinfect*)

EMBASE

('dental pulp cavity'/exp OR 'endodontic procedure'/exp OR endodont* OR fill* OR tooth OR teeth) AND ('sodium hypochlorite' OR naocl OR chlorhexidine OR chx OR edta OR mtad OR saline OR 'etidronic acid' OR hebp OR 'citric acid' OR ozon* OR 'chlorine dioxide' OR clo2) AND (irrigant* OR irrigation OR rinse OR disinfect*) AND [embase]/lim AND 'human'/de

Web of Knowledge

#1 Dental pulp
#2 Root canal
#3 endodont*
#4 fill*
#5 tooth
#6 teeth
#7 "sodium hypochlorite"
#8 NaOCl
#9 chlorhexidine
#10 CHX
#11 EDTA
#12 MTAD
#13 saline
#14 "etidronic acid"
#15 HEBP
#16 "citric acid"
#17 ozon*
#18 "chlorine dioxide"
#19 ClO2
#20 irrigant*
#21 irrigation
#22 rinse
#23 disinfect*
#24 #6 OR #5 OR #4 OR #3 OR #2 OR #1
#25 #19 OR #18 OR #17 OR #16 OR #15 OR #14 OR #13 OR #12 OR #11 OR #10 OR #9 OR #8 OR #7
#26 #23 OR #22 OR #21 OR #20
#27 #26 AND #25 AND #24
#28 #26 AND #25 AND #24 Refined by: TOPIC: (clinical trial)

Cochrane Library (CENTRAL)

#1 MeSH descriptor: [Dental Pulp Cavity] explode all trees
#2 MeSH descriptor: [Root Canal Therapy] explode all trees
#3 endodont*
#4 fill*
#5 tooth
#6 teeth
#7 "sodium hypochlorite"
#8 NaOCl
#9 chlorhexidine
#10 CHX
#11 EDTA
#12 MTAD
#13 saline
#14 "etidronic acid"
#15 HEBP
#16 "citric acid"
#17 ozon*
#18 "chlorine dioxide"
#19 ClO2
#20 irrigant*
#21 irrigation
#22 rinse
#23 disinfect*
#24 #1 OR #2 OR #3 OR #4 OR #5 OR #6
#25 #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17
OR #18 OR #19
#26 #20 OR #21 OR #22 OR #23
#27 #24 AND #25 AND #26
#28 clinical trial
#29 #27 AND #28

REFERENCE

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SUPPLEMENTAL APPENDIX S3. THE RISK OF BIAS ASSESSMENT OF INCLUDED STUDIES

Ercan et al, 2004

Bias	Authors' judgment	Support for judgment
Random sequence generation (selection bias)	Unclear risk	Quote: "The teeth … were randomly divided into two groups." Comment: Insufficient information about the randomization process to permit clear judgment.
Allocation concealment (selection bias)	Unclear risk	The method of concealment was not described. Comment: Insufficient information about the sequence generation process to permit clear judgment.
Blinding of participants and personnel (performance bias)	Unclear risk	The methods to blind participant and operator from acknowledging of interventions were not described. Comment: The information to permit the clear judgment if the outcome was prone to be influenced by lack of blinding.
Blinding of outcome assessment (detection bias)	Unclear risk	There was no information whether the operator was involved in the microbiological outcome assessment or not. Comment: Insufficient information to provide clear judgement.
Incomplete outcome data (attrition bias)	Low risk	No report of missing or incomplete outcome data.
Selective reporting (reporting bias)	Low risk	The study protocol was not provided as well as the prespecified outcomes. However, all expected outcomes in the Methods section were reported.
Other bias	High risk	Half of the participants who had multiple teeth in the same mouth were randomized. The investigation was subjected to the clustering effect or contamination because it was not clear that the individual teeth were a unit of randomization. The total volume, frequency of irrigation, and contact time were not well described. Comment: The antimicrobial effect of each irrigant might be affected by all these factors. The application of inactivated agent before sample collection was not described. Comment: The antimicrobial effect of irrigant (CHX) might remain in the root canal which lead to false interpretation.

Kuruvilla and Kamath et al, 1998

Bias	Authors' judgment	Support for judgment
Random sequence generation (selection bias)	Unclear risk	Quote: "These cases were then randomly assigned into four groups of ten cases each." Comment: Insufficient information about the randomization process to permit clear judgment.
Allocation concealment (selection bias)	Unclear risk	The method of concealment is not described. Comment: Insufficient information about the sequence generation process to permit the judgment.
Blinding of participants and personnel (performance bias)	Unclear risk	The methods to blind participant and operator from acknowledging of interventions were not described. Comment: The information to permit the clear judgment if the outcome was prone to be influenced by lack of blinding.
Blinding of outcome assessment (detection bias)	Unclear risk	There was no information whether the operator was involved in the microbiological outcome assessment or not. Comment: Insufficient information to provide clear judgment.
Incomplete outcome data (attrition bias)	Low risk	No report of missing or incomplete outcome data.
Selective reporting (reporting bias)	Low risk	The study protocol was not provided as well as the prespecified outcomes. However, all expected outcomes in the Methods section were reported.
Other bias	Unclear risk	The application of inactivated agent before sample collection was not described. Comment: The antimicrobial effect of irrigant (CHX) might remain in the root canal, which may lead to false interpretation and affect the result.

Ringel et al, 1982

Bias	Authors' judgment	Support for judgment
Random sequence generation (selection bias)	Unclear risk	Quote: "Thirty teeth were randomly assigned to the experimental group, and the other 30 were assigned to the control group." Comment: Insufficient information about the randomization process to permit clear judgment.
Allocation concealment (selection bias)	Unclear risk	The method of concealment is not described. Comment: Insufficient information about the sequence generation process to permit the judgment.
Blinding of participants and personnel (performance bias)	Unclear risk	The methods to blind participant and operator from acknowledging of interventions were not described. Comment: The information to permit the clear judgment if the outcome was prone to be influenced by lack of blinding.
Blinding of outcome assessment (detection bias)	Unclear risk	There was no information whether the operator was involved in the microbiological outcome assessment or not. Comment: Insufficient information to provide clear judgment.
Incomplete outcome data (attrition bias)	Low risk	No report of missing or incomplete outcome data.
Selective reporting (reporting bias)	Low risk	The study protocol was not provided as well as the prespecified outcomes. However, all expected outcomes in the Methods section were reported.
Other bias	Unclear risk	Eight of the participants who had multiple teeth in the same mouth were randomized. Comment: The investigation was subjected to clustering effect or susceptible to contamination because it was not clear that the individual teeth were a unit of randomization. The total volume, frequency of irrigation, and contact time were not well described. Comment: the antimicrobial effect of each irrigant might be affected by all these factors. The application of inactivated agent before sample collection after irrigation was not described. Comment: The antimicrobial effect of irrigant (CHX) might remain in the root canal, which led to false interpretation.

Rôças et al, 2016*

Bias	Authors' judgment	Support for judgment
Random sequence generation (selection bias)	Low risk	Quote: "Randomization with equal proportion allocation was obtained by drawing lots." Comment: The randomization method was indicated.
Allocation concealment (selection bias)	Low risk	From the e-mail communication with the author: Quote: "Drawing was performed to select the irrigant to be used. Pieces of paper were prepared by one of the investigators and placed in a box. At the time of treatment, the operator picked up one piece containing the information about the irrigant (NaOCl or CHX)." Comment: The judgment assigned to low risk.
Blinding of participants and personnel (performance bias)	Low risk	Quote: "It was not feasible to blind patient and treatment provider because of the recognizable odor of NaOCl." Comment: The methods to blind participant and operator from acknowledging of received intervention did not apply because of treatment limitation. However, the participant was not involved in outcome assessment, while the operator might not influence the treatment because of the same treatment procedure. The judgment assigned to low risk.
Blinding of outcome assessment (detection bias)	Low risk	From the e-mail communication with the author: Quote: "…it was a different person." Comment: The operator was not involved in the microbiological outcome assessment. The judgment assigned to low risk.
Incomplete outcome data (attrition bias)	Low risk	Quote: "Three patients were excluded from the experiment and replaced by another 3 individuals according to the inclusion/exclusion criteria and randomization process." Comment: The excluded data were replaced by the same number and methods. The judgment assigned to low risk.
Selective reporting (reporting bias)	Low risk	The study protocol was not provided as well as the prespecified outcomes. However, all expected outcomes in the Methods section were reported.
Other bias	Low risk	There were no other recognized biases in this study.

*The author provided further information by requested e-mail to evaluate the risk of bias.

Vianna et al, 2006*

Bias	Authors' judgment	Support for judgment
Random sequence generation (selection bias)	Low risk	From the e-mail communication with the author: Quote: "The 32 patients were randomly divided into two groups. Treatment type & codes (random sampling numbers to collect and process samples) were placed in envelopes and one envelope was chosen per patient in the same session of the root canal treatment session" Comment: The judgment assigned to low risk.
Allocation concealment (selection bias)	Low risk	From the e-mail communication with the author: Quote: "Treatment type & codes were placed in envelopes and one envelope was chosen per patient in the same session of the root canal treatment session" Comment: The judgment assigned to low risk.
Blinding of participants and personnel (performance bias)	Low risk	From the e-mail communication with the author: Quote: "Patients did not know the treatment received. The operator knew the regimen used as per treatment allocation. As the irrigation material and protocols were completely distinct, it was impossible to make this a blind procedure to operator." Comment: The operator might not influence the treatment because of the same treatment procedure was performed. The judgment assigned to low risk.
Blinding of outcome assessment (detection bias)	Low risk	Quote: "The endodontist processed the microbiological samples. All samples were codified for laboratory process as per codes provided in the envelopes, making impossible to know to which group it belonged until data was analyzed at the end of the study."
Incomplete outcome data (attrition bias)	Low risk	No report of missing or incomplete outcome data.
Selective reporting (reporting bias)	Low risk	The study protocol was not provided as well as the prespecified outcomes. However, all expected outcomes in the Methods section were reported.
Other bias	Unclear risk	The total volume, frequency of irrigation, and contact time were not well described. Comment: The antimicrobial effect of each irrigant might be affected by these factors.

*The author provided further information by requested e-mail to evaluate the risk of bias.

Xavier et al, 2013*

Bias	Authors' judgment	Support for judgment
Random sequence generation (selection bias)	Unclear risk	Quote: "After accessing the pulp chamber and subsequent first endotoxin sampling, teeth were randomly divided into 4 groups." Comment: Insufficient information about the randomization process to permit clear judgment.
Allocation concealment (selection bias)	Unclear risk	The method of concealment was not described. Comment: Insufficient information about the sequence generation process to permit the judgment.
Blinding of participants and personnel (performance bias)	Unclear risk	The methods to blind participant and operator from acknowledging of interventions were not described. Comment: The information to permit the clear judgement if the outcome was prone to be influenced by lack of blinding.
Blinding of outcome assessment (detection bias)	Unclear risk	There was no information whether the operator was involved in the microbiological outcome assessment or not. Comment: Insufficient information to provide clear judgment.
Incomplete outcome data (attrition bias)	Low risk	No report of missing or incomplete outcome data.
Selective reporting (reporting bias)	Low risk	The study protocol was not provided as well as the prespecified outcomes. However, all expected outcomes in the Methods section were reported.
Other bias	Unclear risk	The total volume, frequency of irrigation, and contact time were not well described. Comment: The antimicrobial effect of each irrigant might be affected by these factors.

*The author provided further information by requested e-mail to evaluate the risk of bias.

Zandi et al, 2016,* 2019†

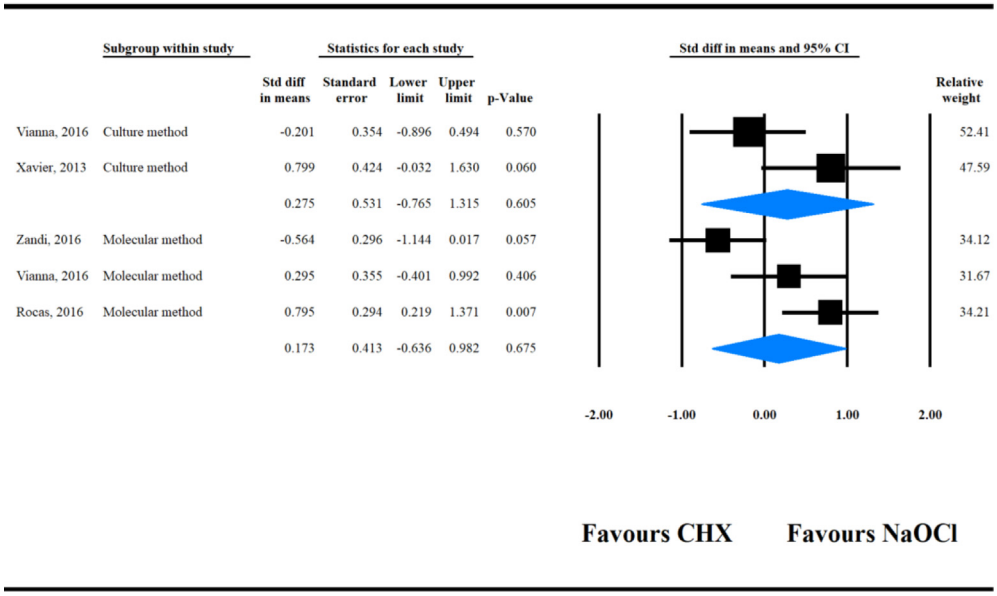
Bias	Authors' judgment	Support for judgment
Random sequence generation (selection bias)	Low risk	From the e-mail communication with the author: Quote: "On admission, cases were randomly distributed into NaOCl and CHX groups by the flipping of a coin." Comment: The randomization method was indicated.
Allocation concealment (selection bias)	High risk	The method of concealment was not performed after communication with the author by e-mail.
Blinding of participants and personnel (performance bias)	Low risk	From the e-mail communication with the author: Quote: "After deciding the groups, each one was treated with the irrigation solution without any blinding. The smell and odor would not allow us to blind." Comment: The methods to blind participant and operator from acknowledging of received intervention were not apply because of treatment limitation. However, the participant was not involved in outcome assessment, while the operator might not influence the treatment because of the same treatment procedure. The judgment assigned to low risk.
Blinding of outcome assessment (detection bias)	Low risk	Primary outcome: From the e-mail communication with the author, the operator was not involved in the microbiological outcome assessment. Secondary outcome: "Root canal fillings, restorations, and crowns were digitally masked…", "Next, the radiographs were coded and randomly mixed." The judgment assigned to low risk.
Incomplete outcome data (attrition bias)	Low risk	Participants who were excluded with reasons during the trial were clearly described.
Selective reporting (reporting bias)	Low risk	The study protocol was not provided as well as the prespecified outcomes. However, all expected outcomes in the Methods section were reported.
Other bias	Low risk	There were no other recognized biases in this study.

*The author provided further information by requested e-mail to evaluate the risk of bias.
†The study published in 2019 by Zandi et al is the continuation of the study in 2016.

SUPPLEMENTAL TABLE S1 - Summary of Findings Table

		Certainty assessment		
Outcome	**Number of teeth (studies)**	**Domain**	**Rate**	**Level of evidence**
Total bacterial number reduction after irrigation during the first visit	105 teeth (4 RCTs)	Study design Risk of bias Inconsistency Indirectness Imprecision Publication bias	Randomized controlled trial Not serious Serious* Serious†,‡ Serious§ Probably present‖	⨁◯◯◯ Very low
Incidence of samples with positive bacterial growth after irrigation during the first visit	205 teeth (6 RCTs)	Study design Risk of bias Inconsistency Indirectness Imprecision Publication bias	Randomized controlled trial Not serious Not serious Serious†,‡ Serious§ Probably present‖	⨁⨁◯◯ low

RCT, randomized controlled trial.
*Considerable heterogeneity (I^2 = 76.336%) was presented in the standardized mean difference analysis.
†The patients who had teeth with either primary or persistent endodontic infection were included in the analysis.
‡The different concentrations of sodium hypochlorite solution and chlorhexidine in solution and gel forms were used during root canal therapy.
§Low information size resulted in the uncertainty of the result.
‖The publication bias was not performed. It was manually assigned to "probably presented".



SUPPLEMENTAL FIGURE S1 – Subgroup analysis based on bacterial detection methods of standardized mean difference of bacterial numbers after irrigation with chlorhexidine (CHX) versus sodium hypchlorite (NaOCl)