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The effect of mouthwashes on SARS-CoV-2 viral load: a systematic review

António Silva

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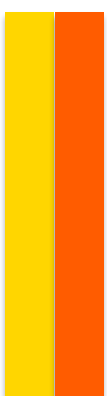

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The effect of mouthwashes on SARS-CoV-2 viral load: a systematic review

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Integrated master's degree thesis presented to the Faculty of Dental Medicine, University of Porto (FMDUP)

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RESUMO

Introdução: A COVID-19 é uma doença causada pelo SARS-CoV-2, um vírus capaz de infetar as células da cavidade oral. Considerando a cavidade oral uma importante porta de entrada e reservatório para o SARS-CoV-2, vários autores aconselham os Médicos Dentistas a pedirem aos seus pacientes que realizem um bocheco com um colutório de forma a reduzir a carga viral oral e assim tornar o ato médico mais seguro. Contudo, não há evidências claras sobre quais os colutórios mais eficazes para a redução da carga viral de SARS-CoV-2.

Objetivo: Realizar uma revisão sistemática de estudos *in vivo* e *in vitro* para avaliar a eficácia de diferentes colutórios na carga viral de SARS-CoV-2.

Métodos: Utilizaram-se as bases de dados *PubMed*, *Web of Science*, *Scopus*, *MedRxiv* e *bioRxiv* para a pesquisa bibliográfica. Foram incluídos estudos *in vitro* e *in vivo* que avaliaram o efeito virucida de colutórios na carga viral de SARS-CoV-2, tendo estes sido selecionados e avaliados por dois revisores independentes. Realizou-se a avaliação do risco de viés no único ensaio clínico randomizado (RCT) incluído.

Resultados: Primeiramente, selecionou-se um total de 504 artigos das diferentes bases de dados, tendo sido vinte incluídos nesta revisão sistemática. Para avaliar a carga viral, os estudos *in vitro* utilizaram ensaios de infetividade em cultura de células e os ensaios *in vivo* avaliaram a carga viral através da reação em cadeia da polimerase de transcrição reversa (RT-PCR). A iodopovidona (PVP-I) foi o colutório mais estudado, mostrando frequentemente reduções eficazes na carga viral em estudos *in vitro,* superiores a 4 $\log_{10}$, conforme estabelecido pela norma europeia EN 14476. Estes resultados foram parcialmente corroborados pelos estudos *in vivo*. O cloreto de cetilpiridínio (CPC) também apresentou bons resultados, embora tendo sido avaliado em poucos estudos *in vitro* e um *in vivo*. Os estudos que avaliaram o gluconato de clorexidina (CHX) e o peróxido de hidrogénio (H_2O_2) não mostraram eficácia na redução da carga viral. Alguns colutórios comerciais como Listerine® Total Care, Listerine® Advanced Gum Treatment, Listerine® Antiseptic e Octenisept® mostraram redução da carga viral em mais de 4 $\log_{10}$, mas em apenas um estudo cada.

Conclusões: Com base nos resultados obtidos nesta revisão sistemática, o bochecho de colutórios à base de PVP-I no contexto médico-dentário parece ser a melhor opção, embora o CPC apresente também bons resultados preliminares. A CHX e H_2O_2 parecem ser ineficazes na redução da carga viral de SARS-CoV-2 e o seu uso com o objetivo de reduzir a carga salivar de SARS-CoV-2 deve ser revisto. De um modo geral, o uso de colutórios de determinadas soluções antiséticas parece reduzir a carga viral de SARS-CoV-2, representando assim uma importante medida de proteção para a equipa médico-dentária. Embora os resultados destes estudos primários sejam relevantes, há necessidade de mais RCT e estudos *in vivo* com os diversos colutórios para avaliar melhor o seu efeito na carga viral de SARS-CoV-2 e na prevenção de infecções.

Palavras-chave: COVID-19; SARS-CoV-2; Colutório; Carga Viral; Virucida

ABSTRACT

Introduction: COVID-19 is a disease caused by SARS-CoV-2, a virus that can infect cells of the oral cavity. Considering the oral cavity a major entryway and reservoir for SARS-CoV-2, several authors advise dentists to ask their patients to perform a preprocedural oral rinse as an additional protective measure. However, there is no clear evidence on which mouthwashes are most effective in reducing the viral load of SARS-CoV-2.

Aim: To perform a systematic review of *in vivo* and *in vitro* studies to assess the effectiveness of different mouthwashes on SARS-CoV-2 viral load.

Methods: PubMed, Web of Science, Scopus, MedRxiv, and bioRxiv databases were used in the search strategy. The inclusion criteria consisted of *in vitro* and *in vivo* studies assessing the virucidal effect of mouthwashes on SARS-CoV-2 or surrogates that were selected and evaluated by two independent reviewers. Risk of bias assessment was performed on the only included randomized controlled trial (RCT).

Results: A total of 504 articles were retrieved from the different databases, being twenty of them included in this systematic review. To assess the viral load, *in vitro* studies used infectivity assays assessing the cell or tissue culture infectious dose (C/TCID50/mL), while *in vivo* assays evaluated viral load via reverse transcription polymerase chain reaction (RT-PCR). Povidone-iodine (PVP-I) was the most studied mouthwash, frequently showing reductions in viral load on *in vitro* assays by more than 4 $\log_{10}$ as established by European norm EN 14476 as effective, these results were partially corroborated by *in vivo* studies. Similarly, cetylpyridinium chloride (CPC) also showed good results, although evaluated in few *in vitro* and one *in vivo* studies. The studies evaluating chlorhexidine gluconate (CHX) and hydrogen peroxide (H_2O_2) showed no effect in viral load reduction in both *in vitro* and *in vivo* studies. Some complex commercial mouthwashes like Listerine® Total Care, Listerine® Advanced Gum Treatment, Listerine® Antiseptic, and Octenisept® showed to reduce viral load by more than 4 $\log_{10}$ but in only one study each.

Conclusions: Based on the current knowledge, PVP-I-based mouthwashes as a pre-rinse in dental context appear to be the best option, although CPC also presented good preliminary results. CHX and H_2O_2 appear to be ineffective in reducing SARS-CoV-2 oral load and their use as a pre-procedural mouthwash aiming to reduce SARS-CoV-2 salivary load should be revised. Overall, the use of specific mouthwashes solutions seems to reduce SARS-CoV-2 viral load, so, their use as a preprocedural rinse may present an important protective measure for dental staff. Although the results of these primary studies are relevant, there is a need for more RCT and *in vivo* studies on mouthwashes to better understand their effect on SARS-CoV-2 viral load and infection prevention**.**

Keywords: COVID-19; SARS-CoV-2; Mouthwash; Viral load; Virucidal

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INDEX OF ABBREVIATIONS

ACE2	Angiotensin-converting Enzyme 2
AS	António Silva
BSA	Bovine Serum Albumin
BSM	Benedita Sampaio-Maia
CCID50	Median Cell Culture Infectious Dose
CDC	Centers for Disease Control and Prevention
CHX	Clorhexidine Gluconate
copies/mL	Copies per milliliter
COVID-19	Coronavirus Disease 2019
CPC	Cetylpyridinium chloride
Ct	Cycle Threshold
EPA	United States Environmental Protection Agency
EN	European Norm
h	Hours
H_2O_2	Hydrogen Peroxide
HIV	Human Immunodeficiency Virus
HVA	Hepatitis Virus A
HVE	Hepatitis Virus E
ISO	International Organization for Standardization
log	Logarithm
MERS	Middle Eastern Respiratory Syndrome
min	Minutes
MJA	Maria João Azevedo
mM	Millimolar
N	Nucleo-capsid gene
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PICO	Population/Patient/Problem; Intervention; Comparison; Outcome
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PVP-I	Povidone-iodine
RCT	Randomized Controlled Trial
RLU	Relative Light Units
RNA	Ribonucleic Acid
RdRP	Ribonucleic Acid dependent Ribonucleic Acid Polymerase gene
RT-PCR	Reverse Transcription Polymerase Chain Reaction
RV-RTPCR	Rapid Viability-Reverse Transcription Polymerase Chain Reaction
S	Seconds
SARS	Severe Acute Respiratory Syndrome
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SD	Standard Deviation
TCID50	Median Tissue Culture Infectious Dose
v-PCR	Viability Polymerase Chain Reaction

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1. INTRODUCTION

INTRODUCTION

Since late 2019, the world has been learning to cope with SARS-CoV-2, which imposed a readjustment in daily activities, habits, and clinical practice. On March 11, 2020, the World Health Organization declared the outbreak of COVID-19 a pandemic (1). After a year, this pandemic led to over 122 million cumulative cases and over 2.7 million deaths worldwide (2).

SARS-CoV-2 belongs to the *Coronaviridae* family, a group of enveloped, crown-shaped, non-segmented, pleomorphic, positive-sense single-stranded, RNA viruses. SARS-CoV-2 is classified as a beta-coronavirus that normally emerges in mammals. Beyond the recent SARS-CoV-2 outbreak, beta-coronavirus have been associated with two other outbreaks, namely severe acute respiratory syndrome (SARS) and middle east respiratory syndrome (MERS) (3, 4).

SARS-CoV-2 is transmitted in different ways, either directly or indirectly. The direct way includes person-to-person transmission or inhalation, while the indirect way of transmission is possible through aerosolization and fomites (5, 6). Noteworthy, smaller orally generated aerosols have shown higher pathogen concentrations than respiratory droplets (7).

The binding of SARS-CoV-2 to human cells mainly occurs via the angiotensin-converting enzyme 2 (ACE2) receptor (8, 9), found in several tissues in the body, namely, lung epithelial alveolar cells, smooth muscle, and epithelium of the small intestine (10). ACE2 is also highly expressed in the oral cavity, mainly in the epithelium of the tongue, but also in the gingival tissue, particularly on the buccal surface of the sulcular epithelium. Considering that the oral cavity may represent a major entryway and a reservoir of SARS-CoV-2 (11, 12), associated with the impossibility of patients wearing a mask during dental procedures normally associated with aerosol generation, some countries advised dentists to reduce their practice to emergency treatments (13). With the evolution of the pandemic, the scientific community adjusted disinfection protocols and preprocedural protocols for dental practice. Widespread use of protective suits was advised, and the use of goggles and shoe covers was reinforced, as well as stricter patient triage ahead of the appointment (5).

Some authors have hypothesized that preprocedural gargling with a mouthwash can act as an additional protective measure, reducing the oral load of SARS-CoV-2. Even before the COVID-19 pandemic, preprocedural gargling was often used in dentistry to reduce microbial load before surgeries or routine procedures. Dentists normally ask their patients to gargle with chlorhexidine-based solutions because of their bactericidal action, but, although chlorhexidine gluconate (CHX) and other mouthwashes are also effective against some viruses, there is still no systematic revised evidence on its effectiveness on SARS-CoV-2 viral load (14, 15). Thus, there is a need to understand if and which mouthwashes are effective against SARS-CoV-2 to improve dental practice safety during the COVID-19 pandemic.

Therefore, in this study, we aim to assess if the use of preprocedural mouthwashes in the dentistry setting is effective in reducing the viral load of SARS-CoV-2 when compared to no using a mouthwash and what mouthwash solutions are more effective in reducing this virus viral load.

Focused question

The main items for conducting and reporting systematic reviews and meta-analyses (PRISMA) were followed to answer the focused (PICO) question: "Is the use of mouthwash, compared to not using mouthwash, effective in reducing SARS-CoV-2 viral load?".

2. MATERIALS AND METHODS

MATERIALS AND METHODS

Protocol and registration

This review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist. Its protocol is registered on the website PROSPERO (International Prospective Register of Systematic Review - Centre for Reviews and Dissemination University of York) (Registration number: CRD42021237418) (Available on the **Appendix**).

Eligibility criteria

Inclusion criteria

We included *in vitro* and *in vivo* studies assessing the virucidal effect of mouthwashes on SARS-CoV-2 or surrogates.

Exclusion criteria

We excluded reviews, letters to the editor, personal opinions, product news, book chapters, case reports, congress abstracts, protocol suggestions, editorials, correspondence articles, recommendations, trial designs, hypotheses, and studies with animals.

Information sources and search strategy

To produce this review, searches were performed in three bibliographic databases, namely MEDLINE (via PubMed), Scopus, and Web of Science. Searches were conducted on January 13th, 2021. To search the databases, the following keywords were used: “mouthwash”, “mouth rinse”, “oral rinse”, “gargle”, “gargle lavage”, “oral irrigation”, “oral lavage”, “coronavirus”, “COVID-19”, “SARS-CoV-2”, and “2019-nCov” (full query available in **Table 1**). This search was complemented with a manual search on MedRxiv and bioRxiv preprint databases, using the keywords “COVID-19” and “mouthwash” (full query available in **Table 1**). Since the first scientific publications on SARS-CoV-2 concern the year 2020, we limited the search to articles published in 2020 and 2021.

TABLE 1. Database search strategy.

Database	Search (January 13th, 2021)
MEDLINE (via PubMed)	(mouthwash* OR "mouth rinse" OR "oral rinse" OR rinse OR gargl* OR "gargle lavage" OR "oral irrigation" OR "oral lavage") AND (COVID-19 OR COVID19 OR sars-cov-2 OR 2019-nCoV OR COVID OR coronavirus)
Scopus	(mouthwash* OR "mouth rinse" OR "oral rinse" OR rinse OR gargl* OR "gargle lavage" OR "oral irrigation" OR "oral lavage") AND (covid-19 OR covid19 OR sars-cov-2 OR 2019-ncov OR covid OR coronavirus)
Web of Science	TS=((mouthwash* OR "mouth rinse" OR "oral rinse" OR rinse OR gargl* OR "gargle lavage" OR "oral irrigation" OR "oral lavage") AND (COVID-19 OR COVID19 OR sars-cov-2 OR 2019-nCoV OR COVID OR coronavirus))
Database	**Search (January 28th, 2021)**
MedRxiv and bioRxiv	COVID-19 AND mouthwash

Study selection

After removing duplicates, the titles and abstracts of the retrieved publications were independently reviewed by two reviewers (AS, BSM). Studies that were not excluded in the screening phase were fully read, with the full-text analysis being independently performed by two investigators. Any divergence was solved by a discussion with a third reviewer.

Data extraction

Data was independently extracted by two reviewers (AS, MJA) using a purposely-built online form. In case of any inconsistency of data collection, a third author resolved it through discussion. The following variables were retrieved from each primary study: author, title, year, country, type of study, sample number and type, characterization of the patients, intervention and control group, virus strain, type of mouthwash, concentration, number of mouthwashes per day, duration of the rinse, duration of the treatment, and decrease in viral load. For *in vitro* studies, cell lineage used, and the existence of interfering substances was also considered.

Risk of bias in individual studies

The assessment of the risk of bias of the included randomized controlled trials was carried out independently by two reviewers (AS, MJA), according to the Cochrane Collaboration tool for assessing the risk of bias in randomized controlled trials. The risk of bias evaluation was classified as "high risk of bias", "low risk of bias", or "unclear risk of bias" if there was any incomplete or unclear data. Disagreements were resolved after discussion and analysis. No risk of bias assessment was performed on *in vitro* studies or observational before-after studies due to a lack of consensually-accepted tools for assessing the risk-of-bias in those specific studies.

Summary measures

Any outcome measures that directly evaluated SARS-CoV-2 viral load were considered. The main outcome measures presented in this systematic review are viral load in logarithmic (log) reduction value, copies per milliliter (copies/mL), and Relative Light Units (RLU). When the primary studies used a mouthwash with known concentration and presented the in a logarithmic scale, such results were interpreted following the European norm EN 14476, which recognizes antiseptics virucidal capacity when achieving a reduction on viral load greater or equal than 4 $\log_{10}$ (16). Therefore, we decided to classify the results of the primary studies, when those were expressed in log scale, according to three levels: considering virucidal activity (viral load reduction) of greater or equal than 4 $\log_{10}$ as a high efficacy (+), a virucidal activity greater or equal than 3 $\log_{10}$ but lower than 4 $\log_{10}$ as a moderate efficacy (±), and a virucidal activity lower than 3 $\log_{10}$ as a low efficacy (-). These cut-off values were determined by the authors of this systematic review.

Synthesis of results

Due to methodological diversity of included primary studies, it was not possible to carry out a meta-analysis.

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3. RESULTS

RESULTS

Study selection

A total of 619 articles were retrieved from bibliographic databases (MEDLINE, Scopus, and Web of Science), and 36 from preprint databases. The study selection process is described below in **Figure 1**.

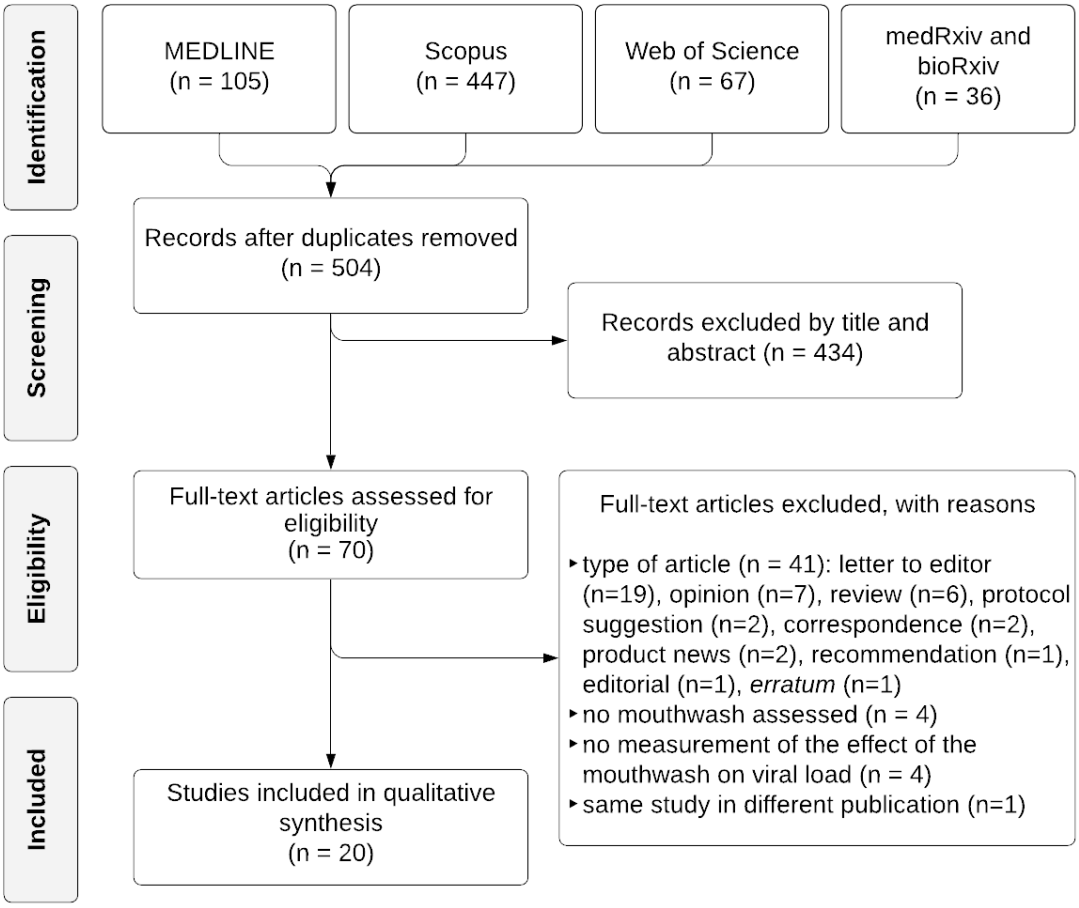

FIGURE 1. PRISMA study selection flowchart.

Study characteristics

The included studies were carried out in different countries, namely South Korea (17), Malaysia (18), Germany (16, 19, 20), Singapore (21, 22), Spain (23, 24), the United Kingdom (25, 26), and the United States of America (27-35). All the studies were conducted in 2020. From a total of twenty included studies, thirteen had been published as peer-reviewed articles (17-23, 27-32) and seven are preprints (16, 24-26, 33-35). Nine of the published articles were performed *in vitro* (18, 20, 22, 27-32) and four were *in vivo* studies (17, 19, 21, 23), one of which was a randomized controlled trial (21) while the remaining were uncontrolled before-and-after studies. All seven included preprints were performed *in vitro*.

All the *in vivo* studies included hospitalized patients with COVID-19 (17, 19, 21, 23), and one also included home-isolated patients (23). The four *in vivo* studies aimed to understand the effect of different mouth rinse solutions in reducing the SARS-CoV-2 viral load, and one (17) also intended to

comprehend the viral dynamics in different body fluids. All the *in vivo* studies quantified viral load via RT-PCR, targeting genes E (17, 19, 21, 23), RNA-dependent RNA polymerase (RdRP) (17, 23), and nucleo-capsid (N) genes (23). Only one of the *in vivo* studies contemplated the use of a control solution (water) (21). *In vivo* studies evaluated the reduction of SARS-CoV-2 in viral titers: two of these studies presented the results in form of a logarithmic reduction value (17, 23), one of them with cycle threshold (Ct) fold changes (21), and lastly, one presented the viral load in copies per milliliter (19). RT-PCR is a technique that can detect small amounts of RNA through DNA amplification. Real-time or quantitative RT-PCR allows indirect quantification through the determination of the Ct, defined as the thermal cycle number at which the signal of DNA amplification increases meaning that the viral RNA was detected. Lower Ct values represent higher quantities of viral genetic material in the sample. When constructing a standard curve with known viral quantity, Ct may be converted in concentration in copies/mL (36).

In vitro papers aimed to understand the effect of different mouthwash solutions on the SARS-CoV-2 viral load. After infecting cellular lineages with the virus, the mouthwash solutions were incubated with the infected cells for a pre-determined period and the effect of the solution was then assessed by endpoint dilution assays. These assays quantify the amount of virus required to produce a cytopathic effect in 50% of infected tissue or cells (TCID50 / CCID50). Regarding the SARS-CoV-2 strains used across studies, fourteen *in vitro* studies used well-characterized SARS-CoV-2 strains, being the most used USA-WA1/2020 (27-30, 32, 33, 35). Only one paper used a SARS-CoV-2 strain directly obtained from an infected patient (24), while one study did not report the SARS-CoV-2 strain employed (16). *In vitro* studies were performed under dirty, clean, or both conditions, being these terms referring to the existence of interfering substances. Ten studies were performed under clean conditions (16, 24, 26-28, 30, 32-35), one was performed in clean and dirty conditions (18), four were performed under dirty conditions (20, 22, 25, 31), and one of the *in vitro* studies did not provide information about the existence of interfering substances (29).

Some studies included the evaluation of more than one mouthwash on their protocol. **Table 2** summarizes the mouthwashes evaluated, concentrations used, study type, and publications' number. Overall, 65% of the studies (n=13) included povidone-iodine (PVP-I) as a test solution, with two being performed *in vivo* (21, 23), and eleven being performed *in vitro* (18, 20, 22, 25-32). Six of the papers investigated the virucidal effect of chlorhexidine gluconate (CHX), two *in vivo* (17, 21), and four *in vitro* (16, 25, 26, 29). Six papers studied the effect of hydrogen peroxide (H_2O_2) on SARS-CoV-2, being one *in vivo* (19), and five *in vitro* (20, 26, 27, 29, 31). Five of the papers focused on the efficacy of cetylpyridinium chloride (CPC), being one *in vivo* (21), and four *in vitro* (24, 25, 31, 34).

TABLE 2. Tested solutions, concentrations, study type, and publications' number.

Test solution	Concentrations	Study type	*N* studies	Publications references
PVP-I	0.45% to 10%	*in vivo*	2	(21, 23)
		in vitro	11	(18, 20, 22, 25-32)
CHX	0.0006% to 0.2%	*in vivo*	2	(17, 21)
		in vitro	4	(16, 20, 26, 29)
H_2O_2	0.0075% to 3%	*in vivo*	1	(19)
		in vitro	5	(20, 26, 27, 29, 31)
CPC	0.05% to 1%	*in vivo*	1	(21)
		in vitro	4	(24, 25, 31, 34)
Other mouthwashes	-	*in vivo*	0	-
		in vitro	10	(16, 20, 24-26, 29, 31, 33-35)

In vivo and *in vitro* studies applied the intervention solution for a pre-determined period, most commonly for 30 seconds (16-22, 25, 27, 28, 31, 32, 34). Three *in vitro* studies included periods of application of the intervention solution of more than 5 minutes (16, 29, 33).

Risk of bias within studies

The only RCT included in this systematic review (21) was evaluated according to the Cochrane Tool for Risk assessment, as presented in **Table 3.** In the Detection Bias domain, the blinding of outcome assessment was marked as "high", as the researchers knew the group each participant was assigned to. The other three *in vivo* studies were "uncontrolled before-after" studies including a low number of participants and for which the assessment of the risk of bias was not feasible.

TABLE 3. Risk of Bias assessment.

	Risk of Bias Domains						
	1.1 Random sequence generation	1.2 Allocation concealment	2.1 Selective reporting	3.1 Other sources of bias	4.1 Blinding (participants and personnel)	5.1 Blinding (outcome assessment)	6.1 Incomplete outcome data
Seneviratne *et al*. (2020)	+	+	+	?	+	−	+

Key: + Low risk of bias − High risk of bias ? Unclear risk of bias

Results of individual studies

Viral load decrease was assessed in these studies in multiple ways, but most commonly via a logarithmic reduction scale. The results of the studies are fully exhibited in **Table 4** and **Table 5**. For studies that measured viral load decrease based on EN 14476 (considering only application times of up to 60 seconds and using mouthwashes with known concentrations), **Tables 6** to **8** can be consulted to compare their results.

Povidone-iodine

Povidone-iodine was the antiseptic most frequently assessed in the primary studies selected for this systematic review. Two *in vivo* studies showed the virucidal efficacy of PVP-I solutions on SARS-CoV-2 **(Table 4).** Seneviratne *et al*. (2020) (21) conducted a RCT comparing the efficacy of three different mouthwashes (PVP-I, CHX, and CPC) when compared to water rinses in reducing salivary SARS-CoV-2 viral load. The rinse with 0.5% PVP-I for 30 seconds was conducted on a group of four hospitalized patients and resulted in a statistically significant reduction of viral load 6 hours post-rinse when compared to water. However, no significant differences were found 5 minutes and 3 hours after the rinse. In an uncontrolled before-after clinical study, Lamas *et al*. (2020) (23) reported that the use of a 60-second rinse with 1% PVP-I led to a significant drop (approximately 5 $\log_{10}$) in SARS-CoV-2 viral load in one of the 4 patients evaluated, sustained for at least three hours.

In vitro studies demonstrated that PVP-I-containing mouthwashes have a virucidal effect on SARS-CoV-2 **(Table 5)**. **Table 6** summarizes the results found in the different studies with application times up to 60 seconds, interpreted following the European norm EN 14476. Concentrations up to 0.75% most commonly showed moderate to high efficacy in reducing SARS-CoV-2 viral load (18, 22, 26-28, 30, 32). The 60-second application of 0.5% presented high efficacy results in all the 3 studies evaluating this condition (18, 26, 30). It is relevant to mention that the majority of test times and concentrations were only evaluated in one paper each. PVP-I used at 1% showed some disparity in results, as one study reported a high efficacy with a 30-second application (22), and the other presented low efficacy with the same time of contact (20). However, Hassandarvish *et al*. (2020) (18) reached high efficacy with application times of 15, 30, and 60 seconds. Concentrations of PVP-I between 1.25% and 2.5% consistently showed moderate to high efficacy results (27, 28, 30, 32). Applying concentrations of PVP-I greater than 2.5% showed low (25) (PVP-I 7.5%), moderate (31) (PVP-I 5%), and high efficacy (22) (PVP-I at 7.5% and 10%) within 30 seconds. The 60-second application also reached moderate to high efficacy results (31) (PVP-I 5%). No study was performed on PVP-I with concentrations greater than 2.5% with 15-second application times.

Two studies included application times greater than 60 seconds. Meyers *et al*. (2020) (31) reported a greater than 4 $\log_{10}$ decrease in viral load after 120 seconds with 5% PVP-I. Xu *et al*. (2020) (29) conducted a study regarding

the virucidal effect of a 30-minute application of PVP-I at different concentrations, measuring the fluorescence intensity from infected cells and presenting the results in relative light units. This study concluded a 5% dilution of an original 10% PVP-I solution was effective at inactivating the virus, resulting in no fluorescence intensity (0 RLU). These results cannot be directly compared to the other studies, or to the EN 14476.

Hydrogen peroxide

Hydrogen peroxide virucidal efficacy was exploited by several authors in primary studies included in this systematic review **(Tables 4 and 5)**. Gottsauner e*t al*. (2020) (19) conducted the only *in vivo* study assessing the virucidal efficacy of a 30-second rinse with H_2O_2 at 1%. Rinsing with this solution showed only a 0.3×10^3 copies per mL decrease in viral load, 30 minutes after the rinse. No statistically significant difference was found between the baseline viral load (1.8×10^3 copies/mL) and 30 minutes after the rinse (1.5×10^3 copies/mL) **(Table 4)**.

Similarly, *in vitro* studies on the virucidal effect of H_2O_2 showed very limited success **(Table 5)**. Considering application times of up to 60 seconds, 1.5% H_2O_2 consistently showed low efficacy (decrease in viral load lower than 3 $\log_{10}$) within 15 seconds (27), 30 seconds (27, 31), and 60 seconds (26, 31). Similarly, 3% H_2O_2 also reached low efficacy results at 15 and 60 seconds (27) **(Table 7)**. Again, two studies considered application times greater than 60 seconds. Meyers *et al*. (2020) (31) reported a decrease in viral load of only up to 2 $\log_{10}$ after 120 seconds with 1.5% H_2O_2. Xu *et al*. (2020) (29) showed that a 30-minute incubation with two dilutions (5% and 50%) of an original 1.5% H_2O_2 solution was effective in reducing viral load. These test conditions led to fluorescence intensity of 0 RLU. These results cannot be directly compared to the other studies, or the EN 14476.

Chlorhexidine Gluconate

Chlorhexidine Gluconate mouthwashes virucidal efficacy was evaluated with *in vivo* **(Table 4)** and *in vitro* studies **(Table 5)**. In a RCT, Seneviratne *et al*. (2020) (21) studied the effect of CHX mouthwashes in a group of six patients and found no reduction of SARS-CoV-2 salivary load, therefore the authors concluded it was necessary a larger number of participants to understand the effect of CHX on SARS-CoV-2 viral load. Yoon *et al*. (2020) (17), while performing an uncontrolled before-after clinical study on the effect of a 30-second 0.12% CHX rinse on two hospitalized patients, stated there was a transient decrease on SARS-CoV-2 viral load for two hours after the rinse. The authors also evidenced that in one patient, one hour-post rinse, no decrease in the viral load was observed.

Considering application times of up to 60 seconds **(Table 7)**, the use of CHX with concentrations lower than 0.2% (0.08% and 0.16%) showed low efficacy within 15, 30, and 60 seconds (16). The use of 0.2% CHX also showed low efficacy after 30 seconds (20) and 60 seconds (26). Meister *et al*. (2020) (20) assessing one CHX mouthwash with an unknown concentration, also reported

a low efficacy on SARS-CoV-2 oral viral load reduction after 30 seconds. Three studies included application times greater than 60 seconds. After 5 and 10 minutes, the use of 0.08% and 0.16% CHX showed low efficacy (16). Xu *et al*. (2020) (29) found that a 30-minute application of a 50% dilution of an original 0.12% CHX solution was effective in inactivating SARS-CoV-2. The 5% dilution had only a moderate virucidal effect, greater than $2x10^4$ RLU, but these results are not comparable to the other studies' results or EN 14476.

Cetylpyridinium Chloride

Cetylpyridinium Chloride *in vivo* virucidal activity was studied in a RCT by Seneviratne *et al*. (2020) (21) on a group of four hospitalized patients **(Table 4)**. When compared to water rinsing, the CPC 0.075% mouthwash reduced salivary SARS-CoV-2 levels within 5 minutes of use. Compared to the control group patients, the effect size of decreasing salivary load with CPC was found to be maintained at 3 hours and 6 hours time points.

In vitro studies demonstrated that CPC-containing mouthwashes have a virucidal effect on SARS-CoV-2 **(Table 5)**. Considering application times of up to 60 seconds **(Table 7)**, concentrations of up to 0.1% showed moderate to high efficacy after 30 and 60 seconds (25, 31, 34). Meyers *et al*. (2020) (31) reported that a 120 seconds application of 0.07% CPC also showed moderate to high efficacy. Muñoz-Basagoiti *et al.* (2020) (24) reported moderate results with a 120 seconds application of CPC at a concentration of up to 10 mM.

Other mouthwashes

Other mouthwashes, either more complex or with less frequently used active compounds, were studied *in vitro* by several authors **(Table 5)**. Listerine® mouthwashes were studied by several authors, although each formulation was only assessed in one study, except for Listerine® Cool Mint® that was assessed by two studies. Overall, results showed a wide range of virucidal effects. When considering application times of up to 60 seconds **(Table 8)**, Listerine® mouthwashes showed low, moderate, and high efficacy. With application times of 30 and/or 60 seconds, Listerine® Antiseptic, Listerine® Advanced Gum Treatment, and Listerine® Total Care achieved high efficacy, reducing SARS-CoV-2 viral load by greater or equal than 4 $\log_{10}$ (25, 26, 31). Listerine® Advanced Defence Sensitive achieved moderate to high efficacy after 60 seconds (26). However, Listerine® Ultra and Listerine® Cool Mint® only showed low efficacy, with application times of 30 seconds (20, 25, 31) and 60 seconds (31). Other mouthwashes like Equate™ (essential oils), Antiseptic Mouthwash (CVS) (essential oils), Dequonal® (Dequalinium chloride, benzalkonium chloride), Octenident® (Octenidine dihydrochloride), ProntOral® (Polyaminopropyl biguanide), Corsodyl (ethanol and CHX), SCD Max (CPC, sodium citric acid, and other active ingredients), a mouthwash containing ethanol (15.7%) and other ingredients, a mouthwash containing zinc sulfate heptahydrate and other ingredients, a mouthwash containing a mix of amyloglucosidase and other ingredients, and an essential iodine solution were also assessed *in vitro* by one primary study each, all showed low efficacy with application times between 30 and 60 seconds (20, 25, 31, 34, 35). OraWize+

effect was assessed by one study and showed low to high efficacy after 60 seconds (26). On the other hand, the use of octenisept® was studied once, showing high efficacy after 15, 30, and 60 seconds (16).

Some studies considered application times greater than 60 seconds. Zoltán (2020) (35) showed that a 90-second application of an Essential iodine solution produced a low efficacy reduction. Meyers *et al*. (2020) (31) concluded that Listerine® Antiseptic could completely eliminate SARS-CoV-2 viral load after a 120-second application. The use of Perio Aid® Intensive Care (CHX- and CPC-containing) for 120 seconds was ineffective (24). Mantlo *et al.* (2020) (33) conducted a study that considered application times of 10, 30, and 60 minutes, concluding that undiluted CupriDyne® led to a low efficacy reduction after 10 and 30 minutes, failing to reduce SARS-CoV-2 viral load by greater than 2 $\log_{10}$. However, the undiluted CupriDyne® incubation after 60 minutes completely eliminated viral load. Xu *et al*. (2020) (29) showed that a 30-minute application of Listerine® Antiseptic Original is effective to moderately effective in reducing SARS-CoV-2 viral load. In this work, a 50% dilution of the mouthwash was effective in inactivating the virus, resulting in fluorescence intensity of 0 RLU, however, a 5% dilution of the same mouthwash resulted in a fluorescence intensity greater than $2x10^4$ RLU. These results are not to be compared to the other studies' results or EN 14476.

Synthesis of results

TABLE 4. *In vivo* efficacy of different mouthwashes on SARS-CoV-2 viral load.

Publication	Study design	Setting	Number of included participants	Assessment of viral load	Product, duration of rinse	Comparison	Results
Seneviratne *et al*. (2020) (21)	Randomized controlled trial	Hospitalized patients with a nasal swab and saliva RT-PCR positive for SARS-CoV-2. Mean age per group ± SD: PVP-I (n = 4): 40.7 ± 11.5; CHX (n = 6): 43.6 ± 8.6; CPC (n = 4): 35.7 ± 8.5; Water (n = 2): 36 ± 14.1 Single rinse performed in a single day.	16	Saliva (passive drool), via RT-PCR	PVP-I (0.5%), 30 s; CHX (0.2%), 30 s; CPC (0.075%), 30 s	Water	Ct values detected in all 16 patients were within the range of 15.6–34.5, with a mean value of 27.7 ± 4.8; Results are presented in form of fold change calculated as a ratio between Ct value at different timepoints and Ct value at baseline. PVP-I: statistically significant increase in fold change was obtained only at 6 h (*ratio* = 1) post-rinsing with PVP-I in comparison with water ($p < 0.01$). In comparison to the water group, the PVP-I group patients had higher fold increases in Ct value after 5 min (*ratio* = 1.1) and 3 h (*ratio* = 1.2) of post-rinsing, but no statistical significance was achieved. CHX: patients demonstrated a varied effect among saliva Ct values after 5-min rinsing and hence further studies with a larger sample size are required to determine its significance. CPC: statistically significant increase in fold change of Ct value at 5 min (*ratio* = 1) and 6 h (*ratio* = 0.9) was observed post-rinsing with CPC mouth-rinse compared to the water group patients ($p < 0.05$). Although the fold changes in Ct values were higher at 3 h (*ratio* = 0.9) in the CPC group, no statistical significance was achieved (*P* = 0.20).
Lamas *et al*. (2020) (23)	Uncontrolled before-after study	Hospitalized and home-isolated patients with positive RT-PCR for SARS-CoV-2 in nasopharyngeal exudate	4	Nasopharyngeal swab and saliva (method not explained), via RT-PCR	PVP-I (1%), 60 s	-	In 2 out of 4 patients, PVP-I resulted in a significant drop (~ 5 $\log_{10}$ and ~ 2 $\log_{10}$ reductions in salivary viral load in each patient) which remained for at least 3 h.

Publication	Study design	Setting	Number of included participants	Assessment of viral load	Product, duration of rinse	Comparison	Results
		with a median age of 63.5 years. Single rinse performed in a single day.					
Gottsauner *et al*. (2020) (19)	Uncontrolled before-after study	Hospitalized patients with a positive test for SARS-CoV-2 within the last 72 h with a median age of 55 years. Single rinse performed in a single day.	10	Oropharyngeal swab, via RT-PCR	H_2O_2 (1%), 30 s	-	Viral load decrease of 0.3×10^3 copies/mL. No significant differences were observed between the baseline viral load and viral load 30 min after the 1% H_2O_2 mouthrinse (P = 0.96).
Yoon *et al*. (2020) (17)	Uncontrolled before-after study	Hospitalized patients diagnosed with COVID-19 with a median age of 55.5 years. One rinse per day on two non-consecutive days (Day 3 and 6 of the study)	2	Saliva (method not specified), via RT-PCR	CHX (0.12%), 30 s	-	The viral load in the saliva decreased transiently for 2 h after using the CHX mouthwash, but it increased again at 2-4 h post-mouthwash. On day 3, viral load was not detected at 1 h and 2 h post rinse, on both patients. One of the patients showed a baseline viral load of 6.86 $\log_{10}$ and the other of 4.87 $\log_{10}$. On day 6, one hour after using the mouthwash, there was no reduction in viral load in one patient.

Note: **CHX:** Chlorhexidine Gluconate; **CPC:** Cetylpyridinium Chloride; **Ct:** Cycle threshold; **h:** hours; **H_2O_2:** Hydrogen Peroxide; **log**: logarithm; **min:** minutes; **PVP-I:** Povidone-iodine; **RT-PCR:** Reverse Transcription Polymerase Chain Reaction; **s**: seconds;

TABLE 5. *In vitro* efficacy of different mouthwashes on SARS-CoV-2 viral load.

Publication	SARS-CoV-2 strain(s); Cellular line	Test mouthwashes (concentrations)	Comparison	Interfering substances	Contact time	Results
A. PVP-I						
Bidra *et al*. (2020) (27)	USA-WA1/2020; Vero 76	PVP-I (0.5%, 1.25%, 1.5%)	Water; Ethanol (70%)	Clean	15 s 30 s	15 s: > 4.33 log_{10} reduction of the infectious virus for all concentrations 30 s: > 3.63 log_{10} reduction of the infectious virus for all concentrations.
Xu *et al*. (2020) (29)	USA-WA1/2020; HEK293T, HeLa	PVP-I (10%) at different final dilutions: 5%, 0.5%, and 0.05%	-	No information available	30 min	Only the 5% dilution of PVP-I was effective in inactivating the viruses (0 RLU).
Pelletier *et al*. (2020) (30)	USA-WA1/2020; Vero 76	Oral Rinse PVP-I antiseptic (0.5%, 0.75%, 1.5%) [(i)]	Water; Ethanol (70%)	Clean	60 s	After incubation with each nasal/oral antiseptic, viral load decrease of > 4 log_{10} infectious viruses for all concentrations.
Frank *et al*. (2020) (32)	USA-WA1/2020; Vero 76	PVP-I (0.5%, 1.25%, 2.5%)	Water; Ethanol (70%)	Clean	15 s 30 s	15 s: the solutions tested were effective at reducing the viral load > 3 log_{10} for all concentrations 30 s: the solutions were effective at reducing the viral load > 3.33 log_{10} for all concentrations
Hassandarvish *et al*. (2020) (18)	SARS-COV-2/MY/UM/6-3, TIDREC; Vero E6	PVP-I (0.5%, 1%)	Water	Clean; Dirty (3.0 g/L BSA + 3 ml/L human erythrocytes)	15 s 30 s 60 s	15 s: 1% PVP-I reduced > 5 log_{10} viral titers. 0.5% PVP-I reduced > 4 log_{10} viral load 30 s: 0.5% and 1% PVP-I reduced > 5 log_{10} viral titers 60 s: 0.5% and 1% PVP-I reduced > 5 log_{10} viral titers.
Meyers *et al*. (2020) (31)	HCoV 229e; HUH7	Betadine® 5%: PVP-I (5%)	-	Dirty (200 µL of 5% BSA)	30 s 60 s 120 s	30s: Decrease in viral load between > 3 log_{10} to < 4 log_{10} 60 s: Decrease in viral load between > 3 log_{10} to > 4 log_{10} 120s: > 4 log_{10} reduction in viral load.

Publication	SARS-CoV-2 strain(s); Cellular line	Test mouthwashes (concentrations)	Comparison	Interfering substances	Contact time	Results
Anderson *et al*. (2020) (22)	hCoV-19/Singapore/2/2020; Vero E6	Antiseptic solution: PVP-I (10%); Antiseptic skin cleanser: PVP-I (7.5%); Gargle and mouthwash: PVP-I (1.0%), 1:2 dilution; Throat spray: PVP-I (0.45%)	PBS	Dirty (0.3 g/L BSA)	30 s	≥ 4 log_{10} reduction of SARS-CoV-2 titers, for all the products.
Bidra *et al*. (2020) (28)	USA-WA1/2020; Vero 76	PVP-I (0.5%, 0.75%, 1.5%)	Water; Ethanol (70%)	Clean	15 s 30 s	15 s: the solutions reduced > 3 log_{10} of the viral load 30 s: the tested solutions reduced > 3.33 log_{10} of the viral load.
Meister *et al*. (2020) (20)	BetaCoV/Germany/Ulm/01/2020, BetaCoV/Germany/Ulm/02/2020, UKEssen; Vero E6	Iso-Betadine® mouthwash 1.0%: PVP-I (1%);	Cell culture medium	Dirty (100 µL mucin type I-S, 25 µL BSA Fraction V, and 35 µL yeast extract)	30 s	Iso-Betadine® mouthwash reduced viral infectivity to up to 3 log_{10}.
Statkute *et al*. (2020)* (25)	England 2; Vero E6	Videne®: PVP-I (7.5%)	-	Dirty (100 µL mucin type I-S, 25 µL BSA Fraction V, and 35 µL yeast extract	30 s	Videne® had an effect of ~ 3 log_{10} reduction.
Davies *et al*. (2020)* (26)	England 2; Vero E6	Povident: PVP-I (0.58%)	PBS	Clean	60 s	≥ 4.1 log_{10} reduction or[(ii)] ≥ 5.2 log_{10} reduction

Publication	SARS-CoV-2 strain(s); Cellular line	Test mouthwashes (concentrations)	Comparison	Interfering substances	Contact time	Results
B. H_2O_2						
Bidra *et al*. (2020) (27)	USA-WA1/2020; Vero 76	H_2O_2 (1.5%, 3%)	Water; Ethanol (70%)	Clean	15 s 30 s	15 s: 1.5% H_2O_2 reduced 1.33 log_{10} infectious virus. 3% H_2O_2 reduced 1.0 log_{10} infectious virus 30 s: 1.5% H_2O_2 reduced 1.0 log_{10} infectious virus. 3% H_2O_2 reduced 1.8 log_{10} infectious virus.
Xu *et al*. (2020) (29)	USA-WA1/2020; HEK293T, HeLa	Colgate® Peroxyl®: H_2O_2 (1.5%) at different dilutions: 0.75%, 0.075%, and 0.0075%	-	No information available	30 min	0.75% and 0.075% Colgate® Peroxyl® were effective in inactivating the viruses (0 RLU).
Meyers *et al*. (2020) (31)	HCoV 229e; HUH7	Peroxide Sore Mouth Cleanser®: H_2O_2 (1.5%); H_2O_2 solution diluted to 1.5% in PBS: H_2O_2 (1.5%); Orajel™ Antiseptic Rinse: H_2O_2 (1.5%); menthol (0.1%)	-	Dirty (200 µL of 5% BSA)	30 s 60 s 120 s	Virus load reduction between < 1 log_{10} to 2 log_{10} for all concentrations and contact times.
Meister *et al*. (2020) (20)	BetaCoV/Germany/Ulm/01/2020, BetaCoV/Germany/Ulm/02/2020, UKEssen; Vero E6	Cavex oral rinse: H_2O_2 (concentration unkown)	Cell culture medium	Dirty (100 µL mucin type I-S, 25 µL BSA Fraction V, and 35 µL yeast extract)	30 s	Viral load decrease between 0.3 log_{10} and 1.78 log_{10}.
Davies *et al*. (2020)* (26)	England 2; Vero E6	Peroxyl®: H_2O_2 (1.5%)	PBS	Clean	60 s	Reduction of the virus titer by 0.2 log_{10}.
C. CHX						
Xu *et al*. (2020) (29)	USA-WA1/2020; HEK293T, HeLa	CHX (0.12%) used in different final dilutions: 0.06%, 0.006%, and 0.0006%	-	No information available	30 min	0.06% CHX was effective in inactivating the viruses (0 RLU). 0.006% CHX had a moderate anti-viral effect (> $2x10^4$ RLU).

Publication	SARS-CoV-2 strain(s); Cellular line	Test mouthwashes (concentrations)	Comparison	Interfering substances	Contact time	Results
Meister *et al*. (2020) (20)	BetaCoV/Germany/Ulm/01/2020, BetaCoV/Germany/Ulm/02/2020, UKEssen; Vero E6	Chlorhexamed® Forte: CHX (concentration unknown); Dynexidin® Forte 0.2%: CHX (0.2%)	Cell culture medium	Dirty (100 µL mucin type I-S, 25 µL BSA Fraction V, and 35 µL yeast extract)	30 s	Viral load decrease between 0.3 log_{10} and 1.78 log_{10}.
Steinhauer *et al*. (2020)* (16)	No available information	CHX: 0.1% and 0.2% (used in different dilutions – 0.08% and 0.16%)	Formaldehyde	Clean	15 s 30 s 60 s 5 min 10 min	Both formulations had > 1 log_{10} reduction of the viral load after 60 s and 5 min (CHX 0.2%) and after 10 min (CHX 0.1%).
Davies *et al*. (2020)* (26)	England 2; Vero E6	CHX Antiseptic Mouthwash: CHX (0.2%); Corsodyl (Alcohol Free Mint Flavour): CHX (0.2%)	PBS	Clean	60 s	CHX Antiseptic Mouthwash: 0.5 log_{10} reduction. Corsodyl: 0.4 log_{10} reduction.
D. CPC						
Meyers *et al*. (2020) (31)	HCoV 229e; HUH7	Crest® Pro-Health™: CPC (0.07%)	-	Dirty (200 µL of 5% BSA)	30 s 60 s 120 s	Crest® Pro-Health™ decreased viral load by at least 3 log_{10} to > 4 log_{10} for all contact times.
Statkute *et al*. (2020)* (25)	England 2; Vero E6	Dentyl® Dual Action: CPC (0.05%-0.1%), Other active ingredients: isopropyl myristate, Mentha Arvensis extract; Dentyl® Fresh Protect: CPC (0.05%-0.1%), Other active ingredients: xylitol;	-	Dirty (100 µL mucin type I-S, 25 µL BSA Fraction V, and 35 µL yeast extract	30 s	Dentyl® mouthwashes completely eliminated the virus (> 5 log_{10} reductions).

Publication	SARS-CoV-2 strain(s); Cellular line	Test mouthwashes (concentrations)	Comparison	Interfering substances	Contact time	Results
Muñoz-Basagoiti *et al*. (2020)* (24)	SARS-CoV-2 isolated from a nasopharyngeal swab; Vero E6	Vitis® CPC Protec: 2.063 mM of CPC; CPC: 10 mM of CPC diluted in distilled water	Culture cell media	Clean	120 s	Viral load decreased by 3 log_{10} for all test solutions.
Green *et al*. (2020)* (34)	HCoV-SARS 229E; MRC-5	Mouthwash containing CPC (0.07%), sodium fluoride, and flavor oil;	-	Clean	30 s 60 s	Viral load decrease of 3.08 log_{10} for all contact times.
E. Other mouthwashes						
Xu *et al*. (2020) (29)	USA-WA1/2020; HEK293T, HeLa	Listerine® Antiseptic Original: Ethanol (20-30%), Thymol 0.064%, Methyl salicylate 0.06%, Menthol (Racementhol) 0.042%, Eucalyptol 0.092% - (50%, 5%, and 0.5% of the original solutions)	-	No information available	30 min	50% dilution of Listerine® Antiseptic was effective in inactivating the viruses (0 RLU). Treatment with 5% Listerine® had a moderate anti-viral effect (> $2x10^4$ RLU).
Meyers *et al*. (2020) (31)	HCoV 229e; HUH7	Listerine® Antiseptic: Eucalyptol (0.092%), Menthol (0.042%), Methyl Salicylate (0.06%), Thymol (0.064%); Listerine® Ultra: Eucalyptol (0.092%), Menthol (0.042%), Methyl Salicylate (0.06%), Thymol (0.064%); Equate™: Eucalyptol (0.092%), Menthol (0.042%), Methyl Salicylate (0.06%), Thymol (0.064%);	-	Dirty (200 µL of 5% BSA)	30 s 60 s 120 s	Listerine® Antiseptic decreased viral load by > 4 log_{10}. After incubation times of 60 s and 120 s, no remaining infectious virus was detected. Listerine® Ultra, Equate™, and Antiseptic Mouthwash showed lower efficacy, (particularly after 30 s). However, these latter mouthwashes decreased infectious virus titers by > 2 log_{10}.

Publication	SARS-CoV-2 strain(s); Cellular line	Test mouthwashes (concentrations)	Comparison	Interfering substances	Contact time	Results
		Antiseptic Mouthwash (CVS): Eucalyptol (0.092%), Menthol (0.042%), Methyl Salicylate (0.06%), Thymol (0.064%)				
Meister *et al*. (2020) (20)	BetaCoV/Germany/Ulm/01/2020, BetaCoV/Germany/Ulm/02/2020, UKEssen; Vero E6	Dequonal®: Dequalinium chloride, benzalkonium chloride; Listerine® Cool Mint®: Ethanol, essential oils; Octenident® mouthwash: Octenidine dihydrochloride; ProntOral® mouthwash: Polyaminopropyl biguanide (polyhexanide)	Cell culture medium	Dirty (100 µL mucin type I-S, 25 µL BSA Fraction V, and 35 µL yeast extract)	30 s	Dequonal® and Listerine® Cool Mint® significantly reduced viral infectivity to up to 3 log_{10}. Octenident® virucidal activities could be observed with reduction factors ranging between 0.3 log_{10} to 1.78 log_{10}; With ProntOral®, one strain was only moderately reduced and the other 2 strains were inactivated.
Statkute *et al*. (2020)* (25)	England 2; Vero E6	Corsodyl: ethanol (7 %), CHX (0.2%), Other active ingredients: peppermint oil; Listerine® Cool Mint®: ethanol (21%), Other active ingredients: thymol (0.064%), eucalyptol (0.092%), methyl salicylate (0.060%) and menthol (0.042 %); Listerine® Advanced Gum Treatment: ethanol (23 %), Other active ingredients: ethyl	-	Dirty (100 µL mucin type I-S, 25 µL BSA Fraction V, and 35 µL yeast extract	30 s	Listerine® Advanced Gum Treatment eliminated the virus (> 5 log_{10} reduction). SCD Max and Listerine® Cool Mint® had a moderate effect (~ 3 log_{10} reduction). Corsodyl was relatively ineffective (< 2 log_{10} reduction).

Publication	SARS-CoV-2 strain(s); Cellular line	Test mouthwashes (concentrations)	Comparison	Interfering substances	Contact time	Results
		lauroyl arginate HCl (0.147%); SCD Max: CPC (0.07-0.1%), sodium citric acid (0.05%), Other active ingredients: sodium monofluorophosphate;				
Steinhauer *et al*. (2020)* (16)	No available information	octenisept®: octenidine dihydrochloride 0.1%, and phenoxyethanol 20% (used in 20% (v/v) and 80% (v/v) concentration)	Formaldehyde	Clean	15 s 30 s 60 s	Reduction of titers by ≥ 4.38 log_{10} was observed for both concentrations and all contact times.
Davies *et al*. (2020)* (26)	England 2; Vero E6	Listerine® Advanced Defence Sensitive: dipotassium oxalate (1.4%); Listerine® Total Care: Eucalyptol, thymol, menthol, sodium fluoride, zinc fluoride; OraWize+ Aqualution Systems stabilised hypochlorous acid (0.01-0.02%)	PBS	Clean	60 s	Listerine® Advanced Defence Sensitive: ≥ 3.5 log_{10} or[(ii)] ≥ 4.2 $log_{10;}$ Listerine® Total Care: ≥ 4.1 log_{10} reduction or[(ii)] ≥ 5.2 $log_{10;}$ OraWize+: ≥ 5.5 log_{10} or[(ii)] 0.4 $log_{10.}$
Muñoz-Basagoiti *et al*. (2020)* (24)	SARS-CoV-2 isolated from a nasopharyngeal swab; Vero E6	Perio Aid® Intensive Care: 1.47 mM of CPC and 1.33 mM of CHX	Culture cell media	Clean	120 s	No impact on SARS-CoV-2 infectivity, when compared to untreated virus.
Mantlo *et al*. (2020)* (33)	USA-WA1/2020; Vero Cells	CupriDyne®: iodine and cuprous iodide (250 ppm, 25 ppm, 2.5 ppm)	Water (boiling and at room temperature)	Clean	10 min 30 min 60 min	CupriDyne® (25 ppm or 2.5 ppm) were not found to cause a statistically significant difference in SARS-CoV-2 titers; CupriDyne® (250 ppm) was shown to effectively inactivate the virus to a statistically significant extent after 10, 30, and 60 min;

Publication	SARS-CoV-2 strain(s); Cellular line	Test mouthwashes (concentrations)	Comparison	Interfering substances	Contact time	Results
						After incubation with undiluted (250 ppm) CupriDyne® for 10 min, viral titers dropped by 1 log_{10}. Viral titers dropped 2 log_{10} after incubation with undiluted CupriDyne® for 30 min. Further incubation with undiluted CupriDyne® for 60 min reduced viral titers below the limit of detection.
Green *et al*. (2020)* (34)	HCoV-SARS 229E; MRC-5	Mouthwash containing ethanol (15.7%), sodium fluoride, and flavor oil. Mouthwash containing zinc sulfate heptahydrate (0.2%), sodium fluoride, and flavor oil. Mouthwash containing a mix of Amyloglucosidase, Glucose Oxidase, Lysozyme, Colostrum, Lactoferrin, Lactoperoxidase, sodium fluoride, and flavor oil.	-	Clean	30 s 60 s	Contact with ethanol, zinc, and enzyme, and protein mouthwashes did not provide a substantial reduction in viral counts. Zinc: after 30 s reduction of 1.17 (± 0.38) log_{10}, after 60 s reduction of 1.83 (± 0.14) log_{10}; Enzymes and proteins: after 30 s reduction of 0.25 (± 0.25) log_{10}, after 60 s reduction of 0.25 (± 0.25) log_{10}; Ethanol: after 30 s reduction of 0.17 (± 0.29) log_{10}, after 60 s reduction of 0.33 (± 0.29) log_{10}.
Zoltán (2020)* (35)	USA-WA1/2020; Vero 76	200 µg elemental iodine/mL at three dilutions (1:1; 2:1, and 3:1)	Water; Ethanol (70%)	Clean	60 s 90 s	60 s: 3:1 dilution reduced viral titer by 2 log_{10}, while 2:1 dilution reduced viral titers by 1.7 log_{10} 90 s: 1:1 dilution reduced viral titer by 2 log_{10}.

Note: ***** preprint article; **~** should be read as "approximately"; **BSA:** Bovine Serum Albumin; **CHX:** Chlorhexidine Gluconate; **CPC:** Cetylpyridinium Chloride; **h:** hours; **H_2O_2:** Hydrogen Peroxide; **(i)** A nasal PVP-I antiseptic (0.5%, 1.25%, 2.5%) was studied as a complement to the oral antiseptic; **(ii)** depending on initial viral concentration (higher, lower); **log:** logarithm; **min:** minutes; **mM:** Millimolar; **PBS:** phosphate buffered saline; **ppm:** parts per million; **PVP-I:** Povidone-iodine; **RLU:** Relative Light Units; **s:** seconds;

TABLE 6. PVP-I *in vitro* effect on SARS-CoV-2 oral viral load. Results interpretation accordingly to EN 14476, considering a reduction on viral load greater or equal than 4 log_{10} as a high efficacy (+), a reduction greater than 3 log_{10} and lower than 4 log_{10} as a moderate efficacy (±), and a reduction lower than 3 log_{10} as a low efficacy (−).

PVP-I											
Concentration	Contact time	Bidra *et al.* (27)	Pelletier *et al.* (30)	Frank *et al.* (32)	Hassandarvish *et al.* (18)	Meyers *et al.* (31)	Anderson *et al.* (22)	Bidra *et al.* (28)	Meister *et al.* (20)	Statkute *et al.** (25)	Davies *et al.** (26)
~ 0.5%[i]	15 s	+		±	+			±			
	30 s	±		±	+		+	±			
	60 s		+		+						+
0.75%	15 s							±			
	30 s							±			
	60 s		+								
1.0%	15 s				+						
	30 s				+		+		−		
	60 s				+						
1.25%	15 s	+		±							
	30 s	±		±							
	60 s		+								
1.5%	15 s	+						±			
	30 s	±						±			
	60 s		+								
2.5%	15 s			±							
	30 s			±							
	60 s		+								
> 2.5%[ii]	15 s										
	30 s					±	+			−	
	60 s					± +					

[i] ranging from 0.45% to 0.58%; [ii] concentrations up to 10%; ~ should be read as "approximately"; * preprint article.

TABLE 7. H_2O_2, CHX, and CPC mouthwashes *in vitro* effect on SARS-CoV-2 oral viral load. Results interpretation accordingly to EN 14476, considering a reduction on viral load greater or equal than 4 log_{10} as a high efficacy (+), a reduction greater than 3 log_{10} and lower than 4 log_{10} as a moderate efficacy (±), and a reduction lower than 3 log_{10} as a low efficacy (−).

Mouthwash	Concentration	Contact time	Bidra *et al.* (27)	Meyers *et al.* (31)	Davies *et al.** (26)	Meister *et al.* (20)	Steinhauer *et al.** (16)	Statkute *et al.** (25)	Green *et al.** (34)
H_2O_2	1.5%	15 s	−						
		30 s	−	−					
		60 s		−	−				
	3%	15 s	−						
		30 s	−						
		60 s							
CHX	< 0.2%[i]	15 s					−		
		30 s					−		
		60 s					−		
	0.2%	15 s							
		30 s				−			
		60 s			−				
CPC	0.05% to 0.1%	15 s							
		30 s		± +				+	±
		60 s		± +					±

[i] includes concentrations of 0.08% and 0.16%; * preprint article.

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TABLE 8. Other mouthwashes *in vitro* effect on SARS-CoV-2 oral viral load. Results interpretation accordingly to EN 14476, considering a reduction on viral load greater or equal than 4 log_{10} as a high efficacy (+), a reduction greater than 3 log_{10} and lower than 4 log_{10} as a moderate efficacy (±), and a reduction lower than 3 log_{10} as a low efficacy (−).

Mouthwash	Contact time	Meyers *et al.* (31)	Meister *et al.* (20)	Statkute *et al.** (25)	Steinhauer *et al.** (16)	Davies *et al.** (26)	Green *et al.** (34)	Zoltán* (35)
Listerine® Antiseptic	15 s							
	30 s	+						
	60 s	+						
Listerine® Ultra	15 s							
	30 s	−						
	60 s	−						
Listerine® Cool Mint®	15 s							
	30 s		−	−				
	60 s							
Listerine® Advanced Gum Treatment	15 s							
	30 s			+				
	60 s							
Listerine® Advanced Defence Sensitive	15 s							
	30 s							
	60 s					± +		
Listerine® Total Care	15 s							
	30 s							
	60 s					+		
Equate™	15 s							
	30 s	−						
	60 s	−						
Antiseptic Mouthwash (CVS)	15 s							
	30 s	−						
	60 s	−						
Dequonal®	15 s							
	30 s		−					
	60 s							
Octenident®	15 s							
	30 s		−					
	60 s							
ProntOral®	15 s							
	30 s		−					
	60 s							
Corsodyl	15 s							
	30 s			−				
	60 s							
SCD Max	15 s							
	30 s			−				
	60 s							

Mouthwash	Contact time	Meyers *et al.* (31)	Meister *et al.* (20)	Statkute *et al.** (25)	Steinhauer *et al.** (16)	Davies *et al.** (26)	Green *et al.** (34)	Zoltán* (35)
octenisept®	15 s				+			
	30 s				+			
	60 s				+			
OraWize+	15 s							
	30 s							
	60 s					− +		
Mouthwash containing ethanol (15.7%), other ingredients	15 s							
	30 s						−	
	60 s						−	
Mouthwash containing zinc sulfate heptahydrate, other ingredients	15 s							
	30 s						−	
	60 s						−	
Mouthwash containing a mix of Amyloglucosidase, other ingredients	15 s							
	30 s						−	
	60 s						−	
Essential iodine solution	15 s							
	30 s							
	60 s							−

* preprint article.

4. DISCUSSION

DISCUSSION

Summary of evidence

In this systematic review, we included primary studies assessing the virucidal effect of mouthwashes regarding SARS-CoV-2, with these studies presenting a diverse set of methodologies and assessing a wide range of mouthwashes. Nevertheless, the use of PVP-I *in vitro* was assessed by most studies (11 primary studies), most of them showing some encouraging results. *In vivo* studies, however, do not seem to agree to such degree with the results of the *in vitro* studies, although serious methodologies limitations were found in those *in vivo* studies. Cetylpyridinium chloride was studied by some authors (1 *in vivo* and 4 *in vitro* papers), also showing some positive results. Although assessed by a limited number of studies, the use of H_2O_2 and CHX appeared to be ineffective both *in vivo* and *in vitro.* Therefore, while it has been shown that SARS-CoV-2 infects and replicates in salivary glands (especially on minor salivary glands which can turn into a source of the virus on saliva (37)) and other cells in the oral cavity, we found that using adequate mouthrinse solutions before dental setting may be beneficial, due to their potential virucidal properties.

The European Union identifies “mouthwashes” as a borderline product directed to the teeth, classifying them as “cosmetic products” since they aim to “exclusively or mainly cleaning them, perfuming them, changing their appearance, protecting them, keeping them in good condition or correcting body odors”. While such classification considers antimicrobial effects as a “secondary” property of mouthwashes (38), the Borderline Manual, updated in September 2020, also states that when a mouthwash or dental gel provides “treatment or prevention of infections, inflammation or other oral cavity diseases” it should not be interpreted as a cosmetic, but a medicinal product (38). Considering mouthwashes as antiseptics, they should follow regulating norms. As stated by Steinhauer *et al*. (2020) (16), the European Standards in the EN 14476 affirm the virucidal efficacy of an antiseptic when it causes a reduction equal or greater to 4 $\log_{10}$ in viral load. Due to the lack of regulation, we decided to compare our results to EN 14476 because it regulates the virucidal capacity of antiseptics and disinfectants although not being specific to oral rinses or oral care products. The International Organization for Standardization (ISO) defines on ISO 16408:2015 the chemical and physical properties of oral rinses, as well as test methods, but the guidelines for microbiological analysis are specific to mold, bacteria, and yeast, lacking virus instructions (39). There seems to be a lack of standardization on the evaluation of mouthwashes regarding virucidal properties.

Overall, only eight of the twenty included studies met, at some point, what is established by the European standards (16, 18, 22, 25-27, 30, 31), reducing SARS-CoV-2 oral viral load by greater or equal than 4 $\log_{10}$. On the other hand, one of the sixteen *in vitro* studies (29) and two of the four *in vivo* studies (19, 21) did not measure the reduction of viral load via a logarithmic reduction, hampering the analysis and interpretation according to the EN 14476 norm.

The included primary studies displayed substantial diversity in their methodologies and results presentation, limiting our capacity of comparing different mouthwashes. PVP-I-based mouthwashes appear to have the potential for reducing SARS-CoV-2 in the oral cavity, in some cases showing *in vitro* effectiveness at concentrations starting at 0.45% and application times of 15 seconds (22), and in the RCT, with a 30-second 0.2% CHX rinse, leading to a statistically significant reduction of viral load, verified after 6 hours (21). Nonetheless, these results have to be cautiously interpreted. The RCT has a high risk of bias and presents its results in an atypical manner, non-comparable to EN 14476. Besides that, it does not seem to exist a dose-response relationship (i.e., studies assessing the effect of higher PVP-I concentrations on SARS-CoV-2 viral load, do not appear to obtain better results) or a time-response relationship.

The use of PVP-I-based mouthwashes as antiseptics is already corroborated in other viruses, like MERS, influenza viruses, and human immunodeficiency virus (HIV). In other *in vitro* studies, 1% PVP-I mouthwashes reduced MERS viral load by 4 $\log_{10}$ within 15 seconds. Influenza viruses and HIV were also inactivated by more than 5 $\log_{10}$ and 4.5 $\log_{10}$, respectively, with application times up to 30 seconds. Conversely, coxsackievirus and poliovirus type 1 were not as sensitive to the action of PVP-I (40). Poliovirus is associated with an oral-oral transmission (41) and coxsackievirus to a saliva transmission route (42), and therefore PVP-I might not represent an effective approach in the reduction of these viruses' transmission. PVP-I application on iodine-allergic patients is usually a concern, but oral use has not been linked to any negative side effects in either adults or infants. Allergies and touch sensitivity to PVP-I are also uncommon (43). The use of PVP-I has been found to have a temporary effect on thyroid activity in some vulnerable patients with no significant effects on their health (44). In addition, PVP-I has low cytotoxicity (44), with some authors suggesting it is lower than CHX cytotoxicity (45). Several primary studies included in this systematic review analyzed PVP-I cytotoxicity, concluding there was no cytotoxicity with concentrations of up to 2.5% (18, 28, 30).

The use of CPC mouthwashes for the reduction of oral SARS-CoV-2 viral load also showed some encouraging results. The only *in vivo* study of these mouthwashes showed a reduction in viral load with a 30-second rinse (21). Also, *in vitro* results are promising, one study demonstrated that CPC could strongly reduce SARS-CoV-2 viral load (greater than 5 $\log_{10}$) with a 30-second application (25). Of note, CPC is also capable of inactivating *influenza* viruses both *in vitro* and *in vivo*, but only after 10 minutes (46).

In the included primary studies, H_2O_2 and CHX-based mouthwashes were found to be ineffective in reducing SARS-CoV-2 viral load. CHX and H_2O_2 are already currently used in some oral care products, however, CHX presents more capability in reducing oral plaque and is considered an excellent broad-spectrum antimicrobial agent (47, 48). Nevertheless, H_2O_2 is also recognized for its activity against anaerobic oral bacteria (49). In the dental setting, the pre-procedural use of H_2O_2 mouthwashes was advised to reduce oral SARS-CoV-2 viral load by national and international government agencies and

professional associations (50-53) so there is a need to reconsider these directives.

Some complex mouthwashes like Listerine® Total Care, Listerine® Advanced, and Listerine® Antiseptic showed promising results in reducing SARS-CoV-2 viral load in the oral cavity, although were evaluated by only one or two studies. These mouthwashes' use as a coadjutant in oral health is well established, possibly aiding in reducing oral plaque and gingivitis (54). In a RCT, an ethanol-free Listerine® (Listerine® Zero) led to a statistically significant reduction in dental plaque (55). In a study by Fine *et al*. (1996) (56), Listerine® Cool Mint® reduced aerobic bacteria counts by 92% and anaerobic bacteria by 88% when compared to a 5% hydroalcohol mouthrinse control. However, Listerine® Cool Mint® showed only an approximately 3 $\log_{10}$ reduction in SARS-CoV-2 viral titers, a reduction inferior to the one proposed on EN 14476.

In vitro infectivity assays, like endpoint dilution assays, were used in most of the *in vitro* studies included in this systematic review. Infectivity assays allow us to quantify virions that are capable of infecting cells (57). All *in vivo* studies performed using RT-PCR tests to quantify the viral load, which may not be capable of distinguishing between infectious and non-infectious virus particles (57-59). RT-PCR poses a quick and easily reproducible way of detecting viruses (57), but it is expensive and incapable of directly quantifying viral load (60). On the other hand, endpoint dilution assays, being more human-labor demanding, are very sensitive, cheap, and allow direct and efficient quantification of viral load (60).

The use of other PCR techniques like viability-PCR (v-PCR) could be addressed to study the infectivity of virus samples collected from patients. Hepatitis viruses A (HVA) and E (HVE) were already studied using this method (61). Randazzo *et al.* (2018) (61) base their approach on capsid integrity to distinguish between infectious and non-infectious viruses. The United States Environmental Protection Agency (EPA), the Centers for Disease Control and Prevention (CDC), and the Lawrence Livermore National Laboratory are currently developing a Rapid Viability-Reverse Transcription PCR (RV-RTPCR) on surfaces and objects (62). This could be an important step to better understand the viability of SARS-CoV-2 on the oral cavity and the respective impact of mouthwashes on the virus infectivity.

There are other protocols for quantifying the viral load on *in vivo* conditions that could be considered in the matter of this thematic. The analysis of aerosols could be also a realistic way to study the impact of dental procedures on the dissemination of viral particles. Choi *et al.* (2018) (63) performed a study on aerosol sampling in the emergency department of a university hospital, collecting a total of forty-four samples, twelve of which positive to known respiratory viruses - influenza A, influenza D, and adenovirus. Lednicky *et al.* (2020) (64) demonstrated the generation of aerosols containing SARS-CoV-2 virions by patients with COVID-19 respiratory manifestations even in the absence of aerosol-generating procedures, which can lead to virus transmission. The authors were also able to quantify the virus generated, detected from a distance higher or equal to two meters. These results highlight

the importance of rubber dam isolation use whenever possible given that rubber dam isolation can reduce aerosol pathogen load by 70% (65).

In addition to the wide diversity of study methodologies, and results' interpretation, a major limitation of this systematic review is the lack of RCTs, with only one meeting the eligibility criteria (21). The validity of the conclusions is affected by the bias of the included primary studies, in this case, regarding the high risk of bias of the RCT. Besides, the other three *in vivo* studies' have important limitations in their designs, including the absence of randomization or even a control group, and a relatively low number of included patients. This prompts a low level of evidence and hampers the precision of their estimates, respectively. Although *in vitro* studies are also part of the tests proposed by the EN 14476, their results cannot be directly transposed to *in vivo* application of these mouthwashes. *In vivo* studies should also be conducted with a better study design, including a higher number of patients and a control solution (e.g. water), allowing a better interpretation of results with a greater level of evidence.

A recurrent inadequacy found in the selected studies was the existence of studies that include application times not feasible in clinical practice. Some *in vitro* studies had application times of 30 minutes (29), and one preprint article also considered an application with a duration of 60 minutes (33). Patients are normally only able to gargle for a short period (66), usually up to 60 seconds, so we find these application times unrealistic and not adequate for clinical practice.

Suggestions for Future Studies

There is a need for more *in vivo* and *in vitro* studies on different mouthwashes that consider adequate and realistic application times, of up to 60 seconds. Well-designed randomized controlled trials with a larger number of patients should be considered a priority when it comes to the design of the *in vivo* studies. Based on results from already published primary studies – reviewed in this systematic review –, future studies should primarily focus on PVP-I and CPC-based mouthwashes. Furthermore, the studies should present their results in form of a logarithmic reduction that can be compared to the goal proposed by EN 14476. Studying mouthwash-induced cytotoxicity should also be a concern when assessing virucidal properties of the different mouthwashes. The study of viral viability post rinse and their presence in aerosols should also be considered to better assess the real impact of virus dissemination in the dental setting. Guidelines for the execution of studies with standardized and comparable methodologies, regarding the evaluation of the effect of mouthwashes on viruses are needed.

Conclusions

In conclusion, the use of PVP-I-based solutions as a preprocedural rinse in dental setting might have some virucidal properties against SARS-CoV-2 in light of the current knowledge, although more randomized controlled trials are necessary. CHX and H_2O_2 appear to be ineffective in reducing SARS-CoV-2

oral load and their use as a pre-procedural mouthwash aiming to reduce SARS-CoV-2 oral load should be revised. More randomized controlled trials together with *in vitro* studies are urgent to further evaluate PVP-I and CPC-based mouthwashes and test other commercially available mouthwashes showing potential results on SARS-CoV-2 load reduction.

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APPENDIX

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#1 PROSPERO Registration

Systematic review

1. * Review title.

Give the title of the review in English

The effect of mouthwashes on SARS-CoV-2 viral load: a systematic review

2. Original language title.

For reviews in languages other than English, give the title in the original language. This will be displayed with the English language title.

3. * Anticipated or actual start date.

Give the date the systematic review started or is expected to start.

15/11/2020

4. * Anticipated completion date.

Give the date by which the review is expected to be completed.

15/05/2021

5. * Stage of review at time of this submission.

Tick the boxes to show which review tasks have been started and which have been completed. Update this field each time any amendments are made to a published record.

Reviews that have started data extraction (at the time of initial submission) are not eligible for inclusion in PROSPERO. If there is later evidence that incorrect status and/or completion date has been supplied, the published PROSPERO record will be marked as retracted.

This field uses answers to initial screening questions. It cannot be edited until after registration.

The review has not yet started: No

Review stage	Started	Completed
Preliminary searches	Yes	Yes
Piloting of the study selection process	Yes	Yes
Formal screening of search results against eligibility criteria	Yes	No
Data extraction	No	No
Risk of bias (quality) assessment	No	No
Data analysis	No	No

Provide any other relevant information about the stage of the review here.

6. * Named contact.

The named contact is the guarantor for the accuracy of the information in the register record. This may be any member of the review team.

António Silva

Email salutation (e.g. "Dr Smith" or "Joanne") for correspondence:

Mr Silva

7. * Named contact email.

Give the electronic email address of the named contact.

up201606687@edu.fmd.up.pt

8. Named contact address

Give the full institutional/organisational postal address for the named contact.

9. Named contact phone number.

Give the telephone number for the named contact, including international dialling code.

10. * Organisational affiliation of the review.

Full title of the organisational affiliations for this review and website address if available. This field may be completed as 'None' if the review is not affiliated to any organisation.

Faculdade de Medicina Dentária da Universidade do Porto (FMDUP)

Organisation web address:

https://sigarra.up.pt/fmdup/pt/web_page.inicial

11. * Review team members and their organisational affiliations.

Give the personal details and the organisational affiliations of each member of the review team. Affiliation refers to groups or organisations to which review team members belong. **NOTE: email and country now MUST be entered for each person, unless you are amending a published record.**

Mr António Silva. FMDUP
Professor Benedita Maia. FMDUP (Faculdade de Medicina Dentária da Universidade do Porto)
Professor Bernardo Pinto. FMUP (Faculdade de Medicina da Universidade do Porto)
Dr Maria Azevedo. Academic Center for Dentistry Amsterdam (ACTA)

12. * Funding sources/sponsors.

Details of the individuals, organizations, groups, companies or other legal entities who have funded or sponsored the review.

None

Grant number(s)

State the funder, grant or award number and the date of award

13. * Conflicts of interest.

List actual or perceived conflicts of interest (financial or academic).

None

14. Collaborators.

Give the name and affiliation of any individuals or organisations who are working on the review but who are not listed as review team members. **NOTE: email and country must be completed for each person, unless you are amending a published record.**

15. * Review question.

State the review question(s) clearly and precisely. It may be appropriate to break very broad questions down into a series of related more specific questions. Questions may be framed or refined using PI(E)COS or similar where relevant.

Is the use of mouthwash, compared to not using mouthwash, effective in reducing Sars-CoV-2 viral load?

16. * Searches.

State the sources that will be searched (e.g. Medline). Give the search dates, and any restrictions (e.g. language or publication date). Do NOT enter the full search strategy (it may be provided as a link or attachment below.)

PubMed, Web of Science and Scopus (search date: January 13th, 2021). MedRxiv and bioRxiv (search date: January 28th, 2021). Restrictions: published between 2020 and 2021

17. URL to search strategy.

Upload a file with your search strategy, or an example of a search strategy for a specific database, (including the keywords) in pdf or word format. In doing so you are consenting to the file being made publicly accessible. Or provide a URL or link to the strategy. Do NOT provide links to your search **results**.

Alternatively, upload your search strategy to CRD in pdf format. Please note that by doing so you are consenting to the file being made publicly accessible.

Do not make this file publicly available until the review is complete

18. * Condition or domain being studied.

Give a short description of the disease, condition or healthcare domain being studied in your systematic review.

This systematic review focus in the effect of the use of mouthwashes on SARS-CoV-2 viral load.

19. * Participants/population.

Specify the participants or populations being studied in the review. The preferred format includes details of both inclusion and exclusion criteria.

In vivo studies involving patients with or without COVID-19 and studies using saliva will be included. We will also include in vitro mouthwash experiments against strains of Sars-Cov-2 or surrogate mouthwash studies.

20. * Intervention(s), exposure(s).

Give full and clear descriptions or definitions of the interventions or the exposures to be reviewed. The preferred format includes details of both inclusion and exclusion criteria.

Usage of mouthwash at any dosage, such as hydrogen peroxide, chlorhexidine digluconate, povidone-iodine, essential oils, or any other antiviral substance.

21. * Comparator(s)/control.

Where relevant, give details of the alternatives against which the intervention/exposure will be compared (e.g. another intervention or a non-exposed control group). The preferred format includes details of both inclusion and exclusion criteria.

Absence of use of any type of mouthwash

22. * Types of study to be included.

Give details of the study designs (e.g. RCT) that are eligible for inclusion in the review. The preferred format includes both inclusion and exclusion criteria. If there are no restrictions on the types of study, this should be stated.

inclusion criteria: in vivo and in vitro studies assessing the effect of mouthwashes on SARS-CoV-2 viral load

exclusion criteria: Reviews, letters to the editor, personal opinions, product news, book chapters, case reports, congress abstracts, protocol suggestions, editorials, correspondence articles, recommendations, trial designs, hypothesis, and studies with animals were excluded.

23. Context.

Give summary details of the setting or other relevant characteristics, which help define the inclusion or exclusion criteria.

24. * Main outcome(s).

Give the pre-specified main (most important) outcomes of the review, including details of how the outcome is defined and measured and when these measurement are made, if these are part of the review inclusion criteria.

Determine the possible efficacy of mouthwash solutions on the reduction of SARS-CoV-2 viral load

* Measures of effect

Please specify the effect measure(s) for you main outcome(s) e.g. relative risks, odds ratios, risk difference, and/or 'number needed to treat.

Any decrease in viral load, virucidal impact against SARS-CoV-2, including PFU (plaque forming units) count, log cell count, virus inactivation percentages, inhibition zone in mm, relative light units

25. * Additional outcome(s).

List the pre-specified additional outcomes of the review, with a similar level of detail to that required for main outcomes. Where there are no additional outcomes please state ‘None’ or ‘Not applicable’ as appropriate to the review

None

* Measures of effect

Please specify the effect measure(s) for you additional outcome(s) e.g. relative risks, odds ratios, risk difference, and/or 'number needed to treat.

Not applicable

26. * Data extraction (selection and coding).

Describe how studies will be selected for inclusion. State what data will be extracted or obtained. State how this will be done and recorded.

After retrieving all the publications, a citation management software (EndNote® 20) will be used to eliminate duplicates. Subsequently, the titles and abstracts of the papers selected from the search results will be independently reviewed by two reviewers. Also in this phase, articles that do not follow the inclusion criteria or meet some of the exclusion criteria will be excluded. Finally, a full-text analysis will be performed by two reviewers on those included by abstract. Any difference will be discussed with a third reviewer and consensus will be found.

The following parameters will be considered: author, title, year, country, type of study, sample number and type, patients' characterization, intervention and control group, virus strain, type of mouthwash, concentration, number of mouthwashes per day, duration of the rinse, duration of the treatment, decrease in viral load. For in vitro studies we will also consider the cell lineage used and the existence of interfering substances. To extract data 2 reviewers are going to use an online questionary, developed by us. In case of any inconsistency of data collection, a third author will resolve it through discussion.

27. * Risk of bias (quality) assessment.

State which characteristics of the studies will be assessed and/or any formal risk of bias/quality assessment tools that will be used.

We intend to use the Cochrane Collaboration tool to assess risk of bias for randomized controlled trials. The risk of bias evaluation will be conducted separately by two reviewers and will be classified as "high risk of bias", "low risk of bias", or "unclear risk of bias" if there is any incomplete or unclear data. In the event of inadequate or unknown information, the author of the study will be contacted for clarification. In case of any inconsistency of data collection, a third author will resolve it through discussion.

28. * Strategy for data synthesis.

Describe the methods you plan to use to synthesise data. This **must not be generic text** but should be **specific to your review** and describe how the proposed approach will be applied to your data. If meta-analysis is planned, describe the models to be used, methods to explore statistical heterogeneity, and software package to be used.

Regarding each form of mouthwash compound tested, a description of the included studies will be presented with separated information for in vitro versus in vivo studies. Data will be extracted from each primary study. The results of the different primary studies will be summarized in a table, where data will be presented on the main variables (variables that define the population, intervention, comparator, and outcome), as well as the

number of participants and the results reported by each primary study regarding the reduction of viral load. Regardless of the possibility of carrying out the meta-analysis, a descriptive analysis of the results of the included primary studies will be carried out, with a comparison of the primary studies that have characteristics similar enough to be compared. In this sense, the results of the comparison between mouthwashes versus their absence concerning the reduction of the viral load will be presented, with the differences between primary studies duly considered. Since the reduction in viral load is a continuous variable, if possible, effect size measures will be pooled by random-effects meta-analysis, with the meta-analytical measure to be calculated and the uncertainty of the estimates assessed through 95% confidence intervals. Cochran's Q and I^2 statistics will be used to assess heterogeneity between primary studies, with significant heterogeneity indicated by p 0.10 associated with Cochran's Q statistic, and severe heterogeneity associated with I^2 50%.

29. * Analysis of subgroups or subsets.

State any planned investigation of 'subgroups'. Be clear and specific about which type of study or participant will be included in each group or covariate investigated. State the planned analytic approach.

Analyze data according to the type of study (in vivo or in vitro) and the method used for its evaluation.

30. * Type and method of review.

Select the type of review, review method and health area from the lists below.

Type of review

Cost effectiveness
No

Diagnostic
No

Epidemiologic
No

Individual patient data (IPD) meta-analysis
No

Intervention
No

Meta-analysis
Yes

Methodology
No

Narrative synthesis
No

Network meta-analysis
No

Pre-clinical
No

Prevention
No

Prognostic
No

Prospective meta-analysis (PMA)
No

Review of reviews
No

Service delivery
No

Synthesis of qualitative studies
No

Systematic review
Yes

Other
No

Health area of the review

Alcohol/substance misuse/abuse
No

Blood and immune system
No

Cancer
No

Cardiovascular
No

Care of the elderly
No

Child health
No

Complementary therapies
No

COVID-19
Yes

For COVID-19 registrations please tick all categories that apply. Doing so will enable your record to appear in area-specific searches

Chinese medicine
Diagnosis
Epidemiological
Genetics
Health impacts
Immunity
Long COVID
Mental health
PPE

Prognosis
Public health intervention
Rehabilitation
Service delivery
Transmission
Treatments
Vaccines
Other

Crime and justice
No

Dental
Yes

Digestive system
No

Ear, nose and throat
No

Education
No

Endocrine and metabolic disorders
No

Eye disorders
No

General interest
No

Genetics
No

Health inequalities/health equity
No

Infections and infestations
Yes

International development
No

Mental health and behavioural conditions
No

Musculoskeletal
No

Neurological
No

Nursing
No

Obstetrics and gynaecology
No

Oral health
Yes

Palliative care

No

Perioperative care
No

Physiotherapy
No

Pregnancy and childbirth
No

Public health (including social determinants of health)
No

Rehabilitation
No

Respiratory disorders
Yes

Service delivery
No

Skin disorders
No

Social care
No

Surgery
No

Tropical Medicine
No

Urological
No

Wounds, injuries and accidents
No

Violence and abuse
No

31. Language.

Select each language individually to add it to the list below, use the bin icon to remove any added in error.
English

There is an English language summary.

32. * Country.

Select the country in which the review is being carried out. For multi-national collaborations select all the countries involved.

Portugal

33. Other registration details.

Name any other organisation where the systematic review title or protocol is registered (e.g. Campbell, or The Joanna Briggs Institute) together with any unique identification number assigned by them. If extracted

data will be stored and made available through a repository such as the Systematic Review Data Repository (SRDR), details and a link should be included here. If none, leave blank.

34. Reference and/or URL for published protocol.

If the protocol for this review is published provide details (authors, title and journal details, preferably in Vancouver format)

Add web link to the published protocol.

Or, upload your published protocol here in pdf format. Note that the upload will be publicly accessible.

No I do not make this file publicly available until the review is complete

Please note that the information required in the PROSPERO registration form must be completed in full even if access to a protocol is given.

35. Dissemination plans.

Do you intend to publish the review on completion?

Yes

Give brief details of plans for communicating review findings.?

36. Keywords.

Give words or phrases that best describe the review. Separate keywords with a semicolon or new line. Keywords help PROSPERO users find your review (keywords do not appear in the public record but are included in searches). Be as specific and precise as possible. Avoid acronyms and abbreviations unless these are in wide use.

mouthwash, COVID-19, SARS-CoV-2, viral load, virucidal

37. Details of any existing review of the same topic by the same authors.

If you are registering an update of an existing review give details of the earlier versions and include a full bibliographic reference, if available.

38. * Current review status.

Update review status when the review is completed and when it is published.New registrations must be ongoing so this field is not editable for initial submission.
Please provide anticipated publication date

Review_Ongoing

39. Any additional information.

Provide any other information relevant to the registration of this review.

This is a resubmission of the protocol in response to a comment. We expanded the description in point 28.

40. Details of final report/publication(s) or preprints if available.

Leave empty until publication details are available OR you have a link to a preprint (NOTE: this field is not editable for initial submission). List authors, title and journal details preferably in Vancouver format.

Give the link to the published review or preprint.

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#2 Oral Communications in National Scientific Meetings

14th Meeting of Young Researchers of University of Porto (IJUP 2021). Online, 5-7 May 2021

Title: "The effect of mouthwashes on SARS-CoV-2 viral load: a systematic review"

Authors: António Silva[1], Maria Azevedo[2,3], Benedita Sampaio-Maia[1,3], Bernardo Pinto[4]

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[3] i3S, Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal
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Oral communication (PARALLEL ORAL SESSIONS XI; Room A1 - Health Sciences XII; May, 7th 14:30-16:00)

Abstract

Since late 2019, the world has been learning to cope with SARS-CoV-2, imposing a readjustment in daily activities, habits, and clinical practice. Binding of SARS-CoV-2 to human cells mainly occurs via angiotensin-converting enzyme 2 receptor which is highly expressed in the oral cavity, mainly in the epithelium of the tongue. Considering the oral cavity a major entryway and SARS-CoV-2 reservoir, several authors suggested that dentists should ask their patients to perform a preprocedural oral rinse as an additional protective measure. Therefore, we performed a systematic review of *in vivo* and *in vitro* studies with the aim of assessing the effectiveness of different mouthwashes on SARS-CoV-2 viral load. Three databases were consulted (PubMed, Web of Science, Scopus), with inclusion criteria being *in vitro* and *in vivo* studies assessing the virucidal effect of mouthwashes on SARS-CoV-2 or surrogates. This search was complemented with a manual search for preprints on MedRxiv and bioRxiv databases. Two independent authors selected and revised a total of 20 articles. To assess viral load, *in vitro* studies used infectivity assays, mostly endpoint dilution assays, while *in vivo* assays evaluate viral load via polymerase chain reaction. Several solutions were tested, namely chlorhexidine gluconate (CHX), cetylpyridinium chloride (CPC), hydrogen peroxide, povidone-iodine (PVP-I), and others. *In vitro* assays show PVP-I and CPC are the most effective in reducing viral load. PVP-I showed the best results *in vivo*. Conversely, CHX showed limited effectiveness in both *in vitro* and *in vivo* studies. Overall, the use of some mouthwashes seems to reduce SARS-CoV-2 viral load, so, their use as a preprocedural rinse may present an important protective measure for dental staff. The results of these primary studies appear relevant, however, there is a need for more randomized control trials to better understand the effect of mouthwashes on SARS-CoV-2 viral load and infection prevention.

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#3 Work Disclosure Form

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Declaração
Mestrado Integrado em Medicina Dentária (MIMD)
Monografia/Relatório de Estágio

Identificação do autor

Nome completo: António Carlos Pacheco Marques da Silva

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Faculdade/Instituto: Faculdade de Medicina Dentária da Universidade do Porto (FMDUP)

Identificação da publicação

Dissertação de Mestrado Integrado (Monografia) ☒ Relatório de Estágio ☐

Título completo: "The effect of mouthwashes on SARS-CoV-2 viral load: a systematic review"

Orientadora: Prof. Doutora Maria Benedita Almeida Garrett de Sampaio-Maia Marques

Coorientadora: Prof. Doutor Bernardo Manuel de Sousa Pinto

Palavras-chave: COVID-19, SARS-CoV-2, mouthwash, viral load, virucidal

Autorizo a disponibilização imediata do texto integral no Repositório da U.Porto: ____ (X)

Não Autorizo a disponibilização imediata do texto integral no Repositório da U.Porto: ___X___ (X)

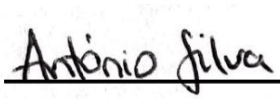
Autorizo a disponibilização do texto integral no Repositório da U.Porto, com período de embargo, no prazo de:

6 meses: _____; 12 meses: _____; 18 meses: _____; 24 meses: ___X___; 36 meses: _____; 120 meses: _____.

Justificação para a não autorização imediata: Aguardo publicação dos resultados em revista internacional

Data 23 / 05 / 2021

Assinatura:



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#4 Statement of Authorship

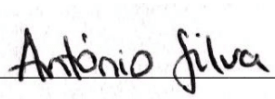
U.PORTO
FACULDADE DE MEDICINA DENTÁRIA
UNIVERSIDADE DO PORTO

DECLARAÇÃO

Monografia/Relatório de Estágio

Declaro que o presente trabalho, no âmbito da unidade curricular "Monografia/Relatório de Estágio", integrada no Mestrado Integrado em Medicina Dentária (MIMD) da Faculdade de Medicina Dentária da Universidade do Porto (FMDUP), é da minha autoria e todas as fontes foram devidamente referenciadas.

Porto, 23 de maio de 2021



António Carlos Pacheco Marques da Silva
(O estudante)

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#5 Advisor Final Submission Statement

U.PORTO
FACULDADE DE MEDICINA DENTÁRIA
UNIVERSIDADE DO PORTO

PARECER
(Entrega do trabalho final de Monografia/Relatório de Estágio)

Informo que o trabalho de Monografia/Relatório de Estágio desenvolvido pelo Estudante **António Carlos Pacheco Marques da Silva**, com o título "**The effect of mouthwashes on SARS-CoV-2 viral load: a systematic review"** está de acordo com as regras estipuladas na Faculdade de Medicina Dentária da Universidade do Porto (FMDUP), foi por mim conferido e encontra-se em condições de ser apresentado em provas públicas.

Porto, 23 de maio de 2021

A Orientadora:

Benedita Sampaio Maia

Maria Benedita Almeida Garrett de Sampaio-Maia Marques
(Professora Auxiliar na FMDUP)

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#6 Co-advisor Final Submission Statement

U.PORTO
FACULDADE DE MEDICINA DENTÁRIA
UNIVERSIDADE DO PORTO

PARECER
(Entrega do trabalho final de Monografia)

Informo que o trabalho de Monografia desenvolvido pelo Estudante **António Carlos Pacheco Marques da Silva**, com o título "**The effect of mouthwashes on SARS-CoV-2 viral load: a systematic review"** está de acordo com as regras estipuladas na Faculdade de Medicina Dentária da Universidade do Porto (FMDUP), foi por mim conferido e encontra-se em condições de ser apresentado em provas públicas.

Porto, 23 de maio de 2021

O Coorientador:

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FACULTY OF **DENTAL MEDICINE**