

Research Article



Antimicrobial efficacy of QMix on *Enterococcus faecalis* infected root canals: a systematic review of *in vitro* studies

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Trial Registration

PROSPERO International prospective register of systematic reviews Identifier: CRD42018096763

Conflict of Interest

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

ABSTRACT

Objectives: This study aimed to summarize the outcome of *in vitro* studies comparing the antibacterial effectiveness of QMix with other irrigants against *Enterococcus faecalis*.

Materials and Methods: The research question was developed by using population, intervention, comparison, outcome, and study design framework. The literature search was performed using 3 electronic databases: PubMed, Scopus, and EBSCOhost until October 2019. The additional hand search was performed from the reference list of the eligible studies. The risk of bias of the studies was independently appraised using the revised Cochrane Risk of Bias tool (RoB 2.0).

Results: Fourteen studies were included in this systematic review. The overall risk of bias for the selected studies was moderate. QMix was found to have a higher antimicrobial activity compared to 2% sodium hypochlorite (NaOCl), 17% ethylenediaminetetraacetic acid (EDTA), 2% chlorhexidine (CHX), mixture of tetracycline isomer, an acid and a detergent (MTAD), 0.2% Cetrimide, SilverSol/H₂O₂, HYBENX, and grape seed extract (GSE). QMix had higher antibacterial efficacy compared to NaOCl, only when used for a longer time (10 minutes) and with higher volume (above 3 mL).

Conclusions: QMix has higher antibacterial activity than 17% EDTA, 2% CHX, MTAD, 0.2% Cetrimide, SilverSol/H₂O₂, HYBENX, GSE and NaOCl with lower concentration. To improve the effectiveness, QMix is to use for a longer time and at a higher volume.

Trial Registration: PROSPERO International prospective register of systematic reviews Identifier: CRD42018096763

Keywords: Chlorhexidine; *Enterococcus faecalis*; QMix; root canal treatment; systematic review

Author Contributions

Conceptualization: Parolia A; Data curation: Lim BSH, Chia MSY; Formal analysis: Jayaraman J; Investigation: Lim BSH, Chia MSY; Methodology: Parolia A, Lim BSH; Project administration: Parolia A; Resources: Parolia A; Supervision: Parolia A; Validation: Jayaraman J, Nagendrababu V; Visualization: Parolia A; Writing - original draft: Lim BSH, Parolia A; Writing - review & editing: Lim BSH, Parolia A, Jayaraman J, Nagendrababu V.

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INTRODUCTION

Microbiota in the root canal system are found in highly organized and complex entities known as biofilms. The persistence of microorganisms inside the root canal system is the most common reason for the failure of root canal treatment [1,2]. The complexity and variability of the root canal system, along with the nature of biofilm, make the root canal disinfection extremely challenging [3,4]. *Enterococcus faecalis* (*E. faecalis*) has been one of the most persistent intra-radicular infections compared with untreated chronic periapical periodontitis [5-7]. It can survive in harsh conditions due to its ability to create biofilms, compete with other microorganisms, invade dentinal tubules, and resist nutritional deprivation [7-10]. Root canal disinfection can be achieved by mechanical and chemical means. However, irrigation plays a crucial role. It can reach areas with anatomical complexities including isthmus, fins of the root canal system as well as facilitate the reduction of microbial biofilms. For this purpose, a wide range of irrigating solutions had been used in endodontics and these include sodium hypochlorite (NaOCl) which is known for its dissolution of organic substances property [11], ethylenediaminetetraacetic acid (EDTA), removal of inorganic debris like smear layer [12], chlorhexidine (CHX), its antibacterial effect [13] and MTAD as a root canal disinfectant [14]. However, no single irrigant has been shown to be effective in meeting the objectives of root canal irrigation such as the dissolution of vital or necrotic pulp tissues, disruption of biofilms, neutralization of endotoxins, and removal of smear layer [15-17].

QMix (Dentsply Tulsa, Tulsa, OK, USA) was introduced as a single irrigant that can remove the smear layer considering its high antimicrobial property. It contains a mixture of a bisbiguanide antimicrobial agent (2% CHX), a polyaminocarboxylic acid calcium-chelating agent (17% EDTA), saline, and a surfactant [16]. QMix has shown to have superior antimicrobial property compared to CHX in reducing *E. faecalis* and the ability to remove the smear layer that is similar to EDTA [18,19]. With this inherent ability to remove the smear layer and antimicrobial action, it may require less time for the dentists to disinfect the root canal system effectively. Many studies have compared the antimicrobial property of QMix with other irrigants against *E. faecalis* showing varying results; some showed stronger antibacterial action at lower volume/timing while some showed contrary results [20-33]. However, to the authors' knowledge, no systematic review has been published to assess the antibacterial efficacy of QMix against *E. faecalis*. Therefore, the aim of this systematic review was to compare the antibacterial effectiveness of QMix with other commonly employed irrigants against *E. faecalis*.

MATERIALS AND METHODS**Protocol registration**

The protocol for this systematic review has been registered with the PROSPERO International prospective register of systematic reviews, registry No. CRD42018096763 and this review followed PRISMA guidelines [34].

Review question

The research question was developed by using the population, intervention, comparison, outcome and study design framework: In the extracted permanent human teeth with *E. faecalis* (P), does QMix irrigant (I) show better antibacterial property (O) compared to the other irrigants (C) from *in vitro* studies (S).

Search strategy

The literature search was performed comprehensively using 3 electronic databases: PubMed, Scopus, and EBSCOhost (Dentistry; Oral Sciences Source) using search strategy (QMix) AND ((root canal) OR endod), from inception to October 2019. The additional literature search was performed from the reference list of the eligible studies. Based on the journals publishing the content relevant to the topic, *Journal of Endodontics*, *International Endodontic Journal*, *Journal of Dentistry*, *Australian Endodontic Journal*, and *Journal of Conservative Dentistry* were hand-searched to identify any relevant studies. The search strategy and the articles retrieved through a combination of key words was shown in **Figure 1**.

Inclusion criteria

Inclusion criteria for this review were: i) Studies performed in the extracted permanent human teeth, ii) studies that compared the antibacterial effect of QMix with at least one irrigant against *E. faecalis*, iii) antibacterial efficacy assessed by either colony forming units (CFUs) or confocal laser scanning microscopy (CLSM), and iv) studies published in English.

Exclusion criteria

Studies were excluded if they were performed *in vivo*, on animals or in bovine teeth.

Study selection and data extraction process

Two reviewers (BL, AP) independently screened the title and abstract of the selected articles based on the specified inclusion and exclusion criteria. The reviewers independently read the articles and extracted the data using the data extraction form exclusively developed

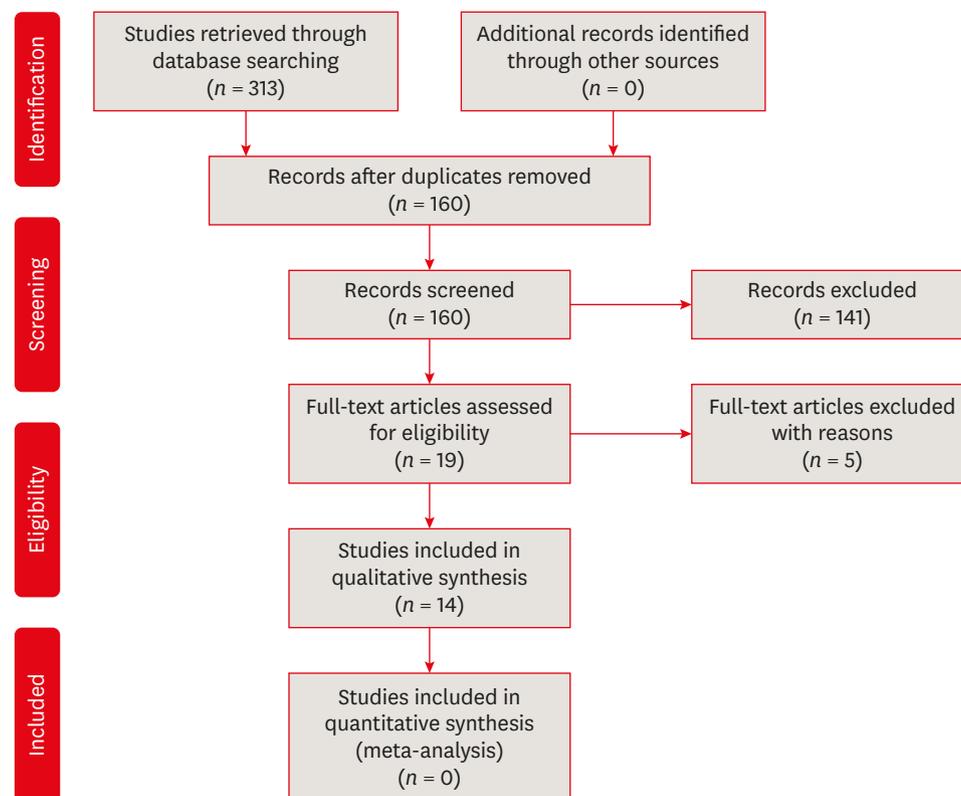


Figure 1. A flowchart of the literature search process.

for this study. This form consisted of the following details: author, year, country, the total number of samples, type of the teeth, interventions, evaluation method, results based on the antimicrobial property, irrigation time, and irrigation volume. Any disagreement between the 2 reviewers was resolved by discussion with a third reviewer (JJ).

Quality assessment of the included articles

The quality of each article was assessed using the revised Cochrane Risk of Bias tool (RoB 2.0, Cochrane Methods, London, UK) [35]. This tool was modified to include the contents based on the methodology employed in the included *in vitro* studies. The quality of included studies was assessed based on the following domains: randomization process, deviations from intended intervention, verification of the presence of *E. faecalis*, the protocol for biofilm formation (21 days), smear layer removal prior to *E. faecalis* inoculation and irrigating regimen (volume, duration). Two authors (BL, AP) independently evaluated and scored the articles based on the above domains. In case of disagreement, a consensus was reached in discussion with another reviewer (JJ).

RESULTS

Study Selection process

A total of 313 studies were identified from the electronic databases. After excluding studies based on title and abstract screening, 19 articles were available for full-text assessment. On careful reading, further 5 studies were excluded for the following reasons: studies were done on agar plate [16], on mixed plaque suspension [18], on bovine teeth [36], QMix as a control variable [37], and QMix used in combination with other irrigants and was not tested individually [38]. Finally, 14 studies were included in the systematic review. The search process employed to identify included studies was shown in **Figure 1**. We did not perform a meta-analysis due to the presence of heterogeneity in the methodology and reporting antibacterial outcomes of the included studies. Most of the studies reported the remaining *E. faecalis* log CFUs with mean and standard deviation [20,23,27,30,31]. However, a limited number of studies reported the data in median and percentiles [21,22,32,33]. Within the mean scores, variations were observed in reporting the log CFU mean scores that were represented in different units [24,26-29]. It was not possible to collate the information from the included studies that rendered difficulty in quantitatively evaluating the extracted data.

Characteristics of included studies

Out of 14 studies, 12 studies included single-rooted teeth [21-31,33]. One study contained both maxillary and mandibular molars [20], and 1 study included mandibular incisors and maxillary second premolars [32]. Five studies were done in dentin blocks or discs with specified dimensions [20,22,27-29], whereas another 9 studies were done in the root canal [21,23-26,30-33]. Six out of these 9 studies mentioned the root length of the tooth sample, at 12 mm [21], 14 mm [23], 15 mm [25,26,31], and 16 mm [33], respectively. Inconsistency was observed in the irrigation protocol in the included studies. One out of 5 studies was done in wells containing dentin blocks and irrigants [20], 3 studies introduced irrigants to the root canal side of the dentin disc [27-29], and 1 study did not mention clearly the placement of irrigants to the dentin disc [22]. Four out of 9 studies performed in root canal employed side vented needle for irrigation [21,23,30,32], whereas 5 studies did not mention the details of irrigation [24-26,31,33]. The samples were obtained using sterile paper points in 6 studies [21,24-26,31,33]. QMix has been compared with various irrigants like EDTA, MTAD, CHX,

NaOCl, cetrimide, SilverSol/H₂O₂, HYBENX, and Gold grape seed extract (GSE) and the antibacterial efficacy was measured by using CFU [20-26,30,31-33] and CLSM [27-29]. The characteristics of the included *in vitro* studies were shown in **Table 1**.

Quality of included studies

The studies were analyzed using the modified Risk of Bias tool and the overall quality of the included studies were found to be “moderate” (**Figure 2**). Most of the studies followed

Table 1. Characteristics of included studies in the systematic review

No.	Author	Year	Country	Total number of samples	Type of teeth	Interventions (groups)	Evaluation method	Results (gain results (group showing significantly higher bacterial reduction))	Irrigation time	Irrigation volume
1	Ma <i>et al.</i> [28]	2011	Canada	12	Single-rooted teeth	1. Sterile water 2. 1% NaOCl 3. 2% NaOCl 4. 6% NaOCl 5. 2% CHX 6. QMix	CLSM	QMix and 6% NaOCl killed more bacteria in 1 min than the other solutions in 3 min	1 and 3 min	50 µL each irrigant
2	Wang <i>et al.</i> [27]	2012	Canada	40	Single-rooted teeth	1. Sterile water (control) 2. 2% NaOCl 3. 6% NaOCl 4. 2% CHX 5. QMix	CLSM	6% NaOCl and QMix were better and no significant difference was found between the 2 agents	1 and 3 min	50 µL each irrigant
3	Wang <i>et al.</i> [29]	2013	Canada	40	Single-rooted teeth	1. Sterile Water 2. 2% NaOCl 3. 6% NaOCl 4. 2% CHX 5. 17% EDTA 6. QMix 7. 2% NaOCl + 2% CHX 8. 2% NaOCl + QMiX 9. 6% NaOCl + QMiX 10. 6% NaOCl + 17% EDTA + 2% CHX	CLSM	6% NaOCl + QMix showed the highest level of bacterial killing in 3 min. At 10 min, combinations of 6% NaOCl + QMix and 6% NaOCl + 17% EDTA + 2% CHX were the most effective antibacterial solutions followed by 2% NaOCl + QMix	3 and 10 min	50 µL each medicament
4	Zhang <i>et al.</i> [20]	2015	China	200	Maxillary and Mandibular molars	1. 17% EDTA 2. 2% CHX 3. 0.2% Cetrimide 4. MTAD 5. QMix 6. Untreated	CFU Inoculation time: cultivated under anaerobic conditions at 37°C for 24 hr	QMix group had the lowest logCFU value	2 min	100 µL each irrigant
5	Liu <i>et al.</i> [21]	2015	China	62	Single-rooted maxillary anterior teeth	1. 17% EDTA/5.25% NaOCl 2. 17% EDTA/2% CHX 3. 17% EDTA/2% CTR 4. MTAD 5. QMix 6. 0.9% Saline	CFU Inoculation time: incubated at 37°C for 48 hr	EDTA/CHX, EDTA/CTR, or QMix exhibited the greatest antimicrobial activity. No significant differences between these 3 groups	NA	5 mL each irrigant
6	Elakanti <i>et al.</i> [23]	2015	India	40	Mandibular premolar teeth	1. 5.25% NaOCl 2. 2% CHX 3. QMix 4. 0.9% Saline	CFU Inoculation time: incubated at 37°C for 24 hr	QMix better than 5.25% NaOCl and 2% CHX	1 min	3 mL each irrigant
7	Cecchin <i>et al.</i> [25]	2015	Brazil	50	Single-rooted teeth	1. 2.5% NaOCl 2. 2% CHX 3. 6.5% GSE 4. QMix 5. DW	CFU Inoculation time: incubated at 37°C for 18 to 24 hr	CHX and GSE better than NaOCl and QMix	30 sec	5 mL each irrigant
8	Bago Jurič <i>et al.</i> [22]	2016	Croatia	65	Single-rooted teeth	1. PDT 2. Nd:YAG 3. QMix 4. 5.25% NaOCl	CFU Inoculation time: incubated at 37°C for 48 hr	NaOCl better followed by PDT, QMix and Nd:YAG	1 min	1 mL each irrigant

(continued to the next page)

Table 1. (Continued) Characteristics of included studies in the systematic review

No.	Author	Year	Country	Total number of samples	Type of teeth	Interventions (groups)	Evaluation method	Results (gain results (group showing significantly higher bacterial reduction))	Irrigation time	Irrigation volume
9	Balić <i>et al.</i> [32]	2016	Croatia	90	Mandibular incisors and maxillary second premolars	1. PIPS with 2.5% NaOCl 2. PIPS with QMix 3. Sonic-activated irrigation with 2.5% NaOCl 4. Sonic-activated irrigation with QMix 5. 2.5% NaOCl needle irrigation 6. QMix solution needle irrigation	CFU Inoculation time: incubated at 37°C for 48 hr	The QMix solution showed significantly greater antimicrobial efficacy than 2.5% NaOCl ($p = 0.04$) when the conventional needle irrigation was used.	30 sec	2 mL each irrigant
10	Vaid <i>et al.</i> [24]	2017	India	190	Single-rooted anterior teeth	1. Normal saline 2. 2.5% NaOCl 3. QMix 4. Normal saline and PAD 5. 2.5% NaOCl and PAD 6. QMix and PAD 7. No irrigation	CFU Inoculation time: incubated at 37°C for 48 hr	Maximum percentage of disinfection (99%) was seen in 15 mL of 2.5% NaOCl solution for 3 min and irradiated with PAD which was similar to 15 mL of 2.5% NaOCl for 3 min and 5 mL of 2.5% NaOCl solution, followed by 5 mL of normal saline, and then, 5 mL QMix for 3 min	3 min	15 mL of Saline/ NaOCl 5 mL of QMix
11	Souza <i>et al.</i> [26]	2017	Brazil	60	Single-rooted teeth	1. DW 2. 2% CHX 3. QMix 4. 6.5% GSE 5. PDT + fiber 6. PDT + no fiber	CFU Inoculation time: incubated at 37°C for 18–24 hr	The greatest bacterial reduction was observed for 2% CHX, QMix and 6.5% GSE, with no statistically significant difference between them.	5 min	NA
12	Ye <i>et al.</i> [30]	2018	China	51	Single-rooted premolars	1. 0.9% NaCl 2. 10 ppm SilverSol with 0.1% H2O2 (SilverSol/H2O2) 3. HYBENX 4. QMix 5. 6% NaOCl	CFU Inoculation time: incubated at 37°C for 48 hr	6% NaOCl > QMix > HYBENX > SilverSol/H2O2 > 0.9% NaCl SilverSol/H2O2 and HYBENX were less adept than QMix at killing biofilm bacteria in root canals.	1 min	6 mL each irrigant
13	Souza <i>et al.</i> [31]	2018	Brazil	60	Single-rooted	1. DW 2. DW + Ultrasonic 3. 17% EDTA 4. QMix 5. 17% EDTA + Ultrasonic 6. QMix + Ultrasonic	CFU Inoculation time: incubated at 37°C for 18–24 hr	The greatest ability to promote bacterial reduction was observed in QMix and QMix + US, with no statistically significant difference between them ($p < 0.05$)	1 min	NA
14	Matos <i>et al.</i> [33]	2019	Brazil	40	Single-rooted	1. EDTA + MA 2. QMix + MA 3. EDTA + PUI 4. QMix + PUI	CFU Inoculation time: incubated at 37°C for 24 hr	QMix + MA and QMix + PUI had superior antibacterial efficacy to EDTA and eliminated 100% of <i>E. faecalis</i>	2 min	3 mL each irrigant

NA, not available; NaOCl, sodium hypochlorite; CHX, chlorhexidine; CLSM, confocal laser scanning microscopy; EDTA, ethylenediaminetetraacetic acid; CFU, colony forming units; MTAD, mixture of Tetracycline isomer, an acid and a detergent; log CFU, log colony forming units; GSE, grape seed extract; DW, distilled water; PDT, photodynamic therapy; PIPS, photon-initiated photoacoustic streaming; PAD, photo-activated disinfection; US, ultrasonic activation; MA, manual agitation; PUI, passive ultrasonic irrigation.

randomisation process [20-22,24-33] with deviation from intended interventions. The verification of the presence of *E. faecalis* was not stated in 2 studies [20,25]. Five studies did not follow the protocol for biofilm formation for 21 days [23,26,28,31,32]. Two studies did not follow the protocol for smear layer removal prior to *E. faecalis* inoculation [21,31]. In the protocol for irrigating regimen, all studies reported information on the volume of irrigants and duration of their use except 3 studies [20,21,26].

Among the results gathered from the included studies, QMix showed higher antibacterial efficacy if not equal to NaOCl as compared to the other endodontic irrigants tested in the

Authors	Year	Country	Randomization process	Deviations from intended interventions	Presence of <i>E. faecalis</i> verified	Protocol for biofilm formation (21 days)	Smear layer removal protocol followed prior to <i>E. faecalis</i> inoculation	Protocol for irrigating regimen (volume, duration)	Overall score
Ma et al. [28]	2011	Canada	+	+	+	-	+	+	-
Wang et al. [27]	2012	Canada	+	+	+	+	+	+	+
Wang et al. [29]	2013	Canada	+	+	+	+	+	+	+
Zhang et al. [20]	2015	China	+	+	-	+	+	-	-
Liu et al. [21]	2015	China	+	+	+	+	-	-	-
Elakanti et al. [23]	2015	India	-	+	+	-	+	+	-
Cecchin et al. [25]	2015	Brazil	+	+	-	+	+	+	-
Bago Jurić et al. [22]	2016	Croatia	+	+	+	+	+	+	+
Balić et al. [32]	2016	Croatia	+	+	+	-	+	+	+
Vaid et al. [24]	2017	India	+	+	+	+	+	+	+
Souza et al. [26]	2017	Brazil	+	+	+	-	+	-	-
Ye et al. [30]	2018	China	+	+	+	+	+	+	+
Souza et al. [31]	2018	Brazil	+	+	+	-	-	+	-
Matos et al. [33]	2019	Brazil	+	+	+	+	+	+	+

Figure 2. Risk of bias assessment of included studies.
+, low risk of bias; -, high risk of bias.

studies. Given the overall moderate risk of bias, standardization is needed to improve the quality and clinical implication of the studies further.

DISCUSSION

The purpose of performing root canal treatment is to remove intracanal microorganisms and to prevent reinfection. Irrigation plays a crucial role in endodontic treatment and facilitates disinfection during and after instrumentation [39]. Thus far, the combination of endodontic irrigation has been commonly employed in root canal treatment to achieve both organic dissolution and removal of inorganic substance. QMix was known for its single irrigant that has both antibacterial and smear layer removal properties [18,19]. Various studies have been published on QMix compared to other commonly used irrigants [20-33]. Although it would be appropriate to test the effectiveness of QMix *in vivo* setting, no study was done in that manner. For the above reason, only *in vitro* studies were included in this systematic review.

Four studies showed that 5% to 6% NaOCl was more effective than QMix against *E. faecalis* biofilm when 1 mL was used for 1 and 3 minutes [22,27,29,30]. However, another study showed no difference between 6% NaOCl and QMix at 1 and 3 minutes of exposure [28]. QMix was more effective than lower concentration of NaOCl (1%, 2%, and 2.5%) at 30 seconds, 1 and 3 minutes' exposure [22,27-29,32]. Similarly, QMix was more effective than 5% to 6% NaOCl when used for one [23] and 10 minutes with a volume of 3 mL [29]. QMix showed better antibacterial property compared to NaOCl, when QMix was employed for longer exposure time [29] and at higher volume [23]. In one study, QMix showed no significant difference compared to 2.5% NaOCl when used with different activation systems [32]. Five studies showed that QMix was more effective than 2% CHX against *E. faecalis* biofilm [20,23,27-29], whereas one study showed no difference between QMix and 2% CHX [26]. The superior antibacterial effect of QMix was probably due to its ability to remove the smear layer, and a gradual antibacterial effect on the dentin bacteria through a synergistic effect [28,29]. QMix showed higher antimicrobial activity compared to 17% EDTA [20,29,31,33], MTAD [20], 0.2% Cetrimide [20], Nd:YAG laser [22], SilverSol/H₂O₂

[30], and HYBENX [30] against *E. faecalis* biofilm. It is interesting to note that QMix had similar antibacterial effectiveness against *E. faecalis* biofilm when compared to GSE [26] and photodynamic therapy (PDT) [22]. Considering all the materials, QMix showed higher antimicrobial activity compared to 17% EDTA, 2% CHX, MTAD, 0.2% Cetrimide, SilverSol/H₂O₂, and HYBENX. Three studies included in the review were conducted by a research team with a potential financial interest in QMix product [27-29]. The above studies have been published after robust peer review, and hence we consider that the results from these studies would not have affected the outcome of this review.

CFU methodology has been widely used for microbiological analysis of bacteria inside the dentinal tubules. Although it was able to provide a reading of the bacterial colony that had invaded the dentinal tubules, it was unable to analyze the spatial distribution and viability of the bacteria. In contrast, CLSM was capable of showing intact undisturbed biological samples with optical sections as thin as 0.3 µm. When used with vital staining techniques, it showed the viability profile and spatial distribution of the examined bacteria [40,41]. In addition, it showed consistent results of ranking when used in various studies on the antibacterial activity of disinfecting agents [29]. The CLSM has the ability to eliminate scattered light and focus on individual bacterial cells inside the dentinal tubules. It has been shown that CLSM is a better method to study the bacterial viability in endodontics [41]. Therefore, in order to critically evaluate both the amount and viability of *E. faecalis* in the root canal system after irrigation, both CFU and CLSM microbiological analysis have been evaluated in this review. By doing so, this had portrayed a clearer picture of the antimicrobial efficacy of QMix irrigant.

In our review, we have modified the revised Cochrane ROB based on the characteristics of the included *in vitro* studies. Six parameters (randomization process, deviations from intended interventions, verification of *E. faecalis*, protocol for biofilm formation [21 days], smear layer removal protocol followed prior to *E. faecalis* inoculation, and protocol for irrigating regimen based on volume and duration) have been used to appraise the quality of included studies as showed in **Figure 2**. All studies satisfied deviations from the intended interventions parameter. The evaluation of *E. faecalis* with CFU counts varies with inoculation time. Out of 10 studies, the inoculation time of *E. faecalis* was 24 hours for 6 studies [20,23,25,26,31,33], whilst in 5 studies, it was done at 48 hours [21,22,24,30,32]. Verification of *E. faecalis* was an essential step in the study design, which helps to confirm the presence and spread of *E. faecalis* biofilm [28]. The average time taken for the formation of mature biofilm is around 21 days, and this duration was crucial to assess the effectiveness of any disinfectant against the mature biofilm [27]. Wang *et al.* [27] showed that mature *E. faecalis* biofilms in dentin canals at 21 days are more resistant to disinfecting solutions than young biofilms. Hence, we included the protocol for biofilm formation at 21 days as one of the domains for assessing the quality of studies. Smear layer removal protocol prior to *E. faecalis* inoculation was essential because the smear layer prevents the penetration of microorganisms into the root canal and reduces the effectiveness of disinfecting agents against *E. faecalis* [29]. Additionally, bacteria remaining in dentinal tubules after root canal preparation may be sealed by the smear layer [42]. In such cases, antibacterial solutions used for root canal disinfection have to penetrate or remove the smear layer to attack the bacteria in the infected dentin. Hence, if the smear layer was not completely removed, in microbial studies, this might show false-positive or false-negative results. Studies have shown that the antimicrobial effect was affected by the volume and concentration of the irrigant. This is apparent in this review as QMix showed better effect when used in higher volume (above 3 mL) and for a longer time (above 10 minutes) [23,29].

The studies included in this review showed substantial differences in their study design. The experimental subject models were not consistent. For example, some studies were done using dentin disc and some in the closed root canal system. Moreover, placement methods of irrigants varied between each study. Studies that were performed in dentin discs, irrigants were added in the wells, whereas experiments done in the root canal employed needle syringe irrigation. The efficacy of irrigants is affected by the vapor lock formation in a closed root canal system surrounded by periodontium [43,44]. Hence, the root canal system should be preferred over the dentin disc to simulate the actual endodontic procedure in clinical scenarios. Despite studies mentioned *E. faecalis* as the most persistent microorganism in intra-radicular infections hence the inclusion criteria, however, biofilm comprising of multiple microorganisms will give a higher impact factor in clinical relevance. It is strongly recommended to use CLSM to study the antibacterial viability properly. Further, *in vitro* and *in vivo* studies are required to check the antibacterial efficacy of QMix against other endodontic pathogens. The clinical relevance of the effect of a root canal disinfection solution considering a single bacterium remains unclear in general.

CONCLUSIONS

This systematic review on the antibacterial efficacy of QMix against *E. faecalis* reveals superior to the usage of single irrigation solution (2% CHX, MTAD, 17% EDTA, 0.2% Cetrimide, SilverSol/H₂O₂, HYBENX, and low concentration NaOCl) and it is a promising alternative to the commonly employed irrigation protocols. To improve the effectiveness, it is recommended to use QMix for a longer time and at a higher volume.

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