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Antibacterial efficacy of antibiotic pastes versus calcium hydroxide intracanal dressing: A systematic review and meta-analysis of *ex vivo* studies

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Abstract

Background:

Conflicting findings on the potency of antibiotic pastes versus calcium hydroxide (CH) have been evident in the literature.

Aims:

To compare the antibacterial efficacy of single antibiotic paste (SAP), double antibiotic paste (DAP), triple antibiotic paste (TAP), and modified TAP (mTAP) with CH on bacterial biofilms.

Methods:

PubMed, Scopus, and Embase were comprehensively searched until August 23, 2021. The study protocol was registered in the PROSPERO. *Ex vivo* studies performed on *Enterococcus faecalis* or polymicrobial biofilms incubated on human/bovine dentin were selected. The quality of the studies was assessed using a customized quality assessment tool. Standardized mean difference (SMD) with a 95% confidence interval (CI) was calculated for the meta-analysis. Meta-regression models were used to identify the sources of heterogeneity and to compare the efficacy of pastes.

Results:

The qualitative and quantitative synthesis included 40 and 23 papers, respectively, out of 1421 search results. TAP (SMD = -3.82; CI, -5.44 to -2.21; $P < 0.001$) and SAPs (SMD = -2.38; CI, -2.81 to -1.94; $P < 0.001$) had significantly higher antibacterial efficacy compared to the CH on *E. faecalis* biofilm. However, no significant difference was found between the efficacy of DAP (SMD = -2.74; CI, -5.56-0.07; $P = 0.06$) or mTAP (SMD = -0.28; CI, -0.82-0.26; $P = 0.31$) and CH. Meta-regression model on *E. faecalis* showed that SAPs have similar efficacy compared to TAP and significantly better efficacy than DAP. On dual-species (SMD = 0.15; CI, -1.00-1.29; $P = 0.80$) or multi-species (SMD = 0.23; CI, -0.08-0.55; $P = 0.15$) biofilms, DAP and CH had similar efficacy.

Conclusions:

Ex vivo evidence showed that antibiotic pastes were either superior or equal to CH. The studied SAPs had considerably higher or similar antibacterial effectiveness compared to DAP, CH, and TAP. Hence, combined antibiotic therapy was not necessarily required for root canal disinfection *ex vivo*.

Keywords: Antibiotic paste, bacterial biofilm, calcium hydroxide, intracanal medicament

INTRODUCTION

Endodontic treatments must strive to eliminate as many bacteria as possible from the root canal system.[1] Chemomechanical preparation plays an essential role in removing bacteria, necrotic tissues, and infected dentin for this aim. Different instrumentation systems leave an unpredictable range of 2.6%–80% of the root canal walls untouched.[2,3] Hence, instrumentation, irrigation, and obturation cannot predictably render canals bacteria-free,[4] and residual bacteria may reside in unaffected areas.[5,6] Most of these species may not survive after the treatment or may persist in low virulence and numbers, insufficient to sustain the periapical inflammation.[7] The microbial etiology of these persistent lesions was reported to comprise different community profiles[8] or single robust species such as *Enterococcus faecalis*.[9]

E. faecalis is commonly identified in persistent endodontic infections.[10,11] The presence of *E. faecalis* in secondary infections is of particular relevance since it is seldom discovered in infected but untreated root canals.[12] There are several unique characteristics for *E. faecalis*, such as inherent antimicrobial resistance[10,13] and ability to withstand extreme environmental conditions.[14] It could sustain viability for 12 months and might serve as a long-term nidus for future infection.[15]

Intracanal medicament (ICM) aids further bacterial elimination after chemomechanical preparation in multivisit endodontic treatments of necrotic teeth.[16] Calcium hydroxide (CH) is the most commonly used ICM in the literature.[1] However, both *in vitro* and *in vivo* studies have shown that CH has limited antibacterial efficacy.[17,18,19] For instance, CH favors the population of *E. faecalis* in multi-species biofilms, as *E. faecalis* survives the high pH of CH.[20,21]

Antibiotic therapy in various formulations is vastly used in medical-related professions to prevent and treat bacterial infections. Given the insufficient spectrum of action of available commercial antibiotic pastes, various antibiotic formulations were developed.[22] It is critical to sterilize the root canal and radicular area during endodontic regenerative procedures since tissue repair and healing are best achieved in a relatively aseptic environment.[23,24] However, the need for a potent antibacterial agent does not solely limit to regenerative procedures.[24] Antibiotic pastes could be used in the treatment of large/persistent periapical lesions[25,26] before/parallel with surgical interventions.[27] A systematic review of 16 articles concluded that even when CH cannot reduce symptoms and heal the periapical lesions, TAP could be effective.[28]

Although numerous studies have compared the antibacterial efficacy of antibiotic pastes with CH, the results are inconsistent.[29,30,31,32] Furthermore, clinical evidence on this issue is limited. The present systematic review and meta-analysis aimed to compare the antibacterial efficacy of single antibiotic paste (SAP), double antibiotic paste (DAP), triple antibiotic paste (TAP), and modified TAP (mTAP) with CH as an ICM on bacterial biofilms from the available *ex vivo* studies.

MATERIALS AND METHODS

Protocol and registration

The protocol of this systematic review was registered in the PROSPERO database (registration number: CRD42021184650), and its report adhered to the preferred reporting items for systematic review and meta-analysis statement.[33]

Formulating the review question

The review question was developed using the PICOS framework: In human/bovine extracted permanent teeth or dentin samples infected with bacterial biofilm (Population), does antibiotic paste (Intervention) provide higher antibacterial efficacy (Outcome) compared to CH (Comparison) in *ex vivo* settings (Study type)?

Eligibility criteria

Ex vivo studies with the following criteria were included: (1) performed on human/bovine extracted permanent teeth or dentin slabs, (2) in press and published papers with full-text available, (3) comprising at least two experimental groups of CH and an antibiotic paste, (4) performed on *E. faecalis* mono-species or polymicrobial (i.e., composed of more than one species) biofilms.

In vivo studies, animal studies, review articles, expert opinions, cross-sectional studies, clinical trials, case reports, and case series were excluded. Furthermore, studies with the following criteria were excluded: (1) assessing the residual antimicrobial efficacy of the medicaments, (2) conducted on immature/deciduous teeth, (3) performed on endotoxins, fungal species, and mono-species bacteria other than *E. faecalis*, and (4) using substrates other than sound dentin.

Search strategy

A combination of medical search heading, Emtree, and free text terms was piloted during the preliminary electronic searches. The search strings were formulated using Boolean operators “OR” and “AND” in three databases: MEDLINE, Scopus, and Embase. No language or date restrictions were applied. The search was last updated on August 23, 2021. The references of the included studies were manually searched for eligible articles. [Supplementary Table 1](#) presents the search queries.

Study selection

Search results were exported to EndNote x9 software (Clarivate, Philadelphia, PA, United States), and the duplicates were automatically removed. Two authors (K.K., M.V.) independently screened titles and abstracts of the identified publications according to the inclusion/exclusion criteria. Potentially appropriate studies were further assessed for eligibility by full-text screening. Disagreements were negotiated with a third author (N.Z.) and resolved.

Data extraction

The same authors (K.K., M.V.) performed the data extraction from the full-text papers covering: (1) general information: first author, year, and country; (2) methodology: bacterial strain, incubation period, type of the teeth, sample dimensions, total sample size, medicament ingredients, concentration, and retention period, outcome measuring technique, depth of dentin, and sampling technique/instrument [

[Supplementary Table 2](#)]; (3) results of culture plate counts, biofilm structural alterations visualized by scanning electron microscopy, colony-forming unit (CFU) counts, viable/dead bacterial cells discovered by confocal laser scanning microscopy, optical density (OD) values, and DNA amounts detected by quantitative polymerase chain reaction.

A third author (O.D.) verified the data sheets and discussed any disagreement between the two authors during the data extraction to achieve consensus. An e-mail was sent to the corresponding author if the desired data were not appropriately mentioned in a manuscript during the data extraction, risk of bias assessment, and meta-analysis. In response, a total of 11 authors supplied the requested information.[\[30,32,34,35,36,37,38,39,40,41,42\]](#)

Risk of bias assessment

A specified bias assessment table was provided inspired by the modified Cochrane risk of bias tool[\[43\]](#) and the tool for before–after studies.[\[44\]](#) The table consisted of 11 items particularly selected for this review to critically assess the studies' methodology.

Two reviewers (K.K., M.V.) independently rated low risk for items that were done and reported accurately, high risk for domains that were not performed/imprecisely reported, not applicable, and not mentioned (NM). In case of disagreement, a third author (N.Z.) was consulted for deliberation. The same authors independently assessed the overall risk of bias. Cohen's Kappa was used to measure the agreement between the two authors using SPSS 25 (IBM corp. Released 2017. IBM Statistics for Windows, Ver. 25.0. Armonk, NY, USA).

Data synthesis and statistical analysis

Standardized mean difference (SMD) with 95% confidence intervals (CIs) was calculated to compare the continuous data on the number of CFUs, percentage of live/dead bacteria, and OD values between the antibiotic and CH groups. Due to the small sample sizes in the studies, the SMDs were computed using Hedges' g statistic. Mean values were calculated from the median in some studies, based on the method proposed by Wan *et al.*[\[45\]](#) The summary estimates were computed using a random-effects model. Statistical heterogeneity in the pooled results was calculated via Chi-square and I^2 statistics, with a $P = 0.05$ significance threshold.

If heterogeneity significantly influenced the summary estimate, random-effects multivariable meta-regression analysis was applied to explore potential sources. [46] When no statistical heterogeneity was observed in the pooled estimate, no further analysis was performed. The first meta-regression model included the variables that were most likely to have an effect on the pooled meta-analytic outcomes: ICM concentration, retention time, and dentin depth. Moreover, the second model compared the efficacy of different antibiotic pastes, while variables of the first model were adjusted. Galbraith plot was employed to display the potential outliers visually. Sensitivity analysis was performed by excluding the outlier study.

Each type of analysis was conducted individually for mono-, dual-, and multi-species biofilm groups, with a restricted maximum likelihood method, using STATA 16 (StataCorp. 2019, College Station, TX, USA).

RESULTS

Literature search and study selection

[Figure 1] displays the flow diagram of the studies. The search resulted in 1417 studies from different databases. Four more articles were added by manual searching. After duplicate removal, 959 records were identified, 46 of which were subjected to full-text screening. Forty and twenty-three studies were included in the qualitative and quantitative synthesis, respectively. Reasons of the exclusion for each synthesis are presented in [Table 1].

Characteristics of the included studies

Table 2 represents a synopsis of included studies. Thirty-five studies (87.5%) were merely conducted on mature/immature *E. faecalis*, 2 (5%) on dual-species mature, and 3 (7.5%) on multi-species mature/immature biofilms. TAP and DAP were the most frequently used antibiotics in 23 (57.5%) and 13 (32.5%) studies, respectively. Out of 40 studies, 3 (7.5%) used spectrophotometry/colorimetry to assess optical density values, 8 (20%) implemented the CLSM approach to quantify the percentage of live/dead bacterial cells, 21 (52.5%) performed culture methods to calculate CFUs, and 8 (20%) used a combination of ≥ 2 different methods.

Risk of bias assessment

Totally, 20 studies (50%) were considered as low overall risk, 6 (15%) were deemed as moderate overall risk, and 14 (35%) were rated as high overall risk of bias. The appraisal of the risk of bias for each study is presented in [Table 3]. Two authors agreed on 88.86% of the items (391/440) with a Cohen's Kappa of 0.82. They were in agreement in 87.5% of the overall scores, yielding a Cohen's Kappa of 0.75.

Meta-analysis and meta-regression on *Enterococcus faecalis* *Enterococcus faecalis*

Triple antibiotic paste versus calcium hydroxide In 31 incorporated comparisons from 13 studies, TAP had significantly higher antibacterial efficacy compared to the CH [[Figure 2a, SMD = -3.82; 95% CI, -5.44 to -2.21; $P < 0.001$]. The effect sizes, however, were statistically heterogeneous ($I^2 = 98.27\%$, $P < 0.001$). One study was excluded from the meta-regression model as an outlier.[30] This model, which is presented in [Table 4], showed that concentration ($P = 0.136$), retention time ($P = 0.150$), and depth of dentin ($P = 0.642$) were not significant predictors for the antibacterial efficacy of TAP.

Double antibiotic paste versus calcium hydroxide There was no statistically significant difference between the DAP and CH in 17 integrated comparisons from 7 studies [[Figure 2b, SMD = -2.74; 95% CI, -5.56-0.07; $P = 0.06$]. However, the pooled data analysis was significantly influenced by heterogeneity ($I^2 = 98.90\%$, $P < 0.001$). The same study was excluded from the meta-regression model.[30] Moreover, another study was dropped[74] so that the model could precisely determine the influence of the variables. The model revealed a strong association between the concentration ($P = 0.021$) and the higher antibacterial efficacy of DAP. In contrast, retention time ($P = 0.167$) and dentin depth ($P = 0.702$) were not significant predictors of efficacy [Table 4].

Modified triple antibiotic paste versus calcium hydroxide No significant difference between mTAP (metronidazole, ciprofloxacin, and clindamycin) and CH was seen in three integrated comparisons from two studies [[Figure 2c, SMD = -0.28; 95% CI, -0.82-0.26; $P = 0.31$] with no statistical heterogeneity ($I^2 = 0.00\%$, $P = 0.48$).

Single antibiotic pastes versus calcium hydroxide In 26 incorporated comparisons from 5 studies, SAPs (including ciprofloxacin, clindamycin, doxycycline, oxytetracycline, erythromycin, metronidazole, and co-amoxiclav) were considerably more effective than CH [[Figure 2d, SMD = -2.38; 95% CI, -2.81 to -1.94; $P < 0.001$] with

substantial heterogeneity among effect sizes ($I^2 = 81.16\%$, $P < 0.001$). One study[40] was dropped due to the collinearity, and the rest of the comparisons fit the meta-regression model [Table 4]. Higher concentration ($P = 0.036$) or retention time ($P = 0.021$) of SAPs was strongly associated with higher antibacterial efficacy. However, the antibacterial efficacy of SAPs was significantly reduced in deeper dentin ($P = 0.002$).

Antibiotic comparison Multiple meta-regression analyses comparing antibiotic pastes with adjusted variables are presented in [Table 5]. All investigated SAPs showed better efficacy compared to DAP ($P < 0.05$). Nonetheless, when compared to mTAP, only clindamycin ($P = 0.034$), erythromycin ($P = 0.021$), and metronidazole ($P = 0.021$) had significantly higher efficacy. Compared to oxytetracycline (as the weakest SAP), metronidazole, erythromycin, and ciprofloxacin were all substantially superior ($P < 0.05$); however, clindamycin, doxycycline, and co-amoxiclav were not ($P > 0.05$). Moreover, the differences between SAPs and TAP were not statistically significant ($P > 0.05$). TAP had significantly higher efficacy than DAP ($P = 0.034$). Nevertheless, there was no significant difference between TAP or DAP compared to mTAP ($P > 0.05$).

Meta-analysis and meta-regression on dual-species biofilm

No statistically significant difference in antibacterial efficacy was seen between the DAP and CH in 5 integrated comparisons from 2 studies [[Figure 3a, SMD = 0.15; 95% CI, -1.00–1.29; $P = 0.80$]. Effect sizes were considerably heterogeneous ($I^2 = 81.58\%$, $P < 0.001$). The meta-regression model indicated that both higher concentration ($P = 0.035$) and retention time ($P < 0.001$) could contribute to increased antibacterial efficacy of DAP. However, in deeper dentin, DAP offered a significantly lower antibacterial efficacy ($P = 0.005$).

Meta-analysis on multi-species biofilm

In 10 incorporated comparisons from 2 studies, the difference between the CH and DAP was not statistically significant [[Figure 3b, SMD = 0.23; 95% CI, -0.08–0.55; $P = 0.15$], with negligible statistical heterogeneity among the data ($I^2 = 12.67\%$, $P = 0.27$).

Sensitivity analysis

The Galbraith plots associated with the studies on *E. faecalis* biofilm are depicted in [Figure 4a-c]. Despite the abundance of outliers, only one study[30] was excluded, and the rest were not removed due to their symmetrical distribution around the regression line. The results of the sensitivity analysis are shown in [Figure 4d and e]. No significant change in the pooled estimate findings or the degree of heterogeneity was detected, confirming the pooled results' robustness.

DISCUSSION

The present systematic review compared the antibacterial efficacy of various antibiotic pastes versus CH on different bacterial strains incubated on human/bovine dentin structure from available *ex vivo* studies. As an overview of the results, TAP and SAPs were significantly superior to CH on *E. faecalis* biofilm, while mTAP and CH displayed similar efficacy. No statistical difference was noticed between DAP and CH in terms of antibacterial potency on mono-, dual-, and multi-species biofilms.

Study findings

On Enterococcus faecalis biofilm

The superiority of TAP compared to CH was in agreement with the results of a recently published systematic review,[81] including both clinical and *in vitro* studies. [82] The antibacterial efficacy of CH directly lies within the diffusion of alkaline hydroxyl ions.[83] After a week of CH introduction inside the canal, pH reaches its maximum values[84,85] and then begins to drop. As the pH falls, the residual bacteria may regrow in the canals treated with CH, while the samples treated with antibiotics may not get affected. However, there was no superiority for DAP/mTAP compared to CH in our review. This unusual phenomenon warrants more investigation as TAP and SAPs were more effective against *E. faecalis* than CH.

Our findings revealed that SAPs were more potent antibacterial agents than CH. One of the included studies employed antibiotics with a minimum inhibitory concentration.[76] Another included study assessed the antibacterial efficacy after only 5 and 10 min of ICM retention.[36] Surprisingly, in both studies, antibiotic groups were significantly more effective than CH. This may indicate a turning point for future research to examine the efficacy of different SAPs, with lower concentrations and reduced retention times.

The comparison of antibiotic pastes with adjusted covariates revealed that each kind of SAPs could reduce *E. faecalis* as effectively as TAP while showing significantly better efficacy than DAP. Combination antibiotic therapy has been used to improve treatment efficacy, broaden the antibiotic range of activity, slow the evolution of drug resistance, and minimize toxicity by lowering the dosage of each active component.[86] However, synergistic effects will not always occur as antibiotics may exhibit inhibitory interactions. The combination of bacteriostatic and bactericidal agents is less effective than the bactericidal agent alone.[87] TAP is a mixture of two bactericides and a bacteriostatic antibiotic. Hence, the probable interactions between minocycline and the bactericidal agents may be responsible for such results obtained comparing the efficacy of SAPs and TAP. Altogether, the underlying philosophy behind most antibiotic interactions is yet to be understood.[88]

Furthermore, the polymicrobial nature of the persistent endodontic infection, with the most predominant species being *E. faecalis* and *Porphyromonas gingivalis*, is different from that of primary infection.[89] Different SAPs, such as metronidazole, could have notable antibacterial efficacy against these species; obligate and facultative anaerobic bacteria.[72] Furthermore, metronidazole is suggested for topical use since it is unlikely to develop resistance.[90] Based on our findings, using combination antibiotic therapy may not be necessary as SAPs could effectively reduce *E. faecalis* colonies.

The ability of an ICM to disperse into the root canal system appears to be critical for its successful antibiofilm efficacy.[83] Based on the findings by Abbott *et al.*,[91] the diffusion of a drug across dentinal tubules is directly related to its concentration, retention time, and the area of the inner canal exposed to the agent.

On polymicrobial biofilms

Of the two studies conducted on dual-species biofilms, one was performed on the *E. faecalis* and *Prevotella intermedia*,[74] while the other one was on *E. faecalis* and *Streptococcus gordonii*[38] combined biofilms. These species were chosen for their capacity to coexist in a biofilm. However, the difference in the bacterial combination could presumably account for part of the heterogeneity of the pooled results. On the other hand, the two studies on the multi-species biofilms were performed on isolated bacteria from mature/immature teeth with necrotic pulps, which were believed to have a similar bacterial population according to a clinical study.[92] Moreover,

these two studies had more methodological characteristics in common (DAP as antibiotic, 7-day retention time, evaluating CFU/mL at the dentin surface), which resulted in low heterogeneity of the pooled results.

Hypothetically, the overall better efficacy of antibiotics compared to CH on *E. faecalis* biofilm might be related to the functioning proton pumps of the bacteria. These pumps maintain cell survival by acidifying the cytoplasm.[20] However, this function might be hindered in polymicrobial biofilms to some extent. Therefore, CH could appear with equal efficacy as antibiotics in such biofilms. Clinical studies have proved this claim by showing equal efficacy of TAP or moxifloxacin compared to CH. [93,94] However, DAP was the only antibiotic investigated on polymicrobial biofilms in this review. Hence, based on the difference between the mono- and polymicrobial biofilms, studying different antibiotic pastes on polymicrobial biofilms is recommended.

In our review, the outcomes of meta-analyses were mostly influenced by substantial heterogeneity. The high degree of statistical heterogeneity was probably explained by focusing on the intrinsic methodological aspects of the studies. The potential confounding factors included bacterial strain, incubation period, type of the teeth, sample dimensions, sample size, cementum removal, ICM vehicle, biofilm development confirmation, sampling technique, and outcome measure technique.

Methodological appraisal of studies

E. faecalis has been reported to be the most prevalent species isolated from root-filled teeth with apical periodontitis.[95] This bacterium, however, is no longer in the spotlight as the only cause of persistent infections,[96] as it is not identified in 100% of the secondary infection cases.[97] *E. faecalis* is simply cultured in the laboratory with little sensitivity to different conditions. Hence, the regular selection of *E. faecalis* by different studies could be explained.[96] Polymicrobial biofilms are also favored for *ex vivo* biofilm investigations, as they more precisely mimic clinical infection. Therefore, this paper systematically reviewed the effect of ICMs on the polymicrobial biofilms as well as the mono-species of *E. faecalis*.

Since single-rooted human and bovine teeth are alike in terms of dentin structure, [98] both were included in this review. Moreover, mature biofilms behave differently than single bacterial strains since they are composed of a complex microbial com-

munity.[99] As a result, studies with immature biofilms were excluded from the final meta-analysis in this review.

Antibiotics' retention time in root canals is vital to eradicate as many bacteria as possible. According to the quantitative results of the included studies, the antibacterial efficacy of SAP and DAP increased with time after application. However, when applied for more than 48 h, antibiotic pastes could induce cytotoxicity and genotoxicity on human stem cells.[100] This issue raises an essential question: Could we benefit from antibiotic pastes in shorter application times? Addressing this question, we included all reported retention times in the meta-analysis to analyze its influence.

Similar to most *ex vivo* systematic reviews, this study used a modified tool for quality assessment.[101] Based on the published evidence, randomization and operator blinding are not regularly performed/reported in *ex vivo* settings.[102] Likewise, no study reported the operator blinding in the present review, and very few reported the randomization procedure or sample size calculation rationale. As performing these steps increases the generalizability of evidence, authors are strongly recommended to use/mention them in their studies.

Four processes should be considered while simulating clinical infection within the dentinal tubules *ex vivo*. First, the smear layer should be removed. This layer seals the tubules' entrance; hence, leaving it intact may decrease the penetration of bacteria[103] and the diffusion of ICMs through the tubules.[104] Second, experiments should be conducted on mature bacterial biofilms, defined as ≥ 3 -week-old incubated biofilm wherein bacteria develop more resistance to the disinfectants.[105,106] All included studies in the meta-analysis followed these two criteria. Third, the cementum layer should be eliminated. Its removal permits easier infiltration of bacteria into the dentinal tubules, resulting in noticeable infection.[107] Fourth, biofilm formation after the incubation period should be verified since *ex vivo* biofilm development depends on various laboratory steps.[96] Cementum removal was NM by most studies, while biofilm development confirmation was rather well performed by the majority of the studies included in the meta-analysis.

Recommendations for the future research

1. *Ex vivo* studies are encouraged to test ICMs on polymicrobial infections. The most acceptable source of such biofilm would be an obturated root canal with a persistent lesion
2. Clinical studies, especially randomized clinical trials, are recommended for testing antibiotic pastes on endodontic outcomes
3. *Ex vivo* studies are suggested to examine new ICMs with minimum concentration and retention time parameters chosen reasonably.

Strengths and limitations

To date, no systematic review has been conducted to compare the antibacterial efficacy of the two commonly-used ICMs via meta-analytic pooling of data. Concerning the limited number of clinical evidence, the abundance of existing *ex vivo* studies, and the lack of consensus toward selecting the proper ICM, only *ex vivo* articles were included.

As strength, a meta-regression analysis was conducted to ascertain the sources of heterogeneity. Moreover, sensitivity analysis indicated the stability of the results.

In addition, data on ICM efficacy against polymicrobial biofilms were obtained and pooled; however, the number of studies on this subject was restricted. Therefore, it is not proper to draw generalized conclusions about polymicrobial biofilms.

Although it has been demonstrated that greater concentrations of CH have enhanced bactericidal activity,[\[108\]](#) it was not possible to calculate/convert the concentrations in all studies. Therefore, as an important limitation, dosage differences for CH were neglected in the meta-analysis.

CONCLUSIONS

Within the constraints of this review, antibiotic pastes were either superior or comparable to CH in terms of overall effectiveness. SAPs, while having the same potency as TAP, exerted significantly better antibacterial efficacy compared to DAP or CH against *E. faecalis* biofilm. Considering the overall superiority of SAPs to other medicaments, a combination of antibiotics (as in DAP, mTAP, or TAP) seems not to be a necessity for root canal disinfection.

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Conflicts of interest

There are no conflicts of interest.

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Figures and Tables

Supplementary Table 1

Search strings using medical search heading, Emtree, and Boolean operators (OR, AND, NOT) and using limits and restrictions in the search ([])

Source	Search string
PubMed	<p>(“antibiotic paste”[Title/Abstract] OR (“ledermix”[Supplementary Concept] OR “ledermix”[All Fields] OR “ledermix”[All Fields]) OR “odontopastes”[All Fields] OR “augmentin”[Title/Abstract] OR “amoxicillin-potassium clavulanate combination”[MeSH Terms] OR (“clindamycin”[MeSH Terms] OR “clindamycin”[All Fields] OR “clindamycine”[All Fields]) OR (“ciprofloxacin”[MeSH Terms] OR “ciprofloxacin”[All Fields] OR “ciprofloxacin”[All Fields] OR “ciprofloxacin s”[All Fields] OR “ciprofloxacin”[All Fields]) OR (“doxycycline”[MeSH Terms] OR “doxycycline”[All Fields] OR “doxycyclin”[All Fields]) OR (“moxifloxacin”[MeSH Terms] OR “moxifloxacin”[All Fields] OR “moxifloxacin”[All Fields]) OR (“metronidazole”[MeSH Terms] OR “metronidazole”[All Fields] OR “metronidazol”[All Fields] OR “metronidazoles”[All Fields]) OR (“polymyxine”[All Fields] OR “polymyxins”[MeSH Terms] OR “polymyxins”[All Fields] OR “polymyxin”[All Fields]) OR “Anti-Bacterial Agents”[MeSH Terms] OR “antibiotic*”[All Fields]) AND (“calcium hydroxide”[MeSH Terms] OR “calcium hydroxide”[All Fields] OR “calcium hydroxide paste”[All Fields]) AND (“antibacter*”[All Fields] OR “antibacterial”[All Fields] OR (“anti infective agents”[Pharmacological Action] OR “anti infective agents”[MeSH Terms] OR (“anti infective”[All Fields] AND “agents”[All Fields]) OR “anti infective agents”[All Fields] OR “antimicrobial”[All Fields] OR “antimicrobials”[All Fields] OR “antimicrobially”[All Fields]) OR (“bacteriostatic”[All Fields] OR “bacteriostatical”[All Fields] OR “bacteriostatically”[All Fields] OR “bacteriostatics”[All Fields]) OR (“bactericidal”[All Fields] OR “bactericidal”[All Fields] OR “bactericidally”[All Fields] OR “bactericidals”[All Fields] OR “bactericide”[All Fields] OR “bactericides”[All Fields] OR “bactericidal”[All Fields] OR “bactericidity”[All Fields]) OR “Biofilms”[MeSH Terms] OR (“biofilm s”[All Fields] OR “biofilmed”[All Fields] OR “Biofilms”[MeSH Terms] OR “Biofilms”[All Fields] OR “biofilm”[All Fields]) OR (“bactericidal”[All Fields] OR “bactericidal”[All Fields] OR “bactericidally”[All Fields] OR “bactericidals”[All Fields] OR “bactericide”[All Fields] OR “bactericides”[All Fields] OR “bactericidal”[All Fields] OR “bactericidity”[All Fields]) OR “anti-biofilm”[All Fields] OR “antibiofilm”[All Fields] OR “anti-biofilm”[All Fields])</p>
Embase	<p>(‘antibiotic agent’/exp OR ‘antibiotic agent’ OR ‘antibiotic’/exp OR ‘antibiotic’ OR ‘demeclocycline plus triamcinolone’/exp OR ‘demeclocycline plus triamcinolone’ OR ‘amoxicillin plus clavulanic acid’/exp OR ‘amoxicillin plus clavulanic acid’ OR</p>

MeSH: Medical search heading

Supplementary Table 2:

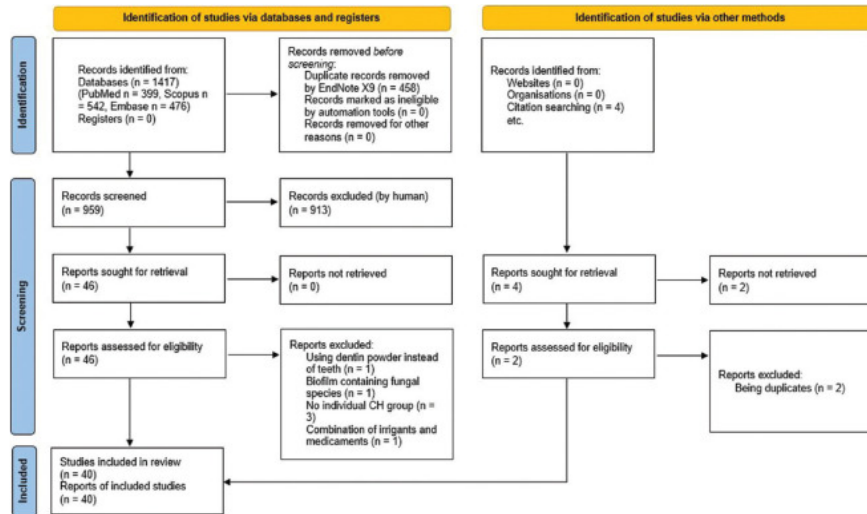
Further study characteristics

Author, year	Country	Bacterial strain	Incubation period (days)	Type of the tooth/dimensions	Total sample size	Further f
Abbaszadegan <i>et al.</i> , 2016	Iran	EF ATCC 29212	21	Human mand. premolar/15 mm RS	108 ^o	-
Adl <i>et al.</i> , 2014	Iran	EF ATCC 11700	21	Human single-rooted teeth/6 mm RS	60	-
Alfadda <i>et al.</i> , 2021	USA	EF ATCC 47077	21	Human mand. premolars/12 mm RS	40 ^o	-
Asnaashari <i>et al.</i> , 2019	Iran	EF ATCC 9854	21	Human single-rooted anterior teeth/NM	62 ^o	-
Balto <i>et al.</i> , 2020	Saudi Arabia	EF ATCC 29212	21	Human single-rooted teeth/6×8 × 0.5 mm DS	100 ^o	-
Carbajal Mejía <i>et al.</i> , 2015	NM	EF ATCC 29212	21	Human single-rooted teeth/8 mm RS	75 ^o	-
Chai <i>et al.</i> , 2013	Malaysia	EF ATCC 29212	21	Human anterior teeth excluding lower incisors/4 mm RS	84	-
Cunha Neto <i>et al.</i> , 2021	Brazil	EF ATCC 29212	5	Human single-rooted teeth/8 mm DS	67 ^o	-
de Freitas <i>et al.</i> ,	Brazil	EF ATCC 29212	21	Bovine incisor	30 ^o	-

^oGroups not within the scope of review are present in the study. SG: *Streptococcus gordonii*, EF: *Enterococcus faecalis*, PI: *Prevotella intermedia*; ATCC: American type culture collection, CH: Calcium hydroxide, CLSM: Confocal laser scanning microscopy, DAP: Double antibiotic paste, DS: Dentin specimen,

DSB: Dual-species biofilm, Mand.: Mandibular, Max.: Maxillary, TAP: Triple antibiotic paste, mTAP: Modified TAP, NM: Not mentioned, PBB: Polybacterial biofilm, RS: Root section, SEM: Scanning electron microscopy, ICMs: Intracanal medicaments

Figure 1



Flow diagram of the identified studies based on The Preferred Reporting Items for Systematic Reviews and Meta-Analyses

Table 1

Reasons for and the number of excluded studies in each synthesis

	Reasons	Number of studies excluded
Qualitative synthesis	Using dentin powder as biofilm formation substrate, [47] biofilm containing a fungal species, [48] absence of individual CH group, [49,50,51] and treatment of samples with different irrigants before medicament placement [52]	6
Quantitative synthesis	Not mentioning the concentrations of antibiotics, [53,54,55,56] lacking an individual antibiotic group, [57,58,59,60] being performed on immature biofilms, [29,37,61,62,63,64,65] and containing inadequately reported data [39,66]	17

CH: Calcium hydroxide

Table 2

Characteristics of the included studies

Author, year	Methodology				
	Intervention and comparison groups (n)	Concentration (mg/mL)	Retention time	Evaluation depth/means of sampling	Measure
Abbaszadegan <i>et al.</i> , 2016[67]	CH (30)	1.5	1, 7, 14 days	NM/GG #5	Percentage reduction of log 10 (CFU/mL+1
	TAP (30)	1.5			
	C+, NS (9) C- (9)				
Adl <i>et al.</i> , 2014[53]	CH (20)	NM	1, 7 days	100, 200 µm/GG #4, #5	CFU
	TAP (20)	NM			
	C, NS (20)				
Alfadda <i>et al.</i> , 2021[34]	CH (10)	750 [†]	7 days	Culture: Surface dentin/#25 H-file	Log 10 CFU/mL, percentage of live cells by CLSM
	TAP (10)	1			
	C+ (5) C- (5)			CLSM: Deep dentin/-	
Asnaashari <i>et al.</i> , 2019[68]	CH (10)	NM	12 days	NM/ProTaper F4	Percentage reduction of CFU/mL
	mTAP, clindamycin (10)	1			

*Due to unavailability of the information/incoherent findings, comparison between the medicaments based on statistical differences was unlikely, ~ No statistical difference, A>B, A was significantly more effective than B in killing bacteria, †Concentrations were converted to mg/mL unit. In the composition of

mTAP, only the new antibiotic part which replaces minocycline was mentioned. EF: *Enterococcus faecalis*, SG: *Streptococcus gordonii*, ATCC: American Type Culture Collection, C+: Positive control, C-: Negative control, CFUs: Colony forming units, CH: Calcium hydroxide, CLSM: Confocal laser scanning microscopy, DAP: Double antibiotic paste, DSB: Dual-species biofilm, DW: Distilled water, GG: Gates Glidden Bur, H-file: Hedstrom file, HPMC: Hydroxypropyl methylcellulose, Log: Logarithm, Mac.: Macrogol, MC: Methylcellulose, TAP: Triple antibiotic paste, mTAP: Modified TAP, NAC: N-acetyl cysteine, NM: Not mentioned, NS: Normal saline, PBS: Phosphate-buffered saline, PG: Propylene glycol, RT-qPCR: Reverse transcription-quantitative polymerase chain reaction, SEM: Scanning electron microscopy, CHX: Chlorhexidine

Table 3

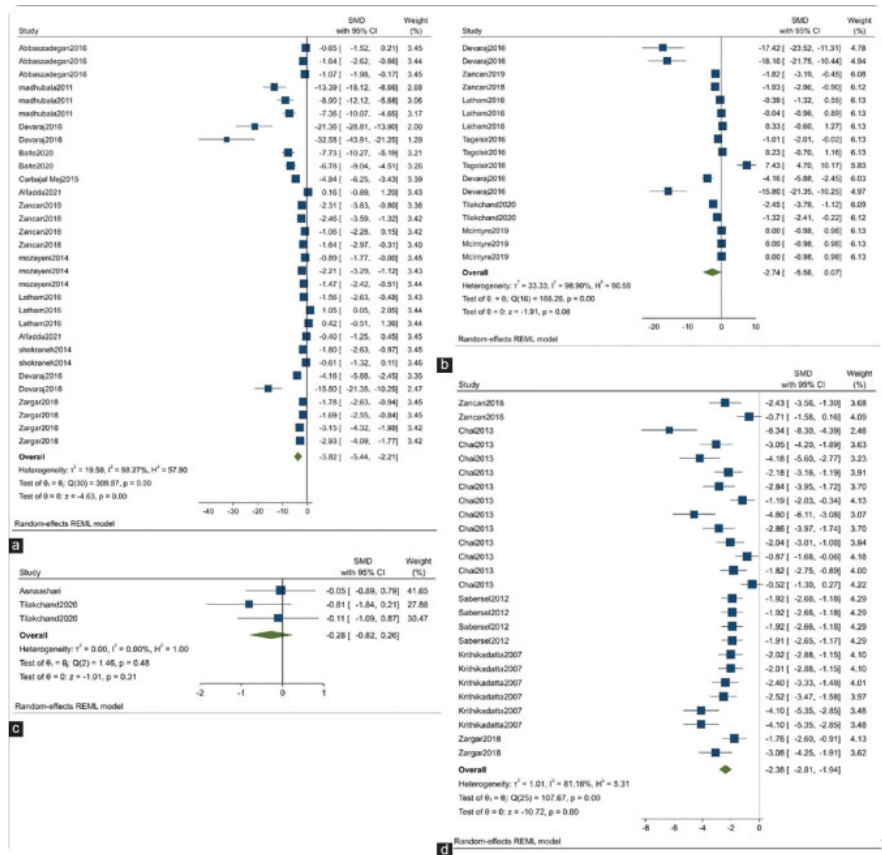
Risk of bias appraisal of the included studies

Author, year	Randomization	Operator blinding	Sample size calculation	Standardized sampling	Depth of dentin	Cementum removal	≥2 domains of bias
Abbaszadegan <i>et al.</i> , 2016[67]	+	NM	NM	-	+	NM	
Adl <i>et al.</i> , 2014[53]	NM	NM	NM	-	+	NM	
Alfadda <i>et al.</i> , 2021[34]	NM	NM	NM	-	+	NM	
Asnaashari <i>et al.</i> , 2019[68]	NM	NM	NM	-	+	NM	
Balto <i>et al.</i> , 2020[69]	+	NM	+	NA	+	NM	
Carbajal Mejía and Aguilar Arrieta, 2016[70]	+	NM	NM	NA	+	NM	
Chai <i>et al.</i> , 2013[36]	NM	NM	NM	-	+	-	
Cunha Neto <i>et al.</i> , 2021[37]	+	NM	+	NA	+	NM	
de Freitas <i>et al.</i> , 2017[57]	+	NM	NM	NA	-	NM	
Devaraj <i>et al.</i> , 2016[30]	NM	NM	NM	-	+	+	
Dewi <i>et al.</i> , 2021[61]	+	NM	NM	+	+	NM	
-	-	----	----	-	---	----	

Dentin samples were considered standardized when their dry mass was equalized between study groups. The control domain was attributed to the presence of a standard positive control group (i.e.: Infected but untreated samples) in the study. The depth of the dentin domain was the depth in which the

antibacterial effectiveness of ICMs was evaluated. +: Low risk, -: High risk, NM: Not mentioned, NA: Not applicable, ICMs: Intracanal medicaments

Figure 2



(a) Forest plot comparing the efficacy of TAP and CH on *E. faecalis* biofilm (negative interval favors antibiotic). (b) Forest plot comparing the efficacy of DAP and CH on *E. faecalis* biofilm. (c) Forest plot comparing the efficacy of mTAP and CH on *E. faecalis* biofilm. (d) Forest plot comparing the efficacy of SAPs and CH on *E. faecalis* biofilm (negative interval favors antibiotic).

Table 4

Meta-regression model comparing the efficacy of antibiotic pastes with calcium hydroxide

Model	Covariate	Coefficient (95% CI)	P
Comparison between TAP and CH on EF biofilm	Concentration	0.001 (-0.000-0.003)	0.136
	Retention time	0.107 (-0.039-0.254)	0.150
	Depth of dentin	0.191 (-0.614-0.995)	0.642
	Constant	-6.290 (-10.536- -2.044)	0.004
Comparison between DAP and CH on EF biofilm	Concentration	-0.003 (-0.007-0.001)	0.021*
	Retention time	-0.113 (-0.275-0.048)	0.167
	Depth of dentin	-0.345 (-2.117-1.426)	0.702
	Constant	3.578 (-0.635-7.791)	0.096
Comparison between SAPs and CH on EF biofilm	Concentration	-0.127 (-0.245- -0.008)	0.036*
	Retention time	-0.506 (-0.938- -0.076)	0.021*
	Depth of dentin	0.864 (0.305-1.422)	0.002*
	Constant	2.017 (-3.186-7.218)	0.447
Comparison between DAP and CH on dual-species biofilm	Concentration	-1.404 (-2.713- -0.095)	0.035*
	Retention time	-0.231 (-0.357- -0.106)	0.000*
	Depth of dentin	0.947 (0.288-1.606)	0.005*
	Constant	2.080 (0.864-3.297)	0.001

* $P < 0.05$, significant at 5% significance level. EF: *Enterococcus faecalis*, CH: Calcium hydroxide, DAP: Double antibiotic paste, TAP: Triple antibiotic paste, SAPs: Single antibiotic pastes, CI: Confidence interval

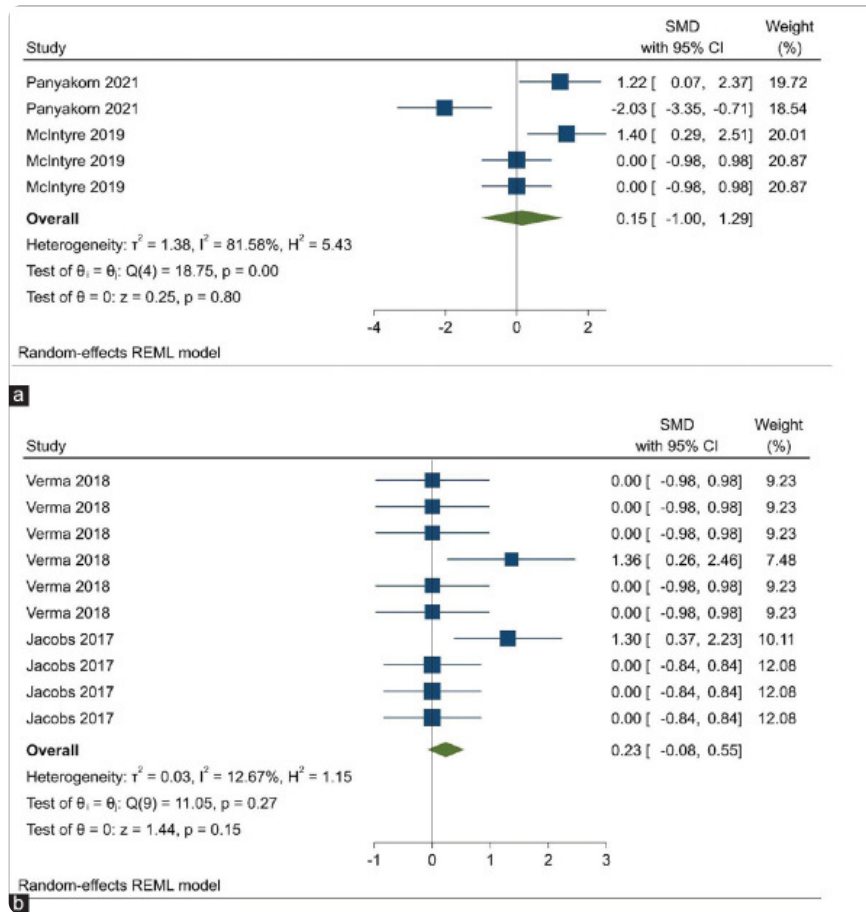
Table 5

Meta-regression analysis comparing the efficacy of antibiotic pastes used on *Enterococcus faecalis* biofilm with adjusted covariates (concentration, retention time, depth)

Comparison	Covariate	Coefficient (95% CI)	P
Between SAPs and DAP	Ciprofloxacin	-4.245 (-7.385- -1.106)	0.008*
	Clindamycin	-5.156 (-7.920- -2.392)	0.000*
	Doxycycline	-4.241 (-7.381- -1.101)	0.008*
	Erythromycin	-7.169 (-10.391- -3.948)	0.000*
	Metronidazole	-6.543 (-9.799- -3.288)	0.000*
	Co-amoxiclav	-4.246 (-7.386- -1.106)	0.008*
Between SAPs and TAP	Ciprofloxacin	1.797 (-2.843-6.438)	0.448
	Clindamycin	1.162 (-1.687-4.011)	0.424
	Doxycycline	1.803 (-2.838-6.443)	0.446
	Erythromycin	0.293 (-2.321-2.906)	0.826
	Metronidazole	0.718 (-1.719-3.154)	0.564
	Co-amoxiclav	1.797 (-2.843-6.438)	0.448
Between SAPs and mTAP	Ciprofloxacin	-2.658 (-6.375- 1.058)	0.161
	Clindamycin	-3.504 (-6.739- -0.269)	0.034*
	Doxycycline	-2.653 (-6.369- 1.063)	0.162
	Erythromycin	-6.363 (-11.781- -0.945)	0.021*

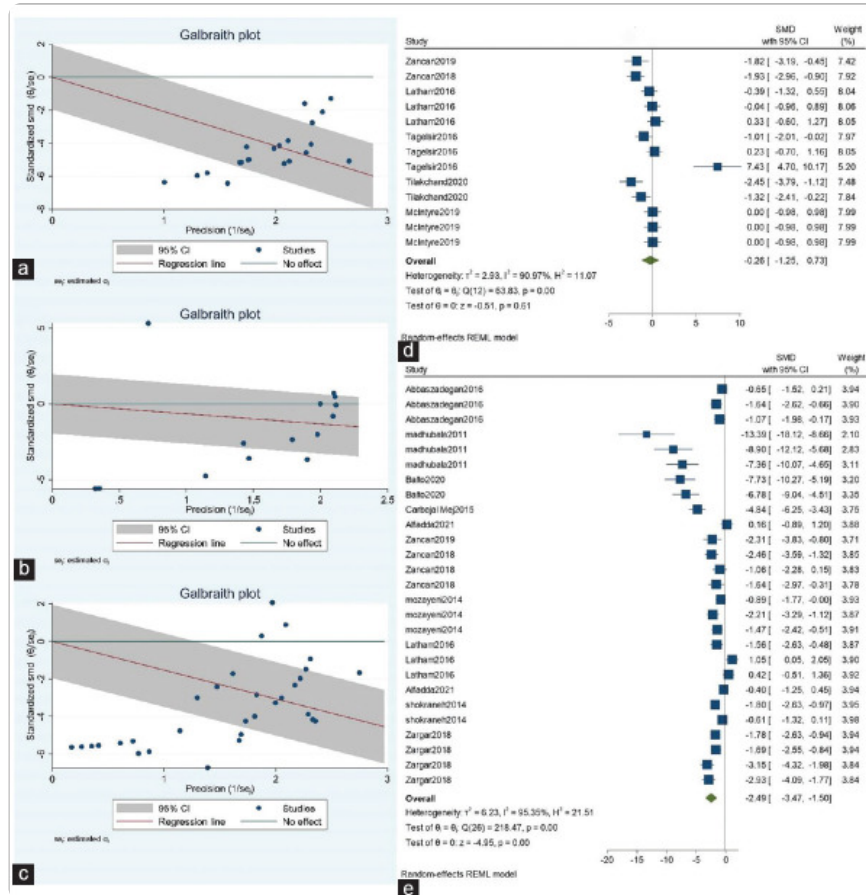
* $P < 0.05$, significant at 5% significance level. DAP: Double antibiotic paste, TAP: Triple antibiotic paste, mTAP: Modified TAP, SAPs: Single antibiotic pastes, CI: Confidence interval

Figure 3



(a) Forest plot comparing the efficacy of DAP and CH on dual-species biofilm (negative interval favors antibiotic). (b) Forest plot comparing the efficacy of DAP and CH on multi-species biofilm

Figure 4



(a) Galbraith plot comparing antibacterial efficacy of SAP and CH on *E. faecalis* biofilm. (b) Galbraith plot comparing antibacterial efficacy of DAP and CH on *E. faecalis* biofilm. (c) Galbraith plot comparing antibacterial efficacy of TAP and CH on *E. faecalis* biofilm. (d) Sensitivity analysis by excluding the outlier from the comparison of DAP and CH. (e) Sensitivity analysis by excluding the outlier from the comparison of TAP and CH