

A systematic review on reduction of bacterial load in root canals of single-canal teeth through use of sodium hypochlorite and chlorhexidine as endodontic irrigants

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Abstract

To avoid failure of endodontic therapy, thorough disinfection of the root canals is paramount. Sodium hypochlorite has been used by dentists for decades but, due to its toxicity and irritative nature, there has been a recent increase in chlorhexidine use. This review has been conducted to determine whether sodium hypochlorite or chlorhexidine causes the greater reduction in bacterial load in mature permanent teeth undergoing root canal treatment.

OVIDMedline, Cochrane Central Trials Database and Cochrane Database of Systematic Reviews were searched on 16 December 2022. Included papers were required to be in-vivo studies using sodium hypochlorite and chlorhexidine as endodontic irrigants independently of each other, on permanent teeth.

The search yielded 182 papers, narrowed to seven by removing irrelevant, inaccessible and duplicate papers, and those that did not fulfil the inclusion criteria. Every included study was a randomised controlled trial, seeing a decrease in bacterial load of root canals between 59.4% and 100.0% through the irrigation in the intervention groups. Four papers reported a difference of less than 5.0% bacterial reduction between the two irrigants. The other three reported chlorhexidine bacteria reductions between 10.6% and 19.0% more than sodium hypochlorite.

There is evidence to suggest that chlorhexidine and sodium hypochlorite reduce bacterial load during endodontic therapy. However, inconsistency of findings makes it difficult to conclude whether chlorhexidine has greater antimicrobial effectiveness than sodium hypochlorite. More high-quality studies are needed to form a judgement regarding which irrigant is preferable to use in standard practice.

Abbreviations

CASP – Critical Appraisal Skills Programme

CFU – Colony Forming Units

CHX – Chlorhexidine

NaOCl – Sodium Hypochlorite

PCR – Polymerisation Chain Reaction

PICO – Population Intervention Comparison Outcome

RCT – Root Canal Therapy

Introduction

The rationale behind endodontic treatment is removal of necrosed pulpal tissue and associated bacteria will ensure that the periapical tissues remain healthy,¹ thereby allowing retention of a non-vital tooth in the mouth. Bacteria associated with pulpal necrosis spread through the pulp and causes damage to the periradicular

tissues, inducing inflammatory processes and leading to apical periodontitis.² Therefore, thorough disinfection of the root canal system during endodontic therapy is crucial to prevent development of this inflammation.

The eradication of bacteria occurs using a combination of mechanical and chemical methods.³ Mechanical instrumentation produces debris, consisting of mineralised collagen matrix, pulpal tissue and bacteria,⁴ which covers the surface of canals undergoing preparation and obstructs the exposed dentinal tubules, allowing accumulation of bacteria to occur. Whilst most bacteria, and their by-products, can be removed with good mechanical instrumentation,⁵ this smear layer formation and anatomical complexities of the canal system make complete disinfection unachievable⁴ without addition of an antibacterial endodontic irrigant.

Sodium hypochlorite (NaOCl) has been a popular choice of irrigant by dentists for decades due to its antibacterial effect and ability to dissolve organic material.⁶ However, NaOCl is toxic to periapical tissues and can cause irritation,⁶ leading researchers to investigate alternative irrigants and their properties, with a focus on improved biocompatibility whilst retaining antibacterial function. Despite its lack of organic tissue dissolving properties, use of chlorhexidine (CHX) as an endodontic irrigant has grown in recent years due to its biocompatibility and substantivity, non-toxic nature and being less irritating to periapical tissues⁷ than NaOCl. However, there is uncertainty amongst researchers and dental professionals as to which irrigant is most effective.

Despite the various attributes and drawbacks of each irrigant, a firm conclusion on their antimicrobial efficacies is yet to be made, mainly due to the inconsistency of results of existing studies. Hence, a review of the evidence comparing the antibacterial effectiveness of NaOCl and CHX endodontic irrigation, in patients with mature permanent teeth needing root canal therapy, is required.

A systematic review was published in 2012,⁸ which included 11 papers dated between 1978 and 2010. Only four trials directly compared NaOCl and CHX as irrigants. In these papers, no primary outcomes, and only one secondary outcome (bacterial growth cultures), were stated.

Another systematic review was published in 2022,⁹ which included seven papers dated between 2009 and 2020. Only two of these trials compared the antimicrobial efficacy of NaOCl and CHX, concluding that there was a significant reduction in bacterial load with use of either irrigant (between 56.0% and 99.8%), but differences of less than 4.0% between the two irrigants. The validity of this conclusion is questionable as it was based on only two studies. Hence a comprehensive review based on the PICO provided in **Table 1** is warranted.

Methods

Search strategy

A search of OVIDMedline, the Cochrane Central Trials Database and the Cochrane Database of Systematic Reviews was conducted on 16 December 2022, using the search strategy detailed in **Figure 1**.

Paper screening and eligibility evaluation

The inclusion criteria led to acceptance of papers that are in-vivo studies, randomised controlled trials, have quantitative methodology and/or results, included permanent/mature teeth, used any concentration of irrigant, and included both irrigants that are being evaluated.

The exclusion criteria led to omission of papers that have qualitative methodology and/or results, no full text available, are in a language other than English, included deciduous/immature teeth, included

endodontic retreatment, included only one or neither of the irrigants being evaluated, only used the irrigants as a final rinse, and when the irrigants are only used in combination with others.

All paper processing was completed independently by the two authors. First, duplicate papers were removed. The titles and abstracts of each remaining paper were read, leading to omissions due to fulfilling the exclusion criteria. Several full texts were inaccessible, again leading to omission. The full texts of the remaining papers were read, further being excluded. Differences in exclusion by the two authors were discussed and settled, producing a list of seven studies^{10,11,12,13,14,15,16} to be included in this review.

Outcome variables and analysis

The primary outcome investigated was reduction in bacterial load. No secondary outcomes were investigated. Only one study¹⁴ contained a full data set. Another¹⁰, contained discrete data. None of the included studies stated standard deviations or confidence intervals. Due to these factors, a meta-analysis was not performed. Therefore, integration of the studies relied upon p-values and mean percentage reduction of bacterial load.

Paper evaluation

The Critical Appraisal Skills Programme (CASP)¹⁷ checklist for randomised controlled trials was used by both authors independently to examine the quality of the papers included in the review to ensure they were of a sufficient calibre. This was used in conjunction with Cochrane Review Group risk of bias 2 tool¹⁸ to produce a risk of bias analysis.

Results

Search outcomes

As shown in **Figure 2**, 182 records were obtained from the literature searches. After removing eight duplicates, 174 papers had their titles and abstracts screened, of which 150 were then excluded. The remaining 24 papers underwent full-text screening, of which seven could not be retrieved. Ten were excluded because they either only used NaOCl and CHX in conjunction, only tested one irrigant, were ex-vivo studies or involved teeth that had been previously treated. Hence seven studies^{10,11,12,13,14,15,16} were included in this review.

Characteristics of papers

Prior to inclusion, each study was quality assessed using the CASP tool for randomised controlled trials. Certain parameters were selected to act as indicators of sufficient quality: a targeted PICO question, equal quality of provided care, magnitudes of bacterial reduction stated (as raw data or percentage), and sufficient applicability to general practice dentistry. Every included paper fulfilled each of these conditions, thereby being deemed appropriate to be included in this review. This type of screening also highlighted certain biases and shortcomings of the papers, which are explored further in the Discussion section and **Figure 3**.

All studies included were randomised controlled trials and investigated single canal teeth that were non-vital or had primary endodontic infection. Sample sizes varied between the studies, with 10,^{11,12,13} 15,¹⁰ 16,¹⁴ 20,¹⁵ and 25¹⁶ teeth per intervention being tested. Across the seven papers, a total of 212 teeth underwent endodontic therapy.

Two papers^{10,11} used 5.25% NaOCl at 2ml volume, one¹⁵ used 5ml 1% NaOCl. All other papers^{12,13,14,16} compared 2.5% NaOCl with volumes of 3ml,^{12,13} 5ml¹⁴ and 15ml.¹⁶ Other than the study which used 0.2%,¹² every study used 2% CHX solution (with one¹⁴ using a gel form) with the volume used ranging from 1ml to 15ml.

Two of the studies,^{11,14} qualified the exposure time as 20 minutes, during which the chemomechanical preparation of the canal occurred; the five other studies did not present these data. Only one paper¹² collected data for the irrigant use independently, the other six studies concentrating on treatment where chemomechanical preparation was undertaken.

Characteristics of outcome measures

As seen in **Table 2**, three papers^{10,12,14} supplied mean values of CFU/mL (colony forming units) pre- and post-irrigation. One of these¹⁰ also recorded the percentage distributions of specific growing and non-growing bacteria. Three studies^{11,14,15} recorded average values for CFU/mL pre- and post-irrigation, as well as recording incidence of positive bacterial presence using a PCR (polymerase chain reaction) technique. The final study¹⁶ measured the incidence of positive microbial presence using 16s rRNA gene-based primers, a type of PCR technique, pre- and post-irrigation.

Summary of findings

Through the irrigative process, every study saw a decrease in bacterial load of between 59.4% and 100.0%.

Based on statistical evidence only, four papers^{11,12,15,16} reported no differences between the bacterial reduction with NaOCl, compared with CHX (no p-values stated, $p > 0.05$, $p > 0.05$, $p > 0.05$, respectively). The remaining three studies^{10,13,14} all found greater reductions in bacteria for CHX compared to NaOCl, with $p = 0.09$,¹⁰ $p < 0.05$,¹³ and $p < 0.01$.¹⁴

Four of the papers^{11,12,15,16} showed less than 5.0% difference between the percentage bacterial reduction of the two irrigants. The other three^{10,13,14} concluded that CHX is more effective than NaOCl, causing between 10.6% and 19.0% more reduction in bacterial load of root canals. However, none of the studies include confidence intervals, or contain a complete enough data set to calculate them, thereby calling into question the precision of the published results.

More information about the characteristics, irrigation protocols and results of each study have been outlined in **Table 2**. A graphical comparison of data can be found in **Figure 4**.

Discussion

Current literature

Previous systematic reviews^{8,9} concluded that there was insufficient evidence to suggest a difference between the antibacterial effectiveness of NaOCl and CHX. This review aimed to address shortcomings in the published reviews but found inconsistency in the findings of the seven included papers. While three papers^{10,13,14} found CHX had greater antibacterial efficacy than NaOCl regarding root canal treatment, the other four studies^{11,12,15,16} did not find a difference.

Review of included studies

CASP was used to quality assess the included papers prior to results collation. Whilst each study was deemed acceptable to be included in the summary, more high-quality research into this area should be conducted.

The studies that were used were broadly compatible as they all measured the bacterial load and showed reductions in presence of bacteria with intervention/control group, despite using three different methods to do so. One paper¹² is, perhaps, less applicable than the others due to the lack of mechanical preparation of the root canals; this was something that was not accounted for in the exclusion criteria.

Every study randomly sorted their participants into two groups, testing either NaOCl or CHX, however, five^{10,12,13,14,15} of these studies failed to declare how the randomisation was undertaken. This lack of information calls into question the soundness of their approach as it could generate randomisation bias. One paper¹¹ asked the participants to choose between groups of envelopes, randomly assigning them to an intervention group depending on what was in their envelope. Another study¹⁶ obtained randomisation by drawing lots.

The only paper¹⁶ to include power calculations, to justify the sample size used, was notably the study with the greatest number of participants – 25% more than the next largest study.¹⁵ The limited number of participants included in each of the studies, and lack of power calculations, casts doubt upon their ability to detect the intended clinical effects. There is also no information in any study about the demographics of each of the treatment groups post-allocation. This may indicate a lack of relevancy of results to a real-world application depending on spread of different social factors. There was no mention of blinding participants or investigators in six of the studies.^{10,11,12,13,14,15} One study¹⁶ stated that it was not feasible to blind the patient or treatment provider because of the recognisable odour of NaOCl. Whilst patient blinding would not be necessary, due to the nature of the study, blinding of the dentist would have eradicated opportunity for performance bias.

Each study only looked at the irrigant effects in single canal teeth, limiting relevance of the summary as endodontic therapy is not only conducted on single canal teeth. Further research, into whether the outcomes of irrigant use are different when all canals of multi-rooted teeth are treated, should be undertaken to remove this limitation.

None of the papers compared irrigants of the same concentration as each other within the individual studies and failed to provide rationale of concentration selection, calling into question the real-life application of their results. The concentrations of the irrigants are not consistent between the studies either, with two papers^{10,11} comparing 5.25% NaOCl and 2% CHX, three papers^{13,14,16} comparing 2.5% NaOCl and 2% CHX, and the remaining two^{12,14} comparing 2.5% NaOCl with 0.2% CHX and 1% NaOCl with 2% CHX respectively. This provides a challenge when evaluating whether the papers' conclusions are capable of integration. Studies testing multiple concentrations of NaOCl and CHX under the same clinical method would be useful to overcome this uncertainty.

Only two studies^{11,14} measured the irrigant exposure time. Ambiguity regarding this begs the question of how easily integrated the results of the studies can be, as the antimicrobial effect of irrigants may be dependent on the period they are within the canal and, therefore, in contact with bacteria. Every study collected bacterial samples directly after irrigation. By doing so, the substantivity of CHX, one of its main advantageous properties, was not considered. Further research investigating antibacterial reduction over time, perhaps a year post-op, would be useful to assess whether substantivity influences the bacterial load within the canals. This was something that was considered as a possible outcome to be measured but was discarded due to the limited literature available.

Whilst deeming bacterial reduction statistically significant or not would appear to make integration of the papers more straightforward, P-values are a very arbitrary way to assess this. The discrepancy between papers that consider bacterial reduction statistically significant and those which have sizeable reduction in terms of magnitude, make conclusion consolidation difficult, therefore, affecting real-world application of the studies.

Despite not directly investigating this, measurements of success of the endodontic treatments would have been interesting to see; potentially giving an indication of a threshold value for bacterial load, beyond which, treatment is successful. It may also have exposed

alternative properties of the irrigants or side effects that increase likelihood of failure.

Although being deemed of high enough quality to be included in this summary, the included papers have issues regarding potential biases and are difficult to integrate due to the variability of clinical method. Only one paper¹⁴ included full datasets that could be used for statistical analysis, making meta-analysis, sensitivity testing and formal integration of the papers impossible. Standardisation of investigative protocol – primarily exposure time, volume and concentration of irrigants used – and data collection could be instrumental in refining our understanding of the effectiveness of endodontic irrigation. More high-quality studies are needed to allow a decision to be made regarding optimisation of technique of this therapy.

Conclusion

Through the selected studies, this summary concludes that use of NaOCl and CHX as endodontic irrigants decrease the bacterial load of root canals in need of root canal therapy, highlighting their importance. However, due to inconsistency of results of the included literature, a firm judgement regarding which irrigant has a greater antibacterial effect cannot be made.

Further to this, more research needs to be conducted into the different properties, as well as side effects, of sodium hypochlorite and chlorhexidine to establish which irrigant should be of standard use by dental practitioners.

Contribution statement

The authors of this review both made substantial contributions to the acquisition, analysis, and interpretation of data for the work; drafting and revising it critically for important intellectual content; and have both approved the final version for inclusion in INSPIRE.

Poppy Daly is the guarantor of this work.

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Poppy Daly

Hello, I'm Poppy. I graduated from University of Bristol in 2024 and am now working as a foundation dentist in Devon. This paper was written when we were in fourth year of university. I am enjoying growing my experience in general practice and getting used to working life! In the future, I plan to specialise

in endodontics. In my spare time, I love exploring new places with my



Lily Lester

Hi! I am Lily, I have recently graduated from University of Bristol and am currently doing a two-year DFT/DCT scheme in North Central London. I am thoroughly enjoying my first year working as a dentist and would consider specialising or further training in the future.

I love spending my spare time outdoors, especially at the beach in my hometown Swansea.

Search conducted 16/12/2022:
Ovid MEDLINE(R) <1946 to present>
Yielded 57 results.

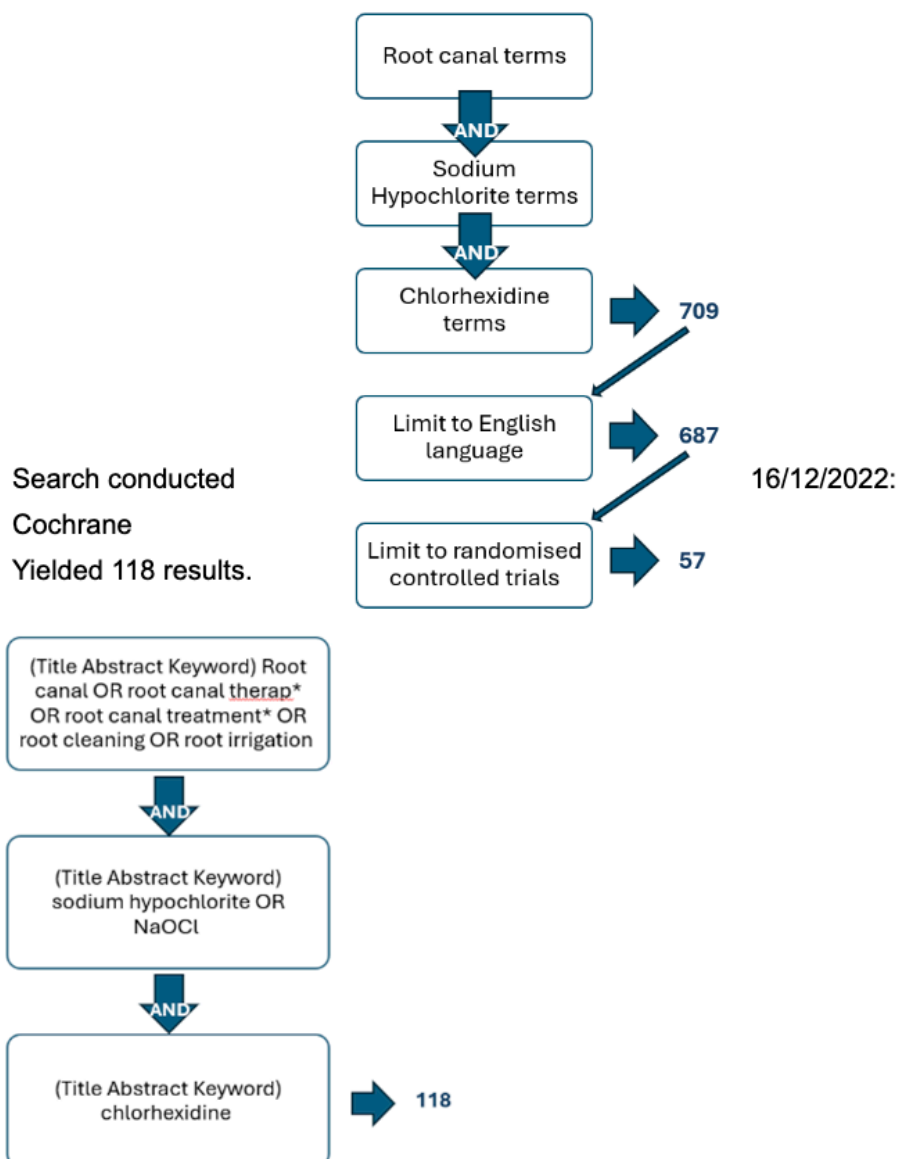
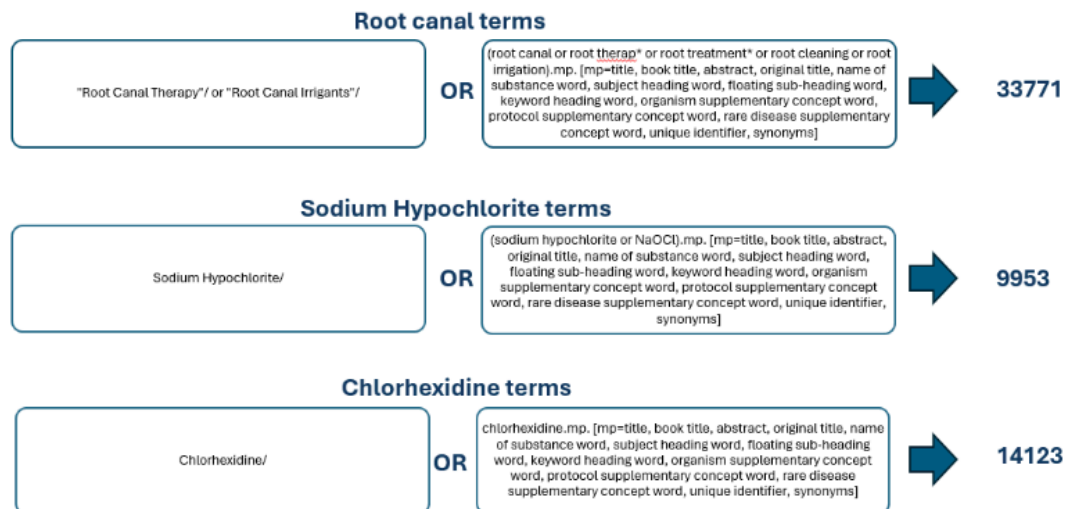


Figure 1. Search strategy depicted with flowcharts.

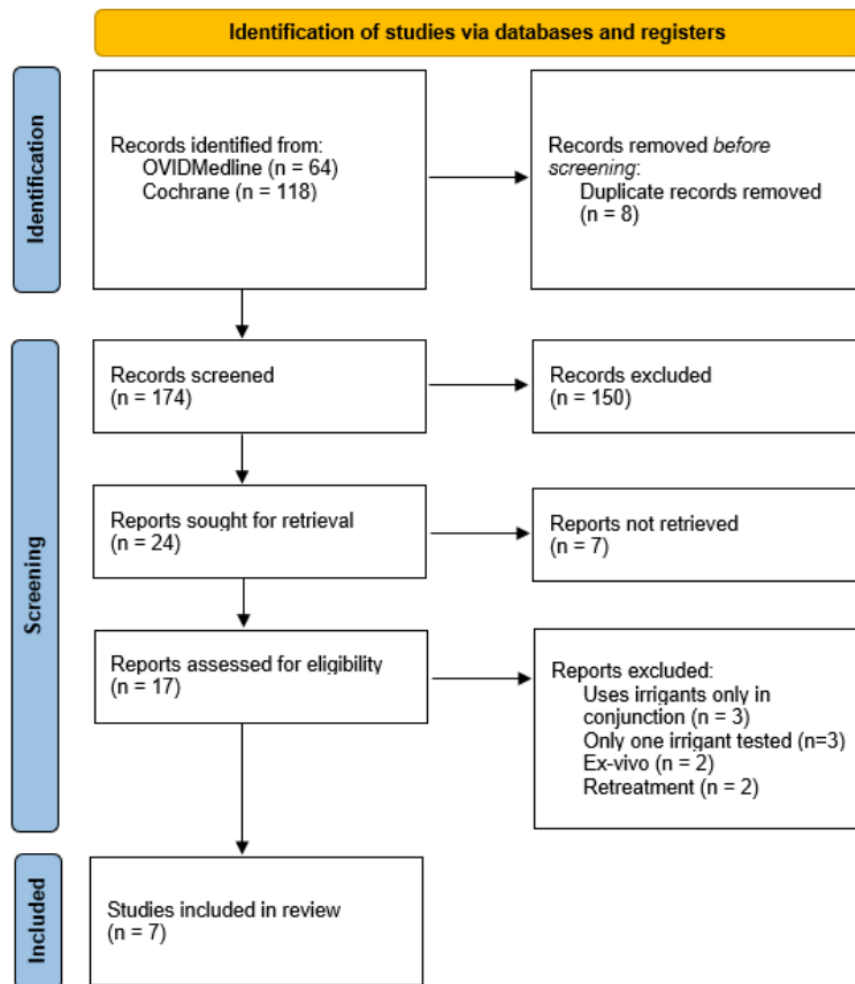


Figure 2. PRISMA flow diagram depicting the search strategy.

	D1	D2	D3	D4	D5	D6	Overall
Ercan et al, 2004	⊖	⊖	⊕	⊕	⊕	⊕	⊖
Khedmat et al, 2018	⊕	⊖	⊕	⊕	⊕	⊕	⊖
Kuruville and Kamath, 1998	⊖	⊖	⊕	⊕	⊕	⊕	⊖
Devi and Kamath, 2001	⊖	⊖	⊕	⊕	⊕	⊕	⊖
Vianna et al, 2006	⊖	⊖	⊕	⊕	⊕	⊕	⊖
Xavier et al, 2013	⊖	⊖	⊕	⊕	⊕	⊕	⊖
Rocas et al, 2016	⊕	⊗	⊕	⊕	⊕	⊕	⊗

Figure 3. Risk of bias assessment of included studies.

- D1 – Bias arising from randomisation process
- D2 – Bias arising from blinding
- D3 – Bias due to deviations from intended intervention
- D4 – Bias arising from incomplete outcome data
- D5 – Bias in measurement of outcome
- D6 – Bias in selection of reported result

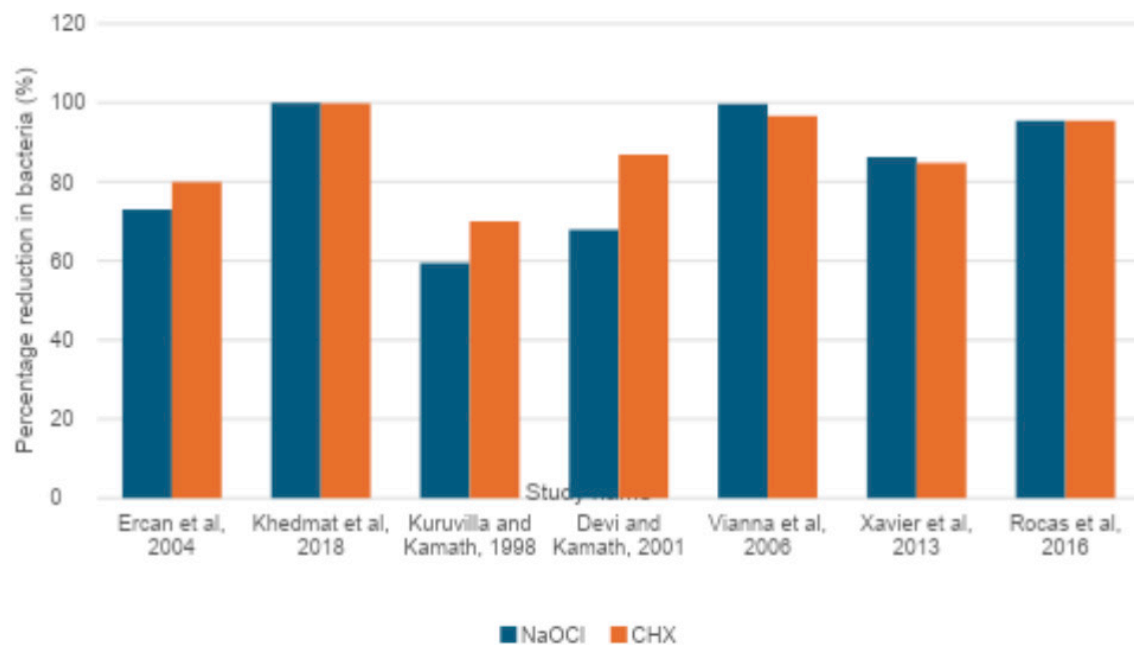


Figure 4. Chart to show percentage reduction in bacteria in canals under NaOCl and CHX irrigation

Table 1. PICO question to be explored in this review

Patient/population/problem	Patients needing root canal treatment
Intervention/exposure	Chlorhexidine irrigation (CHX)
Comparison	Sodium hypochlorite irrigation (NaOCl)
Outcome	Reduction in bacterial load

Table 2. Summary table of the findings from the included studies

Main outcomes	Difference in reduction	CHX was more effective than NaOCl for both sampling periods (p<0.001) No confidence intervals stated	No evidence to suggest a superior irrigant No p-values stated No confidence intervals stated	No evidence to suggest a significant difference between groups (p > 0.05) No confidence intervals stated	Evidence to suggest CHX provided greater (p < 0.05) bacterial reduction than NaOCl No confidence intervals stated	Evidence suggests that NaOCl reduced bacterial load more than CHX (P<0.01). No confidence intervals stated	No evidence of a significant difference between the median percentage values of bacterial reduction found in NaOCl and CHX (P > 0.05) No confidence intervals stated	No evidence to suggest a significant difference in reduction between the groups (p > 0.05) No confidence intervals stated
	Reduction in CHX	CHX saw 10/15 to 0.75/15 (80.0%)	CHX from 5.2x10 ⁵ CFU/ml to 80CFU/ml (99.8%)	Reduction of bacteria with CHX – 70%.	CHX from 5.04 CFU/ml to 0.46 CFU/ml (86.9%)	CHX from 2.3x10 ⁶ to 6.2x10 ⁴ (96.6%) (SYBR) and 3.0x10 ⁶ to 4.3x10 ⁴ (96.6%) (TaqMan)	CHX from 146 EU/ml to 19.76 EU/ml (84.8%)	CHX from 8.77x10 ⁴ to 2.81x10 ³ (95.4%)
	Reduction in NaOCl	NaOCl reduction from a mean of 11.25/15 canals containing bacteria to 1.75/15 (73.0%)	NaOCl levels were reduced from 5.5x10 ⁵ CFU/ml to 70CFU/ml (99.9%)	Reduction of bacteria with NaOCl – 59.4%	NaOCl levels were reduced from 4.96 CFU/ml to 1.67 CFU/ml (67.9%)	NaOCl saw reduction from 2.8x10 ⁶ to 2.0x10 ² RNA copy numbers (99.99%) (SYBR) and 7.6x10 ⁶ to 1.6x10 ⁴ (99.6%) (TaqMan)	NaOCl levels reduced from 114.5 EU/ml to 15.65 EU/ml (86.3%)	NaOCl levels reduced from 3.70x10 ⁵ to 5.49x10 ² (95.5%)
Irrigation protocol	Incubation periods (37°C)	5-7 days	7 days	72 hours	72 hours	7 days	Up to 14 days	NS
	Exposure time	NS	20 mins	NS	NS	20 mins	NS	NS
	Type /concentration /volume	2ml 5.25% NaOCl / 2ml 2% CHX	2ml 5.25% NaOCl / 2ml 2% CHX	3ml 2.5% NaOCl / 3ml 0.2% CHX	3ml 2.5% NaOCl / 3ml 2% CHX	5ml 2.5% NaOCl / 1ml 2% CHX gel	5ml 1% NaOCl / 1ml 2% CHX	15ml 2.5% NaOCl / 15ml 2% CHX
Tooth	Infection status	Pulpal necrosis; apical pathosis; both	Necrotic pulp	Non-vital; definite apical radiolucency	Non-vital; definite apical radiolucency	Non-symptomatic; no response to sensitivity testing	Primary endodontic infection	Necrotic pulp; asymptomatic apical periodontitis
	Type	Incisor and premolar teeth with single canal	Single rooted premolars	Single rooted anterior teeth	Single rooted teeth	Single rooted teeth	Single rooted teeth	Single rooted and single canal teeth
	Sample characteristics	30 (NaOCl n=15; CHX n=15) Age: 20-52 years	20 (NaOCl n=10; CHX n=10) Age: NS	20 NaOCl n=10; CHX n=10) Age: NS	20 (NaOCl n=10; CHX n=10) Age: NS	32 (NaOCl n=16; CHX n=16) Age: 19-63 years	40 (NaOCl n=20; CHX n=20) Age: NS	50 (NaOCl n=25; CHX n=25) Age: 13-52 years (mean 29 years)
	Country of study	NS	NS	NS	NS	Brazil	Brazil	Brazil
	Randomised controlled trial	Ercan et al, 2004	Khedmat et al, 2018	Kuruvilla and Kamath, 1998	Kamath and Devi, 2001	Vianna et al, 2006	Xavier et al, 2013	Rôças et al, 2016

NaOCl – sodium hypochlorite; CHX – chlorhexidine; n – number; NS – not stated