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Research Article

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

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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 isonomer, 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.

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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

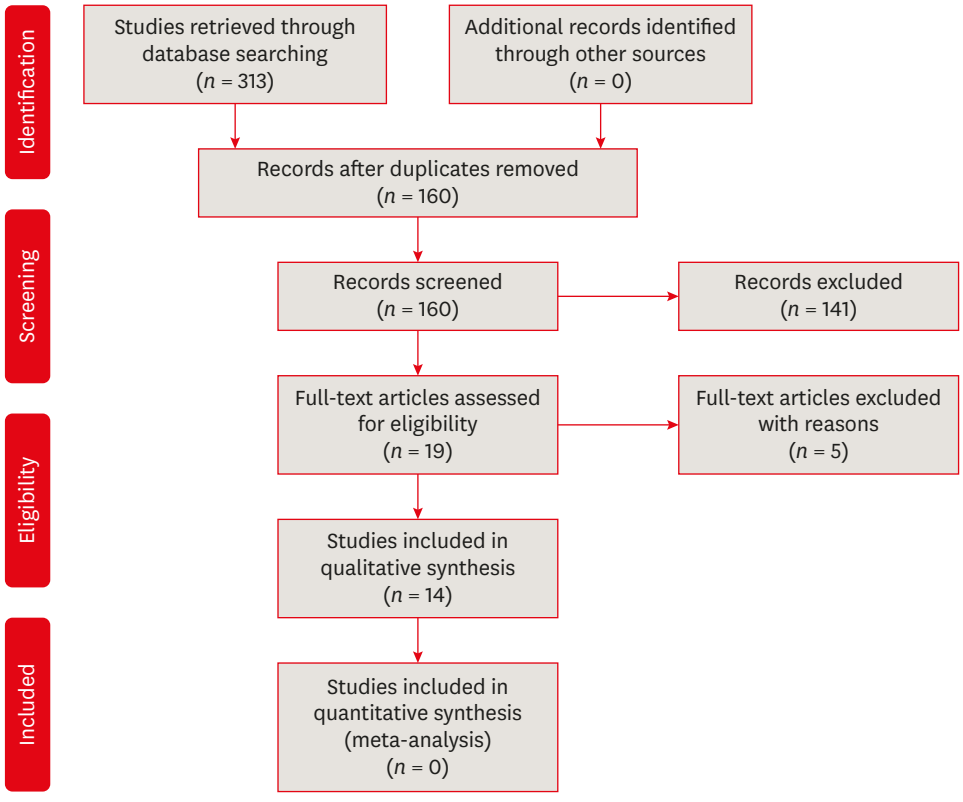

Figure 1. A flowchart of the literature search process.

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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 *et al.* [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 *et al.* [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 *et al.* [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 *et al.* [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 *et al.* [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 *et al.* [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 *et al.* [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 *et al.* [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 *et al.* [32]	2016	Croatia	90	Mandibular incisors and maxillary second premolars	1. PIPS with 2.5% NaOCl 2. PIPS with QMix 3. Sonic-activated irrigation with2.5% 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 *et al.* [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 *et al.* [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 *et al.* [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 *et al.* [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 *et al.* [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 *E. faecalis*	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 isonomer, 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 *E. faecalis* verified	Protocol for biofilm formation (21 days)	Smear layer removal protocol followed prior to *E. faecalis* 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 Jurič *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.

REFERENCES

1. Vieira AR, Siqueira JF Jr, Ricucci D, Lopes WS. Dentinal tubule infection as the cause of recurrent disease and late endodontic treatment failure: a case report. J Endod 2012;38:250-254.
PUBMED | CROSSREF
2. Nair PN, Sjögren U, Krey G, Kahnberg KE, Sundqvist G. Intraradicular bacteria and fungi in root-filled, asymptomatic human teeth with therapy-resistant periapical lesions: a long-term light and electron microscopic follow-up study. J Endod 1990;16:580-588.
PUBMED | CROSSREF
3. Neelakantan P, Romero M, Vera J, Daood U, Khan AU, Yan A, Cheung GSP. Biofilms in endodontics-current status and future directions. Int J Mol Sci 2017;18:E1748.
PUBMED | CROSSREF
4. Song M, Kim HC, Lee W, Kim E. Analysis of the cause of failure in nonsurgical endodontic treatment by microscopic inspection during endodontic microsurgery. J Endod 2011;37:1516-1519.
PUBMED | CROSSREF
5. Love RM. *Enterococcus faecalis*--a mechanism for its role in endodontic failure. Int Endod J 2001;34:399-405.
PUBMED | CROSSREF
6. Wang QQ, Zhang CF, Chu CH, Zhu XF. Prevalence of *Enterococcus faecalis* in saliva and filled root canals of teeth associated with apical periodontitis. Int J Oral Sci 2012;4:19-23.
PUBMED | CROSSREF
7. Zhang C, Du J, Peng Z. Correlation between *Enterococcus faecalis* and persistent intraradicular infection compared with primary intraradicular infection: a systematic review. J Endod 2015;41:1207-1213.
PUBMED | CROSSREF
8. Pinheiro ET, Gomes BP, Ferraz CC, Teixeira FB, Zaia AA, Souza Filho FJ. Evaluation of root canal microorganisms isolated from teeth with endodontic failure and their antimicrobial susceptibility. Oral Microbiol Immunol 2003;18:100-103.
PUBMED | CROSSREF

9. Rossi-Fedele G, Roberts AP. A preliminary study investigating the survival of tetracycline resistant *Enterococcus faecalis* after root canal irrigation with high concentrations of tetracycline. Int Endod J 2007;40:772-777.
PUBMED | CROSSREF
10. Sedgley CM, Lennan SL, Appelbe OK. Survival of *Enterococcus faecalis* in root canals *ex vivo*. Int Endod J 2005;38:735-742.
PUBMED | CROSSREF
11. Zehnder M. Root canal irrigants. J Endod 2006;32:389-398.
PUBMED | CROSSREF
12. Hülsmann M, Heckendorff M, Lennon A. Chelating agents in root canal treatment: mode of action and indications for their use. Int Endod J 2003;36:810-830.
PUBMED | CROSSREF
13. Weber CD, McClanahan SB, Miller GA, Diener-West M, Johnson JD. The effect of passive ultrasonic activation of 2% chlorhexidine or 5.25% sodium hypochlorite irrigant on residual antimicrobial activity in root canals. J Endod 2003;29:562-564.
PUBMED | CROSSREF
14. Johal S, Baumgartner JC, Marshall JG. Comparison of the antimicrobial efficacy of 1.3% NaOCl/BioPure MTAD to 5.25% NaOCl/15% EDTA for root canal irrigation. J Endod 2007;33:48-51.
PUBMED | CROSSREF
15. Siqueira JF Jr, Rôças IN. Optimising single-visit disinfection with supplementary approaches: a quest for predictability. Aust Endod J 2011;37:92-98.
PUBMED | CROSSREF
16. Jose J, Krishnamma S, Peedikayil F, Aman S, Tomy N, Mariodan JP. Comparative evaluation of antimicrobial activity of QMix, 2.5% sodium hypochlorite, 2% chlorhexidine, guava leaf extract and aloevera extract against *Enterococcus faecalis* and candida albicans: an *in-vitro* study. J Clin Diagn Res 2016;10:ZC20-ZC23.
PUBMED | CROSSREF
17. Goldman LB, Goldman M, Kronman JH, Lin PS. The efficacy of several irrigating solutions for endodontics: a scanning electron microscopic study. Oral Surg Oral Med Oral Pathol 1981;52:197-204.
PUBMED | CROSSREF
18. Stojicic S, Shen Y, Qian W, Johnson B, Haapasalo M. Antibacterial and smear layer removal ability of a novel irrigant, QMiX. Int Endod J 2012;45:363-371.
PUBMED | CROSSREF
19. Dai L, Khechen K, Khan S, Gillen B, Loushine BA, Wimmer CE, Gutmann JL, Pashley D, Tay FR. The effect of QMix, an experimental antibacterial root canal irrigant, on removal of canal wall smear layer and debris. J Endod 2011;37:80-84.
PUBMED | CROSSREF
20. Zhang R, Chen M, Lu Y, Guo X, Qiao F, Wu L. Antibacterial and residual antimicrobial activities against *Enterococcus faecalis* biofilm: a comparison between EDTA, chlorhexidine, cetrimide, MTAD and QMix. Sci Rep 2015;5:12944.
PUBMED | CROSSREF
21. Liu Y, Guo L, Li Y, Guo X, Wang B, Wu L. *In vitro* comparison of antimicrobial effectiveness of QMix and other final irrigants in human root canals. Sci Rep 2015;5:17823.
PUBMED | CROSSREF
22. Bago Jurič I, Plečko V, Anić I, Pleško S, Jakovljević S, Rocca JP, Medioni E. Antimicrobial efficacy of photodynamic therapy, Nd:YAG laser and QMiX solution against *Enterococcus faecalis* biofilm. Photodiagnosis Photodyn Ther 2016;13:238-243.
PUBMED | CROSSREF
23. Elakanti S, Cherukuri G, Rao VG, Chandrasekhar V, Rao AS, Tummala M. Comparative evaluation of antimicrobial efficacy of QMix™ 2 in 1, sodium hypochlorite, and chlorhexidine against *Enterococcus faecalis* and Candida albicans. J Conserv Dent 2015;18:128-131.
PUBMED | CROSSREF
24. Vaid D, Shah N, Kothari D, Bilgi P. Additive effect of photoactivated disinfection on the antibacterial activity of QMix 2in1 against 6-week *Enterococcus faecalis* biofilms: an *in vitro* study. J Conserv Dent 2017;20:41-45.
PUBMED | CROSSREF
25. Cecchin D, Farina AP, Souza MA, Albarello LL, Schneider AP, Vidal CM, Bedran-Russo AK. Evaluation of antimicrobial effectiveness and dentine mechanical properties after use of chemical and natural auxiliary irrigants. J Dent 2015;43:695-702.
PUBMED | CROSSREF

26. Souza MA, Dalla Lana D, Gabrielli E, Barbosa Ribeiro M, Miyagaki DC, Cecchin D. Effectiveness of final decontamination protocols against *Enterococcus faecalis* and its influence on bond strength of filling material to root canal dentin. Photodiagnosis Photodyn Ther 2017;17:92-97.
PUBMED | CROSSREF

27. Wang Z, Shen Y, Haapasalo M. Effectiveness of endodontic disinfecting solutions against young and old *Enterococcus faecalis* biofilms in dentin canals. J Endod 2012;38:1376-1379.
PUBMED | CROSSREF

28. Ma J, Wang Z, Shen Y, Haapasalo M. A new noninvasive model to study the effectiveness of dentin disinfection by using confocal laser scanning microscopy. J Endod 2011;37:1380-1385.
PUBMED | CROSSREF

29. Wang Z, Shen Y, Haapasalo M. Effect of smear layer against disinfection protocols on *Enterococcus faecalis*-infected dentin. J Endod 2013;39:1395-1400.
PUBMED | CROSSREF

30. Ye WH, Fan B, Purcell W, Meghil MM, Cutler CW, Bergeron BE, Ma JZ, Tay FR, Niu LN. Anti-biofilm efficacy of root canal irrigants against in-situ *Enterococcus faecalis* biofilms in root canals, isthmuses and dentinal tubules. J Dent 2018;79:68-76.
PUBMED | CROSSREF

31. Souza MA, Hoffmann IP, Menchik VH, Zandoná J, Dias CT, Palhano HS, Bertol CD, Rossato-Grando LG. Influence of ultrasonic activation using different final irrigants on antimicrobial activity, smear layer removal and bond strength of filling material. Aust Endod J 2019;45:209-215.
PUBMED | CROSSREF

32. Balić M, Lucić R, Mehadžić K, Bago I, Anić I, Jakovljević S, Plečko V. The efficacy of photon-initiated photoacoustic streaming and sonic-activated irrigation combined with QMiX solution or sodium hypochlorite against intracanal E. faecalis biofilm. Lasers Med Sci 2016;31:335-342.
PUBMED | CROSSREF

33. Matos FS, Khoury RD, Carvalho CA, Martinho FC, Bresciani E, Valera MC. Effect of EDTA and QMIX ultrasonic activation on the reduction of microorganisms and endotoxins in *ex vivo* human root canals. Braz Dent J 2019;30:220-226.
PUBMED | CROSSREF

34. Moher D, Liberati A, Tetzlaff J, Altman DGPRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med 2009;6:e1000097.
PUBMED | CROSSREF

35. Higgins JP, Sterne JA, Savović J, Page MJ, Hróbjartsson A, Boutron I, Reeves B, Eldridge S. A revised tool for assessing risk of bias in randomized trials. Cochrane Database Syst Rev 2016;10:S29-S31.

36. Giardino L, Andrade FB, Beltrami R. Antimicrobial effect and surface tension of some chelating solutions with added surfactants. Braz Dent J 2016;27:584-588.
PUBMED | CROSSREF

37. Bortoluzzi EA, Carlon D Jr, Meghil MM, El-Awady AR, Niu L, Bergeron BE, Susin L, Cutler CW, Pashley DH, Tay FR. Efficacy of 3D conforming nickel titanium rotary instruments in eliminating canal wall bacteria from oval-shaped root canals. J Dent 2015;43:597-604.
PUBMED | CROSSREF

38. Batinić M, Ročan M, Budimir A, Anić I, Bago I. Comparison of final disinfection protocols using antimicrobial photodynamic therapy and different irrigants after single-file reciprocating instrumentation against intracanal bacterial biofilm: an *in vitro* study. Photodiagnosis Photodyn Ther 2018;24:153-157.
PUBMED | CROSSREF

39. Haapasalo M, Shen Y, Qian W, Gao Y. Irrigation in endodontics. Dent Clin North Am 2010;54:291-312.
PUBMED | CROSSREF

40. Parmar D, Hauman CH, Leichter JW, McNaughton A, Tompkins GR. Bacterial localization and viability assessment in human *ex vivo* dentinal tubules by fluorescence confocal laser scanning microscopy. Int Endod J 2011;44:644-651.
PUBMED | CROSSREF

41. Zapata RO, Bramante CM, de Moraes IG, Bernardineli N, Gasparoto TH, Graeff MS, Campanelli AP, Garcia RB. Confocal laser scanning microscopy is appropriate to detect viability of *Enterococcus faecalis* in infected dentin. J Endod 2008;34:1198-1201.
PUBMED | CROSSREF

42. Drake DR, Wiemann AH, Rivera EM, Walton RE. Bacterial retention in canal walls *in vitro*: effect of smear layer. J Endod 1994;20:78-82.
PUBMED | CROSSREF

43. de Gregorio C, Estevez R, Cisneros R, Heilborn C, Cohenca N. Effect of EDTA, sonic, and ultrasonic activation on the penetration of sodium hypochlorite into simulated lateral canals: an *in vitro* study. J Endod 2009;35:891-895.
PUBMED | CROSSREF

44. de Gregorio C, Estevez R, Cisneros R, Paranjpe A, Cohenca N. Efficacy of different irrigation and activation systems on the penetration of sodium hypochlorite into simulated lateral canals and up to working length: an *in vitro* study. J Endod 2010;36:1216-1221.
PUBMED | CROSSREF