ciência plural

DO ORAL ANTISEPTICS USED IN THE DENTAL ROUTINE HAVE ANTI-VIRAL EFFICACY? A SYSTEMATIC REVIEW OF IN VITRO STUDIES

Os antissépticos orais utilizados na rotina odontológica têm eficácia anti-viral? Uma revisão sistemática de estudos in vitro

¿Los antisépticos orales utilizados en la rutina odontológica tienen eficacia antiviral? Una revisión sistemática de estudios in vitro

Jaiane Carmélia Monteiro Viana ● Federal University of Rio Grande do Norte - UFRN ● DDS ● E-mail: jaiane103@hotmail.com

Guilherme Carlos Beiruth Freire ● Federal University of Rio Grande do Norte - UFRN ● DDS, MS, PhD student ● E-mail: guilherme.beiruth@gmail.com

Carlos Eduardo de Paiva Campos Nogueira Simão ● Federal University of Rio Grande do Norte – UFRN ● DDS, MS Student ● E-mail: carloseduardokadu15@gmail.com

Rayanne Karina Silva Cruz ● Federal University of Rio Grande do Norte - UFRN ● DDS, MS, PhD ● E-mail: rayannescruz@gmail.com

Bruno César de Vasconcelos Gurgel ● Federal University of Rio Grande do Norte - UFRN ● DDS, MS, PhD and Associate Professor IV of Department of Dentistry ● E-mail: bcgurgel@yahoo.com.br

Autora correspondente:
Jaiane Carmélia Monteiro Viana ● E-mail: jaiane103@hotmail.com

Submetido: 02/10/2023
Aprovado: 06/04/2024

ABSTRACT

Introduction: Mouthwashes play an important role in the dental clinic, but their role on viruses requires investigation. **Objective**: to review in vitro studies to identify the effect of different mouthwashes on the main viruses associated with routine dental care. **Methodology:** The following databases were searched in September 2023: PubMed, Embase, Scopus and Web of Science databases; the Cochrane Library and the Virtual Health Library (VHL); and grey literature. In vitro studies that used mouthwashes to reduce the viral load were selected. The PICOS strategy was considered to define eligibility criteria: the Population (viruses involved in the etiology of oral infection), the Intervention (oral antiseptics), the appropriate comparator (positive and negative controls), the Outcomes of interest (reduction of viral load) and the Study design (in vitro studies). **Results:** Considering the eligibility criteria, 19 articles were included in this review. The efficacy of povidone-iodine (PVP-I), chlorhexidine, Listerine®, essential oils, and cetylpyridinium chloride (CPC) rinses were investigated. PVP-I (0.23%) had its effects mainly associated with coronaviruses SARS (Severe Acute Respiratory Syndrome), demonstrating a significant reduction in viral load after 15 seconds of exposure. Chlorhexidine (0.05%; 0.1% and 0.5%) was ineffective against adenovirus, poliovirus, and rhinovirus respiratory viruses. Listerine® demonstrated superior efficacy against HSV-1 and 2 viruses and influenza A, and cetylpyridine chloride also demonstrated virucidal activity against influenza A. **Conclusions:** The type, concentration, and time of exposure to antiseptics varied between studies. PVP-I and chlorhexidine digluconate were the most studied substances, but in general, PVP-I was more effective in reducing viral titers, especially concerning coronaviruses. Other antiseptics such as CPC, H2O2 and Listerine® have also shown significant reduction in viral load, but this is a limited number of studies.

Keywords: Mouthwashes; Virus; Infection Control; Antiviral Agents.

RESUMO

Introdução: Os enxaguantes bucais desempenham um papel importante na clínica odontológico, porém seu papel sobre os vírus requer investigações. **Objetivo**: revisar estudos *in vitro* para identificar o efeito de diferentes colutórios sobre os principais vírus associados ao atendimento odontológico de rotina. **Metodologia:** As seguintes bases foram pesquisadas até setembro de 2023: PubMed, Embase, Scopus e Web of Science; a Biblioteca Cochrane e a Biblioteca Virtual em Saúde (BVS); e literatura cinzenta. Foram selecionados estudos in vitro que utilizaram bochechos com o objetivo de reduzir a carga viral. A estratégia PICOS foi considerada para a definição dos critérios de elegibilidade: População (vírus envolvidos na etiologia da infecção oral), Intervenção (antissépticos orais), Comparador (controles positivos e negativos), os Desfechos de interesse (redução da carga viral) e o desenho do estudo (estudos in vitro). **Resultados:** Considerando os critérios de elegibilidade, 19 artigos foram incluídos para esta revisão. A eficácia da povidona-iodo (PVP-I), clorexidina, Listerine®, óleos essenciais e lavagens com cloreto de cetilpiridínio foram investigadas. O PVP-I (0.23%) teve seus efeitos principalmente associados ao

coronavírus SARS (Síndrome Respiratória Aguda Severa), demonstrando uma redução significativa da carga viral após 15 segundos de exposição. A clorexidina mostrou-se ineficaz contra vírus respiratórios de adenovírus, poliovírus e rinovírus. Listerine® demonstrou eficácia superior contra vírus HSV-1 e 2 e vírus influenza A, e cloreto de cetilpiridinio também demonstrou atividade virucida contra influenza A. **Conclusões:** O tipo, concentração e tempo de exposição aos antissépticos variaram entre os estudos. O PVP-I e o digluconato de clorexidina foram as substâncias mais estudadas, mas no geral, o PVP-I foi mais eficaz na redução dos títulos virais, principalmente no que diz respeito aos coronavírus. Outros antissépticos como CPC, H2O2 e Listerine® também mostraram redução significativa da carga viral, mas trata-se de um número limitado de estudos.

Palavras-Chave: Antissépticos Bucais; Vírus; Controle de Infecção; Agentes Antivirais.

RESUMEN

Introducción: Los enjuagues bucales son importantes en la clínica dental, sin embargo, su efecto sobre los virus requiere investigaciones. **Objetivo:** Revisar estudios in vitro para identificar el efecto de enjuagues bucales sobre los principales virus asociados con la rutina odontológica. **Metodología:** Las siguientes bases de datos fueron investigadas hasta septiembre de 2023: PubMed, Embase, Scopus y Web of Science; Biblioteca Cochrane y Biblioteca Virtual en Salud (BVS); y literatura gris. Se seleccionaron estudios in vitro que utilizaron enjuagues bucales con el objetivo de reducir la carga viral. Se consideró la estrategia PICOS para definir los criterios de elegibilidad: Población (virus implicados en la etiología de la infección oral), Intervención (antisépticos bucales), Comparador (controles positivos y negativos), Resultados de interés (reducción de la carga viral) y diseño del estudio (in vitro). **Resultados:** Considerando los criterios de elegibilidad, se incluyeron 19 artículos. Se investigó la eficacia de povidona yodada (PVP-I), clorhexidina, Listerine®, aceites esenciales y enjuagues de cloruro de cetilpiridinio (CPC). PVP-I (0.23%) mostró sus efectos principalmente asociados al coronavirus SARS (Síndrome Respiratorio Agudo Severo), demostrando una reducción significativa de la carga viral después de 15 segundos. Se ha demostrado que la clorhexidina es ineficaz contra los virus respiratorios adenovirus, poliovirus y rinovirus. Listerine® demostró una eficacia superior contra los virus HSV-1 y 2 y el virus de la influenza A, y el CPC también mostró actividad virucida contra la influenza A. **Conclusiones:** El tipo, la concentración y el tiempo de exposición variaron entre los estudios. PVP-I y digluconato de clorhexidina fueron las sustancias más estudiadas, pero, PVP-I fue más efectiva en la reducción de los títulos virales, especialmente en lo que respecta a los coronavirus. Otros antisépticos como CPC, H2O2 y Listerine® también mostraron una reducción significativa de la carga viral, pero se trata de un número limitado de estudios.

Palabras clave: Enjuagues Bucales; Virus; Control de Infecciones; Agentes Antivirales.

Introduction

The ultrasonic devices, high and low rotation pens, and triple syringes used during dental procedures produce bioaerosols, which are characterized by a heterogeneous composition, including blood, mucous cells, restorative materials, fragments of teeth and large amounts of saliva, in addition to microorganisms, such as bacteria, fungi and viruses[1,2]. The morphological characteristics of viruses - such as their size being between 20 and 400 nanometers - allow them to reach greater distances and to stay longer in the air. In this context, those most relevant to dentistry are the Human Immunodeficiency Virus (HIV), Hepatitis B and C, Herpes Simplex (HSV), Influenza and, more recently, SARS-CoV-2[1,3,4].

Studies point to an association between the spread of microorganisms in the dental office and the development of diseases, such as ophthalmic and respiratory infections, since these can be inhaled or transmitted by direct contact with the conjunctival, nasal, or buccal mucosa[1,5]. Thus, adopting biosafety protocols, including hand washing and the use of personal protective equipment (PPE), such as eye protectors, masks, and face shields, as well as the use of disinfectants and mouthwashes, is important to prevent the environmental spread of viral infections. However, few efficient and specific measures are available to oppose most of these infections, especially when the etiological agent is viral[6].

Many chemical agents have been used for topical use in dentistry to reduce the transmission of microorganisms, including povidone-iodine (PVP-I) and chlorhexidine, which have moderate and weak action, respectively, concerning viruses[7,8]. Essential oils are thought to have natural antiviral agents capable of preventing the invasion of SARS-CoV-2, for example, in the human body[9,10]. Cetylpyridinium chloride (CPC) and antiseptics based on hydrogen peroxide are also both considered virucidal because of their ability to break the integrity of the envelopes of some viruses, such as influenza[11].

Given the current scenario of the SARS-COV-2 pandemic, interest in the investigation of antiseptic agents against viruses has arisen, whether in clinical

application or in vitro studies, to explore their virucidal action in the dental office or as a third step, performed by patients in their daily oral hygiene procedures.

There is a consensus in the literature regarding the effectiveness of using mouthwashes as part of the dental pre-procedure to reduce the microbial load of aerosols, especially for bacteria, but the effect on viruses requires further investigation[12]. Therefore, the objective of this systematic review is to identify the role of different mouthwashes on the main viruses associated with the dental routine, based on in vitro studies.

Methodology

This study consists of a systematic review registered on the OSF REGISTRIES platform (https://osf.io/m5c6v) and conducted in accordance with the recommendations of the Cochrane Handbook and the PRISMA protocol (Preferred Reporting Items for Systematic Review and Meta-analyses). In line with the PICOS strategy, the following research question was formulated: "Do oral antiseptics used in the dental routine have anti-viral efficacy in in vitro studies?"

Search Strategy

Electronic searches were performed in the PubMed, Embase, Scopus and Web of Science databases, and in the Cochrane Library, grey literature, through Google Scholar. In addition, articles were retrieved by manual search. Table 1 shows the words and descriptors included in the search strategy for PubMed, using controlled terms from MeSH (Medical Subject Headings) and free terms, which were adapted for other searches, also considering the DeCS (Health Sciences Descriptors). Searches of the databases and grey literature were carried out until Septiembre 2023.

Table 1. Database search strategy. Natal-RN, 2023.

Database	Search (Sep. 2023)
Cochrane	("Coronavírus Infections" OR "COVID-19" OR Coronavirus OR "SARS-CoV-2" OR "Herpes Labialis" OR "Herpes Simplex, Oral" OR "Herpes Simplex Virus Infection" OR "Herpes Simplex" OR "Stomatitis, Herpetic" OR "Gingivostomatitis, Herpetic" OR Hepatitis OR "Hepatitis C" OR "Hepatitis B" OR "Hepatitis B Virus Infection" OR "Hepatitis, infectious" OR "Infectious hepatitis" OR "Acquired Immunodeficiency Syndrome" OR "Acquired Immune Deficiency Syndrome" OR AIDS OR "Immunodeficiency Syndrome, Acquired" OR "HIV" OR "Influenza, Human" OR Grippe OR "Human Flu" OR "Human Influenza" OR "Influenza in Humans" OR Viruses OR "Virus Diseases" OR "Viral Infections" OR "Respiratory Tract Infections" OR "Infections, Respiratory" OR "Respiratory Infections" OR Virus) AND ("Mouthwashes" OR Mouthwash OR "Rinse, Mouth" OR Rinse OR "Mouth Rinse" OR "Mouth Bath" OR "Mouth Wash" OR "Mouth Washes" OR Gargle OR "Anti-Infective Agents, Local" OR Antiseptics OR "Cetylpyridinium" OR "Cetylpyridinium chloride, zinc acetate drug combination" OR "Cetylpyridinium Chloride" OR "Povidone-Iodine" OR "Povidone iodine" OR "PVP-I" OR "PVP-Iodine" OR "Chlorhexidine" OR "Chlorhexidine Hydrochloride" OR "Hydrochloride, Chlorhexidine" OR "Hydrogen Peroxide" OR "Hydrogen Peroxide (H2O2)" OR Hydroperoxide OR "Essential oils" OR "Oils, Essential" OR Triclosan) AND ("Oral health" OR "Health, Oral" OR "Oral hygiene" OR "Dental Prophylaxis" OR "Prophylaxis, Dental" OR "Health Services, Dental" OR "Dental Health Services")
Embase	("Coronavírus Infections" OR "COVID-19" OR Coronavirus OR "SARS-CoV-2" OR "Herpes Labialis" OR "Herpes Simplex, Oral" OR "Herpes Simplex Virus Infection" OR "Herpes Simplex" OR "Stomatitis, Herpetic" OR "Gingivostomatitis, Herpetic" OR Hepatitis OR "Hepatitis C" OR "Hepatitis B" OR "Hepatitis B Virus Infection" OR "Hepatitis, infectious" OR "Infectious hepatitis" OR "Acquired Immunodeficiency Syndrome" OR "Acquired Immune Deficiency Syndrome" OR AIDS OR "Immunodeficiency Syndrome, Acquired" OR "HIV" OR "Influenza, Human" OR Grippe OR "Human Flu" OR "Human Influenza" OR "Influenza in Humans" OR Viruses OR "Virus Diseases" OR "Viral Infections" OR "Respiratory Tract Infections" OR "Infections, Respiratory" OR "Respiratory Infections") AND ("Mouthwashes" OR Mouthwash OR "Rinse, Mouth" OR Rinse OR "Mouth Rinse" OR "Mouth Bath" OR "Mouth Wash" OR "Mouth Washes" OR Gargle OR "Anti-Infective Agents, Local" OR Antiseptics OR "Cetylpyridinium" OR "Cetylpyridinium chloride, zinc acetate drug combination" OR "Cetylpyridinium Chloride" OR "Povidone-Iodine" OR "Povidone iodine" OR "PVP-I" OR "PVP-Iodine" OR "Chlorhexidine" OR "Chlorhexidine Hydrochloride" OR "Hydrochloride, Chlorhexidine" OR "Hydrogen Peroxide" OR "Hydrogen Peroxide (H2O2)" OR Hydroperoxide OR "Essential oils" OR "Oils, Essential" OR Triclosan) AND (""Oral health" OR "Health, Oral" OR "Oral hygiene" OR "Dental Prophylaxis" OR "Prophylaxis, Dental" OR "Health Services, Dental" OR "Dental Health Services")
PubMed	(("Coronavírus Infections"[Mesh] OR "COVID-19" OR Coronavirus OR "SARS-CoV-2" OR "Herpes Labialis"[Mesh] OR "Herpes Simplex, Oral" OR "Herpes Simplex Virus Infection" OR "Herpes Simplex" OR "Stomatitis, Herpetic" OR "Gingivostomatitis, Herpetic" OR Hepatitis[Mesh] OR "Hepatitis C" OR "Hepatitis B"[Mesh] OR "Hepatitis B Virus Infection" OR "Hepatitis, infectious" OR "Infectious hepatitis" OR "Acquired Immunodeficiency Syndrome"[Mesh] OR "Acquired Immune Deficiency Syndrome" OR AIDS OR "Immunodeficiency Syndrome, Acquired" OR "HIV"[Mesh] OR "Influenza, Human"[Mesh] OR Grippe OR "Human Flu" OR "Human Influenza" OR "Influenza in Humans" OR Viruses[Mesh] OR "Virus Diseases"[Mesh] OR "Viral Infections" OR "Respiratory Tract Infections"[Mesh] OR "Infections, Respiratory" OR "Respiratory Infections") AND ("Mouthwashes"[Mesh] OR Mouthwash OR "Rinse, Mouth" OR Rinse OR "Mouth Rinse" OR "Mouth Bath" OR "Mouth Wash" OR "Mouth Washes" OR Gargle OR "Anti-Infective Agents, Local" OR Antiseptics OR "Cetylpyridinium"[Mesh] OR "Cetylpyridinium chloride, zinc acetate drug combination" OR "Cetylpyridinium Chloride" OR "Povidone-Iodine"[Mesh] OR "Povidone iodine" OR "PVP-I" OR "PVP-Iodine" OR "Chlorhexidine"[Mesh] OR "Chlorhexidine Hydrochloride" OR "Hydrochloride, Chlorhexidine" OR "Hydrogen Peroxide"[Mesh] OR "Hydrogen Peroxide (H2O2)" OR Hydroperoxide OR "Essential oils" OR "Oils, Essential" OR Triclosan)) AND ("Oral health"[Mesh] OR "Health, Oral" OR "Oral hygiene"[Mesh] OR "Dental Prophylaxis" OR "Prophylaxis, Dental" OR "Health Services, Dental" OR "Dental Health Services"[Mesh])
Scopus/ Web of Science	("Coronavírus Infections" OR "COVID-19" OR Coronavirus OR "SARS-CoV-2" OR "Herpes Labialis" OR "Herpes Simplex, Oral" OR "Herpes Simplex Virus Infection" OR "Herpes Simplex" OR "Stomatitis, Herpetic" OR "Gingivostomatitis, Herpetic" OR Hepatitis OR "Hepatitis C" OR "Hepatitis B" OR "Hepatitis B Virus Infection" OR "Hepatitis, infectious" OR "Infectious hepatitis" OR "Acquired Immunodeficiency Syndrome" OR "Acquired Immune Deficiency Syndrome" OR AIDS OR "Immunodeficiency Syndrome, Acquired" OR "HIV" OR "Influenza, Human" OR Grippe OR "Human Flu" OR "Human Influenza" OR "Influenza in Humans" OR Viruses OR "Virus Diseases" OR "Viral

	Infections" OR "Respiratory Tract Infections" OR "Infections, Respiratory" OR "Respiratory Infections") AND ("Mouthwashes" OR Mouthwash OR "Rinse, Mouth" OR Rinse OR "Mouth Rinse" OR "Mouth Bath" OR "Mouth Wash" OR "Mouth Washes" OR Gargle OR "Anti-Infective Agents, Local" OR Antiseptics OR "Cetylpyridinium" OR "Cetylpyridinium chloride, zinc acetate drug combination" OR "Cetylpyridinium Chloride" OR "Povidone-Iodine" OR "Povidone iodine" OR "PVP-I" OR "PVP-Iodine" OR "Chlorhexidine" OR "Chlorhexidine Hydrochloride" OR "Hydrochloride, Chlorhexidine" OR "Hydrogen Peroxide" OR "Hydrogen Peroxide (H2O2)" OR Hydroperoxide OR "Essential oils" OR "Oils, Essential" OR Triclosan) AND ("Oral health" OR "Health, Oral" OR "Oral hygiene" OR "Dental Prophylaxis" OR "Prophylaxis, Dental" OR "Health Services, Dental" OR "Dental Health Services")
Google Scholar	("Virus Diseases" OR "Respiratory Infections") AND ("Mouthwashes" OR "Anti-Infective Agents, Local) AND Antiseptics" OR "Cetylpyridinium Chloride" OR "Povidone-Iodine" OR "PVP-I" OR "Chlorhexidine" OR "Chlorhexidine Hydrochloride" OR "Hydrogen Peroxide" OR "Hydrogen Peroxide (H2O2)" OR "Essential oils" OR "Oils, Essential" OR Triclosan) AND ("Oral health")

Source: the authors.

Eligibility Criteria

The PICOS strategy was used to consider the eligibility of studies for this review:

P = population (viruses involved in the etiology of oral, ophthalmic, respiratory, and systemic infections, such as Herpes Simplex Virus (HSV-1 and 2); Influenza Virus (different subtypes); SARS-CoV-2 and Human Immunodeficiency Virus (HIV)).

I = intervention (mouthwashes including chlorhexidine digluconate (concentrations 0.2% and 0.12%), hydrogen peroxide, cetylpyridinium chloride (CPC), povidone-iodine (PVP-I), Listerine® and active ingredients such as essential oils).

C = comparison (positive controls (70% alcohol, sodium hypochlorite) and negative controls (placebo solutions and water)).

O = outcome (reduction of viral load).

S = study design (in vitro studies). Language, publication date and geographic restrictions were not used.

Exclusion Criteria

Descriptive studies, such as literature reviews, case reports, projects/protocols, opinion articles, letters, posters, and conference abstracts, were excluded. Studies involving pharmaceutical products or manipulations that contained combinations of mouthwashes with other chemical substances, which did not allow the isolated evaluation of the antiseptic, were also not considered.

Screening and Selection

The Rayyan Qatar Computing Research Institute (QCRI) application was used for the selection of studies, and selection was initially carried out by two reviewers (JCMV and CEPCNS) in two phases. In phase one, the independent screening of titles and abstracts was carried out to exclude studies that were irrelevant to the present review based on the inclusion criteria. The agreement between the reviewers was calculated as 71.4%. In phase two, the same reviewers read the texts completely, applying the inclusion and exclusion criteria, as previously established. In case of disagreement in the selection process, a third reviewer (GCBF) was consulted.

Data Extraction and Analysis

The collected data were: 1) author and year of publication; 2) type of virus (enveloped and not enveloped); 3) intervention; 4) temperature and time; 5) assay; 6) reduction of viral load.

Risk of Bias

The analysis of the risk of bias was performed based on an adaptation of a tool developed by Golbach et al. 2016[13], since there is no standard instrument for assessing the risk of bias for *in vitro* studies.

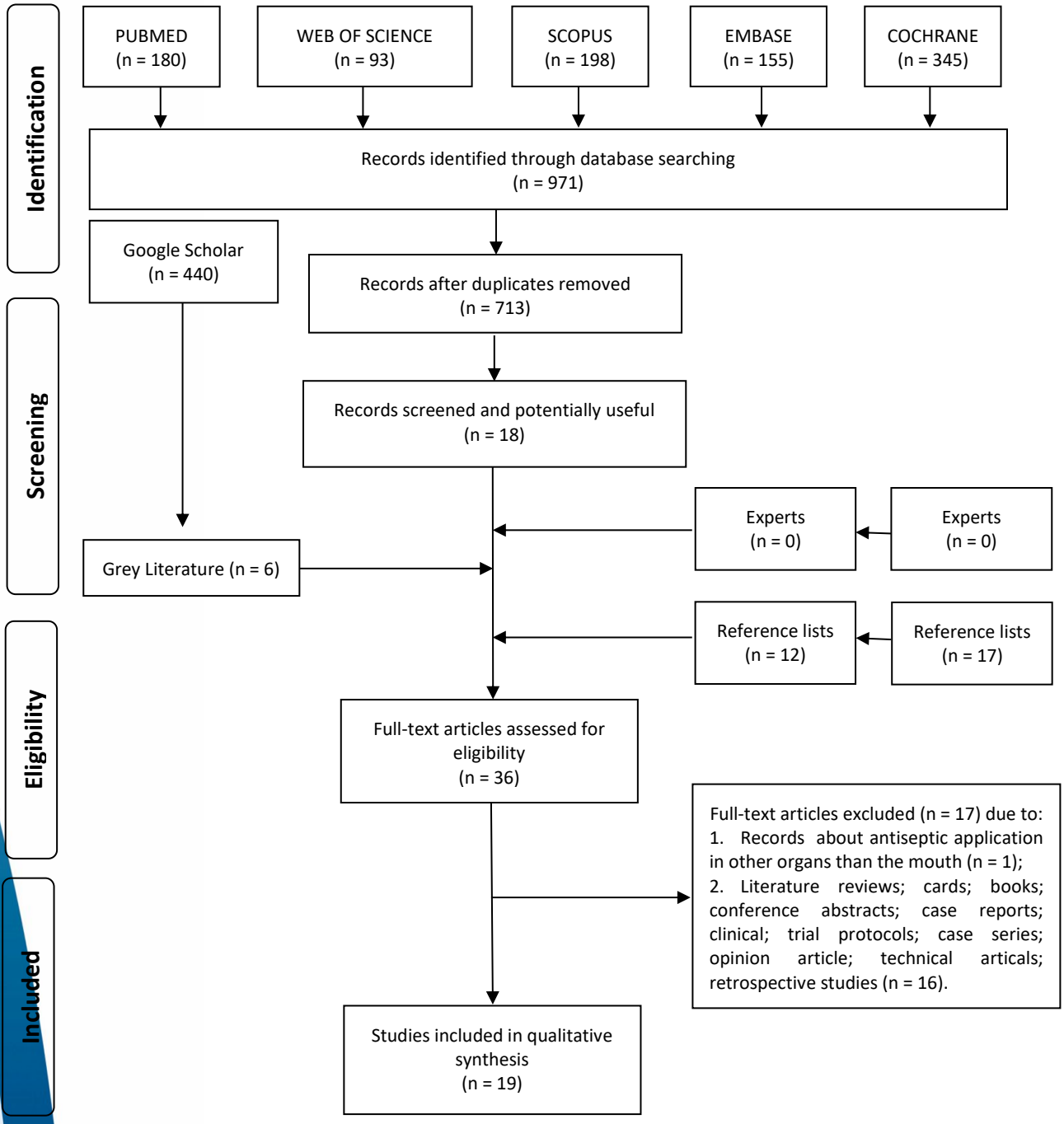
Each of the five domains evaluated considered one or more distinct criteria, which were classified as "low risk", "high risk" or "undefined" (due to insufficient information). Regarding the quality of the articles, aspects such as the type and viral origin were evaluated, as well as the concentration of substances, for example. With regards to the performance bias, the presence or absence of a control group, as well as the randomization of exposures, were analyzed, among other items. Domains relating to the selection, detection, and sponsorship bias of the industry were also analyzed.

Results

A total of 971 articles were retrieved. With the removal of duplicates, 713 articles remained. Then, 695 articles were excluded based on the reading of titles and abstracts, resulting in 18 articles for complete reading. Six more articles from the grey

literature and 12 from the reference list were added, totaling 36 articles eligible for full reading. Of these, 19 were included in the present study once the eligibility criteria were applied (Figure 1). A descriptive and qualitative analysis of the data was carried out (table 2).

Figure 1. Flow diagram for search results. Natal-RN, 2023.



Source: the authors.

Table 2. Characteristics of In Vitro Studies included in the Systematic Review. Natal-RN, 2023.

Study	Virus Type		Intervention	Time, Temperature	Trial (cultivation medium)	Results
	Enveloped virus	Non-enveloped virus				
Frank et al., 2020 [14]	SARS-CoV-2		**Control**: alcohol (70%); water **Test**: PVP-I nasal (0.5%, 1.25%, and 2.5% - dilution 1:1)	0.25 and 0.5 min, (22°C)	MEM + 2% FBS + 50 µg/ml gentamicin; Vero 76 cells (37°C, 5% CO_2, 5 days)	After 0.25 min e 0.5 min of contact time, all of the PVP-I antiseptics tested were effective in reducing greater than 3 and 3.33, respectively. The alcohol revealed the same effectiveness only for the time of 0.5 min
Bidra et al., 2020 [15]	SARS-CoV-2		**Control**: alcohol (70%); water **Test**: PVP-1 bucal (1.0%, 1.5%, 3.0% - dilution 1:1)	0.25 and 0.5 min (22°C)	MEM + 2% FBS + 50 µg/ml gentamicin; Vero 76 cells (37°C, 5% CO_2, 5 days)	After 0.25 min and 05 min of contact time, all of the PVP-I oral antiseptics were effective in reducing greater than 3 and 3.33 log10 CCID50, respectively. The alcohol showed that it took longer for the virus inactivation than the oral PVP-I solutions tested
Kariwa, 2006 [16]	SARS-CoV		**Control**: alcohol (70%) **Test**: Isodine® solution (1%), Isodine Scrub® (1%), Isodine Palm® (0.25%), Isodine Gargle® (0.47%), Isodine Nodo Fresh® (0.23%)	1 and 2 min, (20°C)	MEM + 10% FBS; Vero 76 cells (CO_2, 2 days)	After 1 min treatment with Isodine® and Isodine Gargle® did not completely eliminate the virus infectivity. The other antiseptics reduced the load below detectable levels <40 to <160 TCID 50/ml. Treatment with all the PVP-I products for 2 min completely inactivated the virus
Baqui et al., 2001 [20]	HSV-1 and HIV-1		**Negative Control**: Petri dish with monolayer of uninfected vero cells **Test**: LA, TLA, PX (0.12%) e CHX (0.2%)	0.5 min.	Vero cells (HSV-1; 37°C, 5% CO_2, 5 days) + Lymphoblastoid cell line (MT-2) (HIV-1, 37°, 5 days)	LA and TLA inhibited fully the HSV-1 up to 1:2 dilution, while PX and CHX showed decreased inhibition from 1:2 dilution onwards. LA and TLA completely inhibited HIV-1 only at commercial concentration. PX and CHX completely inhibited HIV-1 virus up to a 1:4 dilution
Vimalana-than, 2014 [26]	H1N1		**Negative Control:** canola oil **Positive Control**: zanamivir **Test**: *Lavandula officinalis, Pelargonium graveolens, Cinnamomum zeylanicum, Salvia officinalis, Eucalyptus globulus, Cymbopogon flexuosus, Thymus vulgaris, Citrus bergamia* e	60 min.	MEM (37 C°, 5% CO_2) + 5% FBS; MDCK and A549 human lung epithelial cells	*Cinnamomum zeylanicum, Citrusbergamia*and *Thymus vulgaris* completely inactivated the virus at high dilutions (<3.1 µL/mL). *Lavandula officinalis* and *Eucalyptus globulus* showed excellent activity at high concentrations (>10 µL/mL), but they were much less effective at low concentrations (<3.1 µL/mL). *Salvia showed* partial activity against the virus. *Pelargonium*

			Cupressus sempervirens			*graveolens* showed good activity against the virus. *Cupressus sempervirens* showed no antiviral effect, even at low dilutions (>10 µL/mL)
Wutzler et al., 2001[17]	H1N1 and HSV-1		**Control**: a drug-free isotonic liposomal preparation containing 4% of the same phosphatidylcholine. **Test**: Aqueous PVP-I solution Betaisodona® containing 10% (m/v) PVP-I and Liposomal PVP-I formulation containing 5% and 4.5% PVP-I	0.5; 1; 1.5; 2; 5; 15 and 30 min. Long-term cytotoxicity 120h	MEM + 0.1 g/ml Tricine and 10% FCS; MDCK cells (influenza A virus); Mixed media containing equal parts of Leibovitz's L-15 medium and lactalbumin hydrolysate + 10% FCS; RTP cells (HSV-1); DMEM + 8% FCS, A549 cells (adenovírus type 8); MEM + 1% non-essential, 20 mM hepes buffer, 500 µg /ml gentamicin + 5% FCS; HeLa cells (HRV-14). All at 37 °C and 5% CO_2	Influenza A and HSV-1 were inactivated by the two PVP-I formulations after 0.5 min. Adenovirus type 8 and HRV-14 needed higher concentrations, 0.23% and 0.45% respectively, and longer exposure time (30 min.) to be inactivated
Eggers et al., 2018 [8]	SARS-CoV, MERS-CoV and H1N1	Rotavirus	**Control:** distilled water **Test:** mouthwash Isodine PVP-I 7% (dilutions 1:300 and 1:3000)	0.25 min for SARS-CoV and MERS-CoV; 0.25 min and 0.5 min for influenza; 0.25 min,0.5 min, 1 min and 2 min for rotavirus (20.0 ± 1.0 °C)	Vero cells E6 for SARS-CoV and MERS-CoV, MDCK cells for influenza A strain, H1N1 and MA104 cells for rotavirus strain WA. All at 37 °C and 5% CO_2	It rapidly inactivated SARS-CoV, MERS-CoV, influenza A virus (H1N1) and rotavirus after 0.25 min of exposure
Bernstein et al., 1990 [21]	HSV-1, CMV, Influenza A, Parainflu-enza type 3 and HBV	Poliovirus type 1	**Control:** placebo contaning only excipients, no CHX. **Test:** CHX (0.12%)	0.5; 5 and 15 min. (37 ° C)	MEM + 2-10% FBS + penicilin (100 units/mL), streptomycin (50 µg/ml) + L-glutamine (2 mmol/L); RK cells (HSV-1); HFF (CMV); RD (poliovirus); CV-1 (parainfluenza) and MDCK (Influenza)	After 30s there were reduction of 59%; 85%; 93%; 97% and 99.7% for parainfluenza, HBV-DNA, influeza A, HSV and CMV respectively, compared to placebo, for the time of 5 min and 15 min. The viral load reduction was greater than 90% and 98%, for all type of viruses, except for poliovirus, on which none of the interventions were effectively
Popkin et al., 2017 [12]	H1N1		**Control:** PBS **Test:** Increasing concentrations of CPC (10µg/mL to 250µg/mL, Sigma-Aldrich, St. Louis, MO) diluted in PBS	10 min. (37°C)	MEM + supplemented with penicillin/streptomycin, L-glutamine, and 10% FCS. MDCK cells were inoculated with influenza virus (32°C, 72h, 5% CO2)	CPC exhibited direct virucidal activity against Influenza A and B viruses, including oseltamivir-resistant viruses. The effective concentration of CPC (EC50) against all influenza viruses ranged from 5 µg/mL to 12.5 µg/ml

Dennison et al., 1995 [24]	HSV-1 and 2, H1N1	Rotavirus and Adenovirus (type 5)	**Control:** Listerine®without virus presence **Test:** Listerine ®	0.5; 2 and 5 min. (37 ° C)	Vero cells (HSV 1 and 2; Adenovirus), MA-104 cells (Rotavirus), MDCK cells (Influenza A)	After 0.5 min Listerine® reduced viral load by 2.2%, 96.3%, 100% and 100% for rotavirus, HSV-1, HSV-2, and influenza, respectively. After 5 min, the test group reduced 33.4% of viral load of adenovirus
Kawana et al., 1997 [18]	Mumps virus, HSV-1, Rubella virus, Measles virus, H1N1 and HIV-1	Rotavirus, Adenovirus (type 5), Poliovirus (type 1 and 3), coxsackie-virus, Rhinovirus,	Control: Absent Test: PVP-I solution; PVP-I gargle; PVP-I cream; CHX; AEG; BAC and BEC	0.25, 0.5, 1, 3, 5 and 10 min. (25°C)	MEM; Cell culture (unspecified)	PVP-I drugs inactivated all viruses in a short period. Rubella, measles, mumps and HIV viruses were all sensitive to antiseptics. In addition to PVP-I, rotavirus was inactivated by BAC and BEC. Adenoviruses, poliovirus and rhinovirus showed sensitivity only to PVP-I, while HSV-1 was inactivated by all solutions in a dose-dependent manner. Adenovirus, rotavirus, rhinovirus and poliovirus were resistant to CHX
Croughan et al., 1988 [25]	HSV-1 and HSV-2		**Control:** present (not specified) **Test:** LA; Bleach, Lysol (dilutions 1%, 0.5%, 0.25%, and 0.1%), alcohol 70%, Alcide desinfectant (after preparation and after 14 days of storage)	Lysol 5 min and 10 min (25 °C); LA 1 min and 5 min (25 °C); Commercial bleach 10 min (25 °C); Alcohol 70% 1 min and 5 min, (25 °C and 37 °C); Alcide desinfectant 1 min and 3 min (25 °C)	MEM + 3% fetal bovine serum, Vero cells (37 °C)	Lysol 1%, 0.5% and 0.1% reduced HSV-1 and 2 titers after 5 min and 10 min. LA showed no cytotoxic effects to cells and inactivated both types of HSV after 5 min at 25°C. Bleach reduced the viral titers of both viral types at a concentration of 2000 ppm. Alcohol 70% reduced the titers of both viral types after 5 min. Alcide disinfectant, both after preparation and after storage, reduced the titers of both viral types after 1 and 3 min
Kaplan, 1987 [19]	HIV		**Control:** culture infected with untreated HIV **Test:** Betadine (dilutions 0.25%, 0.05%); BSS (dilutions 0.25% and 0.125%)	Betadine 0.5 min and 1 min; BSS 1min and 10 min.	RPMI 1640 + 20% inactivated FCS, H-9 cells infected with HIV (37 °C, 5% CO_2, 3-4 days)	Betadine 0.25% inhibited viral replication at all times tested. Betadine 0.05% was ineffective, 0.25% BSS completely inactivated the virus while 0.125% BSS was unable to inactivate the virus even after 10 min
Park, 1989 [23]	HSV-1		**Control:** group without treatment **Test:** CHX 10% (dilutions 0.2%, 0.1%, 0.01%, 0.001%, 0.0008% and 0.0005%)	24 h (37 °C)	MEM + 5% FCS, Vero cells (37 °C and 5% CO_2)	CHX inhibited HSV-1 growth at all dilutions. The higher the concentration, the greater the inhibitory effect. Above 0.001%, CHX had a cytotoxic effect

Shet, et al. 2021 [20]	SARS-CoV-2		**Control:** Placebo **Test:** PVP-I solutions (0.5% (w/v); 5.0% (w/v); 7.5% (w/v) 10.0%(w/v))	<0.15; 0.15; 0.5; 1.0 and 5 min. 20 ± 1 °C	MEM + 2% FBS +1% antibiotic and L-glutamine)	The mean log10 reductions in viral titer for SARS-CoV-2 were consistently higher for 0.5% (w/v) solution and 10.0% (w/v) solution compared with 7.5% (w/v) scrub and placebos
Davies et al. 2021 [21]	SARS-CoV-2		**Control:** Absent **Test**: CHX (Peppermint Flavour); Corsodyl (Alcohol Free Mint Flavour); Listerine® Advanced Defence Sensitive; Listerine® Total Care; OraWize+; Peroxyl and Povident	1 min. (20 ±2 °C)	MEM + 5 % FBS	Listerine® Advanced Defence Sensitive and Total Care formulations, and by commercial mouthwashes containing 0.01–0.02 % hypochlorous acid or 0.58 % povidone iodine demonstrated effective inactivation of SARS-CoV-2. But the use of mouthwashes with hydrogen peroxide or chlorhexidine gluconate to reduce the viral load of SARS-CoV-2 has not been shown to be effective
Bidra, et al. 2020 [44]	SARS-CoV-2		**Control:** Ethanol (70%); Water **Test:** PVP-I (0.5%; 1.25%; 1.5%) and H2O2 (3%; 1.5%)	0.15 and 0.5 min. (22 ± 2 °C)	MEM + 2% FBS + 50 µg/mL gentamicin	SARS-CoV-2 virus was completely inactivated by PVP-I oral antisep- tic rinse in vitro, at the lowest concentration of 0.5 % and at the lowest contact time of 15 seconds. Hydrogen. Hydrogen peroxide (1.5% and 3.0%; 0.5min) was minimally effective as a viricidal agent
Ramjia, et al. 2022 [28]	SARS-CoV-2		**Test:** H2O2 (1.5%); CPC (0.07%); SnF2 dentifrice A e B (0.454%)	0.5 and 1 min. (20 ± 2 °C)	MEM + Vero E6 host cells + 0.3m/l BSA	The 1.5% H2O2 rinse, 0.07% CPC rinse, SnF2 dentifrice A, and SnF2 dentifrice B all produced > 4 log10 re- duction in SARS-CoV-2 titer.
Anderson, E. R. 2022 [29]	SARS-CoV-2		**Control:** 70 % ethanol in distilled water; Distilled water **Test:** CHX (0.2%) CPC (0.07%)	0.5 min.	DMEM) + 10 % FBS+ 0.05 mg ml−1 gentamicin	Two mouthwashes containing 0.07 % CPC were effective at inactivating SARS- CoV- 2, within 30 s with greater than 4.0log10 p.f.u. ml−1 reduction in viral titre. In contrast, mouthwash containing 0.2 % CHX did not have substantial action against SARS- CoV- 2 in vitro.

Legend: MIN, Minutes; PVP-I, Povidone-iodine; MEM, Minimum Essential Medium; FBS, fetal bovine sérum; LA, Listerine® Antiseptic; TLA, Tarter control Listerine® Antisepti; PX, Peridex®; CHX, chlorhexidine; HSV-1, Herpes simplex vírus type 1; HSV-2, Herpes simplex vírus type 2; HIV-1, Human Immunodeficiency Vírus Type 1; H1N1, influenza vírus A; MDCK, Madin-Darby canine kidney cells; HRV-14, human rhinovirus type 14; FCS, fetal calf serum; RTP, rabbit testes primary; DMEM, Dulbecco's modified Eagle's médium; HeLa, Human epithelial-like; CMV, cytomegalovirus; HBV, hepatitis B; RK cells, Rabbit kidney cells; HFF, human foreskin fibroblast; RD, rhabdomyosarcoma; CV-1, African green monkey kidney; CPC, Cetylpyridinium chloride; PBS, Phosphate-Buffered Saline; AEG, alkyldiaminoethyl glycine hydrochloride; BAC, benzalkonium chloride; BEC, benzethonium chloride; BSS (Betadine Surgical Scrub); RPMI 1640, RPMI 1640 medium; H2O2, hydrogen peroxide; SnF2, stannouns fluoride; BSA, bovine serum albumin.

Study Characteristics

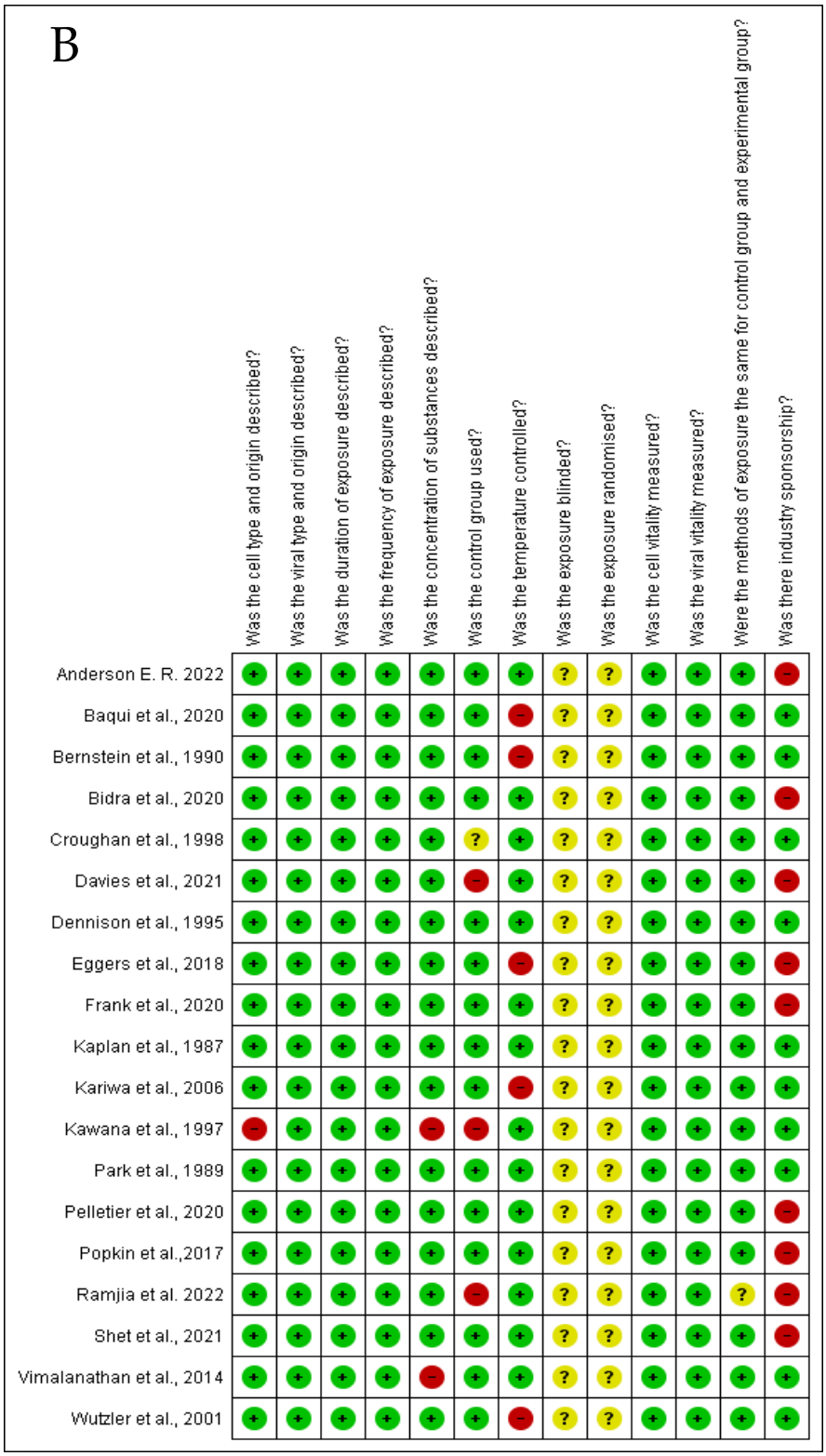
The included studies were published between 1987 and 2023, of which 9 involved the use of PVP-I[8,14-21], 6 involved chlorhexidine[18,21-24,29], four involved Listerine®[21,22,25,26], one involved essential oils[27] and 3 involved CPC[11,28,29].

The viruses investigated were: HSV-1[17,18,22–26]; H1N1[8,11,18,23,25,27]; SARS-CoV-2[8,14-16,20,21,28,29]; SARS-CoV[8,16]; rotavirus[8,18,25] and HIV-1[18,19,22]. Other viruses were tested, such as HSV-2[25,26], adenovirus type 8[17], MERs-CoV[8], cytomegalovirus, hepatitis B virus, parainfluenza type 3, poliovirus[23], measles virus, and rubeola Virus[18].

Risk of Bias

Among the 19 items analyzed for the risk of bias, 5 items were categorized as low risk for all studies (Figure 2A and 2B). The items "blind exposure" and "randomized exposure" were registered as "Unclear risk of bias" in all articles.

Figures 2A and 2B. Risk of bias of the included studies. Natal-RN, 2023.



Source: the authors.

The item "controlled temperature" presented the highest risk of bias (26.3%), with 5 of the 19 studies not controlling the temperature of the experiments. Regarding the "industry sponsorship" variable, 9 of the 19 studies were linked with companies. In addition, 10.5% did not report the concentration of the substances, and

another 5.2% did not record the type and cellular origin, nor the use of a control group. On the other hand, the vitality of the viruses before or after exposure was recorded in 100% of the studies (Figure 2A). The results for each analyzed antiseptic will now be presented.

Povidone-iodine (PVP-I)

Nine articles[8,14-21] used PVP-I, and all of them considered values related to viral titration to determine the effectiveness of the antiseptic on the microorganisms, especially SARS-CoV-2, which was reported in 6 studies[8,14-16,29,21]. Six studies also evaluated the reduction in the values of logarithmic securities[8,14,15,17,20,21].

Among the most relevant findings, Isodine Gargle® (0.47%) applied for 60 seconds was not able to completely reduce the viral infectivity of SARS-CoV, unlike other povidone-based products, such as Isodine® solution (1 %), Isodine Scrub® (1%), Isodine Palm® (0.25%) and Isodine Nodo Fresh® (0.23%), which were employed in the same study[16]. On the other hand, the oral PVP-I solution (0.23%) used by Eggers et al. 2018, demonstrated virucidal activity, reducing the SARS-CoV titration (by more than 4 log10) after 15 seconds of exposure[8]. Data similar were also observed by Davies et al (PVP-I 0.58%)[20] and Shet et al (PVP-I 0.5%)[21].

Chlorhexidine Gluconate

The compounds based on chlorhexidine (0.12% and 0.2%) and those based on essential oils, such as Listerine (Listerine® Antisepetic (LA), Tarter control Listerine® Antiseptic), completely inhibited HIV-1 and HSV-1[18,22]. Regarding the first virus, chlorhexidine demonstrated greater antiviral efficacy when compared to Listerine; even in the presence of dilutions (1:2 and 1:4), it was able to prevent the viability of this microorganism[22]. Adenovirus, rotavirus, rhinovirus, poliovirus type 1, Influenza A and SARS-COV-2, on the other hand, showed resistance or little sensitivity to chlorhexidine[18,21].

Listerine®

The compounds based on Listerine® showed better antiviral efficacy against HSV-1 when compared to chlorhexidine[22]. Dennison et al.[25] investigated the

effectiveness of this antiseptic against a large number of viruses and concluded that the best results were observed against HSV-1, HSV-2 and the influenza virus, while adenovirus revealed low sensitivity to Listerine[30]. As for SARS-COV-2, two different compositions of this antiseptic - Listerine® Advanced Defence Sensitive and alcohol-free Listerine® Total Care - demonstrated efficacy after 1 minute of exposure[21].

Eucalyptus globulus - a species of precursor plant for the essential oil eucalyptol, which is used in the formulation of antiseptics such as Listerine® and Cepacol® - demonstrated better antiviral activity against the H1N1 virus in the vapor phase compared to the liquid phase after 10 minutes[27].

Cetylpyridinium Chloride (CPC)

The antiviral effect of CPC was first tested by a haemagglutination assay against influenza A (H3N2 and H1N1) and B viruses. The concentration required to reduce viral titers by 50% was different for the viral types, requiring higher concentrations against influenza A. Due to the variety of concentrations, a therapeutic index of CPC was determined between 7.7 and 19.2 (CC50 / EC50) - this value is a reason that CC50 represents the cytotoxic concentration of 50% of CPC and EC50 the effective virucidal concentration. Another infection test was performed to confirm the results of the previous test. It was observed that CPC resulted in a reduction of 2 log considering an infectious dose for 50% of cultured cells (TCID50), and the influenza B strain again showed more sensitivity to the substance[11].

Concerning SARS-COV -2, CPC at 0.07% was able to reduce viral titers to insignificant values[28,29].

Discussion

The present study is noteworthy and significant because it investigated the effectiveness of povidone-iodine (PVP-I), chlorhexidine, Listerine®, essential oils, and cetylpyridinium chloride against the main viruses encountered in dental practice, using in vitro studies." Povidone-iodine (PVP-I) and chlorhexidine digluconate were the most frequently investigated antiseptics in studies examining

their effectiveness against viruses in dental practice. However, the type, concentration, and exposure time of the antiseptics varied across studies. PVP-I demonstrated superior efficacy in reducing viral titers, particularly against coronaviruses, compared to other antiseptics. Although cetylpyridinium chloride (CPC), hydrogen peroxide (H2O2), and Listerine® also showed significant reductions in viral load, the number of studies investigating these antiseptics was limited.

Eggers et al.[31] evaluated the virucidal efficacy of PVP-I for oral rinse at 1% and identified that this antiseptic was able to reduce MERS-CoV viral titres in 4 logs compared to the control group (distilled water) after 30 seconds of exposure in a 1:10 dilution, which was corroborated by the findings of Eggers et al.[8] when a similar reduction was achieved for SARS-CoV, after 15 seconds. Such findings suggest that rinsing with a PVP-I mouthwash before dental procedures may represent an alternative for the reduction of viral load, especially coronaviruses, which have their lipid membrane destroyed by this antiseptic, a fact that could contribute to the reduction in rates of transmission of these microorganisms. In addition, enveloped viruses such as HSV and influenza may be considered sensitive, as demonstrated by Wutzler et al.[17], who obtained viral inactivation values higher than 4 logs for HSV-1 and Influenza A, which are some of the viruses that are most sensitive to formulations with PVP-I.

The literature also suggests that non-enveloped viruses, such as adenovirus and rhinovirus, are also susceptible, although they may require higher concentrations of the substance and a longer exposure time[17,18]. However, the results from these in vitro studies require further investigation through controlled clinical studies, since the viruses can be organized into biofilms in the oral cavity (on teeth, restorations, prostheses, and dental implants), which can protect themselves against the action of external agents and the immune system of living organisms[32,33].

On the other hand, a randomized clinical trial evaluating the efficacy of three commercial rinses - PVP-I, chlorhexidine gluconate, and CPC - in reducing the viral load of SARS-CoV-2 present in the saliva of patients diagnosed with COVID-19,

showed significant differences only between the test groups (PVP-I and CPC) in relation to the control group (water). The results showed that only chlorhexidine had no virucidal effect in a sample of 16 patients, with PVP-I and CPC with similar efficacy[34]. It can be suggested that chlorhexidine has a low capacity to inactivate strains of coronavirus, as demonstrated by an integrative review conducted by O'Donnel et al.[35] and by an in vitro study by Meister et al.[36].

Chlorhexidine gluconate is an excellent antiseptic, especially in the treatment and prevention of bacterial infections; however, its virucidal efficacy is still questionable. Bailey et al.[37] and Kawana et al.[18] found that poliovirus and adenovirus were not sensitive to chlorhexidine, unlike HSV. The morphological differences found between these microorganisms may explain the difference in susceptibility to this antiseptic since the first two do not have a viral envelope, which is present in Herpes Simplex.

An in vivo study investigated the antiviral efficacy of the antiseptic Listerine® on HSV after 30 seconds of rinsing and observed that no plaque-forming units per mL (UFP/mL) were identified immediately after the mouth rinse in 18 of the 20 patients of the experimental group. This result was maintained for 9 participants, even after 30 minutes, which demonstrates the residual effect of this product[38]. Similar findings were also shown by Baqui et al.[22] and Dennison et al.[25] in vitro studies, which revealed the effectiveness of Listerine on enveloped viruses such as HSV-1 and influenza viruses.

The effectiveness of a commercial formulation based on CPC with other added components, such as xanthan gum and glycerin, was evaluated by Mukherjee et al[39]. The authors observed that the use of this product by patients as an intraoral topical spray, 3 times a day for 75 days, reduced 55% of respiratory infections. In addition, viruses such as influenza, coronavirus, or rhinovirus were detected only in patients of the placebo group. These results agree with the findings by Popkin et al.[11], which revealed the virucidal efficacy of CPC on the influenza virus after 10 minutes of in vitro exposure. This can be justified by CPC's ability to disintegrate the lipoprotein envelope, which is inherent in most of these microorganisms.

Only two studies included in the present review revealed some cytotoxic effects on the use of mouthwashes[22,24]. Park[24] revealed that the use of chlorhexidine in concentrations greater than 0.001% resulted in undesirable effects, but did not describe them, and Baqui et al.[22] observed that preparations based on chlorhexidine and Listerine®, when diluted 1:10 and 1:100, reduced the levels of cell cytotoxicity to less than 1%. However, an in vitro study that aimed to investigate the cytotoxicity (morphological changes, cell viability, and mitochondrial reductase activity) of some mouthwashes on primary gingival fibroblasts, epithelial cells, and L929 cells, found strong cytotoxic activity inherent to chlorhexidine 0.2%, and moderate cytotoxic activity for cetylpyridine chloride and Listerine®. Although compounds containing chlorhexidine (0.05% and 0.2%) have demonstrated greater antimicrobial efficacy compared to compounds containing CPC, while Listerine® did not reveal any activity on the investigated microorganisms, such as *Porphyromonas gingivalis* and *Fusobacterium nucleatum*, these findings may suggest that, in commercial concentrations, the potential for these substances to cause cell damage is increased, and there is variability between antiseptics, in terms of cytotoxic and antimicrobial effects[40].

With regard to the limitations of the in vitro studies, both the antiviral effect and the cytotoxicity were investigated in cultures of isolated cells, especially Vero cells, distributed in monolayers. However, the oral environment contains cells of different origins and functions, such as immune, epithelial cells, and oral fibroblasts, which are organized in several layers. In addition, the parameters used to analyze viral viability after using rinses also varied among authors. Frank et al.[14] and Bidra et al.[15] considered the reduction of the log value in addition to viral titration, while other studies evaluated only one of these parameters, for example, Kariwa et al.[16] and Eggers et al[8].

Another aspect that also deviated among the authors, making it difficult to compare study findings, was the concentration and dilution of antiseptics: Kariwa et al.[16] used Isodine Gargle (0.47%) and Eggers et al.[41] investigated an oral 0.23% PVP-I solution, for example. Similarly, two other studies evaluated the effectiveness of

chlorhexidine using different representations: Kawana et al.[18] used concentrations of 0.05%, 0.1% and 0.5%, while Baqui et al.[22] used dilutions of 1:2; 1:4, and 1:8.

The methodology and results of in vitro studies provide contributions for the development of clinical trials, reducing the use of pre-clinical studies and minimizing the occurrence of adverse reactions when exposed to these substances. Other limitations include the irreproducibility of the interaction between the experimental and/or control group with the human host because the tests are restricted to inoculation in isolated cell groups, cultivation in tubes, or glass plates, for example[42].

Furthermore, colonization and pathogenesis of viruses in the mouth can be quite dynamic, and this reality is not reproduced in vitro. These microorganisms can cause direct cytopathic effects, hindering the mechanisms of cell renewal and immune response, as well as establishing synergistic relationships with bacteria, in which the virulence, multiplication capacity, and resistance of both are increased[43].

The clinical relevance of these findings may be related to the reduction of viral levels, which could limit the occurrence of cross-contamination, as well as reduce the risk of transmission to healthy individuals, including other patients, dentists, and personal health care workers. The included articles were considered heterogeneous, which made it impossible to perform a meta-analysis, as well as limiting the definition of a dose-response for antiseptics in relation to the different viruses that may be present in the oral environment. In addition, there are other limitations such as the lack of standardization of studies, techniques, and analyses, which could give greater reliability to the results, as well as the fact that it is an in vitro study, in which laboratory conditions do not always match real conditions.

Oral antiseptics have demonstrated efficacy against most viral titers. However, it is still too early to definitively state the differences in susceptibility between enveloped viruses (such as H1N1, HBV, HSV, SARS-CoV) and non-enveloped viruses (such as poliovirus, adenovirus, rotavirus, and rhinovirus), as only a limited number of studies (n=4)[8,18,23,25] have investigated the efficacy of these products on the latter group. Nevertheless, these studies have shown that non-enveloped viruses tend to be more resistant to antiseptics like chlorhexidine[18,23] and Listerine®[25]. This

resistance could be due to either a longer exposure time required or a failure to reduce the viral load18. Additionally, only one study did not find any differences in the performance of mouthwashes against viruses with or without a lipoprotein envelope[8]. Therefore, further clinical studies are needed to assess the role of these agents in reducing virucidal potential in dental practice, particularly in relation to the presence or absence of a viral envelope.

To improve the design of future in vitro studies on oral antiseptics against viruses, researchers should focus on standardizing protocols, comparing multiple antiseptics, using clinically relevant viral strains, simulating the oral environment, investigating potential cytotoxicity, and collaboration among institutions.

Conclusions

The types, concentrations, and durations of exposure to antiseptics varied across studies. Among the substances examined, PVP-I and chlorhexidine digluconate received the most attention. However, PVP-I demonstrated superior effectiveness in reducing viral titers, particularly against coronaviruses.

References

1. Zemouri C, De Soet H, Crielaard W, Laheij A. A scoping review on bio-Aerosols in healthcare & the dental environment. PLoS One. 2017;12(5):1–25. https://doi.org/10.1371/journal.pone.0178007

2. Kobza J, Pastuszka JS, Bragoszewska E. Do exposures to aerosols pose a risk to dental professionals? Occup Med (Chic Ill). 2018;68(7):454–8. https://doi.org/10.1093/occmed/kqy095

3. Zemouri C, Awad SF, Volgenant CMC, Crielaard W, Laheij AMGA, de Soet JJ. Modeling of the Transmission of Coronaviruses, Measles Virus, Influenza Virus, Mycobacterium tuberculosis, and Legionella pneumophila in Dental Clinics. J Dent Res. 2020;99(10):1192–8. https://doi.org/10.1177/0022034520940288

4. Vermisoglou E, Panáček D, Jayaramulu K, Pykal M, Frébort I, Kolář M, et al. Human virus detection with graphene-based materials. Biosens Bioelectron. 2020;166:112436. https://doi.org/10.1016/j.bios.2020.112436

5. California Department of Public Health. California's Aerosol Transmissible Disease Standards and Local Health Departments. Richmond, CA: California Department of Public Health, Occupational Health Branch; 2018. Available from: https://www.cdph.ca.gov/Programs/CCDPHP/DEODC/OHB/CDPH%20Document%20Library/ATD-Guidance.pdf

6. Geller C, Finance C, Duval RE. Evaluation of antiseptic antiviral activity of chemical agents. Curr Protoc Cell Biol. 2011;(SUPP.51):1–22. https://doi.org/10.1002/0471143030.cb2610s51

7. Fernandez MDS, Guedes MIF, Langa GPJ, Rösing CK, Cavagni J, Muniz FWMG. Virucidal efficacy of chlorhexidine: a systematic review. Odontology. 2022;110(2):376-392. https://doi.org/10.1007/s10266-021-00660-x

8. Eggers M, Koburger-Janssen T, Eickmann M, Zorn J. *In Vitro* Bactericidal and Virucidal Efficacy of Povidone-Iodine Gargle/Mouthwash Against Respiratory and Oral Tract Pathogens. Infect Dis Ther [Internet]. 2018;7(2):249–59. https://doi.org/10.1007/s40121-018-0200-7

9. Asif M, Saleem M, Saadullah M, Yaseen HS, Al Zarzour R. COVID-19 and therapy with essential oils having antiviral, anti-inflammatory, and immunomodulatory properties. Inflammopharmacology [Internet]. 2020;28(5):1153–61. https://doi.org/10.1007/s10787-020-00744-0

10. Senthil Kumar KJ, Vani MG, Wang CS, Chen CC, Chen YC, Lu LP, et al. Geranium and lemon essential oils and their active compounds downregulate angiotensin-converting enzyme 2 (ACE2), a SARS-CoV-2 spike receptor-binding domain, in epithelial cells. Plants. 2020;9(6):1–12. https://doi.org/10.3390/plants9060770

11. Popkin DL, Zilka S, Dimaano M, Fujioka H, Rackley C, Salata R, et al. Cetylpyridinium chloride (CPC) exhibits potent, rapid activity against influenza viruses *in vitro* and *in vivo*. Pathog Immun. 2017;2(2):253–69. https://doi.org/10.20411/pai.v2i2.200

12. Marui VC, Souto MLS, Rovai ES, Romito GA, Chambrone L, Pannuti CM. Efficacy of preprocedural mouthrinses in the reduction of microorganisms in aerosol: A systematic review. J Am Dent Assoc [Internet]. 2019;150(12):1015-1026.e1. https://doi.org/10.1016/j.adaj.2019.06.024

13. Golbach LA, Portelli LA, Savelkoul HFJ, Terwel SR, Kuster N, de Vries RBM, et al. Calcium homeostasis and low-frequency magnetic and electric field exposure: A systematic review and meta-analysis of *in vitro* studies. Environ Int. 2016;92–93:695–706. https://doi.org/10.1016/j.envint.2016.01.014

14. Frank S, Brown SM, Capriotti JA, Westover JB, Pelletier JS, Tessema B. *In Vitro* Efficacy of a Povidone-Iodine Nasal Antiseptic for Rapid Inactivation of SARS-CoV-2. JAMA Otolaryngol - Head Neck Surg. 2020;146(11):1054–8.

https://doi.org/10.1001/jamaoto.2020.3053

15. Bidra AS, Pelletier JS, Westover JB, Frank S, Brown SM, Tessema B. Rapid In-Vitro Inactivation of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Using Povidone-Iodine Oral Antiseptic Rinse. J Prosthodont. 2020;29(6):529–33. https://doi.org/10.1111/jopr.13209

16. Kariwa H, Fujii N, Takashima I. Inactivation of SARS coronavirus by means of povidone-iodine, physical conditions and chemical reagents. Dermatology. 2006;212(SUPPL. 1):119–23. https://doi.org/10.1159/000089211

17. Wutzler P, Sauerbrei A, Klöcking R, Brögmann B, Reimer K. Virucidal activity and cytotoxicity of the liposomal formulation of povidone-iodine. Antiviral Res. 2002;54(2):89–97. https://doi.org/10.1016/s0166-3542(01)00213-3

18. Kawana R, Kitamura T, Nakagomi O, Matsumoto I, Arita M, Yoshihara N, et al. Inactivation of Human Viruses by Povidone-Iodine in Comparison with Other Antiseptics. Dermatology [Internet]. 1997;195(2):29–35. https://doi.org/10.1159/000246027

19. Kaplan JC, Crawford DC, Durno AG, Schooley RT. Inactivation of Human Immunodeficiency Virus by Betadine. Infect Control [Internet]. 1987;8(10):412–4. https://doi.org/10.1017/s0195941700066583

20. Shet M, Hong R, Igo D, Cataldo M, Bhaskar S. In vitro evaluation of the virucidal activity of different povidone–iodine formulations against murine and human coronaviruses. Infect Dis Ther, 2021;*10*(4), 2777-2790. https://doi.org/10.1007/s40121-021-00536-1

21. Davies K, Buczkowski H, Welch SR, Green N, Mawer D, Woodford N, et al. Effective in vitro inactivation of SARS-CoV-2 by commercially available mouthwashes. J Gen Virol. 2021;*102*(4). https://doi.org/10.1099/jgv.0.001578

22. Baqui AAMA, Jabra-Rizk MA, DePaola LG, Falkler WA, Meiller TF. *In vitro* effect of oral antiseptics on human immunodeficiency virus-1 and herpes simplex virus type 1. J Clin Periodontol. 2001;28(7):610–6. https://doi.org/10.1034/j.1600-051x.2001.028007610.x

23. Bernstein D, Schiff G, Echler G, Prince A, Feller M, Briner W. *In vitro* Virucidal Effectiveness of a 0.12%-Chlorhexidine Gluconate Mouthrinse. J Dent Res [Internet]. 1990 Mar 8;69(3):874–6. https://doi.org/10.1177/00220345900690030901

24. Park JB, Park NH. Effect of chlorhexidine on the *in vitro* and *in vivo* herpes simplex virus infection. Oral Surgery, Oral Med Oral Pathol. 1989;67(2):149–53. https://doi.org/10.1016/0030-4220(89)90320-4

25. Dennison DK, Meredith GM, Shillitoe EJ, Caffesse RG. The antiviral spectrum of Listerine antiseptic. Oral Surgery, Oral Med Oral Pathol Oral Radiol. 1995;79(4):442–8. https://doi.org/10.1016/s1079-2104(05)80124-6

26. Croughan WS, Behbehani AM. Comparative study of inactivation of herpes simplex virus types 1 and 2 by commonly used antiseptic agents. J Clin Microbiol. 1988;26(2):213–5. https://doi.org/10.1128/jcm.26.2.213-215.1988

27. Vimalanathan S, Hudson J. Anti-influenza virus activity of essential oils and vapors. Am J Essent Oils Nat Prod [Internet]. 2014;2(1):47–53. Available from: http://www.essencejournal.com/vol2/issue1/pdf/8.1.pdf

28. Ramji N, Circello B, Winston JL, Biesbrock AR. Virucidal Activity of Over-the-Counter Oral Care Products Against SARS-CoV-2. Oral Health Prev Dent. 2022;*20*(1), 185-192. https://doi.org/10.3290/j.ohpd.b2960525

29. Anderson ER, Patterson EI, Richards S, Pitol AK, Edwards T, Wooding, D, et al. CPC-containing oral rinses inactivate SARS-CoV-2 variants and are active in the presence of human saliva. J Med Microbiol. 2022;*71*(2). https://doi.org/10.1099/jmm.0.001508

30. De Moraes MDR, Carneiro JRM, Passos VF, Santiago SL. Effect of green tea as a protective measure against dental erosion in coronary dentine. Braz Oral Res. 2015;30(1) https://doi.org/10.1590/1807-3107BOR-2016.vol30.0013

31. Eggers M, Eickmann M, Zorn J. Rapid and Effective Virucidal Activity of Povidone-Iodine Products Against Middle East Respiratory Syndrome Coronavirus (MERS-CoV) and Modified Vaccinia Virus Ankara (MVA). Infect Dis Ther. 2015;4(4):491–501. https://doi.org/10.1007/s40121-015-0091-9

32. Sharma N, Bhatia S, Singh Sodhi A, Batra N. Oral microbiome and health. AIMS Microbiol [Internet]. 2018;4(1):42–66. https://doi.org/10.3934/microbiol.2018.1.42

33. Kuang X, Chen V, Xu X. Novel Approaches to the Control of Oral Microbial Biofilms. Biomed Res Int. 2018;2018. https://doi.org/10.1155/2018/6498932

34. Seneviratne CJ, Balan P, Ko KKK, Udawatte NS, Lai D, Ng DHL, et al. Efficacy of commercial mouth-rinses on SARS-CoV-2 viral load in saliva: randomized control trial in Singapore. Infection [Internet]. 2021;49(2):305–11. https://doi.org/10.1007/s15010-020-01563-9

35. O'Donnell VB, Thomas D, Stanton R, Maillard J, Murphy RC, Jones SA, et al. Potential Role of Oral Rinses Targeting the Viral Lipid Envelope in SARS-CoV-2 Infection. Function [Internet]. 2020 Jan 1;1(1). https://doi.org/10.1093/function/zqaa002

36. Meister TL, Brüggemann Y, Todt D, Conzelmann C, Müller JA, Groß R, et al. Virucidal Efficacy of Different Oral Rinses Against Severe Acute Respiratory Syndrome Coronavirus 2. J Infect Dis [Internet]. 2020 Sep 14;222(8):1289–92. https://doi.org/10.1093/infdis/jiaa471

37. Bailey A, Longson M. Virucidal activity of chlorhexidine on strains of Herpesvirus hominis, poliovirus, and adenovirus. J Clin Pathol. 1972;25(1):76–8.

https://doi.org/10.1136/jcp.25.1.76

38. Meiller TF, Silva A, Ferreira SM, Jabra-Rizk MA, Kelley JI, DePaola LG. Efficacy of Listerine® Antiseptic in reducing viral contamination of saliva. J Clin Periodontol. 2005;32(4):341–6. https://doi.org/10.1111/j.1600-051X.2005.00673.x

39. Mukherjee PK, Esper F, Buchheit K, Arters K, Adkins I, Ghannoum MA, et al. Randomized, double-blind, placebo-controlled clinical trial to assess the safety and effectiveness of a novel dual-action oral topical formulation against upper respiratory infections. BMC Infect Dis [Internet]. 2017;17(1):1–8. https://doi.org/10.1186/s12879-016-2177-8

40. Müller HD, Eick S, Moritz A, Lussi A, Gruber R. Cytotoxicity and Antimicrobial Activity of Oral Rinses *in Vitro*. Biomed Res Int. 2017;2017. https://doi.org/10.1155/2017/4019723

41. Eggers M, Koburger-Janssen T, Ward LS, Newby C, Müller S. Bactericidal and Virucidal Activity of Povidone-Iodine and Chlorhexidine Gluconate Cleansers in an In Vivo Hand Hygiene Clinical Simulation Study. Infect Dis Ther. 2018;7(2):235–47. https://doi.org/10.1007/s40121-018-0202-5

42. Elshafay A, Omran ES, Abdelkhalek M, El-Badry MO, Eisa HG, Fala SY, et al. Reporting quality in systematic reviews of *in vitro* studies: a systematic review. Curr Med Res Opin [Internet]. 2019;35(9):1631–41. https://doi.org/10.1080/03007995.2019.1607270

43. Slots J. Herpesviral-bacterial synergy in the pathogenesis of human periodontitis. Curr Opin Infect Dis. 2007;20(3):278–83. https://doi.org/10.1097/QCO.0b013e3280964da0

44. Bidra, A. S., Pelletier, J. S., Westover, J. B., Frank, S., Brown, S. M., & Tessema, B. Comparison of in vitro inactivation of SARS CoV-2 with hydrogen peroxide and povidone-iodine oral antiseptic rinses. Journal of Prosthodontics. 2020; 29(7), 599-603. https://doi.org/10.1111/jopr.13220

Roling of the funding source: The authors received no financial support for the research, authorship, and/or publication of this article.
Conflict of interest: The authors have no conflicts of interest to declare.