

Is chlorhexidine mouthwash effective in lowering COVID-19 viral load? A systematic review

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Abstract. – OBJECTIVE: This review aims to determine whether there is considerable evidence that mouthwashes containing chlorhexidine (CHX) lower the COVID-19 virus load in saliva.

MATERIALS AND METHODS: A comprehensive literature search was carried out in PubMed/Medline, EMBASE, LILACS, Scopus, Web of Science and Cochrane Library, Google Scholar, Open Gray, and ProQuest electronic databases using the keywords: “coronavirus infections” or “coronavirus” or “covid 2019” or “sars 2” or “sars-cov-2” or “sars-cov-19” or “severe acute respiratory syndrome coronavirus 2” or “coronavirus infection” or “severe acute respiratory pneumonia outbreak” and “CHX” or “CHX Hydrochloride” or “CHX Digluconate.” A manual search of the articles was also conducted utilizing the reference lists of articles. The *in vitro* experimental and clinical studies that tested CHX mouthwash were included. Study selection was not restricted or limited to a specific gender, age, ethnicity of individuals, or time of publication. A mix of keywords and proper truncations were used to search for databases.

RESULTS: Twelve studies (7 clinical and 5 *in vitro*) published between 2020 and 2021 were included in this systemic review. Five randomized controlled trials and one clinical case series demonstrated the effectiveness of CHX in reducing the oral viral load; one was inconclusive. Of the five *in vitro* studies, three showed that CHX is effective against SARS-CoV-2, and two studies denied the effectiveness of CHX. All *in vitro* studies tested CHX activity concentrations of 0.2, 0.12, and 0.1%. One study reported more than a 99.9% reduction in SARS-CoV-2 viral load in a minimal contact time of 30 seconds. CHX exhibited potent antiviral activity at higher concentrations without cytotoxicity.

CONCLUSIONS: Despite differences in the published research, CHX at different concentrations may be effective in lowering the SARS-CoV-2 viral load in saliva.

Key Words:

CHX, Chlorhexidine, Mouthwash, COVID-19, Viral load, Effectiveness, SARS-CoV-2.

Introduction

Coronavirus Disease (COVID-19) affected almost all aspects of human life regardless of the established protocols to minimize its spread worldwide¹. COVID-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV) that disseminated several years ago and was contagious to a lesser degree than COVID-19. SARS-CoV, belongs to β family of coronaviruses that can infect humans². COVID-19 infection includes oral manifestations, mainly taste disorders, oral mucosal lesions, and dry mouth. These manifestations are reduced after a negative polymerase chain reaction (PCR) test. However, other presentations include masticatory muscle pain, tongue, halitosis, and swelling^{3,4}.

The COVID-19 infection occurs through exposure to coughing, sneezing, or close contact with infected persons⁵. The literature reports a viral load of up to 1.2×10^8 copies/mL of COVID-19 virus in the saliva of symptomatic and asymptomatic carriers. Hence saliva plays an essential role in the transmission of the illness through the distribution of virus-infected droplets⁶⁻⁸.

Healthcare professionals, particularly dentists, are at extreme risk of exposure to pathogenic microorganisms, including COVID-19 infection⁹. Accordingly, special attention has been given to dental practice due to the proximity of dentists to patients during dental procedures. Use of ultrasonic scalers, low-speed and high-speed handpiec-

es leads to the production and release of aerosols and droplets^{9,10}. Droplets and aerosols that generate in the presence of the COVID-19 virus in the saliva of the infected patients could infect dental staff^{11,12}. In addition, during the COVID-19 crisis, many dental clinics closed temporarily or permanently due to the failure to provide a virus-free environment regardless of the number of dental emergencies that needed urgent treatment¹³.

Few studies¹⁴⁻¹⁶ have reported salivary viral load reduction in aerosols production following dental procedures. The usage of effective mouthwash has been suggested to reduce oral viral load before dental treatment¹⁴. In addition, preprocedural mouthwashes are widely accepted because of their well-known effectiveness in reducing microbial load¹⁵. One of these mouthwashes is CHX, which is reported¹⁶ to be effective against respiratory viruses, but its efficacy against COVID-19 has not been proven. Therefore, due to the paucity of data in the literature, this systematic review aims to determine whether there is considerable evidence that mouthwashes containing CHX lower the SARS-CoV-2 virus load in saliva.

Materials and Methods

The Preferred Reporting Items for Systematic Reviews and Meta-Analysis Checklist (PRISMA)¹⁷ was used to select studies (Figure 1). In addition, the PICO (population/patient/problem, intervention, control/Comparison/comparator, and outcomes) approach was considered for framing a “foreground” research question¹⁸, as mentioned below.

P = Patient (subjects infected with coronavirus or the contaminated saliva of these individuals), I = Intervention (CHX Mouthwash), C = comparison (compared to a control group through a cross-sectional evaluation or compared to the same individual/saliva at first through a longitudinal assessment), O = outcomes (viral load or % of virus inactivation) and S = study design (clinical trials or *in vitro* experimental studies).

Inclusion Criteria

The *in vitro* experimental and clinical trials that hypothesized CHX reduces viral load in saliva and used CHX mouthwash as an intervention were included in this review. The study was not restricted or limited to a specific sex, age, and race of individuals or the time of publication. However, only the articles published in English were considered for the review.

Exclusion Criteria

The exclusion criteria were applied as follows: a) studies that did not test or used coronavirus-infected individuals/saliva as a sample, b) experiments that used samples other than saliva, c) articles that used other than CHX for decreasing the viral load (as an intervention) or that have used the mouthwash plus another treatment without separating their results, d) studies that lack a viral load evaluation after the intervention or have incomplete data, and e) conference proceedings, expert views, and letters.

Information Sources and Search Strategy

For all the selected electronic databases, a combination of terms and suitable truncations were adapted to search literature in electronic databases of PubMed/Medline, EMBASE, Latin American and Caribbean Literature in Health Sciences (LILACS), Scopus, Web of Science and Cochrane Library. A combination of MeSH keywords was used until June 4, 2021. The search string used for the literature search in this study is shown below:

TI=(“coronavirus infections” or “coronavirus” or “coviid 2019” or “sars2” or “sars-cov-2” or “sars-cov-19” or “severe acute respiratory syndrome coronavirus 2” or “coronavirus infection” or “severe acute respiratory pneumonia outbreak” or “novel cov” or “2019ncov” or “sars cov2” or “cov2” or “ncov” or “covid-19” or “covid19” or “coronaviridae” or “corona virus” or “covid-19 pandemic” or “2019 novel coronavirus disease” or “sars-cov-2 infection” or “covid-19 virus disease” or “2019 novel coronavirus infection” or “2019-ncov infection” or “coronavirus disease 2019” or “coronavirus disease-19” or “2019-ncov disease” or “covid-19 virus infection” or “2019-ncov” or “sars-cov-2” or “sars-cov”) and TI=(“CHX” or “CHX Hydrochloride” or “CHX Gluconate” or “CHX Acetate”). In addition, Google Scholar, Open Gray, ProQuest were utilized to explore the gray literature. Finally, a manual search of the articles was also conducted using the bibliography of retrieved publications.

Study Selection

The study selection was carried out through two stages. In the first stage, three individual reviewers screened the titles and abstracts of all studies independently. Studies that did not match the inclusion criteria were excluded. In the second stage, the same three reviewers reviewed the completed articles they agreed upon in the first stage. Any disagreements about a study and the opinion of the fourth reviewer were considered to finalize the decision.

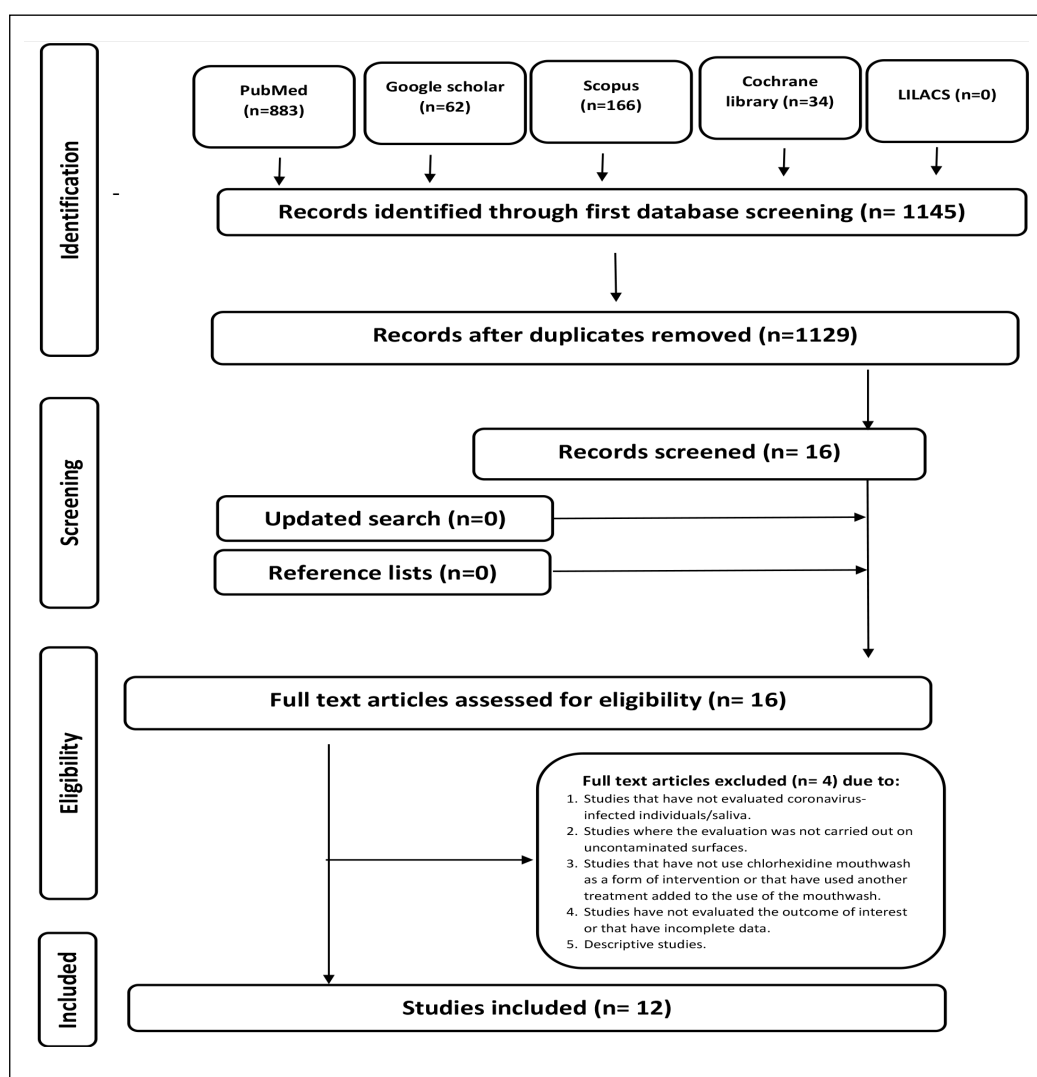


Figure 1. PRISMA Flowchart.

Risk of Bias Assessment

In this review, the risk of bias for clinical studies was carried out using the Newcastle-Ottawa Quality Assessment Scale of the selected articles¹⁹. The assessment was based on the selection of the samples, whether representative or not, presence or absence of the control group to ascertain that both the control and experimental groups had the same method and exposure (Table I²⁰⁻²⁷).

Since there is no standard tool for the *in vitro* studies assessment, risk of bias analysis was done using the previous study approach^{27,28}, which was based on the quality of the report, performance, selection, and detection of bias. The assessment was done using 13 items labeled “low risk,” “high risk,” and “unclear” (Table II).

Data Collection

The data consisted of the study’s characteristics such as title, author name, country, study type, objectives, participants, controls, sample size, concentration of CHX, and main findings. Three reviewers collected the data independently in separate excel sheets. All three data sheets were carefully reviewed, discussed, and combined into one final sheet (Table III).

Statistical Analysis

It was determined that the clinical trials and *in vitro* studies outcomes could not be combined. The individual studies’ findings and features are shown in Table III using the qualitative analysis approach. Based on the type of research design,

Is chlorhexidine mouthwash effective in lowering COVID-19 viral load? A systematic review

Table I. Risk of assessment bias for clinical trials.

Studies	Selection				Comparability	Exposure			Total quality score
	Is the case definition adequate?	Representativeness of the cases	Selection of controls	Definition of controls		Comparability of cases and controls	Ascertainment of exposure	Same method of ascertainment for cases and controls	
Huang and Huang ²⁰	1	1	1	1	1	1	1	1	8
Yoon et al ²¹	1	1	1	1	1	1	1	1	8
Seneviratne et al ²²	1	1	1	1	1	1	1	1	8
Elzein et al ²³	1	1	1	1	1	1	1	1	8
Mukhtar et al ²⁴	1	1	1	1	1	1	1	1	8
Eduardo et al ²⁵	1	1	1	1	1	1	1	1	8
Chaudhary et al ²⁶	1	1	0	0	0	1	0	1	4

Table II. Quality assessment of *in vitro* studies.

Criteria		Jain et al ²⁹	Steinhauer et al ³⁰	Davies et al ³¹	Komine et al ³²	Xu et al ³³
Reporting Quality	Is the cell origin and cell type used reported?	Yes (Low Risk)	Yes (Low Risk)	Yes (Low Risk)	Yes (Low Risk)	Yes (Low Risk)
	Is the duration of exposure reported?	Yes (Low Risk)	Yes (Low Risk)	Yes (Low Risk)	Yes (Low Risk)	Yes (Low Risk)
	Is the frequency of exposure reported?	Yes (Low Risk)	Yes (Low Risk)	Yes (Low Risk)	Yes (Low Risk)	Yes (Low Risk)
	Is the (CHX concentration) magnetic flux density of exposure reported?	Yes (Low Risk)	Yes (Low Risk)	Yes (Low Risk)	Yes (Low Risk)	Yes (Low Risk)
	Environmental background magnetic field reported	Unclear	Unclear	Unclear	Unclear	Unclear
Performance Bias	Is a sham or dummy coil used for control treatment?	Unclear	Unclear	No (Low Risk)	No (Low Risk)	No (Low Risk)
	Is the temperature controlled?	Yes (Low Risk)	Yes (Low Risk)	Yes (Low Risk)	Yes (Low Risk)	Yes (Low Risk)
	Was the exposure blinded?	Unclear	Unclear	Unclear	Unclear	Unclear
	Was the exposure randomized?	Unclear	Unclear	Unclear	Unclear	Unclear
Selection Bias	Is the cell vitality scored/measured?	No	Yes (Low Risk)	Yes (Low Risk)	Yes (Low Risk)	Yes (Low Risk)
Detection Bias	Were the methods the same for control and exposure treatment?	Unclear	Unclear	Yes (Low Risk)	Unclear	Unclear
	Were the data measurements randomized?	Unclear	Unclear	Unclear	Unclear	Unclear
Other Bias	Was there no industry sponsoring involved?	Yes (Low Risk)	Yes (Low Risk)	No	No	Unclear

Table III. Summary and main findings of studies included in this systematic review.

Study Country	Study type	Objective	Participants	Controls	Sample size	CHX concentration	Main findings
Huang and Huang ²⁰ USA	Randomized, prospective cohort study	To investigate the efficacy of CHX mouthwash in posterior oropharyngeal spray to eradicate oropharyngeal SARS-CoV-2 in COVID-19 patients	Patients with confirmed COVID-19 infection.	135 controls	294 Patients	(0.12%)	The CHX used as an oral rinse and posterior oropharyngeal spray is a simple and safe addition to the current COVID-19 prevention guidelines and may have significant effects on controlling the spread of the disease when used with other measures.
Yoon et al ²¹ Korea	Clinical case study	To evaluate the viral dynamics in various body fluid specimens of two patients with COVID-19 before and after the administration CHX mouthwash	Patients with confirmed COVID-19 infection.	No	2 Patients	(0.12%, 15 mL)	SARS-CoV-2 viral load was consistently high in the saliva, and it was relatively higher than that in the oropharynx during the early stage of COVID-19. CHX mouthwash was effective in reducing the SARS-CoV-2 viral load in the saliva for a short-term.
Jain et al ²⁹ India	<i>The in vitro</i> laboratory analysis	To compare and evaluated the antiviral effectiveness of the current 'gold standard' CHX and povidone iodine as a control agent, through an <i>in vitro</i> analysis	-	Povidone iodine	-	0.2% and 0.12%	CHX digluconate in 0.2% concentration inactivated SARS CoV-2 in minimal contact time of 30 seconds. Both CHX and povidone-iodine were found to have antiviral activity against SARS CoV-2 virus.
Steinhauer et al ³⁰ Germany	<i>The in vitro</i> laboratory analysis	To compare the <i>in vitro</i> efficacy of different mouthwash solutions targeting SARS-CoV-2 CHX Di gluconate and OCT	-	-	-	0.1%, 0.2%	OCT mouthwash thus constitutes an interesting candidate for future clinical studies to prove its effectiveness in a potential prevention of SARS-CoV-2 transmission by aerosols.
Seneviratne et al ²² Singapore	Randomized control trial	To evaluate the efficacy of three commercial mouth-rinse viz. PI, CHX and CPC, in reducing the salivary SARS-CoV-2 viral load in COVID-19 patients compared to water.	Confirmed COVID-19 infection	2 out of the 16 patients	16 patients	0.2%	CPC and PI formulated commercial mouth-rinses may have a sustained effect on reducing the salivary SARS-CoV-2 level in COVID-19 patients.
Davies et al ³¹ England	<i>In vitro</i> laboratory analysis.	To evaluate the virucidal efficacy of mouthwashes/oral rinses against SARS- CoV-2, and their applications in reducing risk associated with aerosol generating procedures for infection control in dental practice	-	-	-	0.2%	There is an effective inactivation of SARS-CoV-2 by Listerine Advanced Defense Sensitive and Total Care formulations, containing 0.01–0.02 percent hypochlorous acid or 0.58 percent povidone iodine in <i>in-vitro</i> tests using TCF. Data was in favor of these products, but not for using of hydrogen peroxide or CHX gluconate mouthwashes for reduction of SARS-CoV-2 viral load. Thus, indicating a potential use of these products in the reduction of infectious risk associated with aerosol-generating dental procedures and for SARS-CoV-2 infection control.

Table continued

Table III. (Continued). Summary and main findings of studies included in this systematic review.

Study Country	Study type	Objective	Participants	Controls	Sample size	CHX concentration	Main findings
Komine et al ³² USA, Japan, Europe, China	The <i>in vitro</i> laboratory analysis.	To examine inactivation of SARS-CoV-2 by oral care products in several countries <i>in vitro</i>				0.06 % 0.2%	This study showed that the oral care products containing CPC or delmopinol hydrochloride have antiviral activity against SARS-CoV-2. This supports the recommendation for a preprocedural use of CPC-containing mouthwash for SARS-CoV-2 reduction in aerosol. The mouthwash containing only 0.12 % CHX as antiseptic did not show a sufficient inactivation effect against SARS-CoV-2.
Xu et al ³³ USA	The <i>in vitro</i> laboratory analysis.	To determine the effect of commercially available mouth rinses and antiseptic Listerine Original, povidone-iodine, Colgate Peroxyl, and CHX Gluconate on the infectivity of SARS-CoV-2 virus and of a non-pathogenic, recombinant, SARS-CoV-2 infection vector (pseudotyped SARS-CoV-2 virus)				0.12%	All mouth washes at non-cytotoxic levels exhibited antiviral activity. Colgate Peroxyl and povidone-iodine had greater inhibitory effects on the viruses than CHX or Listerine. All mouth rinses tested inactivated replication competent SARS-CoV-2 viruses. The cytotoxic effects of mouth rinses should be considered when assessing antiviral activities since diluted Listerine and CHX exhibited no cytotoxic effects.
Elzein et al ²³ Lebanon	Randomized-controlled clinical trial	To evaluate the efficacy of two preprocedural mouthrinses in the reduction of salivary SARS-CoV-2 viral load and to compare the results of the mouthwashes to a control group	Confirmed COVID-19 positive patients	For 30 seconds, the control group mouth rinsed with distilled water, the CHX group mouth rinsed with 0.2% CHX and the Povidone-iodine group gargled with 1% Povidone-iodine.	61	0.2%	CHX 0.2% and 1% Povidone-iodine oral solutions are effective preprocedural mouthwashes against salivary SARS-CoV-2 in dental treatments and their use as a preventive strategy to reduce the spread of COVID-19 during dental practice should be considered.

Table continued

Table III. (Continued). Summary and main findings of studies included in this systematic review.

Study Country	Study type	Objective	Participants	Controls	Sample size	CHX concentration	Main findings
Mokhtar et al ²⁴ Qatar	Randomized controlled trial	To determine the average recovery rate, in terms of nasopharyngeal swab test (COVID RT-PCR) for the intervention and control cases, after two weeks of treatment.	Patients of COVID-19, confirmed	Yes	87 (control: 44, MW: 43)		The regular use of potent mouthwash solutions seems to accelerate the recovery of COVID-19 and seems to have no linear relationship with the duration of use. This observed improvement suggests better potential in an earlier stage of the disease, as an addition to the treatment protocols for the hospitalized COVID-19 cases, especially for high-risk populations.
Eduardo et al ²⁵ Brazil	Randomized pilot clinical trial	To investigate whether three types of mouthwash solutions reduce the SARS-CoV-2 viral load in saliva at different time points.	positive SARS-CoV-2 patients	Yes (water)	43 (9 placebo group, 7 CPC and Zn group, 7 HP group, 8 CHX group, 12 HP then CHX group)	0.12%	CPC +Zinc mouthwash and CHX mouthwash provided a significant reduction in the SARS-CoV-2 viral load in saliva for up to 60 mins after rinsing. While HP provided a significant reduction up to 30 mins after rinsing.
Chaudhary et al ²⁶ USA	Randomized control trial	To examine the risk posed to dental personnel from potential patients who report no symptoms of COVID-19, and to investigate the efficacy of a simple preprocedural mouth rinsing on salivary viral load reduction.	Patients who had COVID-19 symptoms and who DID not have COVID-19 symptoms	No	201 four groups: 127 asymptomatic (negative Covid19 symptoms at presentation) 18 pre-symptomatic (asymptomatic at initial presentation) 41 symptomatic 15 post symptomatic (history of COVID-19)	0.12%	Moutrinses are simple and highly efficacious means of reducing the virus from the oral environment for up to 45 minutes and may be a valuable tool in disease mitigation.

CHX=Chlorhexidine, Povidone iodine=PI, Cetylpyridinium Chloride=CPC, Octenidine dihydrochloride =OCT, COVID-19=Coronavirus Disease-19, SARS-CoV-2= Severe acute respiratory syndrome, Zn=Zinc, HP=Hydrogen Peroxide, RT-PCR=Reverse Transcription Polymerase Chain Reaction, mL=Milli Liter.

all 12 papers included in this systematic review were classified as clinical trials or *in vitro* investigations. A total of 7 (58%) clinical trials and 5 (48%) *in vitro* investigations were reviewed further for quality criteria, and the key results were summarized and tabulated. Since this review did not include quantitative analysis of data from the studies, the statistical significance of the *p*-value cannot be computed.

Results

Study Selection

A total of 1,145 articles were identified from the various electronic databases. Subsequently, 1,129 references were duplicated and excluded from the study. Hence a total of 16 full-text articles were assessed for eligibility, and finally 12 studies were included in the systemic review after removing four studies for various reasons.

Characteristics of the Study

All 12 studies were published between 2020 and 2021. Seven were clinical studies, and the others were *in vitro* studies. Five randomized controlled trials^{20,23-26} and one clinical case-series²¹ involving two COVID-19 patients demonstrated that CHX is an effective preprocedural mouthwash in reducing the oral viral load. However, one randomized controlled trial was inconclusive on the effectiveness of CHX in decreasing the viral load in COVID-19 patients²². Of the five *in vitro* studies²⁹⁻³³, three of them^{29,30,33} showed that the CHX is effective against SARS-CoV-2. Contrarily, two studies^{31,32} did not report the effectiveness of CHX against the SARS-CoV-2.

Risk of Bias

The quality assessment of *in vitro* studies was carried out using the previous study's approach^{30,29,31-33} as mentioned in the methodology. Most domains have been labeled as "low risk" (reporting quality and selection bias). "Unclear risk" rate was also high, and it was scattered along the domains; however, "high risk" was marked in three different studies reported by Jain et al²⁹, Davies et al³¹, and Komine et al³² with regards to the scoring of the cell vitality and absence of industry sponsors.

Discussion

Current literature indicates that COVID-19 is easily transmitted by infected saliva through di-

rect contact with the mucosa of the eye, nose, and mouth or indirectly by coughing or sneezing by an infected individual^{34,35,9}. It is essential to reinforce the infection control protocols due to the virus's high survival rates on hands and surfaces that come into contact with infected saliva. Disinfection of the dental clinic environment and personal protective equipment is essential to avoid the risk of cross-infection between patients and the dentist¹. Some studies^{21,26} reported that the CHX provides an efficacious means of reducing the viral load. Nevertheless, using preprocedural CHX as a prevention strategy to decrease the risk of contagion may control the spread of COVID-19^{20,23,30}. All *in vitro* studies included in this article tested CHX activity against SARS-CoV-2 at concentrations of 0.2, 0.12, and 0.1%. Two studies^{31,32} reported that CHX has no antiviral activity with a less than 50% viral reduction. However, Jain et al²⁹ found that the CHX reduced over 99.9% of the SARS-CoV-2 viral load with 30 seconds of contact time. In addition, Xu et al³³ claimed that CHX has a medium antiviral activity with 50% viral load reduction when used at 0.12% concentration. However, it exhibits a potent antiviral activity at higher concentrations without cytotoxicity.

Decreasing the microorganism count in the mouth using commercially available chemical rinses preoperatively in controlling dental plaque is well-known³⁶. Some studies^{37,38} claim that preventing the transmission of viruses and bacteria can be best managed using mouthwashes. Among these chemical agents, some have bactericidal efficiency, while others have virucidal efficiency. Current studies²⁸ indicate using hydrogen peroxide, CHX, and PVP-I as a method or intervention to lessen the possibility of contamination. On the other hand, undesirable side effects of some agents may occur. Additionally, governments worldwide recommended methods for cleaning the environment to prevent the spread of the virus using hydrogen peroxide^{39,40}. As reported in the literature, hydrogen peroxide is widely used as a surgical and oral disinfectant for treating gingivitis^{41,42}.

Despite guidelines for diagnosing and treating new coronavirus pneumonia, the CHX is ineffective against SARS-CoV-2⁹. In this systematic review, we evaluated twelve studies, including seven *in vivo* and five *in vitro*. After assessing the studies with various parameters (CHX concentration, contact times, control), most articles supported CHX mouthwash as having a good antiviral effect against SARS-COV-2. One study³³

claimed that CHX could exhibit a potent antiviral effect at higher concentrations without cytotoxicity, giving CHX a great advantage. On the other hand, two studies^{31,32} have denied CHX as having an antiviral effect against SARS-CoV-2. Despite CHX has an important function in dentistry and antisepsis, it may induce adverse consequences, limiting its usefulness⁴³. However, currently there is little information on how preprocedural mouthwashes diminish SARS-CoV-2 in saliva⁴⁴. Due to the lack of clinical studies with high sample sizes and consistent methodologies, it may be argued that there is insufficient data regarding the effectiveness of CHX in reducing the SARS-CoV-2 virus load. Hence further studies are recommended on this issue.

Conclusions

Based on *in vitro* and clinical investigations, the findings of this review indicate that the use of CHX in different concentrations is likely effective in lowering the SARS-CoV-2 viral load in saliva. However, the degree of scientific evidence associated with CHX is highly frail owing to the absence of a large number of randomized clinical studies and the inclusion of *in vitro* studies.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Ethics Approval

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Research and Innovation Center of Riyadh Elm University (IRB No.: SRP/2021/56/514/500).

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Availability of Data and Materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' Contribution

Ghousia Saeed Rahman: supervision, conception and design of the study, analysis, and interpretation of data, drafting the article, and final approval.

Abdullah Abdulaziz Nasser Alshetan, Sarah Saud Omar Alotaibi, Bayan Mousa Ibrahim Alaskar: data collection, design of the study, analysis, and interpretation of data, drafting the article, and final approval.

Mohammad Abdul Baseer: interpretation of data, drafting the article, and final approval.

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