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Efficacy of different mouthwashes against COVID-19: A systematic review and network meta-analysis

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ABSTRACT

To evaluate the effectiveness of antiseptic mouthwashes in reducing SARS-CoV-2 load clinically and in vitro. A systematic electronic search (MEDLINE/Scopus/Cochrane) was conducted to identify prospective clinical and in vitro studies published between 2019 included and 16 June 2023 assessing the effectiveness of mouthwashes in reducing SARS-CoV-2 load in saliva or surrogates. Data were summarized in tables and a network meta-analysis was performed for clinical trials. Thirty-five studies (14 RCTs, 21 in vitro) fulfilled the inclusion criteria. The risk of bias was judged to be high for 2 clinical and 7 in vitro studies. The most commonly test product was chlorhexidine alone or in combination with other active ingredients, followed by povidone-iodine, hydrogen peroxide and cetylpyridinium chloride. Overall, the descriptive analysis revealed the effectiveness of the mouthwashes in decreasing the salivary viral load both clinically and in vitro. Network meta-analysis demonstrated a high degree of heterogeneity. Among these studies, only chlorhexidine 0.20% was associated to a significant Ct increase in the saliva 5 min after rinsing compared to non-active control ($p = 0.027$). Data from clinical and in vitro studies suggested the antiviral efficacy of commonly used mouthwashes. Large well-balanced trials are needed to identify the best rinsing protocols.

1. Introduction

In early 2020, the World Health Organization (WHO) declared COVID-19, a disease caused by severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2), a pandemic. The oral cavity is one of the first gateways and a site of accumulation and replication for the virus, which has been found to reside in oral cavity epithelium, throat, nose and salivary gland cells [1]. SARS-CoV-2 virus can infect and replicate within salivary gland cells, especially minor ones, thus resulting in the presence of the virus in the saliva also in asymptomatic patients [2]. Furthermore, SARS-CoV-2 RNA was also detected in the saliva in the early phases of the infection before pulmonary clinical manifestations [3]. As saliva droplets seem to play a crucial role in SARS-CoV-2 transmission also from asymptomatic and mild symptomatic patients [4,5], strategies to minimize SARS-CoV-2 spread through the saliva are pivotal in clinical procedures involving the oral cavity.

To protect both healthcare professionals and patients, several infection prevention and control measures have been introduced and implemented since the outbreak of COVID-19. In addition to personal protection equipment (PPE) for healthcare professionals, several European guidelines also recommend the use of preprocedural rinses with antiseptic mouthwashes in order to decrease the viral load contained in patients' saliva [6]. The effectiveness of preprocedural rinsing to reduce bacterial contamination has been extensively demonstrated also prior to the pandemic, especially for professional oral hygiene and surgical procedures involving abundant aerosol production [7–9]. For instance, periprocedural rinsing with 0.12% and 0.20% chlorhexidine (CHX) was found to reduce bacterial, viral, and fungal load in the oral biofilm, thus decreasing the risk of cross infection [10]. As regards the SARS-CoV-2 virus, numerous preprocedural rinsing protocols have been proposed.

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SARS-CoV-2 is an enveloped virus, characterized by an outer lipid membrane that makes the virus highly sensitive to agents that disrupt it. One of these is ethanol at high concentrations of 60–70% (v/v), which has been found to be highly effective against several viral pathogens in vitro, including SARS-CoV-2, but at the same time to damage mammalian cells [11]. The clinical use of 1% hydrogen peroxide (H_2O_2) or 0.2% povidone iodine (PVP-I) mouthwashes has been suggested due to the vulnerability of the SARS-CoV-2 virus to oxidation [12]. CHX, essential oils, and cetylpyridinium chloride (CPC) alone or in combination with other compounds have also been recommended [6,13].

Numerous national and international guidelines have been published so far, suggesting the use of different preprocedural mouth rinse protocols [6,14]. Nevertheless, it can be affirmed that there is no universally accepted preprocedural rinsing protocol for the reduction of SARS-CoV-2 load in the oral cavity, most likely due to evidence showing the effectiveness of several compounds. Furthermore, it is not clear whether and to which extent these interventions are effective in the prevention of viral spread in dental setting. Despite numerous reviews have been published so far on the topic [15–17], to the best of the authors' knowledge, this is the first systematic review and meta-analysis aiming to investigate, both in clinical and in vitro studies, the effectiveness of antiseptic mouthwashes and rinsing protocols in reducing SARS-CoV-2 load.

2. Materials and methods

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement [18] was followed to design and write the present systematic review. The protocol was registered with the international prospective register of systematic reviews (PROSPERO) with registration number CRD42022318922.

2.1. PICOS question

The addressed PICOS (population, intervention, comparison, outcome, study) question was: "Are oral antiseptics able to reduce SARS-CoV-2 load in human oro-pharyngeal fluids and surrogates compared to control in human clinical and in vitro studies?" (Table 1).

2.2. Search strategy, study selection, and data extraction

In human clinical and in vitro studies assessing the effectiveness of mouthwashes in reducing SARS-CoV-2 load were considered. To identify the studies to be included in this systematic review and meta-analysis, an electronical literature search was conducted on the databases MEDLINE (PubMed) online library, Scopus and Cochrane library on paper published between 2019 included and 15 June 2023. A specific search strategy was developed for each database, using a combination of the following MeSH and keywords: "mouthwash", "mouthrinse", "chlorhexidine", "povidone iodine", "cetylpyridinium chloride", "hydrogen peroxide", "delmopinol", "listerine", "essential oils", "cyclodexstrin", "citrox", "SARS-CoV-2", "coronavirus", and "COVID-19″. Details on the search strategy are provided in Table 2. The included papers were selected by two independent reviewers (G.B. and L.Sc.) through 2 screening stages, i.e. abstract-title and full-text. To assess the level of inter-reviewer agreement at both stages, Kappa statistics were calculated with an online tool [19]. Any disagreement was resolved by discussion and, if necessary, a third reviewer (P.B. for in vitro and S.S. for clinical studies) was consulted.

Inclusion criteria were: randomized clinical trials (RCTs) and in vitro studies published in English, investigation/use of SARS-CoV-2, details on mouthwash formulation, concentration and application time, use of a control solution (active and/or non-active products), virus load quantification in oro-pharyngeal fluids and surrogates. For the quantitative analysis, it was also required that they adhered to one collection method

Table 2
Details on the electronic search strategy.

Database	Search strings
MEDLINE (PubMed)	**(mouthwash OR mouthrinse OR chlorhexidine OR cetylpyridinium chloride OR "povidone iodine" OR "hydrogen peroxide" OR delmopinol OR listerine OR "essential oils" OR cyclodextrin OR citrox) AND (SARS-CoV-2 OR coronavirus OR COVID-19) Filters: from 2019 to 2023** (("mouthwashes"[Pharmacological Action] OR "mouthwashes"[MeSH Terms] OR "mouthwashes"[All Fields] OR "mouthwash"[All Fields] OR "mouthwashing"[All Fields] OR "mouthwashings"[All Fields] OR ("mouthrinse"[All Fields] OR "mouthrinsed"[All Fields] OR "mouthrinses"[All Fields] OR "mouthrinsing"[All Fields] OR "mouthrinsings"[All Fields]) OR ("chlorhexidine"[MeSH Terms] OR "chlorhexidine"[All Fields] OR "chlorhexidin"[All Fields]) OR ("cetylpyridinium"[MeSH Terms] OR "cetylpyridinium"[All Fields] OR ("cetylpyridinium"[All Fields] AND "chloride"[All Fields]) OR "cetylpyridinium chloride"[All Fields]) OR "povidone iodine"[All Fields] OR "hydrogen peroxide"[All Fields] OR ("delmopinol"[Supplementary Concept] OR "delmopinol"[All Fields]) OR ("listerine"[Supplementary Concept] OR "listerine"[All Fields] OR "listerine"[All Fields] OR "sodium fluoride"[MeSH Terms] OR ("sodium"[All Fields] AND "fluoride"[All Fields]) OR "sodium fluoride"[All Fields]) OR "essential oils"[All Fields] OR ("cyclodextrine"[All Fields] OR "cyclodextrines"[All Fields] OR "cyclodextrins"[MeSH Terms] OR "cyclodextrins"[All Fields] OR "cyclodextrin"[All Fields]) OR "citrox"[All Fields]) AND ("sars cov 2"[MeSH Terms] OR "sars cov 2"[All Fields] OR "sars cov 2"[All Fields] OR ("coronavirus"[MeSH Terms] OR "coronavirus"[All Fields] OR "coronaviruses"[All Fields]) OR ("covid 19"[All Fields] OR "covid 19"[MeSH Terms] OR "covid 19 vaccines"[All Fields] OR "covid 19 vaccines"[MeSH Terms] OR "covid 19 serotherapy"[All Fields] OR "covid 19 nucleic acid testing"[All Fields] OR "covid 19 nucleic acid testing"[MeSH Terms] OR "covid 19 serological testing"[All Fields] OR "covid 19 serological testing"[MeSH Terms] OR "covid 19 testing"[All Fields] OR "covid 19 testing"[MeSH Terms] OR "sars cov 2"[All Fields] OR "sars cov 2"[MeSH Terms] OR "severe acute respiratory syndrome coronavirus 2"[All Fields] OR "ncov"[All Fields] OR "2019 ncov"[All Fields] OR (("coronavirus"[MeSH Terms] OR "coronavirus"[All Fields] OR "cov"[All Fields]) AND 2019/11/01:3000/12/31[Date - Publication]))))) AND (2019:2023[pdat])
Scopus	**TITLE-ABS-KEY ((mouthwash OR mouthrinse OR chlorhexidine OR "cetylpyridinium chloride" OR "povidone iodine" OR "hydrogen peroxide" OR delmopinol OR listerine OR "essential oils" OR cyclodextrin OR citrox) AND (sars-cov-2 OR coronavirus OR covid-19)) AND PUBYEAR > 2018 AND PUBYEAR > 2018**
Cochrane	(mouthwash OR mouthrinse OR chlorhexidine OR cetylpyridinium chloride OR povidone iodine OR hydrogen peroxide OR delmopinol OR Listerine OR essential oils OR cyclodextrin OR citrox) AND (SARS-CoV-2 OR coronavirus OR COVID-19) in Title Abstract Keyword - with Publication Year from 2019 to 2023, with Cochrane Library publication date Between Dec 2019 and Jun 2023, in Trials

Table 1
Structured PICOS.

P (Population)	Oropharyngeal fluids from COVID-19 positive patients or surrogates	COVID-19 positive patients
I (Intervention)	Exposure to oral antiseptics	Rinsing with oral antiseptics
C (Comparison)	Control solutions	Control solutions
O (Outcome)	SARS-CoV-2 load reduction in the samples	SARS-CoV-2 load reduction in the saliva
S (Study)	*In vitro* studies	Human clinical studies

(passive drool), utilized at least one of the most popular mouthwashes (i.e. CHX, CPC, H_2O_2, PVP-I), and a non-active control.

Exclusion criteria were: animal studies, review, case report and case series, investigation/use of other viruses (e.g. MERS, SARS-CoV-1, influenza virus) or pseudoviruses, less than 15 included patients (for clinical studies), oral/nasal spray and oral gel formulations. The same mouthwash at a different concentration or applied for a different exposure time was not alone considered as a control.

For each included study, relevant data were extracted and recorded on two previously outlined data collection tables for clinical and in vitro studies, respectively.

2.3. Risk of bias assessment

The risk of bias of the included studies was assessed independently and in duplicate by two reviewers (L.Sb. and L.Sc.). Disagreements between the reviewers were solved by discussion. The risk of bias of in vitro studies was assessed by using the Toxicological data Reliability assessment Tool (ToxRTool) [20], comprising 18 criteria.

For RCTs, the revised Cochrane risk-of-bias tool for randomised trials (RoB 2), structured in five bias domains was used [21]. An algorithm estimated the overall risk of the bias according to the results obtained for each domain, i.e. low risk, some concerns, or high risk.

2.4. Network meta-analysis

Network meta-analysis was performed on the included clinical trials reporting on Δ_{Ct} values from saliva samples calculated as the mean difference between immediate (T1; within 15 min post rinsing) follow-up and baseline (T0). Cycle threshold (Ct) values from quantitative reverse transcription polymerase chain reaction (RT-PCR) are commonly used to represent viral load [22]. In case that data were not available, standard errors were approximated using the R-package Metafor [23], starting from mean Δ_{Ct} or mean T0 and T1 and standard deviation values, if given.

Statistical heterogeneity among clinical trials was assessed using the Q test [24] as well as the I^2 index [25] and tau^2 [24]. Network meta-analysis was performed using the Netmeta-package [26] using a random effects model, and based on standardized mean differences (SMDs) and standard errors (SE). Non-active control groups were considered to be the reference. A random effects model was used [24] to account for methodological differences among the studies, and SMDs and 95% confidence intervals (CIs) were computed. Forest and Funnell Plots were created. Statistical significance was defined as a p value < 0.05.

3. Results

Details on the adherence to the updated PRISMA guidelines [18] are presented in **Attachment 1.**

3.1. Study selection process

From the electronic search, 2782 articles were retrieved. After the removal of the duplicates, 1957 titles and abstracts were reviewed. After the first screening phase, 95 studies were considered relevant for the present review (inter-examiner agreement $k = 0.83$). Following full-text reading, 14 clinical and 21 in vitro studies fulfilled the inclusion criteria (inter-examiner agreement $k = 0.93$). Out of these, three clinical studies were eligible for quantitative analysis [27–29]. The reasons for exclusion after full-text screening are reported in Table 3 [30–87].

A flowchart summarizing the selection process is presented in Fig. 1.

3.2. Main results from clinical studies

A summary of the main features of the included clinical studies is reported in Table 4**.** All the included studies were RCTs comparing the use of at least one mouthwash with a non-active control solution. The most frequently investigated mouthwash was found to be chlorhexidine (CHX) at concentration of 0.12% [27,88–93] or 0.20% [28,29,93]. In one study the sequential use of hydrogen peroxide (H_2O_2) 1.5% followed by CHX 0.12% was tested [88]. Mouthwashes containing as active ingredients H_2O_2 [88–92,94,95], povidone-iodine (PVP-I) [28,29,89–92,94,95], cetylpyridinium chloride (CPC) [29,90–92,95–97], CPC in association with zinc lactate (Zn) [88], β-cyclodextrin and Citrox® (CDCM) [98], hypochlorous acid (HOCl) [95], Listerine® [90] and cymenol (Cym) + Zinc chloride ($ZnCl_2$) [93] were also studied.

Table 3
Details on the electronic search strategy.

Reason	Main reason for exclusion	Number	References
1.	Nasal spray(s) alone or in combination with mouthwash(es)	8	[30,31,33,34,40,41,59,64]
2.	Study type (in silico, animal studies, case report/series, non-randomized clinical trials, review, no experimental data)	14	[42,46–48,54,60,62,63,68,71,82,85,86,125]
3.	Off-topic	4	[32,35,45,49]
4.	No longitudinal data on SARS-CoV-2 viral load in human oropharyngeal fluids and surrogates	11	[54,56–58,66–68,70,80,83,87]
5.	Investigation/use of viruses or pseudoviruses different from SARS-CoV-2	5	[50–52,77,81]
6.	Absence of a control group (as defined in the "Materials and methods" section)	4	[37–39,65]
7.	Missing details on mouthwash formulation, concentration and/or application time	10	[36,44,69,72–76,78,85]
8.	Language	1	[43]
9.	Sample Size < 15 patients	3	[53,79,84]

In all the selected studies a rinsing time of 30 or 60 s was adopted. A longer rinsing time was reported only for the combination of H_2O_2 (60 s) and CHX (30 s) [88] and for all the mouthwashes investigated by Farmaha et al. (120 s) [90]. The study design always involved a single rinse except for one study, in which the mouthwash was utilized 3 times daily for 7 days [98]. Saliva samples were collected at least once within one hour in all studies but two [97,98]. As reported in Table 4, the most used saliva collection method was passive drool. All the included studies evaluated the presence of SARS-CoV-2 by RT-PCR. Active viral replication assessment [92,93] and enzyme-linked immunosorbent assay (ELISA) [96,97] were also performed.

CHX 0.12% was found to be effective in reducing saliva viral load both after 5 and 60 min as compared to a placebo solution [27]. Similar results were observed in another study, at 60 and 120 min after rinsing [90], while CHX at the same concentration did not significantly reduced viral load in other investigations [91,92]. In another RCT, in which multiple mouthwashes were tested, CHX 0.12% met minimum acceptance criteria of a $\geq$ 2-fold reduction compared to baseline, which was significant at 30 and 60 min, but not at the early time point [88]. However, the best performances were achieved with H_2O_2 1.5% as well as with CPC-Zn mouthwashes, especially within the first 30 min. The sequential use of H_2O_2 and CHX did not lead to a higher efficacy as compared with the two active ingredients alone [88]. In symptomatic patients no significant differences in saliva viral load reduction among the four different solutions (i.e. CHX 0.12%; H_2O_2 1%; PVP-I 0.5%; saline) were detected after both 15 and 45 min from the rinse [89]. CHX 0.2% and PVP-I 1% were found to be more effective than the non-active compound at 5 min, however no statistically significant difference was found between the two products [28]. After a single administration, PVP-I 0.5% as well as CPC 0.075% exhibited sustained effects in reducing viral load compared to the non-active control for up to six hours [29]. CPC at a similar concentration was found not to be effective

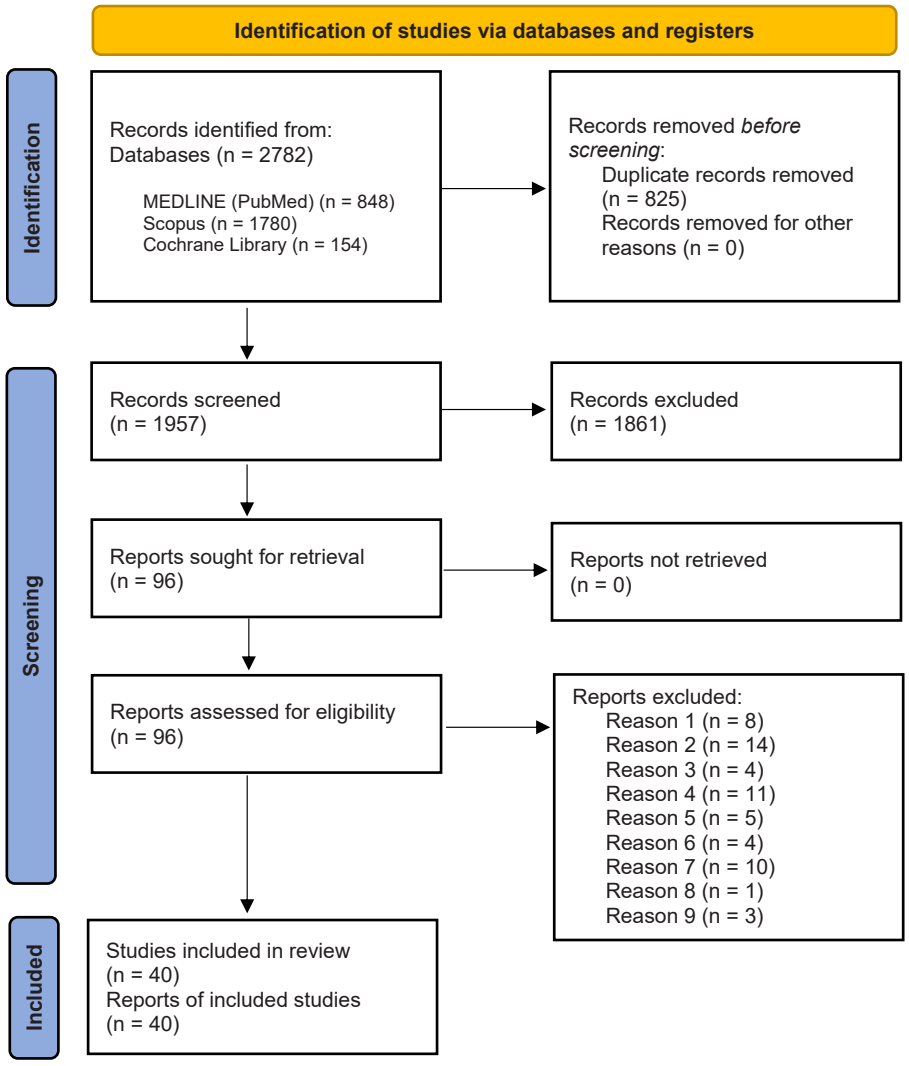

Fig. 1. Flowchart of the selection procedure according to PRISMA 2020 guidelines [18].

in reducing saliva Ct values 2 h after rinsing. However, at the same timepoint, ELISA test showed a significantly higher N protein concentration in the test group compared to the non-active control, indicating an increase in lysed virus [97].

When viral culture was performed, mouthwashes showed modest capacity to reduce viral infectivity in vivo, with viral inactivation detected only in the CPC group one hour after rinsing [92]. Discording results were found concerning CHX 0.12%: while no significant viral inactivation activity was detected in Sánchez Barrueco et al. [92], in another study of the same group the remaining viruses were mostly viable, despite the high viral load reduction detected with RT-PCR [93].

3.3. Main results from in vitro studies

The main features of the included in vitro studies are presented in Table 5. The most commonly test product was CHX alone [99–106] or in combination with other active ingredients, such as CPC [100,101], CPC + F- [100] or ethanol [39,105]. The in vitro behaviour of PVP-I at different dilutions was investigated in 10 articles [99,104–112], followed by H_2O_2 in six articles [100,104–106,109,113]. Other test solutions include: CPC [39,100,101,112,113], CPC + sodium fluoride (F-) [102], CPC + H_2O_2 [100], silver nanoparticles (AgNPs) [114], anionic phthalocyanine derivate (APD) [46,115], octenidine dihydrochloride (OCT) [103], Listerine® Original [106], Listerine® Cool Mint [104], Dequonal® [104], Delmopinol hydrochloride [101], dipotassium oxalate [105], hypochlorous acid [105], thymol [102], Bactidol® [102], SP_T medical gargle [116], and hexadecyl pyridinium chloride [117].

Mouthwash effectiveness was assessed by plaque assay in all studies but three [99,106,115]. The other reported assessment methods were fluorescent assay [106,110,115,117], RT-PCR [99,110,115–117], western blotting [116], and transmission electron microscopy (TEM) [116]. Except for three studies [106,114,115], at least one of the investigated exposure time was comprised between 15 s and 1 min, which is compatible with preprocedural mouth rinsing in dental settings. Contact times above 5 min and up to 72 h were reported in a minority of the studies [103,106,114,115].

A limited efficacy of CHX 0.12% for 30 s was reported in Komine et al. [101], while in combination with CPC presented a high reduction in viral titer comparable to other tested solutions. Even a longer exposure (up to 5 min) of a higher concentration of CHX (0.16%) was found to be of limited efficacy against SARS-CoV-2 [103]. In the same study, favorable results were obtained by OCT, which met the viral titer reduction of $> 4\ \mathrm{Log}_{10}$, required by the European Standard 14476 [118], within a contact time of only 15 s [103]. These findings are in line with what reported by Meister et al. [104], in which three SARS-CoV-2 strains were not highly susceptible to two different commercially available CHX 0.2% mouthwashes, while a significantly reduced viral infectivity was noticed with Dequonal®, PVP-I and Listerine® Cool Mint [104]. By contrast, in Jain et al. [99], CHX 0.2% performed better than PVP-I at the same concentration tested in the former study [104]. As in other

Table 4
Main features of clinical studies.

Ref.	Test (type, concentration, time, N patients)	Non-active control (N patients)	Collection time points	Diagnosis methods (PCR if not specified)	Included N. patient (recruited / virus detected in saliva)	Saliva sample and quantity (mL)	Quantitative analysis on viral reduction (RT-PCR)	Main results
Adl et al., 2023[94]	1. 10 mL H_2O_2 1% (N = 40; 30 s) 2. 10 mL PVP-I 0.25% (N = 40; 30 s)	10 mL saline (N = 40; 30 s)	1. Before mouthwash (T0) 2. 10 min (T1)	Nasal swab	120/53	Spitting (2 mL)	**Δ (T1-T0) viral load (copy/mL)** 1. H_2O_2: 1462850 ± 4884893 2. PVP-I: − 292694 ± 6728707 3. Saline: − 649266 ± 4519460	Among 53 positive patients, 50 were outpatient, while 3 hospitalized. All three mouthwashes reduced the viral load after gargling, but not in a statistically significant way ($p > 0.05$). No significant differences were observed between the three groups ($p = 0.23$)
Alemany et al., 2022[96]	15 mL CPC 0.07% (N = 60; 60 s)	15 mL distilled water with same colorant as test product (N = 58; 60 s)	1. Before mouthwash (T0) 2. 60 min (T1) 3. 180 min (T2)	Not reported	118/105	Saliva passive drool (1–1.5 mL)	**Viral load (copy/mL) mean (SD)** 1. Control: T0: 7.29 (2.18); T1: 7.74 (1.77); T2: 7.66 (1.59) 2. CPC: T0: 6.82 (2.13); T1: 7.55 (1.93); T2: 7.09 (1.75) **Nucleocapsid proteins (pg/mL) mean (SD)** 1. Control: T0: 103.71 (226.41); T1: 160.61 (432.37); T2: 89.14 (248.36) 2. CPC: T0: 237.32 (590.40); T1: 483.60 (769.79); T2: 631.22 (801.87)	No significant difference in viral load between the two groups at 1 h and at 3 h. The level of nucleocapsid protein were significantly higher in CPC group compared with placebo at 1 h and 3 h.
Alzahrani et al., 2023[95]	1. 15 mL PVP-I 1% (N = 15; 30 s) 2. 15 mL H_2O_2 1.5% (N = 15; 30 s) 3. 15 mL CPC 0.075% (N = 15; 30 s) 4. 15 mL HOCl 80 ppm (N = 15; 30 s)	1. 15 mL distilled water (N = 10; 30 s) 2. no-rinse	1. Before mouthwash (T0) 2. 5 min (T1) 3. 30 min (T2) 4. 60 min (T3)	Nasopharyngeal or throat swab	241/55	Saliva passive drool (≥2 mL)	**Mean (SD) viral load (copy/mL)** 1. PVP-I: T0: 7.65 × 109 (6.35 ×109); T1: 1.89 × 1010 (4.52 ×1010); T2: 2.99 × 109 (2.59 ×109); T3: 1.05 × 1010 (2.46 ×1010) 2. H_2O_2: T0: 4.64 × 1010 (8.11 ×1010); T1: 1.59 × 109 (2.76 ×109); T2: 1.34 × 109 (2.18 ×109); T3: 1.63 × 109 (2.31 ×109) 3. CPC: T0: 1.27 × 1010 (2.76 ×1010); T1: 1.34 × 1010 (3.72 ×1010); T2: 1.18 × 1010 (3.57 ×1010); T3: 2.18 × 109 (3.27 ×109)	Salivary viral load reduction over time. Significant reduction at each timepoint (T1, T2, T3) only for H_2O_2, when compared to baseline ($p = 0.0478$, $p = 0.0402$; $p = 0.0485$, respectively). The effect of PVP-I, H_2O_2 and CPC mouth rinses on salivary viral load reduction was significant compared to the no-rinse group at 60 min. Distilled water also showed a significant decrease in viral load compared to the no-rinse group ($P = 0.011$)

(*continued on next page*)

Table 4 (*continued*)

Ref.	Test (type, concentration, time, N patients)	Non-active control (N patients)	Collection time points	Diagnosis methods (PCR if not specified)	Included N. patient (recruited / virus detected in saliva)	Saliva sample and quantity (mL)	Quantitative analysis on viral reduction (RT-PCR)	Main results
							4. HOCl: T0:1.23 × 1010 (3.37 ×1010); T1: 2.17 × 1010 (5.58 ×1010); T2: 1.21 × 109 (1.74 ×109); T3: 7.32 × 1010 (1.6 ×1011) 5. Distilled water: T0: 4.16 × 109 (6.25 ×109); T1: 4.38 × 109 (6.14 ×109); T2: 4.22 × 109 (6.04 ×109); T3: 2.08 × 109 (3.82 ×109) 6. No rinse: T0: 3.42 × 1010 (9.87 ×1010); T1: 9.63 × 1010 (1.97 ×1011); T2: 1.02 × 1010 (2.10 ×1010); T3: 1.44 × 1011 (3.08 ×1011)	
Carrouel et al., 2021 [98]	30 mL β-cyclodextrin 0.1% and Citrox® 0.01% (CDCM group) (N = 88; 60 s 3 times a day for 6 days, i.e. at 9 am, 2 pm and 7 pm)	30 mL placebo (N = 88; 60 s)	The first day: 1. before mouthwash (at 9 am) (T0) 2. at 1 pm (T1) 3. at 6 pm (T2) The following 6 days: 1 sample per day at 3 pm	Nasopharyngeal swab	176/176	Saliva passive drool (2 mL)	**Median SARS-CoV-2 IQR (Log10 copies/ mL of saliva)** Day 1 T0: CDCM: 4.05 (2.94–4.96); placebo: 3.85 (2.97–5.08) Day 1 T1: CDCM: 3.33 (2.29–4.23); placebo: 3.60 (2.07–4.83) Day 1 T2: CDCM: 3.08 (0–4.19); placebo: 3.31 (1.18–4.75) Day 7: CDCM: 0 (0–1.34); placebo: 1.62 (0–1.70)	The first day: 1. Median viral load lower in CDCM than in placebo group at T1 and T2. 2. There was a significant difference in viral load reduction in the before-after comparison within the same pts receiving CDCM versus no difference for the placebo group from T0 to T1 ($p = 0.036$). The percentage median decrease was − 12.58% for CDCM versus − 6.74% for placebo. 3. At T2, significant salivary viral load reduction in both groups compared with T0 (CDCM: $p < 0.001$; placebo: $p = 0.002$). 4. After 7 days: 5. Viral load constantly decreased in both groups till day 7. At day 7, no significant difference in viral load between CDCM and placebo ($p = 0.388$). 6. In both groups significantly lower viral load at day 7 compared to T0 ($p < 0.001$).

(*continued on next page*)

Table 4 (*continued*)

Ref.	Test (type, concentration, time, N patients)	Non-active control (N patients)	Collection time points	Diagnosis methods (PCR if not specified)	Included N. patient (recruited / virus detected in saliva)	Saliva sample and quantity (mL)	Quantitative analysis on viral reduction (RT-PCR)	Main results
Chaudhary et al., 2021 [89]	1. 15 mL H_2O_2 1% (N = 10; 60 s) 2. 15 mL CHX 0.12% (N = 10; 60 s) 3. 15 mL PVP-I 0.5% (N = 10; 60 s)	15 mL saline (N = 10; 60 s)	1. Before mouthwash (T0) 2. 15 min (T1) 3. 45 min (T2)	Not reported	201/82§§	Saliva passive drool	**Median reduction in viral load compared to T0 (%):** 1. CHX: T1: 89%; T2: 70% 2. PVP-I: T1: 61%; T2: 97% 3. H_2O_2: T1: 68%; T2: 72% 4. Saline: T1: 87%; T2: 87%	1. Significantly lower frequency of SARS-CoV-2 detection and viral load in the saliva in asymptomatic and presymptomatic pts than in symptomatic or postsymptomatic ones (p = 0.001, $\chi 2$ test and p = 0.0007, Dunn test with joint ranking, respectively). 2. All 4 solutions reduced SARS-CoV-2 salivary carriage; median reduction ranging from 61% through 89% (mean: 25–74%) at 15 min and from 70% through 97% at 45 min (mean: 30–43%). 3. No differences among the solutions in 15-min reduction in viral load and persistence of reduction at 45 min (p > 0.05). 4. Significant correlation between baseline viral load and reduction at 15 min (p = 0.0073, Spearman rank correlation) and persistence at 45 min (p = 0.0087, Spearman rank correlation). 5. In all pts with baseline viral load < 104 copies/mL of saliva, 100% reduction in viral load both at 15 and 45 min
Costa et al., 2021 [27]	1. 15 mL CHX 0.12% (N = 50; 60 s)§	15 mL Placebo (N = 50; 60 s)§	1. Before mouthwash (T0) 2. 5 min (T1) 3. 60 min (T2)	Nasopharyngeal swab (rapid antigen test)	110/100	Saliva passive drool (1.5 mL)	**Δ_{Ct} (T1-T0):** test: 2.19 ± 4.30; control: − 0.40 ± 3.87 (p = 0.002) **Δ_{Ct} (T2-T0):** test: 2.45 ± 3.88; control: 0.76 ± 4.41 (p = 0.05) **Δ_{Ct} (T2-T1):** test: 0.26 ± 4.16; control: 1.16 ± 4.47 (p = 0.30)	1. Significantly greater Ct reduction in the test group compared to control both at T1 and T2 compared to T0. 2. In the test group, no difference in Ct between T1 and T2 (p = 0.30). 3. Significantly greater proportion of cases with a reduction in viral load in the test group at T1 (test group: 72%; control group: 40%, p = 0.001), while no significant difference at T2 (test group: 76%; control

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Table 4 (*continued*)

Ref.	Test (type, concentration, time, N patients)	Non-active control (N patients)	Collection time points	Diagnosis methods (PCR if not specified)	Included N. patient (recruited / virus detected in saliva)	Saliva sample and quantity (mL)	Quantitative analysis on viral reduction (RT-PCR)	Main results
								group: 60%, $p = 0.09$). 4. No association between reduction in viral load and demographic factors (i.e., cigarette smoking, health status or COVID-19 symptoms) ($p \leq 0.07$).
Eduardo et al., 2021 [88]	1. 20 mL CPC 0.075% + Zn 0.28% (N = 7; 30 s) 2. 10 mL H_2O_2 1.5% (N = 7; 60 s) 3. 15 mL CHX 0.12% (N = 8; 30 s) 4. 10 mL of H_2O_2 1.5% + 15 mL CHX 0.12% (N = 12; 60 s H_2O_2 + 30 s CHX)	20 mL distilled water (N = 9; 60 s)	1. Before mouthwash (T0) 2. Immediately after mouthwash (T1) 3. 30 min (T2) 4. 60 min (T3)	Nasal swab	60/43	Saliva passive drool	**Fold reduction relative to T0:** 1. CPC+Zn: T1: 20.4 ± 3.7; T2: 4.5 ± 0.6; T3: 2.6 ± 0.1 2. H_2O_2: T1: 15.8 ± 0.08; T2: 6.5 ± 3.4; T3: 0.3 ± 1.3 3. CHX: T1: 2.1 ± 1.5; T2: 6.2 ± 3.8; T3: 4.2 ± 2.4 4. H2O2 +CHX: T1: 2.1 ± 0.5; T2: 1.6 ± 0.2; T3: 3.9 ± 0.3	1. Compared to baseline, significant difference in Ct values after treatment: in CPC+Zn group at T1 ($p = 0.004$); in H_2O_2 group at T1 ($p < 0.001$), T2 ($p = 0.016$), and T3 ($p = 0.006$); in CHX group at T2 ($p < 0.001$) and T3 ($p = 0.013$); in H2O2 +CHX group at T1 ($p = 0.004$). 2. H_2O_2 and CPC+Zn mouthwash exhibited higher Ct levels at T1 relative to T0, T2, and T3 while the control and H_2O_2 +CHX groups showed minor changes in Ct level between the timepoints. 3. Based on ΔΔCt values, all treatment groups were observed to have 2-fold or greater viral bioload reduction in saliva immediately after use. 4. Rinsing with H_2O_2 mouthwash or CPC+Zn mouthwash showed the best reduction in viral load at T1. H_2O_2 had a significant viral load reduction only up to 30 min after rinsing. The CHX mouthwash results met the minimum acceptance criteria of a ≥ 2-fold reduction to show its effectiveness in viral load reduction at T1, T2, and T3. The sequential use of the H2O2 mouthwash followed by CHX did not lead to an increase in viral reduction efficacy at T2 (1.6 ± 0.2).

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Table 4 (*continued*)

Ref.	Test (type, concentration, time, N patients)	Non-active control (N patients)	Collection time points	Diagnosis methods (PCR if not specified)	Included N. patient (recruited / virus detected in saliva)	Saliva sample and quantity (mL)	Quantitative analysis on viral reduction (RT-PCR)	Main results
Elzein et al., 2021[28]	1. 15 mL CHX 0.2% (N = 27; 30 s) 2. 15 mL PVP-I 1% (N = 25; 30 s)	15 mL distilled water (N = 9; 30 s)	1. Before mouthwash (T0) 2. 5 min (T1)	Nasopharyngeal swab	70/61	Saliva caught out from throat (2 mL)	**Δ_{Ct} value T1-T0** 1. CHX: 6.37 ± 1.08 2. PVP-I: 4.72 ± 0.89 3. Distilled water: 0.519 ± 0.519	1. Statistically significant difference between ΔCt in distilled water group and both PVP-I and CHX ($p = 0.012$ and $p = 0.0024$, respectively). 2. No significant difference in ΔCt between PVP-I and CHX ($p = 0.24$). 3. Significant mean Ct difference between the paired samples before and after mouthwash with both PVP-I ($p < 0.0001$) and CHX ($p < 0.0001$).
Farmaha et al., 2023[90]	1. 5 mL CHX 0.12% (N = 6; 120 s) 2. 5 mL H_2O_2 1.5% (N = 5; 120 s) 3. 5 mL PVP-I 1% (N = 8; 120 s) 4. 5 mL Listerine® (N = 7; 120 s)	5 mL water (N = 6; 120 s)	1. Before mouthwash (T0) 2. Immediately after (T1) 3. 60 min (T2) 4. 120 min (T3)	Nasopharyngeal swab or saliva sample	410/32	Spitting (~1 mL)	**Mean Ct values** 1. CHX: T0: ~32; T1: ~35; T2: ~36; T3: ~35 2. H_2O_2: T0: ~27; T1: ~32; T2: ~32; T3: ~31 3. PVP-I: T0: ~30; T1: ~32; T2: ~30; T3: ~29 4. Listerine®: T0: ~35; T1: ~38; T2: ~37; T3: ~36 5. Water: T0: ~28; T1: ~29; T2: ~30; T3: ~27	Compared to control, a significant increase in Ct values was observed immediately after rinsing with each of the mouthwashes testes ($p < 0.05$). The Ct values for the Listerine reached the statistical significance cut-off compared to H_2O_2 and PVP-I; a statistically significant difference in Ct values was also observed between CHX group and Listerine group compared to the water group one and two hours after rinse.
Ferrer et al., 2021[91]	1. PVP-I 2% (N = 15; 60 s) 2. H_2O_2 1% (N = 14; 60 s) 3. CPC 0.07% (N = 13; 60 s) 4. CHX 0.12% (N = 12; 60 s)	Distilled water (N = 13; 60 s)	1. Before mouthwash (T0) 2. 30 min (T1) 3. 60 min (T2) 4. 120 min T3)	Nasopharyngeal swab	98/67	Saliva passive drool (≥0.5 mL)	**Median viral load (Log copies/mL)** 1. PVP-I: T0: ~3; T1: ~2.8; T2: ~3; T3: ~2.2 2. H_2O_2: T0: ~3.2; T1: ~3.1; T2: ~3; T3: ~2.8 3. CPC: T0: ~3.9; T1: ~3.7; T2: ~4; T3: ~3.8 4. CHX: T0: ~3.7; T1: ~4; T2: ~3; T3: ~3.1 5. Water: T0: ~4; T1: ~5.1; T2: ~4.1; T3: ~3.1	None of the tested mouthwashes significantly reduced viral load at any timepoint compared with baseline. Compared to control, a significant increase in Ct values was observed immediately after rinsing with each of the tested mouthwashes ($p < 0.05$). The Ct values for the Listerine reached the statistical significance cut-off compared to H_2O_2 and PVP-I.
Sánchez Barrueco et al., 2022[92]	1. 15 mL PVP-I 2% (N = 9; 60 s) 2. 15 mL H_2O_2 1% (N = 6; 60 s) 3. 15 mL CPC 0.07% (N = 10; 60 s) 4. 15 mL CHX 0.12% (N = 9; 60 s)	15 mL distilled water (N = 10; 60 s)	1. Before mouthwash (T0) 2. 30 min (T1) 3. 60 min (T2)	Nasopharyngeal swab	75/44	Saliva passive drool (≥2 mL)	**Median viral load (Log copies/mL)** 1. PVP-I: T0: ~4.9; T1: ~5; T2: ~5.1 2. H_2O_2: T0: ~5; T1: ~5; T2: ~4 3. CPC: T0: ~5; T1: ~4; T2: ~5 4. CHX: T0: ~4; T1: ~5.2; T2: ~5 Water: T0: ~6; T1: ~5.1; T2: ~5	None of the mouthwashes reduced the saliva viral load, either at 30 min after rinsing or at 1 h. Unexpectedly, a significant decrease in the mean values of viral load in saliva was detected 1 h after rinsing with water ($p = 0.05$).

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Table 4 (*continued*)

Ref.	Test (type, concentration, time, N patients)	Non-active control (N patients)	Collection time points	Diagnosis methods (PCR if not specified)	Included N. patient (recruited / virus detected in saliva)	Saliva sample and quantity (mL)	Quantitative analysis on viral reduction (RT-PCR)	Main results
Sánchez Barrueco et al., 2023[93]	1. 15 mL CHX 0.12% (N = 10; 60 s) 2. 15 mL CHX 0.2% (N = 10;60 s) 3. 15 mL Cym + ZnCl2 (N = 11;60 s)	15 mL placebo (N = 12; 60 s)	1. Before mouthwash (T0) 2. 5 min (T1) 3. 15 min (T2) 4. 60 min (T3)	Nasopharyngeal swab	48/43	Saliva passive drool (≥2 mL)	**Median viral load (Log copies/mL)** 1. CHX 0.12%: T0: ~7; T1: ~6; T2: ~5; T3: ~5 2. CHX 0.20%: T0: ~5; T1: ~6; T2: ~5; T3: ~4 3. Cym + ZnCl2: T0: ~5; T1: ~4.6; T2: ~4.2; T3: ~3.9 4. Placebo: T0: ~3.9; T1: ~4; T2: ~3.8; T3: ~3.9	Both in CHX 0.12% and in Cym+$ZnCl_2$, a progressive decrease in salivary viral load was observed over time, being significant with respect to the baseline from 15 min after CHX 0.12% ($p = 0.037$) and with a trend towards significance in Cym+$ZnCl_2$ ($p = 0.054$). In addition, the values were significantly different at 1 h after the mouthwash in both groups (CHX 0.12% group: $p = 0.02$; Cym+$ZnCl_2$ group: $p = 0.04$). In contrast, in both the placebo and CHX 0.2% group, no differences were observed between time-points.
Seneviratne et al., 2021[29]	1. 10 mL PVP-I 0.5% w/v (N = 4; 30 s) 2. 15 mL CHX 0.2% w/v (N = 6; 30 s) 3. 20 mL CPC 0.075% (N = 4; 30 s)	15 mL water (N = 2: 30 s)	1. Before mouthwash (T0) 2. 5 min (T1) 3. 3 h (T2) 4. 6 h (T3)	Nasal swab	36/16	Saliva passive drool (3 mL)	**Fold reduction relative to T0:** 1. PVP-I: T1: 1.1; T2: 1.2; T3: 1 2. CHX: T1: 0.9; T2: 1; T3: 0.9 3. CPC: T1: 1; T2:0.9; T3: 0.9 4. Water: T1: 0.9; T2: 0.8; T3: 0.7	1. All Ct values ranged between 15.6 and 34.5 (mean value 27.7 ± 4.8). 2. No statistical differences in the Ct values were found with regards to any timepoints in all the groups. 3. A statistically significant increase in Ct value fold change at 5 min and 6 h after rinsing with CPC compared to water ($p < 0.05$). 4. In PVP-I group higher fold changes in Ct values 5 min and 3 h post-rinsing compared to water group, but statistically significant increase in fold change only at 6 h ($p < 0.01$). 5. Decreasing salivary load with CPC and PVP-I sustained at 3 h and 6 h compared to control. 6. CHX group pts showed a varied effect among saliva Ct values after 5-min rinsing, while the trends at 3 h and 6 h post-rinsing with CHX were consistent with the other mouthwashes.
Tarrago-Gil et al., 2023[97]	15 mL CPC 0.07% (N = 39; 60 s)	15 mL placebo	1. Before mouthwash (T0)	Nasal swab (rapid antigen test)	80/80	Saliva passive drool	**Mean (SD) Ct values**	**RT-qPCR:** Ct values demonstrated no statistically significant

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Table 4 (*continued*)

Ref.	Test (type, concentration, time, N patients)	Non-active control (N patients)	Collection time points	Diagnosis methods (PCR if not specified)	Included N. patient (recruited / virus detected in saliva)	Saliva sample and quantity (mL)	Quantitative analysis on viral reduction (RT-PCR)	Main results
		(N = 40; 60 s)	2. - 120 min (T1)				1. ORF1ab for CPC: T0: 22.56 (3.85); T1: 22.59 (3.19) 2. ORF1ab for Placebo: T0: 22.82 (5.04); T1: 22.71 (5.08) 3. N gene for CPC: T0: 25.27 (3.63); T1: 25.30 (3.00) 4. N gene for placebo: T0: 26.45 (3.44); T1: 26.22 (3.37)	difference before and after 2 h. **ELISA:** The protein N concentration 2 h after rinsing was significantly higher in the test group than in the placebo group ($p = 0.038$).

CHX: chlorhexidine; CPC: cetylpyridinium chloride; Cym: cymenol; H_2O_2: hydrogen peroxide; HOCl: hypochlorous acid; IQR: interquartile range; NA: not applicable; Zn: zinc lactate; pt: patient; PVP-I: povidone-iodine; § 15 mL gargling for 30 s + 15 mL rinsing for 30 s; §§ out of 82, only the 40 symptomatic patients were included (of these 1 dropped out).

works [104,105], a limited virucidal effect was reported for both CHX and H_2O_2, while CPC alone, or combined with other active ingredients (i.e·H_2O_2, CHX and CHX+F-) showed a higher inactivation of SARS-CoV-2 [100]. Also in Tiong et al. [102] CPC in combination with F- performed better than CHX in vitro. Indeed, in that study a decrease of SARS-CoV-2 load above 99.99% compared to the control was detected with both CPC + F- and Bactidrol® after a contact time of 30 s and 60 s. CPC 0.07% alone, after a contact time of 30 s, demonstrated strong virucidal activity in vitro [113]. When tested at different concentrations and timepoints, it showed a dose- and time-dependent antiviral activity [112]·H_2O_2 at both 1.5% and 3% exhibited minimal virucidal effect after a contact time of 15 s and 30 s. Whereas, at the same time points, complete SARS-CoV-2 inactivation was found with different concentrations of PVP-I, which was comparable to that obtained with ethanol 70% [109]. The in vitro effectiveness of PVP-I was confirmed by other studies using the same assessment method, i.e. plaque assay, and a maximum exposure time of one minute [105,107,108]. The antiviral efficacy of PVP-I was also confirmed by other studies [111,112]. At fluorescent assay, PVP-I 1% and H_2O_2 1.5% showed high antiviral activity, which was however associated with high cytotoxicity also at a dilution of 0.1% and 0.05%, respectively [106]. Despite less effective, Listerine® Original and CHX 0.12% presented no cytotoxic effect [106].

APD was tested in two studies with encouraging results [46,115]. It was found to induce a viral inactivation of 90% independently of the exposure time (i.e. 30 s, 1 min, 5 min) [46]. Moreover, when the viral load reduction was assessed by RT-PCR, a significant decrease was found with ADP 2 mg/mL at a dilution in the range between 1:2 and 1:16 as compared to the non-active control [115].

Other products worth mentioning are dipotassium oxalate and hypochlorous acid, which were effective at inactivating SARS-CoV-2 after a contact time of 1 min [105]. Finally, a long exposure to AgNPs was found to control viral infectivity in vitro, despite viral production was not completely abolished [114].

3.4. Study quality and risk of bias

The quality assessment of the included RCTs is reported in Fig. 2 created using robvis [119]. Two clinical studies presented an overall high risk of bias [29,88]. The main criticisms concerned the randomization process [28,29,88,90] and the deviation from intended intervention [29,88,90,94]. Eight clinical studies were considered at low risk of bias for every RoB2 parameter [27,91–93,95–98].

Risk of bias of the included in vitro studies is presented in Table 6. The toxRTool evaluation found 7 studies not meeting at least one "red criteria" [99,106,110,113–115,117], therefore they were considered not reliable. The remaining ten studies were considered reliable with scores ranging between fifteen and eighteen.

3.5. Network meta-analysis

Network meta-analysis was performed (Fig. 3) estimating the effect of active ingredients from included studies at different concentrations against non-active controls [27–29]. The investigated active products were CHX 0.12% [27], CHX 0.2% [28,29], CPC 0.075% [29], PVP-I 0.5% [29] and PVP-I 1% [28]. Rinsing time of studies included in the network meta-analysis amounted to either 30 s [28,29] or 60 s [27,29]. None of the studies included for quantitative analysis reported on the use of H_2O_2. As only studies reporting data at five minutes post rinsing were identified, the analysis was restricted to this time period. As none of the studies provided data on treatment effects and standard errors, these values were estimated using the Metafor package [23]. Additionally, two multi-arm studies had high variability among participants in the different groups, but lacked statistical comparison between the respective groups. Therefore, these values were estimated using the Metafor package [23].

The heterogeneity of the included studies was high. The I^2 amounted to 94.9 [91.4%– 97.0%], and tau^2 was 5.492. Q-statistics of heterogeneity (within design) amounted to 36.32 ($p < 0.001$) and inconsistency (between design) amounted to 62.19 ($p < 0.001$).

Δ_{Ct} (T1-T0) was found to be increased after rinsing with CHX 0.20% as compared to non-active controls ($p = 0.027$). Whereas, no significant difference was found between non-active controls and PVP-I 1% ($p = 0.087$), CHX 0.12% ($p = 0.789$), CPC 0.075% ($p = 0.612$), or PVP-I 0.5% ($p = 0.485$). A high confidence of interval was observed for PVP-I 1%, even though the SMD appeared promising. CPC alone at low concentration exhibited minor effect against non-active controls, while no data was available on the combination of CPC and CHX.

Funnel plot (Fig. 4) revealed no major asymmetry, giving no indication for publication bias. Egger's test confirmed these trends of publication biases ($p = 0.9702$).

4. Discussion

The present systematic review evaluated the effectiveness of preprocedural mouthwashes in reducing SARS-CoV-2 load. Clinical studies suggested the effectiveness of CHX 0.2% in reducing viral load after

Table 5
Main features of in vitro studies.

Ref.	Test (type, concentration, exposure time)	Control	SARS-CoV2 source	Assessment method	Initial viral concentration	Incubation temperature	Reduction in viral titer compared to non-active control	Main results
Almanza-Reyes et al., 2021 [114]	AgNPs at concentrations of 0.5–0.0004% (24 h; 48 h; 72 h)	Virus + culture medium	SARS-CoV-2 NL/2020 strain (BetaCoV/Netherlands/01)	Plaque assay	Serial two-fold dilutions from 1/2–1/2048	37 °C	**Percentages of infectivity:** 0.03% AgNPs reduces infectivity by 80%	Although AgNPs did not totally abolish viral production, infection was clearly controlled to some extent with a viral load reduction of about 80% at a concentration of 0.03%. A 50% inhibitory concentration was determined by curve fitting (non-linear regression).
Anderson et al., 2022 [126]	1. MW-A: CPC 0.07% + herbal extracts (30 s) 2. MW-B: CPC 0.07% with flavour (with and without saliva) (30 s) 3. MW-C: CHX digluconate 0.2% + ethanol 7% (30 s)	1. EtOH 70% (30 s) 2. Distilled water (30 s)	SARS-CoV-2 variants: 1. USA-WA1/2020 2. alpha: hCoV-19/England/204820464/2020 3. beta: hCoV-19/South Africa/KRISP- EC-K005321/2020 4. delta: SARS-CoV-2/human/GBR/Liv_273/2021, GenBank accession OK392641 5. gamma: hCoV-19/Japan/TY7–503/2021	Plaque assay	1/10 dilution starting from unknown concentration	37 °C	**Log_{10} PFU/mL reduction** 1. variant alpha: MW-A, MW-B and EtOH: 3.11 2. variant beta: MW-A, MW-B, EtOH: 4.11 3. variant gamma: MW-A, MW-B, EtOH: 3.36 4. variant delta: MW-A, MW-B, EtOH: 4.52	Mouthwashes containing CPC 0.07% effectively inactivated SARS-CoV-2 with greater than 4.0 Log_{10} PFU/mL reduction in viral titre. Virucidal activity of CPC was maintained in presence of human saliva. Both CPC 0.07% mouthwashes were as effective as ethanol 70% against four variants. The mouthwash containing CHX digluconate 0.2% did not have substantial action against SARS-CoV-2 in vitro.
Bidra et al., 2020[109]	1. H_2O_2 3% (15 s; 30 s) 2. H_2O_2 1.5% (15 s; 30 s) 3. PVP-I 1.5% (15 s; 30 s) 4. PVP-I 1.25% (15 s; 30 s) 5. PVP-I 0.5% (15 s; 30 s)	1. EtOH 70% (15 s; 30 s) 2. Water (15 s; 30 s)	SARS-CoV-2, USA-WA1/2020	Plaque assay	5.0 Log_{10} $CCID_{50}$/0.1 mL	22 ± 2 °C	**Log_{10} $CCID_{50}$/0.1 mL** 1. H_2O_2 3%: 15 s: ≤ 4.0%; 30 s: ≤ 2.5% 2. H_2O_2 1.5%: 15 s: ≤ 3.67%; 30 s: ≤ 3.33% 3. PVP-I: at all concentrations and timepoint < 0.67 4. EtOH: both after 15 s and 30 s < 0.67 5. Water: 15 s: 5.0; 30 s: 4.3	After 15 s all the three concentrations of PVP-I were equally effective in reducing the SARS-CoV-2 load compared to the water control·H_2O_2 both at 3% and 1.5% concentration showed minimal viricidal effect after 15 and 30 s. Ethanol inactivated the virus similarly to the PVP-I products.
Bidra et al., 2020[108]	1. PVP-I 1.5% (15 s; 30 s) 2. PVP-I 0.75% (15 s; 30 s) 3. PVP-I 0.5% (15 s; 30 s)	1. EtOH 70% (15 s; 30 s) 2. Water (15 s; 30 s)	(SARS-CoV-2) USA-WA1/2020	Plaque assay	5.0 Log_{10} $CCID_{50}$/0.1 mL	22 ± 2 °C	**Log_{10} $CCID_{50}$/0.1 mL** 1. PVP-I: at all concentrations and timepoint < 0.67 2. EtOH: 15 s: 1.5; 30 s: < 0.67 3. Water: 15 s: 3.67; 30 s: 4.0	After 15 s all the three concentrations of PVP-I were equally effective in reducing the SARS-CoV-2 load compared to the water control. EtOH 70% was unable to completely inactivate SARS-CoV-2 after 15 s but was able to inactivate the virus after 30 s. No cytotoxicity was observed with any of the test compounds.
Chen et al., 2022[117]	Hexadecyl pyridinium chloride (0.2, 0.1, 0.05, 0.025, or 0.0125 mg/mL) (30 s; 60 s; 120 s; 300 s)	Culture medium + test solution	SARS-CoV-2 (hCoV-19/Zhejiang/OS2/2020, GISAID, ID: 455692) isolated from a patient at the Zhejiang	1. Plaque assay 2. RT-PCR 3. Immunofluorescence	3×10^3 U/mL	35 °C	**Log $TCID_{50}$/mL reduction** 1. 0.025 and 0.0125: > 10 for each contact time	The disinfection effect of hexadecyl pyridinium chloride is time and concentration-dependent, with the strongest virus-elimination effect at a

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Table 5 (*continued*)

Ref.	Test (type, concentration, exposure time)	Control	SARS-CoV2 source	Assessment method	Initial viral concentration	Incubation temperature	Reduction in viral titer compared to non-active control	Main results
			Provincial Center for Disease Control and Prevention				2. 0.05: < 10 only after 300 s 3. 0.2 and 0.1: ~ 5 for each contact time	concentration of 0.1 mg/mL for 2 min
da Silva Santos et al., 2021 [115]	APD 2.0 mg/mL (30 min)	Virus + culture medium	SARS.CoV2/SP02.2020. HIAE. Br	1. RT-PCR 2. Fluorescent assay	1×10^2 $TCID_{50}$/mL	37 °C	**RT-PCR reduction in viral load at:** 1. 1:2 titers: 99.96% 2. 1:4 titers: 99.88% 3. 1:8 titers: 99.84% 4. 1:16 titers: 92.65% 5. 1:32 titers: 77.42% 6. 1:64 titers: 11.06%	APD, when compared to the control, showed a significant reduction in SARS-CoV-2 load at 1:2, 1:4, 1:8 and 1:16 dilution, and partially inactivated SARS-CoV-2 at 1:32 and 1:64 dilution. Cytotoxic effect was detected only at the initial dilution 2 mg/mL (2:1). No virus neutralization was observed below the 1:128 titer.
Davies et al., 2021[105]	1. CHX 0.2% + EtOH (60 s) 2. CHX 0.2% alcohol-free (60 s) 3. Dipotassium oxalate 1.4% (60 s) 4. Hypochlorous acid 0.01–0.02% (60 s) 5. H_2O_2 1.5% (60 s) 6. PVP-I 0.58% (60 s)	Medium + test solution (60 s)	SARS-CoV-2 England 2 strain	Plaque assay	1.7×10^6 $TCID_{50}$/mL	20 ± 2 °C	**Mean titre reduction (Log_{10} $TCID_{50}$/mL)** 1. CHX + EtOH: 0.5 (0.1–0.9) 2. CHX alcohol free: 0.2 (−0.2 to 0.7) 3. Dipotassium oxalate: ≥ 3.5 (3.2–3.8) 4. Hypochlorous acid: ≥ 5.5 (5.2–5.8) 5. H_2O_2: 0.2 (−0.1 to 0.5) 6. PVP-I: ≥ 4.1 (3.8–4.4)	Dipotassium oxalate demonstrated effective inactivation of SARS-CoV-2 in vitro and by commercial mouthwashes containing 0.01–0.02% hypochlorous acid or 0.58% PVP-I, while both the CHX and H_2O_2 mouthwashes tested in this study resulted to be ineffective against SARS-CoV-2.
Jain et al., 2021[99]	1. CHX 0.2% (30 s; 60 s) 2. CHX 0.12% (30 s; 60 s) 3. PVP-I 1% (30 s; 60 s)	_	SARS-CoV-2 strain isolated from an Indian patient and cultured using VeroE6 cells	RT-PCR	2×10^6 PFU/mL	37 °C	**Relative Ct change** 1. CHX 0.12%: 30 s: 10.5 ± 0.5; 60 s: 11 ± 1.0 2. CHX 0.2%: 30 s: 12.5 ± 0.5; 60 s: 13 ± 0 3. PVP-I: 30 s: 9.5 ± 0.5; 60 s: 11 ± 2	CHX 0.2% inactivated more than 99.9% of SARS-CoV-2 virus after a contact time of 30 s, and was considered as more efficacious than PVP-I 1% utilized for 30 s and 60 s
Koch-Heier et al., 2021 [100]	1. CPC 0.05% + H_2O_2 1.5% (30 s) 2. CHX 0.1% + CPC 0.05% + F- 0.005% (30 s) 3. CPC 0.05% (30 s) 4. CHX 0.1% (30 s) 5. CPC 0.05% + CHX 0.1% (30 s) 6. H_2O_2 1.5% (30 s)	1. SARS-CoV-2 + medium 2. Medium + each test solution	SARS-CoV-2 strain FI-100	Plaque assay	6.25×10^6 PFU/mL	37 °C	**Log_{10} PFU/mL reduction** 1. CPC + H_2O_2: ≥ 1.9 2. CHX+CPC+F-: ≥ 2.0 3. CPC: 0.7 4. CHX: 0.55 5. CPC+CHX: 1.2 6. H_2O_2: 0.3	While a combination of CPC and CHX as well as CPC alone led to a significant reduction of infectious viral particles, H_2O_2 and CHX alone had no virucidal effect against SARS-CoV-2. At the crystal violet staining A reduction resulted from 0.05% CPC and the combination of 0.1% CHX with 0.05% CPC, while no virucidal effect was observed for 0.1% CHX and 1.5% H_2O_2.
Komine et al., 2021 [101]	1. CPC 0.05% (20 s) 2. CHX 0.06% + CPC 0.05% (30 s) 3. CHX 0.12% + CPC 0.05% (30 s)	1. EtOH 70% (20 s) 2. Water (20 s, 30 s, 180 s)	SARS-CoV-2 (JPN/TY/WK-521 strain)	Plaque assay	0.1 mL of virus suspension (viral titer 8.49 Log_{10} PFU/mL)	25 °C	1. EtOH: > 99.99% 2. CPC (0.05%; 0.075%; 0.04%): ≥ 99.99% 3. CHX (0.06% or 0.12%) + CPC: > 99.99%	No cytotoxic or interference effects at dilutions ranging from 1 to 1/100 for any of the tested solutions. All the mouthwashes containing

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Table 5 (*continued*)

Ref.	Test (type, concentration, exposure time)	Control	SARS-CoV2 source	Assessment method	Initial viral concentration	Incubation temperature	Reduction in viral titer compared to non-active control	Main results
	4. CPC 0.075% (30 s) 5. CHX 0.12% (30 s) 6. Delmopinol hydrochloride 0.20% (30 s) 7. CPC 0.04% (20 s)						4. CHX: 42.5% 5. Delmopinol hydrochloride: > 99.99%	0.4–0.075% CPC inactivated SARS-CoV-2 with a reduction of 3.3 to > 4.4 Log_{10} PFU/mL regardless of dosage. Mouthwash containing 0.20% delmopinol hydrochloride inactivated SARS-CoV-2 with a > 5.4 Log_{10} PFU/mL reduction. However, the mouthwash containing only 0.12% CHX as antiseptic did not show a sufficient inactivation effect against SARS-CoV-2 in this study.
Meister et al., 2020 [104]	1. H_2O_2 1.5% (30 s) 2. CHX 0.2% (A) (30 s) 3. Dequonal® (0.015% Dequalinium chloride; 0.035% Benzalkonium chloride) (30 s) 4. CHX 0.2% (B) (30 s) 5. PVP-I 1% (30 s) 6. Listerine® Cool Mint (Eucalyptol 0.091%; Menthol 0.042%; Thymol 0.063%, ethanol (inactive ingredient)) (30 s)	SARS-CoV-2 + medium	1. Strain 1: SARS-CoV-2 isolated from a patient 2. Strain 2: BetaCoV/Germany/Ulm/01/2020 3. Strain 3: BetaCoV/Germany/Ulm/02/2020	Plaque assay	5×10^5 $TCID_{50}$/mL in 1 vol of organic load mimicking respiratory secretions	37 °C	**$TCID_{50}$/mL reduction** 1. H_2O_2: strain 1: 0.78; strain 2: 0.61; strain 3: 0.33 2. CHX (A): strain 1: 1.00; strain 2: 0.78; strain 3: 1.17 3. Dequonal®: strain 1: ≥ 3.11; strain 2: ≥ 2.78; strain 3: ≥ 2.61 4. CHX (B): strain 1: 0.50; strain 2: 0.56; strain 3: 0.50 5. PVP-I: strain 1: ≥ 3.11; strain 2: ≥ 2.78; strain 3: ≥ 2.61 6. Listerine® Cool Mint: strain 1: ≥ 3.11; strain 2: ≥ 2.78; strain 3: ≥ 2.61	Medium control after 30 s exposure time did not reduce viral infectivity. All the three SARS-CoV-2 strains were highly susceptible to various oral rinses. All tested products showed virucidal activity against SARS-CoV-2. In particular, Dequonal®, PVP-I and Listerine® Cool Mint, significantly reduced viral infectivity to up to 3 orders of magnitude to background levels. Evidence that SARS-CoV-2 can be efficiently inactivated by commercially available oral rinses within 30 s was provided.
Okamoto et al., 2022 [112]	1. CPC 0.05% (20 s; 60 s; 300 s) 2. CPC 0.1% (20 s; 60 s; 300 s) 3. CPC 0.3% (20 s; 60 s; 300 s) 4. PVP-I 0.1% (20 s; 60 s; 300 s)	SARS-CoV-2 + medium	SARS-CoV-2/Hu/DP/Kng/19–027,LC528233: SARS-CoV-2 isolated from a patient who developed COVID-19 on the cruise ship Diamond Princess	Plaque assay	10-fold dilution starting from unknown viral stock concentration	Not reported	**% reduction** 1. CPC 0.05%: 91.9% at 20 s; > 97% at 60 s and 300 s 2. CPC 0.1%: > 97% at all timepoints 3. CPC 0.3%: > 97% at all timepoints 4. PVP-I: > 97% at all timepoints	CPC has dose- and time-dependent antiviral activity. In Western blotting after SDS-PAGE under reducing conditions, the molecular weights of four forms of the S protein were unchanged.
Pelletier et al., 2021 [107]	1. PVP-I 1.5% (60 s) 2. PVP-I 0.75% (60 s) 3. PVP-I 0.5% (60 s)	1. EtOH 70% (60 s) 2. Water (60 s)	SARS-CoV-2, USA-WA1/2020 strain	Plaque assay	5.3 Log_{10} $CCID_{50}$/0.1 mL	22 ± 2 °C	**Log_{10} $CCID_{50}$/0.1 mL reduction** 1. PVP-I 1.5%: 4.63 2. PVP-I 0.75%: 4.63 3. PVP-I 0.5%: 4.63 4. EtOH 70%: 4.63	All the oral antiseptics evaluated were effective at reducing > 4 Log_{10} $CCID_{50}$ infectious virus, from 5.3 Log_{10} $CCID_{50}$/0.1 mL to 1 Log_{10} $CCID_{50}$/0.1 mL or less. No cytotoxicity, or cell death, was observed in any of the test wells. Positive control and

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Table 5 (*continued*)

Ref.	Test (type, concentration, exposure time)	Control	SARS-CoV2 source	Assessment method	Initial viral concentration	Incubation temperature	Reduction in viral titer compared to non-active control	Main results
								neutralization controls performed as expected and did not cause cell death.
Ramji et al., 2022[113]	1. H_2O_2 1.5% (30 s) 2. CPC 0.07% (30 s)	Medium alone	SARS-CoV-2 USA-WA1/2020 (NR-52281, BEI Resources; Manassas, VA, USA)	Plaque assay	1:100 dilution for the mouth rinse; 1:10 dilution for the dentifrice	20 ± 2 °C	1. Log10 TCID50/mL reduction 2. H_2O_2: ≥ 4.22 3. CPC: ≥ 4.22	Both tested mouthwashes demonstrated strong virucidal activity in the virucidal efficacy suspension test after a 30 s contact time.
Santos et al., 2021[115]	APD 0.1% (30 s, 60 s, 300 s)	1. SARS-CoV-2 + medium 2. Medium alone	SARS-CoV-2 samples retrieved from oropharynx of patients diagnosed with the new COVID	Plaque assay	5.5 ln $TCDI_{50}$/mL	37 °C	**SARS-CoV-2 inactivation (%)** 1. ADP 30 s: 90% 2. ADP 60 s: 90% 3. ADP 300 s: 90%	Mouthwash 0.1% APD presents 1 reduction Log_{10}, with 90% viral inactivation, with the same percentage of reduction for all the exposure times performed.
Shet et al., 2022[111]	PVP-I 0.5% (15 s; 30 s; 60 s; 300 s)	1. Placebo (15 s; 30 s; 60 s; 300 s) 2. EtOH 70% (15 s; 30 s; 60 s; 300 s) 3. Water (15 s; 30 s; 60 s; 300 s)	SARS-CoV-2, strain USA-WA1/2020	Plaque assay	1:2 dilution from the highest viral suspension	22 ± 2 °C	**Log_{10} $CCID_{50}$/0.1 mL reduction** 1. 15 s: PVP-I 2.8; placebo: 0.63; ethanol 4.0 2. 30 s: PVP-I > 4.0; placebo: 0.17; ethanol: > 4.0 3. 60 s: PVP-I: 3.67; placebo: 0; ethanol: > 4.0 4. 300 s: PVP-I: > 4.0; placebo: 0; ethanol: > 4.0	PVP-I 0.5% demonstrated effective virucidal activity against SARS-CoV-2 at all the timepoints, with a reduction of viral titer up to 2.5 and < 0.67 log_{10} $CCID_{50}$/0.1 mL at 15 s and 30 s, respectively.
Steinhauer et al., 2021 [103]	1. CHX 0.08% (300 s; 600 s) 2. CHX 0.16% (60 s; 300 s) 3. OCT 0.08% (15 s; 30 s; 60 s) 4. OCT 0.02% (15 s; 30 s; 60 s)	SARS-CoV-2 + medium	_	Plaque assay	0.7×10^6 PFU/mL	_	**$TCID_{50}$/mL reduction** 1. CHX 0.08%: 300 s: 0.37; 600 s: 0.76 2. CHX 0.16%: 60 s: 0.4; 300 s: 0.81 3. OCT 0.08%: ≥ 2.02 at 15 s, 30 s, 60 s 4. OCT 0.02%: ≥ 3.02 at 15 s, 30 s, 60 s	The two formulations based on CHX were found to have only limited efficacy against SARS-CoV-2, while OCT demonstrated virucidal efficacy against SARS-CoV-2, meeting the > 4 Log_{10} requirement of EN 14476 within a contact time of only 15 s
Takeda et al., 2022 [116]	SP_T medical gargle (Commercial name) 1. Plaque assay: CPC 0–50 μg/mL (30 min) 2. Plaque assay with saliva: CPC 0–40 μg/mL (30 min) 3. qRT-PCR: CPC 0–25 μg/mL (30 min) 4. Western blotting: CPC 50 μg/mL (10 min) 5. TEM: CPC 10, 50, 250 μg/mL (10 min)	1. Surfactant Triton X-100 2. PBS	SARS-CoV-2 variants: 1. Wuhan (WK-521; EPI_ISL_408667) 2. alpha (QK002; EPI_ISL_768526) 3. beta (TY8–612; EPI_ISL_1123289) 4. gamma (TY7–501; EPI_ISL_833366)	1. Plaque assay 2. qRT-PCR 3. Western blotting 4. TEM	10^7 PFU/mL, 1:2 dilution	Room temperature	**SARS-CoV-2 inactivation** 1. CPC effects depend on viral strain: SARS-CoV-2 Gamma is the most sensitive; SARS-CoV-2 Wuhan is the most resistant. 2. CPC effects are dose depend: 40 μg/mL reduced by 1 log SARS-CoV-2 Wuhan 3. CPC effects are reduced in presence of saliva	**Plaque assay:** CPC significantly suppressed the infectivity of all examined SARS-CoV-2 directly in a dose-dependent manner. CPC (50 μg/mL) treatment completely inactivated SARS-CoV-2 Wuhan strain similarly as Triton X-100 (1%). With saliva: CPC (25–40 μg/mL) significantly inactivate SARS-CoV-2 in saliva, in a dose-dependent manner. **qRT-PCR:** Viral RNA expression level in the cells was significantly reduced by CPC via dose-dependent manner at 24 h postinfection.

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Table 5 (*continued*)

Ref.	Test (type, concentration, exposure time)	Control	SARS-CoV2 source	Assessment method	Initial viral concentration	Incubation temperature	Reduction in viral titer compared to non-active control	Main results
								Western blotting: PBS and CPC might have no effect on the structure of the SARS-CoV-2 virions, whereas Triton X-100 changed the structure. **TEM analysis:** spherical particle structure of SARS-CoV-2 treated with PBS remained unchanged. Most virus particles treated with 10 μg/mL CPC remained unchanged, whereas some disintegrated with 50 μg/mL CPC. In contrast, almost all virus particles treated with 250 μg/mL CPC were clearly disrupted (like 1% Triton X-100).
Tiong et al., 2021[102]	1. CHX 0.12% (30 s, 60 s) 2. CPC 0.075% + F- 0.05% (30 s, 60 s) 3. Thymol 0.05% (30 s, 60 s) 4. Bactidol® (Hexetidine 0.1% + Ethanol 9%) (30 s, 60 s)	1. Salt water (water + sodium chloride 2%) 2. SARS-CoV-2 + medium	SARS-CoV-2 virus isolated from NP/OP swab from a SARS-CoV-2-positive patient	Plaque assay	5×10^4 $TCID_{50}$/mL	37 °C	**Log_{10} $TCID_{50}$/mL reduction** 1. CHX: 4.00 for all the timepoints both in clean and dirty conditions 2. CPC+F-: 5.00 for all the timepoints both in clean and dirty conditions 3. Thymol: 0.5 at 30 s under clean conditions and at 30 s and 60 s under dirty conditions; 0.75 at 60 s under clean conditions 4. Bactidol®: 5.00 for all the timepoints both in clean and dirty conditions 5. Salt water: 0.00 for all the timepoints both in clean and dirty conditions	In this study, Bactidrol® and CPC + F- mouthwashes were the most effective against SARS-CoV-2, decreasing more than the 99.99% of SARS-CoV-2 load compared to the control after 30 and 60 s of exposure, under both clean and dirty conditions. The virucidal activity of the CHX containing mouthwash against SARS-CoV-2 was slightly lower than the virucidal activity of Bactidrol® and CPC based mouthwashes, with a viral inactivation percentage of 99.99% under both clean and dirty conditions. Salt water and thymol did not show any significant reduction in viral load.
Wang et al., 2021[110]	1. PVP-I 2 mg/mL (30 s, 60 s, 120 s or 300 s) 2. PVP-I 1 mg/mL (30 s, 60 s, 120 s or 300 s) 3. PVP-I 0.5 mg/mL (30 s, 60 s, 120 s or 300 s) 4. PVP-I 0.25 mg/mL (30 s, 60 s, 120 s or 300 s) 5. PVP-I 0.125 mg/mL (30 s, 60 s, 120 s or 300 s)	Medium alone	SARS-CoV-2 (hCoV-19/Zhejiang/OS2/ 2020, GISAID, ID: 455692) isolated from a patient in Zhejiang Provincial Centre for Disease Control and Prevention	1. Plaque assay 2. RT-PCR 3. Immunofluorescence microscopy	1×10^6 IU/mL, diluted 1:1000	35 °C	**Log_{10} $TCID_{50}$/mL** Medium ~ 6 at all timepoints 1. 30 s: 2 < PVP-I 0.5, 1 and 2 mg/mL < 3; 5 < PVP-I 0.125 and 0.25 mg/mL < 6 2. 60 s: PVP-I 2 mg/mL < 2; PVP-I 1 mg/mL ~ 2; PVP-I 0.5 mg/mL ~	Viral inhibitory effect at the same concentration was highest when the contact time was 1 min **RT-PCR:** PVP-I at a concentration of 1000 μg/mL had no viral inhibition (CC50 > 2.75 mM). Effect of PVP-I on virus inhibition rate was mainly concentration dependent. The viral titres for the same contact time but different PVP-I concentrations decreased as the concentration of PVP-I

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Table 5 (*continued*)

Ref.	Test (type, concentration, exposure time)	Control	SARS-CoV2 source	Assessment method	Initial viral concentration	Incubation temperature	Reduction in viral titer compared to non-active control	Main results
	6. PVP-I 0.0625 mg/mL (30 s, 60 s, 120 s or 300 s)						3; PVP-I 0.125 and 0.25 mg/mL ~ 6 3. 120 s: PVP-I 2 mg/mL < 2; PVP-I 1 and 0.5 mg/mL ~ 2; PVP-I 0.25 mg/mL ~ 5; PVP-I 0.125 mg/mL ~ 6 4. 300 s: PVP-I 1 and 2 mg/mL < 2;PVP-I 0.5 mg/mL ~ 3; PVP-I 0.25 and 0.125 mg/mL ~ 6.	increased. At high concentrations, prolonging the contact time does not enhance PVP-I virus suppression capability.
Xu et al., 2021[106]	1. Listerine® Original (Eucalyptol 0.092%; Menthol 0.042%; Methyl salicylate 0.060%; Thymol 0.064%; Ethanol 26.9%; 30 min) 2. PVP-I 1% (30 min) 3. H_2O_2 1.5% (30 min) 4. CHX 0.12% (30 min)	SARS-CoV-2 + medium	Pseudotype SARS-CoV-2 (USA_WA1/2020 strain) expressing mNeonGreen	Fluorescent assay	MOI 1:5	37 °C	1. Listerine® original (3% v/v): reduced SARS-COV-2 infection by 40% 2. PVP-I (0.1% v/v): high antiviral activity, but disruption of cell morphology was detectable 3. H_2O_2 (0.05% v/v): high antiviral activity, but disruption of cell morphology was detectable 4. CHX (1.5% v/v): reduced infection by 70% without affecting cells morphology	All undiluted mouthwashes and both 1.5% (v/v) dilutions of HP and PVP-I were highly toxic to HeLa-hACE2 hACE2 and oral epithelial cells. All mouth washes at non-cytotoxic levels exhibited antiviral activity. Highly diluted PVP-I and H_2O_2 significantly inactivated viruses but their antiviral effects were associated with severe cytotoxicity at higher concentrations. Taken together, Listerine® Original and CHX may be better mouth rinse products for SARS-CoV-2 prevention since they did not show cytotoxic effects.

AgNPs: silver nanoparticles; APD: anionic phthalocyanine derivate; CCID50: 50% cell culture infectious dose; CHX: chlorhexidine; CPC: cetylpyridinium chloride; EtOH: ethanol; F-: sodium fluoride; H_2O_2: hydrogen peroxide; NP: nasopharyngeal; OCT: Octenidine dihydrochloride; OP: oropharyngeal; PBS: phosphate buffered solution; PVP-I: povidone-iodine; PFU: plaque-forming unit; TCID50: 50% tissue culture infectious dose; TEM: transmission electron microscopy.

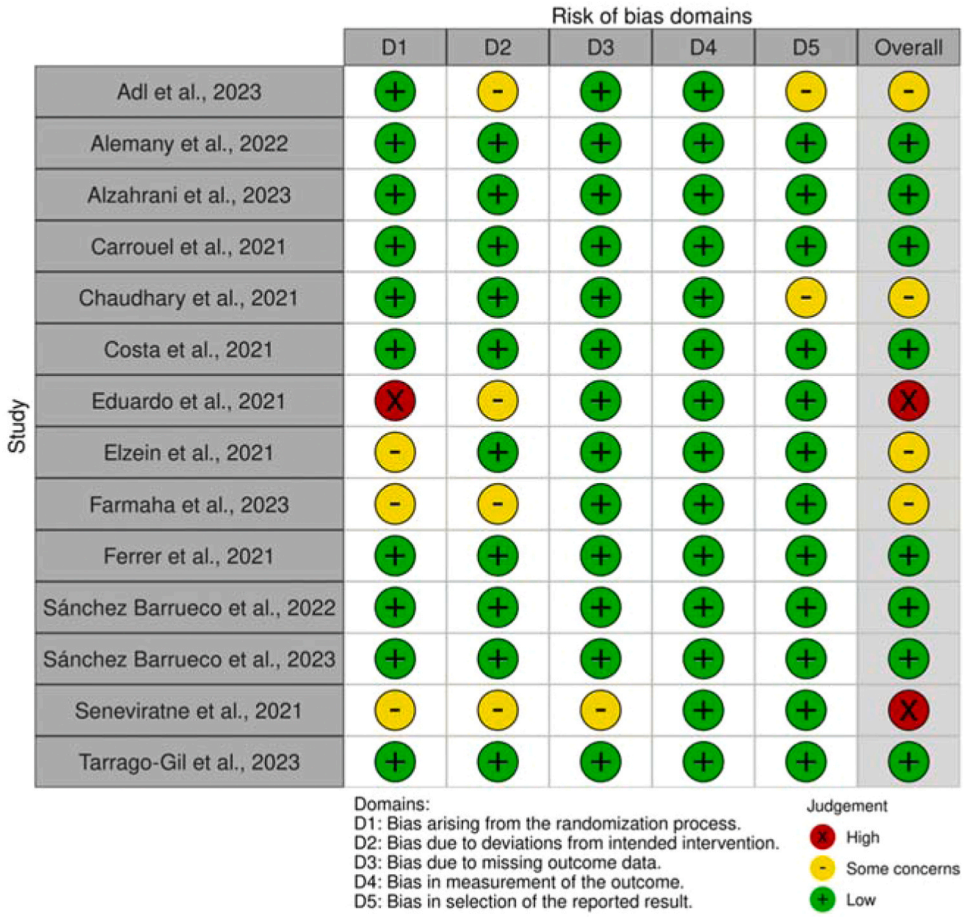

Fig. 2. Risk of bias traffic light plot of ROB 2 assessments created using robvis [119].

5 min, while this evidence was not confirmed by in vitro studies. On the contrary, PVP-I 0.1% tended to reduce the saliva viral load after 5 min from rinsing as compared to non-active control, and these results are in line with in vitro observations [38,120]. Another important difference between clinical and in vitro studies relates to the use of ethanol. As positive control, ethanol 70% proved to be effective against SARS-CoV-2 in vitro [121,122]. As expected, no included clinical study used pre-procedural mouthwashes with alcohol at this concentration. CPC demonstrated virucidal activity in vitro, while inconsistent results were observed in clinical studies regarding CPC-induced salivary viral load reduction. Nevertheless, increased detection of the SARS-CoV-2 nucleocapsid protein in the saliva by ELISA test indicating viral envelope disruption was observed after rinsing with CPC [92,97]. The effectiveness of H_2O_2 mouthwashes remains uncertain, with conflicting results for both clinical and in vitro studies.

In the majority of the included clinical studies, the recruitment of COVID-19 positive patients was based on nasal or nasopharyngeal swab PCR tests and, in all trials, saliva viral load reduction after rinsing was evaluated by means of RT-PCR. Despite this test is rapid and highly sensitive, it allows to detect viral RNA, but does not provide any indication on the infectivity of the virus [123]. As described by Jefferson et al. [124], there is no clear correspondence between the presence of the active virus, which can only be assessed in cell culture systems. However, in a pilot study investigating the effectiveness of H_2O_2 1%, replicating virus could only be determined from one baseline saliva sample out of 5 positive COVID-19 patients with saliva viral load of at least 10^3 RNA copies per mL [53]. SARS-CoV-2 replication in Vero-E6 cell culture after rinsing has been reported only in two studies from the same group [92,93]. Interestingly, the authors detected considerable viral infectivity also with high salivary Ct values [92], suggesting that low-sensitivity SARS-CoV-2 tests could fail to detect cases with infective potential.

Chaudhary et al. focused on a clinically relevant aspect, as they divided patients based on the presence or absence of symptoms [89]. The authors concluded that there are no statistically significant differences between symptomatic and asymptomatic patients and, therefore, preprocedural rinsing is always advisable. These results are confirmed by a recent work of Carrouel et al. [5], in which no differences in median viral load values between symptomatic and asymptomatic patients were found.

The maintenance of the virus inactivation effect over time represents another clinically relevant aspect. Only three of the considered clinical studies evaluated mouthwash efficacy over time after a single rinse [27,88,89]. Chaudary et al. reported the efficacy of CHX, PVP-I and H_2O_2 after forty-five minutes after the initial rinse, with an even increased effect of PVP-I at the latest time point [89]. The long-lasting effect (up to 60 min) of CHX 0.12% was demonstrated also in the other two studies [27,88]· H_2O_2 resulted to be very effective in the short term, but it loses its effectiveness over time [88]. Furthermore, the sequential use of H_2O_2 followed by CHX did not increase the saliva viral load reduction, compared to CHX alone. Overall, these findings suggest that a single preprocedural rinse might be sufficient to achieve a viral load reduction that lasts for the entire time of a normal dental session and it is not necessary to have the patient perform multiple rinses during the session. The mechanical action of rinsing might be sufficient in reducing the salivary viral load, as observed with 30 s of rinsing with distilled water, that showed a significant decrease in viral load compared to the no-rinse group [95].

As regards in vitro studies, they should try to reproduce as much as possible the oral conditions. However, only five out of the fourteen

Table 6
ToxRTool in vitro studies.

Ref./ Criteria	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Total Score	Reliability
Almanza-Reyes et al., 2021 [114]	1	1	0	1	1	1	1	1	1	1	1	0	1	0	1	1	0	1	14	NOT RELIABLE
Anderson et al., 2022 [126]	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	17	RELIABLE
Bidra et al., 2020 [109]	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	18	RELIABLE
Bidra et al., 2020 [108]	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	18	RELIABLE
Chen et al., 2022 [117]	1	0	1	1	1	1	1	1	1	0	0	1	1	1	1	1	0	1	14	NOT RELIABLE
da Silva Santos et al., 2021 [115]	1	0	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	1	12	NOT RELIABLE
Davies et al., 2021 [105]	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	17	RELIABLE
Jain et al., 2021 [99]	1	0	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	16	NOT RELIABLE
Koch-Heier et al., 2021 [100]	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	17	RELIABLE
Komine et al., 2021 [101]	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	16	RELIABLE
Meister et al., 2020 [104]	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	17	RELIABLE
Okamoto et al., 2022 [112]	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	18	RELIABLE
Pelletier et al., 2021 [107]	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	17	RELIABLE
Ramji et al., 2022 [113]	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	16	NOT RELIABLE
Santos et al., 2021 [46]	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	16	RELIABLE
Shet et al., 2022 [111]	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	17	RELIABLE
Steinhauer et al., 2021 [103]	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1	15	RELIABLE
Takeda et al., 2022 [116]	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	18	RELIABLE
Tiong et al., 2021 [102]	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	16	RELIABLE
Wang et al., 2021 [110]	1	1	1	0	1	0	1	1	1	1	1	0	1	1	1	1	1	1	15	NOT RELIABLE
Xu et al., 2021 [106]	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	16	NOT RELIABLE

ToxRTool criteria:
1. Was the test substance identified?
2. Is the purity of the substance given?
3. Is information on the source/origin of the substance given?
4. Is all information on the nature and/or physico-chemical properties of the test item given, which you deem indispensable for judging the data?
5. Is the test system described?
6. Is information given on the source/origin of the test system?
7. Are necessary information on test system properties, and on conditions of cultivation and maintenance given?
8. Is the method of administration given?
9. Are doses administered or concentrations in application media given?
10. Are frequency and duration of exposure as well as time-points of observations explained?
11. Were negative controls included?
12. Were positive controls included?
13. Is the number of replicates (or complete repetitions of experiment) given?
14. Are the study endpoint(s) and their method(s) of determination clearly described?
15. Is the description of the study results for all endpoints investigated transparent and complete?
16. Are the statistical methods for data analysis given and applied in a transparent manner?
17. Is the study design chosen appropriate for obtaining the substance-specific data aimed at?
18. Are the quantitative study results reliable?

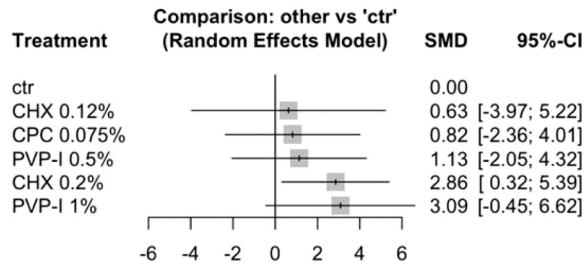

Fig. 3. Forest plot from random-effects meta-analysis of the included clinical studies on Δ_{Ct} (T1-T0).

included studies performed the experiments at 37 °C and used exposure times between 15 and 60 s, similarly to clinical settings [99,100,102,104,115]. The remaining studies performed the incubations at room temperature (22 °C) or used exposure times ranging from 30 min to 72 h. Moreover, viral load tested in in vitro studies is generally higher and difficult to compare with the variable concentrations of the virus retrieved in the human hosts [5]. Therefore, their results might not be translated to clinical situations.

Only two included clinical studies showed an overall high risk of bias [29,88], including one eligible for quantitative analysis [29].

Due to the lack of data consistency and the small or unbalanced number of patients involved in the studies included in the quantitative analysis, network meta-analysis was challenging, and data had to be partially estimated. Hence, results must be interpreted with caution. Furthermore, owing to the high heterogeneity and lack of standard deviation values, no meta-analysis could be performed for in vitro studies. In a recent meta-analysis the effect of PVP-I, CHX and CPC was assessed [17]. Contrary to our quantitative evaluation, the analysis considered not only the immediate but also the long-lasting effect of the mouthwashes. However, the same active agent at different concentrations (e.g. CHX 0.20% and 0.12%) was considered as a unique product. PVP-I was confirmed to be effective against SARS-CoV-2 not only after 5 min from rinsing, but also after hours (i.e. 1 h, 2 h, 3 h and 6 h after rinsing) [17]. In the same meta-analysis, CHX and CPC were found not to be effective in reducing SARS-COV-2 viral load. The clinical efficacy of CHX and PVP-I in reducing the number of negative RT-PCR results in COVID-19 patients was reported in another systematic review, in which both agents gained only moderate effect size on reducing viral load in patients with COVID-19 compared to control [16].

Despite several national and international guidelines recommend preprocedural mouth rinsing [6], their use in dental practices is also related to increased costs and potential adverse reactions to the products. The included clinical studies were largely conducted before the high vaccination coverage of the population and when a high number of COVID-19 positive cases were daily reported. The costs and benefits of the rinsing protocols should be revised in light of the current epidemiological situation and taking into account the immunological and vaccination status of the patients. Finally, it has to be noted that the study was limited to the investigation of mouthwashes. Nonetheless, throat and nose sprays can be effective adjuvants in reducing patients' viral load [49].

5. Conclusion

In conclusion, despite a huge heterogeneity among the studies, data suggested the antiviral efficacy of commonly used mouthwashes. Some included studies presented a high risk of bias and the results have to be considered with caution. Discrepancies between in vivo and in vitro studies were observed, which require attention in future investigations to confirm the transferability of the laboratory data to clinical settings. Clinical data are lacking on the concomitant use of CPC and CHX, which was a promising combination within in vitro experiments. In future studies, it would be interesting to compare the rinsing protocols (active compound, concentration, rinsing duration) that have obtained the most encouraging results so far. However, considering the reduced incidence of COVID-19 positive patients, the acquired immunity after infection, and the high vaccination coverage of the population, a highly relevant question is if these factors have an impact on the efficacy and utility of preprocedural rinsing in dental settings. Moreover, this could provide valuable information for potential future pandemics with similar enveloped viruses, since it is still not known in which patients and in which phases of a pandemic preprocedural rinsing could be beneficial.

Funding

None.

Conflict of Interest

The authors declare no conflict of interest.

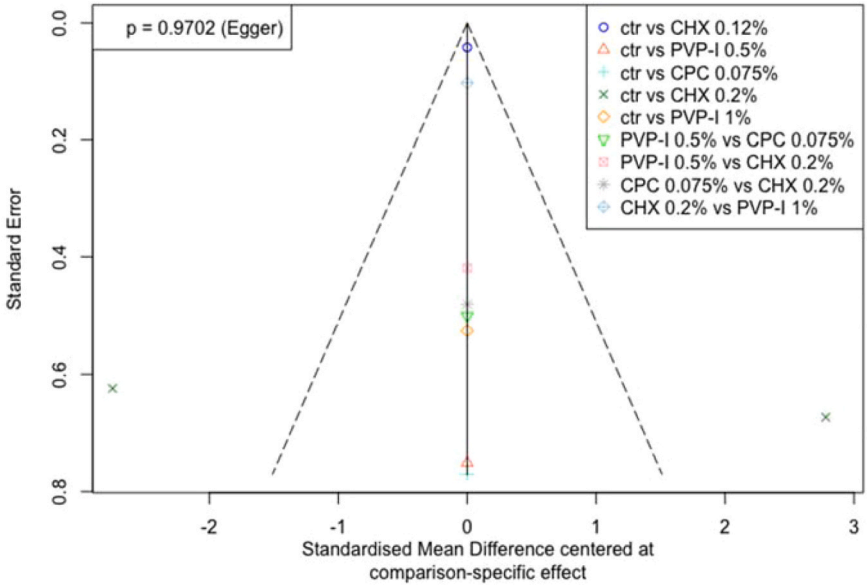

Fig. 4. Funnel plot of the clinical studies included in the network meta-analysis.

Data Availability

Data will be provided upon reasonable request.

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None.

Scientific field of dental science

Dentistry – miscellaneous.

Article type

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PROSPERO registration number

CRD42022318922.

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