

SYSTEMATIC REVIEW

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Antibacterial efficacy of quaternary ammonium compounds (QACs) against *Enterococcus faecalis* in endodontic infections: a systematic review and meta-analysis

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Abstract

Background This systematic review aims to assess the antibacterial efficacy of Quaternary Ammonium Compounds (QACs) against *Enterococcus faecalis* (*E. faecalis*) as a root canal irrigant for endodontic infections.

Materials and methods A comprehensive literature search was conducted utilizing the databases PubMed, Scopus, Cochrane library and Google Scholar for papers from January 2000 up until January 2024. The research question was formulated using the PICO (Population, Intervention, Control and Outcomes) framework. This review focused on the efficacy of QACs (intervention) against *E. faecalis* (population) and compare it with NaOCl (comparator 1) and CHX (comparator 2) for antibacterial activity by using findings of colony forming unit (CFU) and confocal laser microscopy (CLSM) (outcomes). A methodological quality assessment was conducted using a scoring methodology provided by Quin tool to evaluate the studies, and the risk of bias was examined. A systematic screening of the selected publications was performed based on predefined inclusion criteria to identify studies eligible for meta-analysis.

Results Nine research studies papers included varied in their design and had a low to medium risk of bias. The findings demonstrated a decrease in the number of *E. faecalis* bacteria following the inclusion of QACs, leading to improved disinfection and efficacy in combating root canal infections. Furthermore, the meta-analysis findings indicated that there was no statistically significant distinction ($p > 0.05$) in the efficacy of QACs [Quaternary ammonium silane (K21) and Benzalkonium chloride (BAK)] when compared to commercially available NaOCl, however these QACs showed high efficacy against *E. faecalis*.

Conclusion This review found QACs to be promising alternatives to NaOCl and CHX for *E. faecalis* elimination, though no significant difference was observed in meta-analysis due to study heterogeneity. Standardized in vivo studies are needed for validation.

Prospero registration no CRD42023389258.

Clinical trial number not applicable.

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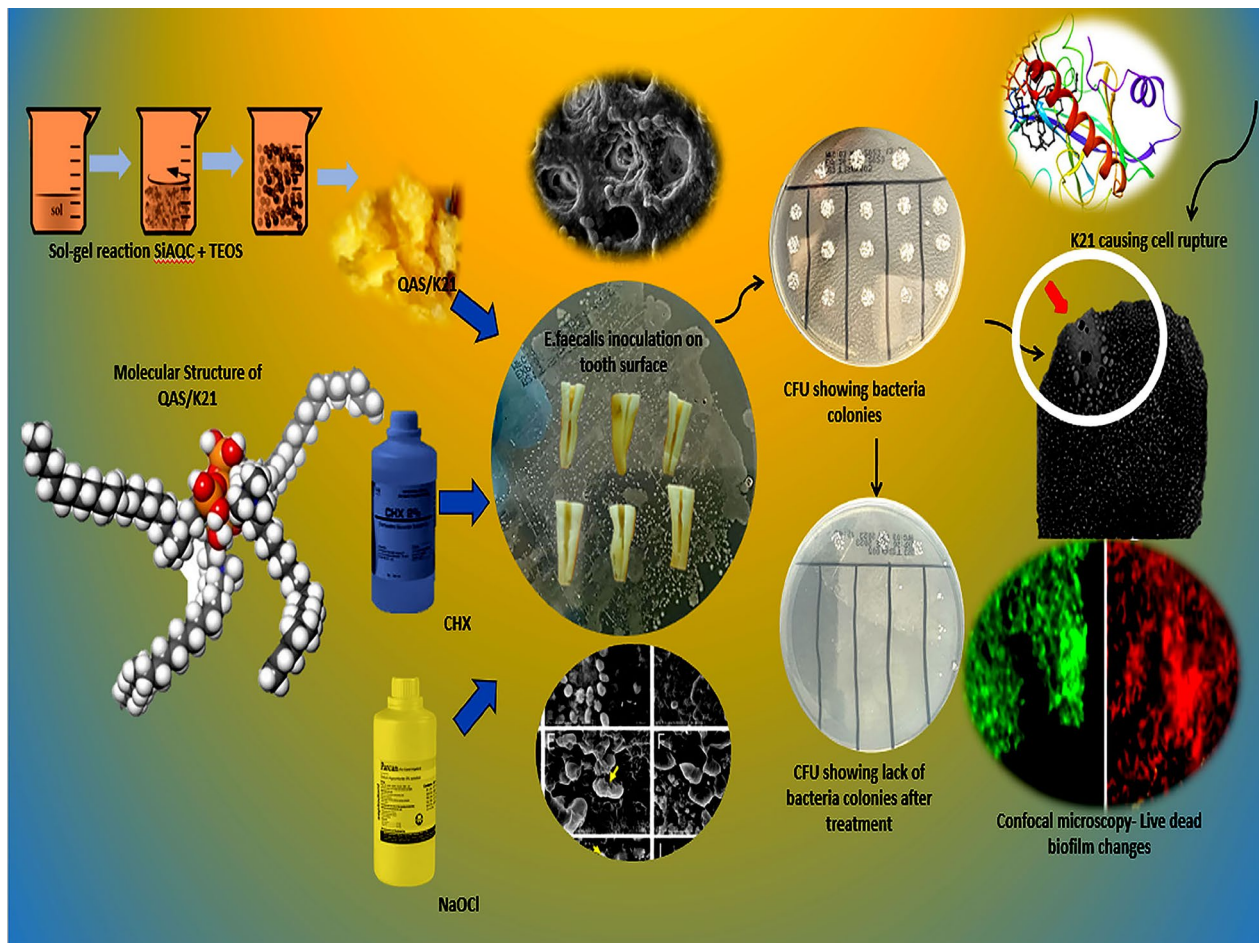
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Graphical Abstract



Keywords Antibacterial, Disinfection, Endodontic infection, Enterococcus faecalis, Infection, Irrigant, Quaternary ammonium compound, And root canal

Introduction

Bacteria present in the oral niches can infiltrate the root canal system leading to inflammation of the dental pulp and the periapical tissues. Endodontic infections may be caused by microflora that is well adapted to the specific ecosystem of low oxygen potential and limited nutritional availability. Microbial communities within the root canals are observed as biofilms that are attached to surfaces [1]. The occurrence of these intricate biofilms was recorded in 70% of the cases [2], and they were found to be firmly connected to the dentin of the root canal wall, spreading into recesses, isthmus and lateral canals. Root canals harbor various microorganisms, including *E. faecalis*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Treponema pallidum*, and other species [3]. The pathogenic microflora is linked to intense inflammatory responses when present in necrotic pulp, leading to pain,

abscess formation, and cellulitis. Resilient bacteria like *E. faecalis* are capable of surviving in challenging conditions and have the ability to withstand antibacterial substances [4]. The success of root canal therapy relies mostly on the effectiveness of chemo-mechanical preparation in eliminating both biofilm-embedded and planktonic bacteria, as well as removing necrotic pulp and toxins generated by microorganisms to facilitate periapical healing [5]. Intracanal medicaments have mostly contributed to root canal disinfection by significantly reducing bacterial counts within the root canal system following chemo-mechanical techniques [6]. *E. faecalis* exhibits a significant level of resistance and plays a crucial role in secondary infections of root canal treated teeth, resulting in a high incidence of treatment failure. It is a predominant microorganism found in persistent intraradicular infections, as opposed to initial chronic periapical periodontitis. *E. faecalis*

demonstrates resilience in very alkaline environments and in the presence of antibacterial substances. The antibacterial medication should be able to eliminate *E. faecalis* biofilms within the root canal system *E. faecalis* [7]. It is crucial to examine various compositions that have minimal harmful effects on periapical tissues and significant antibacterial capabilities. Commonly used antibacterial endodontic irrigants like NaOCl and CHX have also been studied in treatment of endodontic infections. NaOCl has the potential to cause irritation and cytotoxic effects on periapical tissues [8]. Furthermore, it does not eliminate the smear layer, necessitating its use in conjunction with appropriate chelators for that specific purpose [9]. Though it has tissue-dissolving and antibacterial qualities, NaOCl can alter dentin structure, induce allergic reaction, and not clear biofilms [10]. It has also been observed that CHX has a harmful effect on dentin substrates, even when in contact for a brief period of time [11]. CHX exhibits limited solvent capacity and has been demonstrated to exert an adverse impact on collagen fibers inside dentin, hence compromising the mechanical characteristics of dentin and potentially resulting in the debilitation of tooth structure [12], [13]. CHX forms harmful precipitates like para-chloroaniline when used along with NaOCl which may obstruct dentinal tubules and present possible health hazards [14]. Bacteria develop intricate biofilms and adhere to the tooth surface through exopolysaccharides. The bacteria persist in endodontic infections due to an adaptive mechanism inside the environment [15]. These drawbacks highlight a novel biocompatible irrigant with improved antibacterial and tissue-healing properties. Because of its broad spectrum, low cytotoxicity, and ability to attach onto the dentin surface, QAC can be a promising antibacterial irrigant.

QACs are utilized as antibacterial agents in endodontics, demonstrating their promise as novel bioactive biomaterials. These compounds demonstrate a high level of growth inhibition against a wide range of microbes, equivalent to commonly used antibiotics [16]. QACs are surfactants that have both acidic and basic properties. They typically consist of a molecule with one positively charged nitrogen atom and at least one hydrophobic group attached to it [17]. The most frequently utilized QACs for treating endodontic infections as intracanal medicaments include Cetrimide (CTR) [18, 19], Benzalkonium Chloride (BAK) [20], Quaternary ammonium compounds such as dimethylaminododecyl methacrylate (DMADDM) and dimethylaminohexadecyl methacrylate (DMAHDM) [21], as well as a new class of Organosilane quaternary ammoniums known as Quaternary ammonium silane (K21) codenamed as K21 [22, 23] (KHG FiteBac® Technology, GA, USA). The lipophilicity of quaternary ammonium biocides (QACs) is closely linked to their antibacterial activity. The hydrophobicity of the

n-alkyl chain determines the antibacterial properties of QACs against specific bacterial species. The potent antibacterial activity of QACs is due to its lipophilic alkyl chain, which can infiltrate the bacterial cell membrane. K21, containing positive nitrogen atoms, specifically targets membrane lipids, resulting in its lipophilic effects [24], [25]. Results from studies comparing various irrigants for the purpose of eradicating *E. faecalis* colonies in root canal systems need to be analyzed. To the author's knowledge, no systematic review has been published that describes the antibacterial effectiveness of different QACs compared to NaOCl and CHX against *E. faecalis* in endodontic infection. *E. faecalis*. It also provides information on several QACs and identifies the one with the greatest potential to be employed as an efficient endodontic irrigant.

Materials and methods

Study design

The systematic review adhered to the PRISMA Guidelines by Page et al., 2021 [26], and the protocol was registered in PROSPERO on 6 January 2023 (registration number: CRD42023389258). The present systematic review of research studies adheres to the principles set forth by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Fig. 1) [27]. The PICO approach used for the review was as follows:

Population (P)=*Enterococcus faecalis* was used as a test bacterium to evaluate antibacterial effect. Intervention(I)=Quaternary ammonium compounds that were used in selected studies as root canal irrigants. Comparator(s)/control (C)=NaOCl and OR CHX were used as comparative groups as root canal irrigants. Outcome (O)=CLSM or CFU were used for measure of antibacterial activity.

Research question

A research question was formulated following the PRISMA standards. The question in focus was "Does Quaternary Ammonium Compounds (intervention) exhibit antibacterial effect (outcome) against *E. faecalis* (population) compared to sodium hypochlorite (comparator 1) or chlorhexidine (comparator 2) irrigating solutions?"

Eligibility standards

Inclusion criteria Included (a) original articles, (b) studies published in English language only, (c) *in vitro* / *ex vivo* / *in vivo* studies using microbiological assessment as a part of study, (d) quaternary ammonium compound studies reporting *E. faecalis* as a strain for microbiological assessment with quaternary ammonium application, (e) NaOCl or CHX or their combination with either or with QAC studies reporting *E. faecalis* as a strain for

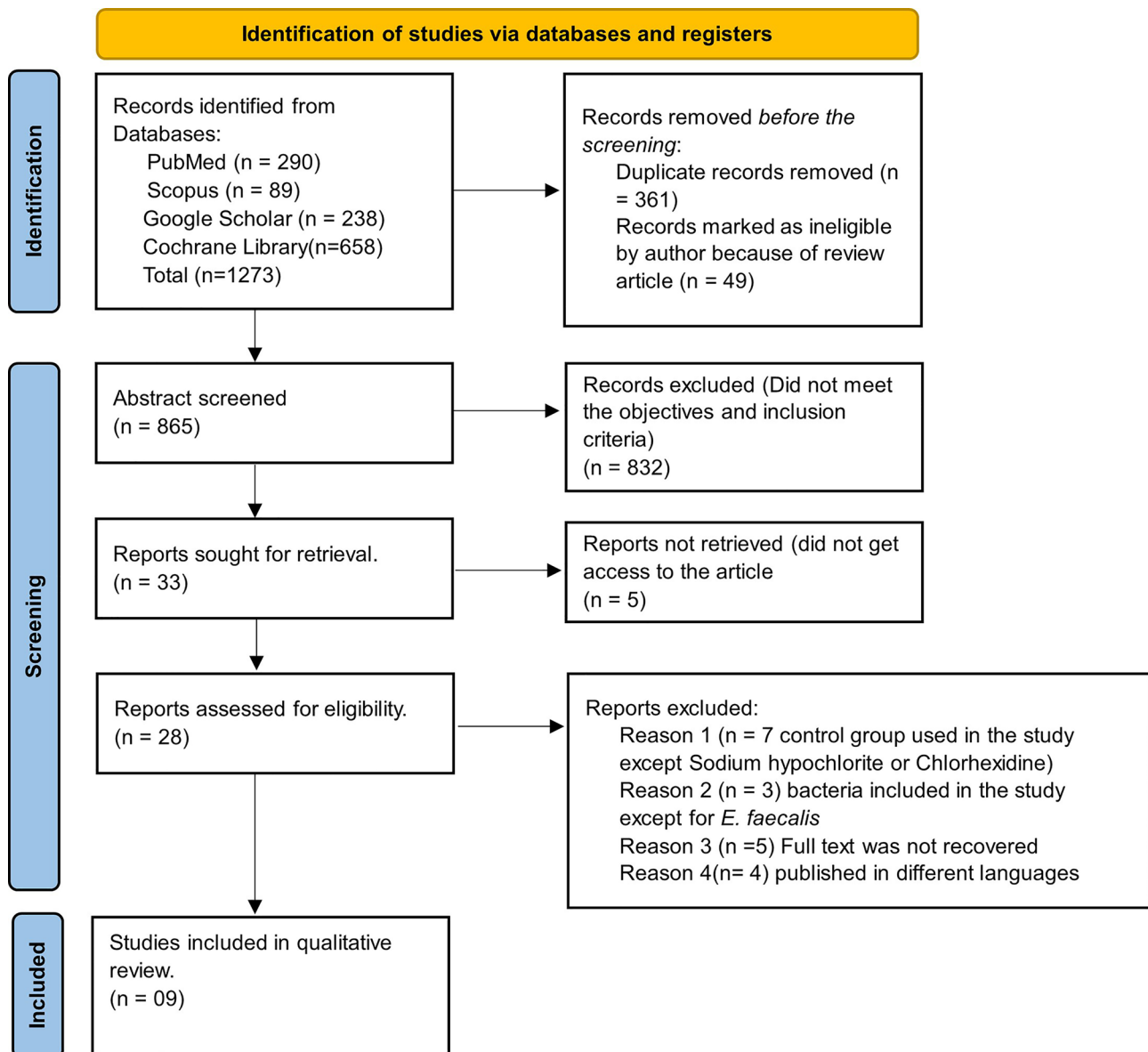


Fig. 1 PRISMA Flowchart of the Study. PRISMA 2020 flow diagram for new systematic reviews which included searches of databases and registers only. From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* 2021;372: n71. <https://doi.org/10.1136/bmj.n71>

microbiological assessment, (f) Quaternary ammonium compound used as irrigant or solution for root canal treatment.

Exclusion criteria included (a) systematic review and review papers, (b) case reports, (c) commentaries, (d) interviews, (e) updates, (f) any intervention except quaternary ammonium compounds and NaOCl or CHX for *E. faecalis* elimination, (g) Quaternary ammonium compound used as sealer or adhesive.

Any disputes regarding these criteria were resolved through consultation with a third-party impartial reviewer.

Information sources and search strategy

To meet the pre-determined inclusion and exclusion criteria, all the titles and abstracts of all the acquired studies were reviewed and screened by two independent reviewers. The databases that were searched for were “PubMed”, “Scopus”, “Cochrane library” and “Google Scholar” to search papers addressing research questions. Table 1 depicts the search strategy used for the study. The search started on 10 January and was performed for papers published between 2000 and 2024. An organized and valid practice was implemented to search the literature to detect the appropriate papers as per the criteria. Papers with available full text available were considered

for further review and analysis. Hand-searching of the papers was also done from the reference lists that could have been skipped during the initial search according to inclusion criteria. The studies were explored using a pre-determined search technique. We started with PubMed and modified our search method for the other databases. The following terms were utilized in the search strategy: “quaternary ammonium,” “quaternary ammonium salt,” “endodontic,” “endodontic irrigant,” “root canal,” “root canal irrigant,” “biofilm,” “bacteria,” “Enterococcus faecalis,” “sodium hypochlorite,” and “chlorhexidine.” Subject header truncations (*) or Boolean operators (“AND”, “OR”, and “NOT”), depending on the requirements of the relevant databases, were used to implement the search. After identifying the studies that were relevant to the study topic, two separate authors (RAB and SUM) reviewed the titles and abstracts of the articles that were found through the literature search. Assisting the third author (UMD) in addressing selection protocol biases and inconsistencies was also a priority. A subsequent round of study selection was finalized by two writers (RAB and SUM). To include studies that met the research objectives, they read each whole publication and assessed it according to the qualifying criteria. An additional author from UMD conducted separate database searches prior to the research analysis to avoid excluding the most recent pertinent study. Because there was no logistical support to retrieve and translate the articles published in languages other than English, the literature search had to be limited to English. Inter-reviewer reliability was assessed during both the study selection and data extraction phases.

The PRISMA flow chart as shown in Fig. 1 delineates the procedural overview of article screening, spanning from the inception of the review to the comprehensive paper assessment stage.

Data assortment

After retrieving all search results by filtering the titles, duplicates were removed using Mendeley Desktop

version 1.19.8. The process of abstract screening was carried out, followed by a thorough reading of the entire document. The reviewers conducted a thorough review of the papers chosen for full text. Any differences regarding the selected papers were managed through discussion.

Search items (Data Extraction)

Formulated data extraction parameters were utilized to obtain data from the selected studies. Data extraction from these studies included author, year of study, type of ammonium salt, type of intervention, data on test group (type of ammonium compound) and control groups (NaOCl and CHX), sample size, model bacteria (with strain information), method of antibacterial measure, outcome of study and statistical power calculation. The antibacterial measurement was done by CFU and or CLSM. The entire data extracted from the selected papers is appended in Table 2.

Criteria for Meta-Analysis

For meta-analysis, CLSM was used as method of outcome measurement. The rationale for including CLSM-based research in the meta-analysis was to uphold methodological consistency and ensure data compatibility for quantitative synthesis. Although CFU is a commonly utilized approach, its reporting in various studies was markedly inconsistent, some presented bacteria count as percentages, some utilized raw CFU values, and a few of them provided statistical presentations without standard deviation or mean values. These differences impeded the correct extraction or conversion of CFU data into a standardized effect size (standardized mean difference) appropriate for meta-analytic comparison. Conversely, CLSM-based investigations consistently documented quantitative and comparable parameters, including mean and standard deviation for live/dead bacterial fluorescence ratios. This consistency facilitated accurate effect size computation and minimized the potential for bias or

Table 1 Search strategy

Date	Database	Search strategy	Number of papers
22.3.23	PubMed	(((((Quaternary ammonium [Mesh Major Topic]) OR (quaternary ammonium salt [Mesh Major Topic])) AND (endodontic [Other term])) AND (root canal irrigants[Title/Abstract])) AND (Biofilm[Other Term])) OR (Bacteria[Other Term])) OR (Enterococcus faecalis[Other Term])) AND (NaOCl[Other Term])) OR (CHX[Other Term]))	290
15.3.23	Scopus	("Quaternary ammonium") AND ("endodontic treatment" OR "root canal irrigants") AND ("Biofilm" OR "Bacteria" OR "Enterococcus faecalis") AND ("NaOCl" OR "CHX")	89
20.2.23	Google Scholar	"Quaternary ammonium" AND "root canal irrigants" OR "Biofilm" OR "Bacteria" OR "Enterococcus faecalis" AND "NaOCl" OR "CHX"	238
26.4.23	Cochrane Library	(Quaternary ammonium NEXT (compound" OR salts")) AND ("endodontic") OR ("irrigant") OR ("root-canal") OR ("irrigant") OR ("root canal sealer") AND ("Enterococcus faecalis") OR ("Enterococcus") OR ("biofilm") OR ("antibacterial")	658

Table 2 Data extraction table of selected studies

Sr. No	Author	Type of ammonium	Time of application	Type of Intervention / application (Drug delivery approach)	Groups (Test and Control together)	Sample Sizes	Model/bacteria (with strain information)	Method of antibacterial measure	Outcome of study	Statistical power calculation Mean / SD	Ref
1.	Daood et al., 2020	K21	2 min	Irrigant	Group A: 6% NaOCl. Group B: 6% NaOCl + 2% CHX. Group C: 3.5% K21. Group D: 2% K21 and Sterile Saline.	Extracted human mandibular third molars (n = 120)	<i>E. Faecalis</i> (ATCC 29212) biofilm	CLSM (confocal laser scanning microscopy) and CFU(Antibacterial activity (log CFU/sample versus exposure time in hours)	K21 groups had higher mean dead bacteria values than NaOCl + CHX and NaOCl groups. The lowest mean CFU/mL readings were with 3.5% K21 specimens. Control groups (7.02 ± 0.36) had a considerably higher count, indicating greater microbial colony multiplication. Statistically, the 6%NaOCl single groups (3.0 ± 0.99) and 2%K21 (3.6 ± 0.44) groups were similar in the count range (p < 0.05).	Dead Bacteria 2%K21- 71.7 ± 5.5 3.5%K21 - 86.7 ± 4.3 6%NaOCl + CHX- 66.4 ± 6.6 6%NaOCl 70.3 ± 3.3 Control 5.67 ± 7.1 Live Bacteria 2%K21- 22.3 ± 5.4 3.5%K21- 13.3 ± 3.3 6%NaOCl + CHX- 33.6 ± 7.1 6%NaOCl - 29.7 ± 6.6 Control - 94.33 ± 2.1 Log CFU/ml 2%K21- 3.6 ± 0.44 3.5%K21 - 2.64 ± 0.11 6% NaOCl + CHX- 4.12 ± 0.28 6% NaOCl- 3.0 ± 0.99 Control- 7.02 ± 0.36	[23]

Table 2 (continued)

Sr. No	Author	Type of ammonium	Time of application	Type of Intervention / application (Drug delivery approach)	Groups (Test and Control together)	Sample Sizes	Model bacteria (with strain information)	Method of antibacterial measure	Outcome of study	Statistical power calculation Mean / SD	Ref
2.	Daood et al. 2019	K21	2 min	Irrigant	The first group, A, used 6% NaOCl and 2% CHX for manual irrigation; the second, B, used 6% NaOCl and 2% CHX for ultrasound irrigation; the third, C, used 6% NaOCl and 2% K21 for manual irrigation; the fourth, D, used 6% NaOCl and 2% K21 for ultrasound irrigation; the fifth, E, and F, used saline for both manual and ultrasound irrigation, respectively.	Eighty healthy, single-rooted anterior teeth were collected from individuals ranging in age from twenty-one to twenty-nine.	<i>E. faecalis</i> (ATCC 29212) biofilm	CLSM (Mean % of Dead and Live bacteria)	The highest reduction of live bacteria were found with 6% NaOCl+2% K21 group compared to other groups.	Mean +SD Dead Bacteria Manual: NaOCl±2% CHX 69.22±5.12, NaOCl ± 2% CHX (ultrasonic) 84.11 ±6.43, NaOCl+2% K21 (manual) 94.64 ± 11.32, NaOCl±2% K21 (ultrasonic) 97.45 + 3.79 Live bacteria NaOCl ± 2% CHX (manual) 29.78+8.12 NaOCl ± 2% CHX (ultrasonic) 15.69 + 5.54 NaOCl ± 2% K21 (manual) 5.36+9.21 NaOCl ± 2% K21 (ultrasonic) 2.33 ± 6.39 Control 98.71 ± 0.71	[42]

Table 2 (continued)

Sr. No	Author	Type of ammonium	Time of application	Type of Intervention / application (Drug delivery approach)	Groups (Test and Control together)	Sample Sizes	Model bacteria (with strain information)	Method of antibacterial measure	Outcome of study	Statistical power calculation Mean / SD	Ref
3.	Daood et al. 2021	K21	2 min	Irrigant	0.5% K21/E, 1% K21/E, 6% NaOCl, 6% NaOCl + 2% CHX, 2% CHX	A total of 135 non-carious single-rooted anterior teeth were removed from patients aged 21 to 31 years.	<i>E. faecalis</i> (ATCC 29212) biofilm	CLSM (Mean % of Dead and Live bacteria)	Compared to control and NaOCl, 0.5% K21-E reduced microbial load. The antibacterial efficacy of 0.5% K21-E was higher than the 6% NaOCl and 2% CHX. With increased concentration of K21, there was decreased viable bacteria ($p < 0.05$). Group 1% K21 exhibited the highest dead cell numbers. Dead cell counts did not significantly differ between the 2% CHX and 6% NaOCl + 2% CHX groups; however, both differed significantly from the 0.5% and 1% K21 groups ($p < 0.05$).	Dead Bacteria 0.5% K21- 90.1 + 11.1 1% K21- 96.2 + 9.1 6% NaOCl- 86.4 + 7.8 Live Bacteria 0.5% K21- 9.9 + 2.4 1% K21- 3.8 + 0.99 6% NaOCl- 15.6 + 2.2 2% CHX- 10.1 + 0.8 6% NaOCl + 2% CHX- 16.7 + 4.1	[22]
4.	Nascimento CA et al. 2014	CTR	1 min and 3 min	Irrigant	2.5% NaOCl, 2.5% NaOCl + 0.2% CTR, 2% CHX, 2% CHX + 0.2% CTR, 0.2% CTR, QMix control	Sample size is not specified, but the bovine central incisor roots were used.	<i>E. faecalis</i> strain ATCC 29212, planktonic and biofilm	CFU (after 1 min application)	The antibacterial action against biofilm was not enhanced by adding CTR to CHX and NaOCl solutions. There was action against planktonic <i>E. faecalis</i> in all of the irrigants and associations that were tested. After 1 and 3 min of direct contact, only NaOCl and NaOCl + CTR were able to remove the biofilm.	Mean Log10 CFU ml-1 2.5% NaOCl- 0 2.5% NaOCl + 0.2% CTR- 0 2% CHX - 4 2% CHX + 0.2% CTR- 4.2 0.2% CTR- 4.2 QMix- 5.5 Control- 7.9	[18]
5.	Oncag et al. 2003	CTR	5 min	Irrigant	5.25% NaOCl, 2% CHX, 2% CHX + 0.2% CTR	Sixty complete, freshly removed permanent teeth	<i>E. faecalis</i> strain ATCC 29212	No Growth of bacteria after 5 min	Compared to a 5.25% NaOCl solution, Cetrexidin and 2% CHX gluconate were safer, more effective, and had greater lasting antibacterial effects.	5.25% NaOCl- 6% 2% CHX- 15% 2% CHX + 0.2% CTR- 10%	[19]

Table 2 (continued)

Sr. No	Author	Type of ammonium	Time of application	Type of Intervention / application (Drug delivery approach)	Groups (Test and Control together)	Sample Sizes	Model bacteria (with strain information)	Method of antibacterial measure	Outcome of study	Statistical power calculation Mean / SD	Ref
6.	Alovisi M et al. 2022	Benzalkonium Chloride (BAK).	2 min	Drug Delivery of Nanodroplets loaded with BAK (NDs-BAK)	BAK 5% NaOCl 2% CHX Positive control, Negative control	A total of 72 single-root teeth from humans (n=20), NDs-BAK (n=20), NaOCl (n=20), CHX (n=20), Positive control (C+) (n=6) Negative control (C-) (n=6)	<i>E. faecalis</i> ATCC 29212 biofilm	CLSM (Red Fluorescence ratio %)	NaOCl had a greater mean red fluorescence ratio (91.23%), while NDs-BAK and CHX groups had similar ratios (68.78% and 65.14%, respectively). NaOCl was more antibacterial than nanodroplets with BAK, which worked like CHX. A statistical difference was found between NDs-BAK and NaOCl ($p < 0.01$) and NaOCl and CHX ($p < 0.001$) in antibacterial activity.	BAK-NDs- 68.78 + 0.0956 NaOCl- 91.23 + 0.1066 CHX- 65.14 + 0.1362 C + - 0.01 + 0 C - Not done	[20]
7.	Tiwari 2020	quaternary ammonium salts (QAMS)-DMAHDM-methylaminododecyl methacrylate and DMAHDM-dimethylaminohexadecyl methacrylate	3 min and 10 min	irrigants	300 µg/ml DMADDM 37.5 µg/ml DMAHDM 2% CHX 5.25% NaOCl Control	Twenty extracted bovine central incisors	<i>E. faecalis</i> , (ATCC 19433) Lactobacillus acidophilus and Actinomyces naeslundii biofilms.	CFU (after 10 min)	The NaOCl group had the strongest antibacterial activity, with about 6 log reductions in CFU ($p < 0.05$). The antibacterial compounds DMADDM and DMAHDM were absorbed in dentine blocks, inhibiting bacterium colonization, and killing bacteria in dentinal tubules.	LogCFUs/ml The result is presented in mean CFUs + Standard deviation (* $p < 0.05$). DMADDM: 5log DMAHDM: 5log NaOCl:3log	[21]
8.	Tiwari 2019	QAMS (DMADDM and DMAHDM)	3 min, 5 min and 10 min	irrigants	QAMS, CHX and NaOCl	Not Mentioned	<i>S. gordonii</i> (ATCC 10558), <i>E. faecalis</i> (ATCC 19433), <i>L. acidophilus</i> (ATCC 4356), and <i>A. naeslundii</i> (ATCC 12104), planktonic and biofilms	CFU, Confocal (values not available)	QAMs at a concentration of 1% exhibited greater efficacy than 2% CHX and were similar in antibacterial activity to 5.25% NaOCl.	There was a reduction of 7 log after 3 min treatment for all the compounds. QAMS were effective than CHX	[43]

Table 2 (continued)

Sr. No	Author	Type of ammonium	Time of application	Type of Intervention / application (Drug delivery approach)	Groups (Test and Control together)	Sample Sizes	Model bacteria (with strain information)	Method of antibacterial measure	Outcome of study	Statistical power calculation Mean / SD	Ref
9.	Baca et al. 2011	CTR	1 min	Irrigant	0.2% CTR 2.5% NaOCl, 2% CHX, 17% EDTA, 7% maleic acid (MA),	Eighty non-carious, unrestored freshly extracted human molars	<i>E. faecalis</i> ATCC 29212 biofilm	CFU (after 1 min application)	When tested against <i>E. faecalis</i> biofilms that had been incubated for three weeks, the antimicrobial activity testing revealed that NaOCl and CTR were far more effective than CHX after only one minute of contact time.	Kill percentage mean \pm standard deviation* 0.2% CTR- 100 2.5% NaOCl- 100	[30]

heterogeneity in the pooled analysis. Consequently, CLSM was used as the sole outcome measure to maintain the internal validity and statistical integrity of meta-analysis. NaOCl was used as comparative group in the selected studies to assess antibacterial efficacy in comparison to QACs. A random-effects model was used to present the data, and a 95% confidence interval (CI) was obtained for the pooled estimate of studies that used ammonium compounds as irrigants. The present inquiry was believed to be better suited to the meta-random-effects analysis method. The average and standard deviation of the number of dead bacteria after being treated with ammonium compound and NaOCl were detected by CLSM. For meta-analysis, the study's sample size was retrieved. Researchers agreed that the random-effect model was the best choice when there was an increased variation in the studies included in the analysis. The percentages of Cochran's Q test (χ^2) and the I² statistic were utilized to ascertain the link between study and estimate heterogeneity. The result is deemed statistically significant if the p-value is less than 0.05. To examine the antibacterial effect of adding an ammonium compound to the overnight culture of *E. faecalis*, the combined results of the investigation were presented using a forest plot (Fig. 2). The number of dead bacteria was measured with CLSM after adding ammonium compound. This transition was recorded in 95% confidence interval (CI). The analysis was performed by using Revman, Cochrane's Review Manager 5.4.1 software, USA.

In Fig. 2, bacterial inhibition caused using NaOCl was considered as the control group, and bacterial inhibition caused by adding ammonium compound was considered as the test group. From the result of Daood et al., 2020 and Daood et al., 2021, it was shown that K21 has better results in bacterial inhibition compared to incorporating NaOCl. On the other hand, Alovisi et al., 2022 confirmed that NaOCl exhibited better antibacterial activity compared to BAK ammonium salt.

Synthesis of findings The statistical technique known as inverse variance approach was employed to generate the meta-analysis results, which were accompanied by a 95% confidence interval (CI). The calculations of a meta-analysis, statistical heterogeneity, p values, z values, and subgroup analysis using a forest plot were validated using Cochrane Review Manager 5.4.1.

Reporting biases

Two autonomous authors assessed the operational quality of chosen studies using a quality evaluation form used by the Cochrane Collaboration. This tool evaluated the following factors: randomization, allocation

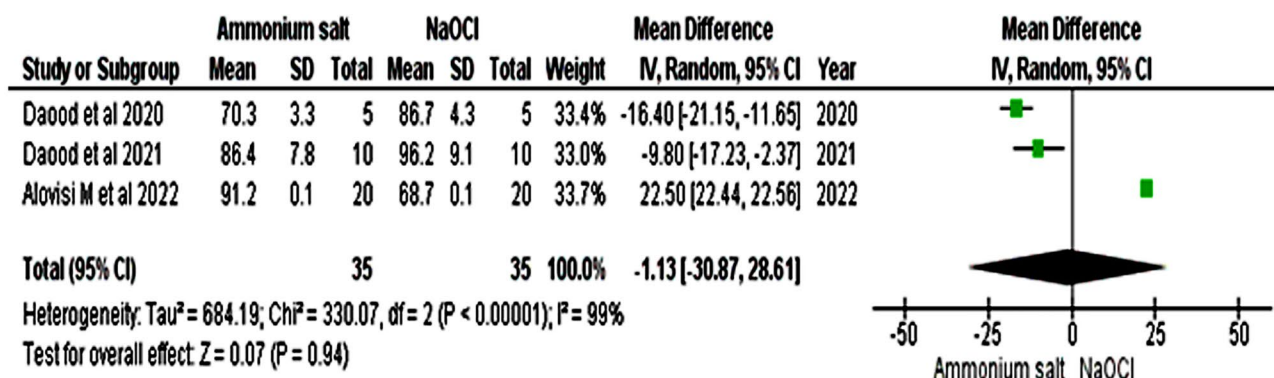


Fig. 2 Forest plot of antibacterial effect against *E. faecalis* of ammonium salt (K21 and BAK) and NaOCl. Meta-analysis evaluated the antibacterial effect between irrigant groups for its efficacy against *E. faecalis*

concealment, extent of follow-up, blinding procedure, publication of only specific outcomes, and other sources of bias [28]. We have identified substantial concerns concerning bias, including a fundamental discrepancy in factors directly related to the consequences, which were not focused on the other areas covered by the QUIN Tool and the information provided in Tables 3 and 4 [29]. The QUIN tool offers a method to assess the potential for bias in in-vitro studies, enabling researchers to assess the reliability of study results. This tool assessed a total of twelve criteria. Each criterion was assigned a score based on its level of specification: properly specified = 2 points, inadequately specified = 1 point, not specified = 0 points, and not applicable criteria were excluded from the calculation. The scores were subsequently aggregated to obtain a cumulative score for the in-vitro investigation. The ratings received were categorized as high risk of bias (50%), medium risk of bias (50–70%), or low risk of bias (>70%). This was computed using the subsequent mathematical equation:

$$\text{Final score} = (\text{Total score} \times 100) / (2 \times \text{number of criteria applicable})$$

The study findings, which encompassed the methodology, sample size, interventions, and outcomes, were documented independently by two authors (RAB and SUM) on a data extraction sheet. Discrepancies were resolved through deliberation and consensus with a third author (UMD), in addition to a thorough examination of the trial report. Two independent reviewers screened the titles, abstracts, and full texts, and any disagreements were resolved through discussion or consultation with a third reviewer. To evaluate the level of agreement, Cohen’s kappa (κ) statistics were calculated, yielding a value of ($\kappa = 0.71$), indicates substantial agreement. This measure ensured consistency and minimized potential bias in study inclusion and data collection.

Results

Study selection

Three electronic databases, “Scopus” (89 papers), “PubMed” (290 papers), “Cochrane library” (658) and “Google Scholar” (238 papers), were searched using a multi-step search approach.

A total of ten papers were selected for final analysis. Initially, a total of 1273 papers were retrieved across all four databases. Following the elimination of duplicates, papers marked by author as ineligible, 865 papers remained for examination. Subsequently, 832 papers were excluded due to their failure to meet the stipulated inclusion criteria, resulting in 33 papers undergoing full-text assessment. Of these, 5 papers could not be accessed, and full texts could not be retrieved. Ultimately, 28 papers were subjected to appropriateness evaluation, of which a portion was not included in the qualitative analysis; specifically, 19 papers were excluded. Among the excluded studies, 7 utilized control groups differing from NaOCl or CHX, 3 studies involved bacteria other than *E. faecalis*, and 5 studies showed incomplete outcomes, and 4 studies were published in languages other than English. The qualitative analysis encompassed a total of 9 papers, of which 3 were deemed suitable for inclusion in the meta-analysis. The study conducted by Daood et al. (2019) utilizing a mixture of NaOCl within both case and control groups was excluded from the meta-analysis due to this particular methodology. Furthermore, six additional studies were omitted from the meta-analysis due to variations in their antibacterial measurement methods, specifically relying on CFU, alongside the absence of consistent documentation regarding the time required for bacterial eradication across all studies.

Analysis of data extraction table of selected studies

In this review, selected studies showed that four QACs (test group) were identified as root canal irrigants including QAMS, BAK, CTR and K21 as described in Table 2. These were compared to CHX and NaOCl (control

Table 3 Quin tool assessment of selected studies

Sr. No	Author	Criteria for Quin Tool Assessment										Pre-sentation of results		
		Clearly stated aims/objectives	Detailed explanation of sample size calculation	Detailed explanation of sampling technique	Details of comparison group	Detailed explanation of methodology	Operator details	Randomization	Method of Measuring outcome	Outcome assessor details	Blinding		Statistical analysis	
1.	Alovisi M et al., 2022	2	0	0	2	2	2	2	2	2	NA	2	2	2
2.	Daood et al., 2019	2	1	1	2	2	2	2	2	NA	2	2	2	2
3.	Daood et al., 2020	2	1	1	2	2	2	2	2	NA	2	2	2	2
4.	Daood et al., 2021	2	1	1	2	2	2	2	2	NA	2	2	2	2
5.	Tiwari et al., 2020	2	1	0	2	2	2	2	2	NA	1	2	2	2
6.	Tiwari et al., 2019	2	0	0	2	2	2	2	2	NA	2	2	2	2
7.	Baca et al., 2011	2	1	1	2	2	2	2	2	NA	2	2	2	2
8.	Nascimento CA, et al. 2014	2	1	0	2	2	2	2	0	NA	2	2	2	2
9.	Oncag et al., 2003	2	1	1	2	2	2	0	0	NA	2	2	2	2

Table 4 Quin tool evaluation of risk of bias

Sr. No	Author	Total Score	Final Score	Risk of Bias
1.	Oncag et al. 2003	12	66.6	Medium
2.	Nascimento CA et al. 2014	11	61.1	Medium
3.	Baca et al. 2011	17	94.4	Low
4.	Daood et al. 2019	16	88.8	Low
5.	Tiwari et al. 2019	12	66.6	Medium
6.	Daood et al. 2020	14	77.7	Low
7.	Tiwari et al. 2020	12	66.6	Medium
8.	Daood et al. 2021	14	77.7	Low
9.	Alovisi M et al. 2022	14	77.7	Low

groups) for their antibacterial activity against *E. faecalis*. Tiwari et al., 2019 and 2020 investigated potential of QAMS, Oncag et al., 2003, Baca et al., 2011 and Nascimento CA et al., 2014 evaluated effect of CTR, Alovisi et al., 2022 studied effect of BAK, while Daood et al., 2019, 2020 and 2021 explored antibacterial effect of K21.

Baca et al., 2011, Nascimento CA et al., 2014, Tiwari et al., 2019 and Tiwari et al., 2020, Daood et al., 2020 applied CFU to measure antibacterial activity whereas Alovisi et al., 2022, Daood et al., 2019, Daood et al., 2020 and Daood et al., 2021 used CLSM to measure the antibacterial activity. Most of the studies used the same strain of *E. faecalis* (ATCC 29212) except study by Tiwari et al., 2019 and 2020 which used *E. faecalis* strain (ATCC 19433).

All the studies used dentin as a primary substrate to allow *E. faecalis* biofilm formation. The source dentin was human permanent teeth, however two of the studies (Nascimento et al., 2014 and Tiwari et al., 2020) used bovine teeth to allow *E. faecalis* biofilm formation.

Application time of irrigation varied across studies with Daood et al., 2019, 2020, 2021, Alovisi et al., 2022 applied for 2 min, Baca et al., 2011 and Nascimento CA et al., 2014 applied for 1 min, Tiwari et al., 2019 and 2020, Nascimento CA et al., 2014 applied for 3 min, Oncag et al., 2023 and Tiwari et al., 2019 applied for 5 min, Tiwari et al., 2019 and 2020 applied for 10 min. For concentration of irrigants in control group, all the studies used 2% concentration of CHX.

Most of the studies used NaOCl concentration in range of 5.25% or 6%, except for Baca et al., 2011 and Nascimento CA et al., 2014 that used 2.5% NaOCl. The concentration of test irrigants varied (information provided in Table 2) in all the studies depending on their manufacturing protocol [18–23, 30].

Risk of Bias in the included studies

All the included studies were evaluated for their risk of bias using the QUIN tool (2022) [29] as depicted in Tables 3 and 4. Out of the 9 studies included, 5 (55.5%) presented low risk of bias, the remaining 4 (44.4%)

presented medium risk of bias. None of the studies presented a high risk of bias. All the research studies explicitly stated their objectives. The studies included detailed descriptions of test groups, control groups, methodological techniques, the statistical presentation, and the outcome of the data. Two studies lacked details regarding sample size calculation and the method used to measure outcomes. Six research studies failed to describe the methods used for sequence generation and allocation concealment. The study did not require information regarding the number of operators, their training and calibration, and the blinding of operators. After the QUIN tool assessment, 3 studies showed a low risk of bias, which we chose for meta-analysis.

Synthesis of meta-analysis

Meta-analysis was conducted with three studies. The meta-analysis evaluated the antibacterial effect of root canal irrigants with and without the use of ammonium compound. We selected three studies because these studies that exhibited similar research criteria and applied CLSM method for investigating bacterial death using ammonium compounds as irrigants in the test group and NaOCl as control group. The ammonium compounds that were considered as per studies were BAK and K21 [22].

The meta-analysis results indicated a substantial level of heterogeneity ($I^2=99\%$) among studies assessing the effect of two different intervention groups on bacterial inhibition. The Chi-squared test further confirms this heterogeneity, showing a significant difference between the effect sizes of the studies ($\text{Chi}^2=330.07$, $p<0.00001$). Despite this significant variation, the overall effect size of the interventions on bacterial inhibition is very small ($Z=0.09$, $p=0.94$), suggesting that the difference between the two intervention groups is not statistically significant. Additionally, the Tau^2 value of 684.19 indicates considerable between-study variance beyond chance. Taking together, while there is substantial variability in the effects observed across studies, the meta-analysis does not demonstrate a significant difference between the two intervention groups (QACs and NaOCl) in terms of their impact on bacterial inhibition.

Discussion

For efficient dentin disinfection and prevention of root canal infection recurrence, it is necessary that root canal irrigating solutions have sustained antibacterial effectiveness. Quats (Quaternary Alkyl Compounds) are a group of chemicals with two fundamental components: a nitrogen atom in the center and four clusters of atoms connected to the nitrogen atom. They exhibit specific physical and chemical characteristics that influence their behavior and toxicity [31]. In this review, as per selection

criteria, four QACs were tested for their efficacy against *E. faecalis* compared to NaOCl and CHX as control groups.

Critical analysis of studies on CTR

CTR, a QAC composed of tetradonium bromide, cetrimonium bromide, and laurtrimonium bromide, is widely used as an antiseptic and disinfectant due to its cationic nature and propensity to break cellular membranes [32]. Oncag et al., 2003 evaluated the antibacterial effects of three concentrations of CHX and NaOCl on human teeth. The in vitro study found that a solution containing 2% CHX and Cetrexidin (0.2% gluconate + 0.2% CTR) was more effective than 5.25% NaOCl. The study concluded that Cetrexidin and 2% CHX outperformed the NaOCl solution in terms of efficacy, duration of antibacterial effects, and safety [19]. These results were in accordance with Turkun et al., 1999 [33] and D'Arcangelo et al., 1999 [34]. Turkun et al., 1999 showed that Cetrexidin exhibited superior antibacterial action compared to 0.2% CHX alone. It is due to lowering of surface tension by CTR that enables better wettability, contact and penetration of CHX within dentin tubules for enhanced antibacterial action. Baca et al., 2011 tested various solutions on dentin blocks for antibacterial efficacy and residual antibacterial activity against *E. faecalis* biofilms. The 3-week *E. faecalis* biofilm was effectively eradicated using 2.5% NaOCl alone or in combinations, and the same result was observed with 0.2% CTR. However, for residual antibacterial activity, results showed that solutions with 2.5% NaOCl had the lowest residual activity (18.10%), while solutions with 2% CHX and 0.2% CTR showed total *E. faecalis* biofilm suppression (100%). The study concluded that 0.2% CTR was the most effective irrigating solution [30]. Weber et al., 2003 also showed that NaOCl exhibited very few residual antibacterial activities. This could be due to presence of high surface tension and free chlorine ions that prevents complete penetration of NaOCl solution inside the dentin tubules [35, 36]. The buffer capability of dentin also inactivates NaOCl hampering its penetration action [37]. Superior results of CTR are due to its cationic nature like CHX, in interacting with dentin and exhibiting enhanced action. It also reduces the surface tension, increasing its penetration within the dentin tubules and anatomic variations in the root canal system [38, 39].

In their study, Nascimento CA et al., 2014 examined the efficacy of NaOCl and CHX, both alone and in combination with CTR and QMix, against both *E. faecalis* planktonic and biofilm forms. After a duration of one minute, CHX showed effectiveness that was similar to CHX + CTR and QMix, as well as NaOCl and NaOCl + CTR. Nevertheless, there was no noticeable distinction between CTR and QMix in relation to the

efficacy of biofilm removal [18]. The investigation determined that the addition of CTR did not augment the antibacterial efficacy of CHX and NaOCl against biofilm which contradicts findings of research done by Baca et al., 2011. One of the factors to be considered is the type of samples used to grow bacteria. Baca et al., 2011 and Oncag et al., 2003 used teeth of human origin while Nascimento CA et al., 2014 used teeth of bovine origin. The findings of study by Nascimento CA et al., 2014 could be affected by inactivation of CHX, CTR irrigants by bovine dentin components [18]. The content and structure of human and bovine dentin can affect root canal irrigants' antibacterial capabilities and penetration into dentinal tubules. Thus, dentin type can affect research results and interpretation of root canal irrigants' efficacy in endodontics against bacterial infections. However, considering the findings of study by Oncag and Baca et al., use of CTR as a quaternary ammonium compound can be used in combination with CHX to enhance elimination of *E. faecalis* from dentinal tubules. Further in vivo RCT studies should be conducted to validate these in vitro results for clinical application.

Critical analysis of studies on K21

K21 is silane modified QAC containing four alkyl or aryl groups, a chloride or bromide anionic ion, and a positively charged nitrogen. K21 links nitrogen to carbon-containing lipophilic chain of 18 carbon atoms. The 18-carbon alkyl chain SiQAC is K21. Propyl and trialkoxysilyl groups bind nitrogen and silicon to SiQAC silane forming a three-dimensional organic silicate network [40] [41].

The findings from the studies by Daood et al. in 2019, 2020, and 2021 suggest that the combination of K21 and NaOCl or CHX as root canal irrigants exhibits strong antibacterial efficacy against *E. faecalis*. In the study from Daood et al., 2019, CLSM analysis showed that the 2% K21 and 6% NaOCl group had the highest bacterial reduction compared to the control and CHX group. This can be attributed to the contact killing of bacteria by K21, which causes leaking of the cytoplasmic content due to its alkyl chain [42]. In the study from 2020, the effect of different concentrations of K21, NaOCl, NaOCl+CHX, and saline against *E. faecalis* was investigated. CLSM analysis showed that the 3.5% K21 group exhibited the highest bacterial reduction compared to the 6% NaOCl group, while the NaOCl+CHX group showed a green zone indicating live bacteria. The CFU results also showed a comparable reduction between 2% K21 and 3.5% K21. The sol-gel based K21 was found to have sustained antibacterial action due to its ability to fully condense when in contact with dentinal fluid and moisture, preventing leaching out of the dentin surface [23]. The research conducted by Daood et al., 2021 found that both

0.5% K21 and 1% K21 were effective in reducing bacterial growth when compared to saline, 6% NaOCl, and 2% CHX. This indicates that K21 has a significant impact on bacterial reduction. The study by Kok et al. in 2021 also supported the prolonged antibacterial effect of 2% K21 compared to 2% CHX against *E. faecalis*. The K21 irrigant outperformed 6% NaOCl alone, 6% NaOCl, and 2% CHX in terms of antibacterial activity. Daood et al.'s 2021 research found that 0.5% and 1% K21 significantly reduced bacterial growth compared to saline, NaOCl, and CHX. K21's hydrophobic tail penetrates bacterial cell membrane, causing membrane integrity breach and cell death [22].

K21 is a promising antibacterial biomaterial in endodontics, effectively decreasing *E. faecalis* biofilm. A concentration of 0.5–1% eliminates most bacterial cells. The crust formed after K21 irrigation shows organic activity and prolonged contact killing, limiting bacteria regrowth. K21 enhances stability through siloxane bridges and apatite crystallites, blocking dentinal tubules and preventing bacterial invasion [40]. A 0.5-1% K21 can be proposed to be used as an antibacterial irrigant during endodontic therapy.

Critical analysis of studies on QAMS and BAK

QAMS (DMADDM and DMAHDM) were assessed as a novel disinfectant for elimination of endodontic bacteria. Tiwari et al., 2019 [43] and 2020 [21] showed that when comparing the DMADDM and DMAHDM compounds to CHX or NaOCl, it was found that at a lower concentration (1% = 10,000 µg/mL), the novel compounds were just as effective as 2% CHX and 5.25% NaOCl in both killing *E. faecalis* and destroying their biofilm. CHX binds to bacteria's cell wall, causing membrane disruption [44]. Bacteria resistance against CHX and cross resistance to DMADDM may share a common mechanism for cell lysis [45]. QAMs form free volume in cell membranes, causing imbalance and intracellular pressure. One of the limitations of DMADDM and DMAHDM is that they can only partially inactivate antibacterial effects by dentine, dentine matrix, and dead bacteria, but can be absorbed in dentine blocks. Hence, more research needs to be done to assess the potential of these monomers as root canal irrigants.

BAK is a QAC used as a disinfectant and antibacterial agent, composed of a benzyl group, nitrogen atoms, methyl and alkyl groups, with its biocidal activity influenced by its chemical chain length. Alovisei et al., 2022 examined the antibacterial effectiveness and penetration of chitosan nanodroplets containing BAK for endodontic disinfection. Three experimental groups were tested utilizing *Enterococcus faecalis*-infected human single-root teeth: NDs-BAK, NaOCl, and CHX. Confocal laser scanning microscopy showed that NDs-BAK and CHX

had similar antibacterial effects and infiltrated dentinal tubules. NaOCl had the strongest antibacterial action, although NDs-BAK performed similarly to CHX, suggesting it could disinfect root canals [20].

Critical analysis of effect of irrigants on *E. faecalis* elimination from root canals

This systematic review and meta-analysis demonstrated the efficacy of QACs as antibacterial agents against *Enterococcus faecalis* in endodontic infections. The meta-analysis did not reveal a statistically significant difference between QACs and sodium hypochlorite (NaOCl); however, the qualitative synthesis indicates that specific QACs, notably K21 and CTR demonstrated encouraging antibacterial characteristics. A comprehensive analysis of the results revealed that although QACs provide benefits like prolonged antibacterial efficacy and diminished cytotoxicity at lower concentrations, their effectiveness is contingent upon factors such as concentration, formulation, contact duration, and the characteristics of the biofilm model. K21 consistently showed superior bacterial reduction and biofilm eradication owing to its capacity to break cell membranes through its lipophilic tail and form a persistent organosiloxane network upon condensation under humid conditions. These attributes facilitate enhanced infiltration and sustained antibacterial efficacy within dentinal tubules. Research conducted by Daood et al., 2020 and Daood et al., 2021 indicated that K21 at concentrations of 0.5–1% surpassed 6% NaOCl and 2% CHX in diminishing *E. faecalis*, while exhibiting enhanced biocompatibility [22, 23]. In contrast, Alovisi et al., 2022 found that BAK performed similarly to CHX but was less effective than NaOCl, possibly due to differences in biofilm penetration or chemical interactions with dentin [20].

Factors such as biofilm maturity, dentin substrate (human vs. bovine), and exposure time significantly influenced irrigant efficacy. For example, Nascimento et al., 2014 observed that CTR did not enhance the antibacterial effect of CHX or NaOCl, contradicting earlier findings by Baca et al., 2011 [18, 30]. This inconsistency may be due to differences in biofilm models or the inactivation of irrigants by bovine dentin components. Additionally, the limited number of studies available for meta-analysis ($n = 3$) restricted the generalizability of the pooled results, emphasizing the need for more high-quality, standardized in vitro and in vivo studies. In a study by Nascimento et al., 2014, the duration of application of irrigant was a critical factor. The biofilm elimination by NaOCl was more pronounced after 1 min compared to other irrigants in comparison to application after 3 min. CHX could not eliminate biofilm completely after 3 min. This observation aligned with Ariaz-Moliz et al., 2010 who examined CHX at 4% concentrations for 2 min [46]. Also,

although NaOCl showed better antibacterial efficacy as per findings by Alovisi et al., but its toxic effects on apical and periodontal tissues pose a risk during root canal treatment [47]. Swallowing it accidentally can lead to pharyngeal oedema and esophageal burns [48].

The effectiveness of CTR-based irrigants like Cetrexidin had variable results. Oncag et al., 2003 indicated that a combination of 0.2% CTR and 2% CHX was more efficacious than 5.25% NaOCl in eliminating *E. faecalis* in human teeth [19]. Baca et al., 2011 demonstrated that 0.2% CTR exhibited 100% residual antibacterial activity, exceeding that of CHX and NaOCl [30]. Nascimento et al., 2014 discovered that CTR did not improve the effectiveness of CHX or NaOCl against biofilms cultivated on bovine dentin, a substrate that may inactivate these agents, thereby elucidating the inconsistency [18].

QAMS (e.g., DMADDM and DMAHDM) were equivalent to NaOCl and CHX at 1% concentrations, but their dentinal interactions and ability to retain antibacterial activity in complex biofilms need further investigation. Similar to CHX, BAK encapsulated in chitosan nanodroplets showed efficacy and tubule penetration in *E. faecalis*-infected root canals. However, NaOCl has higher bacterial lethality, raising questions about efficacy-cytotoxicity balance [21]. Due to its strong resistance, *E. faecalis* are frequently examined in both planktonic and biofilm states. The development of its extracellular matrix is affected by strain origin, environmental factors, and biofilm architecture. In failed root canal cases, diverse virulence gene profiles and drug resistance are noted among *Enterococcus* species. Consequently, the utilization of clinical root canal strains is advised for more pertinent in vitro research [49].

The meta-analysis demonstrated significant heterogeneity exceeding what would be expected by chance. The exceptionally high I^2 score (99%) signified that the majority of variability in study outcomes arose from significant differences in technique, intervention regimens, and outcome measurements, rather than random error. This substantially compromises the integrity and clarity of the pooled results. Although the overall analysis revealed no statistically significant difference across the intervention groups, the marked heterogeneity demanded careful interpretation and highlighted the necessity for additional standardized research in this domain. The meta-analysis revealed substantial between-study variance ($\text{Tau}^2 = 684.19$) and a highly significant Chi-squared test for heterogeneity ($\text{Chi}^2 = 330.07$, $p < 0.00001$), indicating that the variation in effect estimates was predominantly attributable to differences among studies rather than sampling error. Despite this variability, the pooled effect size for bacterial inhibition between quaternary QACs and NaOCl revealed no statistically significant difference ($Z = 0.09$, $p = 0.94$), indicating negligible overall efficacy

variations. Nonetheless, due to the significant clinical and methodological discrepancies amongst research studies, these results should be considered with caution. The variances of specific QAC types, bacterial strains tested, irrigant concentrations and formulations, exposure times, and outcome measures likely influenced the differential antimicrobial efficacy. This indicated that although the meta-analysis offered an aggregated effect estimate, it may not precisely represent the effectiveness of individual QACs in distinct situations. To enhance comprehension of these varied impacts and reduce heterogeneity, subsequent analyses should include subgroup or sensitivity analyses classified by bacterial species, exposure factors, or QAC type.

Research on alternative irrigants used on elimination of *E. faecalis* from root canals

In addition to the QACs investigated, literature has consistently pursued the development of alternative molecules with the aim of eradicating *E. faecalis* from the endodontic system. Puleio et al., 2024 assessed the antibacterial efficacy of a Human Milk Oligosaccharide (HMO) solution including 2'-fucosyllactose and lacto-N-neotetraose against *E. faecalis* in root canals. The HMO solution exhibited negligible bactericidal action, as the majority of samples remained turbid, signifying bacterial persistence, in contrast to the markedly more efficacious NaOCl group. The temporary bacteriostatic action exhibited no duration nor dose dependence. The study indicated that, under present circumstances, HMOs were ineffective against *E. faecalis* and suggested future research employing broader HMO combinations, quantitative assays, and multispecies models to investigate their potential in endodontics [50].

Recent developments in endodontic disinfection have investigated many innovative biomaterials and techniques to efficiently eradicate *E. faecalis*, a potent pathogen linked in root canal failures. Comparatively to triple antibiotic paste and calcium hydroxide, studies have indicated that amoxicillin-clavulanate paste (ACP) exhibited better antibacterial efficacy [51]. Furthermore displaying improved bacterial eradication in a short exposure time was graphene oxide combined with double antibiotic paste (metronidazole and ciprofloxacin) [52]. Especially at higher light intensities, photodynamic treatment (PDT), utilizing methylene blue or photosensitizers like toluidine blue and phycocyanin operated by diode or Er: YAG lasers, greatly reduced bacterial count [53]. Activation of 2% chlorhexidine with Er, Cr: YSGG laser demonstrated more successful than traditional approaches in hard-to-reach isthmus locations [54].

Novel techniques, including nanosilica-mediated antibiotic administration and lipopeptide biosurfactants, have demonstrated efficacy in eliminating *E. faecalis*

biofilms. The use of silica nanoparticles with doxycycline, metronidazole, and ciprofloxacin markedly improved antibacterial effectiveness. Moreover, lipopeptide biosurfactant (LB) exhibited potent antibacterial properties, particularly in conjunction with NaOCl, hence augmenting its efficacy. These methodologies underscore the capacity of innovative drugs to enhance *E. faecalis* biofilm breakdown and facilitate sustained efficacy in root canal therapy [55, 56].

CFU data was excluded from Meta-Analysis because of their inconsistent reporting formats (raw counts, log-transformed values, and percentage reductions), which made reliable standardization impossible. Missing baseline values and statistical information made it impossible to convert the data accurately, making it a limitation to report these results narratively, which could not have maintained the integrity of the analysis.

Limitations of this review

This review is constrained by several important limitations. All the included studies were conducted in vitro, which may not fully replicate the complex biological environment in vivo, thus limiting the clinical applicability of the findings. The heterogeneity among studies in terms of bacterial strains, irrigant concentrations, and exposure durations makes it difficult to draw definitive conclusions. The use of different bacterial strains, which may possess varying virulence factors and resistance mechanisms, further complicates the comparability of results. Moreover, methodological differences, such as varying sample types (human versus bovine dentin), irrigation protocols, and outcome measurement techniques, contribute to the inconsistency across studies.

Variations in dentin substrate are crucial since bovine dentin differs in tube density, mineral composition, and permeability from human dentin, which could influence irrigant flow and antibacterial efficiency. Studies utilizing bovine dentin, for example, may show decreased efficacy due to lower permeability or different dentin-irrigant interactions, hence perhaps under valuating the clinical performance of irrigants such as QACs. Moreover, strain-specific diversity of *E. faecalis* might produce various patterns of resistance. While clinical isolates from persistent infections may express enhanced *E. faecalis* biofilm development, extracellular polymeric substance (EPS) production, and resistance gene expression, laboratory strains often show uniform virulence and sensitivity. All of which could change the observed efficacy of irrigants. This may account for discrepancies among studies using different isolates. Exposure time is also a crucial determinant of efficacy. Among these were studies documenting varying exposure times between one and ten minutes. Shorter contact times could not allow enough penetration or interaction with biofilm components,

thereby underestimating the entire antibacterial ability of an irrigant.

Despite the evaluation of research quality by the QUIN rating system, it is crucial to critically examine methodological shortcomings that may have affected the results of this meta-analysis. A significant issue noted in multiple included studies was the absence of blinding in outcome evaluation. In CLSM evaluations, the lack of blinded image analysis may result in detection bias, as the operator's interpretation of live/dead fluorescence might be subjective. Furthermore, none of the studies stated indicated conducting a sample size calculation, which raises concerns about the sufficiency of statistical power and the risk of Type II errors, perhaps leading to an underestimation of treatment effects. These factors may lead to heterogeneity in the reported outcomes and affect the robustness of the aggregated effect estimates, despite the use of a random-effects model to address variability. Recognizing these limitations is essential for contextualizing the findings and emphasizes the necessity for more carefully constructed future studies with suitable blinding and predetermined sample size calculations to enhance the knowledge base in this field.

The meta-analysis's inclusion criterion limited it to studies using CLSM for bacterial inhibition, excluding many studies using CFU measurement. This limitation may have limited the scope of the evidence. However, CFU-based findings were integrated into the narrative synthesis to enhance the review's interpretive significance.

A significant drawback of this meta-analysis is the inability to conduct subgroup or sensitivity analyses based on the concentrations of QACs. Although fluctuations in QAC concentration are acknowledged to substantially influence antibiotic activity, only three research studies met the inclusion criteria for quantitative synthesis. Performing subgroup analysis with a constrained dataset would lack adequate statistical power and may produce incorrect or misleading outcomes. Thus, the possible impact of concentration-dependent effects was not included in the present meta-analysis. To enhance the interpretability and therapeutic significance of future findings, we advise that subsequent research incorporate a more extensive dataset, facilitating subgroup analysis to assess the influence of QAC concentration and other moderating variables on antibacterial activity.

These limitations underscore the need for future standardized protocols to provide more reliable and clinically relevant evidence on the efficacy of QACs as endodontic irrigants.

Research implications

The study highlights the importance of standardizing study design, including the choice of preparation technique, tooth type, sample size, and method of drug delivery, to ensure consistent results. The review suggests that different strains of *E. faecalis* may exhibit varying virulence, resistance genes, and responses to irrigants, highlighting the need to consider strain-specific factors in research.

Clinical implications

QACs, with special mention of K21, can be incorporated into endodontic procedures at various essential phases of treatment. Clinically, they can be efficiently utilized during intermediate irrigation phases, particularly after mechanical instrumentation, to disrupt *E. faecalis* biofilms and diminish microbial burden inside the canal system. Their capacity to adhere to dentin and deliver sustained antibacterial efficacy renders them ideal for final irrigation methods, where enduring antibacterial sealing is crucial to avert reinfection. They are beneficial in scenarios with anatomical complexities, such as isthmuses, lateral canals, and dentinal tubules, as their low surface tension and deep penetration abilities facilitate more effective disinfection in areas difficult to reach with traditional irrigants.

Update on cytotoxicity of quaternary ammonium compounds

Evaluating the cytotoxicity of QACs is essential prior to its clinical use, given that its biocompatibility is highly reliant on concentration. Despite the inherent limitations of in vitro cell culture models, they offer critical insights into the impact of QACs on cell viability before in vivo testing. Initial studies, including those by Ruiz Oropeza et al., 2011 and Iwata et al., 2015, recorded skin responses and contact dermatitis linked to QACs, underscoring possible hazards [57, 58]. Li et al., 2016 revealed that QACs copolymers (PMT-5% and PMT-10%) exhibited much lower cytotoxicity to three human cell lines than the monomeric QACs agent DTPAC, with relatively high cell viability noted even at dosages effective against *E. coli* and *S. aureus* [59]. Daood et al., 2017 demonstrated that 2%K21 preserved higher cell viability (55.1%) in human dental pulp cells (hDPCs) than 2% CHX (19.3%), whereas deionized water acted as a non-toxic control (80.2%) [60]. In a further investigation, Daood et al. 2019 indicated that mouse fibroblasts exhibited greater survivability with 2% K21 compared to 5% or 10% K21 or 2% CHX, so affirming that cytotoxicity escalates with concentration [42]. Fan et al., 2020 corroborated these findings by demonstrating that K21 demonstrated little cytotoxicity and superior cell compatibility compared to 2% CHX [61]. Recent findings by Daood et al., 2021 shown that lower

doses of 0.5% and 1% K21 yielded significantly greater cell viability than 2% CHX, 6% NaOCl, or their combination [22]. These results highlight the necessity of choosing optimal QACs doses that attain antibacterial activity (MIC and MBC) while maintaining biocompatibility for safe therapeutic application.

Conclusion

The findings of this study indicate that QACs exhibited promising antibacterial effects, comparable to or exceeding that of NaOCl and CHX in some studies. Notably, K21 showed sustained antibacterial activity and prolonged contact killing due to its sol-gel properties, while BAK and CTR demonstrated good penetration into dental tubules with comparable efficacy to CHX. Additional validation via randomized clinical trials and animal studies is necessary to ascertain the safety, effectiveness, and regeneration capacity of K21. Subsequent investigations ought to concentrate on their efficacy in polymicrobial biofilms, interactions with pharmaceuticals and sealants, and influence periapical healing to facilitate their clinical incorporation.

Due to the significant heterogeneity seen in the meta-analysis, it is recommended that future investigations implement standardized protocols for bacterial strains, dentin substrates, exposure durations, and irrigant doses. This standardization would improve the comparability of results across research and bolster the general reliability and statistical power of forthcoming meta-analyses.

However, despite these positive outcomes, the meta-analysis revealed no statistically significant difference between the antibacterial efficacy of QACs and NaOCl, emphasizing the substantial heterogeneity in study designs, bacterial strains, exposure times, and irrigant concentrations among the included studies. Such variability complicates the interpretation and generalization of results.

Abbreviations

BAK	Benzalkonium chloride
CTR	Cetrimide
CHX	Chlorhexidine
CFU	Colony forming unit
CLSM	Confocal laser microscopy
CI	Confidence interval
DMADDM	Dimethylaminododecyl methacrylate
DMAHDM	Dimethylaminohexadecyl methacrylate
<i>E. faecalis</i>	<i>Enterococcus faecalis</i>
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
QACs	Quaternary Ammonium Compounds
K21	Quaternary ammonium silane
NaOCl	Sodium hypochlorite

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Author contributions

Ranjeet Ajit Bapat: Writing – original draft, Visualization, Software, Formal analysis, Data curation, Conceptualization. Sumaiya Zabin Eusufzai: Writing – review & editing, Visualization, Supervision, Formal analysis, Conceptualization. Zohaib Akram: Formal Analysis, review & editing. Tanay Chaulal: Writing – review & editing, Khoo Suan Phaik: Methodology, and Conceptualization. Seow Liang Lin: Writing – review & editing, Supervision, Conceptualization. Umer Daood: Writing – review & editing, Supervision, Conceptualization.

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Data availability

The datasets analyzed in this systematic review are available from the corresponding author upon reasonable request. Due to privacy concerns, the raw data cannot be publicly shared, but de-identified data extracts used for analysis can be provided with appropriate justification.

Declarations

Ethics approval and consent to participate

The ethical committee at IMU University approved the study, protocol number IMU R259/2020. All authors and patients signed the consent forms.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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